Influence of Apolipoprotein E Polymorphism on Apolipoprotein B-100 Metabolism in Normolipemic Subjects

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Abstract

This study examined apolipoprotein (apo) B metabolism in normolipemic subjects homozygous for the apo E_2 (n = 4), apo E_3 (n = 5), or apo E_4 (n = 5) phenotype. Radioiodinated very low density lipoprotein ($VLDL_1$) (ultracentrifuge flotation rate [S_f] 60–400) and $VLDL_2$ (S_f 20–60) were injected into volunteers and the conversion of apo B was followed through intermediate density lipoprotein (IDL) to low density lipoprotein (LDL). Subjects homozygous for E_3 converted ~ 50% of $VLDL_2$ to LDL, the remainder being lost by direct catabolism. Those with the E_2 phenotype produced less $VLDL_1$ but converted more of it to $VLDL_2$ (compared to E_3 subjects). They displayed a characteristic dyslipidemia with the presence of slowly catabolized $VLDL_1$ and $VLDL_2$ remnants. LDL levels were low owing to increased direct catabolism of $VLDL_2$ and IDL and a reduced efficiency of delipidation; only 25% of $VLDL_2$ apo B was directed to LDL production. In contrast, E_4 subjects converted more $VLDL_2$ apo B to LDL than E_3 subjects. About 70% of $VLDL_2$ apo B was found in LDL; direct catabolism of VLDL and IDL was reduced as was the fractional catabolic rate of LDL (0.2 vs. 0.26 in E_3 subjects). These changes in the VLDL → IDL → LDL metabolic cascade can in part be explained by alterations in hepatic LDL receptors with E_2 subjects having higher and E_4 subjects lower activities than those in E_3 homozygotes. (*J. Clin. Invest.* 1991. 88:1490–1501.) Key words: catabolism • modeling • kinetics • synthesis • very low density lipoprotein

Introduction

In humans the gene locus for apolipoprotein E (apo E) is polymorphic with three alleles (E_2, E_3, and E_4) being present in all populations studied so far (1–3). The commonest variant is E_3 for which ~ 70% of Caucasians are homozygous. It codes for a protein of 299 amino acid residues, which although minor in terms of plasma concentration, has an important role in regulating lipoprotein metabolism by virtue of its ability to act as a ligand for specialized lipoprotein receptors. Individuals who possess the E_2 allele synthesize an apo E in which the arginine residue at position 158 is replaced with cysteine as the result of a C → T point mutation. Those with E_4 in contrast generate a product with an arginine at residue 112 compared with cysteine in apo E_3. These mutations in the protein sequence cause charge changes which are readily detected by isoelectric focusing of very low density lipoproteins (VLDL) apoproteins (4) or by isoelectric focusing of plasma followed by detection with immunoblotting (5, 6). Structure–function studies of apo E have revealed that a domain covering amino acid 140–160 is of critical importance in receptor interaction. The E_2 mutation occurs in this region and in vitro studies have demonstrated that the resulting protein has impaired receptor-binding properties (4, 7).

Epidemiological surveys have demonstrated that the apo E polymorphism has a significant impact on plasma cholesterol and apolipoprotein B (apo B) levels (3). Compared to apo E_3 homozygotes, those who are homozygous for the E_2 allele exhibit plasma cholesterol levels that are 10–15% lower while the opposite holds for those who are E_4 homozygotes. Examination of the lipoprotein profile in the various apo E phenotypes has revealed that the gradation in plasma cholesterol from E_2 to E_3 to E_4 homozygotes is due to an increase in the level of low density lipoprotein (LDL). However, E_2 homozygotes have higher VLDL cholesterol levels and display a dyslipidemia that is qualitatively similar to that seen in type III hyperlipidemia. The latter is usually associated with E_2 homozygosity and inheritance of another as yet unidentified mutation that causes an elevation of plasma lipid levels (8). A number of studies have examined the metabolic changes underlying the altered lipoprotein profiles associated with the apo E polymorphism. Chylomicron clearance is reduced in subjects possessing the E_2 allele, whereas E_4 heterozygotes and homozygotes show accelerated clearance (9, 10). Metabolic studies of LDL apo B have shown that type III and, to a lesser extent, E_2 homozygous subjects degrade LDL faster than normal (11, 12). Previous studies from this laboratory have examined VLDL, intermediate density lipoprotein (IDL), and LDL apo B metabolism in a number of genetically determined dyslipidemias. In particular, we found in patients with homozygous familial hypercholesterolemia that the LDL receptor was important not only in LDL catabolism but also in the processing of apo B–containing lipoproteins throughout much of the delipidation cascade from VLDL to LDL (13). Since apo E is important for lipoprotein receptor interaction, we surmised that structural and functional variation in this protein would modulate the metabolism of the lipoproteins within the entire ultracentrifuge flotation rate (S_f)[1] 0–400 spectrum. To investigate this, we conducted VLDL turnover studies in groups of individuals homozygous for the three E variants. The results demonstrate the profound impact that this polymorphism has on apo B metabolism.

