Mevinolin and cholestyramine inhibit the direct synthesis of low density lipoprotein apolipoprotein B in miniature pigs

Murray W. Huff,[1] Dawn E. Telford, Kim Woodcroft, and Wendy L. P. Strong

Department of Medicine, University Hospital, University of Western Ontario, London, Ontario, Canada N6A 5A5

Abstract Previous studies established that following simultaneous injection of ^{125}I-labeled homologous very low density lipoproteins (VLDL) and ^{131}I-labeled homologous low density lipoproteins (LDL) into miniature pigs, a large proportion of LDL apolipoprotein B (apoB) was synthesized directly, independent of VLDL or intermediate density lipoprotein (IDL) apoB catabolism. The possibility that cholestyramine alone (a bile acid sequestrant) or in combination with mevinolin (a cholesterol synthesis inhibitor) could regulate the direct LDL apoB synthetic pathway was investigated. ^{125}I-labeled VLDL and ^{131}I-labeled LDL were injected into miniature pigs (n = 8) during a control period and following 18 days of cholestyramine treatment (1.0 g $kg^{-1}d^{-1}$) or following 18 days of treatment with cholestyramine and mevinolin (1.2 mg $kg^{-1}d^{-1}$). ApoB in each lipoprotein fraction was selectively precipitated using isopropanol in order to calculate specific activity. In control experiments, LDL apoB specific activity curves reached their peak values well before crossing the VLDL or IDL apoB curves. However, cholestyramine treatment resulted in LDL apoB curves reaching maximal values much closer to the point of intersection with the VLDL or IDL curves. Kinetic analyses demonstrated that cholestyramine reduced total LDL apoB flux by 33%, which was due entirely to inhibition of the LDL apoB direct synthesis pathway since VLDL-derived apoB was unaffected. In addition, the LDL apoB pool size was reduced by 30% and the fractional catabolic rate of LDL apoB was increased by 16% with cholestyramine treatment. The combination of mevinolin and cholestyramine resulted in an even more marked inhibition of the direct LDL apoB synthesis pathway (by 90%), and in two animals this pathway was completely abolished. This inhibition was selective as VLDL-derived LDL apoB synthesis was not significantly different. LDL apoB pool size was reduced by 60% due primarily to the reduced synthesis as well as a 40% greater fractional removal rate. These results are consistent with the idea that cholestyramine and mevinolin increase LDL catabolism by inducing hepatic apoB, E receptors. We have now shown that the direct synthesis of LDL apoB is selectively inhibited by these two drugs. —**Huff, M. W., D. E. Telford, K. Woodcroft, and W. L. P. Strong.** Mevinolin and cholestyramine inhibit the direct synthesis of low density lipoprotein apolipoprotein B in miniature pigs. *J. Lipid Res.* 1985. **26:** 1175–1186.

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Metabolic heterogeneity of low density lipoprotein (LDL) formation has been described in a number of apolipoprotein B (apoB) kinetic studies in several species (1–4). Apolipoprotein B, the major structural apolipoprotein of LDL as well as very low density lipoproteins (VLDL), does not exchange between lipoproteins and thus provides a useful marker for the metabolism of these lipoproteins (5). In addition, apoB is the determinant for receptor-mediated catabolism of LDL (6). In normal man, apoB, secreted with triglyceride-rich VLDL from the liver, is converted sequentially to intermediate density lipoproteins (IDL) and then to LDL (7–9). In normolipidemic subjects, all LDL apoB is derived from the catabolism of VLDL and IDL (7). In contrast, in rats (3, 4) and primates (1, 2), a significant proportion of LDL apoB can be secreted by a pathway that is independent of VLDL and IDL catabolism. We have demonstrated in miniature pigs that over 80% of LDL apoB is synthesized directly, the remainder being derived from VLDL (10). This species is unlike the rat in that LDL is the major cholesterol-transporting lipoprotein (11).

A greater understanding of these deviations from absolute precursor-product relationships becomes important because familial hypercholesterolemia in man is characterized by substantial direct production of LDL independent of VLDL catabolism in both homozygous (12) and heterozygous (13) subjects. Factors that control the relative contribution of these two major LDL synthetic pathways are largely unknown. It has been shown that cholesterol feeding increases the secretion of cholesteryl ester-rich LDL from hepatic Golgi apparatus (14) and perfused

Abbreviations: VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; apoB, apolipoprotein B; FCR, fractional catabolic rate; SA, specific activity.

[1] To whom all correspondence should be addressed.

animal livers (15–17). These studies indicate that the primary origin of direct LDL synthesis is hepatic. We therefore reasoned that the direct synthesis of LDL may be related to the availability of hepatic cholesterol. Thus, we hypothesized that if we depleted the supply of liver cholesterol, the direct pathway of LDL formation in miniature pigs might be inhibited.

Cholestyramine has been used in animals (18–20) and man (21) to deplete hepatic cholesterol content, which results in increased hepatic LDL receptor activity and/or increased fractional catabolic rate (FCR) of plasma LDL, thereby lowering plasma LDL concentrations. A further approach to hepatic cholesterol depletion is by selective inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A reductase by mevinolin. Mevinolin alone or in combination with cholestyramine also increased hepatic LDL receptor activity and increased plasma LDL FCR in dogs (22), rabbits (23), and man (24–26). Although total LDL synthesis was reduced in the animal studies (22), the effect of these two drugs on direct versus VLDL-derived LDL synthesis has not been investigated.

The present studies were carried out to determine whether cholestyramine, or both cholestyramine and mevinolin could selectively inhibit the direct synthesis pathway of LDL, a pathway we have demonstrated previously in miniature pigs (10). In addition, we wanted to investigate the effect of these two drugs on VLDL metabolism and the conversion of VLDL apoB to LDL apoB.

METHODS

Animals and drugs

Miniature pigs weighing between 20 and 35 kg were obtained from a local supplier (Hyde Park Farms). They were maintained on Purina pig chow (containing 16% protein and less than 5% fat; Ralston Purina Canada, Longueuil, Quebec). After one week acclimatization, an indwelling silastic catheter was surgically implanted in each external jugular vein. The catheters were tunnelled under the skin and externalized in the middle of the back. A three-way stopcock was attached and the catheter was kept patent by filling it with sterile 7% EDTA-Na_2. The stopcock and externalized portion of the catheter were taped to the pig and held in place with elastic netting. This allowed for ease of sample injection as well as blood sampling throughout the experiment, without any need for anesthetization of the animal. Each pig received cholestyramine (1 g/day per kg body weight; approximately 24 g/day) for 18 days prior to lipoprotein turnover studies. The cholestyramine was mixed with the food, and the animals consumed 750 g/day. The cholestyramine was a gift from Bristol Myers Canada, Ottawa, Ontario. Mevinolin (MK-803), supplied by Merck Frosst Canada, Dorval, Quebec, was fed to each pig (1.2 mg/kg body weight/day) in gelatin capsules for 18 days. The capsules along with a few food pellets were fed by hand immediately before daily feedings to ensure ingestion of the drug. Each pig acted as its own control, and lipoprotein turnover studies were carried out at the end of both control and drug-regimen periods.

