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Effects of apolipoprotein B-100 on the metabolism of a lipid microemulsion model in rats

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Abstract

In previous studies, it was shown that lipid microemulsions resembling LDL (LDE) but not containing protein, acquire apolipoprotein E when injected into the bloodstream and bind to LDL receptors (LDLR) using this protein as ligand. Aiming to evaluate the effects of apolipoprotein (apo) B-100 on the catabolism of these microemulsions, LDE with incorporated apo B-100 (LDE-apoB) and native LDL, all labeled with radioactive lipids were studied after intraarterial injection into Wistar rats. Plasma decay curves of the labels were determined in samples collected over 10 h and tissue uptake was assayed from organs excised from the animals sacrificed 24 h after injection. LDE-apo B had a fractional clearance rate (FCR) similar to native LDL (0.40 and 0.33, respectively) but both had FCR pronouncedly smaller than LDE (0.56, $P < 0.01$). Liver was the main uptake site for LDE, LDE-apoB, and native LDL, but LDE-apoB and native LDL had lower hepatic uptake rates than LDE. Pre-treatment of the rats with 17α-ethinylestradiol, known to upregulate LDLR, accelerated the removal from plasma of both LDE and LDE-apoB, but the effect was greater upon LDE than LDE-apoB. These differences in metabolic behavior documented in vivo can be interpreted by the lower affinity of LDLR for apo B-100 than for apo E, demonstrated in in vitro studies. Therefore, our study shows in vivo that, in comparison with apo E, apo B is a less efficient ligand to remove lipid particles such as microemulsions or lipoproteins from the intravascular compartment. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Apolipoprotein B-100; Low density lipoprotein; Metabolism; Microemulsion; Plasma kinetics; Estradiol

1. Introduction

LDL is a cholesterol-rich lipoprotein produced in vivo via very-low-density lipoprotein (VLDL) delipidation cascade [1,2]. Apolipoprotein (apo) B-100, the protein moiety of low-density lipoprotein (LDL), plays an important role in the intracellular

Abbreviations: apo, apolipoprotein; FCR, fractional clearance rate; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDE, non-protein microemulsion resembling LDL; LDE-apoB, apo B-associated LDE microemulsion; LDL, low-density lipoprotein; LDLR, LDL receptor; NaDC, sodium deoxycholate; VLDL, very-low-density lipoprotein

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assembly of the precursor VLDL [3,4]. In contrast with the other apolipoproteins, which exchange intensively among lipoprotein particles, apo B-100 remains part of the lipoprotein structure during the catabolic process, until the particles are removed from the bloodstream [2]. In this last step, apo B-100 is the ligand for the cellular recognition and uptake of LDL by the specific LDL receptor (LDLR) on the cell plasma membrane [3,5].

In rats, apo B-100 is synthesized predominantly by the liver, whereas apo B-48, a shorter isoform, is mainly produced by the intestine [3]. The latter actually results from an editing mechanism of the apo B-100 mRNA [6,7]. Apo B-100 is a large molecule with polar and apolar domains allowing both the association of the protein with lipids in LDL particle, as well as the interaction of the lipoprotein with LDLR [8,9]. Previous investigations have used protein-free microemulsions resembling LDL, designated LDE, to study LDL metabolism [10,11]. It has been shown that LDE can be removed from circulation through interaction of the apo E, adsorbed by LDE particles, with LDLR [11,12].

In this study, we have evaluated the influence of apo B-100 on LDL metabolism, by modeling the LDL lipid structure with LDE. LDE labeled with radioactive lipid analogues and associated with apo B-100 (LDE-apoB) was injected into the bloodstream of control rats and rats pre-treated with 17α-ethinylestradiol. Administration of this hormone is known to increase LDLR activity [13]. Following injection, plasma kinetics and organ uptake of the LDE-apoB were determined and compared with the metabolic parameters of the LDE and native LDL.

2. Materials and methods

2.1. Animals and treatments

Male Wistar rats, weighing 250–350 g, were fed on a standard commercial chow, with free access to food and water. One group of rats was injected subcutaneously a daily dose of 17α-ethinylestradiol (5 mg/kg body wt.) for 5 days before the experiment [13]. Control rats received 0.5 ml of propyleneglycol in equivalent periods.

