Human apoA-I expression in CETP transgenic rats leads to lower levels of apoC-I in HDL and to magnification of CETP-mediated lipoprotein changes[1]

David Masson,[2,*,†] Jean-Paul Pais de Barros,[*,†] Zoulika Zak,[*,†] Thomas Gautier,[*,†] Naig Le Guern,[*,†] Mahfoud Assem,[§] Jeffrey W. Chisholm,[**] James R. Paterniti, Jr.,[††] and Laurent Lagrost[2,*,†]

Laboratoire de Biochimie des Lipoprotéines,* INSERM U498, Faculté de Médecine, BP87900, 21079 Dijon Cedex, France; Institut Fédératif de Recherche 100,† Faculté de Médecine, BP87900, 21079 Dijon Cedex, France; Department of Pharmaceutical Sciences,§ St Jude Children's Research Hospital, Memphis, TN 38105; CV Therapeutics, Inc.,** 3172 Porter Dr., Palo Alto, CA 94304; and Amylin Pharmaceuticals,†† Inc., San Diego, CA 92121

Abstract **Plasma cholesteryl ester transfer protein (CETP) has a profound effect on neutral lipid transfers between HDLs and apolipoprotein B (apoB)-containing lipoproteins when it is expressed in combination with human apoA-I in HuAI/CETP transgenic (Tg) rodents. In the present study, human apoA-I-mediated lipoprotein changes in HuAI/CETPTg rats are characterized by 3- to 5-fold increments in the apoB-containing lipoprotein-to-HDL cholesterol ratio, and in the cholesteryl ester-to-triglyceride ratio in apoB-containing lipoproteins. These changes occur despite no change in plasma CETP concentration in HuAI/CETPTg rats, as compared with CETPTg rats. A number of HDL apolipoproteins, including rat apoA-I and rat apoC-I are removed from the HDL surface as a result of human apoA-I overexpression. Rat apoC-I, which is known to constitute a potent inhibitor of CETP, accounts for approximately two-thirds of CETP inhibitory activity in HDL from wild-type rats, and the remainder is carried by other HDL-bound apolipoprotein inhibitors. It is concluded that human apoA-I overexpression modifies HDL particles in a way that suppresses their ability to inhibit CETP. An apoC-I decrease in HDL of HuAI/CETPTg rats contributes chiefly to the loss of the CETP-inhibitory potential that is normally associated with wild-type HDL.**—Masson, D., J-P. Pais de Barros, Z. Zak, T. Gautier, N. Le Guern, M. Assem, J. W. Chisholm, J. R. Paterniti, Jr., and L. Lagrost. **Human apoA-I expression in CETP transgenic rats leads to lower levels of apoC-I in HDL and to magnification of CETP-mediated lipoprotein changes.** ***J. Lipid Res.*** **2006.** 47: **356–365.**

Supplementary key words apolipoprotein A-I • cholesteryl ester transfer protein • apolipoprotein C-I

Plasma levels of HDL and especially of apolipoprotein A-I (apoA-I) are inversely correlated with coronary artery disease (1, 2). The beneficial effect of HDL/apoA-I was explained initially in terms of a facilitation of reverse cholesterol transport, a pathway through which cholesterol is transported from peripheral tissues back to the liver. Today, HDL is regarded as a complex entity with several protective effects that are chiefly determined by its apolipoprotein composition (3, 4). Although apoA-I, the major protein component in HDL, is thought to carry a large part of the metabolic specificity of HDL, minor apolipoprotein components in native HDL also contribute significantly to its biological effects (3, 4). This may be the case for apoC-I, a small apolipoprotein molecule that is mainly associated with circulating HDL in fasted plasma samples. Recent studies have demonstrated that HDL-bound apoC-I is able to inhibit significantly the lipid transfer reaction catalyzed by the cholesteryl ester transfer protein (CETP), a plasma factor that mediates the exchange of neutral lipids, cholesteryl esters, and triglycerides between plasma lipoproteins (5, 6). Through its action, CETP leads to the enrichment of proatherogenic apoB-containing lipoproteins with cholesteryl esters at the expense of antiatherogenic HDL. The implication of CETP in HDL metabolism led to the proposal that CETP inhibition might constitute a relevant way to prevent and/or treat atherosclerosis, and new compounds with potentially beneficial impact on plasma cholesterol distribution emerged from the active search for CETP inhibitors (7–9).

Manuscript received 7 July 2005 and in revised form 3 October 2005.

Published, JLR Papers in Press, November 10, 2005.
DOI 10.1194/jlr.M500288-JLR200

Abbreviations: apoA-I, apolipoprotein A-I; CETP, cholesteryl ester transfer protein; LCAT, lecithin:cholesterol acyltransferase; PLTP, phospholipid transfer protein.

[1] In memory of our friend and colleague, Dr. Peter Dolphin (Dalhousie University, Halifax), who contributed to the initiation of the present study.

[2] To whom correspondence should be addressed.
e-mail: laurent.lagrost@u-bourgogne.fr (L.L.);
david.masson@chu-dijon.fr (D.M.)

As expected, pharmacological CETP inhibitors increase HDL cholesterol levels and decrease LDL cholesterol levels in treated subjects (10–12).

Recent intervention studies in animals and humans have demonstrated that direct infusion of either total HDL or apolipoprotein derivatives such as native apoA-I or apoA-I_{Milano} (13), intramural delivery of recombinant apoA-I_{Milano}/phospholipid complexes (14), or oral administration of an apoA-I mimetic peptide synthesized from d-amino acids (15) are able to reduce the extent and progression of atherosclerotic lesions (16, 17). These observations are in good agreement with earlier studies in genetically engineered mice, which directly supported an antiatherosclerotic property for apoA-I. Overexpression of human apoA-I in hypercholesterolemic animal models was proven to reduce the extent of atherosclerotic lesions (18) and to normalize endothelium-dependent arterial relaxation (19). Beyond its antiatherosclerotic effect in vivo, apoA-I overexpression produces significant alterations in the structure and properties of circulating HDL. Thus, in transgenic mice and rats expressing elevated levels of human apoA-I (HuAITg), the HDL protein moiety is mainly human apoA-I, to the exclusion of other apolipoprotein components, including native rodent apoA-I and apoA-II (20–22). Interestingly, the prominence of human apoA-I in HDL from transgenic animals was associated with a significant improvement in the lipid transfer interactions of HDL with human CETP. Although the latter point was explained in terms of a better, species-specific interaction of human CETP with human apoA-I (23), the superiority of human apoA-I over mouse apoA-I in stimulating the lipid transfer process was not directly addressed in comparative studies. In addition, the hypothesis of another inhibitory factor normally associated with HDL could not be excluded (23). Subsequent ex vivo studies with HDL apolipoproteins isolated from wild-type or HuAITg mice confirmed that the ability of HDL to inhibit CETP activity was lost in HuAITg mice (24). Again the precise molecular mechanism accounting for these changes remained to be determined.

The present study was designed to elucidate the molecular basis of the upregulation of CETP activity in animals with elevated levels of human apoA-I expression. To this end, transgenic rats expressing active simian CETP (CETPTg rats) were crossbred with transgenic rats expressing human apoA-I (AITg rats). We demonstrated for the first time that the substantial rise in the specific activity of CETP in plasma of apoAI/CETP transgenic rats is the consequence of concomitant decreases in rat apoA-I and rat apoC-I, two negative modulators of the lipid transfer reaction.

MATERIALS AND METHODS

Animals

All animal studies were conducted in accordance with institutional guidelines (national agreement number: A21.231.006) and were approved by the Ethical Committee of the animal house of the University of Burgundy. CETP-transgenic Fisher rats (CETPTg rats) were characterized previously in our laboratory (25). In this line, the simian CETP gene was placed under the control of the metallothionein promoter. Sprague-Dawley rats expressing various levels (0–595 mg/dl) of human apoA-I (HuAITg rats) have been characterized previously (21). Double transgenic HuAITg/CETPTg rats were obtained by crossbreeding. Wild-type mice and C57Bl6 mice expressing human apoA-I under the control of its natural flanking regions (HuAITg) (20) were used to assess the effect of human apoA-I expression on liver apoC-I mRNA levels.

