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Regulation of hepatic cholesterol and lipoprotein metabolism in ethinyl estradiol-treated rats

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Abstract The regulation of hepatic cholesterol and lipoprotein metabolism was studied in the ethinyl estradiol-treated rat in which low density lipoprotein (LDL) receptors are increased many fold. Cholesterol synthesis was reduced at both its diurnal peak and trough by ethinyl estradiol. The diurnal variation in 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase was abolished, whereas that for acyl coenzyme A: cholesterol acyltransferase (ACAT) was retained. LDL receptor number did not vary diurnally. Feeding these animals a cholesterol-rich diet for 48 h suppressed cholesterol synthesis and reductase activities to levels similar to those found in cholesterol-fed control animals, but ACAT activity was unaffected. LDL receptors were reduced about 50%. Intravenously administered cholesterol-rich lipoproteins suppressed HMG-CoA reductase and LDL receptors in 2 h but had a variable effect on ACAT activity. Intragastric administration of mevalonolactone reduced reductase and increased acyltransferase activity but had little effect on LDL receptors when given 2 or 4 h before death. Although animals fed a cholesterol-rich diet before and during ethinyl estradiol treatment became hypocholesterolemic, free and esterified cholesterol concentrations in liver were high as was ACAT activity. HMG-CoA reductase was inhibited to levels found in control animals fed the cholesterol-rich diet. LDL receptors were increased to a level about 50% of that reached in animals receiving a control diet and ethinyl estradiol. These data demonstrate that key enzymes of hepatic cholesterol metabolism and hepatic LDL receptors respond rapidly to cholesterol in the ethinyl estradiol-treated rat. Furthermore, estradiol increases LDL receptor activity several fold in cholesterol-loaded livers.—**Erickson, S. K., S. Jaeckle, S. R. Lear, S. M. Brady, and R. J. Havel.** Regulation of hepatic cholesterol and lipoprotein metabolism in ethinyl estradiol-treated rats. *J. Lipid Res.* 1989. **30:** 1763–1771.

Supplementary key words 3-hydroxy-3-methylglutaryl coenzyme A reductase • acyl coenzyme A: cholesterol acyltransferase • low density lipoprotein receptor • cholesterol synthesis

The ethinyl estradiol-treated rat has been used extensively to study hepatic lipoprotein and biliary metabolism. In addition to induction of hypocholesterolemia (1, 2) caused by an increase in the number of hepatic low density lipoprotein (LDL) receptors (3), a mild cholestasis develops with reduced bile flow accompanied by reduced bile acid synthesis and secretion (4, 5) and decreased activity of cholesterol-7α-hydroxylase (6), a rate-limiting enzyme for bile acid synthesis. Activity of hepatic acyl coenzyme A:cholesterol acyltransferase (ACAT) activity, the enzyme responsible for intracellular cholesterol esterification, is increased (7) as is hepatic LDL catabolism (8, 9). Reports on in vivo effects of ethinyl estradiol on levels of cholesterol synthesis or on 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, a rate-limiting enzyme for cholesterol biosynthesis, have been contradictory. Administration of estrogens to rats at levels that caused hypocholesterolemia resulted in decreased (1, 10–12) or no change (13) in cholesterol synthesis. HMG-CoA reductase activity was reported to be decreased (11) or elevated (14).

Little has been reported on the influences of ethinyl estradiol on the susceptibility to regulation of hepatic cholesterol and lipoprotein metabolism by other known influences. Therefore, we examined the effects of ethinyl estradiol on the diurnal rhythms of cholesterol synthesis, HMG-CoA reductase activity, and ACAT activity. We then studied the responses of these activities and of cell membrane LDL receptors in the ethinyl estradiol-treated animals to short-term and sustained administration of cholesterol and to increased intracellular sterol synthesis induced by intragastric administration of mevalonolactone, a cholesterol precursor.

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Abbreviations: ACAT, acyl coenzyme A: cholesterol acyltransferase; LDL, low density lipoprotein; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; β-VLDL, β-very low density lipoproteins; MVA, mevalonolactone; CE, cholesteryl esters.

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EXPERIMENTAL PROCEDURES

Materials

Animals. Male Sprague-Dawley rats, 180–200 g (Simonsen, Gilroy, CA or Bantin and Kingman, Fremont, CA) were housed either under normal (lights on 6 AM, lights off 6 PM) or reverse (lights on 3 PM, lights off 3 AM) illumination. They were allowed free access to Purina rat chow and water.

Animals were treated for 3 days either with ethinyl estradiol (1 mg/ml propylene glycol) or the vehicle alone as described previously (9). In some experiments the animals received the chow diet supplemented with 2% cholesterol, 0.3% cholate, and 5% lard for 48 h before being killed. In other experiments, they were fed this diet for 14 days before and during administration of ethinyl estradiol for the final 3 days. In yet others, they were administered 1 mg mevalonolactone/g body weight intragastrically 2 h before being killed.

