Hepatic Catabolism of Rat and Human Lipoproteins in Rats Treated with 17α-Ethynyl Estradiol*

Yu-sheng Chao,† Eberhard E. Windler,§ G. Chi Chen, and Richard J. Havel

From the Cardiovascular Research Institute and Department of Medicine, University of California, San Francisco, California 94143

Rats treated with large amounts of ethynyl estradiol develop a profound hypolipidemia that affects all of the major plasma lipoprotein classes. Net secretion of triglycerides and apolipoprotein A-I from perfused livers of estrogen-treated rats was unaltered, but that of apolipoprotein E was reduced by 75%. The rate of removal of rat and human low density lipoproteins from blood plasma was increased in estrogen-treated rats. The augmented clearance was eliminated in functionally eviscerated animals, indicating that the liver or other splanchnic viscera is the site of the increased removal. Perfused livers from estrogen-treated rats catabolized rat and human 125I-labeled low density lipoproteins at greatly increased rates. Hepatic catabolism of rat high density lipoproteins that contain an appreciable amount of apolipoprotein E was also stimulated by estrogen, but that of rat and human high density lipoproteins that contain predominantly apolipoprotein A-I and little or no apolipoprotein E was unaffected. The stimulated hepatic catabolism of low density lipoproteins was abolished by covalent modification of arginyl residues of component apolipoprotein B with cyclohexanedione. Although these characteristics of the stimulated hepatic lipoprotein catabolism in treated rats resemble those mediated by the low density lipoprotein receptor identified in certain extrahepatic cells, the stimulated hepatic catabolism is not saturated at low (physiological) concentrations of low density lipoproteins in the perfusion medium (approximately 0.05 mg of apoprotein/ml) and continues to increase up to levels of 1.5 mg/ml. Reduction of low density lipoprotein levels in rats induced by administration of orotic acid and stimulation of hepatic cell proliferation following partial hepatectomy produced little or no stimulation of hepatic catabolism of low density lipoproteins.

In a variety of cultured cells, low density lipoproteins (LDL)† of mammalian blood plasma are catabolized after specific binding to a high affinity surface receptor (1). The receptor is repressed in cells grown in the presence of LDL and is induced in the absence of this lipoprotein, especially when the rate of utilization of cholesterol for synthesis of cellular membranes or other purposes is high. Although the concentration of LDL in the extracellular fluid of most organs and tissues is much lower than in blood plasma, the affinity of the LDL receptor for this lipoprotein is such (apparent $K_m \approx 10$ to $15 \text{ ng/ml for apo-LDL}$) that it should be nearly saturated in species, such as humans, in which LDL levels in blood plasma are high.

One approach to this problem is to determine the response of the liver to stimuli that increase the expression of LDL receptor activity in cultured cells. Accordingly, we have quantified LDL catabolism in the liver of rats in which LDL levels have been reduced by administration of orotic acid (5) or estradiol (6–8) or in which hepatic parenchymal cells have been induced to proliferate by partial hepatectomy (9). Here we report experiments which show that the profound hypolipidemia produced in rats by administration of large amounts of estradiol is accompanied by greatly increased hepatic catabolism of LDL and some species of HDL. Concurrent experiments by Kovanen and associates (10) show that cell membranes from livers of rats treated with estradiol exhibit a specific binding site for LDL. Several properties of the binding site are consistent with properties of the augmented catabolic capacity of the liver of estrogen-treated rats for lipoproteins isolated from rat and human blood plasma. The activity of this binding site, therefore, appears to explain the high rates of LDL catabolism that we have observed in our experiments with perfused rat livers and at least partially explains the hypolipidemia of estrogen-treated rats.

**Experimental Procedures**

*Materials—*17α-Ethynyl estradiol, rat serum albumin (Fraction V), and bovine serum albumin were from Sigma Chemical Co. (St. Louis, MO); propylene glycol was from J. T. Baker Chemical Co. (Phillipsburg, NJ); orotic acid and 1,2-cyclohexanedione were from Aldrich Chemical Co., Inc. (Milwaukee, WI); and sodium $[^{131}]I$iodide (carrier-free) was from Amersham/Scarce Corp. (Arlington Heights, IL).

