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[1]

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Abstract We investigated the metabolism of very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), and low density lipoprotein (LDL) apolipoprotein B (apoB) in seven patients with combined hyperlipidemia (CHL), using ^{125}I-labeled VLDL and ^{131}I-labeled LDL and compartmental modeling, before and during lovastatin treatment. Lovastatin therapy significantly reduced plasma levels of LDL cholesterol (142 vs 93 mg/dl, $P < 0.0005$) and apoB (1328 vs 797 μg/ml, $P < 0.001$). Before treatment, CHL patients had high production rates (PR) of LDL apoB. Three-fourths of this LDL apoB flux was derived from sources other than circulating VLDL and was, therefore, defined as "cold" LDL apoB flux. Compared to baseline, treatment with lovastatin was associated with a significant reduction in the total rate of entry of apoB-containing lipoproteins into plasma in all seven CHL subjects (40.7 vs. 25.7 mg/kg · day, $P < 0.003$). This reduction was associated with a fall in total LDL apoB PR and in "cold" LDL apoB PR in six out of seven CHL subjects. VLDL apoB PR fell in five out of seven CHL subjects. Treatment with lovastatin did not significantly alter VLDL apoB conversion to LDL apoB or LDL apoB fractional catabolic rate (FCR) in CHL patients. In three patients with familial hypercholesterolemia who were studied for comparison, lovastatin treatment increased LDL apoB FCR but did not consistently alter LDL apoB PR. We conclude that lovastatin lowers LDL cholesterol and apoB concentrations in CHL patients by reducing the rate of entry of apoB-containing lipoproteins into plasma, either as VLDL or as directly secreted LDL.—**Arad, Y., R. Ramakrishnan, and H. N. Ginsberg.** 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.* 1990. **31:** 567–582.

Supplementary key words kinetic modeling • VLDL • IDL • fractional catabolic rate • HMG-CoA reductase

Elevated plasma and low density lipoprotein (LDL) cholesterol concentrations are considered to be major pathogenic factors in the development of atherosclerosis (1). Patients with either the combination of hypercholesterolemia and hypertriglyceridemia (CHL) or familial hypercholesterolemia (FH) constitute two major groups of patients with elevated plasma LDL cholesterol levels who are at increased risk for atherosclerosis (2, 3). Although individuals in both groups have elevated LDL cholesterol levels, subjects with CHL also have elevated levels of very low density lipoprotein (VLDL) cholesterol and triglycerides (4). In addition, studies of the turnover of apoB in VLDL, intermediate density lipoprotein (IDL), and LDL have demonstrated quite different pathophysiologic mechanisms for the hyperlipoproteinemia present in CHL patients, who have normal fractional catabolic rates (FCR) of LDL and high production rates (PR) of VLDL and/or LDL apoB (5–7), and in FH patients, who have decreased FCR of LDL apoB and either normal or increased PR of LDL apoB (6, 8, 9).

Lovastatin is the first of a family of competitive inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase to be approved for use in the United States. Treatment with lovastatin can dramatically reduce plasma total and LDL cholesterol as well as LDL apoB

Abbreviations: LDL, low density lipoprotein; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; HDL, high density lipoprotein; apoB, apolipoprotein B; CHL, combined hyperlipidemia; FH, familial hypercholesterolemia; SA, specific activity; PR, production rate; FCR, fractional catabolic rate; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; TG, triglyceride; C, cholesterol.

[1]Partial data were presented in abstract form: *Clin. Res.* 1987. **35:** 496A.

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levels in subjects with either FH (10, 11) or nonfamilial hypercholesterolemia (12). It has been postulated that, in vivo, lovastatin reduces plasma LDL cholesterol by inducing increased activity of hepatic LDL receptors secondary to a fall in an intracellular pool of free cholesterol (13). Although studies of LDL apoB turnover in FH subjects showed increased LDL FCR, supporting this hypothesis (14), similar studies in subjects with moderate hypercholesterolemia (15) indicated that reduced PR of LDL apoB, rather than increased FCR of this apolipoprotein, was the basis for the therapeutic effect of lovastatin in that group of patients. Because overproduction of apoB-containing lipoproteins appears to be a common characteristic of subjects with CHL (5–7), we hypothesized that lovastatin might reduce LDL and/or VLDL apoB production in CHL subjects. Since LDL apoB metabolism is closely linked to apoB metabolism in VLDL and IDL, we undertook a study of apoB metabolism in all three lipoprotein classes in subjects classified phenotypically as having CHL, before and during treatment with lovastatin. The primary goals of the study were to determine whether production of LDL apoB is reduced by lovastatin, and, if so, to use multicompartmental analysis to determine the contribution of each of the three possible mechanisms to such a reduction in LDL apoB production: *1)* a reduction in VLDL apoB production; *2)* a reduction in the proportion of VLDL apoB flux that is converted to LDL apoB; and *3)* a reduction in PR of "cold" LDL apoB (LDL apoB that cannot be traced by the injection of radiolabeled VLDL).

METHODS

Patients

The clinical characteristics of seven CHL patients and three FH patients are presented in **Table 1**. All subjects were referred to us for evaluation of hypercholesterolemia. The lipid values shown in Table 1 are those that were present prior to initiation of any diet or drug therapy (means of two to four determinations). Because no single genetic or clinical marker identifying CHL patients is available, our classification was based on a lipid profile that included both an LDL cholesterol concentration greater than 90th percentile for age and sex and a concomitant plasma triglyceride level greater then 250 mg/dl. None of these subjects had xanthomas or xanthelasma. Plasma VLDL cholesterol to triglyceride ratios, at the time of diagnosis, were all less than 0.3, indicating that none of these patients had type III hyperlipidemia.

Three patients were classified as having FH. FH #1, who has been described previously (16), had approximately 40% of normal LDL receptors on his cultured fibroblasts, and had previously undergone a portacaval shunt for severe hypercholesterolemia. After an initial excellent response to surgery, his plasma LDL cholesterol had increased to approximately 70% of pre-shunt levels. FH #2 was studied by Helen Hobbs in the laboratory of Goldstein and Brown and was found to be homozygous for a mutation in the gene for the LDL receptor that resulted in binding of LDL to his cultured fibroblasts that was

TABLE 1. Clinical characteristics

Subject	Sex	Age	Cardiac History	Total Cholesterol	Triglyceride	VLDL C/TG	HDL-C	Medication
		yr			*mg/dl*			
CHL								
1	M	32	none	300	293	0.25	49	none
2	M	44	none	285	294	0.26	37	none
3	F	65	MI, CABG	431	548	0.22	36	digoxin, aspirin
4	M	41	none	320	300	0.26	27	none
5	F	51	angina, HTN	304	366	0.24	30	propranolol, conjugated estrogen
6	M	63	MI, CABG	386	423	0.28	51	propranolol, dipyramidole, aspirin
7	M	60	MI, angina	312	320	0.22	32	furosemide, diltiazam, warfarin, digoxin, nitroglycerine
FH								
1[a]	M	60	MI, CABG	479	169		24	digoxin, diltiazam, aspirin, spironolactone, nitroglycerine
2[b]	M	25	none	500	80		34	none
3	F	41	MI, CHF, HTN	370	92		44	metoprolol, isosorbide dinitrate, aspirin, sucralfate, docusate sodium, dipyramidole

Abbreviations: CHL, combined hyperlipidemia; FH, familial hypercholesterolemia; MI, myocardial infarction; CABG, coronary artery bypass graft; HTN, hypertension; CHF, congestive heart failure.

[a]Post-portacaval shunt.

[b]Homozygous

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2-3% of normal (Hobbs, H., J. L. Goldstein, and M. S. Brown, personal communication). He had previously demonstrated a decrease in LDL cholesterol while taking bile acid-binding resins, and the decision was made, therefore, to attempt therapy with lovastatin. The third patient had previously suffered two myocardial infarctions and had markedly elevated LDL cholesterol levels, normal plasma triglyceride concentrations, and large tendon xanthomas. She had not had LDL receptor studies, but had a clinical and family history compatible with heterozygous FH.