Male New Zealand white rabbits (Bantin and Kingman, Fremont, CA) were housed under normal illumination. They were allowed free access to water and Purina rabbit chow supplemented with 1% cholesterol.

Chemicals. [1, 2-^{3}H]Cholesterol (39–52 mCi/mmol), [^{3}H]-OH (1 Ci/g), D,L-3-hydroxy-3-methyl [3-^{14}C]glutaryl coenzyme A (40–60 mCi/mmol), R,S-[5-^{3}H]mevalonic acid (dibenzylethylenediamine salt, 1–5 Ci/mmol), Na[^{125}I] (approximately 17 Ci/mg) and [1,2-^{3}H]cholesteryl oleate (60–100 mCi/mmol) were from New England Nuclear (Boston, MA). [1-^{14}C]Oleoyl coenzyme A (50–60 mCi/mmol) was from Amersham Corp. (Arlington Heights, IL). Cholesterol, cholesterol oleate, oleoyl coenzyme A, β-NADP, glucose-6-phosphate, mevalonolactone, dithiothreitol, ethinyl estradiol, and glucose-6-phosphate dehydrogenase were from Sigma Chemical Co. (St. Louis, MO), R,S-3-hydroxy-3-methylglutaryl coenzyme A was from P. L. Biochemicals. All other reagents were analytical grade.

Methods

Isolation and administration of human LDL and rabbit β-VLDL. Human LDL were isolated as described previously (15). Rabbit β-VLDL were isolated from rabbits fed a cholesterol-rich diet (16).

Human LDL were injected into rats (lightly anesthetized with diethyl ether) through a femoral vein as a bolus containing approximately 7 mg protein. Control rats received an equal volume of 0.15 M NaCl. The rats were killed 2 or 4 h after injection of the lipoprotein. β-VLDL were injected into unanesthetized rats, previously fitted with an indwelling catheter in a jugular vein 1 h before starting the experiment, as a constant infusion over 2 h of approximately 11 mg protein. The controls received an equal volume of 0.15 M NaCl.

Preparation of liver cell membranes and assay of LDL receptor binding. Liver cell membranes were isolated as described by Kovanen, Brown, and Goldstein (3). EDTA-sensitive binding of β-VLDL was used to measure LDL receptors because β-VLDL bind with higher affinity than LDL and nonspecific binding is relatively low (17). β-VLDL were radioiodinated and direct binding assays in the absence or presence of 30 mm EDTA were carried out by a modification (17) of the method of Kovanen et al. (3).

Preparation of liver microsomes and assay for HMG-CoA reductase and ACAT activities. Microsomes were prepared as described previously (18). HMG-CoA reductase was assayed as described previously (18) except that mevalonolactone was separated by ion exchange chromatography (19). ACAT was assayed as described previously (20) except that a 2-min incubation was used for initial rate measurements and a 15-min incubation was used to estimate changes in microsomal cholesterol substrate availability.[2]

Synthesis of cholesterol and fatty acids from [^{3}H]OH in vivo. Hepatic cholesterol and fatty acid syntheses were assayed in vivo based on the method of Jeske and Dietschy (21) as described previously (22) except that a 1-h incubation time was used.

Other assays. Serum and liver free and total cholesterol concentrations were estimated by an enzymatic procedure (23). Protein was determined by the biuret method (24) or according to Peterson (25) with bovine serum albumin as reference standard.

Statistical analyses. Student's *t*-test was applied to all the data directly.

RESULTS

Effects of ethinyl estradiol on hepatic cholesterol synthesis and its regulation

Hepatic cholesterol synthesis measured in vivo with [^{3}H]OH was suppressed in animals given ethinyl estradiol relative to control animals, both at its diurnal peak (D6, 6th hour of the dark period) and nadir (L6, 6th hour of the light period) (**Table 1**). The diurnal peak value at D6 in the livers from ethinyl estradiol-treated animals was suppressed to levels found at the nadir (L6) in control animals. Thus, although the diurnal rhythm of cholesterol synthesis was present in ethinyl estradiol-treated rats, it was blunted. Incorporation of [^{3}H]OH into fatty acids was not significantly affected by ethinyl estradiol treatment in animals from either the diurnal peak or nadir of synthesis (Table 1).

[2]A 15-min assay time for ACAT estimates the total amount of microsomal cholesterol available to the enzyme for esterification. Although oleoyl-CoA concentrations are above the published apparent K_m for the enzyme (37) at this time, the amount of cholesteryl oleate formed has become constant, suggesting that substrate cholesterol has become rate-limiting (R. Daus and S. K. Erickson, unpublished observations).