*Animals, Diets, and Drugs—*Male Sprague-Dawley rats weighing
300 to 350 g were used in all experiments. Unless otherwise stated, all rats were fed standard Purina Chow ad libitum.

A semisynthetic diet containing orotic acid was a mixture of glucose monohydrate (68%), casein (20%), corn oil (5%), DL-methionine (0.3%), choline chloride (0.2%), orotic acid (2%), plus vitamins and minerals (5). The orotic acid control diet glucose monohydrate was substituted for the orotic acid.

17 α-Ethynyl estradiol was first dissolved in ethanol (100 μg/ml) and then dissolved in propylene glycol to a final concentration of 1 mg/ml. The ethinyl estradiol was injected subcutaneously daily at a dose of 5 mg/kg body weight. Control rats received equal volumes of propylene glycol. The esterified acid was injected subcutaneously daily at a dose of 2% of body weight, whereas control rats gained 10 to 20 g of body weight, whereas control rats gained 10 to 30 g.

Operative Procedures—Partial hepatectomy was performed as described by Higgins and Anderson (9). The lobes removed at this operation constituted 65 to 75% of the liver mass. Control rats were sham operated. After the operation the rats were injected intraperitoneally with 0.3 ml of penicillin G (500,000 units/ml, Squibb and Sons, Inc., Princeton, NJ) and intramuscularly with 0.2 ml of streptomycin sulfate (0.4 g/ml, Pfizer Laboratories, New York, NY). From the operation up to the time of liver perfusion, the rats had free access to water and food.

Rats subjected to functional evisceration were anesthetized with diethylether. The abdominal cavity was opened and the mesenteric arteries, the hepatic artery, and the portal vein were ligated. All procedures have been described previously (3). The radioiodinated lipoproteins, usually containing 200 μg of apo-LDL, were injected into rats. The peritoneal cavity was opened and the mesenteric artery was primarily increased by estradiol, and that the increased catabolic rate did not simply reflect the reduced mass of LDL in treated animals. To measure catabolism of lipoproteins in isolated perfused rat livers (0.41 ± 0.06 (control) versus 0.44 ± 0.10 μg g⁻¹ h⁻¹ (treated); n = 4) was in the serum of the control rats. The rate of removal was reduced after functional evisceration of the estradiol-treated rats as compared with control livers.

Partial Hepatectomy—Expressed per g of liver, the rate of LDL catabolism in isolated regenerating livers was slightly lower 24 h after partial hepatectomy and increased 27% and 20% 48 and 72 h after the operation, respectively (Table I). Based upon an estimated initial weight of 3.4 g after partial hepatectomy, the regenerating livers gained 1.1 g, 2.1 g, and 4.9 g 24, 48, and 72 h after operation, respectively.

**Hepatic Lipoprotein Catabolism in Estradiol-treated Rats**

**RESULTS**

Orotic Acid—In four rats in which addition of 2% erotic acid to the semisynthetic diet for 2 weeks resulted in uniformly fatty livers, plasma concentration of triglycerides was reduced from 47 ± 3.9 (S.D.) to 15 ± 4.8 mg/dl and that of total cholesterol from 45 ± 4.8 to 27 ± 2.6 mg/dl. In another group of four rats, triglycerides of density less than 1.063 g/ml were reduced from 30 ± 5.0 to 5.0 ± 2.3 mg/dl, with little change in cholesterol or triglycerides in lipoproteins of density greater than 1.063 g/ml. The fractional rates of removal of LDL in intact rats were unchanged (11.6 ± 1.45 (control) versus 12.0 ± 1.60 % h⁻¹ (treated); n = 4), as were the catabolic rates in isolated perfused rat livers (0.41 ± 0.06 (control) versus 0.44 ± 0.10 μg g⁻¹ h⁻¹ (treated); n = 4). Net secretion of triglycerides was virtually abolished (6.83 ± 0.62 (control) versus < 0.05 mg h⁻¹ (treated); n = 4). Net secretion of apo-E was reduced by 75% but that of apo-A-I was unchanged in the perfused fatty livers as compared with control livers.