Animals were maintained under controlled conditions of temperature (21 ± 1°C), humidity (60 ± 10%), and lighting (12 h cycle, 8 AM to 8 PM) and had free access to food and water. Heparinized blood samples were collected by jugular vein.

Isolation and delipidation of VLDL and HDL

HDLs were isolated by ultracentrifugation from rat and human plasmas as the $1.070 < d < 1.210$ g/ml plasma fraction with one 7 h, 120,000 rpm run at $d = 1.070$ g/ml followed by two 10 h, 120,000 rpm runs at $d = 1.21$ g/ml using a TL 120.2 rotor in a Beckman optima TLX ultracentrifuge. VLDLs were isolated as the $d < 1.006$ g/ml plasma fraction with one 3 h, 120,000 rpm run using a TL 120.2 rotor in a Beckman optima TLX ultracentrifuge. Plasma densities were adjusted by the addition of solid KBr. Isolated lipoproteins were dialyzed overnight against TBS buffer, and they were subsequently delipidated using butanol-diisopropylether 40:60 (v/v) according to the method of Cham and Knowles (26). Apolipoproteins were dialyzed overnight against TBS buffer.

Apolipoprotein composition of VLDL and HDL

VLDL and HDL apolipoproteins were incubated for 5 min at 70°C in the presence of SDS-containing TBS buffer, and they were separated by electrophoresis in 4–12% NuPage Novex SDS-polyacrylamide gels (Invitrogen) and SDS 8–25% polyacrylamide gradient gels (Phast System; Pharmacia, Uppsala, Sweden), respectively. After electrophoretic migration, gels were stained with Coomassie brilliant blue or silver (6), and apparent molecular weights of individual protein bands were determined by comparison with protein standards.

Measurement of cholesteryl ester transfer activity

Cholesteryl ester transfer activity was determined by quantitating the transfer of radiolabeled cholesteryl esters from biosynthetically labeled LDL-containing tritiated cholesteryl esters (^{3}H-CE-LDL) to unlabeled HDL acceptors, as previously described (27). Briefly, isolated HDL fractions were incubated for 3 h at 37°C with ^{3}H-CE-LDL (2.5 nmol cholesterol) in the presence of 4.5 μg of a partially purified human CETP fraction in a final volume of 50 μl. The purified CETP fraction was devoid of lecithin:cholesterol acyltransferase (LCAT) and phospholipid transfer protein (PLTP) activities. It was prepared from human plasma by a sequential chromatographic procedure (28), and it resulted in an approximately 3,000-fold purification, as compared with total plasma. Following incubation, $d < 1.068$ and $d > 1.068$ g/ml fractions were separated by ultracentrifugation and transferred into counting vials containing 2 ml of scintillation fluid (Optiphase3, Wallac). Radioactivity was quantitated in a Wallac 1410 liquid scintillation counter (Pharmacia). More than 95% of total radioactivity was recovered in $d < 1.068 + d > 1.068$ fractions. Cholesteryl ester transfer rate (%) was calculated from the relative amount of radiolabeled cholesteryl esters transferred to the $d > 1.068$ g/ml fraction after deduction of blank values from control incubations with no CETP added.

Purification of rat apoC-I and preparation of apoC-I-free HDL apolipoproteins

Rat apoC-I was purified by a chromatofocusing method, as previously described (5), with the exception of the 25 mmol/l histidine equilibration buffer, which was adjusted to pH 7.2. Under these experimental conditions, rat apoC-I eluted in the unbound fraction, and bound, apoC-I-free HDL apolipoproteins were eluted with a 25 mmol/l histidine, 2 mol/l NaCl, 0.2 g/l NaN_3, pH 4.0, buffer. Pure apoC-I and apoC-I-free HDL apolipoproteins were dialyzed against TBS buffer, and total proteins were assayed by using a bicinchoninic acid protein assay kit (Pierce) according to the manufacturer's instructions.

Purification of human and rat apoA-I

ApoA-I was purified from human or rat HDL by using the general electrophoretic procedure previously described for other apolipoproteins (29, 30). HDLs were isolated from human or rat serum by sequential ultracentrifugation as the $1.063 < d < 1.210$ mg/ml fraction, after one 2.5 h 90,000 rpm spin at the lowest density, followed by a second 3.5 h 90,000 rpm spin at the highest density in an NVT90 rotor on an XL90 ultracentrifuge (Beckman). Isolated HDLs were delipidated, and HDL apolipoproteins (10 mg) were applied to a discontinuous denaturing polyacrylamide gel. After a 1,000 Vh electrophoresis, the gel portion containing apoA-I was cut off and placed on the top of a vertical agarose gel (0.8% w:v). Electrophoretic transfer in agarose gel was conducted for 600 Vh. After electrotransfer, the apoA-I-containing portion of the agarose gel was cut off, and apoA-I was extracted by a 15 min centrifugation at 250,000 *g* in a 120.1 rotor on a TLX ultracentrifuge (Beckman). Finally, SDS was removed by affinity chromatography on an Extrati-Gel D column (Pierce) according to the manufacturer's instructions.

Chromatofocusing of rat apolipoproteins

Total lipoproteins from rat plasma were delipidated by an ethanol-ether (3:2) mixture, and protein precipitate was recovered in a 25 mmol/l Tris-HCl, 0.02% NaN_3, pH 8.6, buffer (buffer A). Chromatofocusing was conducted on a GOLD HPLC system (Beckman Coulter) equipped with a polybuffer exchanger (PBE) 9.4 (Amersham Pharmacia Biotech) 50 × 4 mm column. Injected samples (90 μl) were eluted at a constant 0.4 ml/min flow rate of buffer A. Under these conditions, only rat apoC-I eluted in the void volume. After each run, the column was washed with buffer A containing 1 mol/l NaCl to release bound proteins. A calibration curve was obtained by injections of known amounts of purified human apoC-I.

Plasma lipid analysis

All assays were performed on a Victor^{2}1420 Multilabel Counter (Wallac). Total cholesterol and triglyceride concentrations were measured by the enzymatic method using Cholesterol-100 and Triglycerides-100 reagents, respectively (ABX Diagnostics).

Plasma lipoprotein profile

Individual plasma samples were injected on a Superose 6 HR 10/30 column (Amersham Biosciences) that was connected to a fast-protein liquid chromatography (FPLC) system (Amersham Biosciences). Lipoproteins were eluted at a constant, 0.3 ml/min flow rate with Tris-buffered saline containing 1 mM EDTA and 0.02% sodium azide. The gel filtration column was calibrated with globular protein standards of known Stoke's diameter (Gel Filtration Calibration Kit, Pharmacia). Total cholesterol and triglyceride concentrations were assayed in individual, 0.3 ml fractions. As shown in previous studies with the same column, human VLDLs were contained in fractions 4–11, human IDLs + LDLs were contained in fractions 12–23, and human HDLs were eluted in fractions 24–44 (25).

RNA isolation and PCR methods

Total RNA was extracted using Trizol reagent (Life Technologies; Carlsbad, CA). Specific mRNAs were analyzed by quantitative real time RT-PCR using the ABI Prism 7900HT (Applied Biosystems; Foster City, CA). Briefly, 5 μg of RNA was reverse transcribed into cDNA using MuMLV retrotranscriptase and oligo dT (Life Technologies). Fifty nanograms of the cDNA mixture were used. Specific cDNAs were amplified using the following primers: mouse apoC-I, sense TCGCTCTTCCTGTCCTGATTG, antisense GACCTGGCACATTACGTGGAT; cyclophiline, sense ATAATGGCACTGGCGGCAGGT, antisense CGCTCTCCTGAGCTACAGAAG. Values were normalized to cyclophiline levels, and relative mRNA levels were evaluated using the ΔΔCt method.

