Effects of lovastatin therapy on guinea pig low density lipoprotein composition and metabolism

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Abstract Lovastatin therapy is known to induce hepatic low density lipoprotein (LDL) receptor mRNA and LDL receptor activity. Yet, in studies in humans and animals it has been difficult to demonstrate an enhancement of the plasma fractional catabolic rate (FCR) of an injected LDL tracer during lovastatin therapy. One explanation may be that the composition of the LDL tracer may also change during therapy, independently affecting LDL clearance. To test this possibility we fed guinea pigs lovastatin, which led to a decrease in their plasma LDL cholesterol levels. Composition studies showed that LDL isolated from lovastatin-treated guinea pigs was slightly cholesterol-depleted and triglyceride-enriched when compared to LDL isolated from control animals. Several independent lines of investigation documented that a substantial increase in hepatic LDL receptor activity occurred in response to the lovastatin treatment. Consistent with this, when a single LDL tracer was injected into control and lovastatin-treated guinea pigs, the FCR was always more rapid in the lovastatin-treated animals. However, when LDL isolated from lovastatin-treated animals (L-LDL) was simultaneously injected intravenously with LDL isolated from control animals (C-LDL) the FCR of the C-LDL was always more rapid than that of the L-LDL. When one compared the FCR of C-LDL determined in control animals with the FCR of L-LDL determined in lovastatin animals there was no difference. Possible explanations for these paradoxical findings are discussed. ▇▇ These data demonstrate that lovastatin therapy affects both hepatic LDL receptor activity as well as the intrinsic metabolic properties of plasma LDL. —Berglund, L., M. F. Sharkey, R. L. Elam, and J. L. Witztum. Effects of lovastatin therapy on guinea pig low density lipoprotein composition and metabolism. *J. Lipid Res.* 1989. **30:** 1591–1600.

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Plasma low density lipoprotein (LDL) levels generally reflect hepatic LDL-receptor activity. When hepatic LDL-receptor activity is impaired, as in familial hypercholesterolemia, plasma LDL levels are increased (1); in contrast, induction of LDL receptor activity is accompanied by a decrease in plasma LDL levels (2,3). Bile acid sequestrant resins and HMG-CoA reductase inhibitors effectively decrease elevated plasma LDL levels (4–7). Treatment with bile acid sequestrants results in increased hepatic LDL-receptor activity (6–10). Studies from this laboratory have shown that in guinea pigs and humans bile acid sequestrant therapy also changes the metabolic properties of the LDL (10,11). LDL isolated from guinea pigs or humans undergoing resin therapy have altered composition and a decreased ability to bind to the LDL receptor. This effect appears to partially oppose the enhanced hepatic LDL receptor activity (10,11) and independently affects overall LDL clearance rates.

In animal models, treatment with lovastatin, an HMG-CoA reductase inhibitor, results in increased hepatic expression of LDL receptor activity (12). In spite of this, both in animal studies and in humans, it has been difficult to consistently demonstrate an increased fractional catabolic rate (FCR) for LDL during lovastatin treatment (13–16). One possible explanation for this apparent inconsistency might be that the composition of the LDL tracer may also change during this form of treatment, independently affecting LDL clearance. In such studies the FCR of LDL isolated during a control period was determined, and subsequently the FCR of LDL isolated during treatment was measured. Such a protocol may not be able to distinguish effects on LDL clearance due to induction of hepatic receptors and those (possibly in the opposite direction) due to intrinsic changes in LDL composition. In order to investigate whether such a mechanism might be present during lovastatin treatment, we used the guinea pig as an animal model as previously described from our laboratory (10). In the present study we demonstrate that treatment with lovastatin induces hepatic LDL receptor activity and substantially lowers LDL levels in guinea pigs. However, in spite of apparently small changes in LDL composition, LDL isolated from lovastatin-treated animals displayed a reduced plasma clearance rate compared to LDL isolated from control animals.

EXPERIMENTAL PROCEDURES

Animals

Male guinea pigs weighing 0.5–1.0 kg were purchased from Simonsen Laboratories (Gilroy, CA) or Charles River Breeding Labs, Inc. (Wilmington, MA). They were fed either Purina guinea pig chow pellets alone or the same chow with the addition of 0.05% or 0.1% lovastatin by weight. Lovastatin, a kind gift from Dr. A. Alberts, Merck Sharp & Dohme, was pelleted into the Purina guinea pig chow by ICN Nutritional Biochemicals (Cleveland, OH). Animals were fed their respective diets for a minimum period of 14 days prior to all experiments. Preliminary experiments showed that this length of time was required for a maximal effect of lovastatin. The control and lovastatin-treated animals consumed approximately equal amounts of pellets and there was no significant difference in the rate of weight increase between the two groups.

Lipoproteins

In order to investigate the effect of lovastatin on plasma lipoprotein levels, equal aliquots of plasma from control and lovastatin-treated animals were centrifuged at 30,000 rpm for 48 h in a Bechman Ti-50.3 rotor at densities of 1.025 and 1.070 g/ml (10). The top and bottom fractions from each tube were harvested, adjusted to the original plasma volume, and cholesterol content was determined. Aliquots of the bottom fractions obtained from the 1.025-g/ml density spin were further precipitated with heparin Mn^{2+}. The resulting supernatant fractions, i.e., the HDL fractions, were assayed for cholesterol content. The cholesterol values obtained from the heparin-Mn^{2+} supernatants matched the cholesterol content of the bottom fractions obtained from the 1.070-g/ml spin. Furthermore, no lipid-staining material could be detected in the beta-zone when the supernatant fractions resulting from the heparin-Mn^{2+} precipitation were subjected to agarose gel electrophoresis.