Two series of experiments were carried out, one in which lipoprotein turnover was studied in four miniature pigs during a control period and again during a period in which the diet contained cholestyramine. The second series of four animals compared a control period and a period in which the diet contained both cholestyramine and mevinolin.

Lipoprotein turnover studies

Studies were conducted essentially as described previously (7, 10). VLDL and LDL for radiolabeling were isolated from approximately 100 ml of plasma, obtained following a 16-hr fast. Blood was collected into sterile tubes containing EDTA (1.5 mg/ml). VLDL (S_f 20–400) was isolated at d < 1.006 g/ml in a Beckman 60 Ti rotor (16 hr, 45,000 rpm at 12°C). The VLDL recovered by aspiration was washed once (in a 50 Ti rotor under the same conditions) through an equal volume of buffered saline (0.15 M NaCl, 1 mM EDTA, 1 mM Tris, pH 7.4). LDL was isolated between density 1.019 and 1.063 g/ml (11) from the VLDL infranatant in the 60 Ti rotor (24 hr, 45,000 rpm at 12°C), and washed once (in the 50 Ti rotor, under the same conditions) at d < 1.063 g/ml. Radioiodination was performed using the iodine monochloride method as modified by Fidge and Poulis (27) as described previously for pigs (10). The lipoproteins were equilibrated to 0.4 M glycine buffer, pH 10, and were labeled with ^{125}I or ^{131}I (Frosst, Montreal, Quebec). Lipoproteins were sterilized by the addition of 100 μg/ml gentamycin sulfate (Schering) and checked for sterility and pyrogenicity. Using methods described previously (7, 27), labeled pig VLDL was found to contain less than 1% free iodine, and approximately 5–12% of the label was associated with lipid. Labeled pig LDL contained less than 1% free iodine and 7–8% of the label was bound to lipid. The percent of iodine bound to apoprotein B was 31–35% for VLDL and 89–96% for LDL (28). In each study, each pig received 20 μCi of ^{125}I-labeled pig VLDL-apoB and 15 μCi of ^{131}I-labeled LDL-apoB. Animals were fasted 16 hr prior to each study. Autologous labeled lipoproteins were infused into one catheter, followed immediately by 10 ml of saline. Following injection, blood samples (12 ml) were obtained from the other catheter at 5, 15, 30, 45 min, 1, 1.5, 2, 3, 4, 6, 12, and 24 hr. Blood samples were taken up to 72 hr in studies of LDL metabolism to enable calculation of LDL apoB kinetic parameters. Pigs received no food until after the 12-hr sample, and were fed following

the 24- and 48-hr samples. This procedure limited the contribution of intestinally derived particles (containing apoB) as discussed previously for studies in man (7, 28), and resulted in constant concentrations of VLDL, IDL, and LDL apoB throughout the turnover studies. The average coefficients of variation of apoB concentrations in VLDL, IDL, and LDL over each turnover study were 9.1% (range 7–11%), 8.1% (range 6–11%), and 7.9% (range 5–9%), respectively. The variations were within the variation of the apoB precipitation method, and no trend in the variation with time was observed. In pigs receiving mevinolin and cholestyramine, approximately 1 hr before injection, pigs were given half the daily dose of mevinolin. The remainder was given along with the daily dose of cholestyramine at the 12-hr feeding.

Blood samples, collected into tubes containing EDTA (1.5 mg/ml), were kept on ice until the separation of plasma. VLDL, IDL, and LDL were isolated from 6.5 ml of plasma by sequential ultracentrifugation in a Beckman 50 Ti rotor. VLDL (d < 1.006 g/ml), IDL (d 1.006–1.019 g/ml) and LDL (d 1.019–1.063 g/ml) were washed once by recentrifugation through a salt solution of the appropriate density. Apoprotein B was isolated from each fraction with isopropanol by minor modifications of methods described previously by Huff et al. (28) and Egusa et al. (29). Seventy to 200 μg of each lipoprotein solution was adjusted to 1 ml with saline, added drop by drop to 1 ml of isopropanol while vortexing, and incubated at room temperature overnight. ApoB was pelleted by centrifugation (1000 *g*, 4°C, 30 min) and the supernatant was aspirated, leaving behind 0.5 ml on top of the pellet. The pellet was washed twice with 2 ml of isopropanol–water 1:1, once with 3 ml of isopropanol, and once with 3 ml of water, leaving behind the last 0.5 ml at each wash. To resolubilize the apoB, samples were incubated at 37°C for 24 hr (VLDL and IDL) or 48 hr (LDL) following the addition of 125 μl of 2 N NaOH. To determine specific activity (SA), samples were counted and protein was determined by the method of Markwell et al. (30) except that NaOH was omitted from the Lowry reagent. The supernatant from the first precipitation was assayed for protein (29) and the difference between this value and total lipoprotein protein was used as a measure of apoB concentration.

Kinetic analyses

Kinetic parameters were calculated from the VLDL or LDL SA disappearance curve. Throughout the study period, VLDL apoB and LDL apoB SA data were best described by a biexponential curve and were analyzed as described by Gurpide, Mann, and Sandberg (31). Curve parameters were calculated by computer using a nonlinear least squares technique (32). The Fisher F statistic was used to test the appropriateness of a two-pool model. The use of the Gurpide model for the study of apoprotein B kinetics has been discussed previously (7, 10, 28, 33). Kinetic parameters calculated from biexponential curves yielded values for flux of material through pool 1, irreversible FCR, and the mass in pool 1. The production rate or flux of VLDL apoB in the Gurpide model is defined as representing input of apoB exclusively into the much larger primary metabolic compartment (pool 1) and excludes any apoB that recycles between the two pools (7). The biexponential nature of the VLDL apoB specific activity curve may reflect a combination of factors including heterogeneity of turnover rates among several populations of VLDL particles and extravascular equilibration (7).

The ^{125}I-labeled apoB specific activity curves for VLDL, IDL, and LDL were compared (**Fig. 1A**); this allowed for the examination of precursor-product relationships among the three lipoprotein fractions. Criteria for precursor-product relationships have been described by Zilversmit (34) and applied to apoprotein B kinetics in man by Reardon, Fidge, and Nestel (7) and Reardon and Steiner (33). Following the injection of a labeled precursor, label must appear in the product, and the peak of the product's specific activity time curve must not occur before the time at which the precursor and product SA curves cross each other. Although VLDL apoB can be heterogeneous with respect to its lipid and apoprotein environment, we have assumed that all possible subpopulations are isolated for labeling and that the initial injected activity is proportional to the apoB mass in each subpopulation. Also, we have assumed that VLDL apoB may be converted to LDL apoB from both the fast and slowly turning over pools represented by the rapid and slow exponentials of the specific activity curve.