RESULTS

Effect of CETP on lipoprotein profile in plasma of wild-type and human apoA-I transgenic rats

As shown in **Table 1**, expression of active CETP decreases total plasma cholesterol, esterified cholesterol, and free cholesterol in both wild-type and HuAITg rats on chow diet. This was due to selective decreases in the cholesterol content of the HDL fraction, which contains the bulk of plasma cholesterol in this species (**Fig. 1** and **Table 2**). Whereas total plasma triglyceride levels were independent of the CETP transgene (Table 1), FPLC analysis revealed that triglycerides tended to redistribute from the VLDL toward the HDL fraction with CETP

TABLE 1. Plasma lipid concentrations in plasma from wild-type, CETPTg, HuAITg, and HuAITg/CETPTg rats

	Rats			
	Wild-type (n = 15)	CETPTg (n = 9)	HuAITg (n = 15)	HuAITg/CETPTg (n = 8)
			g/l	
Total cholesterol	0.73 ± 0.04	0.60 ± 0.05[a]	1.60 ± 0.13	1.06 ± 0.11[b]
Free cholesterol	0.16 ± 0.01	0.12 ± 0.01[a]	0.37 ± 0.03	0.24 ± 0.04[b]
Esterified cholesterol	0.57 ± 0.03	0.48 ± 0.03[a]	1.22 ± 0.10	0.83 ± 0.09[b]
Triglycerides	0.39 ± 0.02	0.41 ± 0.04	0.47 ± 0.07	0.58 ± 0.09

Cholesterol and triglyceride levels were measured as described in Materials and Methods. Values are mean ± SEM. Statistics by Mann-Whitney test.

[a] Significantly different from wild-type rats, $P < 0.05$.

[b] Significantly different from HuAITg rats, $P < 0.05$.

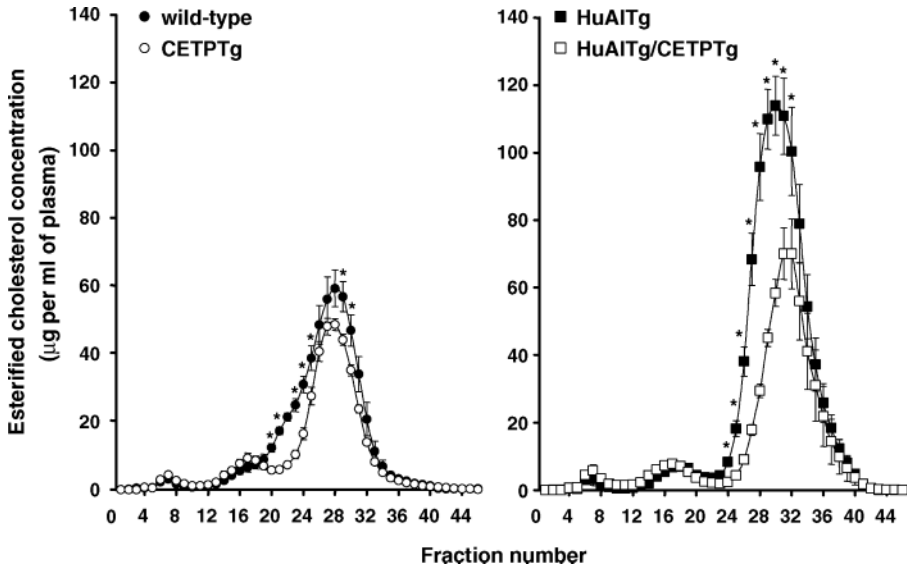

Fig. 1. Cholesterol distribution in plasma from wild-type, transgenic cholesteryl ester transfer protein (CETPTg), transgenic apolipoprotein A-I (HuAITg), and CETPTg/apoAITg rats. Lipoproteins were separated by gel permeation chromatography, and cholesterol was assayed in eluted fractions as described in Materials and Methods. Each point is the mean ± SEM of values from five to six rats. *Significantly different from homologous values in rats expressing CETP, $P < 0.05$; Mann-Whitney test.

expression (**Fig. 2**). Interestingly, changes in both cholesterol and triglyceride redistributions were of greater magnitude when the CETP transgene was expressed in the HuAITg background (Figs. 1, 2). Thus, whereas plasma CETP concentration did not differ significantly between CETPTg and HuAITg/CETPTg rats (2.65 ± 0.49 vs. 3.34 ± 0.71 mg/l, respectively; NS), relative increments in VLDL+LDL-to-HDL cholesteryl ester ratio, as well as in cholesteryl ester-to-triglyceride ratio in VLDL+LDL were 3- to 5-fold greater when human apoA-I was coexpressed with CETP in transgenic animals (**Fig. 3**). Plasma levels of human apoA-I were decreased in HuAI/CETPTg rats as compared with HuAITg rats (human apoA-I: 581 ± 57 mg/dl in HuAITg rats vs. 397 ± 56 mg/dl in HuAITg/CETPTg rats; $P = 0.054$).

Comparison of the ability of native or delipidated HDL from wild-type and human apoA-I transgenic rats to modulate the cholesteryl ester transfer reaction

When constant amounts of purified CETP and radiolabeled LDL were incubated in the presence of increasing concentrations of isolated HDL from wild-type rats, the rate of cholesteryl ester transfer increased gradually until a maximal value was reached, with an HDL cholesterol concentration of 200 nmol/ml (**Fig. 4**). As the amount of wild-type HDL increased above the optimal value, progressive inhibition of the lipid transfer reaction was observed. In contrast, when native HDL from HuAITg mice expressing 360 mg/dl of human apoA-I was used, CETP activity was 2.3-fold higher and was not inhibited at increasing HDL concentration (Fig. 4). These results indicate that the intrinsic property of HDL substrates can markedly influence the CETP-mediated lipid transfer reaction.

The inhibition of CETP by native plasma HDL appeared to reflect a specific property of its apolipoprotein components, and a gradual concentration-dependent inhibition of the lipid transfer reaction could be obtained with increasing concentrations of delipidated HDL apolipoproteins from wild-type rats (**Fig. 5**). Again, HDL apolipoproteins from rats expressing 360 mg/dl of human apoA-I had no inhibitory activity (Fig. 5).

TABLE 2. Cholesteryl esters and triglyceride concentrations in lipoprotein fractions from wild-type, CETPTg, HuAITg, and HuAITg/CETPTg rats

	Wild-type (n = 5)	CETPTg (n = 6)	*P*	HuAITg (n = 5)	HuAITg/ CETPTg (n = 5)	*P*
				mg/l		
VLDL+LDL-CE	62 ± 10	67 ± 7	NS	46 ± 11	66 ± 8	NS
HDL-CE	491 ± 31	351 ± 14	0.02	1016 ± 43	501 ± 72	0.05
(VLDL+LDL)-CE/HDL-CE	0.13 ± 0.03	0.20 ± 0.03	NS	0.05 ± 0.01	0.14 ± 0.02	0.01
VDL+LDL-TG	225 ± 23	178 ± 11	NS	273 ± 35	190 ± 28	NS
HDL-TG	24 ± 7	46 ± 17	NS	28 ± 6	76 ± 25	NS
VLDL+LDL-TG/HDL-TG	13.8 ± 3.9	7.2 ± 2.5	NS	12.9 ± 4.2	3.7 ± 0.9	0.05

Cholesterol and triglyceride levels were measured as described in Materials and Methods in lipoprotein fractions obtained by fast protein liquid chromatography. Values are mean ± SEM. Statistics by Mann-Whitney test.

NS, not significant.

