Physiologic mechanisms of action of lovastatin in nephrotic syndrome

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Abstract The effects of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors on the metabolism of apolipoprotein (apo) B-containing lipoproteins appear to differ according to the predominant lipoprotein profiles present and the condition being treated. In familial hypercholesterolemia, with isolated low density lipoprotein (LDL) elevations, the LDL-apoB elimination rate is increased by up-regulated LDL-receptors. In familial combined hyperlipidemia where very low density lipoprotein (VLDL) and LDL both may be increased and enhanced production of LDL-apoB may be present, HMG-CoA reductase inhibitors seem to diminish increased LDL-apoB production. The drug-induced decreases in LDL-apoB production could be due to decreased production of precursor VLDL-apoB or due to decreased conversion of VLDL-apoB to LDL-apoB after enhanced removal of VLDL by up-regulated LDL-receptors. To distinguish between these possibilities, we assessed the effects of HMG-CoA reductase inhibitors in another condition in which there is both apoB overproduction and accumulation of VLDL and LDL in plasma, the nephrotic syndrome. We used endogenous labeling of apoB with [^{13}C]leucine and a multicompartmental model to calculate the metabolic parameters of apoB-containing lipoproteins. Only subjects with focal segmental glomerular sclerosis (FSGS) were included, as FSGS is a chronic, very slowly progressive form of nephrotic syndrome. A double-blind, randomized, placebo-controlled, crossover design was used. Treatment periods of 6 weeks were separated by a 2-week washout period. Of the four men studied, three had high triglyceride levels and four had high cholesterol levels. Lovastatin (20 mg/day) significantly decreased cholesterol (27.6 ± 6%), LDL-cholesterol (27.6 ± 9%) and plasma apoB (17.9 ± 2.9%) ($P < 0.01$ for all). During the placebo period, calculation of kinetic parameters revealed VLDL-, intermediate density lipoprotein (IDL)-, and LDL-apoB overproduction and decreased VLDL-apoB fractional catabolic rate. Lovastatin significantly decreased LDL-apoB production rate in all cases (34.1 ± 14%, $P = 0.03$). The decreased LDL-apoB was mainly due to a channelling of LDL precursors away from conversion to LDL (conversion of VLDL to LDL decreased from 80.6 ± 8.3% to 55.9 ± 17.2%, $P = 0.05$). Thus, lovastatin decreased LDL-cholesterol in nephrotic subjects mainly by inhibiting LDL-apoB production from VLDL.—**Aguilar-Salinas, C. A., P. H. R. Barrett, J. Kelber, J. Delmez, and G. Schonfeld.** Physiologic mechanisms of action of lovastatin in nephrotic syndrome. *J. Lipid Res.* 1995. **36:** 188–199.

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Hyperlipidemia is one of the components of the nephrotic syndrome. The hyperlipidemia may be implicated in the increased incidence of coronary artery disease observed in subjects with nephrotic syndrome (1). Furthermore, in recent years, hyperlipidemia also has been implicated in the progression of the glomerular damage (2).

Elements in the pathophysiology of the lipoprotein abnormalities associated with the nephrotic syndrome are as follows: *a)* hepatic overproduction of cholesterol, triglycerides, phospholipid and proteins due to decreased oncotic pressure and/or viscosity (3, 4); *b)* decreased activity of lipoprotein lipase (5); *c)* the presence of inhibitors of lipoprotein lipase activity (6); *d)* decreased activity of lecithin cholesterol acyltransferase (7); *e)* increased activity of cholesteryl ester transfer protein (8); and *f)* urinary losses of apoC-II and other unknown macromolecules involved in lipid metabolism (9). However, most of the current information has been obtained from animal models or in vitro studies. The kinetic abnormalities of the apoB-containing lipoproteins in humans have been studied in a small number of subjects by three different research groups (10–12). The kinetics of the three apoB-containing fractions VLDL, IDL, and LDL were reported in only two studies involving four and eight subjects, respectively (11, 12). Overproduction of apoB-containing lipoproteins,

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; apo, apolipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; FSGS, focal segmental glomerular sclerosis; DGUC, density gradient ultracentrifugation; GC-MS, gas chromatography–mass spectrometry; FCR, fractional catabolic rate.

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along with decreased catabolism of VLDL- and LDL-apoB, also were found in some subjects. The remarkable variability among individuals was perhaps due to *a)* the coexistence of other primary lipid abnormalities, *b)* heterogeneity of lipoprotein populations across individuals, *c)* varying etiologies of the nephrotic syndrome, *d)* the presence of varying degrees of renal failure, *e)* the ingestion of drugs such as steroids that affect lipid metabolism, or *f)* undefined factors. Thus, further studies comparing well-defined subgroups of nephrotic subjects with a common etiology of the nephrotic syndrome and well-preserved renal function are needed (12).

The HMG-CoA reductase inhibitors provide important new therapeutic options in the treatment of the hyperlipidemias associated with the nephrotic syndrome. Two physiologic mechanisms have been proposed by which the HMG-CoA reductase inhibitors may achieve their effect of lowering LDL-cholesterol. First, increased LDL-apoB catabolism could follow from the drug-induced suppression of cholesterol synthesis and the consequent up-regulation of LDL-receptors (13). This mechanism seems to operate in heterozygotes for familial hypercholesterolemia. Second, decreased rates of LDL production were noted in other forms of hypercholesterolemia (14), where no effect was observed on LDL clearance. Altered LDL production rates, in turn, could result from two different physiologic mechanisms: either a decreased production of VLDL, the LDL precursor, in the liver, or a decreased conversion of VLDL to LDL due to an increased removal of VLDL (and IDL) from plasma by the up-regulated LDL-receptors. The kinetic studies performed to date have used reinjection of autologous exogenously labeled VLDL and/or LDL. While exogenous labeling may avoid the potential problem of recycling of labels that is a limitation of endogenous labels (15), exogenous labels may fail to detect rapidly turning over pools of LDL precursor lipoproteins such as nascent VLDL (15, 16). If HMG-CoA reductase inhibitor therapy, in fact, increases the clearance of rapidly turning over potential precursors of LDL, such as VLDL and IDL, apparent production rates of VLDL using exogenous labeling could appear spuriously to decrease. The use of endogenous tracer labeling techniques should avoid these problems and should enable the kinetics of all pools of these particles to be better represented. We use here the endogenous labeling method we have previously reported in normal and hypolipidemic subjects (15, 17).

