Three-fold effect of lovastatin treatment on low density lipoprotein metabolism in subjects with hyperlipidemia: increase in receptor activity, decrease in apoB production, and decrease in particle affinity for the receptor. Results from a novel triple-tracer approach

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Abstract To differentiate effects of lovastatin on low density lipoprotein (LDL) receptor activity from effects on LDL metabolic properties, LDL apolipoprotein B (apoB) turnover was studied in eight hyperlipidemic subjects during baseline and lovastatin treatment, in the latter case with LDL tracers isolated during both baseline (C-LDL) and drug treatment (Rx-LDL) conditions. Lovastatin (40 mg/day) significantly lowered total plasma and LDL cholesterol levels (27% and 25%, respectively) as well as plasma triglyceride levels (30%). Using contemporaneous tracers (C-LDL before and Rx-LDL during treatment), lovastatin caused a modest increase in LDL fractional catabolic rate (FCR) (0.410 ± 0.113 vs. 0.339 ± 0.108 pools/day, $P < 0.04$ by paired t). The increase in LDL tracer FCR was higher when C-LDL tracer isolated during the untreated period was injected during lovastatin treatment (0.496 ± 0.177 vs. 0.339 ± 0.108 pools/day, $P < 0.02$). These in vivo studies in humans were confirmed by injecting LDL tracers from two patients into five guinea pigs. The C-LDL tracer was cleared consistently faster than the Rx-LDL tracer (0.082 ± 0.018 vs. 0.057 ± 0.015 pools/h, $P < 0.001$). The results demonstrate three important outcomes of lovastatin treatment in these subjects: LDL receptor activity increased by 49% ($P < 0.02$); LDL apoB production rate decreased by 17% ($P < 0.03$), and LDL particle in vivo affinity for the LDL receptor decreased by 15% ($P < 0.01$). The decrease in LDL particle affinity partially negated the expected effect of increased LDL receptors on LDL clearance. The present study provides an explanation for earlier observations by several investigators using contemporaneous tracers that treatment with HMG-CoA reductase inhibitors resulted in only modest increases in low density lipoprotein functional catabolic rate.**—Berglund, L., J. L. Witztum, N. F. Galeano, A. S. Khouw, H. N. Ginsberg, and R. Ramakrishnan.** Three-fold effect of lovastatin treatment on low density lipoprotein metabolism in subjects with hyperlipidemia: increase in receptor activity, decrease in apoB production, and decrease in particle affinity for the receptor. Results from a novel triple-tracer approach. *J. Lipid Res.* 1998. **39:** 913–924.

Supplementary key words HMG-CoA reductase inhibitors • LDL receptor • apolipoprotein B • lipoprotein metabolism • tracer kinetics • lipid-lowering therapy

The cholesterol-lowering action of HMG-CoA reductase inhibitors is well established and used clinically. On the cellular level, the inhibitory action on HMG-CoA reductase results in secondary increases in the LDL receptor activity (1–5). However, despite increased hepatic LDL receptor activity, which has been demonstrated in humans (6) as well as in animals (7), increased plasma clearance of LDL has not been demonstrated consistently in subjects treated with HMG-CoA reductase inhibitors (8–12). In particular, significant changes in fractional catabolic rates (FCR) of LDL apolipoprotein B (apoB) have not been shown in patients with moderate hypercholesterolemia (9, 11) or with combined hyperlipidemia (10).

Compared to LDL isolated from control animals, LDL isolated from animals treated with lovastatin has been shown to have changes in composition and metabolism that are associated with apparent reduced affinity for the LDL receptor (13). Similar changes in LDL composition have also been found in humans (11, 14–18). Thus, changes in the properties of LDL might

Abbreviations: apo, apolipoprotein; LDL, low density lipoprotein; FCR, fractional catabolic rate; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; C-LDL, tracer before treatment; Rx-LDL, tracer during treatment.

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negate, in part, the effect of an increase in LDL receptor activity. These changes in the characteristics of the LDL could result from effects of HMG-CoA reductase inhibitors on the initial assembly and secretion of apoB-containing lipoproteins or on VLDL metabolism. We have previously found a reduced output of hepatic apoB-containing lipoproteins during treatment with HMG-CoA reductase inhibitors (10, 19), and, recently, VLDL isolated from lovastatin-treated guinea pigs was found to have altered metabolic properties (20). These findings suggest multiple modes of action of HMG-CoA reductase inhibitors, and raise the possibility that the clinical effects of these agents may result from complex changes in apoB metabolism. In the present work, we attempted to better define the changes in LDL metabolism occurring in response to HMG-CoA reductase inhibitor treatment. Each subject was studied during a pretreatment baseline period and then again while on lovastatin, in the latter case using autologous tracers representing both pre-treatment and treatment conditions. This unique design, involving three tracer studies, one baseline and two on treatment, allowed us to separate the effects of lovastatin on LDL receptor activity from those on the metabolic or particle characteristics of LDL.

EXPERIMENTAL PROCEDURE

Subjects

Males and females identified as having LDL cholesterol levels and/or triglyceride levels above the 90th percentile for age (21) prior to any dietary of pharmacological intervention were recruited from a patient population referred to the Arteriosclerosis Research Center at the Columbia Presbyterian Medical Center. Altogether, six men and two women were studied. Their individual clinical characteristics are depicted in **Table 1**. There was no clinical or laboratory evidence of thyroid, kidney, heart, or liver disease or diabetes mellitus in these subjects. Each of the subjects was instructed in the American Heart Association (AHA) Step 1 diet and had been following this diet regimen for at least 6 weeks prior to study. The subjects remained on the Step 1 diet during the entire study period. Dietary compliance was verified at frequent meetings between each subject and a research dietitian, when 3-day food records were submitted to the research dietitian. None of the subjects had received any lipid lowering agent or had taken any other medications known to affect plasma lipids during the study period. Informed consent was obtained from each subject and the study was approved by the Institutional Review Board at the Columbia Presbyterian Medical Center.

TABLE 1. Clinical data and plasma lipid levels of the subjects

Subject	Sex	Age	TC	LDL-C	HDL-C	TG
		yr	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*
1	F	54	242	144	48	254
2	M	43	321	207	28	428
3	M	51	279	203	38	279
4	M	30	275	231	39	75
5	M	55	280	183	26	185
6	M	41	264	180	50	171
7	F	43	412	322	70	100
8	M	27	217	156	31	148

Values were obtained while consuming the AHA Step 1 diet.

