Altered apolipoprotein B metabolism in very low density lipoprotein from lovastatin-treated guinea pigs

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Abstract Previous studies have shown that treatment of guinea pigs with lovastatin alters the composition and the metabolic properties of circulating low density lipoprotein (LDL). Specifically, LDL isolated from lovastatin-treated animals is cleared from plasma more slowly than LDL isolated from control animals, when injected into the guinea pig. In the present study, we examine whether lovastatin also affects the metabolic properties of very low density lipoprotein (VLDL), the metabolic precursor of LDL. VLDL isolated from lovastatin-treated guinea pigs (L-VLDL) and VLDL isolated from untreated (control) guinea pigs (C-VLDL) were radioiodinated and simultaneously injected into eight untreated guinea pigs. Radioactivity associated with apolipoprotein B-100 (apoB) was measured in four plasma density fractions and analyzed using a compartmental model consisting of fast and slow pools for VLDL, fast and slow pools for intermediate density lipoprotein (IDL), and a single slow pool for LDL. The fractional catabolic rate (FCR) for C-VLDL apoB was 2.8 ± 1.0 h^{-1} and for L-VLDL apoB was 5.1 ± 2.0 h^{-1} ($P < 0.002$, paired t test). The fractions of control and lovastatin VLDL apoB converted to LDL averaged 0.15 ± 0.15 and 0.02 ± 0.02, respectively ($P < 0.05$, paired t test). Finally, the FCRs of LDL apoB derived from control and lovastatin VLDL were similar (0.059 ± 0.007 h^{-1} and 0.083 ± 0.038 h^{-1}, respectively; paired t test not significant). These data indicate that L-VLDL was irreversibly removed from the plasma of an untreated guinea pig more rapidly than was C-VLDL. Thus, the metabolic behavior of VLDL apoB is affected by lovastatin. Therefore, changes in lipoprotein particles themselves must be considered in assessing the overall impact of treatment with lovastatin.—**Berglund, L. F., W. F. Beltz, R. L. Elam, and J. L. Witztum.** Altered apolipoprotein B metabolism in very low density lipoprotein from lovastatin-treated guinea pigs. *J. Lipid Res.* 1994. **35:** 956–965.

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It is well known that perturbations of cholesterol metabolism result in changes in hepatic low density lipoprotein (LDL) receptor expression as well as in changes in the composition and biological properties of LDL (1–8). In previous experiments, we have demonstrated that hypolipidemic therapy with either bile acid resins or lovastatin alters the intrinsic metabolic properties of LDL, leading to a decrease in affinity of LDL for the LDL receptor (1–3). In guinea pigs, the decrease in LDL cholesterol and apolipoprotein B-100 (apoB) levels seen with lovastatin primarily reflected a decrease in LDL transport (3). The mechanism behind this effect is not clear. Huff and colleagues (9, 10) have reported a decrease in what has been termed the "direct LDL production pathway" in response to lovastatin in the miniature pig, a species in which this pathway has been reported to dominate LDL transport (10). Similar results have recently been reported in humans (11). However, whether or not a direct LDL production pathway actually occurs or is a construct of the experimental techniques or kinetic models used to describe apoB behavior is under debate (12).

We previously showed that LDL isolated from lovastatin-treated guinea pigs had a decreased fractional catabolic rate (FCR) compared to LDL isolated from untreated guinea pigs (3). Because lovastatin profoundly inhibits hepatic HMG-CoA reductase activity (13), the formation of very low density lipoprotein (VLDL), the precursors of LDL, would likely be affected (14). This, in turn, could result in altered LDL that would have a decreased FCR. In the present study, we test this hypothesis and report the effect of lovastatin on the physical and metabolic properties of very low density lipoprotein (VLDL) in the guinea pig. To directly compare VLDL derived from control and lovastatin-treated animals, the kinetics of these particles were determined in the same animals.

Abbreviations: VLDL, very low density lipoprotein; C-VLDL, VLDL from untreated animals; d, density in g/ml; FCR, fractional catabolic rate; IDL, intermediate density lipoprotein; L-VLDL, VLDL from lovastatin-treated animals; LDL, low density lipoprotein; PBS, phosphate-buffered saline.

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METHODS

Animals

Male Hartley guinea pigs, weighing approximately 0.5 kg, were purchased from Charles River Breeding Labs, Inc. (Wilmington, MA). They were fed either standard, fresh Purina guinea pig chow pellets or the same chow supplemented with 0.1% lovastatin by weight, as previously described (3). Lovastatin, a gift from Dr. A. Alberts, Merck Sharp & Dohme (Rahway, NJ), was pelleted into the chow by ICN Nutritional Biochemicals (Cleveland, OH). The animals were fed their assigned diets for 14 days before being used as either lipoprotein donors or recipients of labeled lipoproteins.

Lipoprotein preparation

For each treatment group, plasma from four to six animals was pooled and centrifuged at d 1.006 g/ml at 8°C for 18 h in a 60 Ti rotor in a Beckman L8-55 ultracentrifuge. The supernate was collected and washed with a second, identical d 1.006 g/ml spin. Isolated VLDL was dialyzed at 4°C against phosphate-buffered saline (PBS) containing 0.3 mM EDTA and filtered through a 0.45-μm filter (Millipore Corp., Bedford, MA). VLDL samples were radioiodinated using the iodine monochloride technique (15, 16), and extensively dialyzed against PBS. Specific activities of the VLDL preparations averaged 804 cpm/ng protein and greater than 96% of the radioactivity was precipitable with trichloroacetic acid (TCA). An average of 22 ± 10% of total radioactivity was extracted by ether and 31 ± 7% was precipitable by isopropanol (17).

Turnover studies

VLDL isolated from control and lovastatin-treated guinea pigs were labeled with either ^{125}I or ^{131}I. The isotope used to label the different VLDL was varied and, in one experiment, two aliquots of the same lovastatin VLDL preparation were labeled with different isotopes. In each experiment, a mixture of the two isotopes was injected into the exposed jugular vein of an untreated guinea pig, as previously described (1). Blood samples (0.2 ml) were obtained by cardiac puncture at 14 times over the following 48 h (5, 15, 30, and 45 min, and 1, 2, 4, 6, 9, 12, 18, 24, 36, and 48 h). Each blood sample was immediately placed into a tube containing solid EDTA, gently mixed, and stored at 4°C until plasma was separated by low speed centrifugation.

