Metabolism of Chylomicron Cholesterol Is Delayed by Estrogen. An *In Vivo* Study in the Rat

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In order to test the effects of estrogen on the clearance of cholesterol of dietary origin from the blood and its elimination from the body *via* the bile in an *in vivo* animal model, the fate of radioactivity from intravenously injected [^{3}H]cholesterol-labeled chylomicrons was investigated in the rat. The labeled lipoproteins were administered intrajugularly to male rats previously given 17α ethinyl estradiol or the vehicle only, and the removal of the radioactivity from the blood and its uptake by the liver and secretion into bile was determined. Experiments were carried out in animals with or without prior drainage (20 hr) of the pool of bile acids in the enterohepatic circulation, to take account of the different demands of the liver for cholesterol in the two conditions. In rats without biliary drainage, estrogen treatment decreased the rate of removal of radioactivity from the blood by about 30% and the recovery of cholesterol in the liver by about 50% in the first 30 min after injection of the labeled chylomicrons. After biliary drainage, however, the recovery of label in the liver after 90 min was similar in estrogen-treated and control animals, although its secretion into bile was markedly reduced in the estrogen-treated group (total biliary secretion in 90 min was 26% of the value found in control rats). In addition, the apolipoprotein E (aopE) content of the serum total lipoproteins was markedly reduced by estrogen. These results provide direct evidence indicating that estrogen retards the elimination of dietary cholesterol from the body *via* the bile in the rat, and this is likely to be mainly due to a reduced level of apoE in chylomicrons. In view of this, we suggest that the hypothesis that estrogen increases the hepatic uptake of chylomicron cholesterol, and its excretion in the bile during contraceptive and hormone replacement therapy should be re-examined. [E.B.M. 2001, Vol 226:112–118]

Key words: chylomicron cholesterol; estrogen; apolipoprotein

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The pre- and postmenopausal difference in the incidence of cardiovascular disease in women directs attention to the effect of estrogen on cardiovascular disease. The hormone has been shown to have many positive effects on risk factors and arterial wall function (1), thus the absence of estrogen is believed to be responsible, at least in part, for the increased rate of cardiovascular disease postmenopausally (2, 3). Hormone replacement therapy containing both estrogens and progestins is recognized to be beneficial in reducing cardiovascular risk in women (4, 5), although a recent study found no reduction in the overall rate of cardiovascular events in postmenopausal patients with established coronary disease (6). The reason for this apparent lack of protection in women with a history of heart disease is not clear, but it is possible that the progestins are responsible. Both estrogens and progestins regulate lipoprotein metabolism at multiple regulatory points, having effects on lipoprotein levels, lipoprotein kinetics, and other mediators of atherogenesis. A better understanding of the mechanism of action of these compounds, therefore, would help to increase the benefit of this therapy (7).

Estrogens are well-known stimulators of hepatic low-density lipoprotein (LDL) catabolism (8–10), and different approaches using experimental animals have consistently found that estrogens induce a 10-fold increase in the catabolic rate of this lipoprotein (8, 11), although the doses of the hormone used were high compared to those given in oral contraceptives or postmenopausal hormone therapy in humans. Chylomicron remnants carry cholesterol from the diet to the liver, where it may be eliminated from the body *via* the bile either unchanged or after conversion to bile acids, or alternatively be returned to the circulation in new lipoprotein. Hepatic uptake is by a receptor-mediated process dependent on apolipoprotein E (12). The particles are believed to bind initially to heparan sulfate proteoglycans and to be internalized subsequently by either the LDL receptor or the LDL receptor-related protein (LRP) (13, 14), although the relative importance of the two receptors is disputed (15–17).

Increased chylomicron clearance during use of oral contraceptives or hormone replacement therapy containing estrogen and progestin has been reported previously (18,

19), although it is not clear which of the hormonal components predominates in this action. In addition, van Beek *et al.* (20) have found decreased removal of chylomicrons from the circulation in post- as compared to premenopausal women. All these studies, however, used oral fat loading tests to follow the disappearance of chylomicrons from the blood, and we are not aware of any previous studies in which the hepatic uptake of chylomicron cholesterol and its excretion in the bile was followed directly in an *in vivo* animal model. Despite this, the positive effect of estrogen on the lipoprotein cascade has been assumed to include increased hepatic catabolism and elimination from the body *via* the bile of cholesterol carried in this type of lipoprotein (21, 22).