**Catabolism of Lipoproteins in Intact Rats**

A measured volume of a tail vein at intervals during the next 24 h. 125I-labeled LDL was injected, but removal remained substantially higher in treated rats than in control rats (Fig. 1). One hour after injection of 125I-LDL only 40% of the injected 125I-LDL remained in the plasma of estradiol-treated rats, whereas 80% to 90% remained in plasma of control rats. In one pair of experiments with human 125I-LDL, trichloroacetic acid-soluble as well as trichloroacetic acid-insoluble 125I was measured in the serum. In the period 1 to 6 h after injection, 4 to 5% of the injected 125I was in the serum of the treated rats as trichloroacetic acid-soluble radioactivity, but only 1.5 to 2% was in the serum of the control rats. The rate of removal was reduced after functional evisceration of the estradiol-treated rats to the level observed in intact control rats (Fig. 2).

In both control and estradiol-treated rats, the rate of removal of human 125I-LDL was inversely related to the mass of LDL injected, but removal remained substantially higher in treated rats over a 50-fold range. Thus, 12 h after injection of 125I-LDL containing 1 mg of protein, 28% of the injected 125I remained in the plasma of a control rat, but only 6% in the plasma of a treated rat. After injection of 125I-LDL containing 50 mg of protein, corresponding figures were 55% and 10%, respectively. The results suggest that catabolism of LDL in the liver or intestine was primarily increased by estradiol, and that the increased catabolic rate did not simply reflect the reduced mass of LDL in treated animals. To measure catabolism directly, liver perfusions were carried out.

In the initial liver perfusions, an equal mass of apo-LDL (at a physiological level) was added to the perfusates of control and estradiol-treated rat livers. The catabolism of rat LDL by

2 P. E. Fielding, personal communication.
Hepatic Lipoprotein Catabolism in Estradiol-treated Rats

TABLE I

Catabolism of apo-LDL in isolated perfused livers after partial hepatectomy

<table>
<thead>
<tr>
<th>Condition</th>
<th>Exp. No.</th>
<th>Rat weight</th>
<th>Serum triglycerides</th>
<th>Serum cholesterol</th>
<th>Liver weight</th>
<th>FCR</th>
<th>Catabolic rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (sham)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>320</td>
<td>62</td>
<td>46</td>
<td>10.2</td>
<td>0.80</td>
<td>0.078</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>335</td>
<td>58</td>
<td>48</td>
<td>12.5</td>
<td>0.70</td>
<td>0.062</td>
</tr>
<tr>
<td>24 h after hepatectomy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>315</td>
<td>36</td>
<td>25</td>
<td>4.2</td>
<td>0.96</td>
<td>0.062</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>320</td>
<td>38</td>
<td>33</td>
<td>4.8</td>
<td>0.27</td>
<td>0.056</td>
</tr>
<tr>
<td>48 h after hepatectomy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>325</td>
<td>44</td>
<td>30</td>
<td>5.3</td>
<td>0.50</td>
<td>0.100</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>320</td>
<td>48</td>
<td>38</td>
<td>5.7</td>
<td>0.52</td>
<td>0.091</td>
</tr>
<tr>
<td>72 h after hepatectomy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>335</td>
<td>46</td>
<td>40</td>
<td>8.1</td>
<td>0.85</td>
<td>0.080</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>340</td>
<td>64</td>
<td>49</td>
<td>8.5</td>
<td>0.75</td>
<td>0.088</td>
</tr>
</tbody>
</table>