Design of the human study

In each subject, two different autologous LDL turnover studies were performed, the first during a period prior to initiation of lovastatin therapy (pretreatment, designated Pre), and a second conducted during a period of lovastatin therapy (treatment, designated Rx), see **Fig. 1**. During the initial turnover study, LDL was isolated during the pretreatment period and designated control-LDL$_1$, (C_1-LDL). The C_1-LDL tracer was then injected into the untreated patient (Pre study, Fig. 1). After completion of the first turnover study and prior to lovastatin treatment, a second control plasma sample was drawn for subsequent isolation of LDL (C_2-LDL). C_2-LDL was not isolated at this stage, as plasma confers extensive antioxidant properties. This plasma sample was stored at 4°C for 8 days. Immediately after collection of the plasma for isolation of C_2-LDL, the subjects were treated with lovastatin, 40 mg/day. Eight days after initiation of lovastatin therapy, a third plasma sample was obtained from the patients and used for preparation of LDL (Rx-LDL). Both the C_2-LDL and the Rx-LDL were then isolated in parallel, differentially labeled with radioiodine, and simultaneously injected into the patient while the lovastatin therapy continued (Rx study).

For each LDL preparation, blood was obtained by venipuncture after a 12–14 h fast. Plasma was isolated by centrifugation at 4°C at 2,000 rpm for 25 min. LDL (density 1.025–1.055 g/ml) was isolated by sequential ultracentrifugation as previously described using sterile techniques (10). The isolated LDL fractions were labeled with ^{131}I or ^{125}I using a modification of the iodine monochloride method (22). For the baseline study (Pre study), C_1-LDL was labeled with ^{131}I as the short half-life of the tracer enabled us to carry out the second turnover study (Rx study) with two tracers (C_2-LDL and Rx-LDL) shortly after completion of the first study. For the second study (Rx study), the C_2-LDL and Rx-LDL were labeled simultaneously, one with ^{131}I and the other with ^{125}I. Whether C_2-LDL was labeled with ^{131}I and Rx-LDL with ^{125}I, or C_2-LDL with ^{125}I and Rx-LDL with ^{131}I was chosen in a random fashion for each pa-

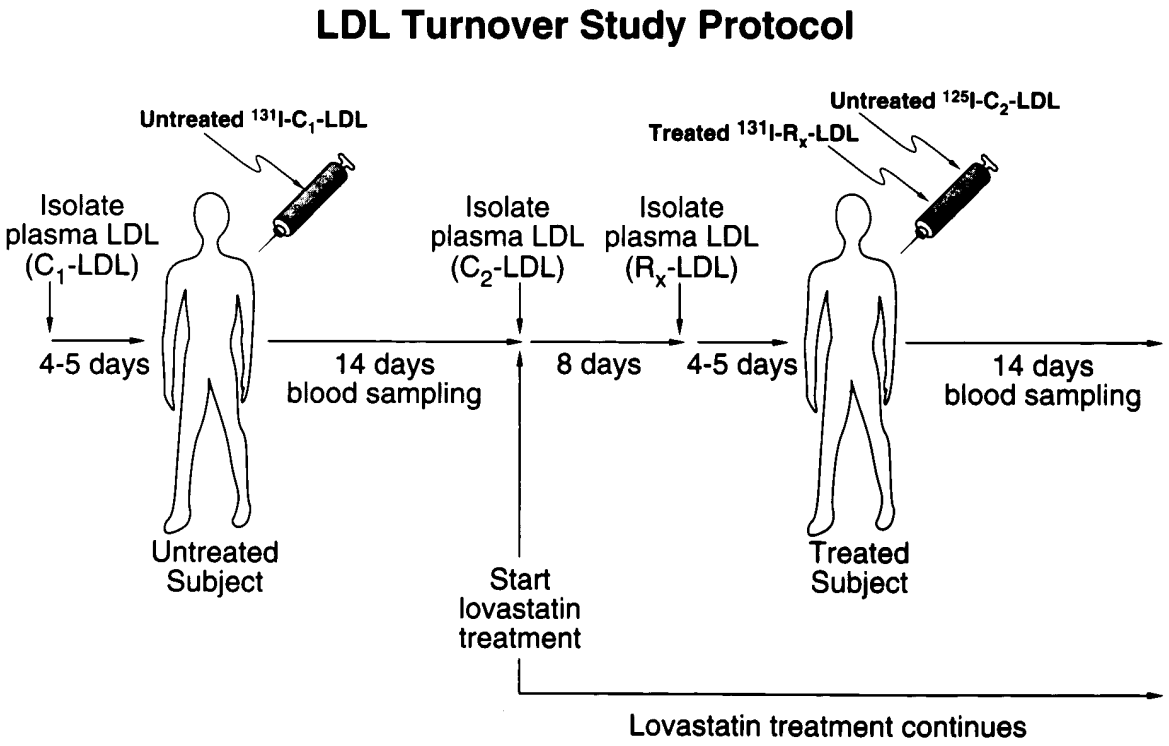

Fig. 1. Schematic model of the study design. In the illustrated case, Rx-LDL was labeled with ^{131}I and C_2-LDL with ^{125}I. This was done for four of the subjects, and in the other four subjects Rx-LDL was labeled with ^{125}I and C_2-LDL with ^{131}I.

tient, as it has been found that the use of iodine isotope might influence LDL clearance (23). Unreacted radioiodine was removed as described (10) and the radiolabeled lipoproteins were passed through 0.22-μm Millipore filters and injected into the study participants within 24 h of labeling. Of the eight subjects, four received a combination of ^{131}I-labeled C_2-LDL and ^{125}I-labeled Rx-LDL, and four received a combination of ^{125}I-labeled C_2-LDL and ^{131}I-labeled Rx-LDL.