Separate aliquots of each plasma sample were centrifuged at densities of 1.006 and 1.025 g/ml in 235-μl ultracentrifuge tubes designed for the 42.2 Ti rotor. Human VLDL was used as a carrier. For the d 1.006 g/ml spin, a 35-μl aliquot of guinea pig plasma was added to a tube containing 75 μl human VLDL and 110 μl PBS. For the d 1.025 g/ml spin, a 35-μl aliquot of guinea pig plasma was added to a tube containing 75 μl human VLDL, 25 μl PBS, 18 μl of a 1.025 g/ml NaBr solution, and 67 μl of a 1.063 g/ml NaBr solution, giving a final density of 1.025 g/ml. All plasma samples obtained during the first 24 h of a single experiment (two or three animals) were spun together at 35,000 rpm at 15°C for 18 h in a 42.2 Ti rotor. On the following day, the two samples obtained during the last 24 h of the experiment were centrifuged in an identical manner.

After centrifugation, the floating fraction was indirectly illuminated with a focused microscope lamp, collected using a fine glass capillary pipette, and transferred to a 12 × 75 mm glass tube. To minimize loss of radioactivity, the pipette was rinsed with PBS and this was also added to the 12 × 75 mm tube. It was important to remove the floating lipoprotein fraction rapidly to prevent diffusion and all tubes in the chilled 42.2 Ti rotor were usually pipetted within 45 min. Subsequently, 50 μl of human plasma was added to each 12 × 75 mm glass tube and PBS was added to adjust the volume to 0.75 ml. The d > 1.006 and d > 1.025 g/ml fractions remaining in the 235-μl, 42.2 Ti tubes were kept at room temperature overnight to facilitate mixing, then transferred to 12 × 75 mm glass tubes. Each 42.2 Ti tube was carefully rinsed with 50 μl of PBS which was also added to the 12 × 75 mm glass tube. The volume was then adjusted to 0.75 ml with PBS.

ApoB-associated radioactivity was determined in each of the four isolated lipoprotein fractions using the isopropanol precipitation method described by Egusa et al. (17). Isopropanol (1 ml) was added to each 12 × 75 mm tube, the tube was vortexed and kept at room temperature overnight. Each tube was then centrifuged at room temperature at 2500 rpm for 30 min. The supernate was removed and radioactivity in the remaining pellet was counted in a double-channel gamma spectrophotometer equipped with programs to adjust for radioactive decay and for ^{131}I overlap in the ^{125}I counting window (1282 Compugamma, LKB Wallac Oy, Turku, Finland). This technique generated four turnover curves for each isotope in each animal: apoB-associated radioactivity in *1)* the d < 1.006 g/ml fraction (VLDL); *2)* the d > 1.006 g/ml fraction; *3)* the d < 1.025 g/ml fraction; and *4)* the d > 1.025 g/ml fraction (LDL), yielding a total of eight kinetic curves for each animal studied.

Analytical methods

Cholesterol and triglyceride contents were measured using standard techniques as described for the Lipid Research Clinics (18). Free and total cholesterol levels were determined enzymatically (1) and the amount of cholesteryl ester was calculated as 1.67 times the difference between these two determinations. Protein was measured as described by Lowry et al. (19), using bovine serum albumin as standard. Usually, the high lipid content of the VLDL fraction required that the tubes and

standards be extracted with ether before protein determination. Phospholipid content was measured using a modification of the procedure described by Bartlett (20). Polyacrylamide gel electrophoresis in the presence of SDS was carried out using 10–15% gradient gels in a horizontal gel system (Pharmacia Phast Gels, Pharmacia Fine Chemicals, Piscataway, NJ). Radiolabeled VLDL fractions derived from control and lovastatin-treated guinea pigs were also subjected to gel chromatography using 1.5% agarose gels (Bio-Rad Laboratories, Richmond, CA) in PBS.

KINETIC MODELING

The kinetic data were analyzed using multicompartmental analysis. All calculations were performed on a MicroVAX II digital computer (Digital Equipment Corp., Maynard, MA) using the SAAM/CONSAM program (21, 22). Only the eight directly measured turnover curves were included in the modeling. Radioactivity in the $1.006 < d < 1.025$ g/ml intermediate density lipoprotein (IDL) fraction was always analyzed together with either that in VLDL (in the $d < 1.025$ g/ml fraction) or that in LDL (in the $d > 1.006$ g/ml fraction). Although estimates of IDL radioactivity could have been calculated and included in the modeling, analysis of only the directly measured fractions has two distinct advantages. First, all data can be included. It was usually found that after about 20 h, the radioactivity in the $d > 1.006$ g/ml fraction was virtually identical to that in the $d > 1.025$ g/ml fraction and at several time points, the radioactivity measured in the $d > 1.025$ g/ml fraction exceeded that measured in the $d > 1.006$ g/ml fraction. If radioactivity in IDL were calculated as $f_{IDL}(t) = f_{d>1.006}(t) - f_{d>1.025}(t)$, then one would likely disregard those data for which $f_{d<1.006}(t) < f_{d>1.025}(t)$ and retain only those points for which $f_{d>1.006}(t) > f_{d>1.025}(t)$. The result would be a biased tail in the decay curve, because the lowest points would be eliminated. The same argument applies to the calculation of $f_{IDL}(t)$ as $f_{d<1.025}(t) - f_{d<1.006}(t)$. Second, consistent weights can be assigned to all data. Specifically, calculated IDL radioactivities would have a different error structure than would the directly measured VLDL and LDL radioactivities, while the $d > 1.006$ and $d < 1.025$ g/ml fractions can be weighted in a manner similar to that for VLDL and LDL (fractional standard deviations of 10% in the current study).

To develop a quantitative description of the tracer kinetics, the turnover data were analyzed with a multicompartmental model. This model, shown in **Fig. 1,** includes *1)* two plasma VLDL compartments, one turning over very rapidly and the other much more slowly; *2)* two plasma IDL compartments, which are also cleared at different rates; and *3)* a single plasma LDL compartment.

