Estrogen Increases Low-Density Lipoprotein Receptor–Independent Catabolism of Apolipoprotein B in Hyperlipidemic Rabbits

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Estrogen has been reported to increase the catabolism of low-density lipoprotein (LDL) apolipoprotein (apo) B by increasing LDL receptor activity. To determine the effect of estrogen on LDL receptor–independent pathways, paired turnover studies of native LDL and chemically modified LDL (methyl-LDL) were performed before and during estrogen administration in female New Zealand rabbits consuming a diet containing 0.5% (wt/wt) cholesterol. Rabbits were matched by plasma cholesterol concentration and assigned randomly to receive estrogen (estradiol cypionate 0.5 mg/kg/wk) or placebo. The residence time of both the native LDL apo B tracer and the methyl-LDL apo B tracer in plasma was decreased by estrogen but not by placebo. Multicompartmental modeling of the paired, double-labeled turnover studies indicated that an increase in fractional catabolic rate (FCR) of the fast-turnover pool, a kinetically distinct LDL subpopulation in plasma, accounted for the observed decrease in residence time in plasma for both tracers. These data support the hypothesis that, in addition to any effect on the LDL receptor, estrogen promotes the activity of LDL receptor–independent pathways.

THE SEX DIFFERENTIAL in cardiovascular disease, the leading cause of death in all Western societies, may be partially attributable to modulation of lipoprotein metabolism by estrogen across age.[1,2] Estrogens in pharmacologic doses decrease low-density lipoprotein (LDL) cholesterol concentration and increase the catabolism of autologous radiolabeled native LDL apolipoprotein (apo) B.[3,4] LDLs isolated from men treated with estrogen during hormonal treatment of prostatic carcinoma have reduced LDL receptor affinity in tissue-culture studies, which is hypothesized to be the consequence of increased expression of hepatic LDL receptors in vivo. This hypothesis is strengthened by the observation that pharmacologic doses of estrogen increase the concentration of hepatic mRNA for the LDL receptor in rabbits.[5] Thus, the pharmacological effects of estrogen are hypothesized to be mediated through an estrogen-induced increase in hepatic LDL receptor activity.[5,6] The effect of estrogen on alternative pathways of LDL catabolism, independent of that mediated by the LDL receptor, remains largely unexplored.

Previously, Goebel et al[7] and Berman,[8] using simultaneous analysis of plasma and urine radioactivity data, proposed a model of LDL apo B catabolism in humans in which two kinetically distinct LDL subpopulations, a rapid-turnover pool and a slow-turnover pool, were identified in plasma. The present study was undertaken to determine whether kinetically distinct LDL subpopulations exist in cholesterol-fed New Zealand white rabbits, and to examine the effect of estrogen treatment on the fractional catabolic rate (FCR) of these subpopulations of LDL apo B. Further, this study estimated the contribution of LDL receptor–independent pathways to the increase in LDL catabolism induced by estrogen, by means of paired turnover studies using native LDL and chemically modified LDL (reductive methylation) performed in cholesterol-fed rabbits before and during estrogen treatment.

MATERIALS AND METHODS

The study was conducted in 10 adult, sexually intact, female New Zealand white rabbits (Hazelton Research Products, Denver, PA). The animals' weights were monitored, and blood samples were drawn each week to monitor lipoprotein concentration and composition. Ketamine (40 mg/kg) was administered intramuscularly as an anesthetic before all blood collections, for restraint. All procedures involving animals were conducted in compliance with state and federal laws, standards of the Department of Health and Human Services, and guidelines established by the Animal Care and Use Committee of Bowman-Gray School of Medicine.

Diet

The rabbits were fed rabbit chow (Prolab High Fiber Rabbit Formula; Agway Country Foods, Syracuse, NY) for the first 3 months after they were purchased. They were then fed an atherogenic diet for 3 months before and during this study. For the atherogenic diet, cholesterol was dissolved in warm corn oil and added to rabbit chow to a final concentration of 0.5% (wt/wt) cholesterol. To control for factors that determine individual variability in response to the atherogenic diet, the animals were matched by total plasma cholesterol concentration after 3 months on the diet and assigned randomly to the estrogen treatment group (n = 5) or the control group (n = 5).

Treatment

Animals assigned to the estrogen treatment group received an intramuscular injection of estradiol cypionate (0.5 mg/kg/wk) each week. Animals assigned to the control group received an intramuscular injection of placebo (2 mL normal saline) each week during the treatment period.

Study Design

LDL and modified-LDL turnover studies were performed during the baseline period and after 6 weeks of treatment in rabbits selected randomly from the control group (n = 3) and the estrogen treatment group (n = 3).