We postulate that if lovastatin, an HMG-CoA reductase inhibitor, can reduce the production of VLDL-apoB by the liver, this action must be particularly evident in clinical pathologies in which there is a hepatic overproduction of apoB, such as the nephrotic syndrome. The objectives of this work were to study the kinetic abnormalities of the apoB-containing lipoproteins in Focal Segmental Glomerular Sclerosis (FSGS), a stable form of the nephrotic syndrome not treated with corticosteroids, using endogenous labeling of apoB with [^{13}C]leucine in order to determine the mechanism of action of lovastatin in these patients.

METHODS

Subjects

Four male patients with FSGS were recruited from the outpatient clinic of the Kidney Center of the Washington University School of Medicine, St. Louis, MO. Absence of response to steroid therapy had been previously documented in all cases. The patients were off steroids for several months before their inclusion in the study. None of the patients received drug therapy for hyperlipidemia, β-blockers, or steroids during the 6 weeks before the study. None of the subjects had a familial history of coronary heart disease. All patients had creatinine clearances greater than 50 ml/min. None of the patients were edematous, however, all were taking diuretics (Furosemide). EKG, plasma glucose, liver, and thyroid tests were in normal range. None of the patients had apoE2 genotype. All subjects completed the entire study. Throughout the studies the patients were asked to follow their normal diets. Each patient was seen by a dietitian regularly. Proteinuria remained stable and in nephrotic range ($>$ 3 g/d) during the course of the study.

Four healthy middle-aged men were included as controls. They are non-blood related members of various kindreds studied in the Lipid Research Clinic of the Washington University School of Medicine, St. Louis, MO. The study protocol was approved by the Human Studies Committee of Washington University School of Medicine and all patients gave written consent. The clinical characteristics of the patients and controls are shown in **Table 1.**

Protocol

A double-blind, randomized, placebo-controlled crossover design was used. Patients were randomly assigned to receive first placebo or lovastatin (20 mg at night). Each drug was given for 6 weeks separated by a 2-week washout period. A physical exam, blood chemistries of liver and thyroid function, and a lipid profile were done at the beginning of each phase of the study. At the end of each phase a primed constant 8-h [^{13}C]leucine infusion was administered to evaluate the kinetics of the apoB-containing lipoproteins. During the kinetic study lovastatin or placebo were continued. For the [^{13}C]leucine infusion, patients were admitted to the General Clinical Research Center at Washington University School of Medicine after fasting for 12 h. A bolus of [^{13}C]leucine (0.85 mg/kg) was administered immediately followed by 0.85 mg/kg per

TABLE 1. Clinical characteristics of patients with focal segmental glomerular sclerosis

Subject	Age	BMI	Urine Albumin	Plasma Albumin	ApoE Genotype
			g/day	*g/dl*	
1	50	46	10.8	3.2	3/4
2	45	37	3.9	2.6	3/3
3	65	27	6.3	2.5	3/3
4	42	34	3.9	3.0	3/4
Controls	40.5 ± 9.11	26.3 ± 2.22		4.35 ± 0.32	3/3 (2) 3/4 (1) 3/2 (1)

BMI, body mass index.

h as a constant infusion. During the study, 37 samples were drawn through a second intravenous catheter in the contralateral arm. All samples were used for determination of plasma leucine enrichment while 28 were used for determination of VLDL-, IDL-, and LDL-apoB leucine enrichment. After 8 h, the tracer infusion was stopped, the patients remained fasting for another 8 h and thereafter they continued their usual diets. Samples were drawn over 84 h as indicated in Fig. 2 for determination of apoB enrichments. ApoB and lipid concentrations were measured in five samples during each kinetic study in order to demonstrate a steady state.

Analytical methods

Isolation of lipoproteins. Blood was collected in EDTA-containing tubes and plasma was separated by low-speed centrifugation. VLDL ($d < 1.006$ g • ml^{-1}), IDL (d 1.006–1.019 g • ml^{-1}), and LDL (d 1.019–1.063 g • ml^{-1}) were isolated from 4 ml of plasma by sequential ultracentrifugation (18) and dialyzed against ammonium bicarbonate (5 mM) for 24 h.

Measurements of lipids and apoB. ApoB concentrations were measured in VLDL, IDL, LDL fractions and a 1-ml concentrate of 50 ml of overnight urine by immunoturbidometry (Behring Diagnostics, Inc., Somerville, NJ). Cholesterol and triglycerides were measured by commercially available tests (WAKO Pure Chemical Industries, Ltd., Osaka, Japan). VLDL-, IDL-, and LDL-apoB pool sizes were determined by multiplying the measured apoB concentrations by plasma volume (body weight multiplied by 0.045).

Density gradient ultracentrifugation (DGUC). To characterize the density distributions and compositions of the apoB-containing lipoproteins, 2 ml of plasma obtained during each of the kinetic studies was fractionated by isopycnic DGUC using a Beckman SW 40 Ti rotor at 40,000 for 40 h at 15°C (19). Briefly, plasma density was increased to 1.063 g/ml by addition of dry, solid KBr. A 0.5-ml cushion of 1.21 g/ml solution was placed at the bottom of the tube followed in order by 2 ml of the density adjusted plasma sample, 1 ml of 1.0464 g/ml solution, 1 ml of 1.0336 g/ml solution, 2 ml of 1.0271 g/ml solution, 2 ml of 1.0197 g/ml solution, 2 ml of 1.0117 g/ml solution, and 2 ml of 1.006 g/ml solution. The gradient was eluted from the top using a peristaltic pump operating at a flow rate of 0.5 ml/min. Cholesterol, triglycerides, and apoB were measured in each 0.5-ml fraction. Cholesterol and triglycerides were measured as previously described. ApoB was measured using an ELISA method involving a partially purified polyclonal anti-human apoB antibody and a monoclonal antihuman apoB antibody.

ApoE genotyping. ApoE genotypes were determined by gene amplification and cleavage with *Hha*I as described by Hixson (20).

Isolation of apoB and plasma amino acids. ApoB was isolated from each lipoprotein fraction by precipitation with butanol–isopropylether (21). Precipitated apoB was dried under nitrogen and hydrolyzed in 12 N HCl for 16 h at 110°C. The HCl was subsequently evaporated. Plasma amino acids were isolated from 0.3 ml of plasma by cation exchange chromatography (22).