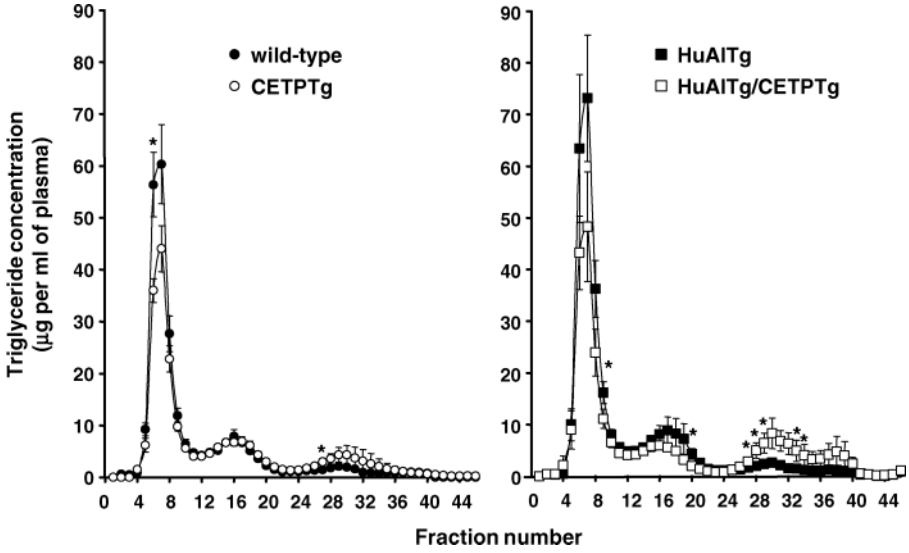

Fig. 2. Triglyceride distribution in plasma from wild-type, CETPTg, HuAITg, and CETPTg/ HuAITg rats. Lipoproteins were separated by gel permeation chromatography, and triglycerides were assayed in eluted fractions as described in Materials and Methods. Each point is the mean ± SEM of values from five to six rats. *Significantly different from homologous values in rats expressing CETP, $P < 0.05$; Mann-Whitney test.

Comparative effects of rat apoA-I and human apoA-I on CETP activity

In accordance with previous studies in HuAI transgenic rats and mice (20–22), human apoA-I expression in apoAITg rats led to the simultaneous reduction in the rat apoA-I content of plasma HDLs. Data from our laboratory confirmed that HDL from rats with elevated plasma human apoA-I levels contained no detectable rat apoA-I (results not shown). To determine whether CETP interacts differently with human and rat apoA-I, apoA-I from both species was purified to homogeneity by preparative electrophoresis, as described under Materials and Methods. As shown in **Fig. 6**, increasing amounts of human apoA-I in the concentration range measured in plasma of apoAITg rats had no significant effect on CETP activity in reconstituted mixtures containing isolated LDL and HDL particles. In contrast, a significant, concentration-dependent inhibition of CETP was observed in the presence of identical amounts of rat apoA-I (Fig. 6).

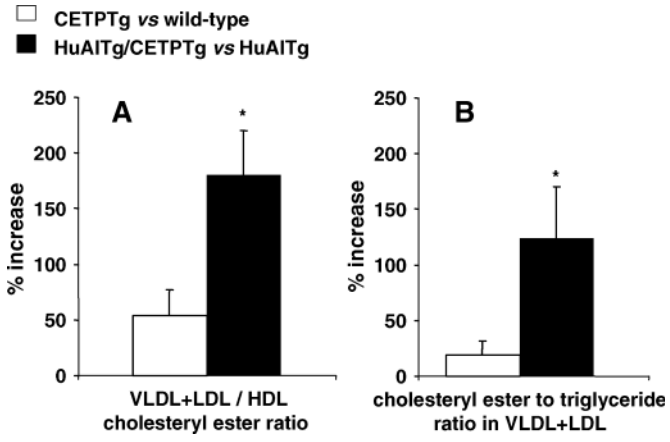

Fig. 3. Changes in apoB-containing lipoprotein-to-HDL cholesteryl ester ratio, and in VLDL+LDL cholesteryl ester-to-triglyceride ratio in wild-type, CETPTg, HuAITg, and CETPTg/HuAITg rats. Lipoproteins were isolated by gel permeation chromatography, and cholesteryl esters and triglycerides were assayed as described in Materials and Methods. Bars indicate the mean ± SEM of values from five distinct rats. *Significantly different from homologous CETPTg versus wild-type values, $P < 0.05$; Mann-Whitney test.

ApoA-I expression in transgenic rats decreases the apoC-I content of HDL and VLDL

Analytical SDS polyacrylamide gradient gel electrophoresis was used to further determine the impact of human apoA-I overexpression on the apolipoprotein profile of plasma HDL and VLDL. **Figure 7** shows that the gradual expression of human apoA-I in transgenic rats led to the progressive disappearance of a number of minor HDL apolipoproteins that were initially present in native particles from wild-type controls. In rats expressing high levels of human apoA-I (360 mg/dl of human apoA-I), only a few discrete bands were detectable in addition to the bulk of apoA-I (Fig. 7). Analysis of the VLDL apolipoprotein composition revealed that human apoA-I enrichment was also associated with decreases in the amounts of minor apolipoproteins in VLDL, including apoC-I (**Fig. 8**). Human apoA-I expression does not alter the hepatic expression of apoC-I in rodents, and apoC-I mRNA levels were similar in livers of wild-type mice and HuAITg mice (1.00 ± 0.13 vs. 1.12 ± 0.38 relative level of expression, respectively; NS). Specific analysis of HDL apolipoproteins by chromatography on a PBE column confirmed further that the relative amounts of apoC-I decreased gradually as the level of human apoA-I expression increased in transgenic rats, with a strong negative correlation between these two parameters in a population of

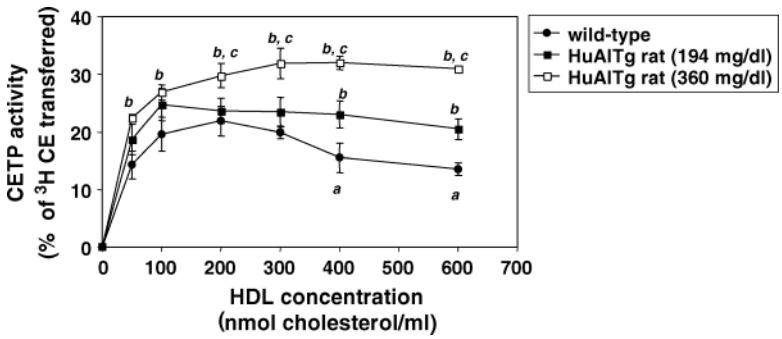

Fig. 4. Concentration-dependent effect of HDL from wild-type and HuAITg rats on CETP activity. Mixtures containing various concentrations of HDL (ranging from 0 to 600 nmol of cholesterol/ml), radiolabeled LDL (50 nmol of cholesterol/ml), and purified CETP (0.17 μg/ml) were incubated for 3 h at 37°C. At the end of the incubation, the percentage of radiolabeled cholesteryl esters transferred was determined after separation of LDL and HDL by ultracentrifugation as described under Materials and Methods. Each point represents the mean ± SD of triplicate determinations. *a*: Significantly different from homologous 200 nmol/ml sample, $P < 0.05$; *b*: significantly different from homologous value in wild-type rats, $P < 0.05$; *c*: significantly different from homologous value in HuAITg rat expressing 1.94 g/l of human apoA-I, $P < 0.05$. Statistics by Mann-Whitney test.

rats with plasma levels of human apoA-I ranging from 0 to 470 mg/dl (**Fig. 9**).

Evaluation of the relative contribution of rat apoC-I to the lipid transfer inhibitory activity associated with HDL

Rat and human apoC-I were purified to homogeneity by the chromatographic procedure described under Materials and Methods. Both rat and human apoC-I are potent blockers of CETP activity, and substantial inhibitions of the cholesteryl ester transfer reaction were observed in the presence of 1.25 μg of either rat apoC-I or human apoC-I (data not shown). As shown in **Fig. 10**, rat HDL apolipoproteins and purified rat apoC-I significantly reduced cholesteryl ester transfer rates in a concentration-dependent manner. Interestingly, the significant reduction in cholesteryl ester transfer rate observed with rat HDL apolipoproteins was not observed with an HDL apolipoprotein extract that differed from total rat HDL apolipoproteins only by its lack of apoC-I (Fig. 10). In fact, analysis of transfer rates indicates that apoC-I in rat HDL accounts for approximately two-thirds of the CETP inhibitory activity that is associated with rat HDL, with the remainder carried by other HDL-bound apolipoprotein inhibitors.