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Lipoprotein Analysis

All blood samples for lipoprotein analysis were collected on the day before the weekly intramuscular administration of estradiol cypionate. Venous blood samples were collected into vacutainer tubes (Becton Dickinson, Rutherford, NJ) containing dry sodium EDTA (1.0 mg/mL blood) from the central vein of the ear of animals fasted overnight (16 hours). Blood was immediately centrifuged at 1,500 × *g* for 30 minutes at 4°C, and the plasma was separated. Plasma was separated by sequential ultracentrifugation into standard density classes[9]: very-low-density lipoprotein (VLDL) as d < 1.006 g/mL, intermediate-density lipoprotein (IDL) as d = 1.006 to 1.019, LDL as d = 1.019 to 1.063, and high-density lipoprotein (HDL) as d = 1.063 to 1.21. Plasma and lipoproteins isolated from sequential ultracentrifugation were characterized by enzymatic assay of cholesterol and triglycerides (Boehringer Mannheim Diagnostics, Indianapolis, IN). Apo B concentration was determined by isopropanol precipitation of apo B from isolated lipoprotein of density 1.019 to 1.063 g/mL as previously described.[10-12]

Lipoprotein Turnover Procedure

To eliminate the effect of estrogen administration on the composition of the LDL tracer used during the turnover studies, pooled LDL was isolated by preparative ultracentrifugation (d = 1.019 to 1.063 g/mL) from the plasma of donor New Zealand rabbits that were not treated with estrogen but were fed an atherogenic diet identical to that eaten by the animals in this study. The donor rabbits were purchased at the same time and from the same breeder as the subjects of this study.

The isolated, pooled LDL was dialyzed against saline (d = 1.006 g/mL) containing EDTA (0.001 mol/L, pH 7.4) to remove potassium bromide. The LDL was then divided into two portions: one was radiolabeled with ^{125}I and the other with ^{131}I by the iodine monochloride method of McFarlane[13] as modified by Bilheimer et al.[14] The free iodide was removed by passing the labeled lipoproteins through a column of Sephadex G-25 followed by dialysis against saline (0.15 mol/L) containing EDTA (0.001 mol/L, pH 7.4) at 4°C with four changes. ^{131}I-LDL was then modified by reductive methylation with formaldehyde plus sodium borohydride using a 60-minute reaction sequence as described by Weisgraber et al.[15] The methylated LDL was further dialyzed against saline (0.15 mol/L) containing EDTA (0.001 mol/L, pH 7.4) at 4°C with four changes. Methylated LDL was characterized by the trinitrobenzenesulfonic acid method, which indicated that greater than 90% of the free-amino groups were modified. Before injection, 91% to 93% of tracer radioactivity precipitated with apo B.[11,12] Two percent to 3% of the total radioactivity of the tracers was lipid-extractable. The tracer preparation was completed and the first turnover studies started within 96 hours after blood collection from the donor rabbits. Two turnover studies were started each day for 3 consecutive days.

The rabbits were given potassium iodide in their drinking water for 3 days before and during the turnover studies to avoid uptake of ^{125}I or ^{131}I by the thyroid. In each study, 12 μCi ^{125}I-LDL and 12 μCi ^{131}I-methyl-LDL were simultaneously injected into the marginal vein of the ear in fasted animals. There was no evidence of extravasation of the tracer during injection. Radioactivity remaining in the tube, syringe, and needle was measured to calculate the actual administered dose. A portion of the same preparation of ^{125}I native LDL and ^{131}I methyl-LDL was injected into each animal studied. Animals were fasted 16 hours before each study and overnight before the 24-, 48-, 72-, and 96-hour samples. Blood samples (3 mL) were drawn at 3 and 10 minutes and 1, 4, 10, 24, 36, 48, 72, and 96 hours from the central vein of the contralateral ear. A 1-mL sample of plasma was counted (Iso-Data Gamma Counter; Iso-Data, Rolling Meadows, IL) with ratio correction for double-label counting to determine plasma radioactivity before and after isopropanol precipitation of apo B–containing lipoprotein to determine apo B–specific count. The final apo B radioactivity count was corrected for isotope decay. Total cholesterol concentration remained constant in the 3-minute and 24-, 48-, 72-, and 96-hour plasma samples, indicating that the subjects were in steady state during the turnover study. All urine was collected and pooled for each rabbit every 12 hours for the first 48 hours of the turnover studies, and the volume and radioactivity were measured. The plasma volume of each rabbit was estimated by the Evans blue dilution method 1 week before the turnover study.[16] Turnover studies were completed twice: at baseline and during the treatment phase of this study.