TABLE II

Serum lipid and protein concentrations in control and estradiol-treated rats

<table>
<thead>
<tr>
<th>Condition</th>
<th>mg/dl</th>
<th>mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control rats (n = 4)</td>
<td>67 ± 11</td>
<td>52 ± 4</td>
</tr>
<tr>
<td>Estradiol-treated rats (n = 4)</td>
<td>8 ± 3</td>
<td>5 ± 3</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control rats (n = 4)</td>
<td>49 ± 2.5</td>
<td>1.3 ± 0.03</td>
</tr>
<tr>
<td>Estradiol-treated rats (n = 4)</td>
<td>3.2 ± 0.06</td>
<td>2.2 ± 0.01</td>
</tr>
<tr>
<td>Phospholipids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control rats (n = 4)</td>
<td>20 ± 1.5</td>
<td>30 ± 1.8</td>
</tr>
<tr>
<td>Estradiol-treated rats (n = 4)</td>
<td>2.2 ± 0.01</td>
<td>30 ± 1.8</td>
</tr>
<tr>
<td>Apo-E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control rats (n = 4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estradiol-treated rats (n = 4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apo-A-I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control rats (n = 4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estradiol-treated rats (n = 4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control rats (n = 4)</td>
<td>60.1 ± 8.7</td>
<td>68.7 ± 6.8</td>
</tr>
<tr>
<td>Estradiol-treated rats (n = 4)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Mean ± S.D.
- Rats were treated with estradiol (5 mg/kg daily) for 5 days.

perfused livers of estradiol-treated rats was increased about 4-fold as compared with control rat livers and that of human LDL was increased approximately 10-fold (Fig. 3). For human 125I-LDL, the rates of appearance of water-soluble 125I (presumably 125I-tyrosine) and chloroform-soluble 125I (radioiodide) were, respectively, 60 ± 10% and 40 ± 8% (mean ± S.D.) of total trichloroacetic acid-soluble 125I for control rats and 65 ± 8% and 55 ± 5%, respectively, for estradiol-treated rats. Bile flow from livers of treated animals was reduced by about 75%. The amount of 125I secreted into the bile during the perfusates was less than 0.5% of the amount of 125I added to the perfusate of control and estradiol-treated rat livers. In perfused livers from estradiol-treated rats the rate of appearance of nonprotein-bound radioiodine in the perfusate was the same for biologically screened (3) and nonscreened radiiodinated rat LDL. This observation makes it unlikely that livers of estradiol-treated rats preferentially remove a population of rapidly catabolized molecules.

![Fig. 1. Removal from blood plasma of 125I LDL in intact control and estradiol-treated rats.](http://www.jbc.org/)

![Fig. 2. Removal from plasma of rat 125I-LDL injected intravenously into two intact and two functionally eviscerated estradiol-treated rats.](http://www.jbc.org/)
Hepatic Lipoprotein Catabolism in Estradiol-treated Rats

The relationship between the mass of LDL in the perfusate and the FCR of apo-LDL is shown in Fig. 4. In control rat livers, production of trichloroacetic acid-soluble \(^{125}\text{I}\) was linear from 1 to 4 h of perfusion (Fig. 3). FCR was, therefore, calculated from the linear increment of total nonprotein-bound \(^{125}\text{I}\) (chloroform-soluble and water-soluble) in the perfusate corrected for the increment observed during circulation of the perfusate through the perfusion apparatus in the absence of a liver (3). Production of trichloroacetic acid-soluble \(^{125}\text{I}\) in perfusates of estradiol-treated rat livers was linear for only 1 to 1.5 h. FCR was, therefore, calculated from the period 0.5 to 1.5 or 2 h of perfusion. Fig. 4 shows that the FCR of human \(^{125}\text{I}\)-LDL was increased several fold by estradiol over an approximately 200-fold range of LDL concentration in the perfusate. The FCR was significantly higher at a perfusate pool size of 0.3 to 0.5 mg (6.89 ± 0.68% h\(^{-1}\)) than at pool sizes of 5 to 100 mg (4.68 ± 0.84% h\(^{-1}\)) (\(p < 0.02\)). At low perfusate concentrations, FCR of rat \(^{125}\text{I}\)-LDL was higher than that of human \(^{125}\text{I}\)-LDL.

The rates of removal of rat HDL (1.09 < \(d < 1.21\) g/ml) and human HDL\(_3\) in intact rats were also increased by treatment with estradiol (Fig. 5). In perfused livers at the same concentration of HDL, catabolism of rat apo-HDL (1.09 < \(d < 1.21\) g/ml) was increased 2-fold by estradiol. By contrast, similar catabolic rates of rat HDL (1.125 < \(d < 1.21\) g/ml) and human HDL\(_3\) were observed in the two conditions (Fig. 6).

