ApoA-II expression in CETP transgenic mice increases VLDL production and impairs VLDL clearance

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Abstract **Apolipoprotein (apo)A-II is a major high density lipoprotein (HDL) protein; however, its role in lipoprotein metabolism is largely unknown. Transgenic (Tg) mice that overexpress human apoA-II present functional lecithin: cholesterol acyltransferase deficiency, HDL deficiency, hypertriglyceridemia and, when fed an atherogenic diet, increased non-HDL cholesterol and increased susceptibility to atherosclerosis. In contrast to humans, mice do not present cholesteryl ester transfer protein (CETP) activity in plasma. To study the in vivo interaction of these two proteins, we crossbred human apoA-II and CETP-Tg mice. CETP×apoA-II-Tg mice fed an atherogenic diet, compared with CETP-Tg mice presented a 2-fold decrease in HDL cholesterol and a quantitatively similar increase in total plasma cholesterol and percentage of free cholesterol, non-HDL cholesterol, and free fatty acids, together with a remarkable 112-fold increase in plasma triglycerides. Plasma triglycerides in CETP×apoA-II-Tg mice were mainly associated with very low density lipoproteins (VLDL), which were also enriched in protein content, and resulted from a combination of higher production rate compared with both of their progenitors and non-Tg control mice, and decreased catabolism compared only with CETP-Tg mice. These results show CETP×apoA-II-Tg mice to be a good model with which to study mechanisms leading to VLDL overproduction and suggest that CETP and, in particular apoA-II, may play a role in the regulation of VLDL metabolism.** —Escolà-Gil, J. C., J. Julve, À. Marzal-Casacuberta, J. Ordóñez-Llanos, F. González-Sastre, and F. Blanco-Vaca. **ApoA-II expression in CETP transgenic mice increases VLDL production and impairs VLDL clearance.** ***J. Lipid Res.*** **2001.** 42: **241–248.**

Supplementary key words atherosclerosis • cholesteryl ester transfer protein • high density lipoprotein • hypertrygliceridemia • very low density lipoprotein • familial combined hyperlipidemia

One method of classifying high density lipoprotein (HDL) particles is according to the content of their major apolipoproteins, apolipoprotein (apo)A-I and apoA-II. The antiatherogenic role of apoA-I has been clearly established in transgenic (Tg) mice (1). Mouse and human apoA-II expression in Tg mice caused, respectively, increased atherosclerosis when the animals were placed on a regular chow diet (2) and inhibition of the atherosclerosis protection shown by human apoA-I-Tg mice when fed a high cholesterol high fat (atherogenic) diet (3). We expressed different levels of human apoA-II in mice to further study the effects of human apoA-II on HDL metabolism and atherosclerosis. When fed an atherogenic diet, Tg mice overexpressing human apoA-II showed a marked reduction in apoA-I levels and lecithin:cholesterol acyltransferase (LCAT) reactivity; increased plasma concentration of cholesterol and triglycerides in very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), and low density lipoprotein (LDL); and increased susceptibility to atherosclerosis (4–6). Also, we have recently demonstrated that overexpression of human apoA-II in apoE-deficient mice causes combined hyperlipidemia by increasing VLDL triglyceride production (7).

Cholesteryl ester transfer protein (CETP) is also involved in lipoprotein metabolism. The action of this protein results in a heteroexchange between HDL cholesteryl ester and VLDL or chylomicron triglycerides (8). CETP plays an important, but complex, role in atherogenesis. Genetic CETP deficiency raises HDL cholesterol and apoA-I levels and decreases LDL cholesterol and apoB, and is therefore suspected of inducing protection against coronary atherosclerosis (8). However, several studies have shown patients with low plasma CETP levels to have a moderate increase in the risk of coronary heart disease (9, 10). Introduction of human or primate CETP transgene in mice resulted in reduced HDL cholesterol and apoA-I lev-

Abbreviations: apo, apolipoprotein; CETP, cholesteryl ester transfer protein; FCHL, familial combined hyperlipidemia; FFA, free fatty acid; HDL, high density lipoprotein; IDL, intermediate density lipoprotein; LCAT, lecithin:cholesterol acyltransferase; LDL, low density lipoprotein; VLDL, very low density lipoprotein.

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els (11, 12) and increased early aortic atherosclerotic lesions in response to an atherogenic diet (13, 14). Conversely, the expression of CETP-Tg mice in hypertriglyceridemic apoC-III-Tg or in LCAT-Tg mice reduced the area of atherosclerotic lesions (14, 15).