For each study, the subjects were admitted to the Irving Center for Clinical Research at the Columbia Presbyterian Medical Center 1–3 days prior to injection for further diet stabilization. All subjects received a saturated solution of potassium iodide (SSKI), twice daily, starting the day prior to injection and continuing throughout each study period. SSKI was not administered between the two turnover periods. In the baseline study (Pre-study), a fasting blood sample was obtained and 25 μCi of autologous ^{131}I-labeled LDL (C_1-LDL tracer) was injected intravenously. In the treatment study, after a fasting blood sample, 75 μCi of the ^{131}I-labeled LDL and 10 μCi of the ^{125}I-labeled LDL samples were injected simultaneously; one was the C_2-LDL tracer and the other was the Rx-LDL tracer. In both studies, blood samples were obtained at 0.5, 1, 2, 6, 12, 24, and 36 h. The patients were given food after the 6 h, 12 h, 24 h, and 36 h samples. Fasting blood samples were then obtained on days 2, 3, 4, 6, 8, 10, 12, and 14. Plasma was isolated by low-speed centrifugation in the cold. All subjects remained at the Clinical Research Center for the first 36 h after injection of tracers, and thereafter stayed at the hospital for varying lengths of time, finishing the sampling protocol as outpatients. All subjects continued to receive lovastatin (40 mg/day) throughout the second turnover study.

Analytical procedures

At the end of each turnover study, plasma samples, stored at 4°C, from each time point were subjected to ultracentrifugation to isolated LDL (density range 1.019–1.063 g/ml) as described (10). The ^{131}I and ^{125}I radioactivities in the isolated LDL samples were determined in a double-channel gamma spectrometer, with correction for cross-over of ^{131}I into the ^{125}I channel. LDL protein concentration was assayed by the Lowry procedure (24), and LDL-apoB specific radioactivity (SA) in each sample was calculated.

Plasma and lipoprotein cholesterol and triglycerides were determined using standard enzymatic techniques using an ABA-100 automated spectrophotometer. Plasma HDL cholesterol levels were measured after precipitation of apoB-containing lipoproteins by dextran

sulfate and magnesium (25). Phospholipid was determined by a modification of the Bartlett procedure (26). In isolated LDL preparations, triglyceride, cholesterol, protein, and phospholipid concentrations were determined. The apoB content in LDL was determined by a specific fluid-phase radioimmunoassay as described previously (10). Our laboratory participates in an ongoing standardization program for measurement of cholesterol and triglycerides supervised by the Centers for Disease Control (CDC). We also participated in the CDC apoprotein standardization program (27).

Nonequilibrium rate zonal ultracentrifugations of $d > 1.006$ g/ml plasma obtained at the start of the baseline (Pre) and treatment (Rx) turnover studies were carried out essentially as described previously (28). Briefly, LDL was isolated from $d > 1.006$ g/ml plasma using a discontinuous NaBr gradient of 1.0–1.3 g/ml. The centrifugation was performed using a Ti-14 zonal rotor at 42,000 rpm, lasted exactly 170 min, and the effluent from the gradient was monitored continuously for absorbance at 280 nm. The LDL-fraction was typically eluted at 180 ± 4.2 ml.

Animal experiments

The isolated and radiolabeled C_2-LDL and Rx-LDL tracers used in the second turnover study (Rx study) were also simultaneously injected into control guinea pigs. Each set of tracers was shipped on ice to the University of California San Diego (UCSD) in La Jolla immediately after radiolabeling. The samples were received within 24 h of shipment, and it was ascertained that they were kept in the cold and not frozen. Within 4 h after arrival at UCSD, the tracers were injected into the jugular vein of control, male Hartley guinea pigs (Charles River Breeding Labs, Inc., Wilmington, MA). All studies were performed with coded tracers, and the investigators at UCSD were blinded as to the identity of the LDL preparation (C_2-LDL or Rx-LDL). Serial blood sampling was performed over the ensuing 24 h by cardiac puncture as described previously (29). 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 as previously described (29), and the apparent FCRs were calculated based on the area under the plasma decay curve.

Cell culture experiments

To assess the interaction of the LDL preparations with the LDL receptor of human fibroblasts, competition experiments were conducted at 4°C, utilizing a standard radioiodinated LDL preparation as tracer (30, 31). Briefly, cells were incubated at 4°C with increasing concentrations of unlabeled C_1-LDL or Rx-LDL in Dulbecco's modified Eagle's medium (pH 7.4) containing 20 mm HEPES, 5% lipoprotein-deficient serum, and 5 μg/ml of ^{125}I-labeled LDL for 2.5 h. Thereafter, the cells were washed, the cell layer was harvested by dissolution in 0.1 m NaOH, and the amount of cell-associated ^{125}I-labeled LDL radioactivity was counted. Protein content was analyzed by the Lowry procedure (24). The amount of LDL bound per mg of total cell protein was calculated, and the results are expressed as percent of displacement of the ^{125}I-labeled LDL in the absence of competitor (C_1-LDL or Rx-LDL). Values presented are averages of duplicate determinations that differed by less than 10%.

Kinetic modeling

For each human subject, each of the three tracer studies was fitted by a two-pool model, one pool representing circulating LDL and the other a noncirculating, extravascular pool in exchange with the circulating pool; all LDL entry was assumed to occur into the circulating pool (32). Each two-pool model has four parameters (initial specific activity, FCR, L_{21} and L_{22}, the last two for kinetics of exchange with the non-circulating pool), for a total of 12 parameters for the three tracers (**Fig. 2**). As two tracers (C_2-LDL and Rx-LDL) were used simultaneously, an attempt was made to fit a single model to all three tracer studies. This integrated model also contained a two-pool configuration for each tracer, but it constrained L_{22}, and possibly L_{21}, to be the same for C_2-LDL and Rx-LDL. If the fits with the integrated model to the three tracer studies were as good as with three separate models, that would provide validity to the experimental approach. Production rates were computed for the baseline condition from the C_1-LDL study and for the treatment condition from the Rx-LDL study by multiplying the respective FCR (in pools/day) and LDL apoB concentration (in mg/dl), scaled by 0.45 for plasma volume (dl/kg). There is no production rate corresponding to C_2-LDL as it was not a contemporaneous tracer.

Statistical analysis

Comparisons of lipid levels pre- and post-treatment and of FCRs in guinea pigs were by paired Student's *t*-test. Comparison of the FCRs from the three studies were by repeated measures ANOVA followed by pairwise contrasts.