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1. *Abbreviations used in this paper:* FCR, fractional catabolic rate; FH, familial hypercholesterolemia; S_f, ultracentrifuge flotation rate; TMU, tetramethylurea.

Methods

Subjects. The study participants were selected from individuals who attended a coronary screening program in health centers in Glasgow. The Institute of Biochemistry received samples for cholesterol assay as part of the screen and those in which the level lay in the range of 5.0–6.2 mmol/liter (195–240 mg/dl) were subjected to apo E phenotyping using the isoelectric focusing/immunoblotting technique described below. Subjects who were found to be homozygous for the presence of apo E_2 ($n = 4$), apo E_3 ($n = 5$), or apo E_4 ($n = 5$) were recruited for the study. For ease of presentation throughout this report subjects are referred to as the E_2, E_3, or E_4 group to denote phenotypically determined homozygosity for the E_2, E_3, or E_4 alleles (14). They were screened for cardiological, renal, endocrine, hepatic, or metabolic disease by routine clinical examination and biochemical testing. Age distribution and body weight index were similar in the three study groups. Each individual's dietary intake was recorded over a 7-d period and this was analyzed for the percent contribution of calories derived from protein, carbohydrates and fat (15).[2] Although daily energy intake (kilocalorie per day) varied considerably, approximately in proportion to differences in body frame, the composition of food was virtually the same in the three groups.

Plasma lipid and lipoprotein levels of study participants given in Table I were measured according to the Lipid Research Clinics protocol (16).

Apo E phenotyping. Apo E isoforms were detected as described by Menzel et al. (5) and Havekes et al. (6). 10 μl of plasma were delipidated, redissolved in 6 M urea containing 0.1 M Tris, pH 10.0, 5% β-mercaptoethanol, and 1% sodium decyl sulfate and applied to a vertical polyacrylamide slab gel of 5% (wt/vol) acrylamide in 8 M urea containing 1% ampholyte (pH range 4–6). The cathode buffer was 0.2 M NaOH and the anode buffer 0.1 M H_3PO_4. Isoelectric focusing was carried out at 3 W per gel for 16 h. Transfer by electrophoresis from the acrylamide slab gels to nitrocellulose membranes was performed as described by Towbin et al. (17). The transfer buffer contained 0.2 M glycine, 0.025 M Tris, and 20% methanol and the current applied was 0.4 A for 3 h. Apo E isoforms bound to the membrane were visualized by immunostaining using a monoclonal or polyclonal apo E-specific antibody and an appropriate IgG-binding second antibody linked to horseradish peroxidase.

Lipoprotein isolation and labeling. The methods for preparation of tracer VLDL subfractions $VLDL_1$ (S_f 60–400) and $VLDL_2$ (S_f 20–60), have been described in detail in previous publications (18). Briefly, 250 ml of plasma was removed by plasmapheresis from subjects who had been fasted overnight and total VLDL of $d < 1.006$ g/ml (S_f 20–400)

2. Intake was analyzed by using the computer program MICRODIET (Department of Computer Sciences, Salford University, Salford, UK).