Kinetic Analysis

Analysis of the apo B–specific plasma counts and urine radioactivity from the paired turnover studies was completed using the SAAM-30 and CONSAM program.[17] The model used to simultaneously describe plasma and urine turnover data is shown in Fig 1 and has been previously applied to LDL apo B kinetic data in humans by Goebel et al[7] and Berman[8] and in baboons by Kushwaha et al.[4] This model is characterized by two plasma compartments of LDL, a rapid-turnover pool, C(3), and a slow-turnover pool, C(1), the sum of which yields the initial LDL apo B radioactivity. All of the injected radiolabeled LDL and methyl-LDL, represented by the triangle in Fig 1, is distributed between these two pools, C(3) and C(1). Simultaneous analysis of plasma and urine radioactivity data is used to characterize the independent contribution of fast and slow catabolic pathways to the total catabolism of LDL apo B. The slow-turnover pool, C(1), exchanges

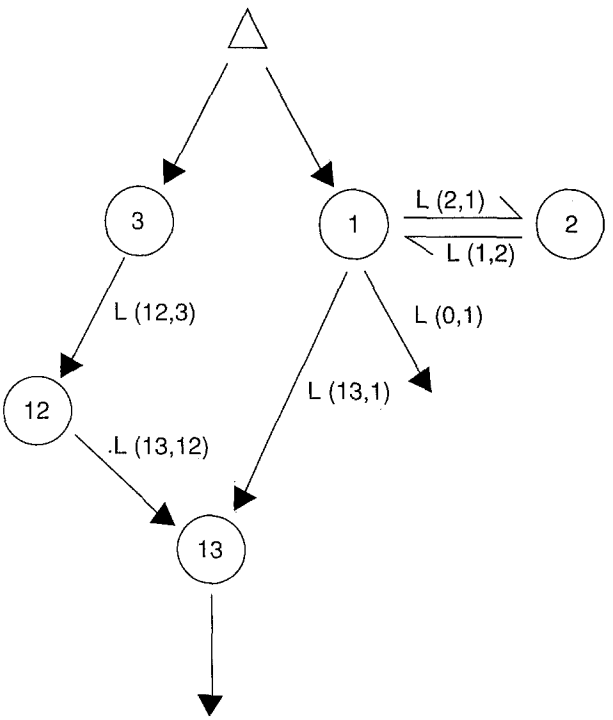

Fig 1. Model used for analysis of native LDL apo B and methyl-LDL apo B kinetic data.

with an extravascular compartment, C(2). The rate constants, L(i,j) (where i and j refer to the compartments), describe the movement of LDL apo B from C(j) to C(i). The model includes a brief delay compartment, C(12), between the rapid-turnover pool, C(3), and the body iodide compartment, C(13). The body iodide pool is assumed to turn over at a rate of 2.5 pools/d.[18] L(0,1) is the rate constant for the removal of plasma radioactivity from the slow-turnover pool that could not be accounted for in urine. This pathway has been previously hypothesized to represent an additional extravascular compartment or incomplete urine collection.[4,8] The value L(0,1) in this study is similar to previously reported values in humans and baboons.

Representative fits of the plasma radioactivity data are shown in Fig 2 for LDL apo B and for methyl-LDL apo B at baseline and during estrogen treatment. Radioactivity is expressed as the percentage of total injected dose of each radioisotope per milliliter of plasma at each sample point in the plasma die-away curves. Representative fits of the appearance of radioactivity in urine in the same rabbit are shown in Fig 3.

Statistical Methods

All values for plasma concentrations are the mean ± SD for each group. Kushwaha and Hazzard[19] have previously shown that the maximal response to this dose of estradiol cypionate in cholesterol-fed rabbits occurs after 6 weeks of treatment. The statistical significance of changes from baseline to 6 weeks of placebo or estrogen administration was calculated using the paired *t* test. The statistical significance of comparisons between the control group and estrogen treatment group was calculated using Student's *t* test.

RESULTS

Five rabbits in the control group and five in the estrogen treatment group completed the study protocol and were included in the analysis. The assignment of individual animals in the estrogen treatment group or the control group was made to establish groups with equal mean ± SD values for plasma cholesterol based on response to the atherogenic diet before initiating estrogen and placebo administration. There was no significant difference in lipoprotein profile (total plasma, VLDL, LDL, or HDL cholesterol and triglyceride concentrations) between groups after randomization (Table 1).

Effect of Estrogen Treatment on Lipoprotein Concentration

The effect of placebo and estrogen administration on the lipoprotein profile of the rabbits is shown in Table 1. In the control group, there was a significant increase in mean total plasma cholesterol concentration versus baseline values during placebo administration ($P = .038$), with an increase in mean apo B concentration ($P = .033$) and an increase in mean LDL cholesterol concentration that approached statistical significance ($P = .09$) during placebo administration. In the estrogen-treated group, there was a significant decrease in mean total plasma cholesterol during estrogen treatment ($P = .038$). This decrease in mean total cholesterol concentration in the estrogen-treated group was entirely accounted for by a decrease in mean VLDL cholesterol during estrogen treatment ($P = .005$). There was an increase in mean apo B concentration ($P = .045$) but no change in mean LDL cholesterol concentration during estrogen treatment. After 6 weeks of treatment, mean cholesterol concentrations were lower in whole plasma and