The major parts of the plasma samples harvested from control and lovastatin-treated animals were pooled separately and subjected to ultracentrifugation. Guinea pig LDL was isolated in the 1.025–1.070 g/ml density range and during the procedure a "wash" was performed at both the upper and lower densities as previously described from this laboratory (10). To monitor the accuracy of each ultracentrifugal spin, the density of the clear solution below the floating lipoprotein fraction was analyzed in a DMA 45 Digital Density Meter (Anton Paar, Graz, Austria). Following isolation, the LDL fractions were dialyzed against phosphate-buffered saline containing 0.3 mM EDTA (PBS) and filtered through a 0.45-μm filter (Millipore Company, Bedford, MA). The isolated LDL frac-

tions were iodinated with ^{125}I or ^{131}I (Amersham, Co., Chicago, IL) using the iodine monochloride method of McFarlane (17), as modified for lipoproteins by Bilheimer, Eisenberg, and Levy (18). The iodinated samples were dialyzed exhaustively against PBS. Specific activities for the iodinated LDL preparations varied from 300 to 700 cpm/ ng protein and more than 97% of the LDL radioactivity was precipitable by trichloroacetic acid. Radiolabeled LDL samples were subjected to density gradient ultracentrifugation, essentially as described by Teng et al. (19) using a density range of 1.025–1.065 g/ml.

Studies of LDL metabolism

LDL isolated from control guinea pigs (C−LDL) and from lovastatin-fed guinea pigs (L-LDL) were always iodinated in parallel using different isotopes, and a mixture of both LDL preparations was simultaneously injected into both control and lovastatin-fed animals. The procedure previously described for preparation of the LDL mixtures, injection, and blood sampling of the guinea pigs by cardiac puncture was followed in detail (10). Plasma radioactivity was determined in a double-channel gamma spectrometer (LKB 1282 Compugamma, Bromma, Sweden) using appropriate decay corrections. The resulting plasma radioactivity decay was analyzed using an interactive curve peeling program developed by William Beltz and Thomas Carew as previously described (10). "Apparent" fractional catabolic rates (FCR) were calculated in the usual manner based on the area under the plasma decay curve, with the assumption that both C-LDL and L-LDL represented kinetically homogeneous populations of particles.

Analytical methods

Plasma triglyceride and cholesterol levels were analyzed using standard procedures as described for the Lipid Research Clinics (20). Free and total cholesterol levels were also determined enzymatically as described (10) and the amount of cholesteryl ester was calculated as 1.67 × mg of the difference between these two determinations. Protein was analyzed as described by Lowry et al. (21), using bovine serum albumin as standard. Phospholipids were determined using a micromodification of the procedure described by Bartlett (22). Polyacrylamide gel electrophoresis in the presence of SDS was conducted using the Pharmacia Phast gel system (Pharmacia Fine Chemicals, Piscataway, NJ) with 10–15% gradient gels. LDL size was determined by non-SDS gel electrophoresis carried out at 150 V for 18 h using 2–16% Pharmacia gradient gels. Agarose gel electrophoresis was carried out as described (23).

Binding assays

Radioactively labeled guinea pig LDL from control and lovastatin-fed animals was incubated with guinea pig fibro-

blasts, cultured from a biopsy of abdominal skin (10). Prior to incubation with labeled LDL, the cells were incubated with lipoprotein-deficient serum for 24–48 h. Binding and degradation of guinea pig LDL was analyzed as described previously, following incubation for 1 h at 4°C or 5 h at 37°C, respectively (10). Partially purified hepatic plasma membranes were prepared from control and lovastatin-treated animals as described (8,10). The membrane fractions were incubated with labeled control guinea pig LDL and the amount bound per mg membrane protein was determined. The protocol previously described was followed in detail (10), except that in some experiments a higher EDTA concentration (12 mM) was used.

Ligand blotting

Hepatic membrane fractions from control and lovastatin-fed guinea pigs were isolated as described (10) and ligand blotting experiments were done to identify the LDL receptor, as previously described from our laboratory (24). On the day of the experiment, the membrane fractions were resuspended on ice in 0.25 M Tris-maleate buffer, pH 6.0, containing 2 mM $CaCl_2$ amd 1 mM phenylmethylsulfonyl fluoride (PMSF) by flushing 10 times through an 18-gauge needle and 10 times through a 22-gauge needle, followed by sonication for two 10-sec bursts. The protein content was measured and the preparations were diluted to a protein concentration of 5 mg/ml with 25 mM Tris-maleate buffer, pH 6.0, containing 0.16 M NaCl, 2 mM $CaCl_2$, 1 mM PMSF, and 1% Triton X-100 (25). The mixture was centrifuged at 100,000 g for 1 h at 4°C and the resulting supernatants were analyzed for protein. Identical amounts of supernatant protein from control and lovastatin-fed animals were loaded on a 3–10% SDS-polyacrylamide gel. Following electrophoresis, the proteins were transferred to a nitrocellulose membrane using an electroblotter (LKB Nova-Blot Unit, Bromma, Sweden). The nitrocellulose membranes were blocked for 18 h at 4°C with bovine serum albumin (50 mg/ml) in PBS. Thereafter, the membranes were incubated at 23°C for 5 h with blocking buffer containing ^{125}I-labeled rabbit β-VLDL (~5 μg of protein and 1 × 10^6 cpm/ml buffer), in the absence or presence of 10 mM EDTA. β-VLDL was isolated from plasma of cholesterol-fed rabbits by centrifugation at 45,000 rpm for 22 h. Following three washes with PBS containing 5 mg/ml bovine serum albumin, either in the absence or presence of 12 mM EDTA, the nitrocellulose membranes were subjected to autoradiography.