RESULTS

Plasma lipid levels and LDL composition

Analyses of serum lipid levels as well as direct measurement of LDL cholesterol levels were performed on

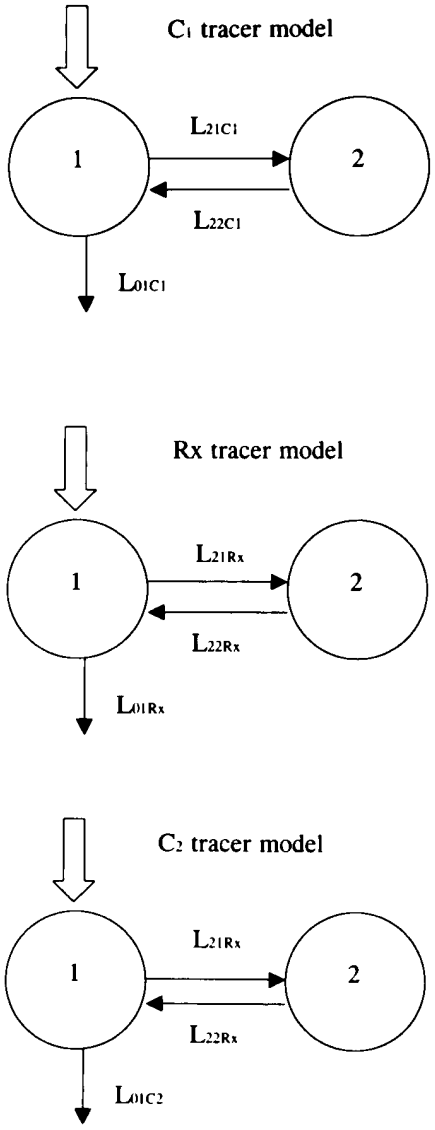

Fig. 2. Kinetic model for LDL apoB metabolism. The model used had two compartments, one circulating and the other noncirculating. L_{21} and L_{22} represent the kinetics of exchange between the two pools, and L_{01} the catabolism from the circulating pool. One two-pool model was used for each tracer study, and the three studies were combined in an integrated model for each subject. The figure illustrates the integrated model with ten parameters, as L_{21} and L_{22} were the same in the Rx and C_2 tracer models. The ten-parameter integrated model was the one used in most of the subjects.

8 consecutive days during each turnover protocol. For the Rx study, it was verified in each patient that LDL cholesterol levels were in steady state at the time of tracer injections. Treatment with lovastatin resulted in a decrease in total plasma and LDL cholesterol levels in all subjects (**Table 2**), with a mean decrease of 27% and 25%, respectively. There was also a significant overall decrease in LDL apoB levels, seen in all subjects, and mean LDL apoB levels fell from 144 mg/dl to 97 mg/dl (Table 2). Also, serum triglyceride levels decreased in all subjects, with a mean reduction of 30%. In contrast, HDL cholesterol levels increased by 7%.

A more detailed compositional analysis was carried out in LDL fractions harvested from five of the subjects (nos 1–5) during the Pre study (C_1-LDL) and during the Rx study (Rx-LDL). The Pre-LDL composition was 35.8 ± 2.6% cholesterol, 6.8 ± 2.7% triglycerides, 29.4 ± 2.9% phospholipids, and 28.0 ± 2.7% protein (% of total weight). There were only minor changes in LDL composition during the study, none of which reached significance. In support of this, no consistent changes were found in the cholesterol/protein, triglyceride/protein, or cholesterol/triglyceride ratios in the isolated LDL fractions (1.26 ± 0.10, 0.25 ± 0.11, and 6.3 ± 2.6 vs. 1.19 ± 0.19, 0.23 ± 0.07, and 5.9 ± 2.5 for Pre-LDL vs. Rx-LDL, respectively). To evaluate whether lovastatin treatment induced changes in LDL size or relative density, the elution pattern of C_1-LDL and Rx-LDL, isolated by nonequilibrium rate zonal centrifugation, was analyzed in three subjects (nos 6–8). No difference was seen in the elution profiles of the LDL fractions (data not shown), and mean elution peaks were 172 ± 15 ml for C_1-LDL and 179 ± 6 ml for Rx-LDL.

Binding of both C_1-LDL and Rx-LDL to human fibroblasts was determined in three of the subjects (nos 6–8). In these experiments, the displacement of normal ^{125}I-labeled LDL by unlabeled C_1-LDL or Rx-LDL was evaluated. An example of a competition experiment is shown in **Fig. 3**. The ratio in EC_{50} between the LDL preparations from each individual was very close to 1.0 (mean 0.98; range 0.88–1.03), indicating very similar competitive binding. Altogether, these results suggest that compared to C-LDL, Rx-LDL did not differ in size or composition nor in its ability to bind in vitro to LDL receptors on cultured fibroblasts. It should be noted that the culture conditions used would maximally upregulate the number of LDL receptors on the cells.

Metabolic studies of LDL

In all eight subjects, each tracer study data were well-fitted by a two-pool model; in no case was a single pool sufficient and in no case was the fit improved with a third pool. These experiments were designed to address two different questions related to the effects of HMG-CoA reductase inhibitors on LDL metabolism. The first concerned the effect of lovastatin on pathways of in vivo LDL metabolism, i.e., would the expected lovastatin-induced increase in LDL receptor activity (4) increase clearance of a "standard" LDL tracer? For this question, untreated LDL tracers (C-LDL) were compared in the same patient before and during therapy.