Table I. Plasma Lipid and Lipoprotein Levels in E_2, E_3, and E_4 Subjects

Subject	Sex	Age	Weight	Total triglyceride	Total cholesterol	VLDL cholesterol	LDL cholesterol	HDL cholesterol	VLDL cholesterol/plasma triglyceride ratio
		yr	*kg*			*mmol/liter*			
$E_{2/2}$									
1	M	47	74.0	1.60±0.79	4.88±0.13	1.03±0.64	2.43±0.43	1.42±0.19	0.64
2	F	39	47.0	1.80±0.27	4.85±0.35	1.07±0.10	2.45±0.26	1.33±0.08	0.59
3	F	45	63.0	1.85±0.27	6.14±0.83	1.41±0.47	3.25±0.49	1.48±0.23	0.76
4	F	57	53.0	1.64±0.08	5.96±0.76	1.25±0.24	3.25±0.59	1.48±0.21	0.76
Mean±SD	*mmol/liter*			1.72±0.10	5.46±0.60	1.19±0.15*	2.84±0.40	1.43±0.06	0.69±0.07‡
	(mg/dl)			(151±9)	(211±23)	(46±6)	(110±15)	(55±2)	(0.30±0.03)
		yr	*kg*			*mmol/liter*			
$E_{3/3}$									
5	M	36	71.0	1.71±0.38	4.96±0.54	0.79±0.21	3.14±0.35	1.01±0.13	0.46
6	M	43	86.0	2.55±0.71	6.23±0.79	1.02±0.25	4.09±0.50	1.36±0.19	0.40
7	M	46	89.0	1.09±0.13	5.76±0.22	0.61±0.14	3.96±0.17	1.19±0.08	0.56
8	F	40	71.0	0.95±0.06	4.97±0.32	0.35±0.08	2.76±0.17	1.86±0.12	0.37
9	F	44	76.0	2.17±0.29	5.63±0.35	0.97±0.21	3.74±0.14	1.20±0.05	0.45
Mean±SD	*mmol/liter*			1.69±0.56	5.51±0.49	0.75±0.25	3.54±0.51	1.32±0.29	0.45±0.06
	(mg/dl)			(148±49)	(213±19)	(29±10)	(137±20)	(51±11)	(0.20±0.03)
		yr	*kg*			*mmol/liter*			
$E_{4/4}$									
10	M	29	85.0	2.00±0.75	6.55±1.00	1.08±0.54	4.37±0.83	1.10±0.22	0.54
11	F	41	62.0	1.49±0.28	6.74±0.35	0.61±0.23	4.66±0.41	1.39±0.19	0.41
12	M	45	79.0	2.01±0.59	6.19±0.34	0.99±0.50	3.96±0.20	1.23±0.14	0.49
13	F	29	50.0	0.90±0.27	5.66±0.40	0.59±0.28	3.35±0.49	1.73±0.09	0.66
14	F	36	48.0	0.93±0.18	5.33±0.46	0.32±0.14	3.45±0.44	1.56±0.11	0.34
Mean±SD	*mmol/liter*			1.47±0.49	6.09±0.53	0.72±0.30	3.96±0.51	1.40±0.23	0.49±0.11
	(mg/dl)			(129±43)	(236±21)	(28±12)	(153±20)	(54±9)	(0.22±0.05)

* Significantly different from E_3 group $P < 0.05$; ‡ $P < 0.01$ by Student's t test.

isolated by centrifugation for 18 h at 36,000 rpm and 10°C in a Ti60 rotor (Beckman Instruments, Inc., Palo Alto, CA). The supernatant was harvested by suction and used for preparation of the subfractions. The VLDL solution was adjusted to a density of 1.118 kg/liter by the addition of NaCl (0.34 g per 2 ml of solution) and layered in a SW40 rotor tube (Beckman Instruments, Inc.). A six-step gradient from 1.0988–1.0588 kg/liter was constructed above the sample and centrifugation carried out at 23°C to separate sequentially $VLDL_1$ (1 h 38 min, 39,000 rpm) and $VLDL_2$ (15 h 41 min; 18,500 rpm). The subfractions were labeled with ^{131}I and ^{125}I, respectively, by a modification of the iodine monochloride method (19) and sterilized by filtration through an 0.45 micron filter (Millipore, Molsheim, France).

Turnover protocol. Subjects were fasted from 8 p.m. and injected with autologous trace-labeled ^{131}I-$VLDL_1$ and ^{125}I-$VLDL_2$ at 8.00 a.m. on the following day. The tracers (50 μCi of each iodine isotope) were administered within 72 h of blood being withdrawn for their preparation. During the first day of the turnover to minimize chylomicron production the volunteers were fasted until 6.00 p.m. Plasma samples were obtained at frequent intervals over the 72 h after injection and then daily in the fasting state for 14 d. The apo B–containing lipoproteins $VLDL_1$ (S_f 60–400), $VLDL_2$ (S_f 20–60), IDL (S_f 12–20), and LDL (S_f 0–12) were prepared by a modification (13) of the cumulative gradient ultracentrifugation (20). From these apo B was isolated by tetramethylurea (TMU) precipitation (21) and its specific activity was determined by radioactivity counting and protein determination (22).

The apo B pool circulating with each of these lipoprotein fractions was determined by replicate analyses of plasma samples collected intermittently throughout the turnover study. Correction was made for possible B protein loss during TMU precipitation by comparing the apo B recovered at the end of the procedure with the values calculated as the difference between total and TMU-soluble (apo E and C) protein contents (21). The composition of each fraction was determined by assay of total and esterified cholesterol, triglyceride, phospholipid, and protein (23). Protein determinations were performed with the addition of sodium dodecyl sulfate to the Biuret reagent (22).

Subjects received potassium iodate in tablet form (180 mg/d) for 3 d before and 1 mo after injection to block thyroidal sequestration of radioactive iodine released by catabolism.