2.2. Preparation of LDE

LDE was prepared from lipid mixture composed of 33% cholesteryl oleate (Nu Check Prep, Elysian, USA), 66% egg phosphatidylcholine (Lipid Products, Surrey, UK) and 1% glyceryl trioleate (Nu Check Prep), and labeled with 5 μCi of [^{3}H]phosphatidylcholine, [^{3}H]glyceryl trioleate, and cholesteryl [^{14}C]-oleyl ester or [^{3}H]cholesteryl oleyl ether (Amersham, UK). Emulsification of the lipids and microemulsion purification was carried out as previously described [11,12]. Briefly, dried lipids were emulsified by sonication in 10 ml of 10 mM Tris-HCl (pH 8), 100 mM KCl for 3 h under N_2 stream at 53°C, using a Branson B-450 Cell Disrupter (Arruda Ultrassom, São Paulo, Brazil) equipped with 1 cm microtip at 125 W with continuous output. After sonication, the crude microemulsion was centrifuged at $155\,000\times g$ ($r_{av.}$ 11.3 cm) for 30 min at 4°C using a Hitachi RBP 40T rotor (Hitachi Centrifuge 70P-72, Tokyo, Japan). The 1 ml upper layer was removed by aspiration. The density of the remainder was adjusted to 1.22 g/ml with KBr and centrifuged for 2 h under identical conditions. The microemulsion particles were recovered from a top layer (20% of total volume) by aspiration and dialyzed overnight against standard buffer. LDE was either dialyzed against 10 mM M Tris-HCl (pH 8) buffer for use in the animal experiments or associated with apo B, as described below.

2.3. Isolation of native LDL and apo B

Plasma was obtained from freshly drawn blood from Wistar male rats weighing 250–350 g. Samples were kept in a solution containing 2.5 mM Na_2-EDTA, 2 mM benzymidine, 7.7 mM phenylmethylsulfonyl fluoride and 1 mM NaN_3. LDL was isolated by sequential ultracentrifugation [14] using a Beckman 40 Ti rotor (Beckman Centrifuge L50-E, Palo Alto USA), at $105\,000\times g$ ($r_{av.}$ 5.9 cm) for 24 h at 10°C. Part of the LDL fraction was used for isolation of apo B, and the remaining volume was labeled with [^{3}H]cholesteryl oleyl ether, as previously described [15], and used in the clearance studies.

LDL fraction was dialyzed against standard buffer (50 mM Na_2CO_3, pH 10, 50 mM NaCl, 1 mM Na_2-

EDTA, 0.15 mM NaN_3) and solubilized with sodium deoxycholate (NaDC) [16]. Following solubilization, apo B-100 was separated from the lipids by gel chromatography on Sepharose CL-4B with the standard buffer containing 10 mM NaDC. Column fractions containing apo B protein were pooled and concentrated to 1 mg of protein/ml by ultrafiltration, using Amicon YM-10 filters (Amicon, Lexington, USA) and stored at 4°C.

2.4. Association of apo B with the LDE

NaDC-solubilized apo B was added to LDE (1 mg apo B protein to 4 mg LDE cholesteryl oleate) at a very slow rate (0.5 ml/h) with continuous dialysis against standard buffer, allowing apo B binding to the microemulsion particles [17]. Removal of NaDC was performed by dialysis over a 24 h period at 4°C and monitored using Na[^{14}C]DC (Amersham, UK). Apo B-associated LDE (LDE-apoB) was diluted in standard buffer, then its density was adjusted to 1.22 g/ml with KBr and centrifuged at $155\,000 \times g$ ($r_{av.}$ 11.3 cm) for 2 h at 4°C using a Hitachi RPS 40T rotor. The LDE-apoB particles were recovered from the 2 ml upper layer and dialyzed against 10 mM Tris-HCl (pH 8) buffer.

2.5. Clearance and tissue uptake in rats

Labeled LDE and LDE-apoB and native LDL were separately injected into the bloodstream of three groups of intact rats to determinate plasma clearance and tissue uptake rates. In tissue uptake studies, cholesteryl oleyl ether was used to trace particles because this lipid is not hydrolyzed by lysosomal enzymes, accumulating in cells, being an appropriated tracer of cumulative tissue uptake of particles [18].