All patients were in stable health at the time of the study. None had secondary causes for the hyperlipidemia such as renal, hepatic, or thyroid disease. The FH subject who had previously had a portacaval shunt had had significantly higher plasma levels of LDL cholesterol before the procedure (16). All patients had been previously treated with various hypolipidemic drugs, but not with lovastatin. All hypolipidemic drugs were discontinued for at least 1 month before the study. However, due to medical considerations, other medications, some of which may have contributed to the subjects' dyslipidemias, were continued throughout the study without change in dosage. The mediations taken by the patients during the study are listed in Table 1. All subjects had been consuming an American Heart Association (AHA) phase 1 diet prior to their baseline study and continued eating this diet throughout the study.

Turnover studies of apoB were performed in the General Clinical Research Center at the Columbia Presbyterian Medical Center. Each patient had the initial turnover study prior to lovastatin and underwent a repeat study during treatment with lovastatin. CHL subjects #1 and #2 received 20 mg lovastatin once daily with dinner while the remaining subjects (both CHL and FH) received 20 mg lovastatin twice daily. Duration of the treatment before the second study varied from 6 weeks to 3 months. Informed consent was obtained before each study. The study was approved by the Institutional Review Board.

Protocol

One week prior to study, 100–250 ml of blood was obtained from each subject after a 14-h fast. VLDL and LDL were isolated by ultracentrifugation as previously described (17, 18), and labeled with ^{125}I (VLDL) or ^{131}I (LDL) by a modification of the iodine monochloride method (19, 20). All procedures were carried out using sterile equipment, and the radiolabeled lipoproteins were passed through 0.45 μm (VLDL) and 0.22 μm (LDL) filters prior to injection into patients. After admission, patients were fed a solid diet of 30% fat, 55% carbohydrates, and 15% protein with a ratio of polyunsaturated to saturated fat of 1.0, and less then 300 mg cholesterol per day. Eight hours before the start of the VLDL turnover study (which began at 8 AM), a liquid formula diet consisting of 75% carbohydrates and 25% protein and providing 60% of the subject's caloric requirements was started. This liquid diet, consumed every 3 h throughout the 48 h of the VLDL turnover study, results in stable plasma cholesterol and triglyceride levels (21). Fifty μCi of autologous VLDL radiolabeled with ^{125}I was injected intravenously and 17 blood samples were obtained for the next 48 h (22). After completion of the VLDL study, 25 μCi of autologous LDL radiolabeled with ^{131}I was injected and 16 blood samples were collected during the next 14 days. Patients were switched back to their solid food diet 2 h after starting the LDL study and were usually discharged from the hospital after the first 2 or 3 days of the LDL protocol. The remainder of the sampling was completed with the subjects as outpatients. Potassium iodide supplements (3 drops of a saturated solution taken twice daily) were started the evening before the VLDL injection and continued until 1 week after last LDL sample was obtained. All subjects continued taking lovastatin during the second turnover study.

Laboratory procedures

VLDL, IDL, and LDL were isolated from each of the 17 samples obtained during the VLDL turnover study by sequential ultracentrifugation in a 50.3 Ti fixed angle rotor (22). ApoB in each lipoprotein fraction from each time point was then isolated using 1,1',3,3' tetramethylurea as previously described (23). ApoB specific activity (SA) in each sample was calculated by obtaining radioactivity and protein mass. LDL was isolated from the 16 samples obtained during the two-week period after injection of ^{131}I-labeled LDL, and apoB SA was determined directly by gamma counting and protein determination (22).

Plasma cholesterol and triglyceride levels were determined as the means of plasma concentrations in 9 (cholesterol) or 18 (triglycerides) time-point specimens. VLDL, IDL, and LDL lipid and apoB concentrations were determined as the means of five plasma samples obtained at 0, 12, 24, 36, 48 h and subjected separately to ultracentrifugation. ApoB mass was determined by specific radioimmunoassay (24). Cholesterol and triglyceride concentrations were determined using enzymatic methods (25, 26).

Compartmental analysis

A multicompartmental model was used to fit the data generated by the injection of ^{125}I-labeled VLDL and ^{131}I-labeled LDL (**Fig. 1**). This model is substantially the same as the one we described earlier (22), with a three-pool delipidation cascade and a remnant pool for VLDL apoB, a single delipidation step plus a remnant pool for IDL apoB, and a single pool exchanging with a noncir-

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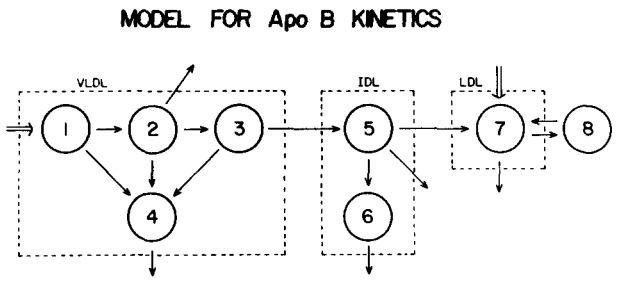

Fig. 1. Multicompartmental kinetic model for apoB metabolism in VLDL, IDL, and LDL. This model stipulates three VLDL pools, one VLDL remnant pool, one IDL pool, one IDL remnant pool, and one intravascular LDL pool exchanging with an extravascular LDL pool. Cold input is allowed into the first VLDL pool and the intravascular LDL pool (see Methods). For a detailed description of the model, see the Appendix.

culating pool for LDL. The model invokes a single site of secretion of VLDL into plasma and a single site of removal of VLDL from plasma, in addition to removal from the VLDL remnant pool. It also allows for de novo entry of apoB-containing particles into LDL ("cold" LDL flux).

Several assumptions were made during the development of this model. The injection of labeled VLDL was assumed to introduce tracer into all the VLDL vascular pools. Since we have evidence for considerable nonuniformity in labeling of different VLDL subfractions (Ramakrishnan, R., Y. Arad, and H. N. Ginsberg, unpublished results), the initial SAs in each VLDL pool were allowed to be different. Initial SA in the IDL and LDL pools were due to contamination of labeled VLDL, but were quite small. At 3 min, they were about 10–15% (IDL) and 1–2% (LDL) of whole VLDL specific activity. Pool sizes of whole VLDL, IDL, and LDL apoB were determined by measurement of plasma apoB concentrations in the different lipoprotein fractions (see above). The mass fractions of the four VLDL and two IDL pools were estimated by the curve-fitting program.

The model has 18 unknown parameters to be estimated: six initial SA (four VLDL pools, the IDL pools with the same specific activity in both, and the one intravascular LDL pool), four mass fractions (three of four VLDL pools, one of two IDL pools), three rate constants in the LDL subsystem, the rate constants of the two IDL pools, the rate constant of the VLDL remnant pool, direct removal from plasma of IDL apoB, and direct removal from plasma of VLDL apoB via the middle VLDL pool. The fractional turnover rates of the individual VLDL pools can be calculated from some of the 18 parameters and the apoB levels of VLDL, IDL, and LDL. Details are given in the Appendix.

The three parameters of the LDL subsystem (the fractional removal from the vascular pool, the fractional conversion of the vascular pool to the noncirculating pool, and the fractional turnover of the latter pool) were determined by fitting a two-pool model to the LDL SA data collected after the injection of ^{131}I-labeled LDL. These parameters were then used in fitting the LDL SA data generated by the injection of labeled VLDL.

The turnover of the remnant pools in VLDL and IDL, especially that of the IDL remnant pool, was too slow to be determined with any confidence from the 48-h study. The fractional turnovers of both remnant pools were therefore fixed for all studies at 0.03 pool per h, which is close to the mean of the remnant VLDL FCR estimated for the individual studies.

The remaining 13 parameters were estimated by fitting the three responses (VLDL, IDL, and LDL) simultaneously with the use of an estimation program developed specially for general pool models (27). For a given set of parameter values, the program solves the pool model to obtain a sum of exponentials for the SA in each pool, for the SA in each observed response, and for the sensitivities of the responses to the parameters being estimated. The program uses these, in turn, to calculate the weighted sum-of-squares criterion, assuming that the errors all have the same coefficient of variation. Estimation of the parameters was performed by minimizing the sum of squares using a modified method of Marquardt (28). A complete mathematical description of the model is given in the Appendix.

Statistical analysis

When many variables are being measured in a study, the proper statistical approach includes the identification of the "primary responses" most relevant to the goal of the investigators at the outset of the study. These were the plasma LDL cholesterol and apoB concentrations, total apoB production, LDL apoB production, VLDL apoB production, percent conversion of VLDL apoB flux to LDL apoB, and production of "cold" LDL apoB. The Student's paired *t*-test was used to compare the variables chosen as the primary responses, before and during lovastatin therapy, in the seven CHL subjects. Carrying out many more statistical tests would have increased the possibility of a type I error (declaring significance when there is none). Although the use of smaller probability levels would counter the risk of a type I error, this approach would likely have reduced the power of the procedure to a point where significant differences would be missed (type II error). We, therefore, limited the number of primary tests of significance to the seven noted above.