The functional role of human apoA-II is still largely unknown and, in contrast to humans, mice do not present CETP activity in plasma. To gain insight into the in vivo effects of the human apoA-II-CETP interaction, we studied lipoprotein metabolism and atherosclerosis susceptibility in control mice, human apoA-II-Tg mice, CETP-Tg mice, and double CETP×apoA-II-Tg mice.

MATERIALS AND METHODS

Mice

Human apoA-II-Tg mice (line 11.1) and cynomolgus monkey CETP-Tg mice (line UCTP-20) were created in the C57BL/6 background as previously described (4, 12). These CETP-Tg mice presented about 8-fold higher CETP activity in plasma than normolipidemic humans as well as reduced HDL cholesterol and increased atherosclerosis susceptibility (12, 13). CETP×apoA-II-Tg mice were obtained by crossbreeding the resulting F1 of the two groups. Also, apoA-II-Tg, CETP-Tg, and control C57BL/6 mice were used in these studies. Mice between 8 and 12 weeks old were fed a regular chow diet (Rodent Toxicology Diet, Universa G. J. B&K, N. Humberside, UK) or an atherogenic diet containing cholate (TD 88051, Harlan Teklad, Madison, WI, of which the composition was 75% mouse chow diet, 7.5% cocoa butter, 1.25% cholesterol, and 0.5% sodium cholate) for 24 weeks and were housed in a temperature-controlled (20°C) room with a 12-hour light/dark cycle and food and water ad libitum. All animal procedures complied with published recommendations for the use of laboratory animals (16).

Plasma lipids, lipoproteins, and apolipoproteins

The methods used for plasma lipid analyses in these mice have been described in detail elsewhere (4–7, 17). Plasma human apoA-II concentrations were measured using a commercial single radial immunodiffusion method (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan).

Enzyme activities

LCAT activity toward endogenous lipoproteins labeled with radioactive cholesterol was measured as previously reported (4). CETP activity was measured as the transfer of labeled cholesteryl oleate from HDL to LDL, using lipoproteins isolated from pools of human normolipidemic individuals and mouse plasma as source of CETP. There were only two changes with respect to the method described to measure human CETP (18). First, we used 10 μl of mouse plasma instead of 20 μl of human plasma and, second, we used a precipitating agent to isolate HDL (17) instead of fast protein liquid chromatography (FPLC). Rates of labeled cholesteryl oleate transfer from HDL to LDL were linear up to 1,500 μM/h. Lipoprotein lipase (LPL) and hepatic lipase (HL) activities against exogenous substrates were measured in postheparin plasmas [5 μl of plasma was used for each of these assays; these plasmas were obtained after 10 min of an intraperitoneal injection of lithium heparin (60 IU/kg of body mass)] using a radiolabeled glycerol [^{3}H]triolein emulsion (Amersham Life Science, Bristol, UK) (19). Intra- and interassay variation of these methods were <4% and <9%, respectively.

Liver lipid content

Liver lipids were extracted with isopropyl alcohol–hexane, dried with nitrogen, and reconstituted with isopropyl alcohol 0.5% sodium cholate prior to lipid measurements (7).

In vivo removal of triglyceride radioactivity after injection of radiolabeled VLDL

The VLDL fraction isolated by ultracentrifugation from each type of mouse was radiolabeled with [^{3}H]triolein exactly as described (20) and 85–90% of the radioactive label was confirmed as bound to VLDL. Approximately 750,000 cpm were injected into fasted mice. Serial blood samples were collected in tubes containing iodoacetate (40 mM) to inhibit CETP activity (21), and plasma [^{3}H] radioactivity was counted. The average radioactivity observed 2 min after injection was defined as 100% of injected radioactivity. Fractional catabolic rate (FCR) was calculated using the reciprocal area under the curve. The amount of radioactivity in the lipoprotein fractions was determined by separation on agarose gel, and lipid extraction was performed as described (22).

In vivo triglyceride production rate

Hepatic triglyceride production rates in plasma were measured as described (23). Briefly, mice were bled to measure baseline plasma triglyceride concentration. Anesthetized mice were injected intravenously with Triton WR-1339 (Sigma, St. Louis, MO) at a dose of 500 mg/kg dissolved in a 15% solution of 0.9% NaCl. Blood was collected after Triton injection and plasma triglycerides were measured and compared with baseline results.

Evaluation of atherosclerosis

The heart and proximal aorta were removed, embedded in OCT compound (Tissue-Tek, Sakura Finetechnical Co., Ltd., Tokyo, Japan), sectioned, stained, and lesion areas quantified as previously described using four sections per animal (5, 7).