Modification of arginyl residues of LDL with 1,2-cyclohex-

![Graph showing production of nonprotein-bound \(^{125}\text{I}\) during perfusion of control (C) or estradiol-treated (E) rat livers with rat \(^{125}\text{I}\)-LDL (A) or human \(^{125}\text{I}\)-LDL (B). Pool sizes of rat (A) and human (B) apo-LDL in the perfusate in these experiments were 0.5 mg and 1.0 mg, respectively. Individual results are shown for two paired experiments with rat \(^{125}\text{I}\)-LDL and mean values (±1 S.D.) are shown for four experiments with human \(^{125}\text{I}\)-LDL.](attachment://graph1.png)

![Graph showing the relationship between pool size of apo-LDL and its fractional catabolic rate (FCR) in perfused control and estradiol-treated rat livers. The FCR was estimated from the period of constant production of total trichloroacetic acid-soluble \(^{125}\text{I}\) (see text). Each point represents one experiment.](attachment://graph2.png)

![Graph showing removal from blood plasma of rat \(^{125}\text{I}\)-HDL (A) and human \(^{125}\text{I}\)-HDL\(_3\) (B) injected intravenously into intact control (C) or estradiol-treated (E) rats. More than 50% of the \(^{125}\text{I}\) in rat HDL was in apo-A-I and 5 to 10% was in apo-E (19). Individual data from two paired experiments for each lipoprotein are shown.](attachment://graph3.png)
Hepatic Lipoprotein Catabolism in Estradiol-treated Rats

FIG. 6. Production of nonprotein-bound $^{125}$I during perfusion of control (○) and estradiol-treated (●) rat livers with rat $^{125}$I-HDL (A) ($0.99 < d < 1.21$ g/ml, apo-E = 5% of total protein); rat $^{125}$I-HDL (B) ($1.125 < d < 1.21$ g/ml, apo-E < 0.5% of total protein); and human $^{125}$I-HDL (C) ($1.125 < d < 1.21$ g/ml, apo-E < 0.5% of total protein). The pool size of rat and human apo-HDL in the perfusate in these experiments was 10 mg. Individual data from two paired experiments for each lipoprotein are shown.

FIG. 7. Removal from blood plasma of human $^{125}$I-CHD-LDL (A) and rat $^{125}$I albumin (C) in intact rats and production of nonprotein-bound $^{125}$I during perfusion of livers with $^{125}$I-CHD-LDL (B). $^{125}$I-CHD-LDL (200 µg of protein) or albumin (200 µg of protein), labeled with $^{125}$I by the iodine monochloride method of McFarlane (12), was injected intravenously into four control (○) or two estradiol-treated (●) rats. Blood samples were obtained from a tail vein and $^{125}$I was determined in 100-µl samples of serum. A portion of the $^{125}$I-CHD-LDL (1 mg of protein) was added to perfusates of livers from two control and two estradiol-treated rats. Production of nonprotein-bound $^{125}$I was measured as described in the text.

FIG. 8. Net secretion of triglycerides, apo-A-I, and apo-E during perfusions of isolated livers from control or estradiol-treated rats. Samples of whole perfusate were collected at hourly intervals for chemical analysis of triglycerides and radioimmunochemical analyses of apoproteins. Each point represents mean values from four experiments. Bars indicate ± S.D.

andenedione (CHD) abolishes its ability to bind to high affinity sites for LDL in human fibroblasts (14). In estradiol-treated rats (but not in control rats), removal of $^{125}$I-CHD-LDL from the blood was much slower than that of $^{125}$I-LDL (Fig. 7A). In
isolated perfused livers, the catabolism of $^{125}$I CHD LDL was not decreased by treatment with the hormone (Fig. 7B). Similarly, the rate of removal of rat $^{125}$I-albumin in intact rats was not affected by treatment with estradiol (Fig. 7C).

The net rates of secretion of triglycerides and apo-A-I into liver perfusates were similar to those reported previously (20, 21) and were not affected by treatment with estradiol (Fig. 8). Net secretion of triglycerides was low during the 1st h of perfusion and increased progressively with time, whereas secretion of apo-A-I was constant during the 4 h of perfusion. Net secretion of apo-E in perfusates of livers from estradiol-treated rats was reduced by 75% (p < 0.02). Secretion of apo-E was also constant from both control and estradiol-treated rat livers.

