

Transgenic mice expressing both human apolipoprotein B and human CETP have a lipoprotein cholesterol distribution similar to that of normolipidemic humans

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Abstract Transgenic mice expressing both human apolipoprotein (apo) B and human cholesteryl ester transfer protein (CETP) have been developed. When fed a normal mouse chow diet, the apoB/CETP double transgenic animals had threefold higher serum CETP activity than humans and had human apoB levels that were similar to those of normolipidemic humans. When compared with nontransgenic mice, the total serum cholesterol levels in the female apoB/CETP transgenic animals were increased significantly. Serum HDL cholesterol levels were decreased significantly in both male and female apoB/CETP transgenic animals. The percentages of the total cholesterol within the HDL, LDL, and VLDL fractions of the apoB/CETP animals were approximately 30%, 65%, and 5%, respectively, similar to the distribution of cholesterol in the plasma of normolipidemic humans. **■** Thus, by expressing both human apoB and human CETP, the lipoprotein cholesterol distribution in the serum of a chow-fed mouse was transformed into one that resembles a human profile.—Grass, D. S., U. Saini, R. H. Felkner, R. E. Wallace, W. J. P. Lago, S. G. Young, and M. E. Swanson. Transgenic mice expressing both human apolipoprotein B and human CETP have a lipoprotein cholesterol distribution similar to that of normolipidemic humans. *J. Lipid Res.* 1995. **36**: 1082–1091.

Supplementary key words cholesteryl ester transfer protein • apolipoprotein B • transgenic mice • lipoprotein cholesterol distribution

Abundant genetic, epidemiologic, and experimental studies have established that apolipoprotein (apo) B and cholesteryl ester transfer protein (CETP) play key roles in human lipoprotein metabolism (1–3). ApoB-100, a 512-kD glycoprotein, is an important structural component of the triglyceride-rich very low density lipoproteins (VLDL) (2, 3). ApoB-100 is also the sole protein component of the cholesterol-rich low density lipoproteins (LDL) and is the ligand responsible for the removal of LDL by the liver. Plasma apoB and LDL cholesterol levels are directly re-

lated to the risk of developing coronary artery disease (CAD) (4). A specific mutation in the apoB gene that interferes with the ability of LDL to bind to the LDL receptor leads to elevated apoB and LDL cholesterol levels and premature atherosclerosis (5–7).

CETP is a 74-kD glycoprotein that mediates the distribution of neutral lipids, including triglycerides and cholesteryl esters, among different classes of lipoproteins (1, 8, 9). Genetic deficiency of CETP in humans results in profound changes in lipoprotein composition and metabolism (10, 11). Humans with complete CETP deficiency have increased levels of HDL cholesterol, increased size of HDL particles, and decreased cholesteryl esters in apoB-containing lipoproteins (12). It has been suggested that CETP deficiency could be anti-atherogenic and may be associated with longevity (11). Resistance to atherosclerosis with CETP deficiency would not be particularly surprising, in view of the well-established inverse relationship between high density lipoprotein (HDL) cholesterol levels and the risk of CAD (13).

The plasma lipoproteins of the normal laboratory mouse are significantly different from those of humans. Whereas normolipidemic humans have 20–30% of their total serum cholesterol in the HDL fraction, mice have approximately 80% and are resistant to the development of atherosclerotic lesions. On a diet rich in fats and cholesterol, C57BL/6 mice and certain other strains de-

Abbreviations: apo, apolipoprotein; CETP, cholesteryl ester transfer protein; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; CAD, coronary artery disease; PCR, polymerase chain reaction.

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velop a marked reduction in HDL cholesterol levels and are susceptible to atherosclerosis (14, 15). Most other mouse strains are resistant to atherosclerotic lesions under the same conditions (15). The resistance of mice to the development of atherosclerosis may, at least in part, relate to the fact that most strains of mice have low levels of apoB-containing lipoproteins (16) and the fact that mouse serum does not contain CETP activity (1).

Over the past several years, transgenic technology has been used to develop mice with altered lipoprotein distribution and metabolism (17, 18). Transgenic mice expressing CETP have been developed by several laboratories (19, 20). In one study, the presence of high levels of monkey CETP activity in these transgenic mice resulted in the redistribution of serum cholesterol from HDL to VLDL and LDL, thus lowering HDL cholesterol levels and raising VLDL and LDL cholesterol levels. These mice were shown to be more susceptible to the development of atherosclerotic lesions (21). More recently, transgenic mice expressing human apoB were developed (22, 23). These mice had plasma apoB-100 levels similar to those of normolipidemic humans. The human apoB-100 was found in the LDL fraction, and the LDL cholesterol levels were increased compared with nontransgenic littermates. However, when fed a chow diet, only approximately 40% of the serum cholesterol in the plasma of these apoB transgenic mice was contained in the LDL lipoprotein fractions. In addition to the CETP transgenic mice, other genetically modified mice, including mice homozygous for apoE null mutations (24, 25) and mice homozygous for an LDL receptor null mutation (26, 27), have been generated and shown to develop atherosclerotic lesions. The LDL receptor-negative mice had an increase in plasma LDL cholesterol, although the amount of HDL cholesterol still appeared to exceed the amount of LDL cholesterol (26). The apoE-negative mice had marked hypercholesterolemia, with most of the cholesterol located in the VLDL and IDL fractions (24, 25). At the current time, no mice have been produced that have a lipoprotein cholesterol distribution similar to that of normolipidemic humans (i.e., a ratio of LDL cholesterol to HDL cholesterol of approximately 2 to 1) when fed a normal chow diet.