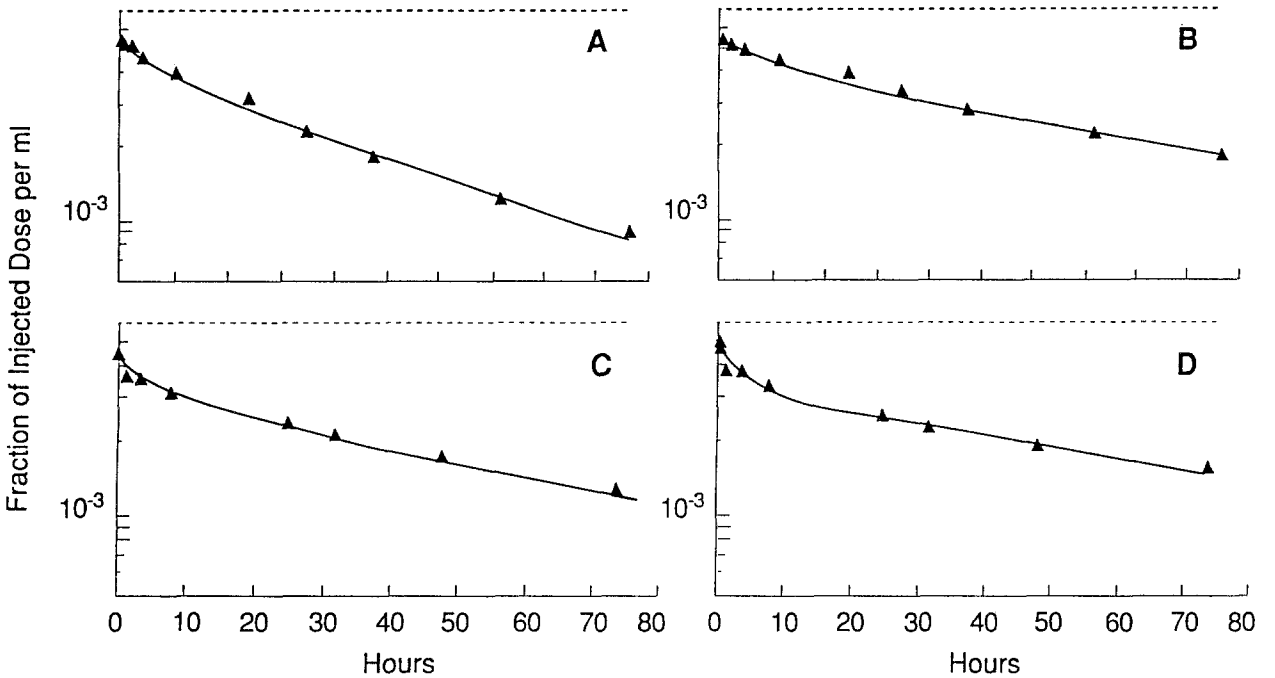

Fig 2. Fit of plasma radioactivity data for LDL apo B at baseline (A) and during estrogen treatment (C) and for methyl-LDL apo B at baseline (B) and during estrogen treatment (D) in a representative cholesterol-fed rabbit. (———) Computer-predicted values; (▲) experimental data expressed as the percentage of total injected dose per milliliter of plasma. Fits were produced from the simultaneous analysis of plasma and urine radioactivity. The fit of urine radioactivity data for the representative rabbit is shown in Fig 3.