Non-fasted male Wistar rats weighing 250–300 g were anesthetized with diethyl ether and a saline-filled polyethylene cannula was inserted through the carotid artery [11]. Clotting was prevented by pretreating the cannula with silicon. The animals were allowed to recover from anesthesia for 2 h in individual cages. Then, radioactively labeled LDE, LDE-apoB or native LDL were injected intraarterially in a volume 0.3 ml with 8×10^3 Bq. After injection, 0.3 ml blood samples were taken into heparinized plastic tubes at 5 and 30 min, and 1, 2, 4, 7, and 10 h. After 24 h the animals were killed by air embolization and organs and tissues (liver, adrenals, spleen, lungs, heart, kidneys, intestine, and adipose and skeletal muscle tissues) were excised and carefully rinsed in ice-cold 150 mM NaCl solution to remove blood as completely as possible without perfusion.

Lipids from plasma and tissue aliquots were extracted with chloroform/methanol (2:1, v/v) [19] and lipid classes were separated by thin layer chromatography in the solvent system hexane/diethyl ether/acetic acid (70:30:1, v/v). Bands corresponding to cholesteryl ester, triacylglycerol, and phospholipid were scraped separately into vials for radioactivity measurement in scintillation solution (PPO/POPOP/Triton X-100/toluene, 5 g/0.5 g/333 ml/667 ml) using a LKB 1211/1212 spectrometer (Wallac, Finland). Plasma clearance kinetics (fractional clearance rate, FCR) of the different isotopes were estimated from bi-exponential curves fitted by least-squares according to Matthews [20]. Total mass of muscle and adipose tissue was calculated according to Caster et al. [21].

2.6. LDE and LDE-apoB density profile

Two hundred µl of cholesteryl ester-labeled LDE and LDE-apoB were completed to 3 ml with 10 mM Tris-HCl (pH 8.0) buffer and adjusted to a 1.101 g/ml density with solid KBr. Solutions were layered with 2.0 ml NaCl solutions of 1.065, 1.050, 1.020 g/ml densities, respectively, and with 1.5 ml of 1.006 g/ml density. Tubes were centrifuged at $192\,000$ g ($r_{av.}$ 11.3 cm) for 24 h at 4°C in a Hitachi RBP 40 T rotor. One to 2 ml aliquots from gradient containing tubes were recovered by aspiration and used for radioactivity measurement and protein content determination (A_{280}).

2.7. Incubation of the microemulsion with rat high-density lipoprotein (HDL)

Rat HDL was isolated from plasma by preparative ultracentrifugation [14]. HDL fraction was dialyzed against 10 mM Tris-HCl (pH 8.0) buffer. LDE and LDE-apoB containing 1 mg of total lipid were incubated with 5 mg of HDL protein for 2 h in a shaking water bath at 37°C. The incubated solution was ad-

Table 1
Chemical composition of LDE and LDE-apoB microemulsions

Constituent	Composition (% wt.)	
	LDE (6)	LDE-apoB (5)
Total cholesterol	31.7 ± 4.2	26.2 ± 8.9
Phospholipid	66.3 ± 4.4	64.2 ± 9.2
Triacylglycerol	1.9 ± 0.4	1.6 ± 0.8
Total protein	–	7.9 ± 1.9
TC/PL	0.96	0.82
PT/PL	–	0.28

Composition is expressed as percentage of total weight of each constituent of the microemulsion. The molar ratio of total cholesterol/phospholipid (TC/PL) and total protein/phospholipid (PT/PL) is also shown. Results are means ± S.D. for the number of rats in parentheses.

justed to 1.05 g/ml density with KBr and centrifuged at 192 000 × g ($r_{av.}$ 11.3 cm) for 24 h at 4°C using a Hitachi RBP 40 T rotor. LDE and LDE-apoB particles were recovered from 1 ml top layer and dialyzed against 10 mM Tris-HCl (pH 8) buffer.

2.8. Analytical methods

Lipid composition of microemulsion was determined by standard methods [22–24]. Total protein from preparations was measured according to Markwell et al. [25]. Serum cholesterol, triacylglycerols and phospholipids were measured by the methods of Siedel et al. [26], Wahlefeld [27] and Bartlett