TABLE 2. Effect of lovastatin treatment on plasma lipid and lipoprotein levels

Subject	Serum cholesterol		LDL cholesterol		HDL cholesterol		Triglycerides		LDL Apo B	
	Pre	Rx	Pre	Rx	Pre	Rx	Pre	Rx	Pre	Rx
	mg/dl		*mg/dl*		*mg/dl*		*mg/dl*		*mg/dl*	
1	204.8 ± 12.6	149.5 ± 13.7	125.6 ± 15.9	75.3 ± 9.0	38.5 ± 2.7	39.8 ± 3.7	216.0 ± 48.8	180.4 ± 26.2	105.8 ± 28.5	46.7 ± 5.1
2	312.0 ± 22.5	230.4 ± 7.7	145.3 ± 12.7	142.9 ± 10.1	27.4 ± 1.4	28.8 ± 1.5	495.0 ± 183.7	278.8 ± 24.9	131.2 ± 30.2	109.1 ± 14.4
3	255.4 ± 15.9	184.4 ± 4.3	154.6 ± 22.7	122.3 ± 8.5	39.4 ± 3.6	44.9 ± 1.9	186.4 ± 38.1	129.7 ± 27.2	139.2 ± 9.8	96.3 ± 7.8
4	265.2 ± 11.1	232.3 ± 8.7	203.6 ± 14.2	180.8 ± 8.7	36.6 ± 2.6	39.4 ± 4.5	105.6 ± 16.3	89.3 ± 23.9	121.0 ± 9.0	109.3 ± 9.9
5	261.2 ± 11.1	172.8 ± 7.0	188.9 ± 6.1	109.6 ± 6.6	24.6 ± 1.3	27.6 ± 2.8	150.7 ± 24.5	113.0 ± 22.0	137.1 ± 12.3	75.7 ± 9.0
6	225.4 ± 15.8	172.9 ± 8.0	100.7 ± 11.8	84.6 ± 7.1	45.5 ± 5.8	53.5 ± 6.9	158.9 ± 36.3	134.2 ± 29.0	101.2 ± 28.9	84.5 ± 11.2
7	399.4 ± 15.6	256.4 ± 8.2	267.6 ± 18.4	157.1 ± 13.0	63.5 ± 4.3	56.3 ± 3.4	84.3 ± 23.7	74.3 ± 16.5	239.1 ± 25.8	152.1 ± 4.1
8	207.9 ± 11.3	157.1 ± 5.4	109.0 ± 8.7	96.8 ± 8.2	29.4 ± 1.6	36.8 ± 3.2	154.3 ± 20.3	93.2 ± 19.4	176.9 ± 21.7	102.1 ± 21.0
Mean levels	266.4 ± 64.2	194.5 ± 39.7	161.9 ± 55.8	121.2 ± 36.8	38.1 ± 6.2	40.9 ± 10.4	193.9 ± 128.5	136.6 ± 66.3	143.9 ± 45.0	97.0 ± 30.5
Change	−72 ± 34, $P < 0.001$		−41 ± 38, $P < 0.02$		+2.8 ± 5.1, $P < 0.2$, NS		−24.8% ± 12%, $P < 0.001$[a]		−46.9 ± 28.1, $P < 0.003$	

The numbers for each individual are based on repeated measurements (n = 6 to 11) during the turnover procedures, and are given as mean ± S.D. for each turnover period (Pre or Rx).

[a] The difference is expressed as percentage using a log transformation of triglyceride levels.

Fig. 3. Displacement of a standard ^{125}I-labeled LDL by C-LDL (○) or Rx-LDL (▲). Human fibroblasts were incubated as described in the text for 2.5 h with varying concentrations of C-LDL or Rx-LDL in medium containing 5 μg/ml of ^{125}I-labeled LDL. Results from one patient are shown and displayed as percent displacement of ^{125}I-labeled LDL in the absence of competitor.

The second question related to whether lovastatin affected the intrinsic metabolic properties of LDL, i.e., would lovastatin therapy change the in vivo interaction of LDL with its receptors? For this question, LDL tracers representing C-LDL and Rx-LDL were compared simultaneously in each subject during treatment.

To answer the first question, we compared the FCR of the untreated LDL tracers (C_1-LDL and C_2-LDL) during the Pre and Rx studies (**Table 3**). Compared to C_1-LDL, the FCR of C_2-LDL increased significantly by an average of 49% ± 42% from 0.339 ± 0.108 pools/day in untreated subjects to 0.496 ± 0.177 pools/day in lovastatin-treated subjects ($P < 0.02$). The increased FCR is consistent with a marked increase in hepatic LDL-receptor number during treatment with lovastatin. Barring a change in non-LDL receptor clearance pathways, the 49% increase in FCR suggests a 49% or greater increase in LDL receptor activity. If all of the FCR were due to LDL receptors, the increase in receptor activity would be 49%; if some of the FCR were by non-receptor pathways, the increase in receptor activity would be correspondingly greater.

To answer the second question, whether lovastatin therapy altered LDL metabolic properties, we compared the FCR of C_2-LDL and Rx-LDL simultaneously injected into subjects while on lovastatin therapy (Table 3). Compared to C_2-LDL, the FCR of Rx-LDL was significantly smaller by an average of 15% ± 11% ($P < 0.01$), interpretable as a 15% or greater decrease in the affinity of LDL particles for the LDL receptor during treatment. These data clearly show that the LDL isolated during lovastatin therapy had different metabolic

TABLE 3. LDL kinetic data of C-LDL and Rx-LDL injected into untreated and lovastatin-treated subjects

Subject	LDL Tracer Fractional Catabolic Rate			FCR Change from A to B	FCR Change from C to B
	A Untreated/C_1-LDL	B Treated/C_2-LDL	C Treated/Rx-LDL		
	pools/day			*%*	*%*
1	0.325	0.640	0.519	97	−19
2	0.532	0.802	0.592	51	−26
3	0.339	0.427	0.386	26	−10
4	0.309	0.304	0.262	−2	−14
5	0.264	0.584	0.414	121	−29
6	0.318	0.405	0.426	27	5
7	0.180	0.280	0.265	56	−5
8	0.448	0.529	0.415	18	−22
Mean ± SD	0.339 ± 0.108	0.496 ± 0.177	0.410 ± 0.113	49 ± 42 $P < 0.02$	−15 ± 11 $P < 0.01$

C-LDL represents LDL isolated from untreated subjects (baseline) and Rx-LDL represents LDL isolated from lovastatin-treated subjects (treated). The combination Treated/C_2-LDL denotes LDL isolated prior to start of lovastatin therapy and reinjected into the same individual after the start of lovastatin treatment.

properties, as previously observed in guinea pigs (13). When comparing the results of the Pre and the Rx studies using contemporaneous tracers, i.e., comparing C_1-LDL in an untreated subject with Rx-LDL in the same subject during treatment, LDL-FCR was only 20% higher during treatment (tracer FCR values 0.410 ± 0.113 pools/day vs. 0.339 ± 0.108 pools/day), although the difference still reached significance ($P < 0.04$). Presumably, this 20% increase is the result of the 49% increase in LDL receptor activity combined with the 15% decrease in LDL particle affinity.