Statistical analysis

All values are given as mean ± standard error. One-way analysis of variance was used to compare differences between groups. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Plasma lipids and enzyme activities in mice fed a chow diet

Compared with control mice on a chow diet, CETP-Tg mice presented hypocholesterolemia, hypoalphalipoproteinemia, and a moderate increase in non-HDL cholesterol, whereas apoA-II-Tg mice presented similar hypocholesterolemia and hypoalphalipoproteinemia, but increased percentage of free cholesterol and moderate hypertriglyceridemia (**Table 1**). Expression of human apoA-II in CETP-Tg mice resulted in a further decrease in HDL cholesterol and a reciprocal increase in non-HDL cholesterol. CETP×apoA-II-Tg mice also displayed increased percentage of free cholesterol and free fatty acids (FFA), and pronounced hypertriglyceridemia compared with their progenitors (Table 1). Interestingly, plasma levels of human apoA-II were only 57.5% in CETP×apoA-II-Tg mice compared with apoA-II-Tg mice. CETP×apoA-II-Tg mice showed slightly lower CETP activity compared with CETP-Tg mice (Table 1). LCAT activity in apoA-II-Tg and CETP×apoA-

TABLE 1. Plasma lipid and protein concentrations in overnight fasted control, CETP-Tg, apoA-II-Tg, and CETP×apoA-II-Tg mice fed a regular chow regular diet for 24 weeks

	Control	CETP-Tg	ApoA-II-Tg	CETP×ApoA-II-Tg
Number of animals	17	15	12	14
Males/females	8/9	5/10	5/7	6/8
Lipids				
Total cholesterol	78 ± 2	49 ± 1[a]	49 ± 3[a]	45 ± 4[a]
HDL cholesterol	69 ± 3	32 ± 1[a]	32 ± 3[a]	13 ± 1[a,b,c]
% Free cholesterol	18 ± 1	20 ± 1	37 ± 3[a]	46 ± 2[a,b,c]
Triglycerides	17 ± 1	14 ± 1	29 ± 2[a]	104 ± 23[a,b,c]
HDL triglycerides	ND	3 ± 1	5 ± 1	7 ± 1[b,c]
FFA	33 ± 3	37 ± 1	35 ± 1	42 ± 1[a,b,c]
Proteins				
Human apoA-II	0 ± 0	0 ± 0	73 ± 6[a]	42 ± 2[a,b,c]
CETP (μM/h)	<10	1,183 ± 36[a]	<10	1,001 ± 43[a,b,c]
LCAT (μM/h)	132 ± 13	115 ± 13	16 ± 4[a]	28 ± 7[a,b]
LPL (U/ml)[d]	93 ± 13	111 ± 19	106 ± 32	106 ± 23
HL (U/ml)[d]	75 ± 10	66 ± 5	79 ± 13	55 ± 2

Unless otherwise specified, results are expressed as mg/dl and as mean ± SEM. CETP-Tg and ApoA-II-Tg mice were not statistically compared. Statistical significance was determined by analysis of variance. LPL, lipoprotein lipase; HL, hepatic lipase; ND, not determined.

[a] Significantly different ($P < 0.05$) from control mice.

[b] Significantly different ($P < 0.05$) from CETP-Tg mice.

[c] Significantly different ($P < 0.05$) from ApoA-II-Tg mice.

[d] Lipolytic activities were determined from six mice in each group.

II-Tg mice was, respectively, only 12.1% and 21.2% that of control mice, whereas LCAT activity in CETP-Tg mice did not differ significantly from that of control mice (Table 1). Postheparin LPL and HL activities did not differ among the different groups of mice (Table 1). No gender differences were found in any of the parameters studied.

Plasma lipids and enzyme activities in mice fed an atherogenic diet

Compared with control mice fed an atherogenic diet, CETP-Tg mice presented hypoalphalipoproteinemia and a mild increase in FFA (**Table 2**). ApoA-II-Tg mice showed several expected effects, that is, hypercholesterolemia and increased levels of non-HDL cholesterol, free cholesterol, total triglyceride (6), and FFA (24) compared with control and CETP-Tg mice. CETP×apoA-II-Tg mice, compared with CETP-Tg mice and apoA-II-Tg mice, respectively, presented increased total cholesterol, increased non-HDL cholesterol, increased FFA levels, a marked increase in triglycerides (112-fold and 7.8-fold), increased HDL-triglycerides (13-fold and 3.3-fold) with no change in percentage of free cholesterol (compared with apoA-II-Tg mice), and a reduction in HDL cholesterol levels compared with both progenitors (Table 2). ApoA-II-Tg mice showed the

TABLE 2. Plasma lipid and protein concentrations in overnight fasted control, CETP-Tg, ApoA-II-Tg, and CETP×ApoAII-Tg mice fed an atherogenic diet for 24 weeks