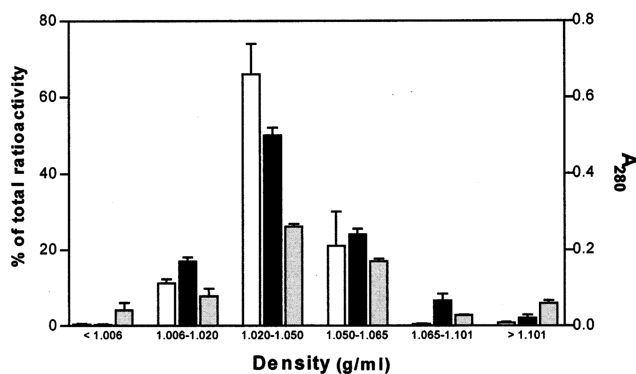

Fig. 1. Effect of apo B-100 on the density distribution profile of LDE and LDE-apoB. Microemulsions labeled with [^{3}H]cholesteryl oleyl ester were submitted to a density gradient ultracentrifugation, and radioactivity of each fraction was determined. Data represent LDE (□) and LDE-apoB (■) radioactivity (% of total) in each gradient fraction. Protein (▩) content of LDE-apoB was determined as A_{280}.

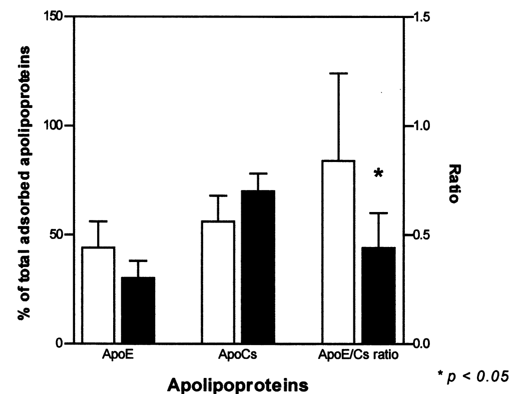

Fig. 2. Association of apo E and apo Cs with LDE or LDE-apoB after incubation with rat plasma HDL. Adsorbed apolipoproteins were separated by SDS-PAGE on 10% gels. Apo E and apo Cs contents were determined by densitometric analysis as % of total proteins incorporated by microemulsion particles. Results are means ± S.D. from three independent experiments with LDE (□) and LDE-apoB (■) microemulsions.

[24], respectively. LDL, apo B, and HDL preparations, as well as apolipoproteins associated with microemulsion particles, were analyzed by SDS-PAGE [28] performed on 8% and 10% polyacrylamide gels.

2.9. Statistical analysis

Differences in FCR and tissue uptake among LDE, LDE-apoB and native LDL were assessed using the one-way ANOVA test. All pairwise multiple comparison procedures were performed by Student-Newman-Keuls method. Student's t-test was used to test differences in lipid composition and the cholesterol ester distribution profile between LDE and LDE-apoB. Two-tailed P-values below 0.05 were considered significant.

3. Results

The chemical analysis of the microemulsions showed that lipid composition of LDE-apoB was similar to that of LDE (Table 1). Both LDE and LDE-apoB microemulsions and native LDL presented compositional data comparable to that previously reported [10–12,17,29,30]. Cholesteryl ester (CE) label of both LDE and LDE-apoB presented a similar distribution profile on density gradient ultracentrifugation. About 80% of particles floated be-