In order to identify and evaluate new pharmaceuticals for the treatment of human lipid disorders, it would be desirable to have a convenient small laboratory animal model that has lipoprotein composition and metabolism similar to those of humans. Here we report the development of double transgenic mice expressing both human CETP and human apoB. These chow-fed double transgenic mice have a lipoprotein cholesterol distribution similar to that of normolipidemic humans, with an LDL/HDL cholesterol ratio of approximately 2 to 1.

MATERIALS AND METHODS

Generation of human CETP and apoB transgenic mice

A gene construct designed to express the human CETP gene was produced as follows. The human CETP cDNA was amplified by polymerase chain reaction (PCR) from a preparation of human liver cDNA (Clontech Laboratories, Inc., Palo Alto, CA) and inserted into pCR 1000 (a TA cloning vector; Invitrogen Corporation, San Diego, CA). This cDNA contained the complete coding region and 18 bases of 5' nontranslated sequence, but not the polyadenylation signal sequence. An approximately 1.75-kb PvuII-KpnI fragment (28) containing the human apoA-I promoter was placed into the KpnI and EcoRI sites of the vector, upstream from the 1.5-kb CETP cDNA sequence. A 0.56-kb SnaBI-BamHI fragment from pSVsport (Gibco-BRL Life Technologies, Inc., Grand Island, NY) encompassing the SV40 small t splice and polyadenylation signal sequence was placed downstream from the CETP coding sequence. SalI and XbaI were utilized to separate the resulting construct from plasmid sequences. The 3.8-kb insert fragment was isolated on an agarose gel and further purified on an Elutip column (Schleicher and Schuell, Keene, NH). This DNA was microinjected into (C57BL/6J × SJL) F2 hybrid zygotes.

Transgenic founders were identified by Southern or slot blot analysis using a ³²P-labeled KpnI-EcoRI fragment encompassing the CETP cDNA. For the Southern analysis, genomic DNA from potential founders was isolated, digested with KpnI, and electrophoresed on a 0.7% agarose gel. The genomic DNA from transgenic founder mice contained a 3.8-kb KpnI fragment that hybridized to the ³²P-labeled cDNA probe. The transgenic founders were bred to (C57BL/6J × SJL) F1 mice to produce G1 animals.

ApoB transgenic mice (line 1102) were generated using a 79.5-kb genomic fragment encompassing the human apoB gene as previously reported (22).

To produce the apoB/CETP double transgenic mice and additional CETP hemizygous mice, apoB hemizygous mice that had been backcrossed once to C57BL/6J were mated with CETP homozygous mice. Thus, the mice used in these experiments were on a mixed C57BL/6J, SJL background.

Serum isolation; cholesterol and triglyceride determinations

Mice were bled through the retroorbital plexus during the light cycle. The blood was centrifuged in an Eppendorf microfuge at 14,000 rpm for 10 min to isolate the serum. Standard enzymatic methodologies were used to determine total cholesterol (Sigma Chemical Co., St. Louis, MO), triglycerides (Boehringer Mannheim Bio-

chemicals, Indianapolis, IN), and HDL cholesterol (Sigma Chemical Co., St. Louis, MO). Human serum was isolated from a normolipidemic 35-year-old male. Total cholesterol, HDL cholesterol, and triglyceride levels were 134 mg/dl, 44 mg/dl, and 115 mg/dl, respectively.

Fast performance liquid chromatography size exclusion analysis

Fifty μ l of serum from individual mice or pooled serum from five mice was chromatographed on a Superose 6 column (Pharmacia Fine Chemicals, Piscataway, NJ) equilibrated with 10 mM Tris-Cl, pH 7.4, 0.15 M NaCl, 0.01% (w/v) EDTA, 0.02% (w/v) NaN_3 . The column was run at a flow rate of 0.5 ml/min. Fractions of 0.5 ml were collected. Aliquots (0.1 ml) from each fraction were assayed for cholesterol and triglyceride as described earlier.

