Expression of simian cholesteryl ester transfer protein (CETP) in C57BL/6 mice causes the animals’ high density lipoprotein (HDL) levels to decrease. The purpose of these studies was to determine how CETP expression caused that reduction. Chemical analysis showed that the HDL of the CETP transgenic mice had about twice as much triglyceride and only about 60% as much cholesteryl ester as the HDL from the C57BL/6 mice. Both strains of mouse had high levels of a circulating lipid. When plasma from the mice was incubated at 37°C for 5 h, the triglycerides in the HDL were hydrolyzed, and apoA-I was shed from the particle. However, apoA-I was shed from the CETP HDL more rapidly than it was shed from the C57BL/6 HDL. Because “free” apoA-I is rapidly cleared by the kidney, increased production of free apoA-I would be expected to shorten the average life span of apoA-I in the mouse. Kinetic analyses indicated that the life span of apoA-I was significantly reduced in the CETP transgenic mice. It was concluded that CETP expression enriched the core of the HDL with triglyceride, which rendered it vulnerable to lipolysis, causing apoA-I to be shed from the particle. That shortened the life span of apoA-I in the CETP mice, which led to lower plasma levels of the protein.

As epidemiological data linking low plasma HDL levels with increased risk of developing coronary artery disease continues to strengthen, so too does interest in the mechanisms that control those levels. HDL originate as small, disc-shaped particles containing apoA-I, phospholipid, unesterified cholesterol, and possibly triglyceride (Marsh and Diffenderfer, 1991). Once in the plasma, the nascent HDL grow in volume as a result of cholesterol uptake and esterification and in surface area as a result of phospholipid and protein uptake (for a comprehensive review of HDL metabolism, see Eisenberg, 1984). The processes responsible for clearance of HDL from the plasma are not well understood. In some instances the whole particle is apparently taken up by the liver (in a manner analogous to that of LDL) (Mahley, 1988); in other instances, the various components move into or out of the particle and are catabolized independently (Glass et al., 1983a, 1985). As a result, it is somewhat difficult to define the average life span of an HDL particle with precision. Since apoA-I is the principal protein component of HDL, its life span is often equated with that of HDL, and in fact, the relationship between plasma apoA-I levels and atherosclerosis is nearly the same as that between HDL cholesterol and atherosclerosis (Stampfer et al., 1991).

We have suggested previously (Melchior et al., 1990; Pape et al., 1991), based on studies with nonhuman primates, that the plasma apoA-I levels are more a function of the life span of the apoA-I-containing particles than of the apoA-I synthesis rate. Those studies indicated that although the apoA-I synthesis rate could vary slightly in response to dietary changes, the degree to which it can vary is limited, and therefore, at least in the cynomolgus monkey, apoA-I synthesis is not a major control point for plasma HDL levels. Those studies also suggested that the life span of apoA-I was related to the size of the particles with which it was associated; the larger the HDL particle, the longer the mean life span of apoA-I and vice versa. If that is correct, factors that influence the size of the HDL may have a potent effect on their plasma concentration.

There are several points in the metabolism of HDL at which the size of the particle is affected. Two enzymes in particular, lecithin:cholesterol acyltransferase and hepatic lipase, are known to have an especially potent effect on the size of the particle (Glomset and Norum, 1973; Hopkins and Barter, 1986); however, a third process, and one that appears from a statistical standpoint to be relatively important (Quinet et al., 1991; Pape et al., 1991), is exchange of core lipids (cholesterol esters and triglycerides) between HDL and other lipoproteins. That process is mediated by cholesteryl ester transfer protein (CETP, also called lipid transfer protein-1 (Quig and Zilversmit, 1990)). CETP is capable of transferring triglycerides, cholesterol esters, phospholipids, and possibly other hydrophobic compounds found in the lipoproteins; however, under normal conditions in humans, CETP mediates a net movement of cholesterol esters from HDL to VLDL, in exchange for triglyceride (Quig and Zilversmit, 1990). Thus, the VLDL become enriched in cholesterol esters, and the HDL gain triglyceride. The VLDL-associated cholesterol is returned to the liver when the VLDL and its metabolic products are cleared from the plasma by the hepatic lipoprotein receptors. The triglyceride contained in the HDL is thought to be removed by hepatic lipase, without destruction of the particle (Shirai et al., 1981; Groot et al., 1983; Barter et al., 1987).
extremely high plasma HDL levels (Takegoshi et al., 1988; Brown et al., 1986; Yamashita et al., 1990, 1991), and those HDL are unusually large, cholesterol ester-rich particles (Yamashita et al., 1991). By contrast, experimental conditions that lead to increased CETP activity in the plasma are associated with a reduction in the size and plasma concentration of the HDL (Franceschini et al., 1989; Melchior and Castle, 1989; Melchior et al., 1990; Quinet et al., 1990, 1991; Pape et al., 1991; Agellon et al., 1991; McPherson et al., 1991; Marotti et al., 1992; Hayek et al., 1992). Thus CETP might well be involved in the process that leads to reduced HDL levels in man; however, the mechanism by which CETP alters the plasma HDL concentration has not been determined.