Analyses

Cholesterol and triglyceride analyses were carried out using enzymatic methods described in kits obtained from Boehringer-Mannheim (Montreal, Quebec). Lipoprotein protein determinations were assayed by the method of Markwell et al. (30). Differences between control and treatment values were analyzed by paired-*t* analysis (35).

RESULTS

The changes observed in plasma and lipoprotein cholesterol and triglyceride concentrations are shown in **Table 1**. Cholestyramine treatment lowered plasma total cholesterol (88 ± 2 vs 98 ± 3 mg/dl, ± SE, $P < 0.05$) which was due entirely to a reduction in LDL cholesterol (42 ± 2 vs 52 ± 2, $P < 0.05$). HDL cholesterol and plasma triglycerides were not affected. Cholestyramine and mevinolin when administered together also resulted in a signifi-

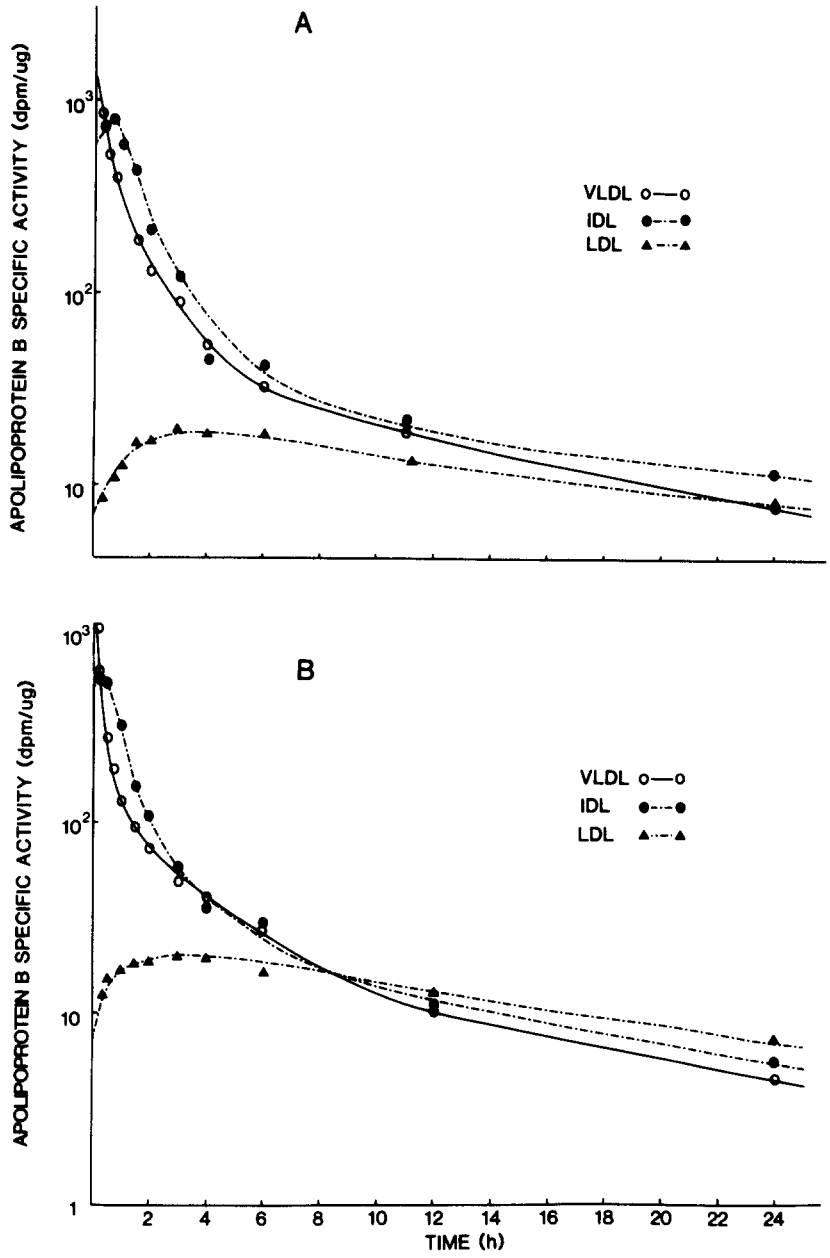

Fig. 1. Apolipoprotein B precursor-product relationships between VLDL, IDL, and LDL fractions following the injection of radiolabeled VLDL. Two curves are shown for animal #3. Curve A was obtained during a control period and curve B was obtained when the pig was treated with cholestyramine.

cant drop in plasma cholesterol (79 ± 11 vs 105 ± 6 mg/dl, $P < 0.025$) and LDL cholesterol (36 ± 6 vs 55 ± 3 mg/dl, $P < 0.025$). HDL cholesterol was significantly higher (41 ± 6 vs 37 ± 8 mg/dl, $P < 0.05$) but plasma triglycerides were not changed.

Autologous iodinated VLDL and LDL were simultaneously injected into miniature pigs. Results from one animal during the control (A) and cholestyramine period (B) are depicted in Fig. 1. ApoB SA curves, calculated from labeled VLDL apoB, were constructed to illustrate the metabolism of apoB from VLDL to IDL and subsequently to LDL. Examination of precursor-product relationships during the control period revealed that the LDL apoB specific activity curve reached its maximal value well before it crossed the specific activity curves of its precursor IDL and VLDL apoB. This indicates that a substantial proportion of LDL apoB enters the circulation via a pathway independent of IDL and VLDL catabolism. Similar curves were obtained for all four animals. The fraction of LDL synthesis derived from VLDL/IDL catabolism

TABLE 1. Plasma and lipoprotein lipid concentrations[a]

Animal	Total Plasma Cholesterol		LDL Cholesterol		HDL Cholesterol		Total Plasma Triglyceride		VLDL Triglyceride	
	C	CY	C	CY	C	CY	C	CY	C	CY
1	86	94	51	45	33	47	36	31	19	13
2	98	90	43	42	39	45	26	24	11	5
3	105	92	62	46	42	40	31	36	7	17
4	99	74	50	33	45	35	30	29	9	11
Mean ± SE	98 ± 3.1	88 ± 3.5	52 ± 3.9	42 ± 2.9	40 ± 2.5	42 ± 2.7	31 ± 2.0	30 ± 2.5	11 ± 2.6	11 ± 2.5
$P <$[b]	0.05		0.05		N.S.		N.S.		N.S.	
	C	CY + M	C	CY + M	C	CY + M	C	CY + M	C	CY + M
5	98	73	73	43	24	29	55	48	24	29
6	92	55	68	23	24	31	55	52	27	16
7	116	82	65	31	47	49	52	48	28	32
8	117	109	59	49	53	54	27	42	21	12
Mean ± SE	105 ± 6.3	79 ± 11.2	55 ± 2.9	36 ± 5.9	37 ± 7.6	41 ± 6.3	47 ± 6.7	48 ± 2.0	28 ± 3.5	20 ± 4.3
$P <$	0.025		0.025		0.05		N.S.		N.S.	