The aim of this study is to investigate directly, using an *in vivo* animal model, the influence of estrogen on the clearance of dietary cholesterol from the blood and its excretion in the bile. As chylomicrons are rapidly converted to chylomicron remnants before their uptake by the liver *in vivo*, we were able to achieve this aim by following the metabolic fate of chylomicrons radiolabeled with [^{3}H]cholesterol in rats treated with 17α ethinyl estradiol. In order to investigate the effects of the hormone on the biliary excretion of chylomicron cholesterol, experiments were carried out with prior drainage of the pool of bile acids in the enterohepatic circulation in conditions of a steady state of biliary bile acid secretion, as described previously (23). However, as biliary drainage increases the hepatic demand for cholesterol (24), the clearance of labeled cholesterol carried in chylomicrons from the blood and its uptake by the liver were also investigated in rats with an intact enterohepatic circulation.

Materials and Methods

Animals and Materials. Male Wistar rats were housed at 25°C under constant day length (12 hr) and allowed free access to food (standard pellet diet) and water for at least 2 weeks before the experiments. Animals (350 ± 24 g) used for *in vivo* experiments, or for the collection of blood for the investigation of the apolipoprotein content, were injected subcutaneously with either 17α ethinyl estradiol (5 mg/kg body weight) in propylene glycol or the vehicle only once per day for three days prior to the experiments. This protocol is similar to those which are commonly used in studies with rats and which have been found to cause marked reductions in plasma cholesterol levels and in biliary secretion in this species (25, 26). The dose of estrogen used, however, is very much higher than the oral contraceptive or postmenopausal replacement dose in humans. 17α ethinyl estradiol and Menhaden fish oil was purchased from Sigma (Poole, Dorset, UK), and sodium taurocholate from Calbiochem (La Jolla, CA). [1α,2α-^{3}H]Cholesterol was supplied by Amersham International (Amersham, Bucks, UK).

Preparation of Radiolabeled Chylomicrons. Rats (300–350 g) were given Menhaden fish oil (1 ml) supplemented with α-tocopheryl acetate (4 mg/ml) as an antioxidant by stomach tube. Approximately 1 hr later, the animals were anaesthetized with sodium pentobarbitol (60 mg/kg body weight), and the thoracic duct was cannulated as described previously (27). A 0.5-ml amount of Menhaden fish oil containing [^{3}H]cholesterol (18 MBq) was then injected into the pyloric region of the stomach, the body wall was sutured, and the rats were placed in restraining cages. The chyle collected after a period of 16–18 hr was layered under 0.9% NaCl (d = 1.006 g/ml) and centrifuged (6×10^5 g·min) in a fixed angle rotor at 12°C, and large chylomicrons (diameter > 100 nm) were harvested from the top 1–1.5 cm by tube slicing. The ratio of triacylglycerol to total cholesterol was approximately 25:1 in all preparations used, and approximately 55% of the radiolabel was in cholesteryl ester with the remainder in unesterified cholesterol.

Experimental Protocol. Animals were prepared with a cannula in the left jugular vein and, in experiments with biliary drainage only, with a second cannula in the common bile duct as described previously (23), then injected intrajugularly with [^{3}H]cholesterol-labeled chylomicrons (10–50 kBq in a volume of 0.5–1.0 ml, containing approximately 0.5 μmol cholesterol and 12.5 μmol triacylglycerol) after either 30 min (without biliary drainage) or 20 hr (with biliary drainage). Biliary drained animals were infused with sodium taurocholate to maintain a steady state of bile acid secretion during the experiment as before (23). Blood samples were taken from the tail vein at various time intervals in all experiments, and bile samples were collected at 15-min intervals in experiments with biliary drainage. After 30 min (without biliary drainage) or 90 min (with biliary drainage), ice-cold KCl (1 *M*) was injected to kill the animal, and the liver was excised. Radioactivity in blood, liver, and bile samples was determined by liquid scintillation counting.

Analytical Methods. For analysis of blood apolipoproteins, blood samples collected from rats treated with 17α ethinyl estradiol or the vehicle only by cardiac puncture were centrifuged at 3000 rpm for 20 min to remove the red blood cells, and the total lipoprotein was isolated from the serum by centrifugation at d 1.25 g/ml (28). After preparation of the samples as described by Mindham and Mayes (29), the apolipoproteins were separated by SDS-PAGE electrophoresis (30). The gels were stained with Coomassie blue, and the bands corresponding to apolipoprotein E (apoE) were quantified by volume analysis using a Bio-Rad HP600 scanning densitometer and Molecular Analytical software.