Even with only seven primary responses, a conservative approach would be to use a significance level smaller than 0.05. We therefore used a modified Bonferroni procedure (29) for evaluating significance. In this procedure, which corrects for the increased likelihood of a type I error when performing multiple statistical tests, the seven *t*-tests are first ranked according to their *P*-values. The most significant *P*-value is compared to $P < 0.5/7$, the next most significant *P*-value is compared to $P < 0.5/6$, and so on.

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TABLE 2. Lipid values before and during treatment with lovastatin

Subject	Total TG		VLDL TG		Total C		VLDL C		IDL C		LDL C		HDL C	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
							mg/dl							
CHL														
1	262	305	173	222	247	229	63	73	19	18	121	98	38	39
2	286	186	207	109	321	251	75	36	34	13	165	131	35	37
3	505	280	313	210	340	256	75	44	19	11	159	123	24	43
4	281	306	179	192	296	223	72	76	36	15	130	74	25	24
5	505	337	359	232	260	189	99	70	12	11	98	65	24	29
6	153[a]	114	86	63	271	164	33	25	19	10	168	88	29	38
7	220	204	167	136	256	160	45	41	13	18	150	71	29	32
Mean	316	248	212	166	284	210	66	52	22	14	142	93	29	35
± SD	± 137	± 81	± 93	± 65	± 35	± 40	± 22	± 20	± 10	± 3	± 26	± 26	± 6	± 7
	Δ = −67 ± 93 ($P < 0.08$)[b]		Δ = −44 ± 61 ($P < 0.08$)		Δ = −81 ± 33 ($P < 0.0005$)		Δ = −13 ± 18 ($P < 0.07$)		Δ = −8 ± 9 ($P < 0.05$)		Δ = −53 ± 24 ($P < 0.0005$)		Δ = 5 ± 7 ($P < 0.09$)	
FH														
1	160	112	69	49	455	337	28	19	20	5	345	247	29	35
2	82	64	25	23	427	320	10	12	19	15	328	255	30	30
3	127	114	59	53	320	221	23	16	15	2	240	151	30	34
Mean	123	97	51	42	401	293	20	16	18	7	304	218	30	33
± SD	± 39	± 28	± 23	± 16	± 71	± 63	± 9	± 4	± 3	± 7	± 56	± 58	± 1	± 3

VLDL, IDL, LDL, and HDL cholesterol and triglyceride concentrations are the means of the concentrations in five ultracentrifuged specimens, with recovery (compared to plasma cholesterol) of 80–95%. Means of nine (cholesterol) or eighteen (triglyceride) time point plasma concentrations were used for "total" values; A, before treatment; B, during treatment.

[a]This patient had a remarkable response to diet therapy resulting in a marked fall in triglyceride levels. His baseline values of plasma total and VLDL triglyceride levels, however, justify his classification as CHL.

[b]Paired *t*-tests, values before versus during lovastatin therapy.

A number of other responses in the CHL subjects were also monitored ("Other observations"). These were not studied as primary objectives but rather for purposes of comparison with previously described data and for the generation of further hypotheses. The results for these other responses should not be accepted as proven until tested independently in subsequent studies. These included plasma concentrations of total and VLDL triglycerides; total, VLDL, IDL, and high density lipoprotein (HDL) cholesterol; apoB concentrations in VLDL and IDL; IDL apoB flux; and VLDL, IDL, and LDL apoB FCR before and during therapy in the CHL group (using Student's paired *t*-test), as well as comparisons between the FH group and the CHL group (using the Wilcoxon rank-sum test, due to the small number of FH subjects). No statistical analysis of the effect of lovastatin therapy on any of the measured variables was performed on the FH group, which had only three subjects.

RESULTS[3]

Plasma lipid and apoB concentrations

Baseline levels. Plasma levels of total and VLDL triglycerides and total, VLDL, IDL, LDL, and HDL cholesterol after dietary therapy but prior to lovastatin treatment and the same parameters during lovastatin therapy are shown in **Table 2**. Differences between the values in Tables 1 and 2 (prior to therapy) are due to the effects of the AHA diet, which the subjects had been consuming for several months prior to the measurements presented in Table 2. Although the CHL patients had rather consistent reductions in total plasma cholesterol while eating the AHA diet, there were variable responses of LDL cholesterol and triglycerides. Hence, there were varying degrees of hypertriglyceridemia, with or without elevated plasma concentrations of LDL cholesterol, prior to lovastatin therapy. Compared to FH subjects, patients with CHL had higher baseline plasma concentrations of total triglycerides (316 vs 123 mg/dl, $P < 0.05$) and VLDL triglycerides (212 vs 51 mg/dl, $P < 0.05$), and significantly lower baseline plasma total cholesterol (284 vs 401 mg/dl, $P < 0.05$) and LDL cholesterol (142 vs 304 mg/dl, $P < 0.05$).

Plasma concentrations of VLDL, IDL, and LDL apoB are depicted in **Table 3**. At baseline, all CHL patients had significantly higher levels of VLDL apoB (194 vs 50 μg/ml, $P < 0.005$) and IDL apoB (104 vs 61 μg/ml, $P < 0.05$) than FH patients.

Primary responses. Lovastatin treatment resulted in decreased plasma LDL cholesterol and LDL apoB in all CHL patients. The decline in LDL cholesterol averaged

[3]As described in Methods, a modified Bonferroni procedure was used to assess the significance of the seven primary responses. The rank order of the responses was: LDL cholesterol concentration ($P < 0.0005$), LDL apoB concentration ($P < 0.001$), total apoB PR ($P < 0.003$), total LDL apoB PR ($P < 0.02$), "cold" LDL apoB PR ($P < 0.03$), VLDL apoB PR ($P > 0.05$), and percent conversion of VLDL apoB to LDL apoB ($P > 0.05$). Using the modified Bonferroni procedure, the critical P values for the five primary responses (out of seven) that were $P < 0.05$ were 0.007, 0.008, 0.01, 0.0125, and 0.017. Hence, the first three primary responses were clearly statistically significant, even with this very conservative approach.

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TABLE 3. ApoB concentration in lipoprotein fractions

Subject	VLDL ApoB		IDL ApoB		LDL ApoB	
	A	B	A	B	A	B
			μg/ml			
CHL						
1	203	112	169	58	1531	988
2	204	201	98	91	1098	972
3	239	141	100	57	1716	1243
4	185	117	151	52	1183	477
5	340	228	68	60	1470	980
6	65	44	62	28	1377	536
7	124	101	78	40	923	383
Mean ± SD	194 ± 87	134 ± 62	104 ± 41	55 ± 20	1328 ± 275	797 ± 327
	Δ = −59 ± 43 [$P < 0.02$][a]		Δ = −49 ± 41 [$P < 0.03$]		Δ = −531 ± 222 [$P < 0.001$]	
FH						
1	63	46	63	35	2048	1700
2	27	21	58	27	2288	1739
3	60	45	61	47	1413	735
Mean ± SD	50 ± 20	37 ± 14	61 ± 3	36 ± 10	1916 ± 452	1391 ± 569

ApoB concentrations are calculated as the means of five ultracentrifuged specimens; A, before treatment; B, during treatment.
[a]Paired *t*-tests.

about 35% (142 vs 93 mg/dl, $P < 0.0005$), and the decline in LDL apoB averaged about 40% (1328 vs 797 μg/ml, $P < 0.001$).

Other observations. Plasma total and IDL cholesterol decreased in the CHL patients during therapy with lovastatin. There were no consistent changes of VLDL and HDL cholesterol or of total and VLDL triglycerides in the CHL group.

All three FH patients showed a decrease in total and VLDL triglycerides and in total, IDL, and LDL cholesterol during treatment. VLDL cholesterol was reduced in two of three FH subjects. HDL cholesterol increased in two of three FH patients. While the absolute decrease in total cholesterol and LDL cholestrol levels of FH patients was larger than that of CHL patients, the groups were similar when changes were compared as percentages of initial levels.