One interesting hypothesis as to how CETP might reduce the size of the HDL has been proposed by Barter et al. (1987). These investigators suggested that by replacing the cholesterol esters in the core of the HDL with triglyceride, CETP renders the core of the particle vulnerable to the action of hepatic lipase. Hydrolysis of those triglycerides by the enzyme then leads to a reduction in the size of the particle. If the proposition that reducing the size of the HDL shortens the mean life span of apoA-I is correct, then one mechanism by which CETP could reduce the plasma apoA-I levels would be via this process.

The purpose of the studies reported here was to evaluate that possibility. We chose as our model C57BL/6 mice that expressed high levels of simian CETP. Those mice have been shown previously (Marotti et al., 1992) to have reduced plasma apoA-I and HDL cholesterol levels, and the size of their HDL was significantly smaller than that of the HDL from nontransgenic C57BL/6 mice. In this study we show that the core of the HDL from the transgenic mice is enriched in triglyceride relative to the HDL of control mice; that that triglyceride is a good substrate for a lipase present in mouse plasma; that coincident with hydrolysis of that triglyceride, apoA-I is lost from the particle; and that the average life span of apoA-I is significantly shorter in the CETP transgenic mice.

**EXPERIMENTAL PROCEDURES**

**Animals and Diets**

We have previously described four lines of transgenic C57BL/6 mice expressing cynomolgus monkey cholesteryl ester transfer protein (Marotti et al., 1992). Males from line UCTP-20, ranging in age from 3 to 7 months, were used for these studies. Male C57BL/6 mice, obtained from the Upjohn colony and of approximately the same age as the UCTP-20 mice, were used as controls. Male ICR mice were used to compare the effect of route of administration on measurements of apoA-I residence time. The mice used in these studies were on a 12-h light/dark cycle and consumed only Purina Rodent Chow (Ralston Purina, St. Louis, MO). All procedures in this study were reviewed by the Upjohn Company Corporate Animal Welfare Committee and are in compliance with the animal welfare act regulations, 9 CFR Parts 1, 2 and 4. To reduce the effects of repeated bleedings, at least 5 h were allowed to elapse between bleedings, and no mouse was bled more than three times during an experiment.

**Analytical Methods**

HDL (if 1.063–1.225 g/ml) were isolated from pooled mouse plasma by ultracentrifugation essentially as described previously (Melchior et al., 1984). The total and nonesterified cholesterol concentrations were determined by the enzymatic method of Allain et al. (1974) and the concentration of esterified cholesterol obtained by subtraction. The triglyceride concentration was determined by the method of Mcgowan et al. (1983). A lipid extract (Folch et al., 1957) of the sample was used to measure phospholipids, and the lipid-phosphorus was quantified by a modification of the Bartlett procedure (Bartlett, 1959). Protein was measured using a modification of the Lowry method (Peterson, 1983); measurement of total protein was quantified by the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA) and greater than 96% of the radioactivity comigrated with apoA-I in every instance. Finally, the plasma was separated from the cells by centrifugation, diluted 1/10 with PBS containing EDTA (1.2 g/liter), chloramphenicol (0.08 g/liter) sodium azide (0.1 g/liter), and gentamicin sulfate (0.08 g/liter) and stored at 4 °C until analyzed. The total radioactivity in the plasma samples was measured with a Beckman 5500 g-counter. The fraction of the protein-bound radioactivity was quantitated by incubation of the plasma samples with 0.37 

**Preparation of Radiolabeled ApoA-I**

Mouse apoA-I, purified as described previously for cynomolgus monkey apoA-I (Melchior et al., 1984, 1989), was dissolved in PBS (1 μg patty mouse plasma and radioiodination with Na125I (American Curityt) using IODO-BEADS (Pierce Chemical Co.) essentially as described by the manufacturer. Briefly, one IODO-BEAD and 2 μl (200 μCi) of Na125I were added to 100 μl of PBS contained in a 1.5-ml Eppendorf centrifuge tube. That mixture was allowed to sit at room temperature for 5 min, after which 100 μl of the apoA-I solution was added, and the tube was vortexed and allowed to sit at room temperature for 20 min. The IODO-BEAD was removed and the apoA-I preparation passed through a PD-10 desalting column (Pharmacia LKB Biotechnology Inc.). That had been previously equilibrated with PBS. The fractions containing the protein-bound radioactivity were identified using a hand-held survey meter equipped with a Na crystal detector. Those fractions were pooled and dialyzed extensively against PBS.