Kinetic analysis. The radioactivity associated with the apo B protein present in each lipoprotein fraction was calculated from the apo B specific activities and the individual pool sizes. These were expressed as a percentage of the total apo B radioactivity (i.e., $VLDL_1$ plus $VLDL_2$, IDL, and LDL) present in the plasma 10 min after injection and the resulting values were used to construct decay curves which were analyzed by the SAAM 30 (24) multicompartmental modeling program. The metabolic model employed (Fig. 1) was basically the same as described elsewhere (13) with the addition of a second subcompartment (compartment 12) in $VLDL_1$ to account for the second exponential seen in E_2 homozygotes. Its main features are as follows: (*a*) apo B synthetic input occurs at the level of $VLDL_1$, $VLDL_2$, and, where indicated, IDL and LDL; (*b*) VLDL is delipidated in a stepwise manner following the concept of Berman et al. (25); (*c*) slowly catabolized remnant subpopulations are present in $VLDL_2$ (compartment 6) and IDL (compartment 9); (*d*) parallel processing pathways exist leading from $VLDL_2$ through IDL to LDL.

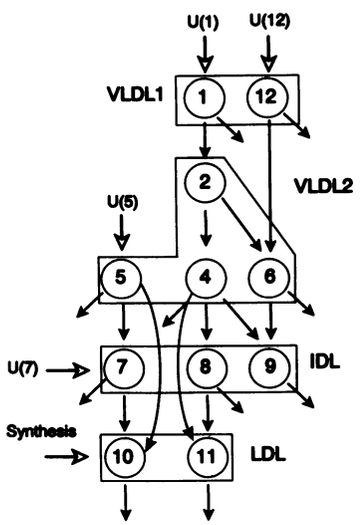

Figure 1. Multicompartmental model for apo B metabolism in $VLDL_1$ (S_f 60–400), $VLDL_2$ (S_f 20–60), IDL (S_f 12–20), and LDL (S_f 0–12). U_1, U_5, U_7, and U_{12} represent de novo input of apo B into $VLDL_1$, $VLDL_2$, and IDL. Synthesis into the LDL density interval was calculated as the difference between the absolute catabolic rate of apo B in this fraction (observed mass × overall FCR) and the input from $VLDL_2$ and IDL.

Rate constants were determined and in combination with B protein pool sizes were used to calculate flux rates and steady-state synthetic inputs. The rate constants, fluxes, and apo B masses were compared in the E_2, E_3, and E_4 homozygotes by Student's *t* test and Mann-Whitney nonparametric test.

Ethical consideration. All subjects participating in the study gave informed consent. The study met the requirements of the Ethical Committee of the Glasgow Royal Infirmary.

Results

The subjects for this study were selected from coronary screening clinic attendees whose plasma cholesterol at the initial visit fell in the range 5.0–6.2 mmol/liter. Mean values for total plasma cholesterol and triglyceride (Table I) in the three groups did not differ significantly from each other or from the population means (±1 SD) for these lipids of 5.8±1.2 and 1.8±1.4 mmol/liter, respectively. Using this selection process we attempted to minimize the effect that gross perturbations in plasma lipid levels would have on apo B kinetics. The distribution of cholesterol in the lipoprotein fractions, however, was characteristically different in the groups. VLDL cholesterol was significantly higher and LDL cholesterol lower in E_2 vs. E_3 homozygotes. Likewise, the VLDL cholesterol/plasma triglyceride ratio was increased in the E_2 individuals. In this small series there was no significant difference in plasma lipoprotein levels between E_3 and E_4 homozygotes, although LDL cholesterol was moderately elevated in the latter as might be expected (3). HDL cholesterol was the same in all three groups. These perturbations in lipid levels were reflected in the distribution of apo B in the four lipoprotein fractions prepared by cumulative ultracentrifugation (Table II). Total apo B concentration, which was calculated as the sum of the apoprotein levels observed in $VLDL_1$, $VLDL_2$, IDL, and LDL, was markedly decreased in E_2 homozygotes. This was due to a much reduced LDL apo B level, which was approximately a third of that seen in E_3 subjects. $VLDL_2$ apo B in contrast was elevated in the E_2 compared with the E_3 group, whereas $VLDL_1$ and IDL apo B concentrations were similar. Individuals in the E_4 group had $VLDL_1$, $VLDL_2$, and IDL apo B levels that were close to those seen in E_3 subjects, although there was a tendency for LDL apo B to be higher in E_4 and the IDL/LDL ratio differed across all three groups (E_2 0.63±0.15, E_3 0.16±0.016, E_2 vs. E_3 $P < 0.05$ by *t* test; E_4 0.13±0.019, E_3 vs. E_4 $P < 0.05$ by *t* test).