Treatment with lovastatin decreased apoB mass in VLDL and IDL in all ten patients, and apoB mass in LDL in all three FH patients. There were no differences in the magnitude of these reductions, expressed as percentage of initial values, between the FH subjects and the CHL subjects.

Compartmental analysis

Key kinetic parameters were obtained by fitting the model shown in Fig 1 to the SA data for apoB in VLDL, IDL, and LDL. The apoB SA data and the computer-generated curves for five of seven CHL subjects and two of three FH subjects studied before and during lovastatin therapy are presented in **Fig. 2**. Individual fractional catabolic rates and fluxes are given in Tables 4–6 and group means for key parameters are depicted in Figs. 3–5. Individual pool mass fractions and rate constants are presented in the Appendix, along with the residual errors for the computer-generated fits of the SA data.

Baseline values. At baseline, VLDL, IDL, and LDL apoB production rates of CHL patients tended to be higher than those of subjects with FH. LDL apoB PR of CHL patients were also high compared to those reported in the literature for normal patients (6, 7). FCR for VLDL and IDL apoB were similar in both groups while the FCR for LDL apoB was significantly lower in the FH patients than in the CHL patients (0.18 vs 0.39 pools per day, $P < 0.005$) (**Table 4**). The LDL apoB FCR in CHL patients was similar to literature normals (6, 7). The percent of VLDL apoB flux converted to IDL and/or LDL apoB was similar between the two groups (**Table 5**). CHL patients had higher PR for total apoB-containing lipoproteins (VLDL apoB plus "cold" LDL apoB) than FH subjects (40.7 vs 18.3 mg/kg · day, $P < 0.03$) (**Table 6**). "Cold" LDL comprised approximately three-fourths of LDL apoB flux at baseline.

Primary responses. The most dramatic effect of lovastatin in CHL patients was on total apoB flux, which is the sum of VLDL flux and "cold" LDL flux. Total apoB flux declined in all seven CHL subjects (40.7 vs 25.7 mg/kg · day, $P < 0.003$) (Table 6). Both LDL apoB flux (23.9 vs 15.1 mg/kg · day, $P < 0.02$) (Table 4, **Fig. 3**) and "cold" LDL flux (18.0 vs 10.4 mg/kg · day, $P < 0.03$) (Table 6, **Fig. 4**), fell in six of seven of the CHL patients following treatment with lovastatin. Lovastatin treatment caused a decrease in VLDL apoB production in five of seven CHL patients but there was no statistically significant effect on the group as a whole (Table 4, **Fig. 5**). Finally, during treatment with lovastatin, the CHL

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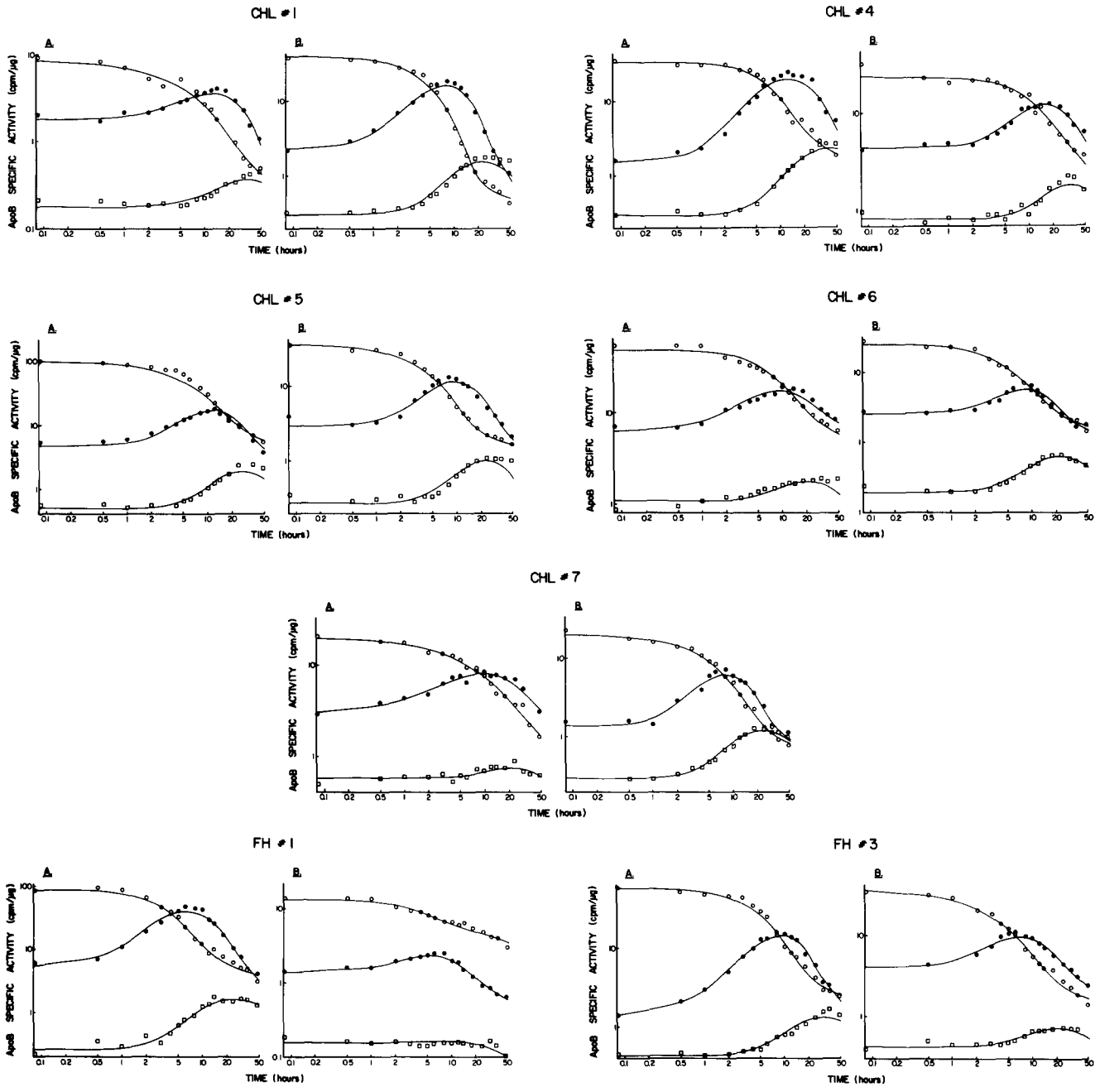

Fig. 2. ApoB specific activity curves in VLDL, IDL, and LDL generated by the injection of radiolabeled VLDL. The actual data (symbols: (○) VLDL; (●) IDL; (□) LDL) and the computer-generated fits (solid lines) of the data are depicted for five of the seven subjects with CHL and for two of the three FH patients before (A) and during (B) lovastatin treatment. The data are plotted as log apoB specific activity versus log time. The residual errors for each curve and for the overall fit of three curves are presented in the Appendix.

patients did not show a significant change in either the percentage or the absolute value of VLDL apoB flux which was converted to LDL apoB (Table 5, Fig. 4).

Other observations. In contrast to the changes in LDL apoB production rates, lovastatin treatment did not result in a consistent change in the LDL apoB FCR of CHL patients (Table 4, Fig. 3). Two patients showed an increase, three had a decrease, and two patients showed no change in LDL apoB FCR during treatment with lovastatin. FH patients had no change in LDL apoB flux during therapy, but LDL apoB FCR increased in all three FH patients. The change in LDL apoB flux was significantly different in the FH group as compared to the CHL group ($P < 0.005$).