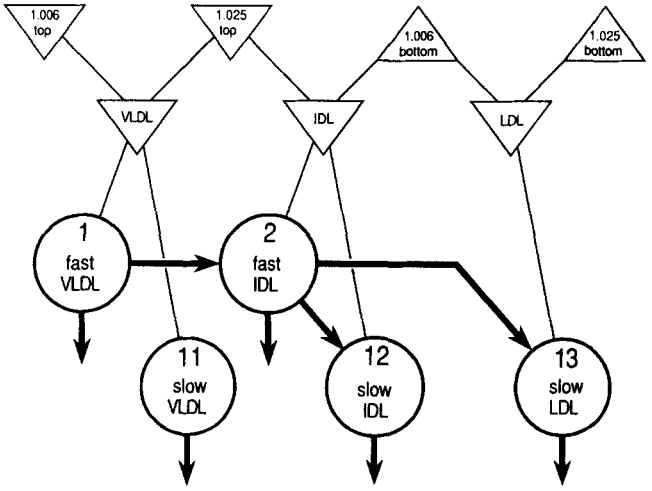

Fig. 1. Multicompartmental model used to quantify the kinetics of either control or lovastatin VLDL in guinea pigs. Circles represent kinetically distinct plasma pools of apoB. Only those pools that could be discerned in the current study are shown. A triangle indicates the sum of the contents of other compartments. Two levels of summing are shown. Triangles in the top row represent the indicated plasma density fractions and are the observed components of the model. The second row of triangles is included to demonstrate how the lipoprotein fractions VLDL, IDL, and LDL relate to the observed fractions as well as to the metabolic pools. Summing always occurs in an upward direction. Thus, $f_{VLDL}(t) = f_1(t) + f_{11}(t)$ and $f_{d<1.025}(t) = f_{VLDL}(t) + f_{IDL}(t) = f_1(t) + f_2(t) + f_{12}(t)$. Note that there is no direct measurement of the IDL pools.

The slowly metabolized pools of VLDL and IDL were necessary to fit the final slopes of the turnover curves. Because these experiments did not have an independent measure of LDL decay, multiple LDL compartments were not necessary to fit the current data. Also indicated in Fig. 1 are the relationships between the measured density fractions (top row of triangles) and the apoB plasma pools (circles). Although IDL apoB radioactivity was not measured directly or included in the analysis, the IDL subsystem (compartments 2 and 12) was required to simultaneously fit either the $d < 1.006$ g/ml and $d < 1.025$ g/ml data or the $d > 1.006$ g/ml and $d > 1.025$ g/ml data. The magnitude of the tail of the VLDL curve was fit by introducing radioactivity directly into compartment 11. Initial radioactivity was distributed among compartments 1, 11, 2, and 13 as needed to fit the early time points of the turnover data. The data for each isotope in each animal were analyzed separately.

To permit the calculation of fractional catabolic rates (FCRs), dummy production rates U_1 and U_{11} (mg/h) were introduced into the fast and slow VLDL compartments, respectively. Defining these production rates does not require that L-VLDL exist naturally in untreated animals, but simply allowed SAAM to calculate apoB masses for all compartments of the model. The values of U_1 and U_{11} were calculated such that $U_1 + U_{11} = 1$ and the initial specific activities would be equal in the two

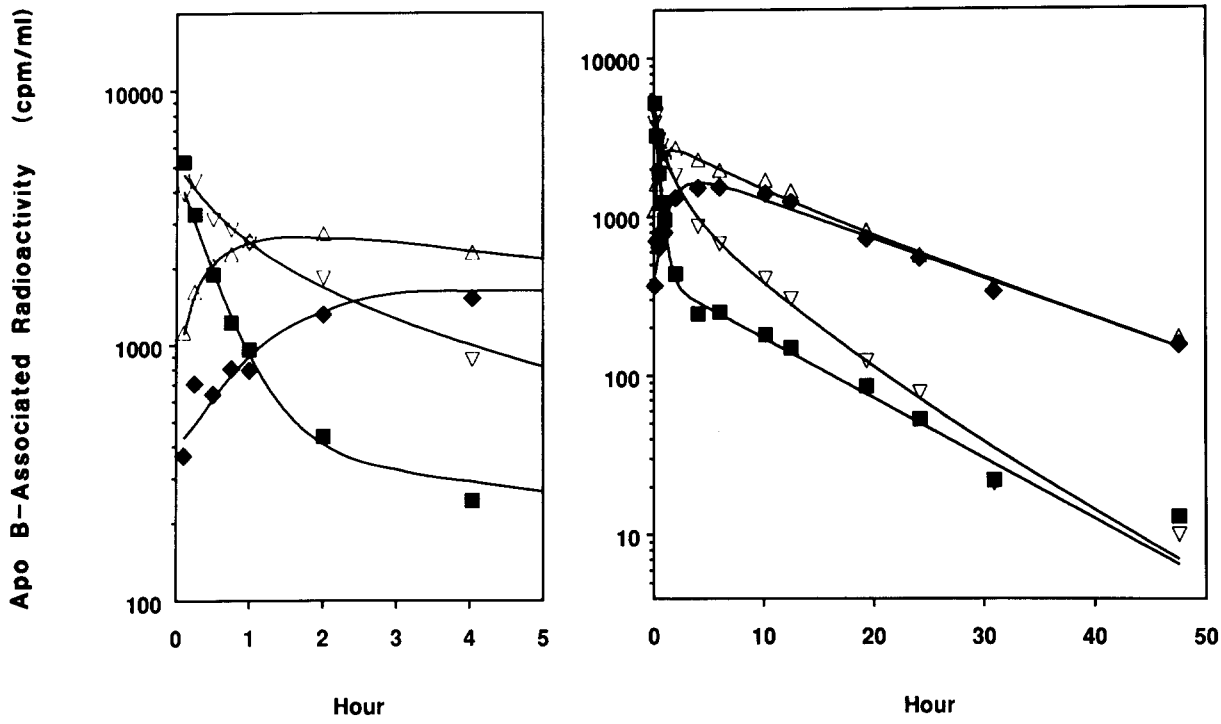

Fig. 2. ApoB kinetics for control VLDL injected into an untreated guinea pig. Discrete symbols represent observed radioactivity in the four measured density fractions: ■, d < 1.006 g/ml (VLDL); ♦, d > 1.025 g/ml (LDL); ▽, d < 1.025 g/ml (VLDL + IDL); △, d > 1.006 g/ml (IDL + LDL). Continuous lines are the best fits of the compartmental model to the observed data. All data are plotted in the right panel. The left panel is an expansion of the first 5 h of data.