Determination of enrichment and calculation of tracer/tracee ratio. Amino acids obtained from plasma samples or from the hydrolyzed apoB precipitates were derivatized to N-acetyl-N-propanol-esters (23). Leucine enrichment was determined by GC–MS using 1.5 m × 2.0 mm glass columns (Supelco, Bellefonte, PA) packed with coated material (Amino Acid Packing, Alltech Associates, Inc., Deerfield, IL) and a Finnigan 3300 quadropule mass spectrometer as described previously (16, 17). Isotope enrichment and tracer/tracee ratios were calculated from the observed ion current ratios *m/z* 371/370 (23, 24). The enrichment of the infused [^{13}C]leucine was 99% (Cambridge Isotope Laboratories, Andover, MA). Because of the non-negligible mass associated with stable isotope tracers it is necessary to transform enrichment data to tracer/tracee ratios (24). Data in this format are analogous to specific activity in radiotracer experiments.

Model of apoB metabolism and calculation of kinetic parameters. A multicompartmental model (**Fig. 1**) was used to describe VLDL-, IDL-, and LDL-apoB leucine tracer/tracee ratios. In multicompartmental modeling,

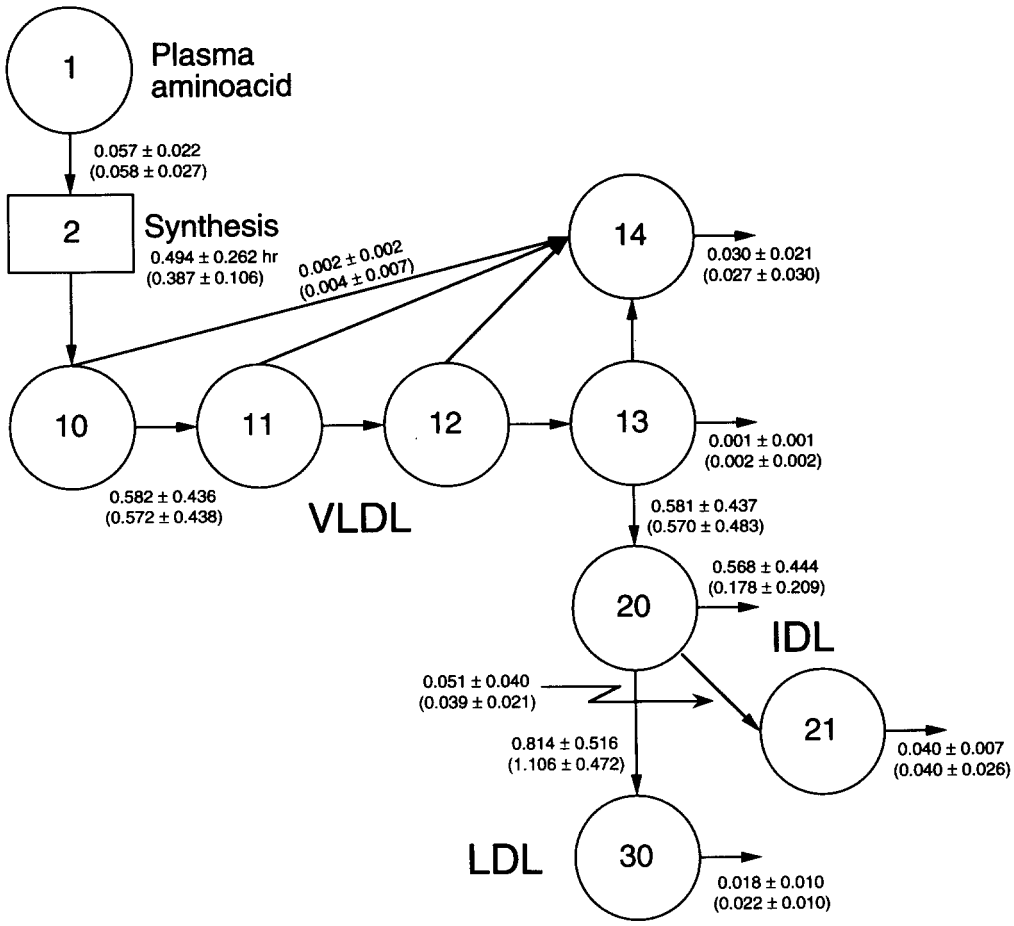

Fig. 1. Multicompartmental model for apoB metabolism. Compartment 1: plasma leucine (forcing function). Compartment 2: delay compartment (synthesis and secretion of VLDL particles). Compartments 10–14: VLDL compartments. Compartments 11–13: delipidation cascade. Compartment 14: slowly turning over VLDL compartment. Compartments 20–21: IDL compartments. Compartment 30: LDL compartment. Numbers on arrows are the mean fractional rate constants ± SD in h^{-1} for the lovastatin and placebo (in parentheses) treatment phases. The numbers next to the delay compartment (compartment 2) represent the time in hours necessary for the synthesis and secretion of apoB.

each compartment or pool represents a group of kinetically homogenous particles. In this study the CONSAM/SAAM programs (25) were used to fit the model to the observed tracer data. Metabolic parameters are subsequently derived from the model parameters giving the best fit. We have previously presented a model that describes the kinetics of apoB in VLDL, IDL, and LDL fractions in normolipidemic and hypolipidemic individuals (16, 17). In these subjects, kinetic heterogeneity of lipoprotein metabolism is less pronounced than in hyperlipidemic subjects. Consequently, we have added structure to the VLDL and IDL sections of the model to account for the more complicated kinetics of apoB seen in patients with nephrotic hyperlipidemia. This model has been applied successfully in subjects with mixed hyperlipidemia (26). The model consists of a precursor compartment of amino acids (compartment 1) and delay compartment (compartment 2) which accounts for the time required for synthesis and secretion of VLDL-apoB into plasma. Plasma leucine tracer/tracee data was fit with a tri-exponential function as described by Parhofer et al. (15). This tri-exponential function was then used as a forcing function to drive the appearance of tracer (amino acid) in the compartmental model. The purpose of the forcing function is to decouple components of the system under investigation. Therefore, we used the tri-exponential function to decouple the kinetics of the amino acid from that of the tracer in apoB. Mathematically, the effect of the forcing function is to replace the amount of tracer in compartment 1 with the value of the tri-exponential forcing function at the same time. In other words, the value of q1(t), the amount of material or tracer in compartment 1 at time t, is replaced by the term q1.FF(t). The model assumes that all apoB enters plasma via compartment 10. Compartments 10 through 14 are used to describe the kinetics of apoB in the VLDL frac-

tion. Compartments 10 through 13 represent a delipidation cascade, as originally described by Phair et al. (27). It is assumed that the residence time of particles in each compartment of the cascade is the same. In addition, the fraction of each compartment in the cascade converted to the slowly turning over VLDL compartment (compartment 13) is the same. VLDL particles in compartment 13 can be converted to IDL or can be removed directly from plasma. The IDL section of the model includes compartments 20 and 21, a rapidly and slowly turning over pool of IDL particles, respectively (28). Particles in compartment 20 can be converted to the slow IDL compartment, to LDL, or can be removed directly from plasma. In this model, LDL-apoB kinetics were described by a single compartment, compartment 20. All LDL-apoB was derived from IDL, compartment 20. After fitting the model to the tracer/tracee data, apoB fractional catabolic rates (FCR), production rates, and conversion rates were determined. The FCR for VLDL-apoB was calculated by dividing the VLDL production rate, i.e., the production rate of apoB into compartment 10 by the mass of apoB in the VLDL fraction. The FCR for IDL was determined by dividing the apoB transport rate from compartment 14 to 20 by the mass of apoB in the IDL fraction. The FCR for LDL is equivalent to the value of the rate constant L(0, 30). In this report, the terms transport rate and production rate are used synonymously, as are FCR and fractional turnover rate.