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TABLE 4. VLDL, IDL, and LDL apoB metabolism

Subject	VLDL FCR		VLDL Flux		IDL FCR		IDL Flux		LDL FCR		LDL Flux	
	A	B	A	B	A	B	A	B	A	B	A	B
	pools/day		*mg/kg · day*		*pools/day*		*mg/kg · day*		*pools/day*		*mg/kg · day*	
CHL												
1	1.9	3.2	17.6	16.0	1.2	2.8	9.4	7.3	0.41	0.42	28.1	18.5
2	2.2	2.5	20.2	22.3	1.4	1.8	5.9	7.2	0.50	0.39	24.5	17.0
3	1.7	2.1	18.0	13.6	2.0	2.0	8.9	5.0	0.51	0.36	39.6	20.4
4	1.2	0.9	10.3	4.7	1.2	1.2	8.4	2.8	0.41	0.52	21.8	11.1
5	3.8	2.8	58.9	28.7	2.3	1.8	6.9	5.0	0.33	0.61	22.0	26.8
6	1.3	4.2	3.8	8.4	1.2	2.3	3.2	2.9	0.30	0.27	18.4	6.5
7	5.4	3.0	30.2	13.6	1.2	3.3	4.4	5.9	0.30	0.30	12.6	5.1
Mean	2.5 ±	2.7 ±	22.7 ±	15.3 ±	1.5 ±	2.2 ±	6.7 ±	5.2 ±	0.39 ±	0.41 ±	23.9 ±	15.1 ±
± SD	1.5	1.0	17.9	8.1	0.4	0.7	2.3	1.8	0.09	0.12	8.5	7.9
	Δ = 0.2 ± 1.7		Δ = −7.4 ± 12.1		Δ = 0.7 ± 0.9		Δ = −1.6 ± 2.6		Δ = −0.01 ± 0.14		Δ = −8.8 ± 7.2	
	[P = ns][a]		[P = ns]		[P = ns]		[P = ns]		[P = ns]		[P < 0.02]	
FH												
1	2.4	3.9	6.9	8.0	2.0	1.7	5.7	2.6	0.25	0.28	23.0	21.7
2	2.5	2.9	3.1	2.8	1.2	2.3	3.0	2.8	0.14	0.25	14.8	19.8
3	3.1	3.7	8.3	7.5	1.8	1.8	4.8	3.7	0.16	0.26	10.0	8.6
Mean	2.7 ±	3.5 ±	6.1 ±	6.1 ±	1.7 ±	1.9 ±	4.5 ±	3.0 ±	0.18 ±	0.26 ±	16.0 ±	16.7 ±
± SD	0.3	0.5	2.7	2.9	0.4	0.3	1.4	0.6	0.06	0.02	6.6	7.1

A, before treatment; B, during treatment; ns, not significant.
[a]Paired t-tests.

VLDL apoB FCR increased in four and decreased in three CHL patients during treatment (Table 4, Fig. 5). There was no consistent effect of lovastatin treatment on VLDL apoB production in FH patients, while VLDL apoB FCR increased in all three FH patients.

Although IDL apoB flux fell in five of seven CHL patients during treatment, the change was not significant (Table 4). IDL apoB FCR and CHL patients was not affected by lovastatin therapy. IDL apoB flux decreased in all three FH patients, but there was no change in their FCR.

In contrast to the results in CHL patients, there was no decrease in "cold" LDL apoB production in FH patients during lovastatin therapy (Table 6).

DISCUSSION

Lovastatin, the first competitive inhibitor of HMG-CoA reductase to be licensed in the United States, has been shown previously to reduce total and LDL cholesterol in patients with FH (10, 11), primary moderate hypercholesterolemia (15), cholesteryl ester storage disease (30), diabetes (31), and dyslipoproteinemia (32). Reduced cholesterol concentrations in these studies were usually accompanied by reductions in LDL apoB levels. Since lovastatin is an inhibitor of HMG-CoA reductase, the rate-limiting enzyme for endogenous cholesterol synthesis, reductions in LDL cholesterol and apoB have been thought to be secondary to increased hepatic LDL receptor activity, which would be, in turn, a response to depletion of a pool of cholesterol in the liver (13). Indeed, lovastatin can increase the activity of LDL receptors in human macrophages (33), and in rabbit (34) and dog (35) livers, and can increase receptor-mediated clearance of LDL apoB in humans with FH (14) and in dogs (35). In contrast, the effects of lovastatin in patients with moderate hypercholesterolemia and normal triglyceride concentration have been shown to result from a reduction in LDL apoB production, and not from an increase in LDL apoB FCR (15). In that study, Grundy and Vega (15) measured only LDL apoB turnover, and could not, therefore, determine whether the observed reduction in LDL production was due to decreased VLDL apoB production, decreased conversion of VLDL apoB to LDL apoB, or reduced production of LDL apoB from nonVLDL sources.

In the present study we have demonstrated that lovastatin reduces LDL cholesterol and LDL apoB concentration in CHL patients. These results are in agreement with East, Bilheimer, and Grundy (36) who showed that lovastatin plus colestipol was effective in CHL patients. We further found that the decrease in LDL apoB concentrations in CHL patients treated with lovastatin was associated with reduced rates of LDL apoB production, and not increased rates of fractional catabolism of LDL apoB.

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TABLE 5. VLDL apoB conversion to LDL apoB

Subject	VLDL Conversion to IDL A	VLDL Conversion to IDL B	VLDL Conversion to LDL A	VLDL Conversion to LDL B
		%		
CHL				
1	54	46	40	44
2	29	32	29	32
3	49	37	49	36
4	82	61	82	54
5	12	17	11	16
6	85	35	70	31
7	14	43	6	27
Mean ± SD	46 ± 30	39 ± 14	41 ± 28	34 ± 12
	Δ = −8 ± 25 [P = ns][a]		Δ = −7 ± 21 [P = ns]	
FH				
1	83	32	69	28
2	100	100	86	92
3	58	49	46	24
Mean ± SD	80 ± 21	60 ± 35	67 ± 20	48 ± 38

All data are based on total VLDL flux. Differences between VLDL apoB to LDL apoB conversion and VLDL apoB to IDL apoB conversion are due to removal of IDL apoB from plasma; A, before treatment; B, during treatment.
[a]Paired *t*-tests.

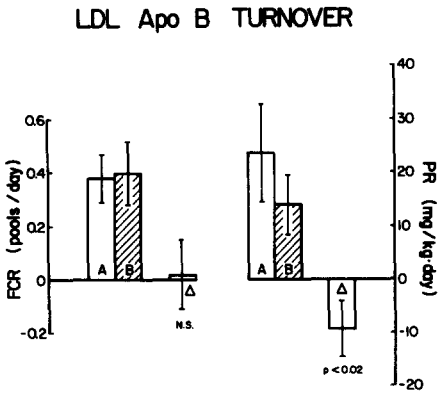

Fig. 3. LDL apoB turnover in CHL patients before (A) and during (B) lovastatin therapy; Δ, change. Data presented as mean ± SD. Lovastatin treatment of CHL patients resulted in a marked fall in LDL apoB PR but no significant change in LDL apoB FCR.

These results are similar to the results reported by Grundy and Vega (15) for patients with primary moderate hypercholesterolemia. Our protocol, however, which simultaneously measured VLDL, IDL, and LDL turnover, allowed us to determine that the decreases in LDL apoB production observed in our CHL patients were associated with decreases in total appearance of apoB-containing lipoproteins in plasma, which occurred in every CHL patient. VLDL apoB production also was reduced in five of seven subjects. In contrast, the effects of lovastatin on the conversion of VLDL apoB to LDL apoB in CHL patients, whether measured as the percent of VLDL apoB flux which was converted to LDL apoB or as the absolute amount of LDL apoB flux which was derived from VLDL apoB, were variable and there were no significant changes in these parameters during therapy for the CHL group as a whole. Based on these results, we conclude that the main effect of lovastatin in CHL patients is via reduced total entry of apoB-containing lipoproteins particles into plasma, rather than through decreased conversion of VLDL apoB to LDL apoB, as Grundy and Vega had proposed (15).

TABLE 6. Total apoB flux and "Cold" LDL synthesis

Subject	"Cold" LDL Flux A	"Cold" LDL Flux B	LDL Flux Derived from "Cold" LDL Production A	LDL Flux Derived from "Cold" LDL Production B	Total ApoB Flux A	Total ApoB Flux B
	mg/kg · day		%		*mg/kg · day*	
CHL						
1	21.1	11.6	75	62	38.7	27.5
2	18.5	9.9	76	58	38.8	32.2
3	30.7	15.5	78	76	48.7	29.1
4	13.4	8.6	61	77	23.7	13.3
5	15.5	22.2	71	83	74.4	50.9
6	15.7	3.9	85	59	19.5	12.2
7	10.8	1.4	85	28	41.0	15.0
Mean ± SD	18.0 ± 6.5	10.4 ± 7.0	76 ± 8	63 ± 18	40.7 ± 18.0	25.7 ± 13.8
	Δ = −7.5 ± 7.0 [P < 0.03][b]		Δ = −12 ± 25 [P = ns]		Δ = −14.9 ± 8.0 [P < 0.003]	
FH						
1	18.2	19.5	79	90	25.12	27.5
2	12.2	17.3	82	87	15.2	20.0
3	6.2	6.8	62	79	14.5	14.4
Mean ± SD	12.2 ± 6.0	14.5 ± 6.8	74 ± 11	85 ± 6	18.3 ± 5.9	20.6 ± 6.6

A, before treatment; B, during treatment; ns, not significant.
[a]Total apoB flux is the sum of VLDL apoB flux and direct LDL production.
[b]Paired *t*-tests.