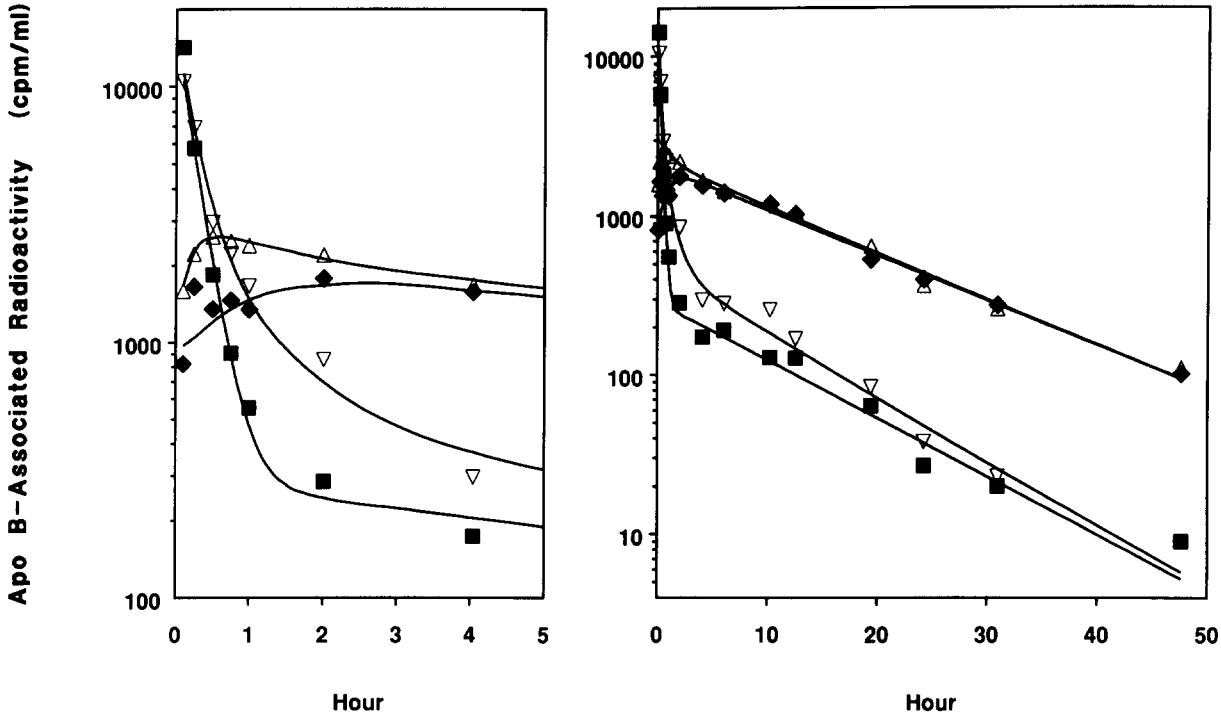

Fig. 3. ApoB kinetics for lovastatin VLDL injected into an untreated guinea pig. Discrete symbols represent observed radioactivity in the four measured density fractions: ■, d < 1.006 g/ml (VLDL); ♦, d > 1.025 g/ml (LDL); ▽, d < 1.025 g/ml (VLDL + IDL); △, d > 1.006 g/ml (IDL + LDL). Continuous lines are the best fits of the compartmental model to the observed data. All data are plotted in the right panel. The left panel is an expansion of the first 5 h of data.

TABLE 1. Compartmental model fractional transfer rates

	Control VLDL	Lovastatin VLDL	Paired t test
		h^{-1}	
$L_{0,1}$	2.03 ± 1.53	4.69 ± 2.12	$P < 0.001$
$L_{2,1}$	0.96 ± 0.71	0.48 ± 0.58	$P < 0.001$
$L_{0,2}$	0.34 ± 0.24	0.66 ± 0.52	$P < 0.05$
$L_{12,2}$	0.10 ± 0.06	0.13 ± 0.11	NS
$L_{13,2}$	0.20 ± 0.16	0.17 ± 0.23	NS
$L_{0,11}$	0.074 ± 0.011	0.058 ± 0.024	NS
$L_{0,12}$	0.091 ± 0.097	0.075 ± 0.056	NS
$L_{0,13}$	0.059 ± 0.007	0.083 ± 0.038	NS

$L_{i,j}$ is the fraction of apoB, either labeled or unlabeled, in compartment j that is transferred to compartment i per hour. $L_{0,j}$ is the fraction of apoB in compartment j that is irreversibly removed directly from compartment j per hour. Compartment numbers correspond to those of Fig. 1. Error terms are population standard deviations for the eight (8) guinea pigs of the current study. Paired t tests were performed on the differences between the control and lovastatin parameters for each guinea pig.

VLDL pools. VLDL FCR was calculated as $(U_1 + U_{11})/(M_1 + M_{11})$, where M_i is the mass of apoB (mg) in compartment i. IDL FCR was calculated as $R_{2,1}/(M_2 + M_{12})$ where $R_{2,1} = M_1 \times L_{2,1}$ is the absolute rate of transfer of unlabeled apoB (mg/h) from compartment 1 to compartment 2 and is therefore the total production rate of IDL apoB. LDL FCR was calculated as $R_{13,2}/M_3$ where $R_{13,2} = M_2 \times L_{13,2}$ is the total production rate of unlabeled LDL apoB. As $R_{13,2} = R_{0,13} = M_3 \times L_{0,13}$, the LDL FCR is numerically equal to $L_{0,13}$. The fractions of radioactivity appearing in IDL and LDL include any radioactivity observed in those fractions immediately following injection (see Table 3) and therefore exceed the fractions of VLDL converted by those amounts.

Representative fits of the model are shown as the solid lines in **Fig. 2** and **Fig. 3.** Individual parameter values for the model shown in Fig. 1 are presented in **Table 1** and **Table 2.**

TABLE 2. Initial distribution of radioactivity

	Control VLDL	Lovastatin VLDL	Paired t test
	percent of injected radioactivity		
VLDL	81 ± 6	88 ± 4	$P < 0.001$
Fast	76 ± 6	86 ± 5	$P < 0.001$
Slow	5 ± 2	2 ± 1	$P < 0.005$
IDL	5 ± 4	2 ± 3	NS
LDL	14 ± 3	10 ± 4	$P < 0.01$

Initial conditions were estimated simultaneously with the fractional transfer rates. Error terms are population standard deviations for the eight (8) guinea pigs of the current study. Paired t tests were performed on the differences between the control and lovastatin parameters for each guinea pig.