[a]Values are expressed as mg/dl. C, control; CY, cholestyramine; M, mevinolin. Each value is the mean of two samples, one taken fasting the morning of the turnover study, the second taken fasting, 24 hr later.
[b]Determined by paired *t*-test; N.S., not significant.

was calculated as described by Zilversmit (34) and applied to lipoprotein kinetics in man (7, 33) and pigs (10). The proportion of LDL apoB derived from VLDL/IDL catabolism equals the ratio of the peak LDL apoB specific activity value to the VLDL apoB specific activity value at the same time. Zilversmit (34) has stated that the penultimate precursor (VLDL apoB) can be used to apply the method, providing it and the immediate precursor (IDL apo B) have the same specific activity values at the time in question. In the present study, the ratio of LDL apoB:VLDL apoB specific activities and LDL apoB:IDL apoB specific activities at peak LDL specific activity values gave almost identical results since, as shown in Figs. 1 and 2, the IDL and VLDL specific activity values at this time are superimposed. In each experiment, all IDL apoB specific activity curves reached maximal values at the point of intersection with the VLDL apoB specific activity curve. Therefore, LDL apoB derived from IDL apoB catabolism originated as a result of VLDL apoB catabolism.

A second method, which does not require knowledge of the exact point of maximal LDL apoB specific activity, was used to verify the amount of LDL apoB derived from VLDL apoB. This involved calculation of the area under the LDL apoB specific activity curve divided by the area under the VLDL apoB specific activity curve, as described by Goldberg et al. (1). In using the latter method, Goldberg et al. (1) assumed that in cynomolgus monkeys only the rapid component of the VLDL apoB specific activity curve was converted to LDL apoB. However, in our experiments, when only the rapid VLDL component was used, values for the fraction of LDL apoB derived from VLDL apoB in four of our sixteen studies exceeded 1.0, suggesting that a portion of the VLDL apoB specific activity curve was not being measured (i.e., the slow component), which was, in fact, contributing to the LDL apoB pool. Also, in preliminary experiments (unpublished) pigs injected with VLDL in which the apoB label was only in the slow VLDL component, labeled apoB was recovered in LDL. Therefore, in miniature pigs, we have utilized the whole VLDL apoB curve to calculate the area. Values obtained for LDL apoB derived from VLDL apoB by the Zilversmit method (34) and the Goldberg method (1), as we have defined it, differed by less than 4%.

Fig. 1B shows the apoB specific activity curves obtained from the same animal during cholestyramine treatment. Inspection of these curves reveals that the maximum LDL apoB specific activity value occurred closer to the point of intersection with the VLDL apoB curve, indicating that a greater proportion of LDL apoB flux was derived from VLDL/IDL apoB catabolism. Compared to the control period, the ratio of LDL apoB:VLDL apoB specific activity was 0.43 ± 0.02 vs 0.22 ± 0.03 ($P < 0.025$). Values for flux of apoB in LDL and VLDL are shown in **Table 2**. From the LDL apoB disappearance curves following injection of labeled LDL, the total flux of LDL apoB was calculated. Cholestyramine significantly lowered the flux of LDL apoB (0.98 ± 0.10 vs 1.59 ± 0.16 mg $hr^{-1}kg^{-1}$, $P < 0.05$). Knowing the proportion of LDL flux derived from VLDL/IDL catabolism, we can calculate that the total flux of LDL derived from VLDL did not change (0.42 ± 0.06 vs 0.37 ± 0.08). However, the independent synthesis of LDL apoB was significantly reduced (0.56 ± 0.05 vs 1.15 ± 0.06, $P < 0.005$) with cholestyramine treatment.

Cholestyramine also reduced the total pool size of LDL apoB (24.2 ± 1.9 vs 34.9 ± 2.1 mg kg^{-1}, $P < 0.05$) and increased the FCR (0.05 ± 0.002 vs 0.04 ± 0.006 hr^{-1})

TABLE 2. Metabolism of very low density and low density lipoprotein apolipoprotein B in miniature pigs treated with cholestyramine[a]

	VLDL									
	Pool Size[b]		FCR		Flux-Total		Flux to LDL[c]		Flux Direct Removal[d]	
Animal	C	CY	C	CY	C	CY	C	CY	C	CY
	mg kg^{-1}		*hr^{-1}*		*mg hr^{-1}kg^{-1}*		*mg hr^{-1}kg^{-1}*		*mg hr^{-1}kg^{-1}*	
1	2.66	2.48	0.42	0.50	1.11	1.25	0.36(32)	0.38(30)	0.75(68)	0.87(70)
2	3.16	2.71	0.32	0.60	1.48	1.63	0.17(12)	0.58(36)	1.31(88)	1.05(64)
3	2.51	2.47	0.72	0.87	1.83	1.76	0.38(20)	0.32(19)	1.45(80)	1.44(81)
4	2.01	2.18	1.09	0.72	2.66	1.90	0.56(21)	0.41(22)	2.10(79)	1.50(78)
Mean ± SE	2.59 ± 0.24	2.46 ± 0.11	0.64 ± 0.170	0.68 ± 0.081	1.77 ± 0.33	1.64 ± 0.14	0.37(21) ± 0.08(4)	0.42(26) ± 0.06(4)	1.40(79) ± 0.28(4)	1.22(73) ± 0.15(3)
$P <$[e]	N.S.		N.S.		N.S.		N.S.		N.S.	
	LDL									
	Pool Size		FCR		Flux-Total		Flux from VLDL[c]		Flux Direct Synthesis[f]	
1	38.4	21.2	0.039	0.045	1.51	0.94	0.36(23)	0.38(40)	1.14(77)	0.56(60)
2	29.4	29.2	0.026	0.050	1.18	1.22	0.17(14)	0.58(48)	1.02(86)	0.64(52)
3	33.7	24.9	0.052	0.058	1.79	0.74	0.38(21)	0.32(43)	1.41(79)	0.42(57)
4	38.12	21.8	0.049	0.048	1.87	1.01	0.56(30)	0.40(41)	1.31(70)	0.61(59)
Mean ± SE	34.9 ± 2.1	24.2 ± 1.9	0.042 ± 0.006	0.050 ± 0.003	1.59 ± 0.16	0.98 ± 0.10	0.37(22) ± 0.08(3)	0.42(43)[g] ± 0.06(2)	1.15(78) ± 0.06(3)	0.56(57)[g] ± 0.05(2)
$P <$[e]	0.05		N.S.		0.05		N.S.		0.005	

[a]Calculations are based on a two-pool model (31). Pool size refers to mass of pool 1; FCR, irreversible fractional catabolic rate; flux of apolipoprotein B represents production and removal rate; CY, cholestyramine; C, control.

[b]The amount of pool 1 in the vascular compartment was 79 ± 3% of the kinetically defined pool size, which did not differ between control and treatment. This was determined by comparing the kinetically defined pool size and the VLDL apoB plasma pool size determined as described in Methods. The plasma volume was assumed to be 42 ml/kg body weight (10).