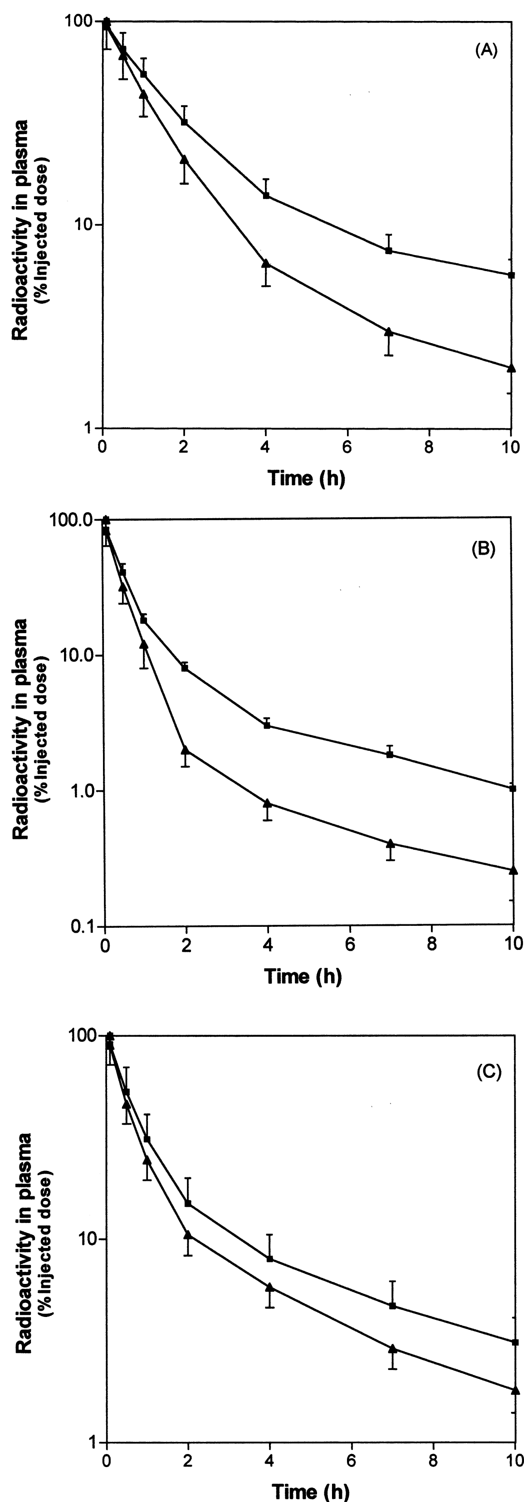

Fig. 3. Removal from plasma of radioactively labeled lipids of the LDE and LDE-apoB microemulsions in rats. Microemulsions were injected into the bloodstream of unanesthetized male Wistar rats. Curves were fitted to the data based on the radioactivity remaining in the plasma in the period of 5 min to 10 h after injection. Results are means ± S.D. from 15 LDE-apoB (■) and 10 LDE (▲) injected rats. (A) Cholesteryl ester, (B) triacylglycerol, and (C) phospholipid.
←

tween 1.020 g/ml and 1.065 g/ml (Fig. 1). Protein content measured as A_{280} of each fraction matched the CE gradient distribution in LDE-apoB.

Both LDE or LDE-apoB incorporated apolipoproteins from HDL fraction in vitro. However, a particular apolipoprotein pattern was observed for each microemulsion. LDE-apoB adsorbed less apo E and more apo Cs than LDE (Fig. 2). Therefore, the apo E/Cs ratio was lower in LDE-apoB compared to that of LDE ($P < 0.05$).

Fig. 3 shows that the decay curves of LDE-apoB radioactive labeled lipids are slower than those of LDE. Table 2 shows accordingly that FCR of the three labels of LDE-apoB is smaller than that of LDE. On the other hand, it is remarkable that the cholesteryl ester FCR of LDE-apoB is similar to that of native LDL.

Liver was the main site of tissue uptake for LDE-apoB, LDE and native LDL particles, as measured 24 h after the injection (Table 3). Liver uptake rate was greatest for LDE and lowest for LDL. In contrast, native LDL had the highest uptake rate in other tissues such as spleen, lungs, and kidneys. Tissue uptake profile of the LDE-apoB was similar to that of LDE, except that of spleen that took up more a LDE-apoB than LDE.

As expected, after estradiol treatment, there was a significant decrease in plasma lipid concentration (data not shown). In estradiol treated rats, removal from plasma of both LDE and LDE-apoB labels was faster than that of controls (Fig. 4). Four hours after injection, only 6% of the injected LDE-apoB remained in the plasma of estradiol-treated rats, compared to 19% in controls. LDE-apoB cholesteryl ester FCR in estradiol-treated rats was approximately two-fold greater than in controls (Table 4). This effect was even greater when LDE was injected: in this case, removal was three-fold greater compared to control animals (Table 4, Fig. 4). As expected from the results described above, tissue uptake of the LDE-apoB lipid tracer was markedly enhanced in estrogen-treated animals (Table 3).

Table 2
Plasma removal of LDE, LDE-apoB and native LDL labeled with radioactive lipids injected into unanesthetized rats

Injected material	Fractional clearance rate (FCR, h^{-1})		
	Cholesteryl ester	Triacylglycerol	Phospholipid
LDE (10)	0.56 ± 0.13[a]	1.16 ± 0.26	0.81 ± 0.16
LDE-apoB (15)	0.40 ± 0.08	0.89 ± 0.12	0.64 ± 0.19
Native LDL (4)	0.33 ± 0.09	–	–
P	0.0009	0.0026	0.0307

Fractional clearance rates (FCR) were calculated using bi-exponential curves fitted to the data based on the radioactivity remaining in the plasma in the period of 5 min to 10 h after injection. Results are means ± S.D. for the number of rats in parentheses.
[a]Different from LDE-apoB and native LDL, $P < 0.05$.