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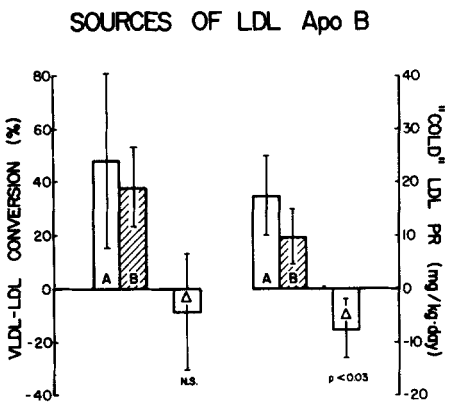

Fig. 4. Sources of LDL apoB in CHL patients before (A) and during (B) lovastatin treatment; Δ, change. Lovastatin treatment caused a significant decrease of direct (nonVLDL apoB-derived) LDL apoB production. On the other hand, the fraction of VLDL apoB flux that was converted to LDL apoB did not change significantly with lovastatin therapy.

Although the small number of FH subjects studied precluded statistical analysis, several observations concerning the effect of lovastatin on apoB metabolism in FH subjects should be noted. For example, in contrast to our findings in CHL patients, the reduction of VLDL and LDL apoB levels during lovastatin therapy in FH patients appeared to be due to increases in VLDL and LDL apoB FCR. VLDL and LDL apoB production showed no consistent changes in response to lovastatin treatment in our FH patients. These results are in agreement with published results in subjects with FH (14, 37), although in the recent report by Vega, East, and Grundy (37), the combined use of lovastatin and colestipol make any conclusions concerning the isolated effects of lovastatin uncertain. Why subjects with FH should respond so differently to inhibition of HMG-CoA reductase, even though they, like the CHL patients, have a large proportion of LDL flux that appears to be independent of VLDL flux, is unclear. One possibility is that the pathophysiologic basis for increased apoB flux through LDL is unique for each of these hyperlipidemic states.

Of particular interest is our finding that "cold" LDL apoB PR dropped in six of seven subjects on lovastatin and accounted for a large fraction of the drop in total LDL apoB PR observed during the lovastatin treatment period. "Cold" LDL apoB (i.e., LDL apoB derived from sources other than circulating VLDL apoB) contributed a major fraction of total LDL apoB flux at baseline, and the decrease in "cold" LDL PR during lovastatin therapy played a significant role in the fall in total rate of appearance of apoB-containing lipoproteins that was observed in every patient with CHL during treatment with lovastatin.

It has been suggested that ultracentrifugation and radiolabeling of VLDL may produce tracers that are not representative of circulating VLDL, and that use of such tracers may lead to misinterpretation of the precursor–product relationship between VLDL and LDL. However, studies of apoB metabolism using either pulse injection or constant infusions of radiolabeled amino acids to endogenously label apolipoproteins have found rates of VLDL apoB production similar to those reported for exogenously labeled tracers in both normal (38, 39) and hypertriglyceridemic (40) subjects. Of particular note is the recent report by Cohn et al. (39) in which nonVLDL-derived LDL flux was demonstrated with a constant infusion of heavy isotope-labeled amino acid to endogenously label apoB.

While "cold" LDL apoB production was significant as estimated with our multicompartmental model in each of the 20 studies, support for "cold" LDL entry into plasma was also found when the primary apoB SA data were viewed in qualitative terms. With IDL as the precursor of LDL, the normal precursor–product relationship requires that the LDL apoB SA curve should reach its peak at the point of its intersection with the IDL apoB SA curve. If the LDL apoB SA peak value is smaller than the IDL apoB SA at that time, it is evidence for entry of unlabeled LDL apoB directly into plasma. This, in fact, is what we found. In none of the 20 studies did the LDL apoB SA curve intersect the IDL apoB SA data at or before the LDL apoB SA peak. In 18 out of 20 studies the LDL apoB SA curve peaked during the 48-h study, the peak time ranging from 18 to 36 h. In every case, the peak LDL apoB SA value was smaller than the IDL apoB SA at that time. This lack of a simple precursor–product relationship between IDL and LDL apoB SA can be seen clearly in Fig. 2, where SA data and fitted curves for seven of ten subjects are presented. The ratio of LDL apoB SA to IDL apoB SA at the time of peak LDL apoB SA in these 18

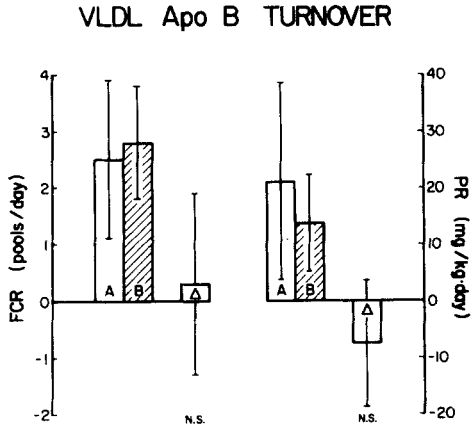

Fig. 5. VLDL apoB turnover in CHL patients before (A) and during (B) lovastatin therapy; Δ, change. Treatment with lovastatin resulted in no statistically significant change in either VLDL PR or VLDL FCR for the CHL group as a whole.

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studies ranged from 0.13 to 0.53 with a mean of 0.3. The presence of the extravascular LDL apoB pool and the remnant IDL apoB pool complicates the interpretation of these ratios, but the degree to which the ratios were smaller than 1.0 suggests strongly that there was significant entry of unlabeled LDL apoB into plasma in these subjects. The values estimated by our compartmental model provide quantification of this "cold" LDL apoB production.

Whether such "cold" LDL flux could represent direct LDL production by the liver has been a controversial issue. Windmueller and Spaeth (41), in a study of perfused rat livers, indicated that less then 5% of LDL could be derived from nonVLDL sources. Perfusion studies in WHHL rabbits suggested that all apoB enters plasma in VLDL (42). In contrast to these findings, other groups have demonstrated secretion of LDL-like particles by cultured hepatocytes (43, 44) and HepG2 cells (45–47) or from perfused pig, monkey, and guinea pig livers (48–52). LDL-like particles have also been isolated from the hepatic Golgi of hypercholesterolemic rats (53). Direct secretion of LDL apoB into plasma has been observed in previous kinetic studies in humans with FH (6, 54), CHL (6, 7), hypertriglyceridemia (22), non-insulin-dependent diabetes (55), hyperapobetalipoproteinemia (5), and type III hyperlipidemia (56). VLDL-independent production of LDL apoB has also been demonstrated in turnover studies of various animal species (57–61.).

Our findings that lovastatin reduced "cold" production of LDL apoB is supported by previous kinetic studies in rabbits (58), miniature pigs (61), and humans with type III hyperlipidemia (56). La Ville et al. (58) found that lovastatin decreased LDL apoB production in rabbits by 66%, without changing VLDL apoB production. The fall in LDL apoB PR that they observed was also too large to be accounted for by changes in VLDL apoB to LDL apoB conversion. Huff et al. (61) showed similar inhibition of direct LDL apoB production by lovastatin in the miniature pig. In a succeeding study, these same investigators demonstrated that the reduction in direct LDL production was within the more dense, LDL_2 subclass (62). Vega et al. (56) studied three patients with familial dysbetalipoproteinemia before and during treatment with lovastatin and found marked inhibition of direct LDL apoB input into plasma during therapy compared to baseline. Finally, we observed a marked reduction in both VLDL apoB production and direct LDL apoB production during lovastatin therapy in a young girl with cholesteryl ester storage disease (30).