[c]Flux of VLDL apoB to LDL apoB is the same as the flux of LDL apoB derived from VLDL apoB. The latter is calculated from the specific activity curves of VLDL and LDL apoB following the injection of radiolabeled VLDL, as described in Methods. Values in brackets are per cent of total flux.

[d]Calculated by subtracting VLDL apoB flux to LDL apoB from total VLDL apoB flux.

[e]Determined by paired *t*-test; N.S., not significant.

[f]Calculated by subtracting the LDL apoB flux derived from VLDL apoB from the total LDL apoB flux. The latter is calculated directly from the LDL apoB specific activity curve following injection of radiolabeled LDL, as described in Methods.

[g]Significantly different from control value, $P < 0.025$.

although the latter did not reach statistical significance ($0.1 > P > 0.05$). Values for VLDL apoB metabolism were largely unchanged (Table 2). Compared to control values, cholestyramine treatment resulted in a VLDL apoB pool size of 2.46 ± 0.11 vs 2.59 ± 0.24 mg kg^{-1}, and total flux of 1.64 ± 0.14 vs 1.77 ± 0.33 mg $hr^{-1}kg^{-1}$. The FCR increased in three of four animals; however, the means were not different (0.68 ± 0.08 vs 0.64 ± 0.17 hr^{-1}) due to a high baseline FCR in the fourth pig. Of the total VLDL apoB flux, we know the amount converted to LDL apoB and by difference the amount of VLDL apoB removed directly from the circulation was calculated. Cholestyramine did not change this value (1.22 ± 0.15 vs 1.40 ± 0.28 mg $hr^{-1}kg^{-1}$).

ApoB specific activity curves of VLDL, IDL, and LDL obtained in one animal during the control period (A) and during treatment with cholestyramine plus mevinolin (B) are depicted in **Fig. 2**. It can be seen from these precursor-product curves that the combination of the two drugs almost abolishes the independent synthesis pathway, indicating that most LDL apoB is derived from VLDL/IDL catabolism. The maximum LDL apoB specific activity occurred at or just prior to the point it crossed the IDL and VLDL apoB curves in all four animals, resulting in

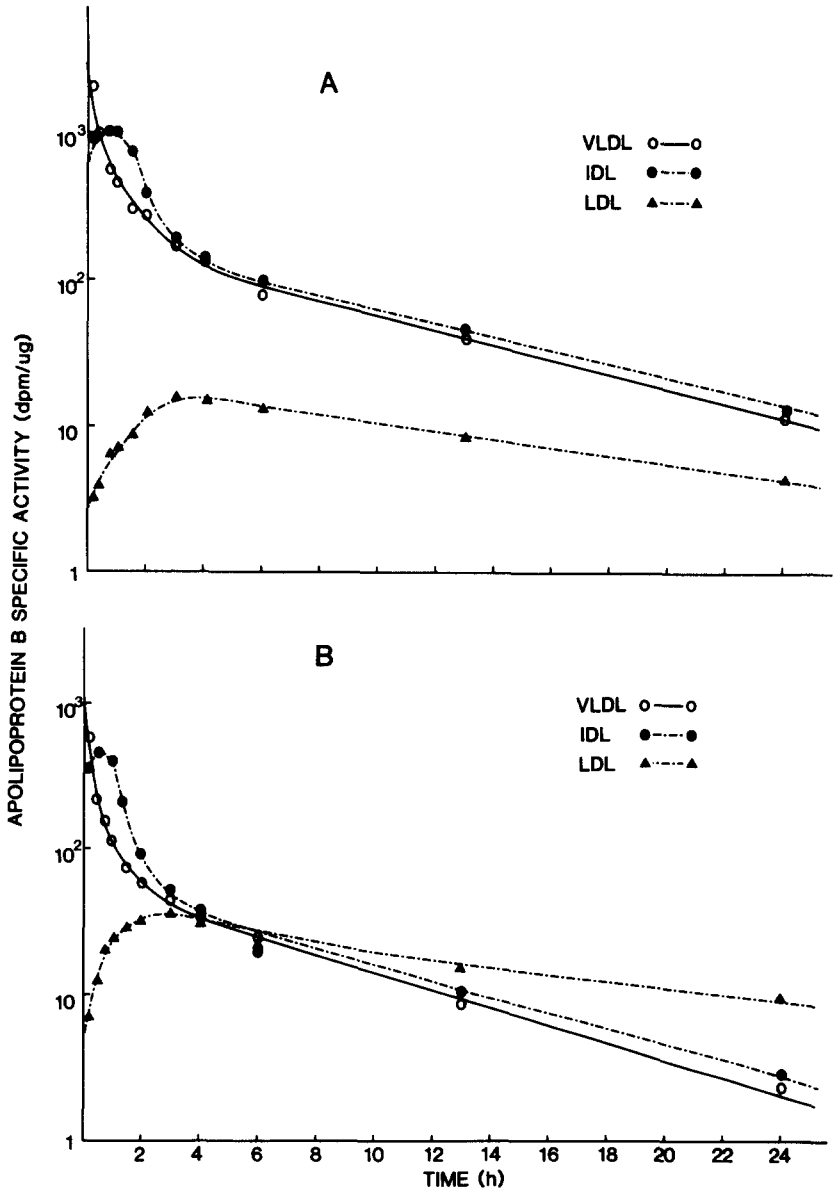

Fig. 2. Apolipoprotein B precursor-product relationships between VLDL, IDL, and LDL fractions following injection of radiolabeled VLDL. Curves are shown for animal #5. Curve A was obtained during a control period and curve B was obtained during treatment with mevinolin and cholestyramine.

ratios of LDL apoB specific activity (maximum):VLDL apoB specific activity to be 0.87 ± 0.06 compared to 0.23 ± 0.09, $P < 0.01$, calculated during the control period (**Table 3**). The two drugs significantly reduced both LDL apoB total flux (0.47 ± 0.06 vs 0.97 ± 0.26, mg $hr^{-1}kg^{-1}$ $P < 0.05$) and the LDL apoB pool size (10.7 ± 1.1 vs 26.5 ± 3.8 mg kg^{-1}, $P < 0.05$). The FCR was increased (0.047 ± 0.004 vs 0.036 ± 0.006 hr^{-1}) as was the flux from VLDL/IDL apoB to LDL apoB (0.39 ± 0.04 vs 0.20 ± 0.06) but these differences were not significant.

In contrast to cholestyramine alone, mevinolin plus cholestyramine significantly decreased the VLDL apoB pool size (1.36 ± 0.11 vs 1.73 ± 0.15, mg kg^{-1}, $P < 0.025$) and increased the FCR (1.09 ± 0.07 vs 0.62 ± 0.06 hr^{-1}, $P < 0.05$). The total VLDL apoB flux was unchanged (Table 3). However, the percentage of total VLDL apoB flux converted to LDL was significantly increased (26 ± 2 vs 16 ± 3, $P < 0.05$) and the percentage of total VLDL apoB flux cleared directly from the circulation was decreased (74 ± 2 vs 84 ± 3, $P < 0.05$). The absolute amounts of each were not significantly different from control values.