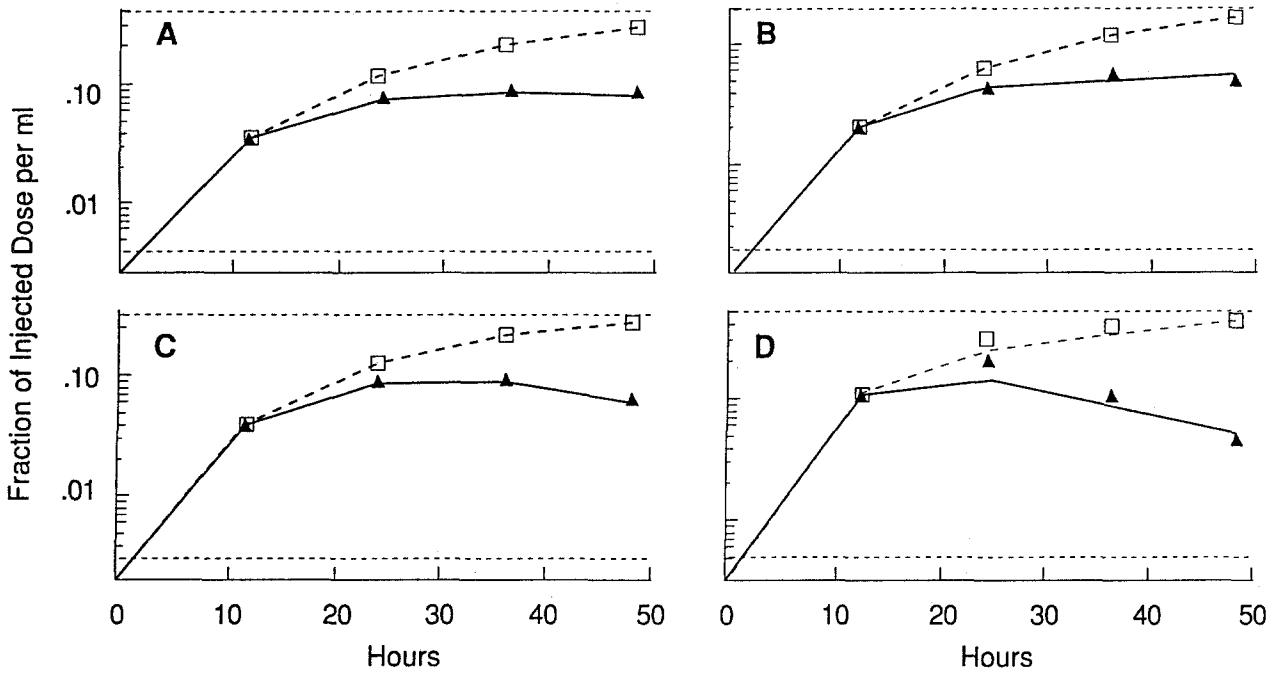

Fig 3. Appearance of radioactivity in urine after injection for LDL apo B at baseline (A) and during estrogen treatment (C) and for methyl-LDL apo B at baseline (B) and during estrogen treatment (D) in a representative cholesterol-fed rabbit. (———) Computer-predicted values; (▲, □) experimental data. Fits were produced from the simultaneous analysis of plasma and urine radioactivity to characterize the independent contribution of fast and slow catabolic pathways. Cumulative data (□) and count at each point (▲) are plotted.

in VLDL in the estrogen treatment group versus the control group (Table 1).

In the estrogen-treated group, mean total triglyceride concentration ($P = .038$) and mean VLDL triglyceride concentration ($P = .015$) decreased during estrogen treatment. There was no significant change in mean triglyceride concentration in whole plasma or any lipoprotein fraction in the control group when comparing baseline concentrations with values obtained after 6 weeks of placebo administration. After 6 weeks of treatment, mean total and VLDL triglyceride concentrations were lower in the estrogen treatment group compared with the control group (Table 1).

Table 1. Influence of Estrogen Treatment on Lipoprotein Profile in New Zealand White Rabbits

Lipoprotein Profile (mmol/L)	Baseline	Placebo	Baseline	Estrogen
Cholesterol				
Total	24.7 ± 5.0	35.3 ± 8.8*	25.8 ± 7.2	18.7 ± 4.1*†
VLDL	13.6 ± 3.9	16.3 ± 4.1	14.6 ± 5.4	7.0 ± 2.7*†
LDL	9.4 ± 2.6	14.7 ± 5.0	9.6 ± 1.0	9.6 ± 2.1
HDL	0.8 ± 0.15	0.66 ± 0.16	0.67 ± 0.08	0.67 ± 0.10
Triglyceride				
Total	1.7 ± 0.5	1.9 ± 0.9	1.6 ± 0.6	0.68 ± 0.1*†
VLDL	0.9 ± 0.2	1.1 ± 0.6	0.98 ± 0.4	0.30 ± 0.04*†
LDL	0.6 ± 0.3	0.7 ± 0.4	0.46 ± 0.2	0.3 ± 0.06
HDL	0.1 ± 0.02	0.1 ± 0.01	0.12 ± 0.02	0.1 ± 0.005
Apo B	3.2 ± 0.8	5.0 ± 1.5*	3.2 ± 0.9	3.8 ± 0.5*

NOTE. Data are the mean ± SD for rabbits on an atherogenic diet at baseline and after 6 weeks of placebo or estrogen administration. No difference was found when comparing baseline parameters between groups.

*$P < .05$ *v* baseline.

†$P < .05$ *v* control.

Effect of Estrogen Treatment on LDL Apo B Metabolism

Figure 4 shows plasma die-away curves of LDL and methyl-LDL in a representative rabbit during the baseline period. As observed by others,[20] LDL receptor–dependent transport is reduced by dietary suppression in the cholesterol-fed rabbit. During the baseline study, 59.8% ± 11.9%