By multiplying the FCRs obtained with the contemporaneous tracers by their respective pool sizes, we could calculate tracee production rates. As seen in **Table 4**, the calculated LDL-apoB production rates decreased during lovastatin therapy in seven of the eight subjects. The average decrease of 17% ± 17% from 21.4 ± 7.9 to 17.2 ± 5.5 mg/kg body weight/day was significant ($P < 0.03$).

To validate our use of three different tracers, an integrated model was fitted to the three tracer studies in each subject. This model constrained some or all of the non-circulating pool kinetics to be the same for C_2-LDL and Rx-LDL and, possibly, for C_1-LDL as well if the treatment had no effect on the non-circulating pool. First, L_{22} was set to be equal for C_2-LDL and Rx-LDL. In seven of eight subjects (all except #4), this constraint did not lead to any deterioration of the fits compared to fitting the three studies separately. In five subjects (#2, 3, 6, 7, 8), L_{21} could also be set equal for C_2-LDL and Rx-LDL with the fits remaining just as good. In three subjects (#1, 2, 7), L_{22} could be set equal for all three tracers; in subject 7, L_{21} could also be set equal for all three tracers. While the number of model parameters is 12 when fitting each data set separately, the number decreases by one with each constraint in the integrated model. These results are summarized in **Table 5** and **Table 6**. Table 5 shows the residual errors with separate fits and with the integrated model. It can be seen that the integrated model fits are as good as the separate fits. In some cases, the residual error is smaller with the integrated model as it has larger degrees of freedom. The fits for two of the subjects (#2 and #7) are shown in **Fig. 4**. Table 6 presents the parameters of the non-circulating pool, with the integrated model for the seven subjects who were fitted well with the model, and for separate fits for patient #4. Except for L_{22} with C_1-LDL, the variability among the seven subjects fitted well by the integrated model ranged from 23% to 42%, about the same as for LDL FCR and LDL apoB production rates given in Tables 3 and 4, further lending credibility to the integrated model and to the triple-tracer approach. In fact, the only aberrant values for L_{21} and L_{22} were found in subject #4, whose data could not be fitted well by the integrated model.

To further confirm the metabolic difference between

TABLE 4. Kinetic data of LDL apoB production rate

Subject	LDL ApoB Production Rate		Change
	Baseline	Treatment	
	mg/kg body weight/day		*%*
1	15.5	11.0	−29
2	31.4	29.0	−8
3	21.2	16.7	−21
4	16.8	12.9	−23
5	16.3	14.2	−13
6	14.5	16.3	12
7	19.4	18.1	−7
8	35.7	19.0	−47
Mean ± SD	21.4 ± 7.9	17.2 ± 5.5	−17 ± 17 $P < 0.03$

TABLE 5. Percent residual errors of the treatment turnover studies with separate fits and the integrated model

	Fitted Separately				Fitted by Integrated Model			
Subject	Number of Parameters	C_1-LDL	C_2-LDL	Rx-LDL	Number of Parameters	C_1-LDL	C_2-LDL	Rx-LDL
1	12	4.3	4.0	4.5	10	4.1	4.0	4.5
2	12	6.8	4.1	5.3	9	6.5	4.0	5.2
3	12	6.0	10.1	12.4	10	6.0	9.9	12.1
4	12	3.9	2.3	2.7	[a]			
5	12	4.6	7.1	7.3	11	4.6	6.9	7.2
6	12	8.7	6.8	7.8	10	8.7	6.7	7.6
7	12	5.0	6.2	7.7	8	4.8	6.0	7.3
8	12	8.2	12.3	12.2	10	8.2	12.0	12.0
Mean		6.2	7.2	8.2		6.1	7.1	8.0

[a]Fit with integrated model was not satisfactory.

the C_2-LDL and Rx-LDL tracers, these two tracers were simultaneously injected into control guinea pigs. Thus, the two tracers could be compared directly in an untreated animal model in vivo. Two different sets of experiments representing different subjects (#1 and 2) were performed, and the tracers were injected into two and three different guinea pigs, respectively (**Table 7**). In one experiment, Rx-LDL was labeled with ^{131}I and C_2-LDL with ^{125}I. In the LDL preparations from the other donor, the assignment of tracers was reversed. Irrespective of the label, Rx-LDL was cleared 33% more slowly than C_2-LDL in the guinea pigs, similar to when these same tracers were injected into their donors (Table 3).

DISCUSSION

In our present studies, utilizing a unique approach with a triple tracer design and two different studies in each individual, we were able to delineate the detailed effects of lovastatin therapy on LDL receptor activity, LDL particle affinity, and LDL apoB production rate. The major conclusions were that lovastatin increased LDL receptor activity, decreased LDL affinity for its receptor, and decreased the LDL apoB production rate. It is important to emphasize that the results regarding receptor activity and particle affinity could only be fully appreciated by the use of the present experimental design using the C_2-LDL tracer. We recognize that one source of error in our studies might be the effect of storage on the C_2-LDL tracer used in the Rx study. In a previous study, we demonstrated that storage of isolated LDL did not alter its metabolic properties (33). In order to further minimize potential modifications of isolated LDL during storage, we stored the sample as plasma in the presence of EDTA, and isolated the C_2-LDL and Rx-LDL tracers in parallel, in contrast to prolonged storage of isolated LDL fractions. Further, the fits in seven of eight subjects using the integrated model were just as good as using separate models (Table 5), indicating that there was no element of rapid or different clearance in the LDL tracer isolated from the stored plasma. Another source of error could be the labeling procedure. In view of our previous observations that the iodination procedure might influence LDL clearance (13, 23), which potentially could have clouded our observations, particular care was taken to minimize these possibilities. Thus, the choice of isotope was random and different among subjects, and the protocol was designed to minimize the time span between labeling and injections (<24 h). We did not find any isotope effect in our studies.