Serum CETP activity determinations and western blot analysis

Serum CETP activity assays were performed using a commercial kit (Diagenescent Technologies, Inc., Yonkers, NY) or a modification of the procedure of Agellon et al. (19). Briefly, this modification was performed by incubating 2.5 μ l of serum with 0.867 μ g HDL (5000 cpm tritiated cholesterol) and 21.7 μ g LDL in 50 mM Tris, pH 7.4, 150 mM NaCl, 2 mM EDTA, 2 mM diethyl-*p*-nitrophenylphosphate. The incubation was adjusted to 50 μ l and incubated at 37°C for 4 h. After the incubation, the volume was adjusted to 550 μ l with TSE (50 mM Tris, pH 7.4, 150 mM NaCl, 2 mM EDTA). The LDL was then precipitated with 200 μ l of a standard precipitation reagent [heparin, 167 units, (Sigma Chemical Co., St. Louis, MO), 333 mM MnCl_2 , 13.3% BSA] on ice for

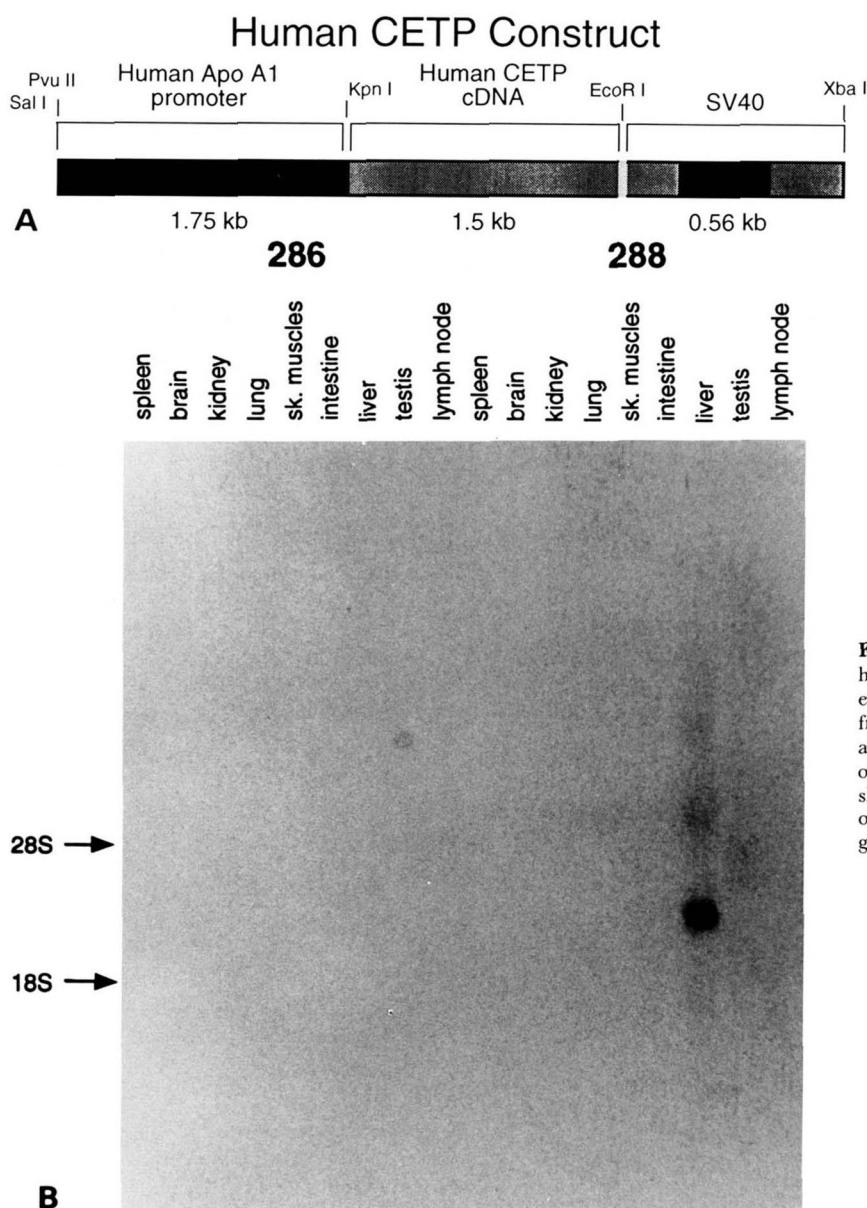


Fig. 1. A: CETP construct containing 1.75-kb of the human apoA-I promoter fused to a cDNA fragment encoding human CETP, which was linked to an SV40 fragment containing the small t intron and the polyadenylation signal sequence. B: Northern blot analysis of RNA isolated from the spleen, brain, kidney, lung, skeletal muscle, intestine, liver, testis, and lymph node of a hemizygous CETP animal (288) and a nontransgenic littermate (286).

5 min. After centrifugation in an Eppendorf microcentrifuge, the radioactivity remaining in the supernatant fluid was determined.

For western blot analysis, 50–120 μ l of mouse serum was passed over an immunoaffinity column constructed with the CETP-specific monoclonal antibody TP2 (29). This column was constructed using a commercial kit (Immunopure IgG Orientation Kit, Pierce, Rockford, IL). The bound antigen was eluted from the column in elution buffer (500 mM acetic acid), dried in a speed vacuum, and resuspended in 10 mM Tris, pH 8.0, 1 mM EDTA. The antigen was then electrophoresed on a Tris-glycine-SDS gel (Novex, San Diego, CA) and western blotted (30) using TP2 as the primary antibody and alkaline phosphatase-conjugated sheep anti-mouse IgG as the secondary antibody. The immunoreactive protein was visualized using Lumi-Phos 530 (Boehringer Mannheim Biochemicals, Indianapolis, IN).