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TABLE 1. Effect of ethinyl estradiol on the diurnal rhythm of hepatic cholesterol and fatty acid synthesis in vivo

Group	Treatment	Cholesterol Synthesis	Fatty Acid Synthesis
		μmol [³H]/g/h	
A. Basal, L6			
1	Control (6)	0.49 ± 0.20	1.56 ± 0.37
2	Ethinyl estradiol (6)	0.23 ± 0.07**	1.20 ± 0.27
B. Peak, D6			
3	Control (10)	1.24 ± 0.54*	2.63 ± 0.79*
4	Ethinyl estradiol (12)	0.43 ± 0.28⁺,°	2.14 ± 1.07⁺⁺

Male Sprague-Dawley rats weighing 200-220 g were housed under normal or reverse illumination. They were treated with ethinyl estradiol as described previously (9) except that a 3-day treatment period was used. Controls received the same volume of propylene glycol. Cholesterol and fatty acid synthesis were measured in vivo by incorporation of [^{3}H]OH over 1 h. The values are the mean ± SD. The number of animals is in parentheses.

*Different from Group 1 at $P < 0.01$.
**Different from Group 1 at $P < 0.02$.
⁺Different from Group 2 at $P < 0.05$.
⁺⁺Different from Group 2 at $P < 0.02$.
°Different from Group 3 at $P < 0.001$.

Feeding the cholesterol-cholate-lard diet for 48 h suppressed hepatic cholesterol synthesis in the control animals by 90% and in ethinyl estradiol-treated animals by 50% when measured at the diurnal peak of synthesis. However, in both cases, cholesterol synthesis was decreased to a similar absolute level (**Table 2**).

Effects of ethinyl estradiol on hepatic HMG-CoA reductase and ACAT activities and their regulation

Treatment with ethinyl estradiol suppressed hepatic HMG–CoA reductase activity in animals assayed at the peak of the diurnal rhythm but not in those assayed at the nadir (**Table 3**). Further, in the ethinyl estradiol-treated animals there was no statistically significant difference in reductase activity at L6 compared with D6; thus, the diurnal variation in the activity of this enzyme as assayed in vitro was abolished.

ACAT activity was increased both at the diurnal peak and nadir by ethinyl estradiol treatment (Table 3). The amount of microsomal cholesterol available for esterification by ACAT was increased by ethinyl estradiol in D6 animals from 1.85 ± 0.52 nmol/mg (n = 12) to 2.90 ± 0.83 nmol/mg (n = 12) ($P < 0.01$). The amount of cholesterol available to ACAT in the D6 control animals (n = 12) was 1.85 ± 0.52 nmol/mg whereas that in L6 control animals (n = 6) was 0.93 ± 0.88 nmol/mg ($P < 0.01$). The ethinyl estradiol-treated animals also showed diurnal differences in microsomal substrate cholesterol availability: 2.90 ± 0.83 nmol/mg for D6 animals (n = 12) as compared with 1.54 ± 0.78 nmol/mg for L6 animals (n = 6) ($P < 0.01$). These data suggested that changes in ACAT activity were due in part to diurnal variation in substrate availability.

Feeding the cholesterol-cholate-lard diet for 48 h led to further inhibition of HMG-CoA reductase activity, about 73%, in the ethinyl estradiol-treated animals (**Fig. 1**, top panel, left). Reductase activity was inhibited by 59% within 2 h after injection of a bolus of human LDL (Fig. 1, middle panel, left). It was inhibited 84% within 2 h after intragastric administration of mevalonolactone (Fig. 1, bottom panel, left). The increases in hepatic ACAT activities in the ethinyl estradiol-treated animals after the cholesterol-rich diet or LDL bolus (Fig. 1, top and middle panels, right) were not statistically significant; however, the increase induced by mevalonolactone administration was (Fig. 1, bottom panel, right).

In animals administered the vehicle with no ethinyl estradiol, feeding the cholesterol-cholate-lard diet caused 90% inhibition of hepatic HMG-CoA reductase activity (Fig. 1, top panel, left). The enzyme activity was inhibited 90% by intragastric administration of mevalonolactone (Fig. 1, bottom panel, left). Hepatic ACAT activity was increased significantly by both the cholesterol-cholate-lard diet and by administration of mevalonolactone (Fig. 1, top and bottom, panels, right). In these short-term experiments there was little effect of an LDL bolus on either HMG-CoA reductase

TABLE 2. Effect of a cholesterol-rich diet on hepatic cholesterol synthesis in vivo in rats treated with ethinyl estradiol

Group	Treatment	Cholesterol Synthesis
		μmol [³H]/g/h
1	Control (7)	1.36 ± 0.64
2	Ethinyl estradiol (9)	0.43 ± 0.33*
3	Control + cholesterol-rich diet (9)	0.14 ± 0.06**
4	Ethinyl estradiol + cholesterol-rich diet (9)	0.20 ± 0.06⁺

A cholesterol-cholate-lard diet was fed for the last 48 h of ethinyl estradiol treatment to half the animals. All animals were killed at the diurnal peak (D6) of synthesis. The values are the mean ± SD. The number of animals is in parentheses.

*Different from Group 1 at $P < 0.01$.
**Different from Group 1 at $P < 0.001$.
⁺Different from Group 2 at $P < 0.05$ by one-tail t test.