	Control	CETP-Tg	ApoA-II-Tg	CETP×ApoAII-Tg
Number of animals	22	19	25	17
Males/females	12/10	9/10	12/13	8/9
Lipids				
Total cholesterol	203 ± 12	200 ± 9	256 ± 20[a]	396 ± 62[a,b,c]
HDL cholesterol	63 ± 4	49 ± 2[a]	64 ± 6	24 ± 5[a,b,c]
% Free cholesterol	19 ± 1	22 ± 1	33 ± 1[a]	36 ± 2[a,b]
Triglycerides	7 ± 1	5 ± 2	72 ± 19[a]	561 ± 141[a,b,c]
HDL triglycerides	ND	1 ± 1	4 ± 1	13 ± 2[b,c]
FFA	27 ± 1	31 ± 1[a]	39 ± 2[a]	59 ± 6[a,b,c]
Proteins				
Human apoA-II	0 ± 0	0 ± 0	111 ± 7[a]	107 ± 9[a,b]
CETP (μM/h)	<10	1,059 ± 44[a]	<10	1,010 ± 70[a,c]
LCAT (μM/h)	176 ± 21	107 ± 12[a]	44 ± 6[a]	48 ± 4[a,b]
LPL (U/ml)[d]	90 ± 11	121 ± 8	104 ± 22	105 ± 3
HL (U/ml)[d]	70 ± 7	85 ± 10	72 ± 6	76 ± 13

Unless otherwise specified, results are expressed as mg/dl and as mean ± SEM. CETP-Tg and ApoA-II-Tg mice were not statistically compared. Statistical significance was determined by analysis of variance. LPL, lipoprotein lipase; HL, hepatic lipase; ND, not determined.

[a] Significantly different ($P < 0.05$) from control mice.

[b] Significantly different ($P < 0.05$) from CETP-Tg mice.

[c] Significantly different ($P < 0.05$) from ApoA-II-Tg mice.

[d] Lipolytic activities were determined from six mice in each group.

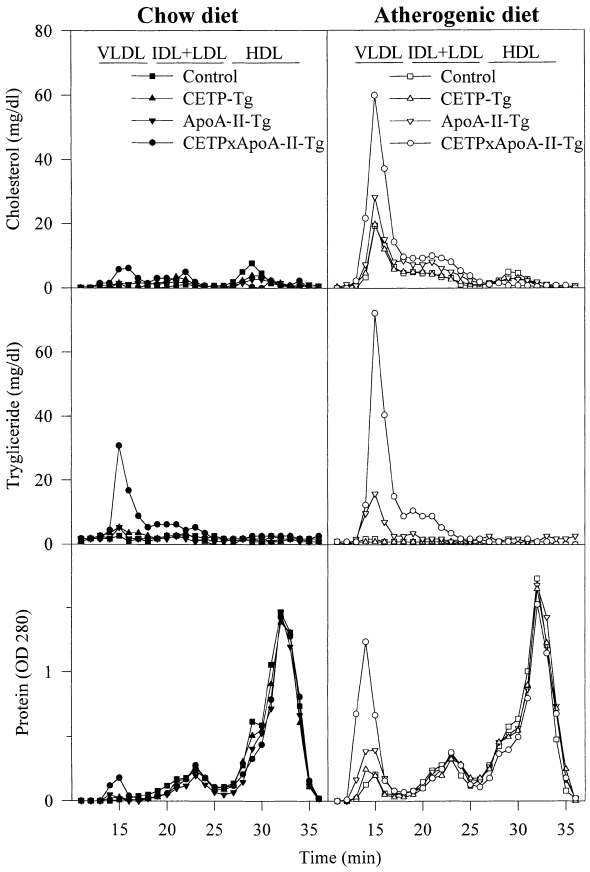

Fig. 1. Plasma lipoprotein profiles of each group of mice fed a regular chow diet or an atherogenic diet. Pooled plasmas (200 μl) of six to eight overnight fasted mice from each group were isolated by FPLC. Fractions were collected and assayed for cholesterol, triglyceride, and protein. The positions of elution of the VLDL, IDL/LDL, and HDL are represented by horizontal lines.

highest plasma concentration of the human protein when fed an atherogenic diet (Tables 1 and 2) and, in contrast with the findings in mice fed a chow diet, CETP×apoA-II-Tg and apoA-II-Tg mice presented similar levels of human apoA-II and CETP activity. LCAT activities were 61%, 25%, and 27% in CETP-Tg, apoA-II-Tg mice, and CETP×apoA-II-Tg mice, respectively, compared with the activity found in non-Tg control mice fed an atherogenic diet (Table 2). Postheparin LPL and HL activities did not differ among the different groups of mice (Table 2). No gender differences were found in any of the parameters studied.