DISCUSSION

This study demonstrates for the first time that a combination of mevinolin (a cholesterol synthesis inhibitor) and cholestyramine (a bile acid sequestrant) administered to miniature pigs inhibits the direct pathway of LDL synthesis (by approximately 90%), whereas VLDL-derived LDL synthesis is largely unchanged. In addition, the combination of drugs increased the FCR of LDL apoB by 40%, resulting in a lowering of the LDL pool size by 60%. We have also demonstrated that cholestyramine alone selectively inhibits the direct LDL synthesis pathway. However, the percentage reduction in this pathway (30%) was less than with both drugs given together, and the increase in LDL FCR was only 16%. This resulted in a 30% reduction in LDL pool size. This potentiation of effect by combining mevinolin and cholestipol (similar to cholestyramine) has been demonstrated in studies in dogs (22) and man (26) in which the FCR of labeled human LDL was increased 110% and 70%, respectively. However, only total LDL synthesis was measured and only the dog experiments using labeled human LDL demonstrated a decreased relative LDL synthetic rate (22). Mevinolin alone given to rabbits lowers plasma LDL by an increase in FCR (23, 26) and a decrease in total LDL synthesis (36).

It has been suggested, based on theoretical grounds, that cholestyramine, by enhancing the conversion of cholesterol to bile acids, results in a reduction of hepatic cholesterol (37). This has the effect in animal studies (18, 19, 22) of increasing the number of hepatic LDL receptors, thereby reducing the plasma LDL concentrations and resulting in an increase in plasma LDL FCR. The effectiveness of bile acid sequestrants has long been known to be blunted by the compensatory increase in hepatic cholesterol biosynthesis. Thus, the addition of an HMG-CoA reductase inhibitor, such as mevinolin, would be expected to maximize the reduction in hepatic cholesterol, produce an enhanced number of hepatic LDL receptors, and as discussed above, potentiate both the lowering of plasma LDL and increase in LDL FCR. These effects have been clearly demonstrated in dogs (22) and in one hypercholesterolemic man (26). The increase in hepatic LDL receptors in hypercholesterolemic man (given only mevinolin) has been determined indirectly from the clearance of modified LDL (26).

In normal man, all LDL apoB is derived from the catabolism of VLDL and IDL apoB (7, 8). However, in familial homozygous and heterozygous hypercholesterolemic subjects (12, 13) and in familial combined hyperlipidemia (38), a substantial proportion of LDL apoB is synthesized by a pathway independent of the VLDL/IDL catabolic cascade. Although it has been demonstrated that in dogs (22) total LDL synthesis is reduced by mevinolin in combination with cholestyramine, the idea that reduction of hepatic cholesterol with these two drugs may selectively reduce the direct synthesis pathway of LDL has never been explored. We have previously demonstrated that LDL metabolism in the miniature pig is characterized by a substantial direct synthesis pathway (10). The results of the present study, that this direct pathway is inhibited, and not VLDL-derived LDL synthesis, clearly support our hypothesis. Recently, LaVille et al. (36) demonstrated in rabbits that mevinolin alone reduced total LDL synthesis whereas VLDL flux was not affected. They speculated that the direct synthesis pathway of LDL was reduced but, in contrast to our study, they could not determine whether reduced VLDL-derived LDL synthesis was responsible for the observed effects.

Factors that control or regulate direct LDL apoB synthesis are not clear. It is interesting to note that the combination of mevinolin and cholestyramine decreased VLDL apoB pool size, increased the FCR, but had little effect on VLDL apoB flux. It is possible that the direct synthesis pathway occurs in situations where there is a need to transport hepatic cholesterol, but a relatively low requirement to transport triglyceride as VLDL. Thus, the lipoprotein synthesized by the liver is cholesteryl ester-rich LDL rather than triglyceride-rich VLDL. Therefore, in the present study, by presumably reducing the need to transport hepatic cholesterol, the direct LDL synthesis pathway was inhibited. Presumably, there was sufficient cholesterol for normal VLDL synthesis. Although VLDL cholesterol concentrations were very low (2–4 mg/dl) no consistent change in VLDL lipid composition was ob-

TABLE 3. Metabolism of very low density and low density lipoprotein apolipoprotein B in miniature pigs treated with cholestyramine and mevinolin[a]

	VLDL									
	Pool Size[b]		FCR		Flux-Total		Flux to LDL[c]		Flux Direct Removal[d]	
Animal	C	CY + M	C	CY + M	C	CY + M	C	CY + M	C	CY + M
	mg kg^{-1}		*hr^{-1}*		*mg hr^{-1}kg^{-1}*		*mg hr^{-1}kg^{-1}*		*mg hr^{-1}kg^{-1}*	
5	1.85	1.25	0.48	1.16	0.88	1.67	0.17(19)	0.49(29)	0.71(81)	1.18(70)
6	1.29	1.14	0.59	1.24	0.75	1.47	0.10(14)	0.42(29)	0.65(86)	1.05(71)
7	1.81	1.37	0.80	0.91	1.75	1.29	0.37(22)	0.30(24)	1.38(78)	0.99(76)
8	1.97	1.66	0.61	1.05	1.38	1.75	0.14(10)	0.38(22)	1.24(89)	1.37(78)
Mean ± SE	1.73 ± 0.15	1.36 ± 0.11	0.62 ± 0.06	1.09 ± 0.07	1.19 ± 0.23	1.55 ± 0.10	0.20(16) ± 0.06(3)	0.39(26)[g] ± 0.04(2)	0.99(84) ± 0.18(2)	1.15(74)[g] ± 0.08 (3)
$P <$[e]	0.025		0.025		N.S.		N.S.		N.S.	

	LDL									
	Pool Size		FCR		Flux-Total		Flux from VLDL[c]		Flux Direct Synthesis[f]	
5	33.6	13.8	0.033	0.050	1.74	0.68	0.17(10)	0.49(71)	1.57(90)	0.19(29)
6	32.8	10.4	0.020	0.042	0.55	0.44	0.10(16)	0.42(97)	0.45(84)	0.02(3)
7	20.1	9.8	0.046	0.040	0.77	0.38	0.37(48)	0.30(80)	0.40(52)	0.08(20)
8	19.3	8.7	0.045	0.056	0.83	0.39	0.14(17)	0.38(98)	0.69(83)	0.01(2)
Mean ± SE	26.5 ± 3.8	10.7 ± 1.1	0.036 ± 0.006	0.047 ± 0.004	0.97 ± 0.26	0.47 ± 0.06	0.20(23) ± 0.06(9)	0.39(87)[h] ± 0.04(6)	0.77(77) ± 0.27(9)	0.08(13)[h] ± 0.041(7)
$P <$[e]	0.05		N.S.		0.05		N.S.		0.025	

[a,c,d,e,f] Same as footnotes to Table 2. CY, cholestyramine; M, mevinolin; C, control.