The cholesterol and triacylglycerol content of the chylomicron preparations was assayed enzymatically using kits supplied by Boehringer Mannheim GmbH (Mannheim, Germany). For determination of radioactivity in cholesterol and bile acids in bile, samples were mixed with propan-2-ol (1:5 v:v), the lipids were separated by thin-layer chromatography, the appropriate bands were scraped into scintillation vials, and the radioactivity was determined by liquid scintillation counting (23). Significance limits were calcu-

lated using Student's *t*-test or the ANOVA repeated measures test.

Results

Effect of Estrogen on Plasma Lipid and apoE Levels. The body weight of the rats used was not significantly different in estrogen-treated and untreated rats in experiments both with and without biliary drainage. As expected from previous work (8), plasma total cholesterol levels were markedly reduced in estrogen-treated as compared to control rats (Table I). Total cholesterol in the $d < 1.006$ g/ml plasma fraction, however, was not significantly changed.

Estrogen treatment tended to decrease plasma triacylglycerol concentrations, but because of relatively large variations between individual animals, the difference did not reach statistical significance.

The apolipoprotein content of the total lipoprotein fraction of the serum was decreased in the animals treated with estrogen, and this change was particularly dramatic in the case of apoE (Fig. 1). Quantification of the bands by optical density volume analysis showed that apoE was reduced from 15.0 ± 4.6 units ($n = 3$) in the control rats to 0.25 ± 0.23 units ($n = 3$) in those given 17α ethinyl estradiol.

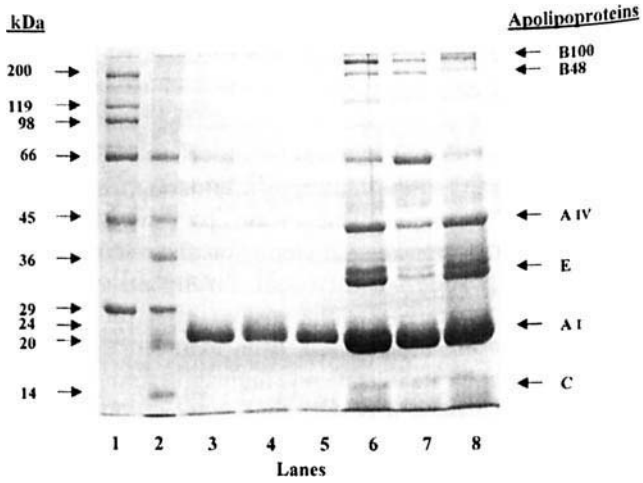

Figure 1. Effect of estrogen on the apolipoprotein content of rat serum. Rats were injected with 17α ethinyl estradiol (5 mg/kg body weight) in propylene glycol or propylene glycol only once per day for 3 days, and the total lipoprotein fraction ($d < 1.25$ g/ml) was isolated from the serum obtained from the blood collected by cardiac puncture. The apolipoproteins from equal volumes of serum were then separated by SDS–PAGE electrophoresis using 10% gels and stained with Coomassie blue. Lane 1, high molecular weight standards; lane 2, low molecular weight standards; lanes 3–5, rats injected with 17α ethinyl estradiol; lanes 6–8, rats injected with propylene glycol only.

Effect of Estrogen on the Removal of Chylomicron Cholesterol from the Blood. The effects of estrogen treatment on the removal of chylomicron cholesterol from the blood in experiments with or without prior biliary drainage are shown in Fig. 2. In the shorter-term experiments without biliary drainage (Fig. 2A), there was delayed clearance of labeled chylomicron cholesterol in estrogen-treated as compared to control rats, and the difference was highly significant (ANOVA repeated measures, $P < 0.01$). Estimation of the decay rates for chylomicrons in the two groups after curve fitting showed that there was a decrease of about 30% in the estrogen-treated animals.