Analysis of the radiolabeled apoA-I preparations indicated that in every case greater than 98% was precipitated by 10% trichloroacetic acid, approximately 93% co-purified with mouse apoA-I, and greater than 95% co-purified with free apoA-I on agarose electrophoresis. The specific radioactivity of the final preparations averaged 0.37 ± 0.10 μCi/μg of protein.

**ApoA-I Turnover Studies**

Four experiments were conducted. Experiment 1 was a study designed to determine if the route of administration affected the estimates of mean residence time. Experiments 2–4 were studies to compare the mean residence time of apoA-I in UCTP-20 mice with that in C57BL/6 mice. Twelve ICR mice were used for experiment 1 (6 received isotope intravenously; 6 received it intraperitoneally); 12 ICR mice were used for experiment 2 (6 UCTP-20 and 6 C57BL/6); 36 mice were used for experiments 3 and 4 (9 UCTP-20 and 9 C57BL/6 mice for each experiment). All of the mice were given 250 μl of a 5 μg/ml Na125I solution intraperitoneally the day before the Na125I-labeled apoA-I was injected, to inhibit uptake of radioactive iodine by the thyroid gland. Immediately prior to injection, the radiolabeled apoA-I was diluted with sterile PBS to a final concentration of 2.0 ± 1.3 μCi/ml (mean ± S.D. for the four experiments), and 250 μl of that solution was injected into the mice. Blood samples (70 μl) were taken from the peribronchial sinuses of subgroups of the mice at 0.167, 0.5, 1, 3, 5, 7, 10, 24, 48, and 72 h after isotope injection. At least two mice were used for each time point in experiments 1 and 2, and at least three mice were used for each time point in experiments 3 and 4. To reduce the effects of repeated bleedings, at least 5 h were allowed to elapse between bleedings, and no mouse was bled more than three times during an experiment. The plasma was separated from the cells by centrifugation, diluted 1/10 with PBS containing EDTA (1.2 g/liter), chloramphenicol (0.08 g/liter) sodium azide (0.1 g/liter), and gentamicin sulfate (0.08 g/liter) and stored at 4 °C until analyzed. The total radioactivity in the plasma samples was measured with a Beckman 5500 g-counter. The fraction of the protein-bound radioactivity was quantitated by incubation of the plasma samples with 0.37 

**Lipase activity was measured by adding human HDL that contained [9,10-3H]triolein (DuPont NEN) to fresh mouse or human plasma and incubating that mixture at 37 °C for several hours. Ten μl of the HDL solution (10 μg of HDL cholesterol, 4.96 × 104 dpm of [9,10-3H]triolein) was added to 190 μl of the plasma. The HDL was labeled with the [9,10-3H]triolein by adding 15 μCi of the isotope dissolved in synthetic bile, to 30 ml of plasma, exactly as previously (Pape et al., 1991), and then isolating the HDL by ultracentrifugation. The free fatty acids were extracted by the method of Belfrage and Vaughan (1969), and the associated radioactive was quantified by liquid scintillation counting. Less than 0.5% of the radioactivity was extractable from the HDL in the absence of a source of lipase. To measure the effect of the incubation on the HDL triglyceride level, separate aliquots of the plasma were added to dialysis bags and dialyzed against PBS at 37 °C for the same period of time. Afterward, the VLDL + LDL triglyceride was removed by polyethylene glycol precipitation (Chesebro and Svehag, 1989) and the HDL triglycerides quantified using the McGowan method (McGowan et al., 1983).**

**Preparation of Radiolabeled ApoA-I**

Mouse apoA-I, purified as described previously for cynomolgus monkey apoA-I (Melchior et al., 1984, 1989), was dissolved in PBS (1 μg patty mouse plasma and radioiodination with Na125I (American Curityt) using IODO-BEADS (Pierce Chemical Co.) essentially as described by the manufacturer. Briefly, one IODO-BEAD and 2 μl (200 μCi) of Na125I were added to 100 μl of PBS contained in a 1.5-ml Eppendorf centrifuge tube. That mixture was allowed to sit at room temperature for 5 min, after which 100 μl of the apoA-I solution was added, and the tube was vortexed and allowed to sit at room temperature for 20 min. The IODO-BEAD was removed and the apoA-I preparation passed through a PD-10 desalting column (Pharmacia LKB Biotechnology Inc.). That had been previously equilibrated with PBS. The fractions containing the protein-bound radioactivity were identified using a hand-held survey meter equipped with a Na crystal detector. Those fractions were pooled and dialyzed extensively against PBS.