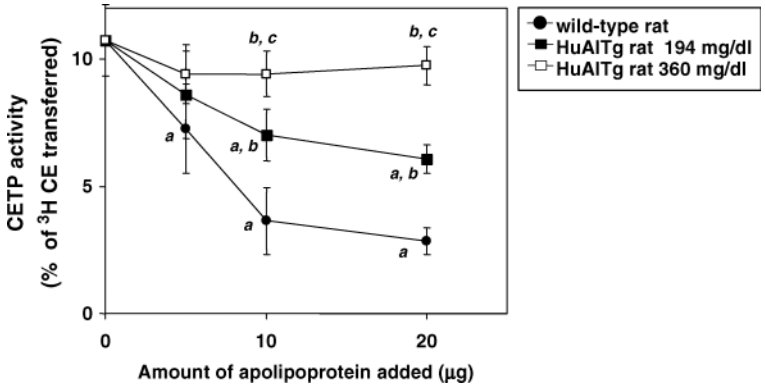

Fig. 5. Concentration-dependent effect of delipidated HDL apolipoproteins from wild-type and HuAITg rats on CETP activity. HDLs were ultracentrifugally isolated from the plasma of wild-type and HuAITg rats and delipidated as described under Materials and Methods. Delipidated apolipoprotein preparations were added to incubation mixtures containing human HDL (200 nmol of cholesterol/ml), radiolabeled LDL (50 nmol of cholesterol/ml), and purified CETP (0.17 μg/ml). At the end of the incubation, the percentage of radiolabeled cholesteryl esters transferred was determined after the separation of LDL and HDL by ultracentrifugation as described under Materials and Methods. Each point represents the mean ± SD of triplicate determinations. *a*: Significantly different from mixtures with no delipidated apolipoproteins added $P < 0.05$; *b*: significantly different from homologous values in wild-type rats, $P < 0.05$; *c*: significantly different from homologous value in HuAITg rat expressing 1.94 g/l of human apoA-I, $P < 0.05$. Statistics by Mann-Whitney test.

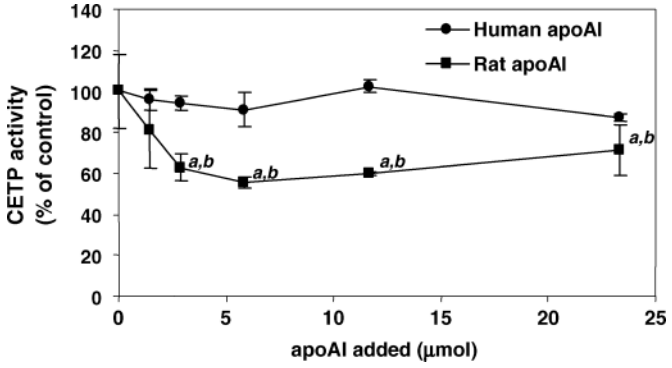

Fig. 6. Comparative effects of rat or human apoA-I on CETP activity. Rat and human apoA-I were purified as described under Materials and Methods, and they were added to incubation mixtures containing human HDL (200 nmol of cholesterol/ml), fluorescent-labeled liposomes, and purified CETP (0.17 µg/ml). Cholesteryl ester transfer rates were determined as described in Materials and Methods. Each point represents the mean ± SD of triplicate determinations. *a*: significantly different from control mixture with no apolipoprotein A-I added, $P < 0.05$ *b*: significantly different from homologous mixture containing human apoA-I, $P < 0.05$. Statistics by Mann-Whitney test.

DISCUSSION

Detailed analysis of plasma lipoprotein profiles of transgenic rats expressing either CETP or both CETP and human apoA-I revealed that CETP, when expressed in combination with human apoA-I, has a much more profound effect on neutral lipid transfers between HDL- and apoB-containing lipoproteins. These observations in transgenic rats are in very good agreement with earlier observations by Hayek and colleagues (23) in apoA-I/CETP transgenic mice. In both studies, plasma CETP expression levels were comparable in CETPTg and HuAITg/CETPTg animals, indicating that the observed changes in lipoprotein phenotype were due to a better interaction of CETP with human apoA-I-containing HDL than wild-type rodent HDL. Ex vivo observations with HDL isolated from mouse plasma (24) or from rat plasma (present study) demonstrated that the differences between the properties of human apoAITg and wild-type HDL relate to differences in the CETP-inhibitory potential of the particles. Earlier studies in mice suggested that CETP might be activated more efficiently by human apoA-I than by mouse apoA-I (23). In the present study, a significant, concentration-dependent inhibition of CETP was observed in the presence of rat apoA-I. Although this

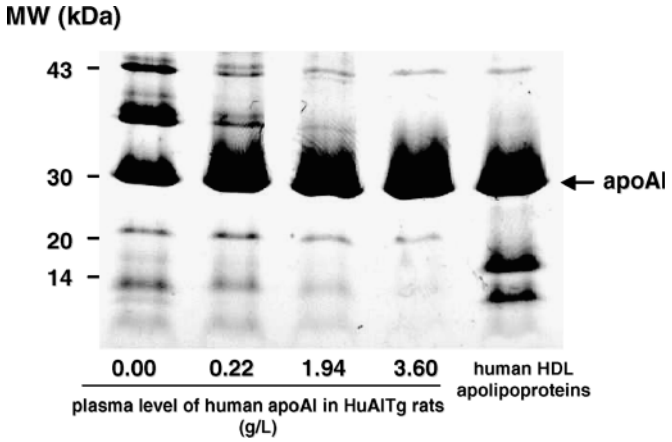

Fig. 7. SDS polyacrylamide gradient gel electrophoresis of HDL apolipoproteins from humans, wild-type rats (0.0 g/L of human apo A-I), and HuAITg rats. HDL apolipoproteins were incubated for 5 min at 70°C in SDS-containing TBS buffer. Subsequently, HDL apolipoproteins were separated by SDS electrophoresis in 8–25 polyacrlyamide gradient gels (Phast System, Pharmacia). Finally, proteins were stained with Coomassie brilliant **b**lue as described under Materials and Methods.

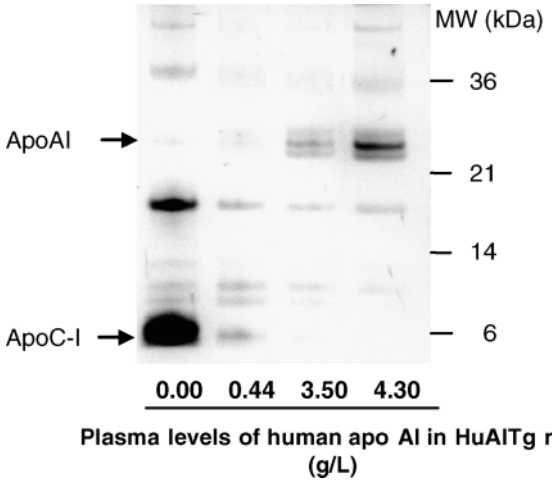

Fig. 8. SDS polyacrylamide gradient gel electrophoresis of VLDL apolipoproteins from wild-type rats and HuAITg rats. Delipidated VLDL apolipoproteins were incubated for 5 min at 70°C in SDS-containing TBS buffer and were separated by electrophoresis on 4–12% NuPage Novex SDS-polyacrylamide gels according to the manufacturer's instructions. After electrophoretic migration, gels were silver stained, and apparent molecular weight of individual protein bands were determined by comparison with protein standards.

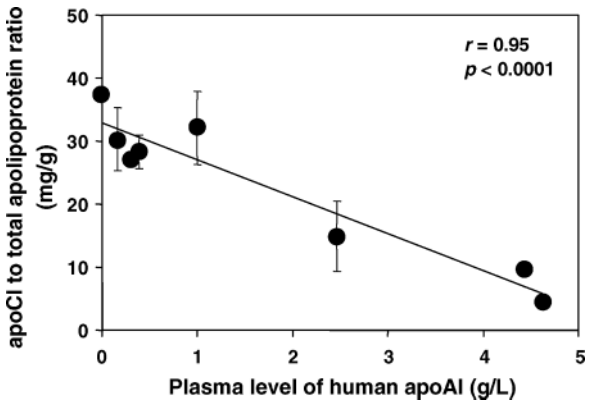

Fig. 9. Correlation between human apoA-I expression level and apoC-I content of HDL in HuAITg rats. Human apoA-I and apoC-I were quantified as described under Materials and Methods. Coefficient of correlation (*r*) was calculated by using linear regression analysis. Error bars indicate mean ± SD.