RESULTS

Effect of lovastatin on VLDL composition

We have previously shown that feeding 0.1% lovastatin to guinea pigs significantly lowered LDL levels but did not alter VLDL cholesterol levels (3). To determine whether lovastatin also altered VLDL composition, preparations of VLDL were prepared from two groups of control and lovastatin-treated guinea pigs (**Table 3**). No consistent differences in composition were observed between VLDL isolated from control animals (C-VLDL)

TABLE 3. Composition of VLDL isolated from control and lovastatin-treated guinea pigs

	Control VLDL A	Lovastatin VLDL A	Control VLDL B	Lovastatin VLDL B
	percent of total weight			
Esterified cholesterol (EC)	1.3	0.6	0.4	0.6
Free cholesterol (FC)	5.3	4.0	2.3	2.6
Total cholesterol (TC)	6.6	4.6	2.7	3.2
Triglyceride (TG)	72.4	74.1	78.8	79.7
Phospholipid (PL)	7.9	9.9	10.2	9.0
Protein	13.2	11.4	8.3	8.1
Ratios:				
Lipid/protein (w/w)	6.58	7.77	11.05	11.35
TG:protein (w/w)	5.48	6.50	9.49	9.84
TC:protein (w/w)	0.50	0.40	0.33	0.40
EC:protein (w/w)	0.10	0.05	0.05	0.07
FC:protein (w/w)	0.40	0.35	0.28	0.32
PL:protein (w/w)	0.60	0.87	1.23	1.11

Preparations A represent pools of control and lovastatin VLDL prepared from four to six guinea pigs. Preparations B represent the average VLDL compositions from three control and three lovastatin-treated guinea pigs, respectively.

and VLDL isolated from lovastatin-treated animals (L-VLDL). Agarose gel chromatography of C-VLDL and L-VLDL demonstrated that the peak for L-VLDL eluted somewhat later than that for C-VLDL, indicating a slight difference in size between the two VLDL samples (**Fig. 4**). SDS-PAGE of the isolated VLDL fractions did not reveal any difference in apolipoprotein composition. In particular, there was no observable difference in apolipoprotein E (apoE) content (data not shown).

Effect of lovastatin on VLDL apolipoprotein B metabolism

In these experiments we addressed the question whether lovastatin would influence the intrinsic metabolic properties of VLDL. We therefore directly compared clearance of C-VLDL and L-VLDL in the same animal. The recipient guinea pigs were always fed standard chow without lovastatin. Plasma apoB kinetics were determined in eight guinea pigs simultaneously injected with both C-VLDL and L-VLDL. These experiments consisted of three different pairs of VLDL preparations. In two of the experiments, control VLDL was labeled with ^{131}I and lovastatin VLDL was labeled with ^{125}I. In the third experiment, the isotopes were reversed. In a fourth experiment, separate aliquots of VLDL from a lovastatin-treated animal were labeled with the ^{125}I and ^{131}I and plasma decay curves were determined in a single recipient. The kinetic results for lipoproteins labeled with the two isotopes in the latter experiment were similar (data not shown).

Representative kinetic curves for the four measured density fractions of one guinea pig are shown in Fig. 2 for C-VLDL and in Fig. 3 for L-VLDL. Because the initial decay is very rapid for both VLDL fractions, the data for the first 5 h after injection are also plotted on an expanded scale in the left panel of each figure. It is clearly seen that the initial component of VLDL decay is more rapid for L-VLDL than for C-VLDL. Also apparent from inspection of Fig. 2 and Fig. 3 is the greater fraction of apoB-associated radioactivity transferred to LDL ($d > 1.025$ g/ml) from C-VLDL than from L-VLDL. These two observations were found in all animals studied, whether L-VLDL was labeled with ^{125}I or with ^{131}I. To ease the direct comparison of C-VLDL and L-VLDL kinetics, the VLDL ($d < 1.006$ g/ml) and LDL ($d > 1.025$ g/ml) data for both isotopes are plotted in **Fig. 5.**

Results obtained using the model shown in Fig. 1 and described in Kinetic Modeling are summarized in **Table 4.** These results confirm the visual inspection of the data in that *1)* L-VLDL was irreversibly removed from the VLDL fraction at a much greater rate than was C-VLDL; and *2)* a greater fraction of C-VLDL apoB was converted to LDL ($d > 1.025$ g/ml) than of L-VLDL. Results not as readily apparent from visual inspection of the data were that *1)* a greater fraction of C-VLDL apoB was converted to IDL than was L-VLDL apoB; and *2)* the fractional

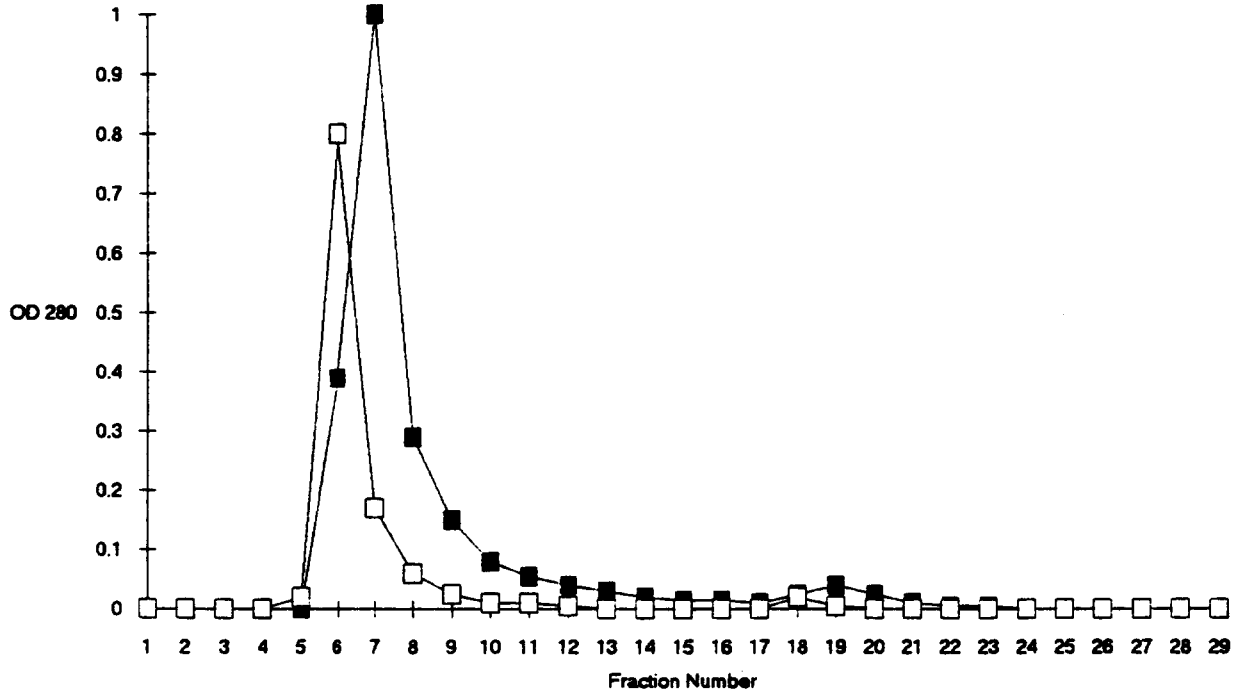

Fig. 4. Agarose gel chromatography of VLDL from control guinea pigs (□) and from guinea pigs treated with lovastatin (■). Compositions of the VLDL used in this analysis are shown in Table 3 as Preparation A.