The composition of the four apo B–containing lipoprotein fractions is given in Table III. Compared with E_3 individuals, those with E_2 showed a high unesterified cholesterol content in $VLDL_1$ and an enrichment of cholesteryl ester at the expense of triglyceride in $VLDL_2$. The percentage of free cholesterol in LDL was decreased in E_2 homozygotes, and in this lipoprotein fraction there was also a graded decrease in triglyceride content comparing E_2 with E_3 and E_4.

Apo B kinetic studies. The metabolic behavior of apo B in $VLDL_1$, $VLDL_2$, IDL, and LDL after simultaneous injection

Table II. Apo B Concentrations in E_2, E_3, and E_4 Subjects

Subject	$VLDL_1$	$VLDL_2$	IDL	LDL	Total Apo B
			mg/dl		
$E_{2/2}$					
1	1.3	7.6	7.1	17.0	33
2	2.8	9.5	13.4	21.3	47
3	3.8	7.6	16.8	24.8	53
4	2.6	9.5	15.1	19.8	47
Mean±SD	2.6±0.9	8.6±1.0*	13.1±3.7	20.7±3.2*	45±8*
$E_{3/3}$					
5	3.3	6.3	8.7	57.8	76
6	2.4	7.3	13.8	86.6	110
7	3.7	5.8	11.3	76.3	97
8	1.8	2.6	8.1	50.5	63
9	4.9	7.5	12.6	66.0	91
Mean±SD	3.2±1.1	5.9±2.0	10.9±2.2	67.4±14.4	87±18
$E_{4/4}$					
10	4.1	7.5	13.6	82.8	108
11	1.8	4.6	13.3	103.3	123
12	4.6	5.0	8.1	58.3	76
13	1.3	2.9	7.7	64.1	76
14	0.2	3.5	10.1	88.1	102
Mean±SD	2.4±1.7	4.7±1.6	10.6±2.5	79.3±16.4	97±18

* Significantly different from E_3 group, $P < 0.02$ by Student's t test.

of $VLDL_1$ and $VLDL_2$ tracers is shown in Fig. 2. In order to examine intergroup differences more closely, the observed data at each time point were averaged within group and are presented as a mean and standard error for the E_2, E_3, and E_4 subjects. Individual decay curves and apo B masses were used in multicompartmental modeling to generate the kinetic parameters given in Tables IV and V.

$VLDL_1$ apo B radioactivity was cleared rapidly from the plasma of E_3 and E_4 homozygotes (Fig. 2 *a*). The decay curve was monoexponential and only ~ 1% remained at the last time point on the first day, 14 h after injection. Clearance of this lipoprotein was slower in E_2 subjects with ~ 10% remaining at 14 h. The decay curve in the last group was biexponential containing a slow component which accounted for up to 10% of the apo B present in the $VLDL_1$ tracer. This feature necessitated the addition to the model of a second compartment (compartment 12) in $VLDL_1$ (Fig. 1). $VLDL_1$ apo B appeared in $VLDL_2$ with a peak radioactivity ~ 5 h after injection in all subjects (Fig. 2 *b*). Transfer of $VLDL_1$ to $VLDL_2$ was greater and catabolism of apo B from $VLDL_2$ was slower in E_2 compared with E_3 and E_4 subjects. The latter was true whether label in this fraction was introduced directly as a $VLDL_2$ tracer or derived from $VLDL_1$.

The metabolism of IDL derived from both tracers (Fig. 2 *c*) was similar in all three groups. Radioactivity peaked at 8–10 h and decayed in a multiexponential fashion, slightly faster in E_3 than in E_2 or E_4 subjects. Substantial differences however were observed in the metabolic behavior of LDL apo B. Peak radioactivity values of 20% for the $VLDL_1$ apo B tracer and ~ 35% for the $VLDL_2$ apo B tracer were observed in LDL for E_3 and E_4 homozygotes. In contrast, < 5% and ~ 12% of apo B radioactivity derived from the $VLDL_1$ and $VLDL_2$ tracers, respectively, was seen in the LDL fraction of E_2 subjects. The decay curve for LDL apo B in E_3 homozygotes appeared to decline more sharply than that for the E_4 group, particularly over the first 5 d of the turnover.