**DISCUSSION**

In confirmation of previous reports (6–8), we have found that administration of large amounts of ethinyl estradiol to male rats produces a profound hypolipidemia that involves all major lipoprotein classes and the major apoproteins of LDL and HDL (4, 8). Unlike Davis and Rolcianu (8), we have found that the reduction of plasma phospholipid concentrations is comparable to that of cholesterol and triglycerides. Two observations indicate that the reduced levels of LDL and, at least in part, those of HDL are the result of augmented hepatic lipoprotein catabolism. First, net secretion of triglycerides (and presumably VLDL) from the liver was unaffected by estrogen administration. As LDL are derived primarily from VLDL in the rat (22), LDL formation should not be greatly reduced by estrogen unless the fraction of VLDL converted to LDL is altered. Second, the fractional rate of removal of both rat and human LDL from the blood was greatly augmented in estrogen-treated rats even when large amounts were injected. Hay and associates (4) reported earlier that removal from plasma of LDL of density 1.006 to 1.040 g/ml was increased in rats treated with estradiol benzoate. LDL in this density range contain appreciable amounts of non-B apoproteins and the extent to which their results reflect effects on VLDL-remnant removal is unclear.

The increased rate of removal of LDL was virtually abolished in hormone-treated, functionally eviscerated rats, and perfused livers from treated rats catabolized rat LDL at a rate approximately 4-fold that of untreated control animals. Thus, the liver appears to be primarily responsible for the augmented rate of catabolism. The rate of removal of LDL from plasma of rat LDL (1.09 < d < 1.21 g/ml) was substantially increased in hormone-treated rats. Whether increased removal of LDL from plasma can explain the profound reduction of HDL concentration is less certain because, as discussed below, increased hepatic catabolism of HDL is apparently confined to HDL species that contain apo-E.

Our observations of the effects of estrogen treatment upon the catabolism of different lipoproteins in the perfused liver are consistent with the findings of Kovanen and associates (10) on the binding of lipoproteins to membranes of livers prepared from similarly treated animals. Thus, their observations of a 3- to 10-fold increase in binding to liver membranes for human LDL is consistent with our observation of a 10-fold increase in catabolic rate in the perfused liver. Likewise, they observed no increase in binding of human HDL to liver membranes and we observed no effect of estrogen on the catabolic rate of human HDL.

The relationship between the binding and catabolism of lipoproteins in the liver of estrogen-treated rats to the "LDL pathway," as defined in cultured human fibroblasts and other cultured cells, is uncertain. The two systems have several features in common. First, the augmented binding and catabolism evidently are confined to lipoprotein species that contain apo-B and apo-E (23). Thus, we found that catabolism of rat LDL (1.09 < d < 1.21 g/ml), in which apo-E comprises about 5% of the apoprotein mass, was increased 2- to 3-fold, whereas that of rat HDL (1.25 < d < 1.21 g/ml) and human HDL, which contain less than 0.5% apo-E, were unaffected by treatment with estrogen. Second, covalent modification of the arginyl moiety of apo-LDL, which prevents binding of LDL to the high affinity LDL receptor in cultured fibroblasts (14), also abolished the increased rate of LDL catabolism in the perfused liver as well as the increased rate of removal in vivo. These similarities to the fibroblast receptor have also been found for LDL binding to liver membranes (10).

The characteristics of the estrogen-induced increase in LDL catabolism in the perfused liver differ from those of the fibroblast receptor in several respects. First, the rate of catabolism is not readily saturated at low LDL concentrations (approximately 0.05 mg of LDL-protein/ml), but increases up to levels of at least 0.5 to 1.5 mg/ml. This difference is also reflected in the apparent $K_m$ for LDL binding in liver membranes, which is about 0.3 mg of LDL protein/ml at 37°C (10).