RNA isolation and northern blot analysis

RNA was prepared by acid guanidinium thiocyanate-phenol-chloroform extraction (31) using an RNA STAT-60 kit (TEL-TEST "B", Inc., Friendswood, TX). Ten μ g of total RNA was subjected to electrophoresis on a 1% agarose formaldehyde gel and blotted onto Biotrans (ICN Biomedicals, Inc., Costa Mesa, CA). Blots were hybridized to the 1.5-kb KpnI-EcoRI human CETP cDNA probe.

ApoB quantitation

Total human apoB levels were determined by radial immunodiffusion (RID plates made by The Binding Site, San Diego, CA). Samples were quantitated using a human apoB standard curve. No cross-reactivity with murine apoB could be detected with serum from nontransgenic mice, CETP transgenic mice, or LDL-receptor knockout mice (26).

The relative amounts of human apoB-100 and apoB-48 were determined by western blot analysis using 125 I-labeled C1.4 antibody as previously described (22).

RESULTS

Development of CETP transgenic mice

To produce mice expressing both apoB and CETP, transgenic mice containing either human CETP or human apoB were developed and mated. The transgenic mice expressing human CETP were produced from (C57BL/6 \times SJL) F2 hybrid zygotes using the construct illustrated in Fig. 1A. The apoA-I promoter fragment was used to direct expression of the transgene in the liver. Seven founder transgenic mice were identified. Transgenic mice were bred to (C57BL/6J \times SJL) F1 mates to produce lines of transgenic offspring. Of these lines, two

contained detectable CETP activity in serum (data not shown). Line CETP28 had plasma CETP activity similar to that seen in humans. Line CETP4 had activity levels that were approximately threefold those observed in humans and was most extensively characterized. Western blot analysis of serum using a CETP-specific antibody demonstrated CETP in the serum from both lines of mice (data not shown). Northern analysis of RNA isolated from brain, intestine, kidney, liver, lung, lymph node, spleen, skeletal muscle, and testis indicated that the human CETP mRNA was expressed exclusively in the liver of line CETP4 animals (Fig. 1B).

Lipid levels in CETP transgenic mice

To determine the effect of CETP activity on lipid levels, total serum cholesterol levels, serum HDL cholesterol levels, and serum triglyceride levels were determined in the CETP4 mice and were compared with those of nontransgenic littermates. Total serum cholesterol levels were decreased 33% in hemizygous CETP4 males and 20% in hemizygous females when compared to nontransgenic mice (Table 1). HDL cholesterol levels in male CETP4 mice were 45% lower than those of nontransgenic littermates (Table 1). HDL levels in female transgenics were 27% lower than those of nontransgenic littermates (Table 1). Lipoprotein cholesterol profiles of pooled samples were determined by Superose 6 chromatography (Fig. 2). Consistent with the HDL levels measured by precipitation methods (Table 1), a lower percentage of the total cholesterol could be found in the HDL fractions when com-

TABLE 1. Serum cholesterol and triglyceride levels

Genotype	Sex	n	Total Cholesterol	HDL	Triglyceride
			mg/dl		
Non-TG	M	12	106 \pm 6	73 \pm 3	142 \pm 31
	F	12	76 \pm 6	51 \pm 2	119 \pm 23
CETP	M	11	71 \pm 3 ^a	40 \pm 3 ^a	152 \pm 10
	F	14	61 \pm 2 ^b	37 \pm 2 ^a	96 \pm 10
ApoB ^c	M	11	112 \pm 7	53 \pm 2 ^a	149 \pm 14
	F	6	164 \pm 21 ^a	49 \pm 5	142 \pm 30
ApoB \times CETP	M	12	113 \pm 7	23 \pm 2 ^a	240 \pm 16 ^d
	F	12	127 \pm 4 ^a	30 \pm 2 ^a	146 \pm 16

See Methods for details concerning analysis. Data are expressed as means \pm SEM. Differences in total cholesterol levels, HDL levels, and triglyceride levels between transgenic groups and nontransgenic controls were evaluated using Student's *t* test. Differences were considered significant when *P* < 0.05; n, number of animals.

^a*P* < 0.0001.

^b*P* = 0.013.

^cSix apoB males and three apoB females were generated from an intercross of apoB hemizygous animals. These progeny were determined to be hemizygous by Southern blot analysis.

^d*P* = 0.01.