There was little difference in $VLDL_1$ apo B pool size in the three groups (Table IV *a*) in concordance with their similar plasma triglyceride levels (Table I). Apo B in this flotation interval was synthesized at about 800 mg/d and cleared at a rate

Table III. Composition of Apo B-containing Lipoproteins in E_2, E_3, and E_4 Subjects

	Free cholesterol	Cholesteryl ester	Triglyceride	Phospholipids	Protein
			g/100 g		
$VLDL_1$					
$E_{2/2}$	4.7±0.5*‡	19.9±3.9	50.9±4.2	17.3±1.2	7.2±1.2
$E_{3/3}$	1.5±1.8	16.2±3.2	57.4±4.1	15.4±2.6	9.4±2.0
$E_{4/4}$	3.6±1.7	14.4±5.8	57.0±4.3	17.7±2.7	7.5±1.0
$VLDL_2$					
$E_{2/2}$	8.6±1.4	30.5±3.7‡	27.5±5.2§	21.5±1.4	12.0±1.4
$E_{3/3}$	6.4±1.9	22.4±4.3	36.9±2.9	20.4±2.3	14.1±1.4
$E_{4/4}$	7.0±2.2	22.6±2.1	34.8±2.8	21.2±0.8	14.5±0.9
IDL					
$E_{2/2}$	9.5±1.6	38.3±2.2	11.6±2.8	23.7±1.0	17.0±0.8
$E_{3/3}$	8.9±3.0	35.6±4.7	14.3±1.7	22.1±1.7	19.0±1.7
$E_{4/4}$	9.4±2.5	37.0±1.7	11.4±2.0	23.2±1.2	19.0±1.4
LDL					
$E_{2/2}$	8.4±1.6‡	38.2±1.9	7.9±1.2‡	22.8±0.7	22.8±0.9
$E_{3/3}$	11.6±1.6	37.0±2.0	6.0±0.9	22.0±0.5	23.4±1.4
$E_{4/4}$	11.2±1.2	36.5±0.7	4.4±0.6§	21.9±1.7	26.0±2.4

* Mean±1 SD. Significantly different from E_3 group, ‡ $P < 0.05$, § $P < 0.02$ by Student's t test.