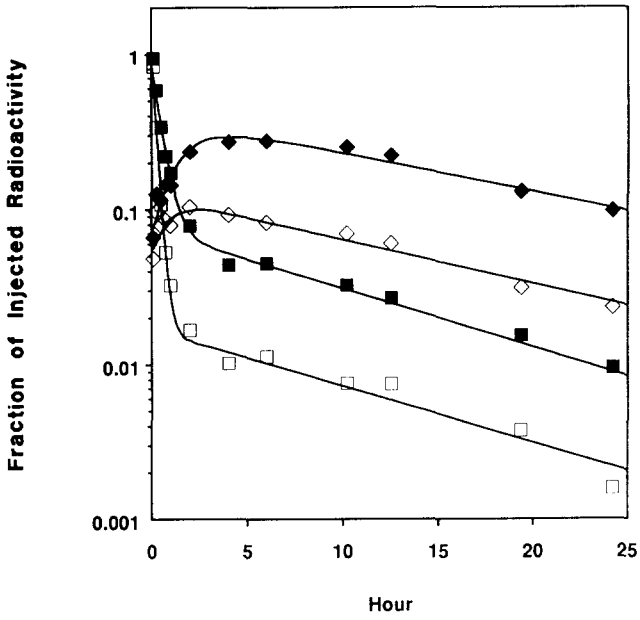

Fig. 5. Comparison of kinetics of apoB as measured in VLDL and LDL following the injection of ^{131}I-labeled control VLDL and ^{125}I-labeled lovastatin VLDL into the same untreated guinea pig. Discrete symbols represent observed radioactivities: ■, ^{131}I in VLDL (d < 1.006 g/ml); ♦, ^{131}I in LDL (d > 1.025 g/ml); □, ^{125}I in VLDL (d < 1.006 g/ml); ◊, ^{125}I in LDL (d > 1.025 g/ml). Continuous lines are the best fits of the compartmental model to the observed data. Only data for the first 24 h are shown.

catabolic rates (FCRs) of apoB in the LDL compartment were similar for the two labels.

DISCUSSION

We have previously shown that LDL isolated from lovastatin-treated guinea pigs have different in vivo metabolic properties than LDL isolated from control donors (3). In this study we compared the metabolic behavior of VLDL apoB derived from lovastatin-fed guinea pigs (L-VLDL) to that derived from control guinea pigs (C-VLDL). To make a direct assessment of the importance of the source of VLDL for its clearance rate, three different pairs of C-VLDL and L-VLDL were injected simultaneously into eight untreated guinea pigs. In all animals, L-VLDL apoB was irreversibly cleared from plasma at a greater rate than C-VLDL apoB and a reduced fraction of L-VLDL apoB was converted to IDL-associated and LDL-associated apoB than was C-VLDL apoB. Taken together, these findings indicate that lovastatin alters the intrinsic metabolic properties of VLDL as we previously observed for LDL (3). As lovastatin and other similar compounds inhibit hepatic HMG-CoA reductase activity (13, 23), it is not unlikely that a decrease in endogenous cholesterol production might affect the synthesis and composition of hepatic lipoproteins. Indeed, it has been reported that lovastatin therapy in humans reduces the cholesterol to protein ratio in VLDL (24). However, we could not document any consistent changes in the gross composition of L-VLDL in the guinea pig other than an apparently smaller size observed on agarose gel chromatography. Also in other studies, lovastatin treatment has not resulted in major alterations of VLDL composition (10). Although our methods to detect such changes were not sufficiently sensitive, alterations in apoB metabolism suggest that important changes must have occurred in the VLDL or in its apolipoprotein content or conformation in response to lovastatin treatment. VLDL metabolism is obviously complex (12, 25), involves rapid delipidation steps (26, 27) and multiple interactions with enzymes and other lipoproteins. Any of these could be altered. Thus, whatever the mechanisms responsible, it is clear that lovastatin alters the intrinsic metabolic properties of both VLDL and LDL.

We previously found in lovastatin-treated guinea pigs that LDL levels were lowered because of an apparent decrease in LDL transport despite the fact that a marked increase in hepatic LDL receptor activity had occurred (3). Taken at face value, the present data suggest that intrinsic changes in L-VLDL result in less being converted to LDL. This is compatible with the reported decrease in LDL transport in humans treated with lovastatin (11, 28, 29). However, caution must be urged in making this interpretation in guinea pigs as VLDL samples must first be studied in lovastatin-treated animals to fully characterize these complex metabolic events (see below).

The present data could not distinguish a few features of apoB metabolism that have been suggested elsewhere.

TABLE 4. Results from compartmental model

	Control VLDL	Lovastatin VLDL	Paired *t* test
VLDL FCR (h^{-1})	2.8 ± 1.0	5.1 ± 2.0	$P < 0.01$
IDL FCR (h^{-1})	0.44 ± 0.19	0.71 ± 0.47	NS
LDL FCR (h^{-1})	0.059 ± 0.007	0.083 ± 0.038	NS
Fraction of radioactivity appearing in IDL	0.32 ± 0.22	0.11 ± 0.08	$P < 0.01$
Fraction of radioactivity appearing in LDL	0.27 ± 0.12	0.12 ± 0.04	$P < 0.01$
Fraction of VLDL converted to IDL	0.38 ± 0.30	0.11 ± 0.10	$P < 0.01$
Fraction of VLDL converted to LDL	0.15 ± 0.15	0.02 ± 0.02	$P < 0.05$

FCRs were calculated as described in the text.

These are shown as shaded components in **Fig. 6.** This figure is identical to Fig. 1 except for the addition of these elements. First, it could not be determined whether slow VLDL was derived from fast VLDL or was produced de novo. Second, as LDL was only labeled endogenously via conversion from labeled VLDL, the presence of a fast LDL pool may not have been reflected in the kinetic data. Third, slow VLDL may be converted to slow IDL. Yamada et al. (30) have shown kinetic heterogeneity related to apoE content in the VLDL, IDL, and LDL of the rabbit. In their model, slow, apoE-deficient VLDL is converted directly to slow, apoE-deficient IDL. Although not necessary to fit the present data, the existence of this pathway could not be ruled out.