RESULTS

Induction of hepatic LDL-receptor activity

Treatment of hamsters and rabbits with lovastatin has been found to result in induction of LDL-receptor mRNA

(12). To determine whether lovastatin treatment of guinea pigs also induced hepatic LDL receptors, we compared the ability of guinea pig LDL (obtained from control animals) to bind to partially purified hepatic membranes isolated from control and lovastatin-fed animals. Under the conditions used, no difference could be detected in total binding to membranes from either group. However, as shown in **Fig. 1**, EDTA-sensitive binding was increased in membranes isolated from lovastatin-fed animals. Such EDTA-sensitive binding represents classic LDL-receptor binding (8, 26, 27).

To further document the effect of lovastatin therapy on LDL receptor activity, ligand blotting experiments using ^{125}I-labeled β-VLDL were performed. This ligand is known to bind to the LDL receptor protein with high affinity (28). As demonstrated in **Fig. 2**, β-VLDL bound to a 130,000 dalton protein in membrane preparations from control or lovastatin-fed animals, consistent with that previously reported for the LDL-receptor (29). Furthermore, when identical amounts of protein from both control and lovastatin-treated animals were subjected to ligand blotting studies, there was an increased binding of β-VLDL to the 130,000-dalton band in the lovastatin-treated animals, indicating increased LDL-receptor expression.

Effect of lovastatin treatment on plasma lipoprotein levels and LDL composition

Inclusion of lovastatin in the diet (0.05% by weight) for adult, male guinea pigs resulted, in general, in a decrease in total plasma cholesterol levels of 15–25%. However, this decrease differed somewhat from animal to animal. When 0.1% lovastatin was included in the diet, a consistent decrease in total plasma cholesterol of 45% or more was
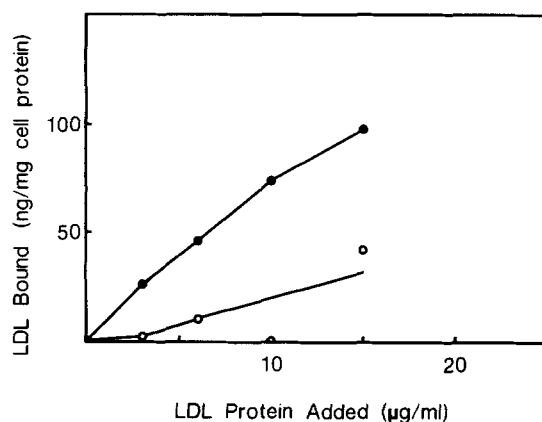

Fig. 1. Binding of ^{125}I-labeled guinea pig LDL to hepatic membranes from control and lovastatin-treated guinea pigs. Partially purified hepatic membranes were prepared from guinea pigs fed control pellets (○) or 0.1% lovastatin pellets (●). Radiolabeled control guinea pig LDL was incubated with membranes for 1 h at 37°C and bound LDL was separated from free LDL by ultracentrifugation (10). Binding was determined in the presence or absence of 12 mM EDTA and the figure shows the amount of EDTA- sensitive binding (i.e., total binding minus binding in the presence of 12 mM EDTA).
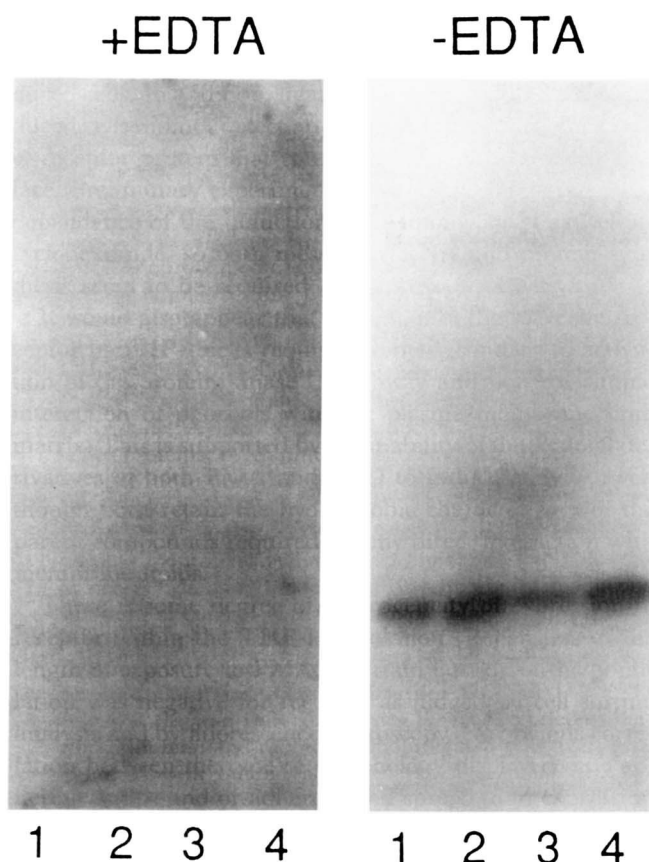
+EDTA   −EDTA