Statistical analysis

Results are presented as means ± standard deviations. Paired *t*-tests were used to compare results obtained during lovastatin and placebo therapy. Pearson correlation coefficient was used to analyze the relation between body mass index and VLDL-apoB production rate. All statistical analyses were calculated using Instat 2 (GraphPad Software).

RESULTS

Lovastatin was well tolerated by all subjects participating in the study. Biochemical and hematologic monitoring revealed no clinically significant changes from the baseline attributable to therapy. One patient first received lovastatin and then placebo while the sequence was reversed in the other three.

Plasma lipids and apolipoproteins (Table 2)

Placebo period. The subjects had elevated total plasma cholesterol and LDL-cholesterol concentrations (311 ± 22 and 186 ± 22 mg/dl, respectively). Three of the four patients (subjects 1, 3, 4) had triglyceride levels greater than 250 mg/dl. HDL-cholesterol was low (< 35 mg/dl) in the three hypertriglyceridemics subjects. All subjects had elevated levels of plasma apoB (157.9 ± 31 mg/dl).

TABLE 2. Serum lipid and apolipoprotein levels during placebo and lovastatin therapy

Subject	Cholesterol	Triglycerides	Plasma ApoB	LDL-Chol	HDL-Chol
			mg/dl		
1					
Placebo	290 (240 ± 9)	1468 (807 ± 43)	124 (123 ± 4)	169 (166 ± 4)	20
Lovastatin	229 (200 ± 6)	1038 (736 ± 26)	108 (107 ± 3)	137 (147 ± 5)	24
2					
Placebo	301 (241 ± 9)	601 (355 ± 29)	195 (152 ± 6)	174 (139 ± 4)	30
Lovastatin	217 (201 ± 6)	301 (327 ± 24)	128 (128 ± 6)	130 (108 ± 4)	30
3					
Placebo	312 (256 ± 6)	207 (112 ± 9)	156 (156 ± 5)	218 (189 ± 3)	61
Lovastatin	233 (206 ± 4)	173 (117 ± 10)	122 (123 ± 3)	148 (136 ± 2)	58
4					
Placebo	343 (300 ± 10)	627 (541 ± 48)	199 (200 ± 7)	185 (144 ± 6)	30
Lovastatin	246 (239 ± 6)	420 (444 ± 19)	158 (158 ± 4)	138 (121 ± 4)	33
Group					
Placebo	311 ± 22.8	725.7 ± 530	158 ± 31	186.5 ± 22	36.2 ± 17
Lovastatin	229 ± 10[a]	483 ± 383[b]	129 ± 21[a]	138.2 ± 7[a]	36.2 ± 14
Controls (n = 4)	179 ± 20	61 ± 16	99 ± 17	120 ± 21	47 ± 7

Results are in mg/dl and represent means ± SD of values taken during the indicated treatment periods. Numbers in parentheses represent values obtained during the days of infusions. The means ± SD of five samples are provided, documenting the steady state.

[a] $P < 0.05$ placebo versus lovastatin.

[b] $P < 0.05$ obtained after log transformation of the triglyceride concentrations.

Lovastatin period. After 4 weeks of therapy (20 mg/day), plasma triglyceride levels were decreased in all patients (26.2 ± 7%, $P < 0.05$ after log transformation of triglyceride concentrations). Total-cholesterol and LDL-cholesterol also both decreased significantly, by 28.7 ± 4% and 30.6 ± 9%, respectively (both $P < 0.01$). Plasma apoB was decreased 17.8 ± 3.9% ($P < 0.01$). HDL-cholesterol levels were not modified by therapy (35.2 ± 17 vs. 36.2 ± 14 mg/dl). Thus, the falls in total-cholesterol were due to falls of the cholesterol associated with apoB-containing lipoproteins. The greater falls in cholesterol relative to apoB suggested that the compositions of apoB-containing lipoproteins may have been altered (see below).

In both phases of the study urine apoB was measured. In none of the subjects were any detectable levels of apoB found. Consequently, the apoB urine concentration in all subjects was less than 0.02 μg/dl.

Kinetics of apoB-containing lipoproteins

Plasma leucine tracer/tracee ratios of free leucine were similar in all subjects and did not change with lovastatin therapy (not shown). **Figure 2** shows VLDL- and LDL-apoB leucine tracer/tracee ratios in subject #2 during placebo and lovastatin therapy. The symbols represent observed values while the lines represent the best fit of the multicompartmental model. There is a good agreement between the model-derived fits and the observed data.

Placebo period. The production rates of IDL- and LDL-apoB were increased in all patients compared with the four normolipidemic controls (**Table 3**). Mean VLDL-apoB production rates also were increased but the differences were of borderline statistical significance ($P = 0.07$) due to subject #3 who had a VLDL-apoB PR in normal range. Direct IDL-apoB or LDL-apoB production rate pathways were not necessary for fitting the curves to the model in any subject. Consequently, the increased LDL-PR was a consequence of the increased VLDL-PR.

FCRs for VLDL-apoB were decreased and FCRs of IDL-apoB and LDL-apoB were higher than the controls in three of the four nephrotic subjects. In each case subject #3 had values close to those of controls (Table 3). The proportion (percent) of VLDL-apoB converted to LDL-apoB was not different from the controls.

Lovastatin therapy. The most consistent changes associated with lovastatin therapy were decreases in LDL-apoB PR (34.14 ± 14%, $P = 0.03$) (Table 3) due to consistent decreases in conversion of VLDL-apoB to LDL-apoB (80.7 ± 8.3 vs. 55.9 ± 17.2, $P = 0.05$). Other parameters responded differently in the various subjects, e.g., VLDL-apoB PR and IDL-apoB PR increased in subject #1, decreased in subject #4, and stayed constant in subjects #2 and #3. There were no significant changes in the mean FCRs for any of the lipoproteins, because LDL-apoB FCRs fell in three subjects and rose in one (#3).