In previous experiments we have found that in some situations the choice of iodine isotope used for LDL labeling could affect its clearance rate, ^{131}I-labeled LDL being cleared at a somewhat faster rate than ^{125}I-labeled LDL (3, 31). In the present experiments, we therefore varied the isotopes between the two VLDL fractions. In six of the eight animals studied, C-VLDL was labeled with ^{131}I. In spite of this, C-VLDL was always cleared more slowly than L-VLDL. To further address this issue, L-VLDL was labeled with both ^{125}I and ^{131}I and a mixture of the two tracers was injected into one animal. The degree of clearance of the two tracers was similar, further arguing against a significant isotope effect on VLDL clearance under the conditions used in these experiments.

In apparent contrast to our previous study on the effects of lovastatin on LDL metabolism (3), no significant difference in clearance between the two LDL apoB labels was found in the present study. The similarity of the LDL FCRs for the two isotopes suggests that the metabolic properties of LDL were determined by the metabolic state of the guinea pig more than by the inherent properties of the VLDL from which the LDL was derived. Thus, control guinea pigs appear to make "control LDL" out of "lovastatin VLDL." However, the heterogeneity of LDL cannot be addressed by following only the apoB tracer derived from VLDL, and the effect of lovastatin on LDL metabolism is more complex than merely an effect on VLDL clearance. Previous studies in animals (32) and man (33) support the in vitro observation that HMG-CoA reductase inhibitors lead to stimulation of hepatic LDL receptor mRNA and activity (7). In vivo metabolic studies, however, have led to several suggestions regarding the mechanisms by which these agents lower LDL levels. These include increased removal of LDL particles (7, 11, 34, 35), decreased LDL transport due to decreased VLDL production (11, 28, 29, 36), decreased LDL transport due to decreased "direct production" of LDL (9–11), and decreased LDL transport due to increased removal of precursors (present study).

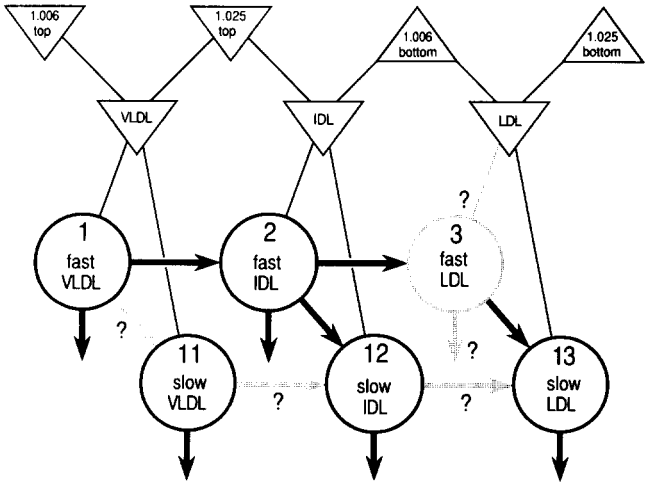

Fig. 6. Hypothetical multicompartmental model that might be used to quantify the kinetics of VLDL in guinea pigs. Circles represent kinetically distinct plasma pools of apoB. Shaded circle and arrows indicate those components that could be discerned in the current study but have been suggested in the literature. See text for further discussion.

In the current study, VLDL apoB isolated from lovastatin-treated guinea pigs had enhanced removal from plasma within the VLDL density range ($d < 1.006$ g/ml). This phenomenon could contribute to the decreased VLDL levels seen with lovastatin therapy as well as to decreased LDL production, since the fractional conversion of VLDL apoB to LDL is greatly reduced. An increase in direct removal of VLDL apoB as well as a reduction of the conversion of VLDL apoB to LDL has also been reported during combined lovastatin and fish oil treatment of miniature pigs (37). Thus, the changes in VLDL may contribute to reductions in both VLDL and LDL levels. These results were obtained from complex kinetic studies and complex models to evaluate data obtained in heterogeneous populations. Most studies have assumed that the intrinsic metabolic properties of the tracers have not changed between the control and treated states. Our studies in guinea pigs suggest that lovastatin affects the intrinsic metabolic properties of both VLDL and LDL as well as properties of the host's metabolic pathways. Thus, from a physiological standpoint, it is important to consider the effects of a given perturbation on both VLDL and LDL as well as metabolic pathways in a homologous system. Further studies on these complex issues are warranted.