Analysis of the radiolabeled apoA-I preparations indicated that in every case greater than 98% was precipitated by 10% trichloroacetic acid, approximately 93% co-purified with mouse apoA-I, and greater than 95% co-purified with free apoA-I on agarose electrophoresis. The specific radioactivity of the final preparations averaged 0.37 ± 0.10 μCi/μg of protein.
ApoA-I Metabolism in CETP Transgenic Mice

TABLE I
Plasma lipid and apolipoprotein concentrations in CETP transgenic mice (UCTP-20) and C57BL/6 mice

| Mouse strain | Cholesterol | Triglyceride | Phospholipid | ApoB | Apo-A-I |
|--------------|-------------|--------------|--------------|------|---------|
|              | Total VLDL  | HDL          | Total VLDL   | HDL  |          |
|              | mg/dl       | mg/dl        | mg/dl        | mg/dl| mg/dl   |
| UCTP-20      | 61 ± 3*     | 22 ± 3       | 39 ± 3       | 46 ± 3| 23 ± 2  |
| C57BL/6      | 96 ± 2*     | 14 ± 1       | 82 ± 4       | 46 ± 2| 29 ± 2  |
|              | 117 ± 7     | 27 ± 2       | 90 ± 4       | 205 ± 8| 19 ± 1  |
| Prob > t     | <0.01       | <0.01        | <0.01        | <0.01| <0.01   |

* Mean ± S.E.

The effects of the CETP gene on the plasma lipoprotein profile and the apo-A-I mean residence time were tested for significance using an independent sample t test. The null hypothesis was not rejected unless the probability of obtaining a larger value of t, assgn ignored, was less than 0.05 (Snedecor and Cochran, 1967).

RESULTS
Table I shows the major plasma lipid and apolipoprotein concentrations of C57BL/6 mice and transgenic C57BL/6 mice expressing simian CETP. Those data indicate that CETP expression caused an increase in VLDL + LDL levels (whether measured as VLDL + LDL-associated cholesterol, phospholipids, or apoA) and a marked decrease in the HDL levels (whether measured as HDL-associated cholesterol, phospholipids, or apoA1). Note, however, that in both cases the triglycerides changed in the direction opposite that of the other lipids, i.e. the levels of VLDL + LDL-associated triglycerides were slightly lower, and the levels of HDL-associated triglycerides were slightly higher in the transgenic mice than in the C57BL/6 controls.

To evaluate the effect of CETP expression on HDL composition in more detail, HDL from both control and transgenic mice were isolated by ultracentrifugation and their composition determined. The results of those analyses are shown in Table II. The most striking difference in the HDL from the two strains of mouse was the molecular mass. Those calculations indicated that the HDL from UCTP-20 mice were, on average, 86 kDa smaller than the HDL from the C57BL/6 mice. Nonetheless, the chemical compositions (expressed as wt%) of the HDL from the two strains of mouse were quite similar. The major exception was the cholesteryl ester:triglyceride ratio. That ratio averaged 2.1 ± 0.01 in HDL from the transgenic mice versus 6.2 ± 0.06 in the C57BL/6 controls. Thus, CETP expression was associated with a reduction in the cholesteryl ester content and an increase in the triglyceride content of the HDL.

SDS-PAGE analysis of the HDL apoprotein distribution indicated that it did not change appreciably as a result of CETP expression (not shown). ApoA-I was the principal protein in both cases and was determined by immunochemical analysis to constitute between 60 and 70% of the total protein mass on both types of HDL (Table II). Note, however, that the mass of protein/mole of UCTP-20 HDL was significantly lower than the mass of protein/mole of C57BL/6 HDL. That difference in protein mass appeared to be due primarily to differences in the apoA-I content of the HDL. Those calculations were based on the assumption that all of the circulating apoA-I was HDL-associated. To confirm that this was the case, fresh plasma from UCTP-20 and C57BL/6 mice was analyzed by agarose-electro-
Composition of HDL from CETP transgenic mice (UCTP-20) and C57BL/6 mice

The values in the table are the mean values obtained from eight pools of mouse plasma (four C57BL/6 pools and four CETP pools). Each pool consisted of the plasma from 10 mice of a given strain. The molecular masses were calculated from the Stokes radii (r) and particle density (d) assuming: 

\[ M_{\text{HDL}} = \frac{4}{3} \pi r^3 \times 10^{-27} \times (6 \times 10^{27}) \text{ d} \]  

(Shen et al., 1977). The HDL Stokes radii were measured by fractionating plasma on nondenaturing gradient gels, transferring the proteins to nitrocellulose paper, and probing with antibody monospecific for mouse apoA-I (Marotti et al., 1992). Those radii averaged 46 ± 5 and 50 ± 10 angstroms in UCTP-20 and C57BL/6 mice, respectively. In all instances a single band was identified. The particle density was calculated from the composition exactly as described by Schumaker (1973). The densities and molecular weights of the components were assumed to be: apoA-I, d = 1.373, M_s = 27,712; other proteins, d = 1.373, M_s = 8,280; cholesteryl ester, d = 0.958, M_s = 650; phospholipid, d = 1.031, M_s = 750; triglyceride, d = 0.915, M_s = 860. The calculated density of UCTP-20 HDL was 1.133 g/ml; that of C57BL/6 HDL was 1.126 g/ml. The calculated molecular masses were: UCTP-20, 268 kDa; C57BL/6, 354 kDa. Note that these molecular masses are larger than reported previously (Marotti et al., 1992) because the Shen model was not used for the earlier calculations.