Why should an agent whose only known biochemical effect is the inhibition of HMG-CoA reductase cause an apparent reduction in the rates of entry of apoB into plasma? One possibility relates to the nature of "cold" LDL apoB production. It has been suggested that what is determined kinetically to be "cold" (or direct) LDL apoB production actually derives from a fraction of secreted VLDL apoB that binds to the endothelium during the first pass through the heart, lungs, and peripheral arterial circulation. These VLDL particles may then be converted to LDL by interaction with lipoprotein lipase before they appear in the peripheral venous circulation. Since we sample the venous circulation, we may "see" these particles as directly secreted LDL during our tracer studies. The reduced "cold" LDL apoB production we found with lovastatin treatment might then be the result of increased activity of LDL receptors in the heart, lungs, and peripheral tissue (which would cause greater removal of apoB-containing particles during the first pass). Even our demonstration that lovastatin reduced VLDL apoB PR in several CHL patients could be interpreted as increased removal of nascent VLDL, or VLDL rapidly converted to LDL within the hepatic sinusoids, by upregulated hepatic LDL receptors.

Both of the possibilities described above are consistent with the proposals by Beltz et al. (63) and Vega et al. (56). The latter suggested that an intravascular VLDL pool (V_0) exists which has an extremely rapid turnover and which serves as a precursor to the "direct" LDL pool. They proposed that increased LDL receptor activity secondary to lovastatin treatment had resulted in increased removal of V_0, thus creating an apparent decrease in "direct" LDL production. Some indirect support for this mechanism comes from studies by Goldberg et al. (60, 64). They demonstrated direct production of LDL apoB in cynomolgus monkeys injected with radiolabeled VLDL and LDL (60). However, when lipoprotein lipase activity was inhibited by infusion of antibodies to that enzyme, it appeared that direct LDL apoB production, as assessed by endogenous labeling of lipoproteins with [^{3}H]leucine, was blocked (64). This result suggested that "cold" LDL apoB production in the chow-fed cynomolgus monkey might actually derive from a V_0 pool of VLDL. However, such a V_0 pool has never been isolated, thus remaining purely theoretical. In addition, LPL inhibition may generate altered VLDL remnants which, after uptake by the liver, could affect the direct production of LDL. Finally, use of the chow-fed monkey as a model for LDL production in hyperlipidemic humans has obvious limitations.

A closer inspection of our data does not support the hypothesis that increased removal of V_0 can explain our findings. If a lovastatin-induced upregulation of LDL receptors caused a greater fraction of the precursors of "directly secreted" LDL particles to be removed rapidly from plasma, we should have observed a direct relationship between the magnitude of the increase in LDL apoB FCR and the magnitude of the decrease in "cold" LDL apoB production in our subjects. In fact, we observed an inverse relationship between these two values (**Fig. 6**). This correlation held up for the CHL group alone, as well

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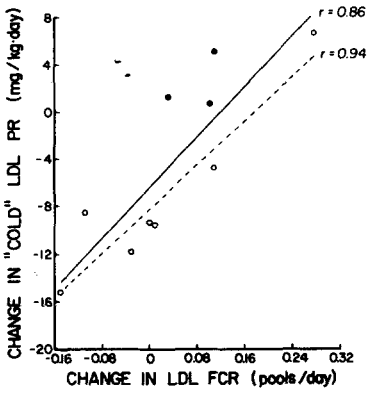

Fig. 6. Relationship between the effect of lovastatin therapy on direct LDL apoB PR (Δ in mg/kg · day) and its effect on LDL apoB FCR (Δ in pools/day) in CHL (open circles) and FH (closed circles) patients. Larger increases of LDL apoB FCR are associated with smaller decreases in direct LDL apoB PR. This relationship holds for the CHL group alone (r = 0.94, dashed line) as well as for all ten patients (r = 0.86, solid line) and strongly argues against increased activity of LDL receptors as the cause for decreased direct LDL apoB PR (see Discussion).

as for all ten patients combined. Some of the largest reductions in "cold" LDL apoB production occurred in subjects in whom the LDL apoB FCR fell. This observation is supported by the changes in the percent of total LDL apoB production that is derived from "cold" LDL apoB production (Table 6). In the two CHL patients (#4 and #5) who had an increase in LDL apoB FCR, and in the three FH patients (each of whom increased his/her LDL apoB FCR during therapy), the percent of LDL apoB flux derived from " cold" LDL apoB production also increased, whereas those CHL patients whose LDL apoB FCR decreased had a decrease in the percentage of LDL apoB flux derived from "cold" LDL apoB production. These findings contradict the above hypothesis postulating increased LDL receptor activity as the cause for reduced "cold" LDL apoB PR. We would note, however, that our interpretation of these correlations rests upon the assumption that the autologous VLDL and LDL tracers isolated before lovastatin therapy interact with LDL receptors similarly to the respective tracers isolated during lovastatin therapy. Berglund, Elam, and Witztum (65) have reported that lovastatin may alter the in vivo catabolism of LDL in guinea pigs. If similar alterations occur in humans, interpretation of the data in Fig. 6 would be very complex.

An alternative explanation for the effects of lovastatin on apoB metabolism that we observed, and the one that we favor is that lovastatin treatment directly influenced the total secretion of apoB-containing lipoproteins. Although we have not, at present, determined the mechanism for such an action, several possibilities exist. The inhibition of HMG-CoA reductase and the resultant decrease in intrahepatic free cholesterol might serve as a signal for decreased transcription of apoB DNA and/or reduced apoB synthesis. Alternatively, when cholesterol production is curtailed and less cholesterol, relative to apoB, is available for lipoprotein synthesis, apoB degradation might increase in the hepatocyes. Finally, the type of lipoprotein assembled in the liver may be altered during lovastatin therapy. The crucial role of lipid availability in the regulation of the type of lipoprotein particle that may be assembled and secreted by the liver is supported by demonstrations of both increased direct LDL apoB production, concomitant with reduced VLDL triglyceride production, after weight loss (22), and decreased nonVLDL-derived LDL apoB flux, concomitant with increased VLDL apoB flux, after feeding (39). Although measurements of total body cholesterol synthesis during lovastatin treatment have not demonstrated consistent reductions (66), probably due to increased HMG-CoA reductase mass (67), treatment with lovastatin may reduce the quantity of cholesterol available for lipoprotein assembly, thereby restricting lipoprotein secretion by the liver. Indeed, Khan, Wilcox, and Heimberg (68) recently demonstrated that lovastatin treatment inhibited VLDL secretion by perfused rat livers. The decreased VLDL apoB production in five of seven CHL patients treated with lovastatin in this study and our previous demonstration of reduced VLDL and LDL apoB production in a patient with cholesteryl ester storage disease during lovastatin therapy (30) support the idea that reduced "cold" LDL production in lovastatin-treated CHL patients results from actual reductions in the numbers of apoB-containing lipoproteins secreted by the liver. The clinical and biological implications of this finding need further investigation.

APPENDIX

The model shown in Fig. 1 for apoB kinetics after injection of labeled VLDL is described mathematically by the following differential equations:

$$\frac{dy_1}{dt} = -l_{11}y_1 \qquad y_1(0) = u_1$$

$$\frac{dy_2}{dt} = l_{22}\,(y_1 - y_2) \qquad y_2(0) = u_2$$

$$\frac{dy_3}{dt} = l_{33}\,(y_2 - y_3) \qquad y_3(0) = u_3$$

$$\frac{dy_4}{dt} = \frac{l_{44}}{3}\,(y_1 + y_2 + y_3) - l_{44}y_4 \qquad y_4(0) = u_4$$

$$\frac{dy_5}{dt} = l_{55}\,(y_3 - y_5) \qquad y_5(0) = u_I$$

$$\frac{dy_6}{dt} = l_{44}\,(y_5 - y_6) \qquad y_6(0) = u_I$$

$$\frac{dy_7}{dt} = l_{75}y_5 - l_{07}y_7 + l_{78}\,(y_8 - y_7) \qquad y_7(0) = u_L$$

$$\frac{dy_8}{dt} = l_{88}\,(y_7 - y_8) \qquad y_8(0) = 0$$

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The observed specific activities are described by the following:

$$z_1 = SA_{VLDL} = m_1y_1 + m_2y_2 + m_3y_3 + m_4y_4$$
$$z_2 = SA_{IDL} = m_5y_5 + (1 - m_5)y_6$$
$$z_3 = SA_{LDL} = y_7$$

Here, y_i is the apoB SA in pool i as a function of time; dy_i/dt is the rate of change in y_i with time; and $y_i(0)$ is the initial SA in pool i. Pools 1–3 are the three VLDL cascade pools; pool 4 is the VLDL remnant pool; pool 5 is the main IDL pool; pool 6 is the remnant IDL pool; pool 7 is the circulating LDL pool; pool 8 is the noncirculating (extravascular) LDL pool. Rate constants are denoted l_{ij}, which is the apoB mass flux (tracee) into pool i from pool j divided by the apoB mass in pool i; l_{ii} is the apoB mass flux through pool i divided by the apoB mass in pool i (equal to the FCR for VLDL and IDL pools); l_{0i} is the apoB mass flux from pool i to the outside divided by the apoB mass in pool i; l_{i0} is the apoB mass flux into pool i from outside (de novo synthesis) divided by the apoB mass in pool i. The term m_1 is the apoB mass in pool 1 as a fraction of total VLDL apoB mass; similarly for m_2, m_3, and m_4. The term m_5 is the apoB mass in pool 5 as a fraction of total IDL apoB mass. The observed SAs are denoted by z_1, z_2, and z_3: z_1 is the apoB SA in whole VLDL obtained as the mass-weighted average of the individual SAs in the four VLDL pools, i.e., each SA is multiplied by the corresponding mass fraction and added up. Values are determined similarly for z_2, which is the apoB SA in whole IDL. Since there is only one circulating LDL pool, z_3 equals y_7.