For investigation of the effects of estrogen on the excretion of chylomicron cholesterol *via* the bile, biliary drained rats infused with taurocholate to maintain a steady state of bile acid secretion (23) were used. However, when rats are biliary drained, the demand for cholesterol from lipoproteins in the liver is changed. Previous work has shown that about 20% of lipoprotein cholesterol is secreted into bile as bile acid within 6 hr in rats injected after 36–48 hr biliary drainage, compared to 1–2% in the same period when the dose was given immediately after surgery (24). For this reason, we also investigated the effect of estrogen on the removal of chylomicron cholesterol from the blood in the biliary drained rats. In this case, a consistent difference in the removal of the radioactivity from the blood was observed at all time points studied between 20 and 60 min after the administration of labeled chylomicrons, although this did not reach statistical significance (ANOVA repeated measures test) (Fig. 2B).

Effect of Estrogen on the Recovery of Chylomicron Cholesterol in the Liver. The recovery of radioactivity (% administered dose) in the liver was lower in estrogen-treated rats in experiments without biliary drainage (mean ± SEM; control rats, 66.8% ± 6.7%, $n = 4$; estrogen-treated rats, 26.6% ± 3.7%, $n = 5$, $P < 0.01$), and the specific activity of liver total cholesterol was also significantly decreased (% administered dose/μmol; control rats, 0.76 ± 0.16; estrogen-treated rats, 0.29 ± 0.05, $P < 0.05$), although the cholesterol content of the tissue remained unchanged. In the longer experiments with biliary drainage, however, the recovery of radioactivity in the liver after 90

Table I. Rat Body Weights and Plasma Lipid Levels in Estrogen-Treated and Untreated Rats

Parameter	Untreated rats	Estrogen-treated rats
Body weight (g) (experiments without biliary drainage)	363 ± 20 (4)	357 ± 13 (5)
Body weight (g) (experiments with biliary drainage)	345 ± 8 (3)	354 ± 15 (4)
Plasma total cholesterol (μmol/ml)	1.13 ± 0.14 (5)	0.21 ± 0.04 (6)
Total cholesterol in the $d < 1.006$ g/ml plasma fraction (μmol/ml)	0.12 ± 0.03 (4)	0.08 ± 0.03 (5)
Plasma triacylglycerol (μmol/ml)	2.06 ± 0.21 (4)	1.19 ± 0.63 (4)

Note. Rats were injected with 17α ethinyl estradiol (5 mg/kg body weight) in propylene glycol or with propylene glycol only once per day for 3 days. Data shown are the mean ± SEM, and the numbers of animals used are shown in parentheses.

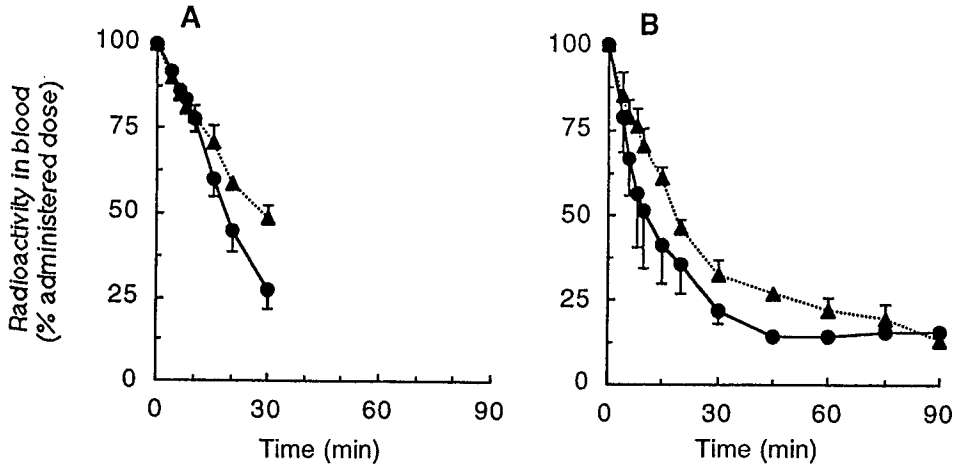

Figure 2. Effect of estrogen on the clearance of radioactivity from the blood after intravenous administration of cholesterol-labeled chylomicrons. Blood samples were taken from the tail vein at the times indicated after the administration of [^{3}H]cholesterol-labeled chylomicrons to control rats (●) or rats injected with 17α ethinyl estradiol (▲), (A) without biliary drainage and (B) with biliary drainage. Each point is the mean from 3–5 rats and error bars show the SEM. Significance values (ANOVA repeated measures test), estrogen-treated versus control rats are as follows: without biliary drainage (A), $P < 0.01$, and with biliary drainage (B), $P = 0.39$.

min was not appreciably lowered by estrogen (control rats, 41.4% ± 3.4%, $n = 3$; estrogen-treated rats, 52.8% ± 3.8% [range] $n = 2$), and the hepatic total cholesterol content and specific activity (% administered dose/μmol; control rats, 0.46 ± 0.10; estrogen-treated rats, 0.51 ± 0.15 [range]) were also similar in the two groups.