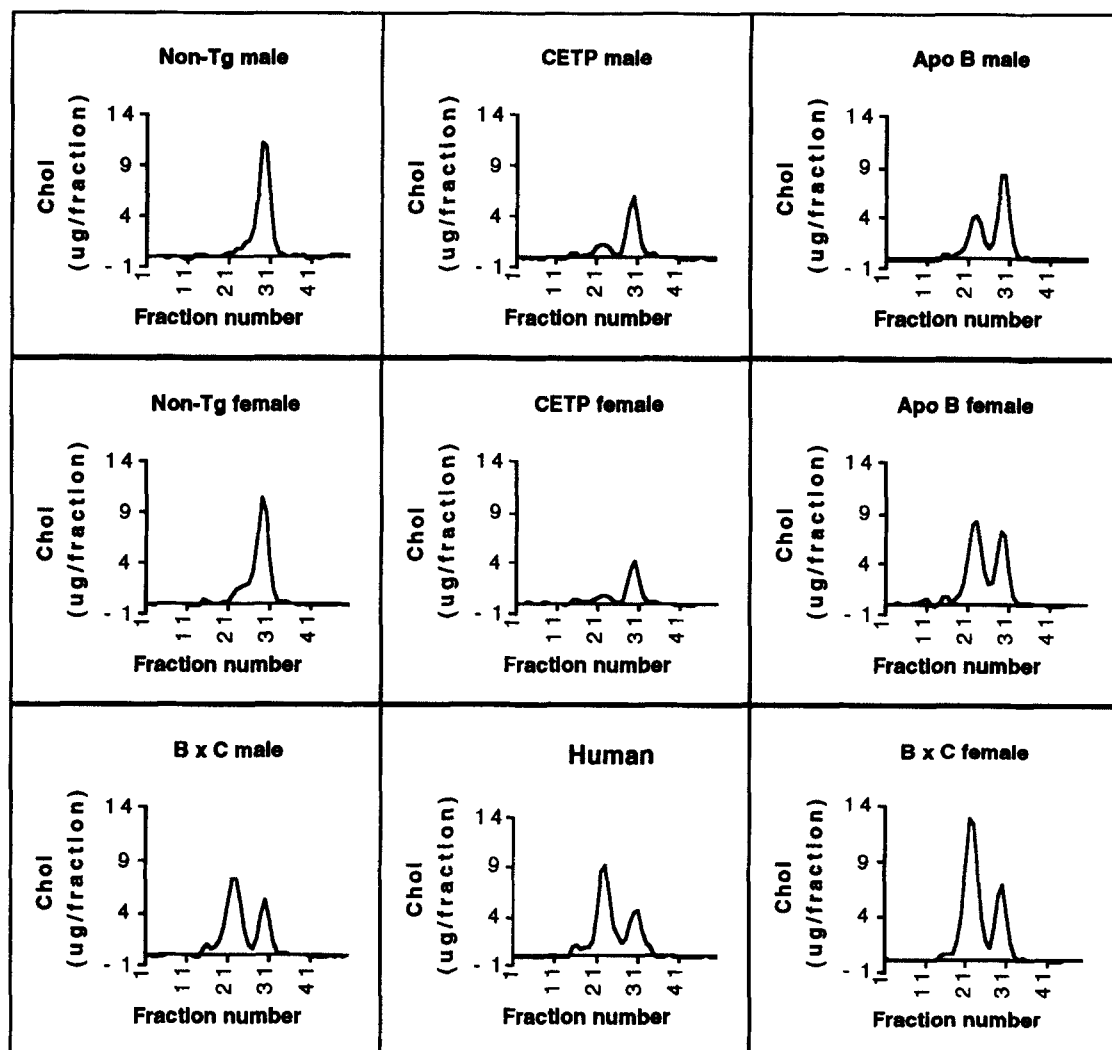


Fig. 2. Lipoprotein cholesterol profiles of pooled serum samples (five mice each) from male and female nontransgenic (non-tg), CETP transgenic (CETP), apoB transgenic (ApoB), and apoB/CETP double transgenics (B×C). A sample from a human male is shown for comparison.

pared with nontransgenic littermates (Fig. 2, Table 2). This decrease in the percentage of total cholesterol in HDL was not as dramatic as the absolute decrease in HDL cholesterol levels. This was due to the fact that the decrease in HDL cholesterol was not accompanied by as dramatic an increase of LDL and VLDL cholesterol. To assess the variability of the phenotype within a group, lipoprotein cholesterol profiles were also determined for individual mice. The results of these studies (Table 3) indicated only slight variability between animals within each group.

Serum triglyceride levels were not significantly different when CETP4 mice and nontransgenic mice were compared (Table 1). Lipoprotein triglyceride profiles were determined by Superose 6 chromatography (Fig. 3). The profiles for both CETP4 mice and nontransgenic mice

TABLE 2. Percentage of total cholesterol in HDL, LDL, and VLDL (pooled samples)

Genotype	Sex	HDL	LDL	VLDL
%				
Non-Tg	M	85	13	2
	F	79	19	2
CETP	M	73	22	5
	F	71	21	7
ApoB	M	59	38	3
	F	41	56	4
B X C	M	33	62	6
	F	30	67	4
Human	M	34	60	6

The percentage of total cholesterol in HDL (fractions 27–40), LDL (fractions 19–26), and VLDL (fractions 14–18) was determined from the FPLC cholesterol profiles in Fig. 2.

TABLE 3. Percentage of total cholesterol in HDL, LDL, and VLDL (individual males)

Genotype	n	HDL	LDL	VLDL
		%		
Non-Tg	3	84 ± 3	13 ± 2	2 ± 1
CETP	5	65 ± 2	24 ± 2	11 ± 1
ApoB	4	68 ± 2	31 ± 1	2 ± 1
ApoB × CETP	6	25 ± 2	64 ± 3	11 ± 2

FPLC cholesterol profiles from individual males (not shown) were analyzed as in Table 2; n, number of profiles analyzed. Data are expressed as means ± SEM.

had similar percentages of triglyceride in VLDL, LDL, and HDL (Table 4 and Table 5).