| Mouse strain | Protein | Cholesterol | Cholesteryl ester | Phospholipid | Triglyceride |
|--------------|---------|-------------|------------------|-------------|-------------|
|              | Total   | ApoA-I      |                  |             |             |
| UCTP-20      | 44.6 ± 2.2 | 27.5 ± 1.4 | 17.1 ± 0.8      | 3.3 ± 0.1   | 15.6 ± 0.5  |
| Wt. %        | 8.3 ± 0.4  | 2.7 ± 0.1   | 5.6 ± 0.4       | 22.7 ± 0.3  | 64.4 ± 1.1  |
| Mol/mol HDL  | 41.6 ± 1.0 | 28.0 ± 0.6  | 13.6 ± 0.6      | 3.6 ± 0.1   | 18.7 ± 0.2  |
| C57BL/6      | 9.4 ± 0.2  | 3.6 ± 0.1   | 5.6 ± 0.2       | 32.5 ± 0.2  | 102 ± 1     |
| Wt. %        | 41.6 ± 1.0 | 28.0 ± 0.6  | 13.6 ± 0.6      | 3.6 ± 0.1   | 18.7 ± 0.2  |
| Mol/mol HDL  | 9.4 ± 0.2  | 3.6 ± 0.1   | 5.6 ± 0.2       | 32.5 ± 0.2  | 102 ± 1     |
| Prob > t     | 0.049     | <0.001      | 0.686            | <0.001      | <0.001      | <0.001      |
| (Mol/mol HDL)|          |             |                  |             |             |

DISCUSSION

We have shown previously (Marotti et al., 1992) that transgenic C57BL/6 mice expressing cynomolgus monkey CETP have an altered apoA-I metabolism. Not only did those mice

Theoretically, the estimate of apoA-I whole-body residence time obtained by following the turnover of radiolabeled apoA-I should be the same regardless of the metabolic compartment into which the tracer is injected (Shipley and Clark, 1972; Rescigno and Gurpide, 1973). To test that premise, ^125I-labeled apoA-I was injected into some mice intravenously and into others intraperitoneally and their plasma decay curves and mean residence times calculated (experiment 1). The results of that experiment are shown in Fig. 2. Note that radiolabeled apoA-I administered intraperitoneally appeared in the plasma within minutes after injection, and peak plasma radioactivity levels were reached between 1 and 3 h later. From that point on, the decay of radioactivity from the plasma of mice given the isotope intraperitoneally was essentially the same as that of mice given the isotope intravenously. Furthermore, essentially all of the protein-bound radioactivity in the plasma comigrated with mouse apoA-I on SDS-PAGE, regardless of whether it was administered intravenously or intraperitoneally. Finally, the area under the intraperitoneal curve was quite similar to the area under the intravenous curve, and the residence times calculated from those areas were also quite similar (Fig. 2).

Phoresis immunoblotting. Those analyses (Fig. 1, t = 0) showed that more than 95% of the plasma apoA-I from both types of mouse migrated a. That was taken as an indication that it was HDL-associated.

Previous studies (Ishida et al., 1990) indicated that incubation of mouse HDL at 37 °C caused pre-β apoA-I to be produced. To determine what effect incubation might have on pre-β apoA-I production in the UCTP-20 mouse, fresh plasma from those mice was incubated at 37 °C for up to 5 h. The results of those experiments are shown in Fig. 1 and indicate that, even with incubation, approximately 95% of the apoA-I from the C57BL/6 mice remains HDL-associated, but 20% or more of the apoA-I from the CETP-transgenic mice disassociates from the HDL. That suggested that surface components were being lost from the HDL, as might occur if the core of the particle were contracting. Given the relatively high triglyceride content of UCTP-20 HDL (Table II), one mechanism by which the core of the HDL could contract would be a result of that triglyceride being hydrolyzed by a plasma lipase. To test that possibility, fresh plasma from UCTP-20 mice, C57BL/6 mice, and humans was incubated at 37 °C for 5 h and the effect of that incubation on the HDL triglycerides measured. The results of those studies are contained in Table III and show that the mice had relatively high levels of a circulating lipase and that a significant fraction of the HDL triglycerides of both UCTP-20 mouse and C57BL/6 mice was hydrolyzed during the incubation.

Given the relatively high rate at which pre-β apoA-I was produced when the UCTP-20 plasma was incubated at 37 °C and the fact that those particles are small enough to pass through the glomerular membrane (Glass et al., 1983a, 1983b; Neary and Gowland, 1988; Horowitz et al., 1993), the question arose as to whether the average life span of an apoA-I molecule might be shorter in the CETP transgenic mice than in the C57BL/6 controls. To evaluate that possibility, the mean apoA-I residence times were measured in both strains of mouse by injecting radiolabeled apoA-I and following its decay from the plasma.