Effect of Estrogen on the Excretion of Chylomicron Cholesterol in the Bile. The recovery of radioactivity expressed as a percentage of the administered dose in six bile samples collected at 15-min intervals in experiments carried out with biliary drainage is reported in Fig. 3. Excretion of the label in bile was markedly lower in estrogen-treated than in control animals at all time points after 15 min, both on a cumulative basis (Fig. 3A) and in each 15-min sample (Fig. 3B). After 90 min the total amount of radioactivity excreted was 3.42% ± 1.1% ($n = 3$) in control rats and 0.89% ± 0.16% ($n = 4$) in the estrogen-treated group; 75%–80% of the radioactivity in bile was in bile acids and the remaining 20%–25% in cholesterol in both the control and estrogen-treated groups.

Discussion

Chylomicron clearance has been reported to be increased in women during the use of oral estrogen–progestin (18, 19, 31) and to be decreased after menopause (20). Thus, it has become commonly accepted that one of the beneficial effects of estrogen is enhanced removal of chylomicrons from the circulation (4, 21, 32), through the up-regulation of the LDL receptor (21, 33). However, it is not clear which hormonal component in oral formulations, estrogen or progestin, predominates in this action (22). Furthermore, these studies in women were restricted to the measurement of the disappearance of chylomicron particles labeled with vitamin A from the blood using oral fat loading tests, and they provide no information about the effects of estrogen on the uptake of the cholesterol they carry by the liver, or its excretion from the body in the bile. In order to study these processes directly, it is necessary to use an animal model, as in the present work.

Part of the label (approximately 45%) in the chylomicrons used in our experiments was in unesterified cholesterol, which has the capacity to exchange with plasma lipoproteins and tissues, and it is possible that this may affect the results obtained. However, extensive studies have shown that the exchange process takes time to occur, and that in rats only about 5% of labeled unesterified cholesterol from chylomicrons is transferred to high-density lipoprotein (HDL) in 30 min (34). As much of the radioactive dose (Fig. 2) has already been removed from the blood at this time in our study, it is unlikely that a significant amount of the

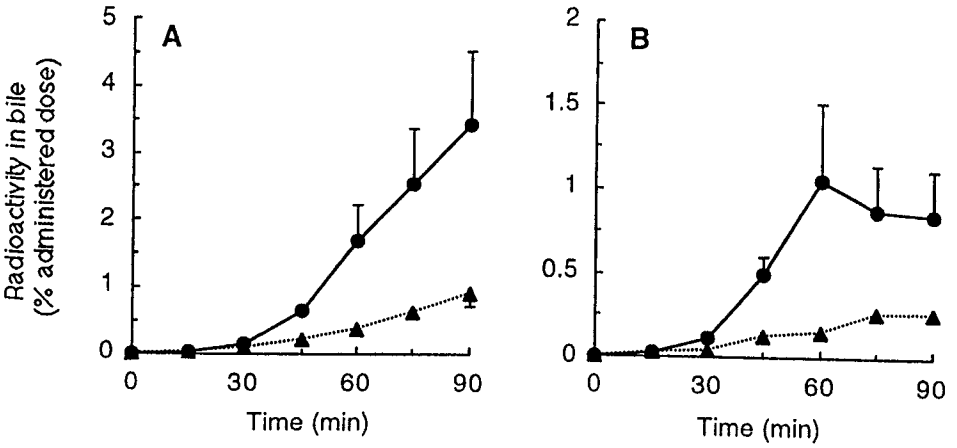

Figure 3. Effect of estrogen on the secretion of radioactivity into bile after intravenous administration of cholesterol-labeled chylomicron remnants. Bile samples were collected at 15-min intervals for 90 min after intravenous administration of [^{3}H]cholesterol-labeled chylomicrons to control rats (●) or rats injected with 17α ethinyl estradiol (▲). (A) Cumulative secretion; (B) secretion in each 15-min sample. Each point is the mean from 3 (control) or 4 (estrogen-treated) rats, and error bars show the SEM.