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TABLE 3. Effect of ethinyl estradiol on the diurnal rhythm of hepatic HMG-CoA reductase and ACAT activities

Group	Treatment	HMG-CoA Reductase	ACAT
		nmol MVA/min/mg protein	*nmol CE/min/mg protein*
A. Basal, L6			
1	Control (10)	0.210 ± 0.111	0.091 ± 0.025
2	Ethinyl estradiol (10)	0.216 ± 0.164	0.173 ± 0.082***
B. Peak, D6			
3	Control (9)	0.539 ± 0.177**	0.156 ± 0.051*
4	Ethinyl estradiol (12)	0.134 ± 0.094°	0.283 ± 0.138°°,+

Microsomes were prepared and assayed for the enzyme activities. Values are the means ± SD. The number of animals is in parentheses.
*Different from Group 1 at $P < 0.02$.
**Different from Group 1 at $P < 0.001$.
***Different from Group 1 at $P < 0.01$.
°Different from Group 3 at $P < 0.001$.
°°Different from Group 3 at $P < 0.01$.
+Different from Group 2 at $P < 0.05$.

or ACAT in control animals (Fig. 1, middle panels), perhaps due in part to the low number of LDL receptors.

Feeding the cholesterol–cholate–lard diet to animals given the vehicle alone increased the amounts of microsomal cholesterol available for esterification by ACAT from 1.18 ± 0.39 nmol/mg (n = 9) for controls fed a normal diet to 1.97 ± 0.90 nmol/mg (n = 9) ($P < 0.05$), but it had little effect in the ethinyl estradiol-treated animals: 2.00 ± 0.64 nmol/mg for animals fed the control diet (n = 12) as compared with 2.22 ± 0.67 for those fed the cholesterol-rich diet (n = 9), suggesting that cholesterol availability to the enzyme was already close to saturation. Administration of