4. Discussion

The lipid structure of LDL can be artificially modeled by very well defined procedures [10,11,31,32]. These protein-free microemulsion models mimic the lipid physical behavior of native LDL. In previous studies, we had demonstrated that the metabolism of LDE had some similarities with the metabolism of endogenous LDL in rats [11] and in human subjects [12]. We have hypothesized that in the plasma compartment, LDE is capable of assimilating apolipoproteins from the plasma lipoproteins, similarly to triglyceride-rich emulsions [33,34]. In vitro experiments have confirmed that LDE is able to incorporate various exchangeable apolipoproteins, mainly apo E, donated by HDL [11,12]. These apo E molecules modulate the LDE metabolism, allowing the recognition and uptake of the microemulsion particles by LDLR on cell surface. Dependence of LDE removal on LDLR was also demonstrated by an increased LDE clearance in patients with acute myelogenous leukemia, where LDLR of neoplastic cells are upregulated [35].

Phospholipid-cholesterol ester-apoprotein B complexes resembling human LDL have been proposed as a model to study the molecular organization and interactions, and metabolism of the LDL [17,29,36,37]. In vitro experiments have demonstrated that the reconstituted LDL (r-LDL) was able to bind to LDLR in human fibroblasts with the same affinity as the native LDL [36].

The present experiments were designed to observe in the rat the plasma kinetics and tissue uptake of the LDE associated to murine apo B-100 (LDE-apoB).

Table 3
Tissue uptake of the [^{3}H]cholesterol oleyl ether of injected LDE, LDE-apoB, and native LDL into control or estradiol-treated rats

Tissues	Tissue uptake (% of injected dose)			
	Control			Estradiol
	LDE (10)	LDE-apoB (8)	Native LDL (3)	LDE-apoB (5)
Liver	48.64 ± 5.05	43.34 ± 4.78	39.35 ± 9.19[a]	50.20 ± 2.17[b]
Spleen	0.95 ± 0.31	1.38 ± 0.30[a]	2.54 ± 0.44[a,b]	2.50 ± 0.37[b]
Adrenal	0.58 ± 0.21	0.55 ± 0.07	0.25 ± 0.09[a,b]	1.42 ± 0.51[b]
Intestine	0.53 ± 0.20	0.54 ± 0.24	0.88 ± 0.41	0.44 ± 0.11
Lung	0.23 ± 0.10	0.26 ± 0.07	1.01 ± 0.40[a]	0.43 ± 0.11[b]
Kidney	0.20 ± 0.12	0.25 ± 0.07	0.43 ± 0.16[a,b]	0.28 ± 0.05
Heart	0.15 ± 0.11	0.17 ± 0.06	0.12 ± 0.08	0.37 ± 0.08[b]
Muscle	3.14 ± 1.47	4.14 ± 1.95	4.73 ± 0.40	6.82 ± 1.03[b]
Adipose	2.18 ± 1.32	2.79 ± 0.95	1.61 ± 0.14	3.47 ± 0.84

Estradiol-treated rats were injected subcutaneously with 17α-ethinylestradiol (5 mg/kg body wt.) during 5 days before the experiment. Organs and tissues were excised for lipid extraction and radioactivity measurement 24 h after microemulsion injection. Values are means ± S.D. for the number of rats in parentheses.
[a]Different from LDE in control rats, $P < 0.05$.
[b]Different from LDE-apoB in control rats, $P < 0.05$.