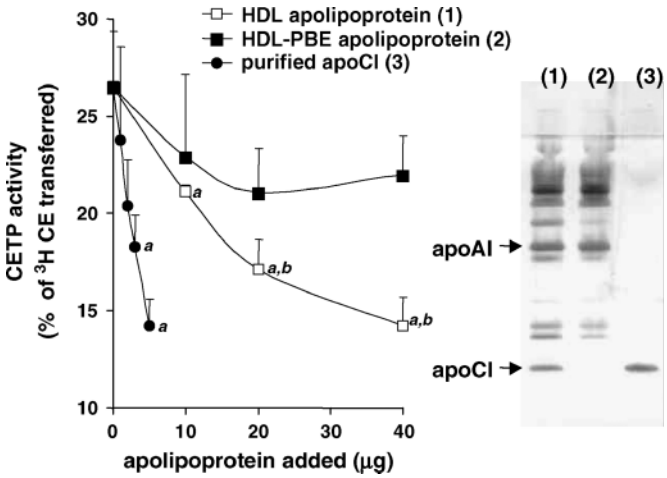

Fig. 10. Determination of the relative contribution of apoC-I to the CETP inhibitory activity associated with plasma HDL. HDLs were ultracentrifugally isolated from plasmas of wild-type rats and were subsequently delipidated as described in Materials and Methods. ApoC-I was selectively removed from total apolipoproteins by passage through a polybuffer exchanger column (see Materials and Methods). Delipidated apolipoprotein preparations were added to incubation mixtures containing human HDL (200 nmol of cholesterol/ml), radiolabeled LDL (50 nmol of cholesterol/ml), and purified CETP (0.17 μg/ml), and cholesteryl ester transfer rates were determined as described under Materials and Methods. Each point represents the mean ± SD of triplicate determinations. *a*: Significantly different from incubation mixtures with no apolipoprotein added, $P < 0.05$; *b*: significantly different from homologous samples receiving the same amounts of apoC-I-free HDL apolipoproteins, $P < 0.05$. Statistics by Mann-Whitney test.

effect might relate to a direct inhibition of CETP by rat apoA-I, it might also be due to the displacement of human apoA-I by rat apoA-I. A similar mechanism, based on the displacement of human apoA-I, was proposed to explain the inhibitory potential of human apoC-III in a previous study (31). Beyond the modulating potential of apoA-I, the present study demonstrates that the reduction in HDL apoC-I in transgenic rats overexpressing human apoA-I contributes predominantly to the loss of the CETP-inhibitory potential of rat HDL. In this case, CETP inhibition was proven to be a direct and specific property of apoC-I (6, 32).

ApoA-I, the major apolipoprotein component of HDL, has pleiotropic effects in vivo, including activation of LCAT and stimulation of the reverse cholesterol transport pathway (33–35). Despite close similarities between the primary structures of rat and human apoA-I, the two proteins cannot be considered identical in terms of HDL structure and function (36). The replacement of mouse apoA-I by human apoA-I in transgenic mice was shown to produce dramatic changes in HDL particle size distribution and was associated with a reduction in the selective uptake of HDL cholesteryl esters by the liver (22). HDLs from HuAITg mice are less efficient in mediating cell cholesterol efflux than wild-type HDL (31). As well, human PLTP functions optimally when coexpressed with human apoA-I in PLTP/apoAI transgenic mice (37), human LCAT has a preference for HDL-containing human apoA-I (38), and earlier in vitro studies demonstrated that rat apoA-I interacts more readily with rat LCAT than do the human components (39). Because CETP-mediated changes are much more pronounced in apoAITg/CETPTg rodents than in their CETPTg counterparts (23) and present study)], it is conceivable that human CETP works optimally with human apoA-I. In fact, the present study suggests that rodent apoA-I may contribute directly to CETP activity inhibition, a property that is not shared by human apoA-I. Alternatively, the hypothesis of a displacement of a CETP activator (human apoA-I) by a neutral factor (rat apoA-I) remains plausible. Nevertheless, the results shown in Fig. 10 indicate that the latter hypothesis could not account for more than 30% of the total inhibitory activity associated with HDL.

In contrast to previous studies in HuAITg animals with no plasma CETP activity, in which overexpression of apoA-I caused only minimal changes in the structure and metabolism of apoB-containing lipoproteins (22, 40), CETP expression in transgenic rats and mice produced profound alterations in the neutral lipid content of VLDL and LDL [(23) and present study]. In particular, the changes were characterized by a significant increase in the cholesteryl ester-to-triglyceride ratio of VLDL+LDL. The latter point is important to consider, inasmuch as kinetic studies in rabbits and humans demonstrated that the majority of cholesteryl esters initially generated in the HDL core are actually transferred toward the apoB-containing lipoprotein fraction during their intravascular transport (41–43). It is worth noting that similar lipoprotein changes were achieved in both apoAITg/CETPTg mice (23) and apoCIKO/CETPTg mice (6), when compared with their CETPTg counterparts. Increases in the apoB-containing lipoprotein-to-HDL cholesteryl ester ratio were similar in these animals, and they are reflective of the CETP-mediated neutral lipid transfer activities in these models. On the basis of the profound changes that human apoA-I overexpression induces in the apolipoprotein pattern of circulating rodent HDL, we hypothesized that the apoA-I-mediated removal of apoC-I from HDL might also contribute significantly to the magnification of the lipoprotein phenotype in HuAITg/CETPTg rats. In the present study, we demonstrate that the loss of apoC-I in HDL not only contributes to the rise in CETP activity in apoAITg rats but also is the major factor that accounts for the observed difference in lipoprotein phenotype in HuAITg/CETPTg rats compared with CETPTg rats. Rat and human apoC-I display very similar structures and a weak electronegativity with a cluster of lysine residues in the C terminus of the protein in both species (44). These similarities may explain why, in the present study, both rat and human apoC-I exert a potent inhibitory effect on CETP activity. Our results are further supported by in vivo studies in which the specific CETP activity was substantially reduced in CETPTg mice expressing human apoC-I (45) and the CETP-mediated effects on the plasma lipoprotein profile were magnified by apoC-I deficiency in apoCIKO/CETPTg mice (6). Although the molecular mechanism

that links human apoA-I expression to the reduction in the apoC-I content of rat HDL remains to be further clarified, we observed that apoC-I mRNA levels were unchanged in HuAITg mice, as compared with wild-type mice. These data are in good agreement with observations made with other murine apolipoproteins in HuAITg rodents (20–22), indicating that the significant reduction of apolipoprotein secretion by liver of HuAITg rats occurs at a postranscriptional level. In further support of the latter view, we observed that not only HDL apoC-I but also VLDL apoC-I was significantly reduced in HuAITg rats. Because apoC-I is secreted mainly in VLDL (46), the present observations suggest that apoC-I and human apoA-I may compete for the association with VLDL, thus contributing, at least in part, to the significant reduction in apoC-I levels in the plasma. In addition, a displacement of apoC-I by apoA-I could occur in the plasma compartment, producing lipoprotein-free apoC-I that could predictably show an accelerated catabolic rate.

In conclusion, our work in HuAITg/CETPTg rats demonstrated that the overexpression of human apoA-I in rats results in fewer CETP inhibitors at the HDL surface. ApoC-I redistribution accounts for the majority of differences in neutral lipid exchanges measured with HDL from either HuAITg animals or wild-type controls. These observations contribute to a more complete picture of the effect of apoA-I overexpression on the quantity and quality of circulating HDL, which has been identified as a promising strategy in the treatment of atherosclerosis. In this context, apoA-I_{Milano}, a human apoA-I variant with potent antiatherosclerotic properties (47, 48), has received considerable attention over the last decade. As compared with apoA-I, apoA-I_{Milano} contains fewer helical segments, accounting for significant differences in the ability of the two proteins to interact with lipids and to determine HDL structure (49, 50). Whether apoA-I_{Milano} also has the ability to displace apoC-I from human HDL and to modulate CETP activity or whether it differs from native apoA-I in this respect deserves further attention.