FPLC lipoprotein profiles

FPLC analyses revealed that CETP×apoA-II-Tg mice, fed either a regular chow diet or the atherogenic diet, exhibited a marked increase in apoB-containing lipoproteins (**Fig. 1**). These data demonstrated that the bulk of triglycerides concomitant with large amounts of proteins was in the VLDL fraction, particularly in the atherogenic diet. In the latter, a significant proportion of human apoA-II was associated with VLDL in apoA-II-Tg and CETP×apoA-II-Tg mice (4.2 and 9.2 mg/dl, respectively) and the HDL cholesteryl ester/triglyceride ratios of the different lines (when calculated in mM) were 132 in control mice, 17 in apoA-II-Tg mice, 19 in CETP-Tg mice, and 5 in CETP×apoA-II-Tg mice.

Liver lipid content

Liver lipid content was measured in mice fed an atherogenic diet. Liver total cholesterol, free cholesterol, FFA, and triglyceride content were increased in CETP×apoA-II-Tg mice compared with control mice (**Table 3**). Most of these parameters were also significantly increased in apoA-II-Tg and in CETP-Tg compared with control mice. Only liver triglyceride content, however, increased almost significantly ($P < 0.08$) in CETP×apoA-II-Tg mice compared with CETP-Tg mice (Table 3).

VLDL metabolism

Autologous VLDL containing radiolabeled [^{3}H]triolein was injected intravenously and the disappearance of total radioactivity from plasma was measured to investigate the mechanisms underlying the severe hypertriglyceridemia in the CETP×apoA-II-Tg mice fed an atherogenic diet (**Fig. 2A**). The decay of radiolabeled triglyceride in apoA-II-Tg mice was slightly, but not significantly, increased compared with that of control mice. Progressive increased decay of VLDL triolein radioactivity was observed in CETP×apoA-II-Tg mice and in CETP-Tg mice (Fig. 2A). The percentage of radiolabeled [^{3}H]triolein associated with HDL throughout the experiment is shown in the inset of Fig. 2A. Less than 10% of radiolabeled [^{3}H]triolein was bound to HDL at the indicated times both in control and apoA-II-Tg mice, but reached around 60% in CETP×apoA-

TABLE 3. Liver lipid concentrations in overnight fasted control, CETP-Tg, ApoA-II-Tg, and CETP×ApoA-II-Tg mice fed an atherogenic diet for 24 weeks

	Control	CETP-Tg	ApoA-II-Tg	CETP×ApoA-II-Tg
Number of animals	8	8	10	7
Males/females	4/4	4/4	5/5	4/3
Liver weight (g)	2.6 ± 0.2	2.4 ± 0.2	2.1 ± 0.3	2.5 ± 0.2
Liver cholesterol	13 ± 2	22 ± 1[a]	19 ± 2[a]	22 ± 2[a]
Liver free cholesterol	2.7 ± 0.3	4.6 ± 0.3[a]	3.9 ± 0.4[a]	4.3 ± 0.3[a]
Liver triglycerides	4.1 ± 0.5	6.4 ± 0.7[a]	8.4 ± 1.4[a]	9.3 ± 1.5[a,b]
Liver FFA	3.4 ± 0.2	4.2 ± 0.4[a]	3.7 ± 0.2	4.6 ± 0.4[a]

Unless otherwise specified, results are expressed as mg/g and as mean ± SEM. CETP-Tg and ApoA-II-Tg mice were not statistically compared. Statistical significance was determined by analysis of variance.

[a] Significantly different ($P < 0.05$) from control mice.

[b] Almost significantly different ($P < 0.08$) from CETP-Tg mice.