Density distributions and compositions of apoB-containing lipoproteins

Placebo period. **Figure 3** and **Fig. 4** show the DGUC profiles of the apoB-containing lipoproteins observed in one representative patient (#2) and one control. The flotation densities of LDLs peaked from fraction 21 to fraction 24 in the four patients, while LDLs peaked at fraction 19 in normals, suggesting that the LDLs of the patients had higher densities and/or were smaller in size. The cholesterol, triglyceride, and apoB contents of VLDL, IDL, and LDL were individually summed for fractions 1–3, 4–15, and 16–27, respectively, in order to calculate ra-

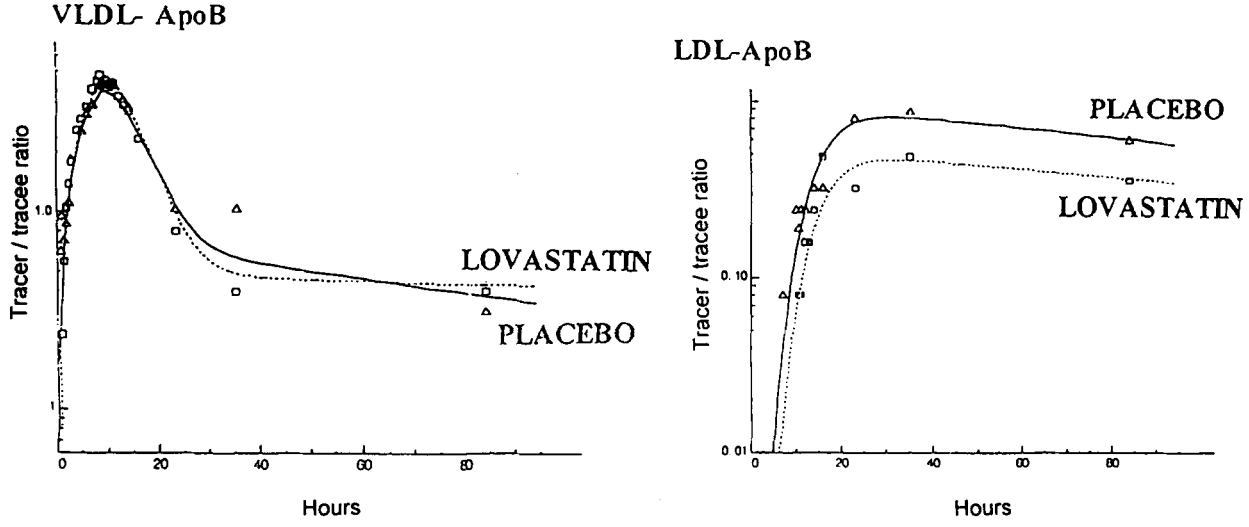

Fig. 2. Observed values (symbols) and calculated fits (lines) to the VLDL- and LDL-apoB tracer/tracee ratio using the multicompartmental model in a representative study.

TABLE 3. Kinetic parameters during placebo and lovastatin therapy

Subject	VLDL-ApoB		IDL-ApoB		LDL-ApoB		% VLDL→LDL
	PR	FCR	PR	FCR	PR	FCR	
1							
Placebo	32.9	1.37	26.3	13.3	25.7	0.827	78
Lovastatin	45.7	2.14	44.8	24.8	18.2	0.785	40
2							
Placebo	31.1	2.43	31.1	15.1	23.3	0.435	75
Lovastatin	27.3	2.40	26.2	17.1	10.3	0.210	38
3							
Placebo	17.6	7.83	17.6	6.9	16.7	0.237	95
Lovastatin	18.9	5.85	17.9	5.1	14.2	0.266	75
4							
Placebo	49.7	1.9	47.0	21.8	37.1	0.606	75
Lovastatin	32.5	2.01	32.5	16.4	23.4	0.453	71
Group							
Placebo							
Mean ± SD	32.8 ± 11.3[c]	3.43 ± 2.6[a]	30.5 ± 10.6[a]	14.2 ± 5.2	25.7 ± 7.3[a]	0.526 ± 0.21	81 ± 8
Lovastatin							
Mean ± SD	31.1 ± 9.7	3.1 ± 1.5	30.4 ± 9.8	15.8 ± 7.0	16.5 ± 4.8[b]	0.428 ± 0.22	56 ± 17[b]
Controls (n = 4)							
Mean ± SD	20.2 ± 3.9	8.4 ± 2.6	6.8 ± 2.9	6.5 ± 5.7	13.1 ± 3.3	0.352 ± 0.08	71 ± 19

PR, production rate, mg/kg/day; FCR, fractional catabolic rate, pools/day. Group results are means ± SD.
[a] $P < 0.05$ placebo versus controls.
[b] $P < 0.05$ placebo versus lovastatin.
[c] $P < 0.07$ versus controls.