[b] The amount of pool 1 in the vascular compartment was 86 ± 4% of the total kinetically defined pool size, which did not differ between control and treatment. Calculated as described in footnote to Table 2.

[g] Significantly different from control value, $P < 0.025$.

[h] Significantly different from control value, $P < 0.01$.

served with either drug treatment. One might expect, however, that if VLDL apoB flux was increased, hepatic cholesterol would enter the circulation as part of the VLDL particles. We have shown that a diet high in sucrose increased VLDL apoB flux and inhibited the direct LDL synthesis pathway (Huff, M. W. and D. E. Telford, unpublished observations).

We have previously demonstrated that in miniature pigs a substantial proportion of VLDL apoB flux is cleared from the circulation presumably by the liver without conversion to LDL (10). This shunt pathway for VLDL apoB flux has also been demonstrated in rats (3, 4), rabbits (39), monkeys (1, 2), and hypertriglyceridemic man (7). There is evidence in the rat that VLDL remnants are removed by the liver via the chylomicron or apoE receptor (40, 41), whereas studies in the Watanabe rabbit (which lacks an LDL receptor) indicate that VLDL remnants are normally cleared by the LDL receptor (39). The receptor responsible for VLDL remnant clearance in other species including swine is not known. Cholestyramine or cholestyramine and mevinolin treatment increased the VLDL apoB FCR in seven of eight animals, suggesting increased hepatic receptor activity. Cholestyramine and mevinolin together are known to stimulate the hepatic LDL receptor in dogs (22), and cholestyramine alone in dogs is known to stimulate the hepatic LDL receptor but not the chylomicron (apoE) receptor (42). Therefore, it is possible that in the miniature pig the LDL receptor mediates hepatic VLDL remnant uptake. It should be noted that in the cholestyramine plus mevinolin experiments the percent of total VLDL flux converted to LDL was significantly increased, whereas the percent removed directly from the circulation was significantly decreased. Therefore, if the LDL receptor mediates VLDL catabolism in this species, the cholestyramine plus mevinolin treatment shifts the activity of this receptor process to favor LDL formation.

It has been suggested that the direct synthesis pathway of LDL apoB in monkeys (1) could be due to *i*) particles secreted from hepatocytes as VLDL but quickly modified to LDL-like particles before entering the circulation, (and thus are not labeled with plasma VLDL) and *ii*) a very small but very rapidly turning-over subpopulation of VLDL which is not labeled when plasma VLDL is labeled. For the results of the present experiments to be consistent with these hypotheses, we would have to speculate that mevinolin and cholestyramine resulted in the secretion of VLDL from the liver of altered composition such that *i*) all VLDL entered the circulation before being converted to LDL and *ii*) the small rapidly turning-over pool of VLDL became much larger and turned over more slowly, so that it became labeled as part of the VLDL tracer. We saw no consistent evidence of altered VLDL composition. Although VLDL apoB pool size was increased, total flux (turnover) was not decreased, but was, in fact, increased slightly. Further studies will be required to determine directly whether either of these explanations for the LDL direct synthesis pathway is valid.

The findings of the present study clearly demonstrate that the deviations from absolute precursor-product relationships of VLDL to LDL metabolism in the miniature pig can be altered to become closer to absolute. Mevinolin and cholestyramine selectively inhibited the direct LDL synthesis pathway without altering the conversion of VLDL to LDL. It remains to be determined whether these drugs can correct the deviations from absolute precursor-product relationships of VLDL-LDL metabolism in hypercholesterolemic man. It is possible that inhibiting the LDL direct synthesis pathway in hypercholesterolemic man may correct the overproduction of these atherogenic lipoproteins observed in these individuals (38, 43).

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REFERENCES

1. Goldberg, I. J., N. A. Le, H. N. Ginsberg, J. R. Paterniti, Jr., and W. V. Brown. 1983. Metabolism of apoprotein B in cynomolgous monkey: evidence for independent production of low-density lipoprotein apoprotein B. *Am. J. Physiol.* **244:** E196–E201.
2. Illingworth, D. R. 1975. Metabolism of lipoproteins in nonhuman primates. Studies on the origin of low density lipoprotein apoprotein in the plasma of the squirrel monkey. *Biochim. Biophys. Acta.* **388:** 38–51.
3. Faergeman, O., T. Sata, J. P. Kane, and R. J. Havel. 1975. Metabolism of apoprotein B of plasma very low density lipoproteins in the rat. *J. Clin. Invest.* **56:** 1396–1403.
4. Fidge, N. H., and P. Poulis. 1978. Metabolic heterogeneity in the formation of low density lipoprotein from very low density lipoprotein in the rat: evidence for the independent production of a low density lipoprotein subfraction. *J. Lipid Res.* **19:** 342–349.
5. Schaefer, E. J., S. Eisenberg, and R. I. Levy. 1978. Lipoprotein apoprotein metabolism. *J. Lipid Res.* **19:** 667–687.
6. Brown, M. S., P. T. Kovanen, and J. L. Goldstein. 1981. Regulation of plasma cholesterol by lipoprotein receptors. *Science.* **212:** 628–635.
7. Reardon, M. F., N. H. Fidge, and P. J. Nestel. 1978. Catabolism of very low density lipoprotein B apoprotein in man. *J. Clin. Invest.* **62:** 850–860.
8. Sigurdsson, G., A. Nicholl, and B. Lewis. 1975. Conversion of very low density lipoprotein to low density lipoprotein. A metabolic study of apolipoprotein B kinetics in human subjects. *J. Clin. Invest.* **56:** 1481–1490.