Theoretically, the estimate of apoA-I whole-body residence time obtained by following the turnover of radiolabeled apoA-I should be the same regardless of the metabolic compartment into which the tracer is injected (Shipley and Clark, 1972; Rescigno and Gurpide, 1973). To test that premise, ^125I-labeled apoA-I was injected into some mice intravenously and into others intraperitoneally and their plasma decay curves and mean residence times calculated (experiment 1). The results of
Fig. 1. Effect of incubation on production of pre-β apoA-I. Fresh plasma from C57BL/6 mice (□) and UCTP-20 mice (■) was incubated at 37 °C for the indicated time and then analyzed by agarose electrophoresis-immunoblotting to measure the percent of total plasma apoA-I that migrated pre-β.

Table III
Comparison of lipase activities in plasma from CETP transgenic mice (UCTP-20), C57BL/6 mice, and humans

| Plasma source | n | Total TG  | HDL TG | Free fatty acid radioactivity |
|---------------|---|----------|--------|-------------------------------|
|               |   | Before   | After  | Before           | After  | Before         | After  |
| UCTP-20       | 8 | 103 ± 17 | 63 ± 11| 38 ± 6         | 22 ± 4| 228           | 10,131 ± 555|
| C57BL/6       | 4 | 133 ± 13 | 105 ± 10| 28 ± 2         | 9 ± 1 | 228           | 13,181 ± 1,424|
| Human         | 9 | 119 ± 29 | 121 ± 25| 20 ± 2         | 28 ± 3| 228           | 445 ± 29 |

a Total triglyceride.

b HDL-associated triglyceride.

have significantly lower plasma apoA-I levels, but the apoA-I-containing particles that were present were smaller than those from the nontransgenic controls. Because the presence of the cynomolgus monkey CETP cDNA was the only known difference in the genetic composition of the CETP transgenic mice compared with the C57BL/6 controls, it was concluded that the changes in apoA-I metabolism were caused by the presence of CETP in the plasma of those mice. The purpose of this study was to determine how CETP caused those changes in size and plasma concentration of the apoA-I-containing lipoproteins.

Analysis of the HDL from both strains of mouse showed that the HDL from the UCTP-20 mice had a much higher triglyceride:cholesteryl ester ratio than did the HDL from the C57BL/6 mice; those HDL were more vulnerable to lipolysis than the HDL of the C57BL/6 controls; and, apoA-I was shed from the UCTP-20 HDL more readily than from the C57BL/6 HDL. Therefore, we concluded that the HDL were smaller in the UCTP-20 mice because the core of those HDL were enriched with a lipid that was subject to hydrolysis in the plasma, at the expense of one that was not. That, given the high levels of circulating lipase in the mouse, had the net effect of reducing the volume of the HDL core (Shen et al., 1977) which, in turn, caused "excess" surface components (apoA-I and phospholipid) to be shed from the particle.

The most obvious explanation for the altered lipid content of the HDL from UCTP-20 mice is the fact that they have such high plasma CETP activity, the assumption being that the triglyceride present in the HDL originated in triglyceride-rich VLDL from the liver or intestine and was transferred into the HDL by CETP. However, it should be noted that these studies provide no definitive information regarding the origin of the HDL triglyceride, and it is conceivable that some may have been present in the nascent particle. Previous studies (Winkler and Marsh, 1989a, 1989b; Marsh and Diffenderfer, 1991), for example, have shown that nascent HDL from rats are rich in triglyceride, and the triglyceride content of that nascent HDL depended to a large extent on the metabolic condition of the liver (during periods of high hepatic triglyceride production the nascent HDL were more "enriched" in triglyceride than during periods of low hepatic triglyceride production). The same may be true of mice, although we have no information regarding the nascent HDL of these mice. Nonetheless, we have observed that UCTP-20 males appear to develop a fatty liver more rapidly than do the C57BL/6 males (mild hepatic fatty metamor-
ApoA-I Metabolism in CETP Transgenic Mice

**Fig. 2.** Comparison of plasma radioactivity decay curves of $^{125}$I-labeled apoA-I administered intravenously and intraperitoneally. $^{125}$I-Labeled apoA-I (250 μl) was administered to 12 ICR mice: 6 received the tracer via the tail vein (V); 6 received it intraperitoneally (O). Blood samples, taken from the peri-orbital sinuses of the mice at various times between 10 min and 72 h after injection, were analyzed as described under “Experimental Procedures,” and PBR was plotted versus time. Two mice were used for each time point, and the mean PBR of the two is shown. The area under the curves (AUC) and the mean residence times of apoA-I ($R_1$) were calculated as described under “Experimental Procedures” and are shown in the inset. It was concluded from these data that accurate estimates of the $R_1$ could be obtained when the $^{125}$I-apoA-I was administered intraperitoneally.