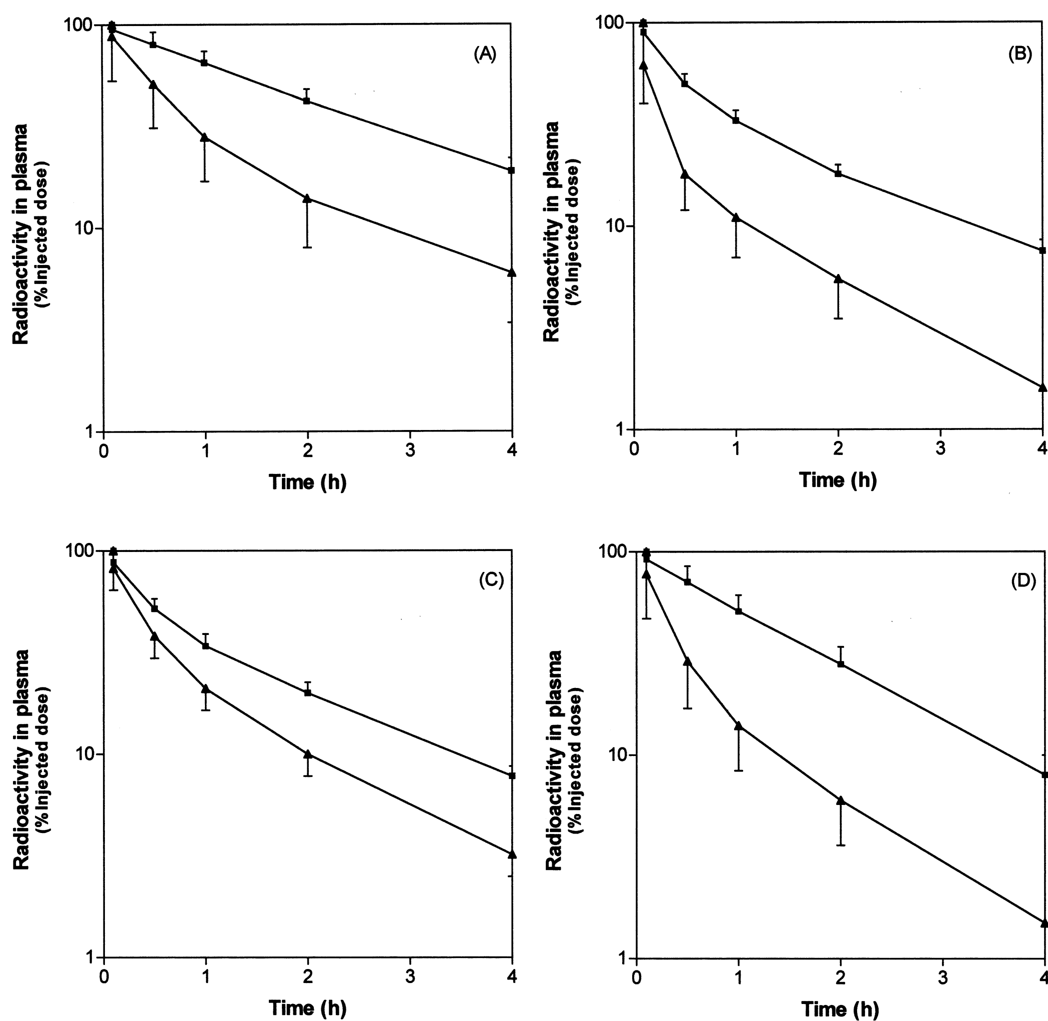

Fig. 4. Effects of estradiol treatment on removal from plasma of radioactively labeled lipids of the LDE and LDE-apoB microemulsions in rats. Male Wistar rats were injected subcutaneously with 17α-ethinylestradiol (5 mg/kg body wt., 5 days) before the experiment. Microemulsions were injected into the bloodstream of unanesthetized rats. Curves were fitted to the data based on the radioactivity remaining in the plasma in the period of 5 min to 4 h after injection. Results are means ± S.D. from three control (■) and seven estradiol-treated (▲) rats. LDE-apoB (A) cholesteryl ester, (B) triacylglycerol, and (C) phospholipid. LDE (D) cholesteryl ester.

When LDE-apoB was injected into the circulation of the animals, the FCR of the cholesteryl ester moiety of the microemulsion resembled that of the injected native LDL, as well as the organ distribution of the radioactive tracer. This strongly suggests that both artificial and natural lipoproteins undergo the same intravascular metabolic events. It is noteworthy that in the rat the cholesteryl ester moiety of the emulsion is a reliable marker of plasma clearance of the microemulsions and native LDL particles because it cannot be selectively removed from the particles. This is due to the virtual absence of cholesterol ester transfer protein (CETP) in this species [38].

The fact that LDE was removed from plasma faster than LDE-apoB and native LDL may be ascribed to the differences in apolipoprotein profile on the particle surface. This conceivably alters the binding characteristics of the particles to the cellular receptors. It is well known that apo B-100 is the only apolipoprotein in native LDL and is the ligand of the lipoprotein particles to LDLR that remove them from plasma circulation [2,3]. Apo B-100 is the main protein of LDE-apoB and native LDL, and is the presumable ligand to the removal sites. In contrast, LDE uptake by the receptor sites is dependent on exchangeable apolipoproteins incorpo-

rated from circulating native lipoproteins, mainly apo E which is the LDE ligand to LDLR (B,E receptor) [11,12].