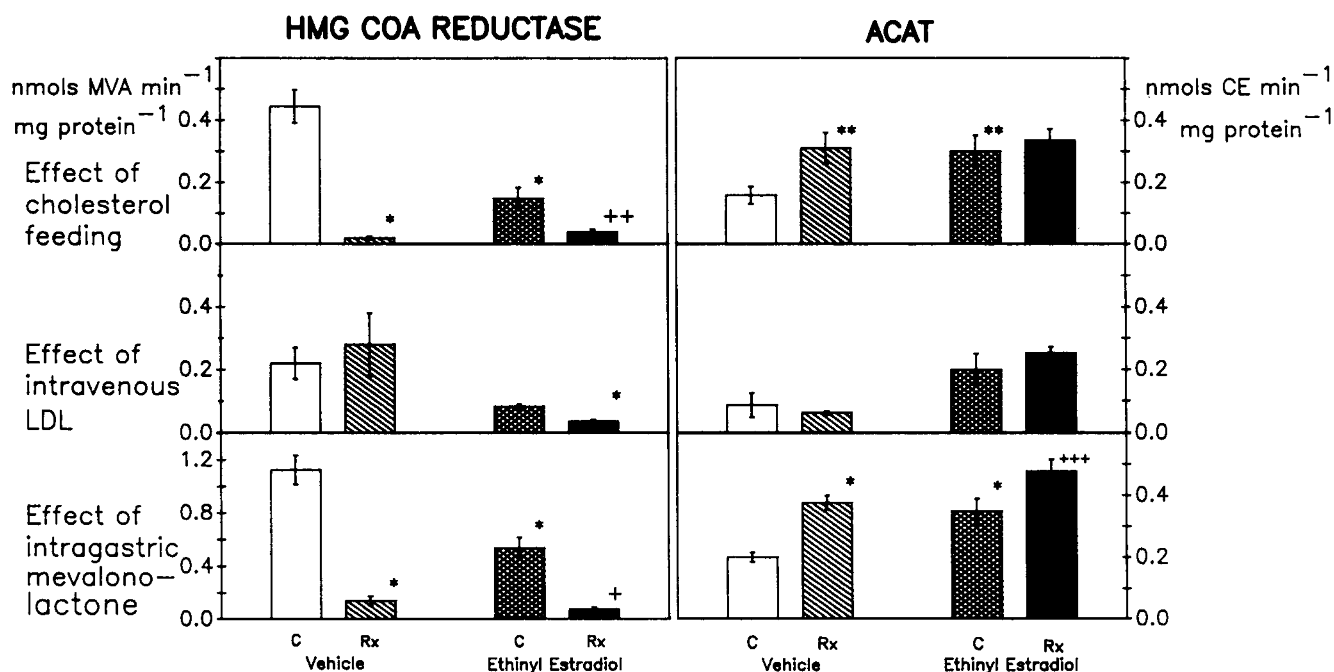

Fig. 1. Effect of ethinyl estradiol on the regulation of hepatic HMG-CoA reductase and ACAT activities. Animals were injected with vehicle or vehicle containing ethinyl estradiol for 3 days. Top panels: During the second and third days animals were fed a control diet (six animals received the vehicle and nine animals received ethinyl estradiol) or a cholesterol–cholate–lard diet (nine animals received the vehicle and eight animals received ethinyl estradiol) They were killed at D6. Middle panels: Three control animals received a bolus of 0.15 M NaCl and three control animals received a bolus of 0.15 M NaCl containing human LDL (11 mg cholesterol) 2 h before they were killed at L6. Nine ethinyl estradiol-treated animals received a bolus of 0.15 M NaCl and twelve ethinyl estradiol-treated animals received a bolus of 0.15 M NaCl containing human LDL (11 mg cholesterol) 2 h before they were killed at L6. Bottom panels: Twelve animals received a bolus of 0.15 M NaCl and twelve animals received a bolus of 0.15 M NaCl + mevalonolactone (1 mg MVA/g body weight) intragastrically. The animals were killed 2 h later at D6. The values are the mean ± SD. *Different from vehicle control ($P < 0.001$). **Different from vehicle control ($P < 0.05$). +Different from ethinyl estradiol control ($P < 0.001$). ++Different from ethinyl estradiol control ($P < 0.005$). +++Different from ethinyl estradiol control ($P < 0.05$).

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a bolus of LDL to the ethinyl estradiol-treated animals also did not significantly alter the amount of cholesterol available to ACAT: 1.08–0.24 nmol/mg for controls (n = 6) and 1.34 ± 0.33 nmol/mg for those receiving LDL (n = 6). Administration of mevalonolactone increased the cholesterol available for esterification by ACAT in liver microsomes from the animals receiving the vehicle alone from 2.04 ± 0.16 to 3.99 ± 0.57 nmol/mg ($P < 0.01$, n = 3 in each group), but had a variable effect in the ethinyl estradiol-treated animals: 4.94 ± 0.31 nmol/mg for those receiving vehicle (n = 3) and 5.61 ± 1.53 nmol/mg for those receiving mevalonolactone (n = 3).

Regulation of hepatic LDL receptors in the ethinyl estradiol-treated rat

The number of hepatic LDL receptors was also measured in some of the animals used to assay HMG-CoA reductase and ACAT. Based on earlier work (17, 26), we have used the maximal EDTA-sensitive binding of β-VLDL to hepatic membranes as a measure of the number of functional LDL receptors present.

EDTA-sensitive membrane binding sites in rat liver for rabbit β-VLDL are increased about tenfold by ethinyl estradiol treatment (17). The number of EDTA sensitive-binding sites for β-VLDL was similar at the diurnal peak and nadir of cholesterol metabolism in livers from ethinyl estradiol-treated animals, 1.58 ± 0.11 and 1.72 ± 0.08 μg β-VLDL/mg protein, respectively. Thus, in ethinyl estradiol-treated rats, there was no apparent correlation of LDL receptor number with the diurnal rhythms of other key enzymes of cholesterol metabolism.

β-VLDL binding sites were reduced 38% by the cholesterol–cholate–lard diet after 48 h of feeding (**Table 4**). No change in binding affinity was observed.

β-VLDL binding sites were reduced by 49% 2 h after administration of a bolus of LDL (7 mg protein; Table 4); by 4 h, receptor number had returned to control level. No differences in affinity were detected at either time. When β-VLDL (11 mg protein) were infused at a constant rate into two animals over 2 h, β-VLDL binding sites were reduced by 51% (Table 4 and **Fig. 2**), again with no change in affinity. In these experiments, there was little effect of β-VLDL infusion on HMG-CoA reductase activity (0.02 and 0.03 nmol MVA min^{-1} mg protein $^{-1}$ compared with 0.02 and 0.03 nmol MVA min^{-1} mg $protein^{-1}$ for two control animals infused with saline). The very low levels of reductase activity in the control animals suggested that, in this experiment, the enzyme was already maximally inhibited. However, ACAT activity was increased from 0.24 and 0.42 nmol CE min^{-1} mg $protein^{-1}$ to 0.59 and 0.63 nmol CE min^{-1} mg $protein^{-1}$ with

TABLE 4. Regulation of LDL receptors in hepatic cell membranes from ethinyl estradiol-treated rats