labeled unesterified cholesterol from the chylomicrons is catabolized by this route. Thus, the disappearance of radioactivity from the blood and its appearance in the bile in the experiments described here can be taken to represent the fate of both the unesterified and esterified cholesterol carried in the administered chylomicrons. The results indicate that removal of chylomicron cholesterol from the circulation, its uptake by the liver, and secretion into bile in rats is delayed by a pharmacological dose of estrogen. This is the first demonstration using an *in vivo* model showing that dietary cholesterol is taken up by the liver and eliminated from the body at a slower rate in rats treated with estrogen as compared to control animals. Previous work from our laboratory has shown that, despite the decreased bile flow in rats treated with 17α ethinyl estradiol, the biliary output of both cholesterol and bile acid mass is not significantly changed (25). Thus, the decreased elimination of chylomicron cholesterol *via* the bile is a specific effect, and is not due to a general lowering of biliary lipid output.

Clearance of chylomicron cholesterol tended to be slower in estrogen-treated rats both in animals with and without biliary drainage, and the effect was highly significant in the latter case (Fig. 2A). In rats with biliary drainage, however, because of the relatively high variability in the results obtained with individual animals, the difference did not reach statistical significance (Fig. 2B). Recovery of radioactivity in the liver was also significantly lower in rats without biliary drainage, and the magnitude of this change cannot be accounted for by the difference in the amount of label remaining in the blood estrogen-treated and control rats after 30 min. The reason for this discrepancy is not clear; however, we have found evidence to suggest that uptake by non-hepatic tissues was enhanced in the former group. Recovery of radioactivity in the testicles, for example, was increased about 8.5-fold, from 0.034% of the administered label in control rats to 0.29% in the estrogen-treated group. Clearly, this only represents a small proportion of the radioactivity not accounted for in the liver and plasma, but it is possible that similar changes also occur in other non-hepatic tissues. In addition, chylomicron remnants have been shown to be taken up by the artery wall (35), and in a different study we have found that uptake by macrophages is increased by estrogen (unpublished results). It is likely, therefore, that tissues other than the liver take up a greater proportion of chylomicron cholesterol in estrogen-treated as compared to control animals, and this may explain the discrepancy in the total amount of label recovered in the liver and the blood in the two experimental groups.

Few previous studies with animal models have examined the effects of estrogen on the hepatic uptake of chylomicrons, although a number of investigations, often reporting conflicting results, have used estrogen treatment to try to clarify the role of the LDL receptor in the process. Using a rat liver perfusion model, Arbeeny and Rifici (36) found that the removal of chylomicron remnants from the perfusate was unaffected by estradiol treatment, while that of very low density lipoprotein (VLDL) remnants was increased 2-fold, suggesting that the two types of remnants are cleared by different mechanisms. In contrast, Wade *et al.* (37) found a marked increase in the binding of chylomicron remnants *in vitro* to rat liver membranes from estrogen-treated as compared to control rats, while Szanto *et al.* (33) have suggested from the results of their experiments with rats *in vivo* that estradiol treatment increases chylomicron remnant clearance. This conclusion, however, was based on differences in the cholesterol levels in the $d < 1.006$ g/ml plasma fraction, which contains VLDL as well as chylomicrons and chylomicron remnants. It is possible, therefore, that the observed decrease in cholesterol in triacylglycerol-rich lipoproteins was due to a change in VLDL rather than chylomicron remnant levels.

The decreased rate of removal of chylomicron cholesterol from the blood observed in estrogen-treated animals in our experiments could be caused, not by a lower rate of uptake by the tissues but by expansion of the plasma pool size of cholesterol-chylomicrons/chylomicron remnants causing a dilution of the label, or by increased competition for lipoprotein lipase (LPL)-mediated chylomicron triacylglycerol hydrolysis or for receptor-mediated uptake, because of increased endogenous VLDL levels. It is difficult to measure cholesterol concentrations in chylomicrons/chylomicron remnants specifically, because of the technical problems of separating them from VLDL. However, these lipoproteins are cleared from the blood very rapidly in the rat, and circulating levels are usually very low (38). Chylomicron production is also low in animals kept on a low-fat diet, as in our experiments. It is unlikely, therefore, that there would be sufficient chylomicron cholesterol in the blood in the estrogen-treated rats to cause significant dilution of the administered label. Furthermore, total cholesterol levels in the $d < 1.006$ g/ml fraction were not increased by estrogen treatment, and plasma triacylglycerols, which are found mainly in the $d < 1.006$ g/ml fraction (39), tended to be decreased rather than increased (Table I). These data indicate that endogenous VLDL levels are not raised by estrogen.