Lipid levels in human apoB transgenic mice

Human apoB-100 transgenic mice were produced by introducing a 79.5-kb fragment containing the entire apoB

gene (including 19-kb of the 5' flanking sequence and 17.5-kb of the 3' flanking sequence) into a (C57BL/6 × SJL) F2 hybrid embryo (22). In the line used in this study, line 1102 (22), the total apoB levels were similar to those observed in normal human serum (Table 6). The total cholesterol levels in male transgenic animals were slightly, but not significantly, higher than those in nontransgenic males (Table 1). Total cholesterol levels in female transgenic animals, however, were approximately twofold higher than those in female nontransgenic animals (Table 1). In both male and female apoB transgenic mice, there was a significant increase in the amount of cholesterol in the LDL compared with nontransgenic mice, as determined by Superose 6 size exclusion chromatography (see Fig. 2 and Tables 2 and 3). In the serum of males, 38% of the cholesterol was within the LDL, compared to 13% in the nontransgenic mice. In females, 50% of cholesterol was within the LDL, compared to 19% for the nontransgenic mice. In males, the HDL cholesterol levels were

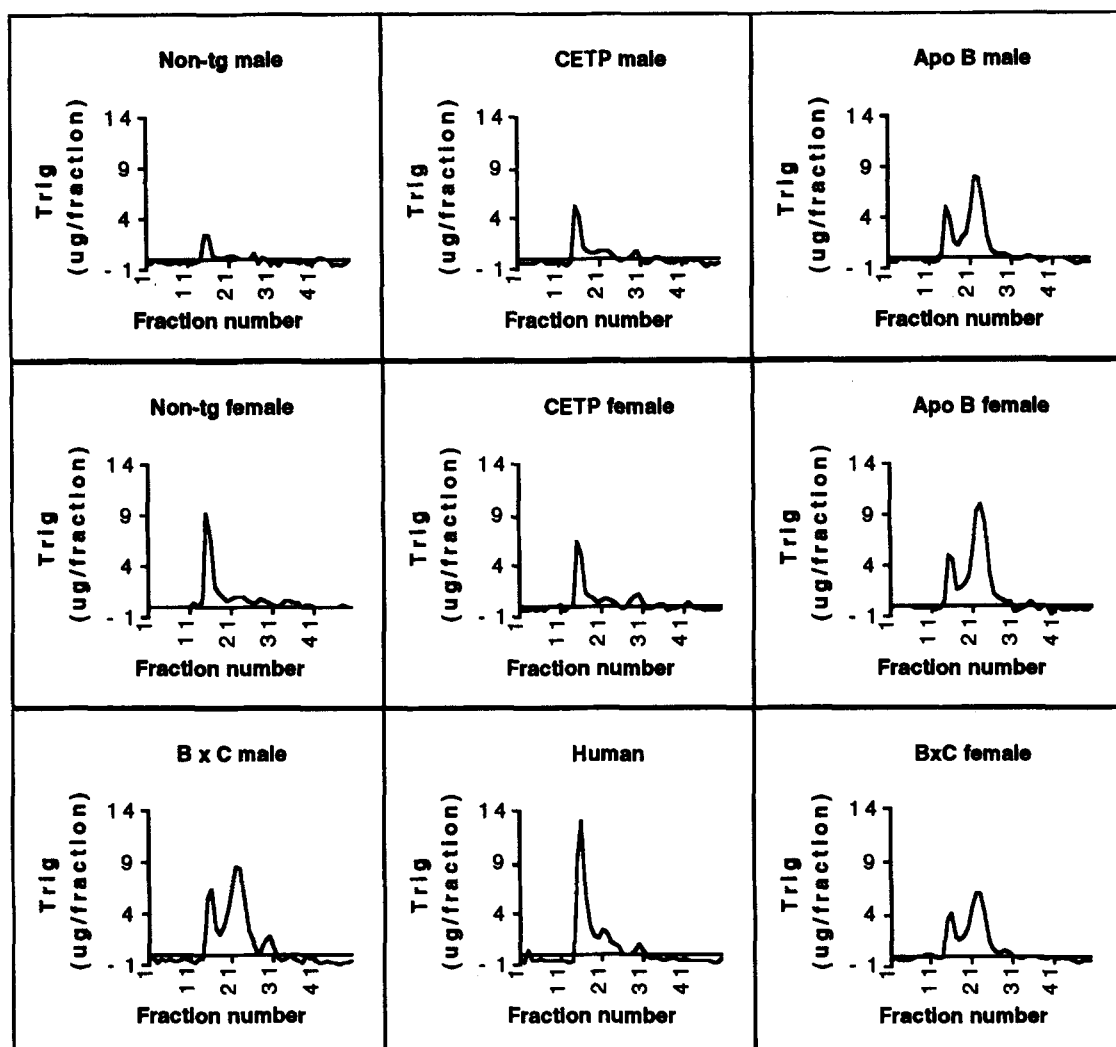


Fig. 3. Lipoprotein triglyceride profiles of pooled serum samples (five mice each) from male and female nontransgenic (non-tg), CETP transgenic (CETP), apoB transgenic (ApoB), and apoB/CETP double transgenics (B×C). A sample from a human male is shown for comparison.