Treatment	Serum Cholesterol	Hepatic Cholesterol		LDL Receptors	K_D
		Free	Ester		
	mg/dl	*mg/g liver*		*μg β-VLDL/mg protein*	*μg β-VLDL/ml*
A. Ethinyl estradiol					
+ control diet (3)	2 ± 1	1.85 ± 0.38	0.85 ± 0.28	1.65 ± 0.24	0.85 ± 0.31
+ cholesterol-rich diet fed last 48 h (3)	7 ± 4	2.72 ± 0.12**	6.40 ± 1.80*	1.03 ± 0.12**	0.87 ± 0.09
B. Ethinyl estradiol[a]					
+ vehicle, 2 h (3)	17 ± 3	1.70 ± 0.08	2.05 ± 0.16	1.72 ± 0.12	0.60 ± 0.08
+ LDL 2 h (6)	22 ± 13	1.64 ± 0.04	2.15 ± 0.84 (n = 3)	1.05 ± 0.13**	0.88 ± 0.32
+ vehicle, 4 h (3)	–	1.78 ± 0.31	1.65 ± 0.40	1.69 ± 0.14	0.97 ± 0.24
+ LDL, 4h (3)	–	1.71 ± 0.54	1.57 ± 0.69	1.56 ± 0.57	1.06 ± 0.03
C. Ethinyl estradiol[b]					
+ vehicle, 2 h (2)	8, 8	1.89, 1.72	3.69, 2.16	2.00, 1.75	1.06, 0.93
+ β-VLDL, 2 h (2)	515, 252	2.75, 2.18	4.97, 3.34	1.00, 0.90	1.06, 0.99
D. Ethinyl estradiol[c]					
+ vehicle, 2 h (3)	22 ± 3.5	1.24 ± 0.25	1.07 ± 0.13	1.09 ± 0.20	0.80 ± 0.16
+ MVA 2 h (3)	20 ± 4	1.09 ± 0.24	1.23 ± 0.25	1.17 ± 0.21	1.19 ± 0.16***
Ethinyl estradiol					
+ vehicle, 4 h (3)	10, 10 (n = 2)	2.03 ± 0.05	1.52 ± 0.29	0.87 ± 0.35	0.54 ± 0.21
+ MVA, 4 h (3)	13 ± 2	2.03 ± 0.07	1.96 ± 0.31	0.94 ± 0.35	0.60 ± 0.10

The values are the means ± SD unless otherwise indicated. The number of animals is in parentheses.
[a]LDL was given intravenously as a bolus of 11 mg cholesterol. Controls received an equal volume of 0.15 M NaCl.
[b]β-VLDL was given intravenously as an infusion of 92.1 or 97.4 mg cholesterol over 2 h. Controls received an equal volume of 0.15 M NaCl.
[c]MVA was given intragastrically as a bolus of 1 mg/g body weight. Controls received an equal volume of 0.15 M NaCl.
*Different from control at $P < 0.001$.
**Different from control at $P < 0.02$.
***Different from control at $P < 0.05$.

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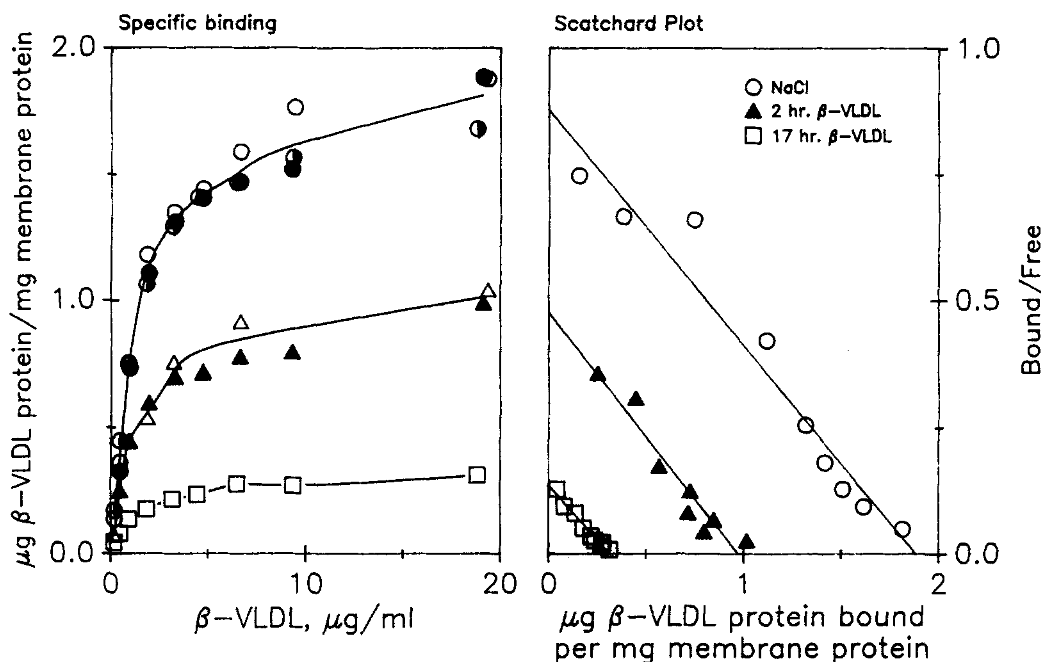

Fig. 2. Effect of β-VLDL infusion on functional hepatic LDL receptors in ethinyl estradiol-treated rats. β-VLDL was given as a continuous infusion for 2 h or 17 h. Controls received the same volume of 0.15 M NaCl. Left panel: specific EDTA-sensitive binding. Values for all experiments are shown. Control (○, ◑, ●); β-VLDL, 2h (△, ▲); β-VLDL, 17 h (□). Right panel: Scatchard plots. Values are means for each group in left panel.

an increase in microsomal cholesterol available for esterification from 2.22 and 2.25 nmol/mg protein in controls to 4.78 and 4.86 nmol/mg protein in β-VLDL-infused animals.