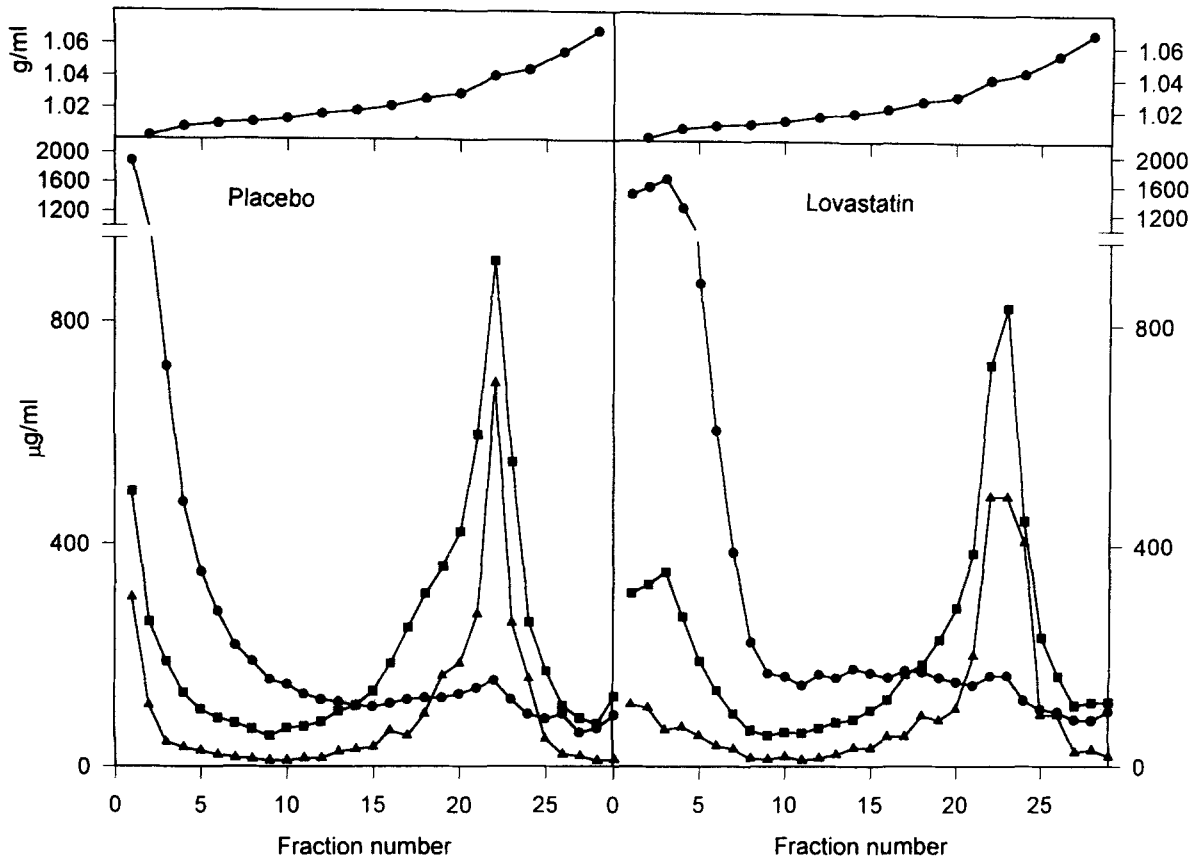

Fig. 3. Density gradient ultracentrifugation from subject #2 during placebo and lovastatin periods. The upper graphs correspond to the density gradients. Triglycerides concentrations are shown as circles, cholesterol as squares, and apoB as triangles.

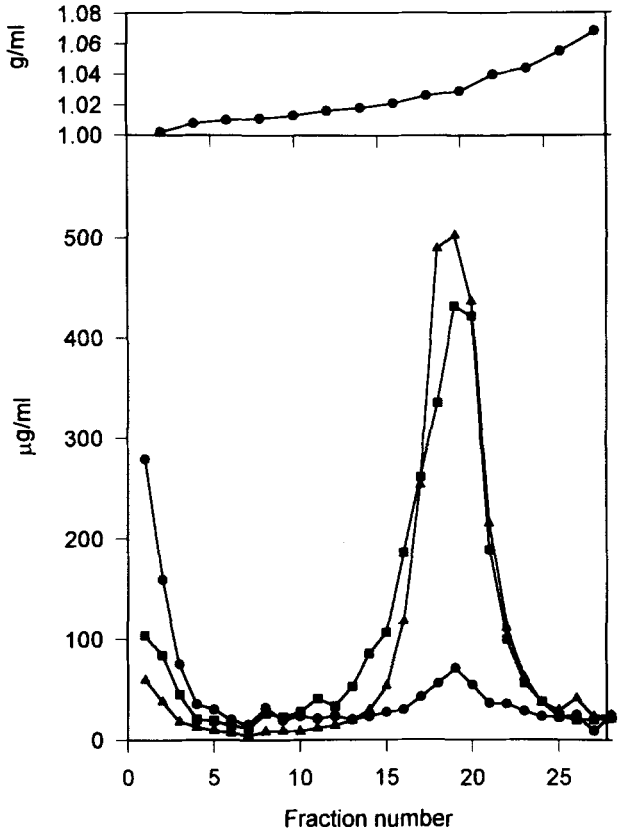

Fig. 4. Density gradient ultracentrifugation from a normal subject. The upper graph corresponds to the density gradient. Triglycerides concentrations are shown as circles, apoB as squares, and cholesterol as triangles.

tios of lipids to apoB, and lipids to each other as indexes of composition (**Table 4**). Particles in the VLDL and IDL density ranges had greatly increased absolute amounts of apoB, cholesterol, and triglycerides in all patients compared to controls (Figs. 3 and 4) and the lipid to apoB ratios in subjects #1, #2, and #4 were increased (Table 4). LDL fractions also contained increased absolute amounts of triglycerides, cholesterol, and apoB in all patients. LDL-triglyceride/apoB ratios were high, and LDL-cholesterol/triglyceride ratios were low in subjects #1, #2, and #4 (Table 4). Thus, the abnormal concentrations of total-cholesterol and total-triglycerides in the plasmas of three of the subjects were due to the accumulation of increased numbers of VLDL, IDL, and LDL particles that were enriched in both cholesterol and triglycerides for VLDL and IDL and triglycerides for LDL. The compositions of the lipoproteins in subject #3 had increased cholesterol/triglyceride ratios compared with the other patients, while other ratios were lower.

Lovastatin period. During lovastatin therapy, cholesterol, triglyceride, and apoB levels fell in plasma in all patients (Table 2). In three of four patients (#1-3) levels of lipids and apoB decreased in VLDL fractions 1-3, increased in IDL fractions 4-10, and decreased in LDL fractions 16-27, (see Fig. 3 and **Fig. 5**). Patient #4 had decreases in the levels of IDL components. Ratios of cholesterol/triglyceride fell in nearly all fractions in all patients, reflecting a greater decrease of cholesterol relative to triglycerides (Table 4), while triglyceride/apoB ratios rose in the VLDL of subjects #s 1-3, and cholesterol/apoB ratios fell in the IDL of all four subjects.

DISCUSSION

The objective of this work was to study the metabolism of the apoB-containing lipoproteins in a "lipoprotein overproduction" syndrome such as the nephrotic syndrome and to determine the mechanism of action of lovastatin. We used endogenous labeling of apoB with [^{13}C]leucine. Only patients with FSGS, a chronic, relatively stable form of nephrotic syndrome, were included. This selection criterion eliminated many of the sources of variability related to the various etiologies and therapies of the nephrotic syndrome (31, 32). The rates of progression of the nephrotic syndrome in these patients were slow as documented by the stable levels of proteinuria during the study. None of the subjects had apoE2 genotype, eliminating the possibility of dysbetalipoproteinemia as a factor in their kinetic abnormalities. The increased levels of apoB-containing lipoproteins at baseline reflected increased numbers of lipid-rich particles (Table 4 and Figs. 3 and 4) (33, 34). Lovastatin caused falls in total triglycerides and cholesterol (Table 2), reflecting decreases of "lighter" VLDL and LDL fractions in all subjects (29, 30); lipid levels in "heavy" VLDL and IDL rose selectively in three of four subjects and fell in one (Fig. 5 and Table 4).