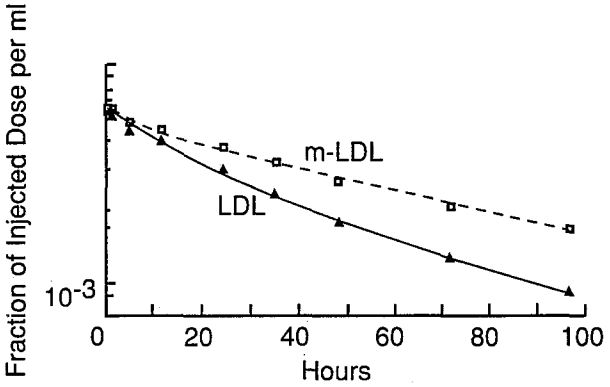

Fig 4. Fit of plasma radioactivity data for native LDL apo B (▲) and methyl-LDL apo B (□) after simultaneous intravenous injection of ^{125}I-LDL and ^{131}I methyl-LDL during the baseline period in a representative cholesterol-fed rabbit. (———) Computer-predicted values; (▲, □) experimental data expressed as the percentage of total injected dose per milliliter of plasma. Fits were produced from the simultaneous analysis of plasma and urine radioactivity for native LDL and methyl-LDL at baseline.

of the total FCR of LDL apo B was attributable to LDL receptor–independent processes. The contribution of the LDL receptor to the total FCR of LDL was decreased from the 66% reported in chow-fed rabbits[14] to 40% in these cholesterol-fed rabbits. There was no difference in the estimated residence time of the native LDL tracer and methyl-LDL tracer in the control group compared with the estrogen-treated group at baseline.

As described previously for native LDL apo B in humans and baboons,[4,7,8] the analysis indicated kinetic heterogeneity for the native LDL apo B tracer in rabbits. In addition, the analysis indicated kinetic heterogeneity for the methyl-LDL apo B tracer. In this study, the tracers were derived from pooled LDL from donor rabbits consuming the experimental diet and not treated with estrogen. Because distribution of the tracer to the fast and slow pools is determined by the attributes of the tracer, the distribution should be the same in all animals receiving each tracer preparation. For all turnover studies, the best fit of the native LDL and methyl-LDL turnover data was provided with 21% of the initial tracer radioactivity distributed to the fast pool and 79% to the slow pool.

The control rabbits did not receive estrogen. One of the purposes of including control rabbits in this study was to verify that the injected radiolabeled lipoproteins were not denatured and that there was no significant change in LDL metabolism between baseline and the second turnover study, due to the passage of time or other uncontrolled factors. Plasma die-away curves for LDL and methyl-LDL were identical in control rabbits when comparing the turnover study during the baseline period with the turnover study during placebo administration. There was no change in the estimated residence time of native LDL tracer and methyl-LDL tracer in the control group with placebo administration. Figure 5 compares the plasma data and best fit for the native LDL tracer and the methyl-LDL tracer in a representative rabbit from the control group.

In contrast, during estrogen treatment, a more rapid removal of both native LDL tracer and methyl-LDL tracer was observed during the initial component of the biexponential die-away curve, compared with the baseline turnover study. The estimated residence time of the native LDL tracer decreased from 47.5 ± 4.9 hours at baseline to 30.7 ± 1.5 hours during estrogen treatment ($P = .005$). Figure 6A compares the plasma data and best fit of the native LDL tracer at baseline and during estrogen treatment in a representative rabbit from the estrogen treatment group. The estimated residence time of the methyl-LDL tracer decreased from 88.6 ± 18.2 hours at baseline to 15.1 ± 3.8 hours during estrogen treatment ($P = .005$). Figure 6B compares the plasma data and best fit of the methyl-LDL tracer at baseline and during estrogen treatment in the same rabbit.

There was no significant difference in mean rate constants for the LDL apo B kinetic model (Table 2) comparing the control group with the estrogen-treated group at the time of randomization, and no significant change in the rate constants in the control group with placebo administration. The decrease in residence time of both the native LDL apo

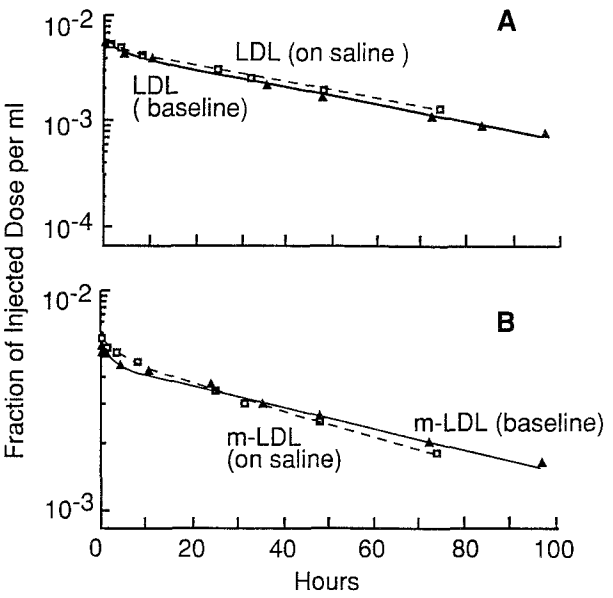

Fig 5. Fit of plasma radioactivity data for native LDL apo B (A) and methyl-LDL (m-LDL) apo B (B) at baseline (▲) and during placebo administration (□) in a representative cholesterol-fed rabbit. (———) Computer-predicted values; (▲, □) experimental data expressed as the percentage of total injected dose per milliliter of plasma. Fits were produced from the simultaneous analysis of plasma and urine radioactivity.