We undertook these studies in subjects with hyperlipidemia with increases in LDL cholesterol and/or plasma triglyceride levels. This is a relatively common hyperlipidemic pattern and the exact underlying mechanisms have not been clarified. Previous studies on similar subjects with mixed hyperlipidemia or moderate hypercholesterolemia have not always demonstrated an

TABLE 6. Kinetics of non-circulating pool

	L_{21}			L_{22}		
Subject	C_1-LDL	C_2-LDL	Rx-LDL	C_1-LDL	C_2-LDL	Rx-LDL
1	0.14	0.18	0.27	0.43*	0.43*	0.43*
2	0.11	0.15†	0.15†	0.27*	0.27*	0.27*
3	0.12	0.21†	0.21†	0.39	0.49*	0.49*
4	0.87	0.06	0.14	3.57	0.15	0.41
5	0.06	0.29	0.44	0.13	0.51*	0.51*
6	0.18	0.22†	0.22†	0.49	0.39*	0.39*
7	0.16†	0.16†	0.16†	0.40*	0.40*	0.40*
8	0.06	0.16†	0.16†	0.22	0.29*	0.29*

All values are given as pools/day. Within each subject, the dagger (†) values were found to be equal and the star (*) values were found to be equal.

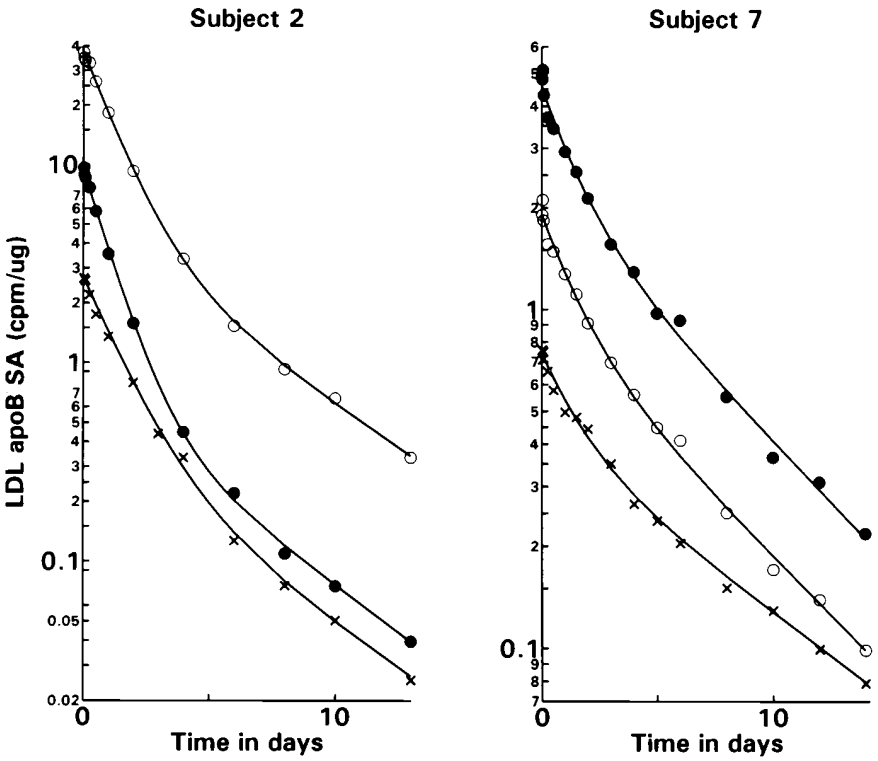

Fig. 4. Plasma decay data and fitted curves of LDL apoB with two different tracers (C-LDL and Rx-LDL) injected in the same individual during baseline and lovastatin therapy, resulting in three different LDL apoB decay curves (C_1-LDL during baseline, crosses; C_2-LDL during treatment, closed circles; Rx-LDL during treatment, open circles). The solid lines are best fit curves with the integrated model. Data are shown for subjects #2 and #7.

increase in LDL catabolism during treatment with HMG-CoA reductase inhibitors, suggesting that several mechanisms may be operating (9–12). This is in contrast to subjects with familial hypercholesterolemia (FH), where administration of HMG-CoA reductase inhibitors has been associated consistently with an increase in LDL catabolism (8, 10). The discrepancy in the effects of HMG-CoA reductase inhibitors on LDL metabolism may be due to a combination of underlying causes contributing to the dyslipidemic pattern in mixed hyperlipidemia; FH subjects, by comparison, uniformly have a profound reduction in LDL receptor number, while the metabolism of triglyceride-rich lipoproteins is normal (8, 10, 34). Other explanations are also possible; effects of drug treatment on LDL composition and metabolic properties could be contributory. In a series of studies in animals and humans, primarily subjects with FH, we previously addressed these issues using cholestyramine as a model perturbation, and demonstrated that treatment with this drug causes profound changes in LDL composition resulting in decreased affinity for the LDL receptor (29, 33). Thus, measurement of in vivo LDL clearance with an autologous tracer isolated during cholestyramine treatment would tend to underestimate the actual effect of treatment on LDL receptor activity. Similar effects of lovastatin treatment on the in vivo affinity of LDL for its receptor have

TABLE 7. Fractional catabolic rates of C-LDL and Rx-LDL injected into control guinea pigs

	Fractional Catabolic Rate		
	C-LDL	Rx-LDL	Difference
	pools $\times$ *hr*$^{-1}$		%
Experiment 1			
Animal #1	0.085	0.067	−21
Animal #2	0.091	0.067	−26
Mean	0.088	0.067	−24
Experiment 2			
Animal #1	0.090	0.058	−36
Animal #2	0.095	0.066	−31
Animal #3	0.047	0.028	−40
Mean	0.077	0.051	−34
Mean (Exp 1 + 2)	0.082 ± 0.02	0.057 ± 0.017	−31
Difference (Rx vs Pre)		−0.024 ± 0.006 $P < 0.001$	

been found in normolipidemic guinea pigs although, interestingly, LDL compositional and binding properties when tested in vitro were considerably less affected than observed during cholestyramine treatment (13).