ApoB kinetics

The combined use of endogenous labeling and multicompartmental analysis of the obtained data is currently considered very useful for the study of lipoprotein kinetics (15, 35, 36). Because of its safety, this approach allows the performance of several studies in the same subject over relatively short periods of time. Also a better representation of all subpopulations that constitute the apoB-containing lipoproteins may be achieved. The model used is a modification of a previously validated model (16, 27), that was used successfully in patients with mixed hyperlipidemia (26). One limitation of this procedure is that kinetic parameters may be affected by recycling of the tracer. This is especially true for the slowly turning over particles. However, the forcing function includes not only the infused tracer but also the recycled tracer, thus accounting for recycling. Furthermore, using the study subjects as their own controls decreases the impact of any recycling on the conclusions obtained, as lovastatin did

TABLE 4. Indexes of lipoprotein composition during placebo and lovastatin therapy

Subject	VLDL			IDL			LDL		
	TG/ApoB	Chol/ApoB	Chol/TG	TG/ApoB	Chol/ApoB	Chol/TG	TG/ApoB	Chol/ApoB	Chol/TG
1									
Placebo	13.2	2.9	0.22	11.3	2.9	0.25	1.3	1.4	1.1
Lovastatin	16.3	3.5	0.21	9.6	2.8	0.29	1.2	1.4	1.2
2									
Placebo	7.9	2.1	0.26	10.6	4.3	0.41	0.7	2.1	3.0
Lovastatin	16.7	3.5	0.21	14.8	3.9	0.26	1.1	2.3	2.1
3									
Placebo	3.0	1.1	0.36	1.3	1.2	0.90	0.2	1.1	4.4
Lovastatin	4.1	1.0	0.25	1.7	0.9	0.51	0.4	1.4	3.7
4									
Placebo	17.5	6.2	0.35	15.4	7.0	0.45	1.0	3.4	3.3
Lovastatin	11.3	3.1	0.28	15.3	6.8	0.44	1.4	4.1	3.0
Group									
Placebo									
Mean ± SD	10.4 ± 6.3	3.0 ± 2.2	0.29 ± 0.6	9.65 ± 5.9	3.8 ± 2.4	0.5 ± .27	0.8 ± .46	2.0 ± 1.02	3.0 ± 1.3
Lovastatin									
Mean ± SD	12.1 ± 5.8	2.7 ± 1.19	0.23 ± .03	10.3 ± 6.3	3.6 ± 2.4[a]	0.37 ± .11	1.02 ± .43	2.3 ± 1.27	2.5 ± 1.08
Normals	3.0	0.6	0.21	1.1	0.37	0.6	0.33	2.6	8.0

VLDL, IDL, and LDL consisted of fractions 1-3, 4-15, and 16-27, respectively (see Figs. 3 and 4). Data are mass ratios, e.g.,

$$\text{VLDL TG/apoB} = \frac{\text{TG in fraction 1-3}}{\text{apoB in fractions 1-3.}}$$

[a] $P < 0.05$ placebo versus lovastatin.

not discernably affect leucine metabolism over the course of the studies. Consequently, any recycling of the tracer would affect both studies in the same way and the differences observed could be attributed to drug intervention.

Placebo phase. Production rates of VLDL-apoB were increased several fold in three of the four subjects. One of four patients studied by Vega and Grundy (11) and six of eight reported by Warwick et al. (12) also had high VLDL-apoB production rates. As body mass is related to VLDL production, we pooled together the previously described 12 patients reported by Vega and Grundy (11) and by Warwick et al. (12) with our own patients and found a small ($r = 0.519$, n = 16) but significant correlation ($P < 0.05$) between BMI and VLDL-apoB production rate. This correlation remained significant when we analyzed only our data and those of Vega and Grundy (11) ($r = 0.551$, n = 12, $P < 0.05$). (Both data sets included subjects with high and normal BMIs.) The correlations suggest that obesity may contribute to the VLDL-apoB overproduction seen in the nephrotic syndrome as it may in diabetes mellitus (37).

In addition to overproduction, VLDL-apoB accumulation was also due to a decreased VLDL-apoB FCR in three of the four patients that was only ~40% of the value obtained in normolipidemic controls (Table 3). The decreased FCR was observed only in VLDL-apoB and not IDL- or LDL-apoB, suggesting a specific abnormality in VLDL catabolism (11). Decreased clearance of VLDL due to down-regulation of LDL-receptors was unlikely because FCRs of the LDLs in these three subjects were high. Decreased FCR of VLDL-apoB also has been described in non-nephrotic hypertriglyceridemic subjects (38). Possible mechanisms for the catabolic defect of VLDL-apoB include *a)* abnormalities in VLDL structure or composition that may render them poor substrates for lipolytic enzymes (39) or transfer proteins, *b)* decreased activities of lipoprotein lipase (LPL) (5), *c)* the existence of circulating inhibitors of LPL (6), or *d)* urinary losses of apoC-II, a cofactor of LPL (9). Another possibility is a down-regulation of the specific VLDL-receptor recently described by Yamamoto (40). We cannot distinguish between these mechanisms on the basis of our studies.

Patient #3 exhibited nearly normal LDL-apoB kinetics, but along with the other subjects he had overproduction of IDL-apoB and LDL-apoB (Table 3) that were the consequences of increased conversion from VLDL-apoB pools, rather than the direct overproduction of IDL- or LDL-apoB (i.e., it was not necessary to invoke any direct IDL-apoB or LDL-apoB production pathway to explain the kinetic data in any of the cases). In cases #1, #2, and #4 the increased conversion was due to normal fractional conversion from enlarged VLDL pools; in case #3 it was due to a greater fractional conversion of a nearly normal VLDL pool. Decreased clearance of IDL-apoB and LDL-