TABLE 4. Percentage of total triglyceride in HDL, LDL, and VLDL (pooled samples)

Genotype	Sex	HDL	LDL	VLDL
%				
Non-Tg	M	10	30	60
	F	19	21	60
CETP	M	17	28	55
	F	22	20	57
ApoB	M	7	68	25
	F	3	74	23
B × C	M	12	62	26
	F	8	66	26
Human	M	8	29	63

The percentage of total triglyceride in HDL (fractions 27–40), LDL (fractions 19–26), and VLDL (fractions 14–18) were determined from the FPLC profiles in Fig. 3.

reduced 27% compared with those of nontransgenic controls (Table 1). In females, no significant HDL reduction was observed compared with nontransgenic controls (Table 1). Consistent with this data, in both males and females, the percentage of total cholesterol carried by HDL particles was decreased compared with the nontransgenic animals of the same sex (59% vs. 85% in males, 41% vs. 79% in females; see Fig. 2 and Table 2). Lipoprotein cholesterol profiles for individual males demonstrated only slight variability in lipoprotein cholesterol distribution among different human apoB transgenic animals (Table 3).

Serum triglyceride levels were not significantly different between apoB transgenic mice and nontransgenic mice (Table 1). As shown previously (22), however, the LDL of the human apoB transgenic mice were enriched in triglycerides compared to nontransgenic mice. As shown in Table 4, the human apoB transgenic mice had 68% (males) and 74% (females) of their total triglyceride in LDL, as compared with 30% (male) and 21% (female) in nontransgenic mice.

TABLE 5. Percentage of total triglyceride in HDL, LDL, and VLDL (individual males)

Genotype	n	HDL	LDL	VLDL
%				
Non-Tg	3	19 ± 5	38 ± 7	44 ± 2
CETP	5	21 ± 3	22 ± 3	57 ± 3
ApoB	4	15 ± 2	65 ± 4	20 ± 5
ApoB × CETP	6	13 ± 2	59 ± 4	28 ± 3

FPLC triglyceride profiles from individual males (not shown) were analyzed as in Table 4; n, number of profiles analyzed. Data are expressed as means ± SEM.

TABLE 6. Human apoB levels

Genotype	Sex	n	ApoB
mg/dl			
ApoB ^a	M	10	112 ± 7
	F	6	127 ± 7
ApoB × CETP	M	10	137 ± 9
	F	11	159 ± 5
Human	M		145

Data are expressed as means ± SEM. Differences in apoB among groups were evaluated using Student's *t* test. Difference between apoB males and apoB females, *P* = 0.19; difference between apoB males and apoB × CETP females, *P* = 0.047; difference between apoB × CETP males and apoB × CETP females, *P* = 0.033; and difference between apoB females and apoB × CETP females, *P* = 0.0016.

^aSix apoB males and three apoB females were generated from an intercross of apoB hemizygous animals. These progeny were determined to be hemizygous by Southern blot analysis.

Lipid levels in apoB/CETP transgenic mice

Mice expressing both the human CETP and the human apoB transgenes were obtained by crossing homozygous CETP4 mice with hemizygous apoB transgenic mice. In males, total cholesterol levels in the double transgenics were similar to those of nontransgenic mice and transgenic animals expressing only human apoB-100 (Table 1). In female mice, however, total cholesterol levels in the double transgenic mice were 67% higher than those of nontransgenic mice, and 22% lower than serum cholesterol levels in animals expressing only human apoB (Table 1). ApoB levels in the apoB/CETP animals were increased compared with apoB single transgenics (Table 6). The serum apoB-100 to apoB-48 ratios in the apoB transgenic mice and the apoB/CETP transgenic mice were greater than 5 as determined by quantitative western blot analysis (data not shown).

Superose 6 cholesterol distribution profiles from the double transgenic mice were similar to the cholesterol distribution profile of a normolipidemic human (Fig. 2 and Tables 2 and 3). The percentage of total cholesterol within the LDL was much higher in the double transgenics than in mice expressing human apoB-100 alone or in mice expressing human CETP alone (Fig. 2 and Tables 2 and 3). In addition, HDL cholesterol levels were reduced in these double transgenic animals when compared with nontransgenic animals or animals expressing human apoB or CETP alone. The serum levels of CETP activity in the double transgenic animals were similar to those in CETP single transgenic mice (data not shown).