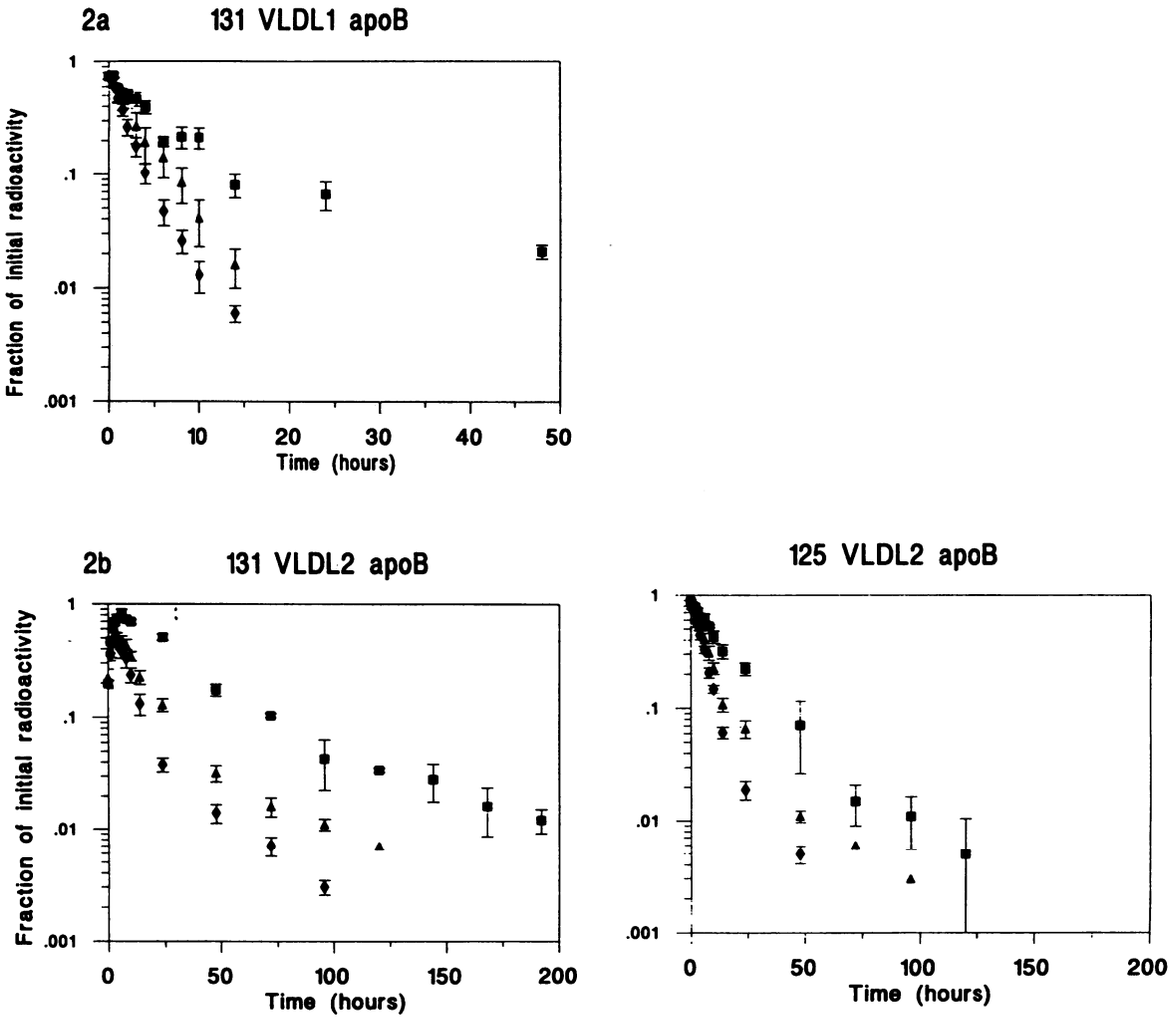

Figure 2. Apo B radioactivity decay curves in (*a*) $VLDL_1$, (*b*) $VLDL_2$, (*c*) IDL, and (*d*) LDL (*opposite page*) in E_2, E_3, and E_4 subjects. Tracers of ^{131}I-$VLDL_1$ and ^{125}I-$VLDL_2$ were injected simultaneously and their metabolism was followed through IDL to LDL. The data were calculated for individual subjects and are here presented as a mean±SE for each group: (■) E_2, (♦) E_3, (▲) E_4.

of 10 pools/d in E_3 subjects. It had two metabolic fates, on average two-thirds were catabolized directly from plasma whereas the remainder was lipolysed to $VLDL_2$. The results in E_4 homozygotes did not differ significantly from those seen in the E_3 group. However, E_2 homozygotes exhibited low apo B synthetic rates and greatly reduced direct catabolism of $VLDL_1$ (Table IV *a*, Fig. 2 *b*). It is noteworthy that the $VLDL_1$ to $VLDL_2$ transfer rate was not affected by apo E phenotype. Approximately 10% of the $VLDL_1$ apo B mass in E_2 subjects was placed in the slowly metabolized second compartment [M (12), Table V *a*]. Material from this pool was cleared directly from the plasma or delipidated to $VLDL_2$. The mathematical model (Fig. 1) was constructed so that apo B from remnant compartment 12 in VLDL, when delipidated, appeared in the $VLDL_2$ remnant pool (compartment 6).

Apo E phenotype appeared not to influence the rate of input of apo B into $VLDL_2$ whether the material was derived from direct synthesis or from delipidation of $VLDL_1$ (Table IV *b*). The overall pool size of $VLDL_2$ apo B did not differ in the three groups although the remnant compartment (compartment 6) was specifically increased in the E_2 subjects ($P < 0.02$ by Mann-Whitney test, Table V *c*). This was apparently due to increased diversion of apo B from the delipidation chain into the slowly metabolized compartment {cf. L(6, 2)/[L(4, 2) + L(6, 2)] in E_2 vs. E_3 and E_4 subjects in Table V *b*} as well as a contribution of material derived from $VLDL_1$ remnant catabolism [by L(6, 12), Table V *a*]. Direct catabolism of $VLDL_2$ apo B was reduced (although not significantly, $P = 0.11$) in E_2 homozygotes compared to E_3 subjects (Table IV *b*) while on-going delipidation to IDL was the same in all three groups. In no subject was there a requirement for de novo apo B synthesis at the level of IDL (Table IV *c*); the plasma pool of IDL apo B which was ~ 300–500 mg in all subjects was derived entirely from $VLDL_2$ catabolism. The metabolic fate of this fraction differed according to apo E phenotype. The transfer rate of IDL to LDL was reduced significantly by 66% in the E_2 group compared to E_3 and E_4 homozygotes. In contrast, direct IDL apo B catabolism was increased in E_2 subjects (Tables IV *c* and V *d*). This redirection was found to be a feature of both parallel pathways of apo B delipidation [Table V *d* L(10, 7), L(11, 8)]. The differences in E_4 vs. E_3 subjects were not as dramatic. There was a tendency for reduced direct IDL apo B catabolism in E_4 ho-