B tracer and the methyl-LDL apo B tracer during estrogen administration was entirely accounted for in the model by an increase in the mean rate constant for the rapid-turnover pool. L(12,3), the rate constant of the rapid-turnover pool,

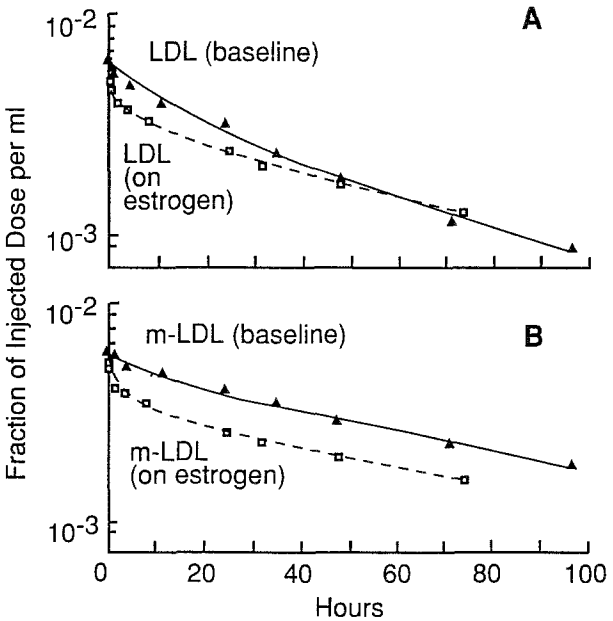

Fig 6. Fit of plasma radioactivity data for native LDL apo B (A) and methyl-LDL apo B (B) at baseline (▲) and during estrogen administration (□) in a representative cholesterol-fed rabbit. (———) Computer-predicted values; (▲, □) experimental data expressed as the percentage of total injected dose per milliliter of plasma. Fits were produced from the simultaneous analysis of plasma and urine radioactivity.

Table 2. Rate Constants for LDL Apo B Model 1

Group	L(0, 1)	L(13, 1)	L(1, 2)	L(2, 1)	(12, 3)	L(13, 12)
Estrogen-treated						
LDL						
Baseline	.006 ± .002	.010 ± .001	.373 ± .008	.093 ± .019	.041 ± .001	.038 ± .017
Estrogen	.008 ± .002	.004 ± .001*†	.672 ± .568	.150 ± .125	.104 ± .005*†	.076 ± .004*†
m-LDL						
Baseline	.002 ± .001	.006 ± .001	.168 ± .172	.030 ± .039	.029 ± .005	.019 ± .003
Estrogen	.002 ± .002	.007 ± .001	.145 ± .186	.028 ± .040	.278 ± .072*†	.212 ± .048*†
Controls						
LDL						
Baseline	.006 ± .002	.010 ± .006	.518 ± .462	.097 ± .089	.048 ± .008	.024 ± .004
Placebo	.004 ± .003	.011 ± .001	.412 ± .511	.081 ± .099	.047 ± .002	.020 ± .007
m-LDL						
Baseline	.003 ± .001	.006 ± .001	.037 ± .026	.007 ± .004	.029 ± .005	.020 ± .007
Placebo	.005 ± .002	.006 ± .001	.139 ± .023	.037 ± .006	.029 ± .004	.022 ± .004

NOTE. Twenty-one percent of injected radioactivity was distributed to initial condition compartment 3, IC(3), and 79% IC(1). IC(3) = 1 − IC(1). Data are the mean ± SD rate constants from the simultaneous analysis of native LDL and m-LDL at baseline and after 6 weeks of estrogen or placebo administration (n = 3 per group). Units are pools per hour. No difference was found when comparing parameters at baseline between groups, and no difference was found when comparing parameters in the control group at baseline and during placebo administration.

*$P < .05$ *v* baseline.

†$P < .024$ *v* control group.

and L(13,12), the rate constant of the delay compartment between the rapid-turnover pool and the body iodide compartment, were increased for both the native LDL and methyl-LDL during estrogen treatment compared with the baseline study and with the control group.

DISCUSSION

Estrogen administration decreased total plasma cholesterol concentration in this group of cholesterol-fed female New Zealand white rabbits. The decrease in total cholesterol concentration was entirely accounted for by a decrease in VLDL cholesterol with no significant change in the mean concentration of IDL, LDL, and HDL. As observed by others, estrogen increased the catabolism of native LDL tracer. In addition, estrogen increased the rate of removal of methyl-LDL tracer from plasma. This observation is believed to be the first to suggest that LDL receptor–independent clearance of LDL is increased by estrogen. Chemically modified LDL, in this case methyl-LDL, in which the receptor-recognition site in apo B has been blocked, is catabolized only by LDL receptor–independent pathways.[15,21-23] The estrogen-induced decrease in residence time of both the native LDL and the methyl-LDL tracer was entirely accounted for in the kinetic model by an increase in the FCR of the rapid-turnover pool, a kinetically distinct LDL subpopulation in plasma.