| DOSE | AUC   | $R_1$ |
|------|-------|-------|
| IV   | $2.17 \times 10^6$ | 22    |
| IP   | $1.95 \times 10^6$ | 24    |

**Fig. 3.** Plasma radioactivity decay curves from CETP transgenic mice (UCTP-20) and C57BL/6 mice. $^{125}$I-ApoA-I was administered to 48 mice intraperitoneally: 24 were C57BL/6 mice (O), and 24 were UCTP-20 mice (I); these were the mice used for experiments 2–4. Blood samples were taken at the times indicated and analyzed as described under “Experimental Procedures.” Eight mice were used for each time point, and the mean ± S.E. PBR was normalized by dose and plotted. The mean PBR of the C57BL/6 mice was compared with that of the UCTP-20 mice at each time point by independent sample t test, and the values of the UCTP-20 mice were found to be significantly ($p < 0.05$) lower than those of the C57BL/6 mice at all sampling times after 3 h. The area under the curves (AUC) and the mean residence times calculated from these curves are shown in the inset.

| STRAIN | AUC   | $R_1$ |
|--------|-------|-------|
| UCTP-20 | 2.09  | 16    |
| C57BL/6 | 3.70  | 23    |

Phosphorylation is evident in males of both strains at 6 months of age but appears to be slightly more advanced in the UCTP-20 mice. Therefore, the altered lipid content of the HDL reported here may not be solely a consequence of an altered plasma metabolism but could be due in part to an altered hepatic metabolism of HDL occurring either in the hepatocyte or space of Disse. Regardless of its origin, however, it appears from these data that it is the replacement of cholesterol esters with triglyceride which is largely responsible for the small HDL in the UCTP-20 mice.

A second question regarding CETP expression in these mice concerned its effect on apoA-I concentrations. The apoA-I concentrations reported here (Table I) confirm our preliminary observations (Marotti et al., 1992) that CETP expression is associated with a reduction in the plasma apoA-I levels. There are three ways that CETP expression could reduce the apoA-I concentrations: 1) it could reduce the apoA-I production rate; 2) it could shorten the average life span of a molecule of apoA-I; 3)
it could cause a redistribution of apoA-I-containing lipoproteins out of the plasma compartment and into the extravascular space.

To gain some insight into which of these mechanisms might be contributing to the reduction in plasma apoA-I levels in the transgenic mice, we injected radiolabeled apoA-I and followed its turnover in the plasma of these mice for 72 h. However, whereas measuring changes in the chemical composition of the HDL is a relatively simple and straightforward process, quantifying changes in apoA-I metabolism is somewhat more complex and requires certain assumptions for interpretation of the data. In addition, we chose a somewhat unconventional approach for administration of the tracer.

A fundamental assumption of any turnover study is that the tracer is tracing the metabolism of the substance of interest (i.e. the tracee). Two observations support the assumption that the radiolabeled apoA-I was tracing the metabolism of endogenous apoA-I in these mice. First, 96% of the PBR in the plasma at any time comigrated with native apoA-I during SDS-PAGE. Thus, it was apoA-I, and not some radiolabeled impurity, whose turnover we were following. Second, 99-98% of the PBR migrated with the apo lipoproteins during agarose electrophoresis (i.e. was HDL-associated) throughout the study. Thus, the free apoA-I that we injected did equilibrate with HDL-associated apoA-I in the plasma and presumably traced its metabolism.

Finally, there is the question regarding the effects of route of administration of the tracer on the interpretation of the results. We chose to administer the radiolabeled apoA-I intraperitoneally. Our reasoning was that apoA-I moves back and forth between the vascular compartment and the interstitium relatively easily (Sloop et al., 1987), and therefore isotope administered this way should equilibrate with that in the rest of the system relatively rapidly. Comparison of the plasma radioactivity decay curve of apoA-I administered intravenously with that of apoA-I administered intraperitoneally indicated that this was the case. Even so, as Shipley and Clark (1972) point out, when measuring the residence time, it matters not into which compartment the tracer is injected, nor how rapidly or slowly the tracer is administered, as long as one is sampling from the compartment from which the tracer and tracee are irreversibly lost. Most models of apoA-I metabolism indicate that that is the plasma compartment.

Note that if all of the administered isotope entered the plasma compartment before entering peripheral pools, this method would be the equivalent of infusing tracer intravenously over a period of a few hours but with the advantage that apoA-I damaged either by the purification or labeling process has the opportunity to be cleared by the immune system prior to entering the metabolic compartment of interest. However, this method has the distinct disadvantage that one is never certain precisely what fraction of the injected material entered the system. Although that knowledge is not necessary for calculation of residence times (as long as it is the same for mice of a given strain), it is required for a precise estimate of the apoA-I production rate.