The model equations express the assumptions in Fig. 1 as well as those stated in the text. In the differential equation for y_2, the coefficients of y_1 and y_2 adding to zero (being equal in magnitude and of opposite sign) means there is no de novo synthesis of apoB into pool 2; similarly for pools 3 to 6 and 8. In the differential equation for y_4, the coefficients of y_1, y_2, and y_3 being equal means the mass flux through pool 4 is derived equally from the three cascade pools. The equations for y_6 express the assumptions that the rate constant for pool 6 is the same as for pool 4, and that the initial SA in pool 6 is the same as in pool 5. The differential equation for y_7 expresses the assumptions that label may enter pool 7 from pool 5 or from pool 8 but not from other pools, and that there may be direct LDL apoB production (l_{07}–l_{75}).

The model for apoB kinetics after injection of labeled LDL is given by just the equations for y_7 and y_8 except that there is no term with y_5. The parameters for this model are estimated from the LDL SA data following labeled LDL injection and, except for u_L, used in the study with labeled VLDL (i.e., parameters l_{07}, l_{78}, and l_{88}). This is done for each study separately.

Mass fluxes are calculated as follows:

$$\text{LDL apoB production} = l_{07}M_L$$
$$\text{VLDL apoB production} = l_{11}m_1M_V$$
$$\text{VLDL-LDL apoB flux} = l_{75}M_L$$
$$\text{Direct LDL apoB production} = (l_{07} - l_{75})\,M_L$$

$$\text{Mean residual error} = 100\sqrt{\frac{\sum^{n}\left[\frac{(\text{fitted SA} - \text{observed SA})}{\text{fitted SA}}\right]^2}{n - 13}}$$

where the summation is over the n observed SAs (VLDL, IDL, LDL together) and 13 is the number of parameters being estimated. The mean residual error varied from 8.5% to 31.6% in the 22 studies, with 11 under 12%, and 10 in the 12–20% range. Of the more than 300 data points, only one residual exceeded twice the mean residual error.

The adequacy of the model was tested by augmenting the model in a variety of ways: addition of a fourth pool to the VLDL cascade, removal from pool 1 or from pool 3, conversion from pool 1, 2, or 3 to IDL or LDL, de novo synthesis into pool 2, into pool 3, or into IDL, pathway from remnant VLDL to IDL, allowing l_{44} to vary, allowing l_{66} to differ from l_{44}, allowing $y_6(0)$ to be different from $y_5(0)$.

No augmentation led to a better fit of the data for any of the studies. We conclude that the model we used is adequate. It is possible that one or more of the augmentations are physiologically correct; it is just that our kinetic data do not require them. It is possible that when more extensive data on VLDL subfractions are available, the model would have to be augmented, but for now, the model we have is adequate. No augmentation of the LDL protein portion was tried, since the LDL apoB SA data from labeled LDL were well fitted by a two-pool model. It is possible that a different structure for LDL can lead to a better fit to the LDL apoB SA data from labeled VLDL injection.

APPENDIX TABLE 1. Mass fractions and fractional rate constants for VLDL

	M1		M2		M3		L11		L22		L33	
Subject	A	B	A	B	A	B	A	B	A	B	A	B
CHL-1	31	34	6	36	37	16	6	9	33	9	3	9
CHL-2	33	25	28	25	13	19	7	10	8	10	5	4
CHL-3	27	27	33	27	24	19	6	8	5	8	3	4
CHL-4	38	35	18	18	14	17	3	3	7	5	7	3
CHL-5	1	26	84	36	13	10	372	11	5	8	3	5
CHL-6	34	44	13	19	23	34	4	10	10	22	5	4
CHL-7	6	23	52	38	37	31	88	13	10	8	2	4
FH-1	48	64	19	16	15	0	5	6	13	24	144	11
FH-2	69	71	24	13	3	13	4	4	11	22	80	23
FH-3	24	17	25	19	36	39	13	22	12	19	5	5

M1, M2, M3: Percent of total VLDL apoB mass in each of the three pools in the VLDL cascade (Fig. 1). The mass fraction of pool 4 is the remainder. L11, L22, L33: Fractional rate constants, in pools/day, for the three pools in the cascade. A, before treatment; B, during treatment.

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APPENDIX TABLE 2. Mass fractions and fractional rate constants for IDL and LDL

	M5		L55		L78		L87	
	A	B	A	B	A	B	A	B
CHL-1	100	78	1	4	0.12	0.16	0.17	0.33
CHL-2	100	83	1	2	0.03	0.10	0.23	0.13
CHL-3	100	74	2	3	0.36	0.38	0.31	0.61
CHL-4	100	48	1	2	0.12	0.12	0.33	0.08
CHL-5	47	59	5	3	0.22	0.13	0.08	0.24
CHL-6	22	43	5	5	0.11	0.24	0.14	0.33
CHL-7	39	53	3	6	0.25	0.15	0.54	0.38
FH-1	65	41	3	4	0.09	0.33	0.38	0.83
FH-2	41	36	3	6	0.72	0.62	0.96	0.99
FH-3	50	44	4	4	0.03	0.17	0.38	0.52

M5: Percent of total IDL apoB mass in pool 5 (Fig. 1). The mass fraction of pool 6 is the remainder. L78, L87: Fractional rate constants for the exchange between the intravascular and extravascular LDL apoB pools 7 and 8. A, before treatment; B, during treatment.

APPENDIX TABLE 3. Initial apoB SA in VLDL cascade pools

	u1/uv		u2/uv		u3/uv	
	A	B	A	B	A	B
CHL-1	130	84	866	192	13	11
CHL-2	183	38	115	350	18	4
CHL-3	30	33	271	333	3	3
CHL-4	44	78	442	321	4	8
CHL-5	46	40	107	234	5	4
CHL-6	66	183	399	18	52	18
CHL-7	87	43	55	217	146	4
FH-1	39	84	393	8	4	848
FH-2	42	60	284	290	4	79
FH-3	167	52	178	434	28	5

Individual specific activities are expressed as percent of whole VLDL specific activity, u1, u2, and u3 are the intital specific activities in the three cascade pools, respectively; uv is the specific activity of whole VLDL apoB. A, before treatment; B, during treatment.

APPENDIX TABLE 4. Residual errors: overall and individual error for computer fits of apoB SA data

	Overall		VLDL		IDL		LDL	
	A	B	A	B	A	B	A	B
CHL-1	11.7	12.0	15.4	11.9	8.9	8.8	11.5	14.1
CHL-2	15.7	32.4	11.4	26.6	23.5	34.3	10.0	34.0
CHL-3	19.9	17.0	19.2	14.0	20.6	22.0	19.8	13.9
CHL-4	13.9	10.8	15.6	13.3	17.0	7.3	6.8	10.9
CHL-5	14.5	13.2	14.7	6.7	14.3	14.6	14.6	15.5
CHL-6	11.0	8.6	11.1	8.7	9.8	9.9	11.8	7.2
CHL-7	10.0	10.4	13.3	11.1	8.5	14.0	9.3	5.7
FH-1	13.6	9.0	15.8	10.5	11.2	6.2	13.8	9.4
FH-2	16.3	10.7	14.9	10.6	12.1	7.3	19.8	12.7
FH-3	10.4	8.8	12.0	12.4	7.8	7.4	11.1	6.6

A, before treatment; B, during treatment.

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