The increased catabolism of native and methyl-LDL apo B induced by estrogen was not due to differences in the tracer preparation. Because native LDL is both biochemically and kinetically heterogeneous,[24-32] even when great care is taken to standardize the conditions under which LDL is modified, heterogeneous particles are produced[15,22,33] and there is variability in products formed from preparation to preparation. One method for standardizing between preparations of tracers is to administer identical lipoprotein tracer preparations to untreated control subjects. By this criterion, there was no evidence that the change in catabolism of native LDL tracer and methyl-LDL tracer with estrogen treatment was due to differences in the tracer preparation.

There may be important differences in LDL isolated from estrogen-treated rabbits versus untreated rabbits attributable to an estrogen-induced decrease in apo E and apo B concentrations,[34-36] which may directly influence the catabolism of tracer LDL.[7,13,37] To control for potential estrogen-induced changes in LDL composition in this study, the tracers were derived from pooled LDL from donor rabbits who were not treated with estrogen, and therefore, during the turnover studies, control native and methyl-LDL are being traced. Thus, comparison of tracer catabolism during baseline versus the treatment phase can be made while reducing the confounding effect of changes in the tracer used in the two studies.

Little is known regarding the components of LDL receptor–independent pathways, in part due to the methodological shortcomings that complicate the precise determination of the contribution of LDL receptor–independent pathways to total LDL catabolism. One method to estimate the contribution of the LDL receptor and the LDL receptor–independent pathways to the total FCR of LDL is to determine the difference between the FCR of native LDL and chemically modified LDL.[15,21,22] This method depends on the assumptions that the chemical modification is stable, that it completely blocks catabolism by the LDL receptor, and that it has no effect on the interaction of the tracer with all other pathways.[22] New properties may be acquired by the tracer during modification that activate rapid clearance of the tracer by Kupffer cells, as seen following acetoacetylation of LDL.[38] Methyl-LDL does not bind to the high-affinity LDL receptor, the acetyl-LDL receptor, the scavenger receptor, or other components of the reticuloendothelial system. There was no evidence of accelerated clearance of

the methyl-LDL tracer from plasma during estrogen treatment analogous to the accelerated clearance of acetoacetylate-modified LDL in rats.

Attempts to identify the mechanisms responsible for LDL receptor–independent catabolism have not provided clear answers. Mechanisms that may be responsible for LDL receptor–independent catabolism include adsorptive endocytosis, pinocytosis, and the uptake of modified LDL by high-affinity receptors of the reticuloendothelial system.[22,23,39] The interrelationship of these processes is certain to be complex, and the precise mechanism responsible for the increased catabolism of methyl-LDL during estrogen treatment remains uncertain. As observed by Spady and Dietschy,[20] catabolism of LDL by LDL receptor–independent pathways occurs mainly in parenchymatous cells, with no evidence of increased uptake of methyl-LDL by organs of the reticuloendothelial system.[19] A working hypothesis has been that LDL receptor–independent processes are unregulated. This hypothesis is challenged by the results of the present study, which suggest that estrogen increases the catabolic rate of LDL receptor–independent pathways. Further studies of tissue- and cell-specific uptake of native LDL and methyl-LDL in rabbits before and during estrogen treatment are needed to determine the anatomic components of LDL receptor–independent pathways modulated by estrogen.

The results of the current study appear to be discordant with a previously reported study by Chao et al[40] examining the catabolism of cyclohexanedione (CHD)-modified human LDL in male rats treated with a pharmacological dose of estrogen,[40] however, major differences in the experimental methods confound the comparison of results of these studies. First, the modulation of lipoprotein metabolism by estrogen is affected by the dose, the preparation administered, and the duration of treatment. In the previous experiment, chow-fed rats were treated with ethinyl estradiol for 5 days, which produced a profound hypolipidemia (total cholesterol was 52 ± 4 mg/dL in the control group *v* 5 ± 3 mg/dL in the estradiol-treated group) and weight loss (~5% body weight). Second, in the previous experiment, the contribution of LDL receptor–independent pathways in rats was estimated with CHD-modified human LDL. Because human LDL binds poorly to rat cell receptors, metabolic studies using human LDL in rats should be interpreted cautiously.[41] Third, obstacles remain that confound the comparison of metabolic studies using LDL tracers modified by different chemical methods. Theoretically, both methyl-LDL and CHD-treated LDL should have the same catabolic rate, but in rabbits the FCR of CHD-treated LDL is faster than that of methyl-LDL.[42] All chemically modified LDL tracers prepared to date yield slightly different estimations of the contribution of LDL receptor–independent pathways. At present, there is insufficient evidence to determine the cause of this discrepancy.

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