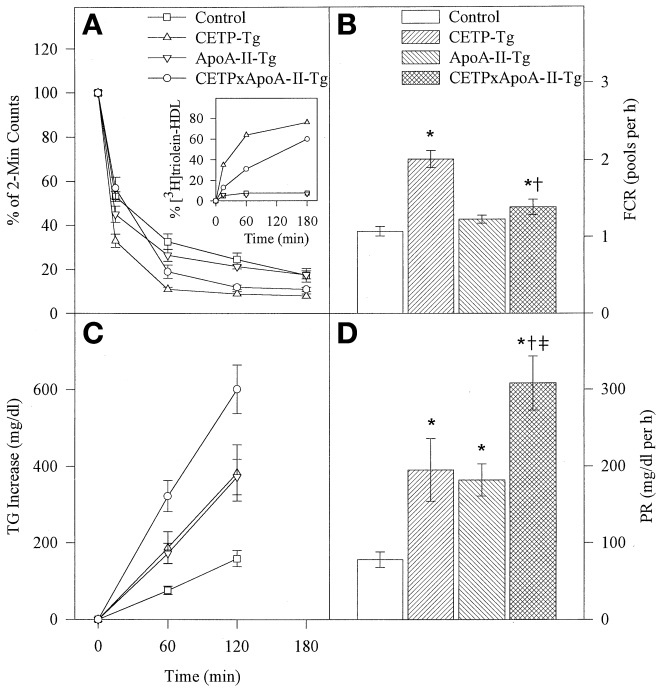

Fig. 2. A: Plasma clearance of injected [^{3}H]triolein-VLDL in overnight fasted mice fed an atherogenic diet. The radioactivity present in 50 μl of plasma obtained from each mouse was measured and the percentage of radioactivity remaining 2 min after injection is shown. The percentages of radiolabeled [^{3}H]triolein-HDL are shown in the inset. B: The data of plasma [^{3}H]-triolein clearance were used to calculate the fractional catabolic rates (FCR) and expressed as pools per h. C: In vivo production of triglyceride (TG). Overnight fasted mice were bled immediately prior to and after Triton WR-1339 iv injection. TG were measured and their change with respect to the baseline result is shown. D: The hepatic triglyceride production rates (PR) are expressed as mg/dl/h. In all cases, each value represents the mean ± SEM of data from six to eight mice. * Significantly different ($P < 0.05$) from control mice; † significantly different ($P < 0.05$) from CETP-Tg mice; ‡ significantly different ($P < 0.05$) from apoA-II-Tg mice.

II-Tg mice and around 80% in CETP-Tg mice. FCR of the radiolabeled triglyceride was enhanced ≈2-fold in CETP-Tg mice compared with control and apoA-II-Tg mice (Fig. 2B). Interestingly, the expression of the human apoA-II transgene in CETP-Tg mice resulted in a significant 30% delay in [^{3}H]triglyceride clearance compared with CETP-Tg mice (Fig. 2B).

Rates of triglyceride production were ≈2.4-fold higher in CETP-Tg and apoA-II-Tg mice than in control mice (Fig. 2D). Human apoA-II expression in CETP-Tg mice enhanced triglyceride production to rates 1.7-fold that of their progenitors and 4-fold that of control mice (Fig. 2D).

Susceptibility to atherosclerosis

Aortic atherosclerotic lesions were examined in mice fed a regular chow diet for 24 weeks. The mean lesion area of two male control mice (144 ± 144 μm^2), five male CETP-Tg mice (203 ± 152 μm^2), five male apoA-II-Tg mice (226 ± 82 μm^2), and six male CETP×apoA-II-Tg mice (212 ± 202 μm^2) were not significantly different. In contrast, significant lesions were observed in five of eight female CETP×apoA-II-Tg mice (**Fig. 3A**). The lesion size of female CETP×apoA-II-Tg mice was significantly increased (803 ± 353 μm^2, $P < 0.05$) compared with 10 female CETP-Tg mice (32 ± 19 μm^2), 7 female apoA-II-Tg mice (109 ± 54 μm^2), and 2 female control mice (0 ± 0 μm^2).

Atherosclerotic lesion area of mice fed an atherogenic diet for 24 weeks was also analyzed (Fig. 3B). As in previous studies (13), the mean lesion areas in male and female CETP-Tg mice were increased with respect to those of control mice. Mean lesion areas in male and female apoA-II-Tg mice were also 2.7- and 4.1-fold larger, respectively, than those of their non-Tg control mice counterparts (5). Male CETP×apoA-II-Tg mice presented an ≈2-fold increase in mean lesion area compared with male CETP-Tg and apoA-II-Tg mice. However, no significant differences were observed among aortic lesion areas of female CETP×apoA-II-Tg, CETP-Tg, and apoA-II-Tg mice.

DISCUSSION

This study was performed with the mice in fasting conditions because the concentration of human apoA-II in