1 2 3 4   1 2 3 4

Fig. 2. Ligand blot of ¹²⁵I-labeled β-VLDL binding to hepatic membrane obtained from control guinea pigs (lanes 1 and 3) or lovastatin-fed guinea pigs (lanes 2 and 4). Equal amounts of soluble proteins (100 μg) were solubilized in Triton X-100, separated on a gradient SDS-polyacrylamide gel, and subsequently blotted onto a nitrocellulose membrane. The filters were then exposed to equal amounts of ¹²⁵I-labeled β-VLDL. The left panel represents binding of radiolabeled β-VLDL in the presence of 12 mM EDTA and the right panel binding in the absence of EDTA. The apparent molecular weight of the radioactively labeled protein band was estimated to be 130,000 using molecular weight markers.

observed. This decrease was accounted for solely by a 58% decrease in LDL cholesterol (density 1.025–1.070 g/ml) levels, which decreased from 28.5 mg/dl to 11.8 mg/dl (**Table 1**). No significant changes were detected in the cholesterol levels of other lipoprotein density classes. In the animals used in these experiments the initial, pretreatment cholesterol levels were consistently lower than that observed by us in previous experiments (10), and in addition varied somewhat between various experiments. This was found to be the case irrespective of the use of different control chows or whether animals were obtained from different sources. In all cases, however, there was a consistent and similar degree of reduction of plasma cholesterol levels in response to treatment with 0.1% lovastatin, irrespective of the initial plasma cholesterol level.

In previous experiments, it was demonstrated that treatment of guinea pigs with a bile acid sequestrant changed the composition of their plasma LDL particles. In the present study, the LDL fraction in the control animals had a considerably lower cholesterol:protein ratio than that reported earlier by us for other populations of guinea pigs (10), presumably reflecting the overall lower cholesterol levels in the animals used. As seen in **Table 2**, the cholesterol:protein ratio in LDL decreased somewhat further during lovastatin treatment. This change was seen in all groups of guinea pigs fed lovastatin, but was quantitatively of a small magnitude. However, a difference was noted for guinea pigs obtained from different sources. The Charles River guinea pigs initially had a higher cholesterol:protein ratio in their LDL and a relatively larger decrease in this ratio in response to treatment with lovastatin than the animals obtained from Simonsen Labs. In all animals, lovastatin treatment reduced the LDL content of both cholesteryl esters and free cholesterol, but the decrease was more pronounced for the latter, resulting in a decrease in the free cholesterol:phospholipid ratio. In addition, small but consistent increases in the triglyceride:protein ratio occurred in all animals. Although the changes in LDL composition that occurred in response to lovastatin treatment were small in magnitude, nevertheless important changes may have occurred in the organization of both the surface and core of the LDL particles. There was also a slight difference in modal densities of C-LDL (1.048 g/ml) and L-LDL (1.054 g/ml) when analyzed by density gradient centrifugation, consistent with the observation that L-LDL was slightly more protein-enriched. No difference in size could be detected between C-LDL and L-LDL using non-SDS-polyacrylamide gel electrophoresis (**Fig. 3**). SDS-PAGE of the LDL preparations indicated that apoB-100 was almost the only apoprotein in both LDL fractions, but no differences were detected in the apoprotein content of the two LDL fractions. When heavily loaded gels from both C-LDL and L-LDL were run, small amounts of a protein with molecular weight of 44,000 were seen, consistent with our previous observations (10). ApoE was not detected in the ultracentrifugally isolated LDL samples (data not shown).

TABLE 1.   Total and lipoprotein cholesterol levels in plasma of control and lovastatin-fed guinea pigs

	Control	Lovastatin-fed
	mg/dl	
Plasma	38.9 ± 6.1	20.7 ± 3.4
d < 1.025 g/ml	6.4 ± 1.9	5.7 ± 1.3
1.025 < d < 1.070 g/ml	28.5 ± 4.3	11.8 ± 3.4
d > 1.070 g/ml	4.1 ± 1.2	3.2 ± 1.4

Cholesterol levels were analyzed from animals representing both control and lovastatin-treated groups. The results are the mean ± SD from four different experiments, each pool made up of three to five animals. The lipoprotein classes were isolated from the individual animals as described in Methods.

TABLE 2. Composition of LDL fractions (d 1.025–1.070 g/ml) isolated from control and lovastatin-treated guinea pigs

	C-LDL	L-LDL	C-LDL	L-LDL
	A	B	C	D
	% of total weight			
Cholesteryl ester	38.1 ± 2.0	37.7 ± 1.1	43.3 ± 1.0	40.9 ± 0.5
Free cholesterol	4.2 ± 0.5	3.1 ± 0.1[a]	5.5 ± 0.8	3.6 ± 1.3[a]
Triglycerides (TG)	8.9 ± 0.8	10.5 ± 0.3[a]	7.0 ± 0.9	10.3 ± 1.8[a]
Phospholipids (PL)	12.8 ± 2.0	12.3 ± 0.7	12.7 ± 1.8	12.5 ± 1.0
Protein	36.0 ± 0.8	36.4 ± 0.7	31.5 ± 1.8	32.7 ± 1.7
Ratios:				
Lipid/protein (w/w)	1.78	1.75	2.18	2.06
TG:protein (w/w)	0.25	0.29	0.22	0.32
Total chol:protein (w/w)	0.76	0.70	1.00	0.86
Esterified chol:prot (w/w)	0.64	0.62	0.83	0.75
Free chol:protein (w/w)	0.12	0.09	0.18	0.11
PL:protein (w/w)	0.36	0.34	0.40	0.38
Esterified chol/free chol (mol/mol)	5.42	7.31	4.73	6.82
Free chol/PL (mol/mol)	0.64	0.49	0.84	0.56