Second, LDL catabolism was only marginally increased in livers in which the cells were rapidly proliferating after partial hepatectomy. By contrast, the activity of the LDL receptor in cultured cells rapidly increases under similar conditions (1). Third, we failed to induce an increase in the catabolic rate of LDL in perfused livers from animals in which the concentration of LDL was greatly reduced by treatment with orotic acid. Cultured cells grown in the absence of LDL manifest greatly increased receptor activity within 24 to 48 h (1). However, orotic acid treatment may have failed to induce increased LDL catabolism because livers from orotic acid-treated rats continue to secrete some apo-E in discoidal HDL. Retention of lipid in the liver of orotic acid-treated rats could also influence LDL catabolism.

Recently, it has been found that acetoacetylated, acetylated, and succinylated LDL are rapidly removed from the blood of rats and are primarily taken up by Kupffer cells (24, 25). By contrast, the augmented hepatic uptake of LDL in estradiol-treated rats appears to be mediated by the parenchymal cells. In preliminary autoradiographic studies, we have observed early localization of radioiodinated LDL at the surface of hepatocytes, followed by rapid movement into the cells. Little or no radioactivity was observed in association with Kupffer cells or endothelial cells of hepatic sinusoids. Our observations are consistent with the increased binding of LDL to liver membranes observed by Kovanen and associates (10) and further suggest that LDL are catabolized after they are taken up into hepatic parenchymal cells.

In perfused livers of both normal and estradiol-treated rats, trichloroacetic acid-soluble radioiodine first appeared in the perfusate about 30 min after $^{125}$I-LDL was added and then proceeded at a constant rate for at least 1 h. The effect of estradiol upon LDL catabolism is, thus, consistent with stimulation of the normal mechanism by which LDL are taken up and catabolized by the liver. However, the amount of LDL bound specifically to liver membranes from untreated rats is insufficient to permit reliable assessment of apparent binding constants or other properties of the interaction (10). It is, therefore, uncertain whether the LDL binding site and the augmented rate of hepatic catabolism of LDL as observed in estradiol-treated rats represent the pathway by which LDL are normally metabolized in rat liver. As stimulation of HDL catabolism in the liver of estradiol-treated rats is a function of the content of apo-E, this pathway could also participate in

---

4. Y.-S. Chao, A. L. Jones, E. Windler, and R. J. Havel, unpublished observations.
Hepatic Lipoprotein Catabolism in Estradiol-treated Rats

The catabolism of those HDL particles that contain apo E. It could also function in the catabolism of remnants of chylomicrons and VLDL, in which the major protein components are apo-B and apo-E (15).

The secretion of apo-A-I from isolated perfused livers (Fig. 8) and from the small intestine into mesenteric lymph is not affected by estradiol, yet the concentration of HDL and apo-A-I in plasma of treated rats was profoundly reduced. Given the amount of apo-E in rat HDL (1.09 < d < 1.21 g/ml), approximately 5% of the total protein mass, it can readily be calculated that most of these HDL particles contain no apo-E. The amount of apo-E in rat HDL as they exist in vivo is difficult to ascertain because this protein readily dissociates from HDL when they are isolated by preparative ultracentrifugation (26). When rat HDL are separated by chromatography on 10% agarose gel, apo-E appears to be associated mainly with large HDL particles and the major component apoprotein of HDL (apo-A-I) with smaller particles (26). Possibly, smaller HDL may intermittently acquire a molecule of apo-E and, thus, become subject to augmented hepatic uptake.

Net secretion of apo-E was reduced to about 25% by administration of estradiol. Inasmuch as the perfused liver can take up lipoproteins that it secretes, the observed net secretion rate clearly represents a minimal value for the rate at which the liver delivers lipoproteins into the perfusate. The reduced net secretion of apo-E in estradiol-treated rats may, therefore, not reflect the unidirectional flux of apo-E into the perfusate, but rather augmented uptake of apo-E mediated by the LDL binding site on the plasma membrane of hepatocytes.

Acknowledgments—We are grateful to Esther Chou, Agnes Frank, and Anneliese Merlino for their expert technical assistance.

REFERENCES

5 E. Windler, Y.-S. Chao, and R. J. Havel, unpublished observations.
Hepatic catabolism of rat and human lipoproteins in rats treated with 17 alpha-ethinyl estradiol.
Y S Chao, E E Windler, G C Chen and R J Havel