This research was supported by an International HDL Research Awards Program grant (L.L.) and by the Institut National de la Santé et de la Recherche Médicale, the Conseil Régional de Bourgogne, and the Fondation de France. The authors thank Novartis Pharmaceuticals Corporation for giving access to the human apoA-I transgenic rat line.

REFERENCES

1. Tribble, D. L., and R. M. Krauss. 1993. HDL and coronary artery disease. *Adv. Intern. Med.* **38:** 1–29.
2. Gordon, D. J., and B. M. Rifkind. 1989. High-density lipoprotein—the clinical implications of recent studies. *N. Engl. J. Med.* **321:** 1311–1316.
3. Rutledge, J. C., A. E. Mullick, G. Gardner, and I. J. Goldberg. 2000. Direct visualization of lipid deposition and reverse lipid transport in a perfused artery: roles of VLDL and HDL. *Circ. Res.* **86:** 768–773.
4. Shah, P. K., S. Kaul, J. Nilsson, and B. Cercek. 2001. Exploiting the vascular protective effects of high-density lipoprotein and its apolipoproteins: an idea whose time for testing is coming, part II. *Circulation.* **104:** 2498–2502.
5. Gautier, T., D. Masson, J. P. de Barros, A. Athias, P. Gambert, D. Aunis, M. H. Metz-Boutigue, and L. Lagrost. 2000. Human apolipoprotein C-I accounts for the ability of plasma high density lipoproteins to inhibit the cholesteryl ester transfer protein activity. *J. Biol. Chem.* **275:** 37504–37509.
6. Gautier, T., D. Masson, M. C. Jong, L. Duverneuil, N. Le Guern, V. Deckert, J. P. Pais de Barros, L. Dumont, A. Bataille, Z. Zak, et al. 2002. Apolipoprotein CI deficiency markedly augments plasma lipoprotein changes mediated by human cholesteryl ester transfer protein (CETP) in CETP transgenic/ApoCI-knocked out mice. *J. Biol. Chem.* **277:** 31354–31363.
7. Sugano, M., N. Makino, S. Sawada, S. Otsuka, M. Watanabe, H. Okamoto, M. Kamada, and A. Mitsushima. 1998. Effect of antisense oligonucleotides against cholesteryl ester transfer protein on the development of atherosclerosis in cholesterol-fed rabbits. *J. Biol. Chem.* **273:** 5033–5036.
8. Rittershaus, C. W., D. P. Miller, L. J. Thomas, M. D. Picard, C. M. Honan, C. D. Emmett, C. L. Pettey, H. Adari, R. A. Hammond, D. T. Beattie, et al. 2000. Vaccine-induced antibodies inhibit CETP activity in vivo and reduce aortic lesions in a rabbit model of atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* **20:** 2106–2112.
9. Okamoto, H., F. Yonemori, K. Wakitani, T. Minowa, K. Maeda, and H. Shinkai. 2000. A cholesteryl ester transfer protein inhibitor attenuates atherosclerosis in rabbits. *Nature.* **406:** 203–207.
10. de Grooth, G. J., J. A. Kuivenhoven, A. F. Stalenhoef, J. de Graaf, A. H. Zwinderman, J. L. Posma, A. van Tol, and J. J. Kastelein. 2002. Efficacy and safety of a novel cholesteryl ester transfer protein inhibitor, JTT-705, in humans: a randomized phase II dose-response study. *Circulation.* **105:** 2159–2165.
11. Brousseau, M. E., E. J. Schaefer, M. L. Wolfe, L. T. Bloedon, A. G. Digenio, R. W. Clark, J. P. Mancuso, and D. J. Rader. 2004. Effects of an inhibitor of cholesteryl ester transfer protein on HDL cholesterol. *N. Engl. J. Med.* **350:** 1505–1515.
12. Clark, R. W., T. A. Sutfin, R. B. Ruggeri, A. T. Willauer, E. D. Sugarman, G. Magnus-Aryitey, P. G. Cosgrove, T. M. Sand, R. T. Wester, J. A. Williams, et al. 2004. Raising high-density lipoprotein in humans through inhibition of cholesteryl ester transfer protein: an initial multidose study of torcetrapib. *Arterioscler. Thromb. Vasc. Biol.* **24:** 490–497.
13. Nissen, S. E., T. Tsunoda, E. M. Tuzcu, P. Schoenhagen, C. J. Cooper, M. Yasin, G. M. Eaton, M. A. Lauer, W. S. Sheldon, C. L. Grines, et al. 2003. Effect of recombinant ApoA-I Milano on coronary atherosclerosis in patients with acute coronary syndromes: a randomized controlled trial. *J. Am. Med. Assoc.* **290:** 2292–2300.
14. Kaul, S., V. Rukshin, R. Santos, B. Azarbal, C. L. Bisgaier, J. Johansson, V. T. Tsang, K. Y. Chyu, B. Cercek, J. Mirocha, et al. 2003. Intramural delivery of recombinant apolipoprotein A-IMilano/phospholipid complex (ETC-216) inhibits in-stent stenosis in porcine coronary arteries. *Circulation.* **107:** 2551–2554.
15. Navab, M., G. M. Anantharamaiah, S. Hama, D. W. Garber, M. Chaddha, G. Hough, R. Lallone, and A. M. Fogelman. 2002. Oral administration of an Apo A-I mimetic peptide synthesized from D-amino acids dramatically reduces atherosclerosis in mice independent of plasma cholesterol. *Circulation.* **105:** 290–292.
16. Shah, P. K., J. Nilsson, S. Kaul, M. C. Fishbein, H. Ageland, A. Hamsten, J. Johansson, F. Karpe, and B. Cercek. 1998. Effects of recombinant apolipoprotein A-I(Milano) on aortic atherosclerosis in apolipoprotein E-deficient Mice. *Circulation.* **97:** 780–785.
17. Chiesa, G., and C. R. Sirtori. 2003. Recombinant apolipoprotein A-I (Milano): a novel agent for the induction of regression of atherosclerotic plaques. *Ann Med.* **35:** 267–273.
18. Rubin, E. M., R. M. Krauss, E. A. Spangler, J. G. Verstuyft, and S. M. Clift. 1991. Inhibition of early atherogenesis in transgenic mice by human apolipoprotein AI. *Nature.* **353:** 265–267.
19. Deckert, V., G. Lizard, N. Duverger, A. Athias, V. Palleau, F. Emmanuel, M. Moisant, P. Gambert, C. Lallemant, and L. Lagrost. 1999. Impairment of endothelium-dependent arterial relaxation by high-fat feeding in ApoE-deficient mice: toward normalization by human ApoA-I expression. *Circulation.* **100:** 1230–1235.
20. Rubin, E. M., B. Y. Ishida, S. M. Clift, and R. M. Krauss. 1991. Expression of human apolipoprotein A-I in transgenic mice results in reduced plasma levels of murine apolipoprotein A-I and the appearance of two new high density lipoprotein size subclasses. *Proc. Natl. Acad. Sci. USA.* **88:** 434–438.