Column A and column B represent LDL preparations isolated from guinea pigs obtained from Simonsen Labs; and columns C and D represent LDL preparations from guinea pigs purchased from Charles River Labs. Each value represents the mean ± SD of values obtained from three separate pools of LDL, and each pool was made up of LDL from pooled plasma of six animals.

[a]Analysis of variance indicated a significant change in triglyceride and free cholesterol content ($P < 0.05$) in L-LDL versus C-LDL, irrespective of the source of guinea pigs.

Interaction of C-LDL and L-LDL with fibroblasts

Both binding and degradation of C-LDL and L-LDL to guinea pig fibroblasts were determined in five different experiments using three different preparations of both LDL fractions. In none of these experiments were we able to demonstrate any consistent difference between degradation of C-LDL versus L-LDL in the guinea pig fibroblasts. Similarly, repeated experiments failed to reveal any consistent differences in experiments in which LDL binding to fibroblasts were performed. Because these techniques might not be sensitive enough to detect subtle compositional differences that may nevertheless have important metabolic consequences in vivo (10), we also compared the in vivo metabolic properties of C-LDL and L-LDL.

Metabolic studies of LDL

In these experiments we addressed two different aspects of LDL metabolism. The first issue concerned the effect of lovastatin treatment on the metabolic pathways of the treated guinea pigs; i.e., did enhanced hepatic LDL-receptor activity result in increased clearance of an injected LDL tracer. The second issue addressed was the question of whether the changes in LDL composition induced by lovastatin, as described above, would independently influence the intrinsic metabolic property of the LDL; i.e., would L-LDL have the same plasma disappearance rate as C-LDL when studied in the same animal.

In an initial experiment a single LDL tracer, derived from a control animal, was labeled and injected into three control and three lovastatin-fed animals. The clearance

of the injected LDL tracer was considerably faster in the lovastatin-treated animals compared to control animals (0.155 ± 0.029 pools/h vs. 0.093 ± 0.011 pools/h, mean ± SD), consistent with the induction of hepatic LDL-receptor activity demonstrated above.
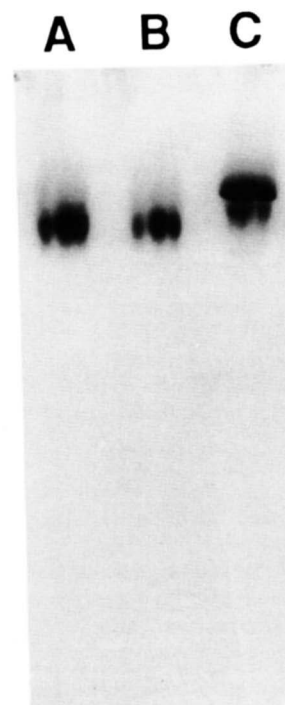

Fig. 3. Non-SDS polyacrylamide gel electrophoresis of isolated LDL fractions: (A) control guinea pig LDL; (B) lovastatin guinea pig LDL; (C) control human LDL.

Having established that the induced hepatic LDL receptors were capable of effecting enhanced removal of LDL from plasma, we next addressed the issue of whether L-LDL had a different plasma removal rate than C-LDL. We followed the approach previously used in our studies of cholestyramine in the guinea pig (10). Briefly, C-LDL and L-LDL were iodinated in parallel using either ^{125}I or ^{131}I. A mixture of the two iodinated samples was then simultaneously injected into control and lovastatin-treated animals, and the plasma decay rates of C-LDL and L-LDL were compared. A typical experiment is shown in **Fig. 4**. Consistent with the earlier experiment noted above, the FCR for the C-LDL tracer was always faster in the lovastatin-treated animals than when the same tracer was injected into the control animals (**Table 3**). This was also true when L-LDL was used as tracer in both groups of guinea pigs. However, in each individual animal the clearance of L-LDL was always slower than that of the simultaneously injected C-LDL, in both control as well as lovastatin-fed guinea pigs (Fig. 4 and Table 3). In a second experiment, in which different C-LDL and L-LDL preparations were used, again

Fig. 4. Plasma decay curves of LDL isolated from control animals (C-LDL) (O) and lovastatin-treated animals (L-LDL) (△) simultaneously injected into a control guinea pig (top panel) and a lovastatin-treated guinea pig (bottom panel). The same mixture of control-LDL and lovastatin-LDL was injected into both guinea pigs. Similar results were obtained with two other pairs of control and lovastatin-treated guinea pigs.