In the present study of patients with varied hyperlipidemia, we were able to demonstrate a decrease in LDL cholesterol as well as in LDL-apoB levels in response to lovastatin therapy. We could not detect any significant changes in the composition of LDL isolated during baseline and treatment periods in the present study. As discussed above, compositional changes in LDL from animals treated with lovastatin were modest and limited to small changes in the relative contents of triglyceride and free cholesterol (13). In previous human studies, similar modest changes in lipoprotein composition during administration of HMG-CoA reductase inhibitors have been found (14–18). The elution pattern of rate zonal centrifugation of LDL harvested during baseline and lovastatin treatment was also unchanged in the present study, arguing against any major changes in overall LDL density. This is in agreement with other studies in subjects with familial combined hyperlipidemia (35, 36). Finally, in vitro receptor binding studies did not reveal any differences in affinity between the different LDL isolated during baseline and treatment periods; a finding similar to that previously observed in lovastatin-treated guinea pigs (13). These results suggest that the in vitro binding assays might not detect subtle alterations in LDL conformation/composition that affect in vivo metabolism (13, 29, 33).

One of the primary goals of the present study was to evaluate the changes in metabolic properties of LDL induced by lovastatin treatment. In our protocol, each subject was studied with the same tracer (C_1-LDL and C_2-LDL) under two conditions (baseline and lovastatin treatment); further, each subject was studied simultaneously with two different tracers (C_2-LDL and Rx-LDL) under one condition (lovastatin treatment). It is important to emphasize that clearance of C_2-LDL and Rx-LDL was directly compared in the same treated environment. These two pairs of tracer studies (with control LDL appearing in both studies) allowed us to separately quantify the impact of lovastatin-induced increases in LDL receptor activity versus those changes due to alterations in LDL particle properties in each individual. Thus, the results using the same tracer (C_1-LDL and C_2-LDL) under baseline and lovastatin treatment indicated a 49% or greater increase in LDL receptor activity during lovastatin treatment, consistent with a major induction of hepatic LDL receptor activity. A direct comparison of the FCRs of C_2-LDL and Rx-LDL measured in the same study provided evidence for a 15% or greater decrease in clearance of LDL particles isolated during lovastatin treatment consistent with a decreased affinity in vivo for the LDL receptor. This difference clearly indicated that important changes in the LDL particle population occurred during HMG-CoA reductase inhibitor treatment, and that treatment affected the in vivo reactivity of LDL towards LDL receptors. This could be a reflection of a change in the distribution of subpopulations of LDL particles with different physico-chemical properties. Our findings of similar lipid composition, size, and relative density in the LDL fraction isolated before and during lovastatin therapy suggest that there was a change in receptor affinity over a broad spectrum of particle size or density. The underlying molecular basis for the changes in LDL could not be determined in the present studies: an increase in LDL receptor activity could result in preferential removal of VLDL (20) or LDL particles via the LDL receptor than via other mechanisms. This would lead to a greater proportion of LDL particles in circulation removed via non-LDL receptor mechanisms compared to baseline, resulting in lower affinity of LDL as a whole for the LDL receptor. In addition, a decrease in LDL apoB production could accompany changes in the type of apoB-containing lipoproteins assembled and secreted by the liver, e.g., direct LDL production, and/or a change in the flux from VLDL (10), so that a disproportionate decrease in particles with a higher affinity could result in a lower receptor affinity for the LDL as a whole. Thus, the increased receptor activity and the decreased LDL apoB production could, separately or together, contribute to the lower-affinity LDL.

The implications of the present study are that both the metabolic properties of circulating LDL particles as well as the number of LDL receptors are affected by lovastatin treatment. It has been well established that the LDL fraction is heterogeneous, particularly in subjects with mixed hyperlipidemia and varying degrees of hypertriglyceridemia (35–38). It is conceivable that LDL heterogeneity might contribute to the varying results in previous pre- and posttreatment studies utilizing contemporaneous LDL tracers (39, 40). Alternatively, there might be variation in the degree of change in LDL receptor number in different types of patients. However, our findings clearly suggest that there was a significant difference in LDL FCR during lovastatin treatment utilizing tracers representing baseline conditions (C-LDL) versus tracers representing treatment conditions (Rx-LDL). Using a more conventional study design, in which contemporaneous tracers representing baseline and lovastatin treatment (i.e., C_1-LDL and Rx-LDL) had been injected only once (C_1-LDL during baseline and Rx-LDL during treatment), would have underestimated the degree of LDL receptor up-regulation. The present triple-tracer study design clearly demonstrated that LDL receptor activity increased signifi-

cantly, by 50% or more, during lovastatin treatment, but that the effects of this increase were partially negated by a decrease in LDL particle affinity. We also observed a significant reduction in LDL apoB production rates, confirming our previous study in a different group of hyperlipidemic subjects, where lovastatin treatment also decreased LDL apoB production rates (10). It is important to emphasize that this 17% decrease represented a true decrease in LDL apoB production rate during lovastatin treatment, as this parameter is estimated using contemporaneous tracers. The individual contributions of increased LDL receptors and reduced LDL apoB production rates to the reduction in plasma LDL cholesterol levels during treatment may vary in different hyperlipidemic situations. Thus, it is possible that under some circumstances, HMG-CoA reductase inhibitors may more substantially affect hepatic lipoprotein production or alternatively the pathway from VLDL, via IDL, to LDL (10, 19, 20, 41–43).

In conclusion, utilizing an integrated model based on experiments with tracers representing both baseline and treatment conditions, we have demonstrated for the first time in humans that while LDL receptors increased during lovastatin treatment, the affinity of LDL particles for the receptor decreased. It is important to emphasize that use of contemporaneous tracers, i.e., C-LDL during baseline and Rx-LDL during lovastatin treatment, yields results that reflect the true net changes resulting from treatment. Thus, the reduced LDL apoB production rate, as well as the modestly increased LDL FCR observed during therapy, represent the true physiological responses to lovastatin treatment. However, using the present study design, we could clearly show that the net increase in LDL FCR during lovastatin treatment was a result of two opposing mechanisms, an increase in LDL receptor activity and a decrease in the LDL particle affinity for these receptors. These results contribute to our understanding of the complex physiologic effects of HMG-CoA reductase inhibitors on various stages in lipoprotein metabolism, and offer insights into the reasons for the apparently divergent results of the effect of HMG-CoA reductase inhibitors on LDL metabolism found in several previous metabolic studies.

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