21. Swanson, M. E., T. E. Hughes, I. S. Denny, D. S. France, J. R. Paterniti, Jr., C. Tapparelli, P. Gfeller, and K. Burki. 1992. High level expression of human apolipoprotein A-I in transgenic rats raises total serum high density lipoprotein cholesterol and lowers rat apolipoprotein A-I. *Transgenic Res.* **1:** 142–147.
22. Chajek-Shaul, T., T. Hayek, A. Walsh, and J. L. Breslow. 1991. Expression of the human apolipoprotein A-I gene in transgenic mice alters high density lipoprotein (HDL) particle size distribution and diminishes selective uptake of HDL cholesteryl esters. *Proc. Natl. Acad. Sci. USA.* **88:** 6731–6735.
23. Hayek, T., T. Chajek-Shaul, A. Walsh, L. B. Agellon, P. Moulin, A. R. Tall, and J. L. Breslow. 1992. An interaction between the human cholesteryl ester transfer protein (CETP) and apolipoprotein A-I genes in transgenic mice results in a profound CETP-mediated depression of high density lipoprotein cholesterol levels. *J. Clin. Invest.* **90:** 505–510.
24. Masson, D., N. Duverger, F. Emmanuel, and L. Lagrost. 1997. Differential interaction of the human cholesteryl ester transfer protein with plasma high density lipoproteins (HDLs) from humans, control mice, and transgenic mice to human HDL apolipoproteins. Lack of lipid transfer inhibitory activity in transgenic mice expressing human apoA-I. *J. Biol. Chem.* **272:** 24287–24293.
25. Zak, Z., L. Lagrost, T. Gautier, D. Masson, V. Deckert, L. Duverneuil, J. P. De Barros, N. Le Guern, L. Dumont, M. Schneider, et al. 2002. Expression of simian CETP in normolipidemic Fisher rats has a profound effect on large sized apoE-containing HDL. *J. Lipid Res.* **43:** 2164–2171.
26. Cham, B. E., and B. R. Knowles. 1976. A solvent system for delipidation of plasma or serum without protein precipitation. *J. Lipid Res.* **17:** 176–181.
27. Lagrost, L., L. Perségol, C. Lallemant, and P. Gambert. 1994. Influence of apolipoprotein composition of high density lipoprotein particles on cholesteryl ester transfer protein activity. Particles containing various proportions of apolipoproteins AI and AII. *J. Biol. Chem.* **269:** 3189–3197.
28. Lagrost, L., A. Athias, P. Gambert, and C. Lallemant. 1994. Comparative study of phospholipid transfer activities mediated by cholesteryl ester transfer protein and phospholipid transfer protein. *J. Lipid Res.* **35:** 825–835.
29. Meunier, S., P. Gambert, J. Desgres, and C. Lallemant. 1986. Preparative electrophoresis of human apolipoprotein E: an improved method. *J. Lipid Res.* **27:** 1324–1327.
30. Lagrost, L., P. Gambert, S. Meunier, P. Morgado, J. Desgres, P. d'Athis, and C. Lallemant. 1989. Correlation between apolipoprotein A-IV and triglyceride concentrations in human sera. *J. Lipid Res.* **30:** 701–710.
31. Ohnishi, T., and S. Yokoyama. 1993. Activation of human plasma lipid transfer protein by apolipoproteins. *Biochemistry.* **32:** 5029–5035.
32. Dumont, L., T. Gautier, J. P. de Barros, H. Laplanche, D. Blache, P. Ducoroy, J. Fruchart, J. C. Fruchart, P. Gambert, D. Masson, et al. 2005. Molecular mechanism of the blockade of plasma cholesteryl ester transfer protein (CETP) by its physiological inhibitor apolipoprotein CI. *J. Biol. Chem.* **280:** 38108–38116.
33. Albers, J. J., J. Lin, and G. P. Roberts. 1979. Effect of human plasma apolipoproteins on the activity of purified lecithin:cholesterol acyltransferase. *Artery.* **5:** 61–75.
34. Atger, V., M. de la Llera Moya, M. Bamberger, O. Francone, P. Cosgrove, A. Tall, A. Walsh, N. Moatti, and G. Rothblat. 1995. Cholesterol efflux potential of sera from mice expressing human cholesteryl ester transfer protein and/or human apolipoprotein AI. *J. Clin. Invest.* **96:** 2613–2622.
35. Golder-Novoselsky, E., A. V. Nichols, E. M. Rubin, and T. M. Forte. 1995. Human apo A-I in transgenic mice is more efficient in activating lecithin:cholesterol acyltransferase than mouse apo A-I. *Biochim. Biophys. Acta.* **1254:** 217–220.
36. Gong, E. L., C. S. Tan, M. I. Shoukry, E. M. Rubin, and A. V. Nichols. 1994. Structural and functional properties of human and mouse apolipoprotein A-I. *Biochim. Biophys. Acta.* **1213:** 335–342.
37. Jiang, X.-C., O. L. Francone, C. Bruce, R. Milne, J. Mar, A. Walsh, and J. L. Breslow. 1996. Increased prebeta-high density lipoprotein, apolipoprotein AI, and phospholipid in mice expressing the human phospholipid transfer protein and human apolipoprotein AI transgenes. *J. Clin. Invest.* **98:** 2373–2380.
38. Francone, O. L., E. L. Gong, D. S. Ng, C. J. Fielding, and E. M. Rubin. 1995. Expression of human lecithin-cholesterol acyltransferase in transgenic mice. Effect of human apolipoprotein AI and human apolipoprotein all on plasma lipoprotein cholesterol metabolism. *J. Clin. Invest.* **96:** 1440–1448.
39. Furukawa, Y., T. Urano, Y. Hida, H. Itoh, C. Takahashi, and S. Kimura. 1992. Interaction of rat lecithin-cholesterol acyltransferase with rat apolipoprotein A-I and with lecithin-cholesterol vesicles. *J. Biochem. (Tokyo).* **111:** 413–418.
40. Walsh, A., Y. Ito, and J. L. Breslow. 1989. High levels of human apolipoprotein A-I in transgenic mice result in increased plasma levels of small high density lipoprotein (HDL) particles comparable to human HDL3. *J. Biol. Chem.* **264:** 6488–6494.
41. Goldberg, D. I., W. F. Beltz, and R. C. Pittman. 1991. Evaluation of pathways for the cellular uptake of high density lipoprotein cholesterol esters in rabbits. *J. Clin. Invest.* **87:** 331–346.
42. Schwartz, C. C., J. M. VandenBroek, and P. S. Cooper. 2004. Lipoprotein cholesteryl ester production, transfer, and output in vivo in humans. *J. Lipid Res.* **45:** 1594–1607.
43. Ouguerram, K., M. Krempf, C. Maugeais, P. Maugere, D. Darmaun, and T. A. Magot. 2002. A new labeling approach using stable isotopes to study in vivo plasma cholesterol metabolism in humans. *Metabolism.* **51:** 5–11.
44. Jackson, R. L., J. T. Sparrow, H. N. Baker, J. D. Morrisett, O. D. Taunton, and A. M. Gotto, Jr. 1974. The primary structure of apolopoprotein-serine. *J. Biol. Chem.* **249:** 5308–5313.
45. Gautier, T., D. Masson, M. C. Jong, J. P. Pais de Barros, L. Duverneuil, N. Le Guern, V. Deckert, L. Dumont, A. Bataille, Z. Zak, et al. 2005. Apolipoprotein CI overexpression is not a relevant strategy to block cholesteryl ester transfer protein (CETP) activity in CETP transgenic mice. *Biochem. J.* **385:** 189–195.
46. Cohn, J. S., M. Tremblay, R. Batal, H. Jacques, L. Veilleux, C. Rodriguez, L. Bernier, O. Mamer, and J. Davignon. 2002. Plasma kinetics of VLDL and HDL apoC-I in normolipidemic and hypertriglyceridemic subjects. *J. Lipid Res.* **43:** 1680–1687.
47. Chiesa, G., E. Monteggia, M. Marchesi, P. Lorenzon, M. Laucello, V. Lorusso, C. Di Mario, E. Karvouni, R. S. Newton, C. L. Bisgaier, et al. 2002. Recombinant apolipoprotein A-I(Milano) infusion into rabbit carotid artery rapidly removes lipid from fatty streaks. *Circ. Res.* **90:** 974–980.
48. Weisgraber, K. H., S. C. Rall, Jr., T. P. Bersot, R. W. Mahley, G. Franceschini, and C. R. Sirtori. 1983. Apolipoprotein A-IMilano. Detection of normal A-I in affected subjects and evidence for a cysteine for arginine substitution in the variant A-I. *J. Biol. Chem.* **258:** 2508–2513.
49. Franceschini, G., G. Vecchio, G. Gianfranceschi, D. Magani, and C. R. Sirtori. 1985. Apolipoprotein AIMilano. Accelerated binding and dissociation from lipids of a human apolipoprotein variant. *J. Biol. Chem.* **260:** 16321–16325.
50. Calabresi, L., G. Vecchio, F. Frigerio, L. Vavassori, C. R. Sirtori, and G. Franceschini. 1997. Reconstituted high-density lipoproteins with a disulfide-linked apolipoprotein A-I dimer: evidence for restricted particle size heterogeneity. *Biochemistry.* **36:** 12428–12433.