TABLE 3. Comparison of fractional catabolic rate (FCR) of C-LDL and L-LDL injected into control and lovastatin-fed guinea pigs

| | Fractional Catabolic Rate | |
	C-LDL	L-LDL
	pools/h	
Experiment 1		
Control (n = 3)	0.140 ± 0.008	0.106 ± 0.008
Lovastatin-fed (n = 3)	0.172 ± 0.008	0.134 ± 0.006
Experiment 2		
Control (n = 3)	0.099 ± 0.026	0.078 ± 0.024
Lovastatin-fed (n = 3)	0.155 ± 0.021	0.117 ± 0.010

FCR values are the mean ± SD from all animals in the respective groups. Two different preparations of C-LDL and L-LDL were prepared, labeled using different isotopes, mixed, and simultaneously injected into the same animals, either control or lovastatin-fed guinea pigs. Examination of experiment 1 by analysis of variance indicated a significant effect of treatment, vertical comparisons, ($P < 0.0001$) as well as of type of LDL, horizontal comparisons ($P < 0.0001$); for experiment 2, there was also a significant effect of treatment ($P < 0.005$) and also of LDL type ($P < 0.04$).

similar findings were made (Table 3). Thus in every animal tested, the L-LDL had a slower clearance than the simultaneously injected C-LDL. However, lovastatin-treated animals always cleared a given tracer faster than did control animals.

The FCRs from both experiments, involving six control and six lovastatin-fed animals, are summarized in **Table 4**. The horizontal values compare the FCR of C-LDL and L-LDL in the same animals. The mean decrease in FCR for L-LDL compared to C-LDL was very similar in both control and lovastatin-fed guinea pigs (24% vs 23%). The vertical columns in Table 4 show the clearance of each tracer injected into either control or lovastatin-treated animals. In both cases, treatment with lovastatin was found to increase the LDL clearance 37%.

Control studies

In the present experiments the LDL fractions were labeled with either ^{125}I or ^{131}I. Previously no significant isotope effect had been detected (10,11), but we investigated this again in the present study as LDL was affected differently by the present perturbation. Separate aliquots of C-LDL (and L-LDL) were each labeled with both ^{125}I and ^{131}I, respectively. Mixtures of ^{125}I-labeled C-LDL and ^{131}I-labeled L-LDL, as well as ^{125}I-labeled L-LDL and ^{131}I-labeled C-LDL and mixtures of the same type of LDL labeled with both isotopes (e.g., ^{125}I-labeled L-LDL and ^{131}I-labeled L-LDL) were simultaneously injected into control animals. This protocol allowed us to evaluate both the effect of choice of isotope on a chosen LDL fraction as well as allowing a direct comparison of the FCR of C-LDL versus L-LDL. The experiment demonstrated that under the present conditions there was a small but significant increase in plasma clearance rate of a given LDL when it was labeled with
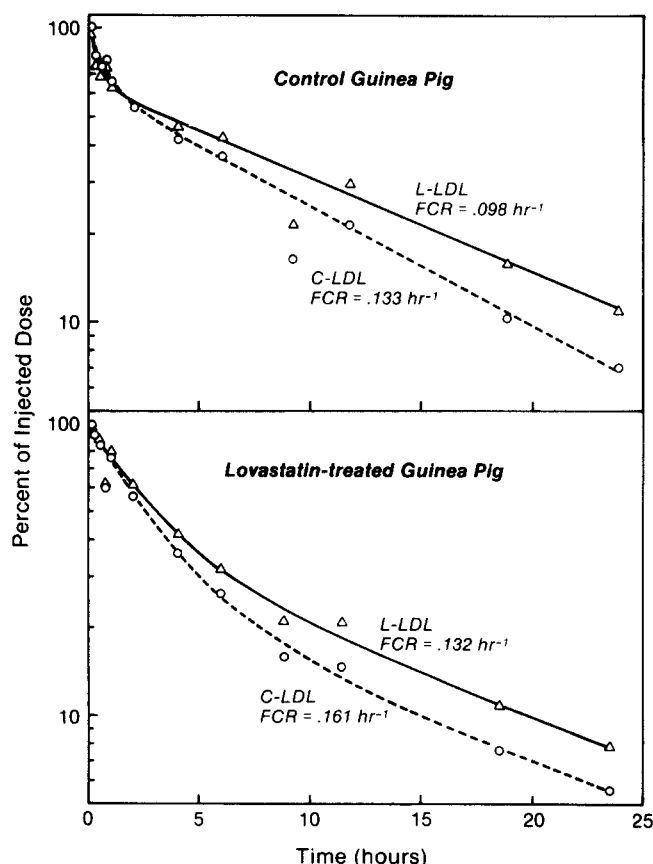
TABLE 4. Analysis of combined fractional catabolic rate (FCR) data from the two experiments shown in Table 3

	C-LDL	L-LDL
Control (n = 6)	0.119 ± 0.022	0.092 ± 0.022
Lovastatin-fed (n = 6)	0.163 ± 0.017	0.126 ± 0.012

The table contains pooled data derived from Table 3. Analysis of variance indicates both a significant effect of treatment (vertical comparisons) and of type of LDL (horizontal comparisons). However, the mean FCR of C-LDL in control guinea pigs was not different from that of L-LDL in lovastatin-treated guinea pigs (P = 0.626 by nonpaired Student's t-test).

[131]I (**Table 5**). However, this effect accounted for only a 10% difference in FCR. When a multivariate analysis was performed, a significant difference in the clearance rate of C-LDL and L-LDL was still apparent even after the tracer effect was accounted for. This finding confirms the results shown above and underscores the fact that the intrinsic metabolic behavior of LDL isolated from lovastatin-treated guinea pigs is different than LDL isolated from control guinea pigs.