It has been reported that affinity of LDLR for apo E is several times greater than that of apo B-100 [9]. This property is due to multiple binding sites for apo E molecules present in LDLR. Indeed, the LDLR affinity correlates well with the number of active apo E molecules in the apo E-rich lipoproteins. When one active apo E molecule is present, the affinity of the particle by LDLR is equivalent to the apo B-100-containing LDL [39]. Lipid microemulsions with adsorbed apo E also exhibit higher capability of binding to LDLR in fibroblasts than native LDL [40,41]. Moreover, we have demonstrated that LDE is able to displace LDL uptake by mononuclear cells [12].

The finding that FCR of radiolabeled lipids from LDE was higher than LDE-apoB can be, therefore, attributed to higher affinity of the LDLR by apo E, which is more abundant in LDE as shown in experiments carried out in vitro. It is noteworthy that both LDE and LDE-apoB adsorbed mainly apo E and apo Cs when they were incubated with rat HDL. However, LDE-apoB particles assimilated less apo E and more apo Cs than LDE. The differences in apo E/apo Cs ratio are probably due to competition of these apolipoproteins for adsorption on the microemulsion surface as it was observed on a triglyceride-rich emulsion model [42]. It has been observed that apo Cs can inhibit the apo E-dependent recognition of triglyceride-rich lipoproteins by LDLR [43,44]. In VLDL, for example, remnant particles with a high apo Cs content are less efficiently taken up by the liver [45]. Consequently, these particles have greater residence time in plasma circulation and therefore are more easily transformed in LDL. Thus, the lower apo E/apo Cs ratio on LDE-apoB surface reduces the affinity of LDLR by the microemulsion particles, delaying their removal from plasma. According to our results, apo B-100 seems to play an important role on the acquisition balance of apo E and apo Cs by cholesterol-rich microemulsions in vivo, driving the metabolic fate of the recombinant lipoprotein.

An interesting finding in our experiments is the greater trend of LDE to be picked up by the liver than LDE-apoB and native LDL. In contrast, the uptake of the apo-B containing particles was greater in peripheral tissues than that of LDE. Possibly, as the affinity of LDLR for LDE is greater, given the apo E-dependent uptake mechanism, the entry of this microemulsion into the organ that has the major expression of LDLR is facilitated, compared with the apo-B containing particles.

The involvement of LDLR with both LDE and LDE-apoB clearance was confirmed in rats pretreated with 17α-ethinylestradiol that enhances cell LDLR expression [46,47]. This estradiol-induced effect accelerates LDL removal from plasma and reduces plasma lipid concentrations, mainly total cholesterol, by LDLR-dependent and -independent pathways [48]. Increased removal from the plasma and tissue uptake of LDE-apoB observed in estradiol-treated rats strongly suggests that the microemulsion clearance is mediated by the highly expressed LDLR on cells, as in native LDL [49,50]. Nonetheless FCR of the LDE-apoB particles was still lower than that of LDE, supporting the hypoth-

Table 4
Plasma removal of LDE and LDE-apoB labeled with radioactive lipids injected into control or estradiol-treated rats

Groups	Fractional clearance rate (FCR, h^{-1})			
	LDE-apoB			LDE
	Cholesteryl ester	Triacylglycerol	Phospholipid	Cholesteryl ester
Control (3)	0.42 ± 0.06	0.80 ± 0.08	0.67 ± 0.07	0.63 ± 0.12[a]
Estradiol (7)	0.96 ± 0.25	2.25 ± 0.82	1.20 ± 0.26	1.94 ± 0.81[a]
P	0.0067	0.0167	0.0101	0.0167

Rats were injected subcutaneously with 17α-ethinylestradiol (5 mg/kg body wt.) during 5 days before the experiment. Fractional clearance rates (FCR) were calculated using bi-exponential curves fitted to the data based on the radioactivity remaining in the plasma in the period of 5 min to 4 h after injection. Results are means ± S.D. for the number of rats in parentheses.
[a]Different from LDE-apoB, $P < 0.05$.

esis that LDLR preferentially trap apo B-free particles through incorporated apo E, which is highly expressed in estradiol-treated rats [51].

In conclusion, our results show in vivo that the receptor mechanism of plasma clearance is slower when apo B-100 is used as particle ligand in comparison with apo E.

This finding is conceivably related with the greater affinity of LDLR for apo E shown previously in in vitro experiments.

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