In the present study we followed radioactivity clearance from the animals using a hand-held survey meter, and in no instance did we detect retention of radioactivity in the abdomen of a mouse, i.e. the loss of "whole-body" radioactivity generally paralleled loss from the plasma. Nonetheless, we chose not to report the apoA-I production rates calculated from these curves. However, one can compare those rates (i.e. determine their ratio) by comparing the area under the apoA-I-specific radioactivity decay curves (expressed as cpm/mg of apoA-I), for the production rates are inversely proportional to those areas. When that was done for experiments 2-4, the respective ratios (UCTP-20:C57BL/6) were 1.28, 1.21, and 0.98. That indicates that the apoA-I production rates were not decreased in the UCTP-20 mice. That conclusion is in agreement with the hepatocyte studies, which also indicated that CETP expression had no effect on the apoA-I production rate.

The question remained as to whether the apoA-I was redistributed among the metabolic compartments in the UCTP-20 mice. Although we cannot rule out completely that some redistribution might have taken place as a result of CETP expression, we would argue that that is not a likely explanation for the reduced plasma apoA-I levels, for if CETP simply caused a redistribution of apoA-I-containing lipoproteins into the extravascular compartment, then the plasma levels of the protein would decrease, and its plasma residence time would also decrease, but the whole-body mass and whole-body residence time would remain unchanged. Three separate apoA-I turnover experiments indicated that the whole-body residence time was, indeed, reduced in the UCTP-20 mice relative to the C57BL/6 controls. That was taken as an indication that the principal mechanism by which CETP expression reduced the plasma apoA-I concentration in the UCTP-20 mice was by shortening the average life span of apoA-I in those mice.

These conclusions contrast with those of Hayek et al. (1992) who studied apoA-I turnover in transgenic mice expressing both human CETP and human apoA-I (HuAICETP/Tg) mice. Those investigators reported that expression of human CETP caused a reduction in the plasma levels of the human apoA-I but had minimal effect on the plasma residence time of that apoA-I in their transgenic mice. They concluded that the reduction in plasma levels of the human apoA-I must have been due to some other mechanism. The reason(s) for this contradiction in our respective conclusions cannot be established unequivocally because there were major differences in the experimental designs of the two studies. The C57BL/6 mice used in the present study were, for example, expressing simian, rather than human, CETP. Moreover, the simian CETP gene was the only extraneous gene present, and it was being expressed in UCTP-20 mice at levels substantially higher than the CETP levels expressed in the HuAICETP/Tg mice. In addition the studies reported here were concerned only with the effect of CETP expression on the metabolism of intrinsic apoA-I. Under those circumstances, the most reasonable explanation for the lower apoA-I levels in the UCTP-20 mice was a shortened plasma residence time; however, in an animal expressing an extrinsic apoA-I at very high levels, CETP expression might have an entirely different effect. Therefore it is not unreasonable that our respective conclusions are different.

The premise that CETP exerts its effect on plasma apoA-I concentrations by shortening the apoA-I residence time implies that expression of the gene has some direct effect on the circulating HDL, rather than on some aspect of intracellular processing or secretion of apoA-I. In support of that deduction is the observation that after a 5-h incubation, the levels of "pre-β" apoA-I (apoA-I in the plasma which is not associated with HDL) are much higher in UCTP-20 plasma than in the C57BL/6 plasma. That implies that pre-β apoA-I is produced in vivo at a faster rate in the UCTP-20 mice than it is in the C57BL/6 mice; yet, when the levels of pre-β apoA-I in fresh UCTP-20 plasma were compared with those in fresh C57BL/6 plasma, there were no apparent differences. That would suggest that the pre-β apoA-I is cleared fairly rapidly from the plasma and thus does not accumulate. Apparently, because the pre-β apoA-I particles are of relatively low molecular weight, they are able to traverse the glomerular membrane (Glass et al., 1983a, 1983b; Neary and Gowland, 1988; Horowitz et al., 1993), and a significant fraction is cleared by renal filtration.

When the observations reported here are examined together with the in vitro studies of Barter and co-workers (Barter et al.,
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1987; Newnham and Barter, 1990; Clay et al., 1992) and Kunitake and co-workers (Kunitake et al., 1992; Hennessy et al., 1993), a picture evolves of what must be occurring in these mice. Apparently, CETP expression causes the HDL of the UCTP-20 mice to become enriched with triglyceride and depleted of cholesterol esters. As those triglycerides are hydrolyzed by the circulating lipase, the core of the particle shrinks, producing pre-β apoA-I. The latter particles presumably have a very short life span. The net effect of this process is a reduction in the plasma apoA-I pool size and an increase in its rate of turnover. The resultant lipoprotein profile is one in which the plasma apoA-I levels are decreased, and the HDL are reduced in size.

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