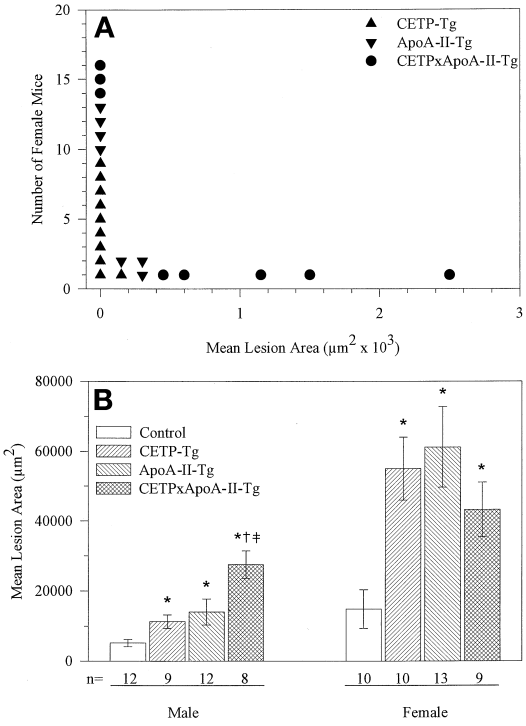

Fig. 3. A: Atherosclerotic lesion areas of female CETP-Tg, apoA-II-Tg, and CETP×apoA-II-Tg mice fed a chow diet for 24 weeks. Each symbol represents the average area of lesion of four proximal aortic sections from each mouse. Note that the CETP×apoA-II-Tg mice developed significant atherosclerotic lesions compared with CETP-Tg and apoA-II-Tg mice. B: Mean area of atherosclerotic lesion of male and female mice fed an atherogenic diet for 24 weeks. Bars represent mean ± SEM in μm^2. n, number of mice. * Significantly different ($P < 0.05$) from control mice; † significantly different ($P < 0.05$) from CETP-Tg mice; ‡ significantly different ($P < 0.05$) from apoA-II-Tg mice.

the plasma of Tg mice did not change in relation to the postprandial period and neither did the degree of hypertriglyceridemia compared with control mice (data not shown). These findings contrast sharply with those of Boisfer et al. (25) who described an important postprandial increase in human apoA-II and degree of hypertriglyceridemia in their independently generated apoA-II-Tg mice. It is possible that the differences in results obtained by our groups are due to the fact that the 3-kb genomic apoA-II injected into mice were not the same; ours was obtained by digestion with *Msp*I (4) and theirs by digestion with *Hind*III (25). This leaves our construction 260 base pairs (bp) longer at the 5′ end but 174 bp shorter at the 3′ end compared with that used by Boisfer et al. (4, 25). We speculate that this could be important in the regulation of human apoA-II synthesis in Tg mice by altering the balance of transcription factor activators such as SERBP-1 (26), RXR-PPAR (27, 28), and USF/HNF-4 (29), some of which are well known to be differently activated or inhibited by fed/fasting conditions (30, 31).

In the present study, expression of human apoA-II in primate CETP-Tg mice caused severe hypertriglyceridemia, particularly in those on an atherogenic diet. In previous studies, we attributed the increase in triglycerides in apoA-II-Tg mice to their functional LCAT deficiency (4–6). However, LCAT-deficient mice did not present pronounced hypertriglyceridemia (23, 32, 33) and, consequently, the increase in plasma triglycerides found in this study in CETP×apoA-II-Tg mice seems not to be due to decreased LCAT.

To our knowledge, only one other study to date (34) has crossbred human apoA-II and CETP-Tg mice. These animals were maintained on a regular chow diet and the study was focused on the inhibition by apoA-II of HL action on HDL (34). In our double Tg mice fed a regular chow diet, the expression of each transgene influenced the other. Human apoA-II and CETP activities were both decreased in the double Tg mice compared with the single Tg mice. The reasons for these observations are unknown and a similar finding, albeit milder, was shown in mice fed an atherogenic diet.

Because hypertriglyceridemia was more severe in CETP×apoA-II-Tg mice fed an atherogenic diet, and in this situation, high FFA concentrations were concomitant (a situation of interest for human pathology), our metabolic studies were focused on mice fed an atherogenic diet. [^{3}H]Triolein-VLDL clearances of apoA-II-Tg mice were similar to those of control mice. In contrast, CETP-Tg mice showed a marked increase in [^{3}H]triolein VLDL FCR, which may be accounted for by the introduction by CETP of a new pathway that presumably involves the transfer of VLDL triglyceride to HDL, where it is likely to be hydrolyzed by HL (12, 34). The decrease in [^{3}H]-triolein-VLDL clearance in CETP×apoA-II-Tg mice compared with CETP-Tg mice could be due to a slower transfer rate of [^{3}H]triolein from VLDL to HDL as a result of the decreased HDL pool and/or to decreased CETP and HL reactivity toward apoA-II-containing HDL particles (34–36). In this context, the increased HDL triglyceride content observed in CETP×apoA-II-Tg mice compared with their progenitors is very likely to reflect an impairment of the HL reactivity toward apoA-II-enriched HDL particles.