DISCUSSION

Three different types of evidence support the conclusion that hepatic LDL-receptor activity increased during lovastatin treatment in guinea pigs. First, there was an increase in EDTA-sensitive binding of LDL to hepatic membranes isolated from lovastatin-treated animals; second, ligand blotting experiments showed increased binding of β-VLDL to a 130 kD protein in membrane preparations from lovastatin-treated animals; and third, in all in vivo experiments a given LDL tracer was always cleared more rapidly in lovastatin-treated animals.

We also observed that, as is the case in some other animal species and humans, lovastatin therapy in guinea pigs reduces LDL levels. However, the reduction in LDL cholesterol levels in the guinea pigs was accompanied by much smaller changes in LDL composition than was seen in previous studies in which LDL levels were lowered to a comparable degree by cholestyramine (10). Compared to C-LDL, the L-LDL had small decreases in relative content of free cholesterol and increases in triglyceride, whereas relative cholesteryl ester content remained virtually unchanged. However, no significant difference in size was detected between C-LDL and L-LDL particles. This was in contrast to the effect that cholestyramine treatment had on LDL composition, where smaller, more dense particles accumulated within the LDL range (10,11). Nevertheless, although small in magnitude, the consistent compositional changes seen in the L-LDL fraction are compatible with a decreased content of free cholesterol in both the surface and core of LDL, important changes that

could lead to altered metabolic properties (D. Small, personal communication). It has also recently been shown that small changes in LDL-triglyceride content affect the interaction of LDL with the LDL receptor (30,31).

Feeding lovastatin to guinea pigs not only induced hepatic LDL-receptor activity but also increased the guinea pigs' ability to clear LDL. This was demonstrated by a marked increase in LDL-FCR in treated animals, compared to control animals, when the same LDL tracer was injected into both groups (Table 4). This is similar to previous reports in dogs and rabbits where a single LDL tracer was also used in control and treated animals (8,32). However, an additional effect occurred, in that the LDL isolated from lovastatin-treated guinea pigs was altered in its in vivo metabolic properties. L-LDL was consistently cleared more slowly than C-LDL when compared in the same animals (Table 3). The absolute degree to which the L-LDL was slower was partially influenced by the procedure used for radiolabeling of the respective LDL fractions (e.g., the choice of radioisotopes). However, even when this was taken into account, there was a significant difference in metabolism between C-LDL and L-LDL when compared in the same animal. Thus, at steady state, LDL isolated from lovastatin-treated animals showed different kinetic properties than control LDL.

In view of these findings it was somewhat surprising that the differences seen in vivo between C-LDL and L-LDL were not detected in the binding or degradation studies performed in cell culture despite repeated measurements using several LDL preparations. This discrepancy between in vivo and in vitro behavior of the same LDL was seen previously even with the LDL isolated from cholestyramine-treated guinea pigs, a situation in which considerable compositional

TABLE 5. Comparison of clearance rates of LDL fractions, labeled with the same isotope

LDL Fraction	Fractional Catabolic Rate	
	Exp. 1 (n = 6)	Exp. 2 (n = 4)
	pools · h⁻¹	
[125]I-Labeled L-LDL	0.121	0.127
[125]I-Labeled C-LDL	0.139	0.143
Difference (%)	15	13
[131]I-Labeled L-LDL	0.141	0.139
[131]I-Labeled C-LDL	0.150	0.158
Difference (%)	6	14

Experiment 1 represents mean values from two sets of guinea pigs, each consisting of three animals. The two sets were injected with identical mixtures of L-LDL and C-LDL, the only difference being that the isotope labeling was switched between the two sets. Thus, in experiment 1, three animals were injected with a mixture of ^{125}I-labeled L-LDL and ^{131}I-labeled C-LDL and three animals were injected with a mixture of ^{131}I-labeled L-LDL and ^{125}I-labeled C-LDL. In experiment 2, mixtures of ^{125}I-labeled L-LDL and ^{131}I-labeled L-LDL and of ^{125}I-labeled C-LDL and ^{131}I-labeled C-LDL were injected into two sets of control guinea pigs, each set consisting of two animals.

changes occurred (10). Several factors may contribute to this apparent difference. First, there may simply be a difference in the ability to detect such differences between the in vitro and in vivo experimental protocols. A conformational change in apoB might have occurred that would be too small to detect in the fibroblast system. For example, when 5% of apoB's lysine residues are glycated, decreased uptake by fibroblasts is not consistently detectable in cell culture, yet such a change reliably decreases the FCR of LDL by 5–20% when injected in vivo in guinea pigs or rabbits (33,34). Alternatively, the explanation may not be methodological, but real. Subtle changes in LDL could occur that would be expressed in differences in in vivo metabolism that would not be seen in vitro. For example, the isolated LDL fractions which were used in the in vitro studies with cultured guinea pig fibroblasts lacked detectable amounts of apoE. In vivo, a portion of LDL probably binds apoE (35). In vivo, L-LDL may have a decreased ability to bind apoE, and consequently, when injected into a recipient animal, may acquire fewer apoE molecules on its surface and consequently have a decreased rate of clearance (35). Thus, the apparent differences between the cell culture and in vivo experiments might not necessarily be in conflict when one considers the different conditions used in these experiments. Further studies are required to explain these findings.