When β-VLDL were infused into one estradiol-treated rat for 17 h (Fig. 2) hepatic LDL receptors were reduced to about 10% of the number found in an estradiol-treated rat infused with 0.15 M NaCl. HMG-CoA reductase decreased 97% from 0.031 nmol MVA min^{-1} mg $protein^{-1}$ to 0.001 nmol MVA min^{-1} mg $protein^{-1}$ and ACAT activity increased from 0.61 nmol CE/min per mg protein to 0.81 nmol CE min^{-1} mg $protein^{-1}$ with a concomitant increase in microsomal substrate cholesterol available to the enzyme from 3.88 nmol/mg protein to 6.24 nmol/mg protein. In the rat infused with β-VLDL for 17 h, serum cholesterol was 318 mg/dl compared with 2 mg/dl in the estradiol-treated control rat infused with an equal value of 0.15 M NaCl. Hepatic cholesterol concentration in the rat infused with β-VLDL was 144 μg/mg protein (free/ester = 0.35) as compared with 39 μg/mg protein (free/ester = 0.81) in the control rat infused with 0.15 M NaCl.

Little effect on β-VLDL binding sites was noted 2 h after intragastric administration of mevalonolactone (Table 4), in contrast to its effects on HMG-CoA reductase and ACAT (Fig. 1, bottom panels). Although the effects on ACAT and HMG-CoA reductase persisted for 4 h (data not shown), the number of β-VLDL binding sites after 4 h remained indistinguishable from that in animals not treated with mevalonolactone. Binding affinity at the 2-h time point was slightly increased (Table 4), whereas that at the 4-h time point was the same as control.

Effect of pretreatment of animals with a cholesterol-rich diet on ethinyl estradiol-induced changes in hepatic cholesterol metabolism

The ability of estradiol to induce LDL receptors was investigated in animals pretreated with the cholesterol-rich diet. Although plasma cholesterol levels fell to the same low level in the two groups, hepatic free cholesterol and, to a greater extent, esterified cholesterol concentrations were increased in the animals receiving both the diet and ethinyl estradiol (**Table 5**). HMG-CoA reductase activity was suppressed by about 85% in these animals whereas ACAT activity was increased about twofold above that of animals on the control diet that were given ethinyl estradiol. The number of β-VLDL binding sites (Table 5) was about fivefold greater than the number found in livers of animals fed a chow diet (17); however, they were only about 50% of the number found in liver membranes from animals on the control diet given ethinyl estradiol (Table 5). There were no detectable changes in binding affinity.

DISCUSSION

Our data show that diurnal regulation of hepatic cholesterol metabolism is altered in the ethinyl estradiol-treated rat. The diurnal increase in cholesterol synthesis,

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TABLE 5. Effect of ethinyl estradiol on hepatic enzymes and LDL receptors in rats maintained on a cholesterol-rich diet

	Control (n = 6)	Cholesterol-Rich Diet (n = 6)
Serum cholesterol, mg/dl	18 ± 11	17 ± 6
Liver cholesterol		
Free, mg/g liver	1.52 ± 0.27	2.43 ± 0.51**
Ester, mg/g liver	0.62 ± 0.22	29.40 ± 7.64*
HMG-CoA reductase, nmol MVA/min/mg protein	0.166 ± 0.093	0.020 ± 0.010**
ACAT, nmol CE/min/mg protein	0.274 ± 0.076	0.596 ± 0.076*
LDL receptors, μg β-VLDL/mg protein	1.47 ± 0.22	0.68 ± 0.05*
LDL receptor K_D, μg β-VLDL/ml	0.86 ± 0.27	0.82 ± 0.12

Animals received either a control diet or one containing cholesterol, cholate, and lard for 14 days. They were then given ethinyl estradiol during the last 3 days. The number of animals is in parentheses. Values are mean ± SD.
*Different from control at $P < 0.001$.
**Different from control at $P < 0.02$.

although present, was severely blunted while the increase for HMG-CoA reductase was no longer apparent. ACAT activity was increased at both the diurnal peak and nadir, and its diurnal variation was similar in magnitude to that of control animals. We are aware of no reports of diurnal variation of LDL receptors in control animals, but we found no diurnal variation of hepatic LDL receptors in ethinyl estradiol-treated rats. The tenfold enrichment of cholesteryl esters in hepatic VLDL of ethinyl estradiol-treated rats (26) may reflect, in part, the increased ACAT activity in their livers. Re-uptake of these particles by the liver may contribute to the decreased rates of hepatic cholesterol synthesis and of HMG-CoA reductase activity.