Lipoprotein triglyceride determinations were also performed in double transgenic mice. Serum triglyceride levels were increased by 69% in double transgenic males compared with nontransgenic males but were not significantly different between the female double transgenics and nontransgenics (Table 1). As shown previously (22)

and in Fig. 3 and Tables 4 and 5, the triglyceride distribution profiles of serum from mice expressing only the human apoB transgene differed from that observed with human serum in that there were high levels of triglyceride in the LDL. In this study, the triglyceride distribution profile for mice expressing both human apoB and CETP was determined. The double transgenic mice also had significant levels of triglyceride in LDL (62% in males, 66% in females; Fig. 3 and Tables 4 and 5). These levels were not significantly different from those observed in transgenic mice expressing only human apoB (68% in males, 74% in females). In humans, as well as nontransgenic mice and CETP single transgenic mice, most of the triglycerides are contained within the VLDL fractions.

DISCUSSION

Many laboratories have used transgenic technology to develop mouse models for the study of atherosclerosis and lipoprotein metabolism. In this report, we have examined the effect of CETP activity on the lipoprotein cholesterol profile of transgenic mice expressing high levels of human apoB. As demonstrated previously by others (20), the presence of plasma CETP activity in mice redistributes cholesterol from HDL to VLDL and LDL, thus lowering HDL cholesterol and elevating LDL and VLDL cholesterol. Our CETP transgenic mice expressed high levels of CETP activity. These animals had lower HDL cholesterol levels and higher VLDL-LDL cholesterol levels than nontransgenic animals. Similarly, the serum of the apoB/CETP transgenic mice had a higher percentage of total serum cholesterol in the combined LDL and VLDL fractions than in transgenic mice expressing human apoB alone. Although these results are probably due to redistribution of lipids in the plasma compartment (19, 20), the increase in VLDL and LDL cholesterol in mice expressing CETP could, at least in part, be a result of LDL-receptor down-regulation (32).

The percentage of total cholesterol in the HDL fraction was significantly lower in the double transgenic animals than in either nontransgenic, apoB single transgenic, or CETP single transgenic mice. The marked decrease in HDL cholesterol in the apoB/CETP animals might, at least in part, be due to the increased number of apoB-containing lipoproteins, which serve as acceptors for the CETP-mediated transfer of HDL cholesterol. However, it is important to note that human apoB transgenic animals tended to have lower serum HDL levels than nontransgenic mice. Although the metabolism of apoB- and that of apoA-I-containing lipoproteins are known to be interrelated, the mechanism by which human apoB expression lowers HDL cholesterol levels is not known.

In an initial report describing the line 1102 human apoB transgenic mice (22), the total cholesterol levels were

increased in a mixture of male and female apoB transgenic animals when compared with nontransgenic littermates. In the current study, we demonstrated that female transgenic mice expressing either human apoB alone or both human apoB and CETP had higher total serum cholesterol levels than their nontransgenic littermates. Interestingly, the male apoB transgenic mice had a slight but not statistically significant increase in total serum cholesterol compared with nontransgenic littermates. The female transgenic mice expressing either human apoB alone or both human apoB and CETP tended to have higher serum levels of total cholesterol than the male animals, although these differences were not statistically significant. Consistent with this result and with the FPLC lipoprotein cholesterol profiles, the females had higher levels of human apoB than male mice.

The triglyceride distribution profiles in mice expressing only the apoB transgene showed substantial amounts of triglyceride in the LDL. This result is in contrast to the triglyceride distribution profile of normal human serum, where the triglycerides are predominantly in the VLDL. One potential explanation for the high levels of triglyceride in LDL is that in the absence of CETP activity, the triglycerides on apoB-containing lipoproteins are not transferred to HDL in exchange for cholesteryl ester. However, as shown here, mice expressing both human apoB and CETP had triglyceride profiles similar to those seen in mice expressing human apoB alone. Thus, apoB expression causes the accumulation of triglyceride-rich LDL, and the addition of CETP activity does not significantly alter this. One possible explanation for the triglyceride-rich LDL is that these may represent nascent lipoproteins synthesized by the liver. In the setting of markedly increased apoB production by the liver, it is quite conceivable that the nascent lipoproteins could have the size and density of LDL.

The percentage of total cholesterol in HDL, LDL, and VLDL in mice expressing both human CETP and apoB-100 was similar to that of a normolipidemic human (33%, 62%, and 6%, respectively, in male mice; 30%, 67%, and 4%, respectively, in female mice; 34%, 60% and 6%, respectively, in a human male). This lipoprotein cholesterol profile was significantly different from that of nontransgenic, human apoB transgenic, and CETP transgenic mice, in which most of the cholesterol was found in the HDL fractions. We believe that these double transgenic animals may prove to be a useful model for testing pharmaceutical compounds designed to alter the distribution of cholesterol among different classes of lipoproteins. Further studies will be required to determine clearance rates for the various lipoproteins in the transgenic animals, and to determine the effects of reference pharmaceuticals on lipoprotein levels and lipoprotein metabolism in these animals.

It has been reported that CETP transgenic mice have increased susceptibility to developing atherosclerosis on a high-fat diet (21). This is also true for the human apoB transgenic mice on a high-fat diet (33). In both of these transgenic models, the increased susceptibility to atherosclerosis was observed under the conditions of a high-fat diet, which has the effect of lowering HDL cholesterol levels. In future studies, it will be particularly interesting to compare the susceptibility of the CETP, the apoB, and the apoB/CETP mice to the development of atherosclerotic lesions. ■

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