Whatever the explanation, the changes in LDL produced by lovastatin treatment did retard the plasma clearance of L-LDL compared to C-LDL at a given level of LDL-receptor activity. The net effect of this was in large part to counteract the increased potential for hepatic receptor-mediated LDL clearance due to induction of hepatic receptors. Under steady state conditions and using homologous tracers, the FCR of C-LDL in control animals (0.120 ± 0.028 pools/h) was very close to the FCR of L-LDL in lovastatin-treated animals (0.126 ± 0.012 pools/h). The observed decrease in LDL levels in plasma cannot be fully accounted for by the small net increase in LDL clearance observed during lovastatin treatment. This suggests that a decrease in LDL production rate also

occurred. Conventional analysis of these data (36), using the respective homologous tracers under steady state conditions as indices of LDL FCR (i.e., C-LDL in control animals and L-LDL in treated animals), would indicate that the rate of LDL apoB production decreased 49% (**Table 6**). There are several possible factors that might contribute to such a decrease. First, the induction of hepatic LDL-receptor activity could result in an increased removal of IDL and VLDL remnants (37). Second, because of inhibition of cholesterol synthesis, the hepatic excretion of VLDL might be reduced, and/or VLDL of altered composition may be secreted that may have a different metabolic fate. Finally, lovastatin therapy might inhibit the "direct" synthesis of LDL. Huff and coworkers (14) reported that combined lovastatin and cholestyramine treatment of miniature pigs did not result in an enhanced LDL FCR. It is most interesting to note that (similar to our protocol) their protocol used autologous LDL as tracers during the control and treatment periods. They interpreted their results as indicating decreased direct production of LDL. Obviously there is a need to further investigate the effect of lovastatin on the clearance rate of LDL precursors.

The calculations shown in Table 6 that indicate a decreased production of LDL rest on the assumptions that LDL are composed of a kinetically homogeneous population of particles and that the radiolabeled tracer used is representative of the LDL fraction from which it is derived, i.e., it is a true tracee for the entire LDL pool. However, as we (10,11,38–40) and others (41–46) have previously suggested, LDL is not physically or kinetically homogeneous and indeed, a rapidly cleared LDL subfraction might be present that would not be detected by the present techniques. Such a rapidly cleared LDL subfraction, interacting with enhanced hepatic LDL-receptor activity, could account in part for the decreased LDL levels. Finally, it should be noted that both of these two possibilities may be operative, i.e., decreased LDL production and increased clearance of an LDL subfraction may contribute jointly to decreased LDL levels.

TABLE 6. Estimate of LDL turnover parameters in control and lovastatin-treated guinea pigs

Parameter	Control	Treated	% Change
LDL cholesterol pool size (mg)	9.24	3.97	− 57
LDL cholesterol flux (mg × hr^{-1})	1.10	0.50	− 55
FCR (pools × hr^{-1})	0.120	0.126	+ 5
LDL-apoB pool size (mg)	10.24	5.04	− 51
LDL-apoB production rate (mg × hr^{-1})	1.24	0.63	− 49

Data were calculated using the FCR of the C-LDL tracer in control animals and the FCR of the L-LDL tracer in lovastatin-treated animals. LDL cholesterol pool sizes were calculated using the measured LDL cholesterol values assuming a plasma volume of 43.5 ml/kg body weight. LDL-apoB pool sizes were calculated using the cholesterol:protein ratios of C-LDL and L-LDL described in Table 2. The calculations assume: that all LDL-cholesterol leaves the plasma compartment associated with LDL-protein; that all LDL-protein consists of apoB; and that all particles within the LDL density range can be considered homogeneous from a kinetic standpoint. The validity (or lack thereof) of these assumptions is discussed in the text. The values in the table represent mean values from the six control and six lovastatin-treated guinea pigs cited in Table 4.

Lovastatin treatment in humans also effectively reduces LDL cholesterol levels (7,13,42,47,48). Although the reported changes in LDL composition are small (13), similar to the present findings in guinea pigs, nevertheless they may affect the intrinsic metabolic properties of LDL in humans as well. It has been reported that in many human subjects lovastatin therapy increases the FCR of LDL to only a minor degree (7,49,50). In those studies autologous C-LDL was isolated prior to therapy and its FCR was determined in the subject. Subsequently, the individual was treated with lovastatin and autologous L-LDL isolated and injected into the subject during treatment. Thus, any changes in FCR noted between the two studies would reflect changes in both the lipoprotein degradation pathways, as well as intrinsic changes in the LDL particles themselves. In some subjects, intrinsic changes in LDL might "counteract" the increased rate of LDL clearance expected from the induction of hepatic LDL receptor activity. Although definitive proof that such changes occur in humans in response to lovastatin will have to await further studies, studies by Grundy and Vega (13,15) already indicate that similar phenomena may be occurring in some human subjects. Further studies are needed to test this hypothesis.

In summary, we have obtained evidence that lovastatin therapy produced changes in LDL particles that independently influence the metabolic behavior of the LDL. Since cholestyramine therapy of guinea pigs and humans also affects the intrinsic metabolic behavior of LDL, it seems reasonable to expect that other interventions might also result in changes in LDL composition that would affect its metabolism. The finding that changes in the kinetic behavior of LDL occurred, despite only minor compositional changes, adds further complexity to the issue of LDL heterogeneity.

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