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REFERENCES

1. Witztum, J. L., S. G. Young, R. L. Elam, T. E. Carew, and M. Fisher. 1985. Cholestyramine-induced changes in low density lipoprotein composition and metabolism. I. Studies in the guinea pig. *J. Lipid Res.* **26:** 92–103.
2. Young, S. G., J. L. Witztum, T. E. Carew, R. W. Krauss, and F. T. Lindgren. 1989. Colestipol-induced changes in LDL composition and metabolism. II. Studies in humans. *J. Lipid Res.* **30:** 225–238.
3. Berglund, L., M. F. Sharkey, R. L. Elam, and J. L. Witztum. 1989. Effects of lovastatin therapy on guinea pig low density lipoprotein composition and metabolism. *J. Lipid Res.* **30:** 1591–1600.
4. Brown, M. S., and J. L. Goldstein. 1986. A receptor-mediated pathway for cholesterol homeostasis. *Science.* **232:** 34–47.
5. Mahley, R. W., and T. L. Innerarity. 1983. Lipoprotein receptors and cholesterol homeostasis. *Biochim. Biophys. Acta.* **737:** 197–222.
6. Witztum, J. L., G. Schonfeld, S. W. Weidman, W. E. Giese, and M. A. Dillingham. 1979. Bile sequestrant therapy alters the compositions of low-density and high-density lipoproteins. *Metabolism.* **28:** 221–229.
7. Bilheimer, D. W., S. M. Grundy, M. S. Brown, and J. L. Goldstein. 1983. Mevinolin and colestipol stimulate receptor-mediated clearance of low density lipoprotein from plasma in familial hypercholesterolemia heterozygotes. *Proc. Natl. Acad. Sci. USA.* **80:** 4124–4128.
8. Shepherd, J., C. J. Packard, S. Bicker, T. D. Lawrie, and H. G. Morgan. 1980. Cholestyramine promotes receptor-mediated low-density-lipoprotein catabolism. *N. Engl. J. Med.* **302:** 1219–1222.
9. Huff, M. W., D. E. Telford, K. Woodcroft, and W. L. Strong. 1985. Mevinolin and cholestyramine inhibit the direct synthesis of low density lipoprotein apolipoprotein B in miniature pigs. *J. Lipid Res.* **26:** 1175–1186.
10. Huff, M. W., and D. E. Telford. 1989. Regulation of low density lipoprotein apoprotein B metabolism by lovastatin and cholestyramine in miniature pigs: effects on LDL composition and synthesis of LDL subfractions. *Metabolism.* **38:** 256–264.
11. Arad, Y., R. Ramakrishnan, and H. N. Ginsberg. 1990. Lovastatin therapy reduces low density lipoprotein apoB levels in subjects with combined hyperlipidemia by reducing the production of apoB-containing lipoproteins: implications for the pathophysiology of apoB production. *J. Lipid Res.* **31:** 567–582.
12. Shames, D. M., and R. J. Havel. 1991. De novo production of low density lipoproteins: fact or fancy. *J. Lipid Res.* **32:** 1099–1112.
13. Alberts, A. W. 1988. Discovery, biochemistry and biology of lovastatin. *Am. J. Cardiol.* **62:** 10J–15J.
14. Ginsberg, H. N., N. A. Le, M. P. Short, R. Ramakrishnan, and R. J. Desnick. 1987. Suppression of apolipoprotein B production during treatment of cholesteryl ester storage disease with lovastatin. Implications for regulation of apolipoprotein B synthesis. *J. Clin. Invest.* **80:** 1692–1697.
15. McFarlane, A. S. 1958. Efficient trace-labeling of proteins with iodine. *Nature.* **182:** 53–57.
16. Bilheimer, D. W., S. Eisenberg, and R. I. Levy. 1972. The metabolism of very low density lipoprotein proteins. I. Preliminary in vitro and in vivo observations. *Biochim. Biophys. Acta.* **260:** 212–221.
17. Egusa, G., D. W. Brady, S. M. Grundy, and B. V. Howard. 1983. Isopropanol precipitation method for the determination of apolipoprotein B specific activity and plasma concentrations during metabolic studies of very low density lipoprotein and low density lipoprotein apolipoprotein B. *J. Lipid Res.* **24:** 1261–1267.
18. Lipid Research Clinics Program. 1974. Manual of Laboratory Operations. DHEW Publication, NIH 75-628, National Institutes of Health, Bethesda, MD.
19. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
20. Bartlett, G. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234:** 466–468.
21. Berman, M., and M. F. Weiss. 1978. SAAM Manual. National Cancer Institute, National Institutes of Health, U.S. Dept. of Health, Education, and Welfare, Bethesda, MD.
22. Berman, M., W. F. Beltz, P. C. Greif, R. Chabay, and R. C. Boston. 1983. CONSAM User's Guide. National Cancer Institute, National Institutes of Health, U.S. Dept. of Health and Human Services, Bethesda, MD.
23. Grundy, S. M. 1988. HMG-CoA reductase inhibitors for treatment of hypercholesterolemia. *N. Engl. J. Med.* **319:** 24–33.
24. Nozaki, S., G. L. Vega, R. J. Haddox, E. T. Dolan, and S. M. Grundy. 1990. Influence of lovastatin on concentrations and composition of lipoprotein subfractions. *Atherosclerosis.* **84:** 101–110.
25. Shepherd, J., and C. J. Packard. 1987. Metabolic heterogeneity in very low-density lipoproteins. *Am. Heart J.* **113:** 503–508.
26. Zech, L. A., S. M. Grundy, D. Steinberg, and M. Berman. 1979. Kinetic model for production and metabolism of very low density lipoprotein triglycerides. Evidence for a slow production pathway and results for normolipidemic subjects. *J. Clin. Invest.* **63:** 1262–1273.
27. Beltz, W. F., Y. A. Kesäniemi, N. H. Miller, W. R. Fisher, S. M. Grundy, and L. A. Zech. 1990. Studies on the metabolism of apolipoprotein B in hypertriglyceridemic subjects using simultaneous administration of tritiated leucine and radioiodinated very low density lipoprotein. *J. Lipid Res.* **31:** 361–374.
28. Vega, G. L., R. M. Krauss, and S. M. Grundy. 1990. Pravastatin therapy in primary moderate hypercholesterolemia: changes in metabolism of apolipoprotein B-containing lipoproteins. *J. Intern. Med.* **227:** 81–94.
29. Vega, G. L., C. East, and S. M. Grundy. 1988. Lovastatin therapy in familial dysbetalipoproteinemia: effects on kinetics of apolipoprotein B. *Atherosclerosis.* **70:** 131–143.
30. Yamada, N., D. M. Shames, J. B. Stoudemire, and R. J. Havel. 1986. Metabolism of lipoproteins containing apolipoprotein B-100 in blood plasma of rabbits: heterogeneity related to the presence of apolipoprotein E. *Proc. Natl. Acad. Sci. USA.* **83:** 3479–3483.
31. Khouw, A. S., S. Parthasarathy, and J. L. Witztum. 1993. Radioiodination of low density lipoprotein initiates lipid peroxidation: protection by use of antioxidants. *J. Lipid Res.* **34:** 1483–1496.
32. Ma, P. T. S., G. Gil, T. C. Südhof, D. W. Bilheimer, J. L. Goldstein, and M. S. Brown. 1986. Mevinolin, an inhibitor of cholesterol synthesis, induces mRNA for low density lipoprotein receptor in livers of hamsters and rabbits. *Proc. Natl. Acad. Sci. USA.* **83:** 8370–8374.
33. Reihnér, E., M. Rudling, D. Stahlberg, L. Berglund, S. Ewerth, I. Björkhem, K. Einarsson, and B. Angelin. 1990.

Influence of pravastatin, a specific inhibitor of HMG-CoA reductase, on hepatic metabolism of cholesterol. *N. Engl. J. Med.* **323:** 224–228.
34. Malmendier, C. L., J. F. Lontie, C. Delcroix, and T. Magot. 1989. Effect of simvastatin on receptor-dependent low density lipoprotein catabolism in normocholesterolemic human volunteers. *Atherosclerosis.* **80:** 101–109.
35. Vega, G. L., C. East, and S. M. Grundy. 1989. Effects of combined therapy with lovastatin and colestipol in heterozygous familial hypercholesterolemia. Effects on kinetics of apolipoprotein B. *Arteriosclerosis.* **9:** I135–I144.
36. Cortner, J. A., M. J. Bennett, N. A. Le, and P. M. Coates. 1993. The effect of lovastatin on very low-density lipoprotein apolipoprotein B production by the liver in familial combined hyperlipidaemia. *J. Inherit. Metab. Dis.* **16:** 127–134.
37. Huff, M. W., D. E. Telford, and P. H. Barrett. 1992. Dietary fish oil plus lovastatin decreases both VLDL and LDL apoB production in miniature pigs. *Arterioscler. Thromb.* **12:** 902–910.