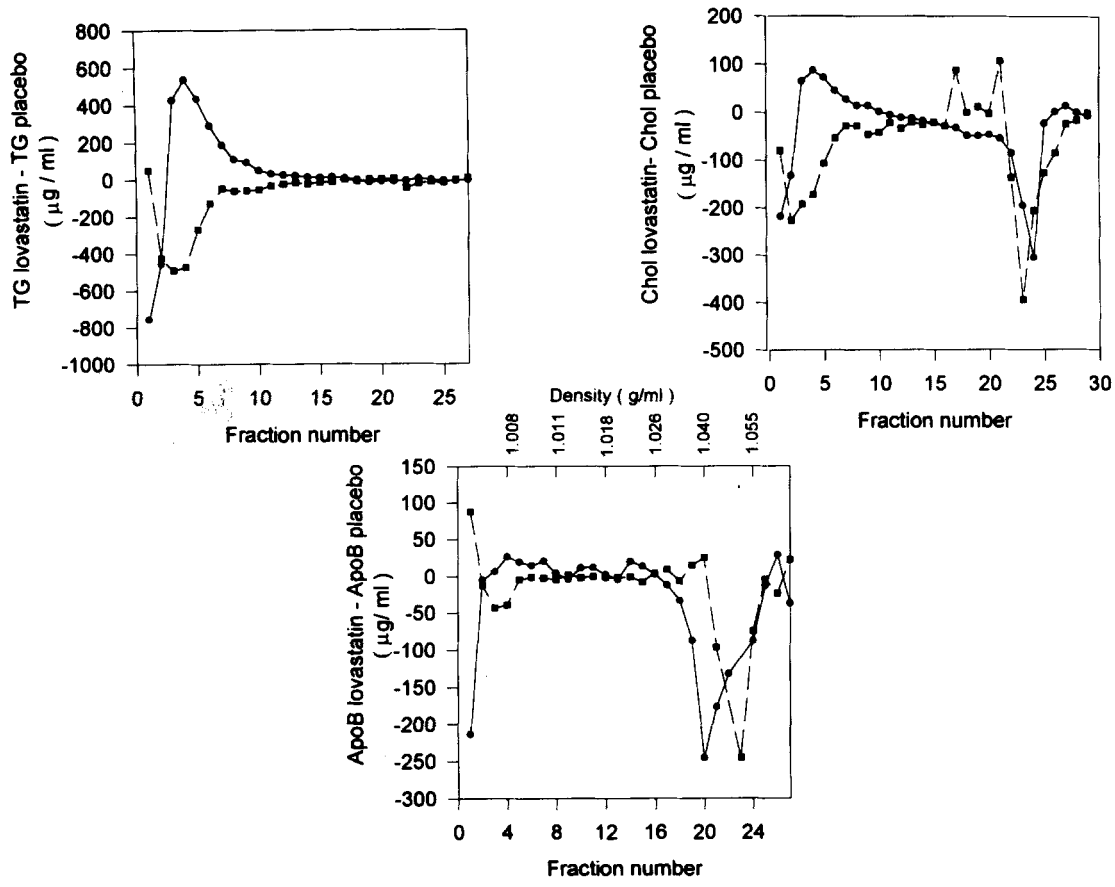

Fig. 5. Mean differences in triglyceride, cholesterol, and apoB between DGUC profiles done during the placebo and lovastatin phases. The mean differences observed in patients 1–3 are shown as filled circles. The difference between the lovastatin and placebo DGUC profiles observed in patient 4, who had a different response from patients 1–3, is shown as filled squares.

apoB were unlikely causes of IDL and LDL accumulation as the FCRs of these lipoproteins were similar or higher than those observed in the normolipidemic controls. (A correction of the FCR for urinary losses was not necessary as significant amounts of urine apoB were not detected in our patients.) Vega and Grundy (11) and Warwick et al. (12) reported compatible data in patients with moderate elevations of LDL-apoB. On the other hand, decreased LDL-apoB FCRs were reported by Warwick et al. (41) in another set of subjects with higher LDL-apoB concentrations. Subject #3 appeared to behave as did their subjects. However, when one analyzes the data in both reports by Warwick et al. (12, 41) there is not a negative correlation between LDL-apoB and LDL-apoB FCR, as would be expected were LDL-apoB levels primarily determined by rates of LDL-apoB clearance in nephrotic patients. No correlation was observed even when pooling the data from Vega and Grundy (11), Warwick et al. (12, 41), and this report. On the other hand, there is a significant correlation ($r = 0.407$, $P < 0.05$) between LDL-cholesterol and LDL-apoB production rates when the data of the four reports described above are pooled. Consequently, we believe that high LDL levels in most subjects with the mixed lipemias of nephrosis are due to increased LDL production rates. However, a subset of nephrotic subjects, e.g., our subject #3, may also have abnormal LDL catabolism.

Lovastatin phase. Two physiologic mechanisms by which the HMG-CoA reductase inhibitors achieve their effects of lowering LDL-cholesterol have been proposed (42). The first is increased LDL-apoB catabolism. The proposed sequence is as follows: the drug induces suppression of hepatic cholesterol synthesis and cholesterol levels in intracellular regulatory pools followed by the up-regulation of LDL-receptors and increased clearance of LDL from plasma. This mechanism seems to account for the drug-induced decrease in LDL in heterozygotes for familial hypercholesterolemia (43), in polygenic hypercholesterolemia, and in mixed lipemia (triglycerides 180–300 mg/dl, LDL-cholesterol > 200 mg/dl) (26).

The second postulated mechanism is an HMG-CoA reductase-induced decrease in LDL-apoB production rate, which could result from either a lowered hepatic production of VLDL-apoB or diminished conversion of VLDL- and IDL-apoB particles to LDL, as VLDL-apoB and IDL-apoB precursors are diverted from VLDL → LDL conversion pathways to VLDL clearance pathways,

probably due to up-regulation of the LDL-receptors. Because lovastatin decreased the fractional conversion of VLDL to LDL and also uniformly decreased LDL-apoB production in all our patients (Table 3), we believe this to be one consistent physiologic response to HMG-CoA reductase inhibitors in nephrotic patients with mixed lipemia. However, not all parameters were affected identically in all our patients despite our attempts to choose very similar subjects for this study. Lovastatin also decreased VLDL-apoB production rate in subject #4, increased VLDL-apoB production in subject #1, and increased LDL-apoB clearance in another, subject #3, suggesting that more than one physiologic response could be stimulated by lovastatin and that more than one physiologic mechanism could be operating in individual patients (see also ref. 44). The small number of patients does not permit us to assess the extent of heterogeneity of physiologic responses in the nephrotic population.

In summary, in this report in which endogenous labeling of apoB was used to study the kinetics of apoB-containing lipoproteins in patients with nephrotic syndrome, the main abnormalities observed were increased VLDL-apoB production rate and a remarkable decrease in VLDL-apoB fractional catabolic rate. Both abnormalities resulted in overproduction of IDL- and LDL-apoB. Lovastatin decreased LDL-apoB production rate in subjects with nephrotic syndrome. The reduction in LDL-apoB production rate was explained by decreased conversion of VLDL to LDL, probably due to removal of VLDL by up-regulated LDL-receptors.

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