It is clear that estrogen treatment up-regulates hepatic LDL receptor activity (8–10), but the present findings, obtained from direct studies on the rate of removal of labeled chylomicron cholesterol in an *in vivo* model in the rat, indicate that this is accompanied by decreased rather than increased uptake of chylomicron cholesterol by the liver. This is consistent with the findings of Eriksson *et al.* (40) whose studies on the metabolism of lipoprotein remnants in humans showed that the clearance of chylomicron remnants is not affected by variation in the expression of LDL receptors. The hepatic uptake of chylomicron remnants by the LDL receptor or the LRP is mediated by apoE, although evidence from recent work has indicated that the process also involves ligands such as LPL and hepatic lipase (HL) (13, 41–44). LPL also has an important role in the conversion of chylomicrons to chylomicron remnants (38, 44). The

findings of the present study demonstrate that the apoE content of the plasma lipoproteins was markedly reduced in the estrogen-treated as compared to the control rats (Fig. 1), and this is consistent with previous work (45). As circulating chylomicron remnants obtain most of their apoE by transfer from HDL (46), these results suggest that the reduction in the rate of clearance of chylomicron cholesterol caused by estrogen may be due to decreased apoE levels in the particles. However, slower conversion of chylomicrons to chylomicron remnants because of decreased LPL activity and/or a reduction in HL activity, which has been shown to be brought about by estrogen (47), may also contribute to the effect. In contrast to estrogen, progestins have been found to increase the level of HL (48) but to have no influence on the activity of LPL (49) and plasma levels of apoE (50). Thus, with progestins increased hepatic chylomicron remnant uptake may result, and this might explain why combined female hormone therapy is characterized by an enhanced removal of both LDL and chylomicron remnants, leading to the beneficial effects of estrogen replacement therapy (2). This idea is supported by the findings of Khokha *et al.* (51) who reported that the progestin, *d*-norgestrel, increases the removal of very low density lipoprotein (VLDL) remnants from the blood in rats.

Contraceptive steroids are known to cause cholestasis in women, and this is thought to be responsible for the increased risk of the development of cholesterol gallstone disease associated with oral contraceptives (52, 53). Rats treated with 17α ethinyl estradiol at dose levels comparable to those used in the present investigation have also been shown to become cholestatic (54, 55), and it has been suggested that this model is suitable for the study of the events associated with intrahepatic cholestasis caused by contraceptive steroids in humans (54). In previous studies, we have found that a decreased rate of clearance of chylomicron cholesterol from the blood is usually associated with a decreased rate of excretion *via* the bile (23, 56). In the current work, however, although we found a delay in the appearance of label from chylomicron cholesterol in the bile (in the form of both bile acids and cholesterol) in estrogen-treated as compared to control rats; the total amount of radioactivity recovered in the liver after 90 min was not changed. Thus, after estrogen-treatment there appears to be a similar amount of cholesterol delivered by chylomicrons within the liver, but less secreted into bile, suggesting that the hormone influences the intrahepatic processes responsible for the secretion of cholesterol of dietary origin into bile, leading to a delay in its removal from the body.

Although a number of previous studies in humans have suggested that the removal of chylomicron particles from the blood is increased by both exogenous and endogenous estrogen (18–20), these experiments were unable to address the uptake of chylomicron cholesterol by the liver and its excretion in the bile, and the assumption that estrogen treatment leads to increased catabolism *via* these pathways (21) appears to be based on extrapolation of its effects on the metabolism of other lipoproteins. The present work clearly demonstrates that the elimination of dietary cholesterol from the body in the bile in the rat is retarded by estrogen, and that this is likely to be mainly due to a marked reduction in the apoE content of chylomicrons. Because of the differences in lipoprotein metabolism between the rat and the human, and the high pharmacological dose of estrogen used in our experiments, we cannot assume that these findings apply to humans. Nevertheless, they suggest that the hypothesis that estrogen increases the hepatic uptake of chylomicron cholesterol and its excretion in the bile during contraceptive and hormone replacement therapy should be re-examined.

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