9. Ginsberg, H. N., N. A. Le, J. Melish, and W. V. Brown. 1981. Effect of a high carbohydrate diet on apoprotein B catabolism in man. *Metabolism.* **30:** 347–353.
10. Huff, M. W., and D. E. Telford. 1985. Direct synthesis of low-density lipoprotein B in the miniature pig. *Metabolism.* **34:** 36–42.
11. Mahley, R. W., K. H. Weisgraber, T. Innerarity, H. B. Brewer, and G. Assmann. 1975. Swine lipoproteins and atherosclerosis. Changes in the plasma lipoproteins and apoproteins induced by cholesterol feeding. *Biochemistry.* **14:** 2817–2823.
12. Soutar, A. K., N. B. Myant, and G. R. Thompson. 1977. Simultaneous measurements of apolipoprotein B turnover in very low and low density lipoproteins in familial hypercholesterolemia. *Atherosclerosis.* **28:** 247–256.
13. Janus, E. D., A. Nicholl, R. Wootton, P. R. Turner, P. J. Magill, and B. Lewis. 1980. Quantitative studies of very low density lipoprotein conversion to low density lipoprotein in normal controls and primary hyperlipoproteinemic states and the role of direct secretion of low density lipoprotein in heterozygous familial hypercholesterolemia. *Eur. J. Clin. Invest.* **10:** 149–159.
14. Swift, L. L., P. D. Soulé, and V. S. LeQuire. 1982. Hepatic Golgi lipoproteins: precursors to plasma lipoproteins in hypercholesterolemic rats. *J. Lipid Res.* **23:** 962–971.
15. Noel, S. P., L. Wong, P. J. Dolphin, L. Davy, and D. Rubinstein. 1979. Secretion of cholesterol-rich lipoproteins by perfused livers of hypercholesterolemic rats. *J. Clin. Invest.* **64:** 674–683.
16. Guo, L. S., R. L. Hamilton, R. Ostwald, and R. J. Havel. 1982. Secretion of nascent lipoproteins and apolipoproteins by perfused livers of normal and cholesterol-fed guinea pigs. *J. Lipid Res.* **23:** 543–555.
17. Johnson, F. L., R. W. St. Clair, and L. L. Rudel. 1983. Studies on the production of low density lipoproteins by perfused livers from nonhuman primates. *J. Clin. Invest.* **72:** 221–236.
18. Hui, D. Y., T. L. Innerarity, and R. W. Mahley. 1981. Lipoprotein binding to canine hepatic membranes: metabolically distinct apo-E and apo-B, E receptors. *J. Biol. Chem.* **256:** 5646–5655.
19. Chao, Y., T. T. Yamin, and A. W. Alberts. 1982. Effects of cholestyramine on low density lipoprotein binding sites on liver membranes from rabbits with endogenous hypercholesterolemia induced by a wheat starch-casein diet. *J. Biol. Chem.* **257:** 3623–3627.
20. Slater, H. R., C. J. Packard, S. Bicker, and J. Shepherd. 1980. Effects of cholestyramine on receptor-mediated plasma clearance and tissue uptake of human low density lipoproteins in the rabbit. *J. Biol. Chem.* **255:** 10210–10213.
21. Shepherd, J., C. J. Packard, S. Bicker, T. D. V. Laurie, and H. G. Morgan. 1980. Cholestyramine promotes receptor-mediated low density lipoprotein catabolism. *N. Engl. J. Med.* **302:** 1219–1222.
22. Kovanen, P. T., D. W. Bilheimer, J. L. Goldstein, J. J. Jaramillo, and M. S. Brown. 1981. Regulatory role for hepatic low density lipoprotein receptors in vivo in the dog. *Proc. Natl. Acad. Sci. USA.* **78:** 1194–1198.
23. Chao, Y. S., P. A. Kroon, T. T. Yamin, G. M. Thompson, and A. W. Alberts. 1983. Regulation of hepatic receptor-dependent degradation of LDL by mevinolin in rabbits with hypercholesterolemia induced by a wheat starch-casein diet. *Biochim. Biophys. Acta.* **754:** 134–141.
24. Tobert, J. A., G. D. Bell, J. Birtwell, I. James, W. R. Kukovetz, J. S. Pryor, A. Buntinx, I. B. Holmes, Y-S. Chao, and J. A. Bolognese. 1982. Cholesterol-lowering effect of mevinolin, an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase in healthy volunteers. *J. Clin. Invest.* **69:** 913–919.
25. Illingworth, D. R. 1984. Mevinolin plus colestipol in therapy for severe heterozygous familial hypercholesterolemia. *Ann. Int. Med.* **101:** 598–604.
26. 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 hypercholesterolemic heterozygotes. *Proc. Natl. Acad. Sci. USA.* **80:** 4124–4128.
27. Fidge, N. H., and P. Poulis. 1974. Studies on the radioiodination of very low density lipoprotein obtained from different mammalian species. *Clin. Chim. Acta.* **52:** 15–26.
28. Huff, M. W., N. H. Fidge, P. J. Nestel, T. Billington, and B. Watson. 1981. Metabolism of C-apolipoproteins: kinetics of C-II, C-III_1, and C-III_2 and VLDL-apolipoprotein B in normal and hyperlipoproteinemic subjects. *J. Lipid Res.* **22:** 1235–1246.
29. 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.
30. Markwell, M. A. K., S. M. Haas, L. L. Bieber, and N. E. Tolbert. 1978. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal. Biochem.* **87:** 206–210.
31. Gurpide, E., J. Mann, and E. Sandberg. 1964. Determination of kinetic parameters in a two-pool system by administration of one or more tracers. *Biochemistry.* **3:** 1250–1255.
32. Bard, Y. 1974. Nonlinear Parameter Estimation. Academic Press, New York.
33. Reardon, M. F., and G. Steiner. 1982. The use of kinetics in investigating the metabolism of very low and intermediate-density lipoproteins. *In* Lipoprotein Kinetics and Modeling. M. Berman, S. M. Grundy, and B. V. Howard, editors. Academic Press, New York. 99–112.
34. Zilversmit, D. B. 1960. The design and analysis of isotope experiments. *Am. J. Med.* **29:** 832–848.
35. Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. 6th Edition. Iowa State University Press, Ames, IA. 94–97.
36. LaVille, A., R. Moshy, P. R. Turner, N. E. Miller, and B. Lewis. 1984. Inhibition of cholesterol synthesis reduces low-density-lipoprotein apoprotein B production without decreasing very-low-density-lipoprotein apoprotein B synthesis in rabbits. *Biochem. J.* **219:** 321–323.
37. Goldstein, J. L., T. Kita, and M. S. Brown. 1983. Defective lipoprotein receptors and atherosclerosis. *N. Engl. J. Med.* **309:** 288–296.
38. Kissebah, A. H., S. Alfarsi, and D. J. Evans. 1984. Low density lipoprotein metabolism in familial combined hyperlipidemia. Mechanism of the multiple lipoprotein phenotypic expression. *Arteriosclerosis.* **4:** 614–624.
39. Kita, T., M. S. Brown, D. W. Bilheimer, and J. L. Goldstein. 1982. Delayed clearance of very low density and intermediate density lipoproteins with enhanced conversion to low density lipoprotein in WHHL rabbits. *Proc. Natl. Acad. Sci. USA.* **79:** 5693–5697.
40. Noel, S. P., and R. Dupras. 1983. The kinetic parameters of the uptake of very low density lipoprotein remnant cholesteryl esters by perfused rat livers. *Biochim. Biophys. Acta.* **754:** 117–124.
41. Cooper, A. D., M. A. Shrewsbury, and S. K. Erickson.

1982. Comparison of binding and removal of remnants of triglyceride-rich lipoproteins of intestinal and hepatic origin by rat liver in vitro. *Am. J. Physiol.* **243:** G389–G395.
42. Mahley, R. W., D. Y. Hui, T. L. Innerarity, and K. H. Weisgraber. 1981. Two independent lipoprotein receptors on hepatic membranes of dog, swine and man. *J. Clin. Invest.* **68:** 1197–1206.
43. Kesaniemi, Y. A., G. L. Vega, and S. M. Grundy. 1982. Kinetics of apolipoprotein B in normal and hyperlipidemic man: review of current data. *In* Lipoprotein Kinetics and Modeling. M. Berman, S. M. Grundy, and B. V. Howard, editors. Academic Press, New York. 181–205.