Inhibition by dietary cholesterol of cholesterol synthesis and HMG-CoA reductase in estradiol-treated rats was similar to that of control animals. HMG-CoA reductase in ethinyl estradiol-treated rats also could be inhibited by administered lipoproteins and mevalonolactone. Thus, mechanisms for regulation of HMG-CoA reductase are intact in the ethinyl estradiol-treated rat although the apparent sensitivity to regulation appeared to be reduced.

Dietary cholesterol had little effect on ACAT activity in the ethinyl estradiol-treated animals, and the amount of microsomal cholesterol available for esterification by the enzyme was not increased. A bolus of LDL also failed to alter ACAT activity or availability of microsomal cholesterol to the enzyme; however, infusion of β-VLDL, which delivered a larger quantity of cholesterol in 2 h, did increase ACAT activity. Moreover, hepatic ACAT in the ethinyl estradiol-treated rats also increased in response to intragastric administration of a bolus of mevalonolactone. Thus, the ability of the enzyme to respond was intact, but like HMG-CoA reductase, its sensitivity to regulation appeared to have been reduced by ethinyl estradiol.

We found that hepatic LDL receptors were susceptible to rapid regulation by administered lipoproteins in estradiol-treated rats, but not to an amount of mevalonolactone that regulated HMG-CoA reductase and ACAT activities. This suggested that β-VLDL binding sites, unlike HMG-CoA reductase and ACAT activities, were unable to respond to rapid regulation by products of MVA.

Koelz and co-workers (27) and Spady, Turley, and Dietschy (28) have shown that hepatic cholesterol homeostasis of normal rats is maintained primarily by changes in cholesterol synthesis and the activity of HMG-CoA reductase. In their experiments, LDL were administered as a constant infusion (28) and LDL uptake and cholesterol synthesis were measured 6 h later. They found no evidence for down-regulation of hepatic LDL receptors as reflected by rate of hepatic LDL clearance under conditions where sterol synthesis varied over a 110-fold range. Since we found that ethinyl estradiol treatment alone decreased cholesterol synthesis and HMG-CoA reductase and induced ACAT, the ability of these enzymes to respond further to dietary cholesterol may not have been sufficient to maintain cholesterol homeostasis, in contrast to the normal rat. Furthermore, bile secretion is suppressed in these animals (4,5), and their livers secrete a cholesterol-enriched VLDL (26), a large portion of which are taken up rapidly by the liver because of the increased LDL receptor number. Thus, the remaining avenue for maintenance of cholesterol homeostasis by these animals in the face of a dietary or lipoprotein challenge would be down-regulation of LDL receptors.

ACAT activity in the animals fed the cholesterol-rich diet and then given ethinyl estradiol was about twice that found in the ethinyl estradiol-treated animals on a normal diet (Fig. 1 and Table 5) or in those subsequently fed the cholesterol-rich diet (Fig. 1). This suggests that the mechanisms for regulating ACAT activity by long-term (14 day) cholesterol feeding and ethinyl estradiol may be different and are additive.

We have shown that hepatic LDL receptors remain inducible by ethinyl estradiol despite massive increases in hepatic free and esterified cholesterol levels produced by feeding the cholesterol–cholate–lard diet. Further, this induction of receptors abolished the hypercholesterolemia

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(ca. 170 mg cholesterol/dl) normally observed in animals fed this diet (29). However, the increase in functional LDL receptors was about half that which would have occurred in ethinyl estradiol-treated animals on a normal diet, and it therefore seems likely that the high concentrations of hepatic cholesterol attenuated the induction of receptors by estradiol. This interpretation is consistent with the observation that the number of LDL receptors in the liver of these animals was similar to that of the animals treated with ethinyl estradiol and then fed cholesterol.

The mechanism(s) for rapid regulation of LDL receptor activity is unknown. On the basis of studies with cultured cells, it seems unlikely that changes in synthesis could account for this; however, the turnover time for the rat liver receptor in vivo is unknown. In addition to regulation at the genetic level, LDL receptor function might be altered in the short term by post-translational modification such as phosphorylation-dephosphorylation. A phosphorylation-dephosphorylation mechansism for short-term reversible regulation has been demonstrated for HMG-CoA reductase (30,31) and proposed for ACAT (32). Although the LDL receptor protein can be phosphorylated (33), the role that phosphorylation plays in its activity or regulation is unknown.

Regulation of HMG-CoA reductase and the LDL receptor is achieved in part at the level of gene transcription via homologous sterol-sensitive regulatory elements (34,35). Ma et al. (36) have shown that mRNA for the LDL receptor is increased by administration of pharmacological doses of ethinyl estradiol to rabbits, consistent with a pretranslational effect. Our observation that ethinyl estradiol induces enhanced expression of LDL receptors in cholesterol-loaded livers raises the possibility that estrogens increase that number of LDL receptors by affecting a different gene regulatory element or that they modify post-transcriptional events in a manner different from sterols.

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