CETP-Tg and apoA-II-Tg mice had higher triglyceride production rates compared with control mice. However, plasma triglyceride levels in CETP-Tg mice were similar to those of non-Tg controls, suggesting that in the former, increased triglyceride clearance compensated the effects of increased triglyceride production. Expression of apoA-II in CETP-Tg mice caused a synergistic and marked increase in triglyceride production which, combined with a decreased VLDL triglyceride clearance (respect to CETP-Tg mice), may explain the hyperlipidemic phenotype of CETP×apoA-II-Tg mice. Plasma FFA in CETP×apoA-II-Tg mice, especially when fed an atherogenic diet, were increased compared with the other mouse groups. The increase in FFA reaching the liver is a major inductor of VLDL synthesis and secretion (37) and could be the cause of the greater triglyceride production found in human apoA-II-Tg mice fed an atherogenic diet. It should be noted that even though the increase in FFA in these mice was moderate compared with non-Tg control mice, this situation is expected to last the 12 h of daylight when mice are usually fasted; hence, it could have an impact on liver triglyceride concentration (which, in fact, was increased in CETP×apoA-II-Tg mice). Indeed, the liver is one of the sites where human apoA-II may function to increase VLDL synthesis and secretion. Conversely, human apoA-II may act, directly or indirectly, upon adipose tissue, causing an increased flux of fatty acids to the liver and a rise in VLDL synthesis and secretion. Similar or additional mechanisms may be involved in the increase in triglyceride production found in CETP-Tg mice compared with control mice. CETP expression resulted in increased plasma triglycerides in LDL receptor-deficient mice overexpressing human apoC-III (38) and in apoE-deficient mice (39), but none of these studies ascertained the cause of such an increase. It is also of note that the cynomolgus monkey (whose CETP gene was used for developing the CETP-Tg mice used in this work) fed a high cholesterol diet showed a 2- to 4-fold increase in plasma CETP activity and, in this situation, apoB secretion rate increased 4-fold without changes in liver apoB mRNA levels (40).

A major effect of CETP and apoA-II expression on the concentration and distribution of other apolipoproteins is unlikely to explain the results of this work because in previous studies, the concentration of apoE was shown to be similar in apoA-II-Tg and non-Tg (5), and mouse apoA-II tended to be lower in the human apoA-II-Tg mice (5), which would anticipate, in view of the investigations performed in apoA-II-knockout mice (41), a lower production of triglycerides and FFA rather than the contrary. Moreover, major alterations in apoC and/or apoE concentration or distribution in plasma would be expected to impair VLDL triglyceride catabolism at least as much as VLDL triglyceride synthesis and secretion.

CETP×apoA-II-Tg mice present some phenotypic features that are similar to familial combined hyperlipidemia

(FCHL) in humans, in which VLDL overproduction is characteristic (42). Because apoA-II and CETP have not been identified as "major" FCHL genes in genome-wide scans (42), they could be "modifier" FCHL genes. We have discussed in detail the lines of evidence supporting this hypothesis in the case of apoA-II (7).

The present study suggests a pro-atherogenic potential of CETP expression. However, apoA-II expression in CETP-Tg mice did not consistently increase atherosclerosis susceptibility and the results obtained varied depending on gender (even though males and females did not present significant differences in plasma lipids and lipoproteins) and diet. This contrasts with earlier studies that showed reduced atherosclerotic lesions in mice coexpressing CETP and apoC-III that also resulted in severe hypertriglyceridemia (14), but is consistent with the progression of atherosclerosis generally observed after expressing CETP in apoE- and LDL receptor-deficient mice expressing or not expressing apoC-III (38, 39). Therefore, the final effect of CETP expression on atherosclerosis may depend on its cause.

Several characteristics of the CETP×apoA-II-Tg mice need to be noted before attempting to apply these results to human pathophysiology: *i*) these mice, when fed a regular chow diet, have CETP activities approximately 8-fold higher than those of normolipidemic humans (18), and *ii*) the CETP used in this study was from the cynomolgus monkey and did not increase in response to a cholesterol-rich diet as does human CETP, as it lacks the flanking regions of the gene that contain an LXR element (43). However, the high structural homology at amino acid level (95%) between human and primate CETP renders a species-specific effect unlikely (40), and the lack of increase in CETP in response to an atherogenic diet led to apoA-II×CETP-Tg mice presenting plasma levels of the Tg proteins that were around 3- and 6-fold higher, respectively, than those of humans with FCHL (44, 45).

In summary, in the animal model used in this study, apoA-II and CETP directly or indirectly stimulate VLDL production, with the former inhibiting the increase in VLDL triglyceride catabolism induced by the latter. This combination of mechanisms induces severe hyperlipidemia in mice fed a high-cholesterol high-fat diet and this suggests that CETP and, in particular apoA-II, may play a role in the regulation of VLDL metabolism. CETP×apoA-II-Tg mice may serve as an important model for a better understanding of FCHL.

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