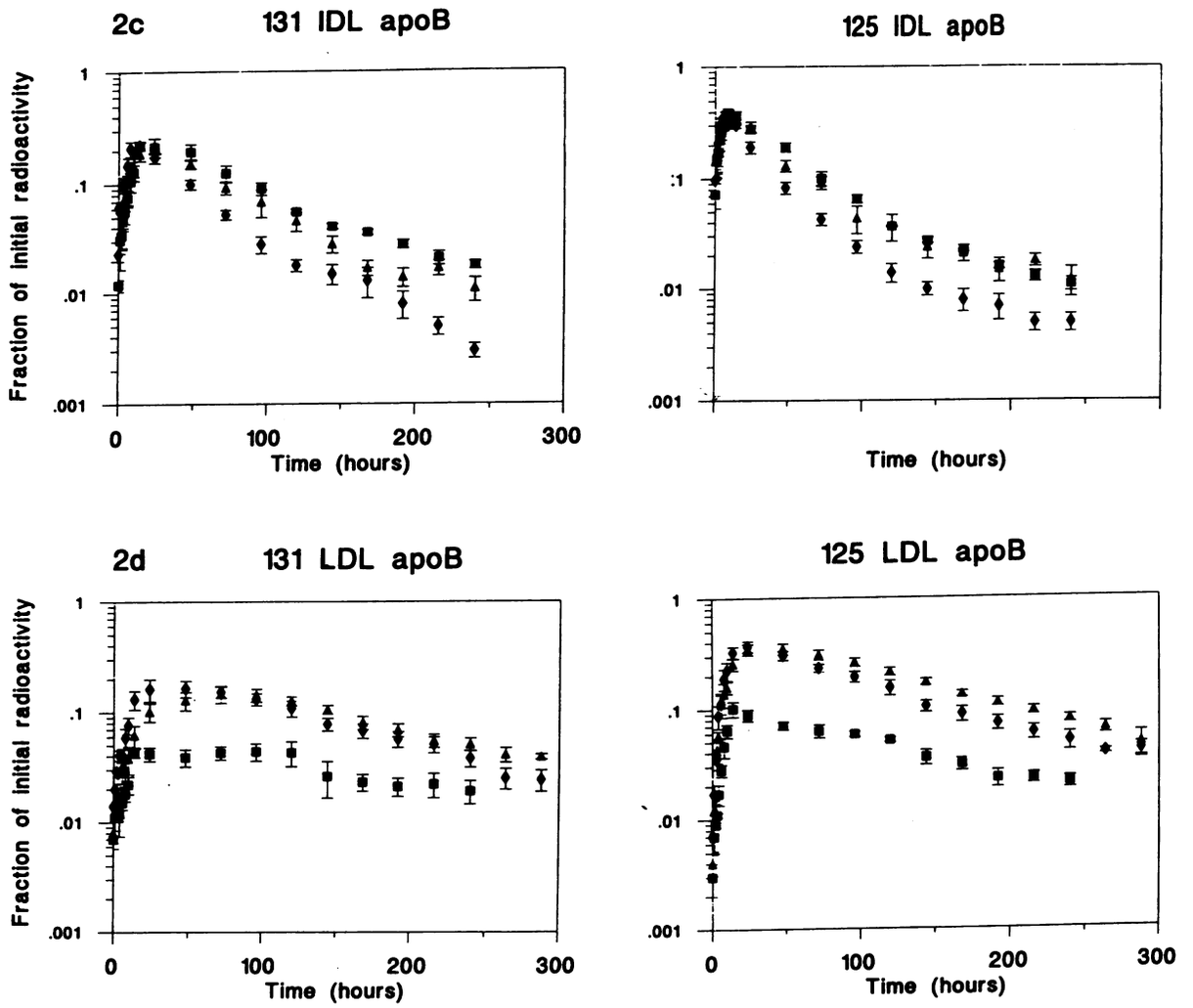

Figure 2 (Continued)

mozygotes (Table IV *c*). This can be seen in the individual fractional rates of delipidation and catabolism for compartments 7 and 8 in Table V *d*. When the proportion of $VLDL_2$ turnover in milligrams per day directed towards LDL apo B was calculated (i.e., LDL production/$VLDL_2$ synthesis, Tables IV *b* and *d*), there was a clear gradation across the phenotypes. In E_2 homozygotes 23±8% of $VLDL_2$ apo B turnover was used to make LDL. This was significantly different from the 50±13% in E_3 subjects ($P < 0.02$ by Mann-Whitney test). Homozygotes for E_4, however, converted significantly more $VLDL_2$ apo B to LDL (70±14%, $P < 0.05$) compared to the E_3 group.

The main cause of the lower LDL apo B plasma concentration in E_2 subjects was reduced synthesis from VLDL (Table IV *d*). Both LDL subcompartments (compartments 10 and 11) were reduced in mass compared to E_3 (Table V *e*). There was little direct synthesis of apo B in the LDL flotation interval of the E_2 group and the catabolic rate for the apoprotein was similar to that seen in E_3 homozygotes. In E_4 homozygotes the overall fractional catabolic rate (FCR) of LDL apo B was reduced by 23% (Table IV *d*) owing to a specific decrease in the elimination constant for the faster catabolized compartment 10 (Table V *e*). In the majority of E_2 and E_3 individuals, L(0, 10) exceeded L(0, 11) by ~ 63%, whereas in the E_4 group the rate of LDL apo B catabolism from these two compartments was virtually identical. This is in keeping with the flat nature of the LDL apo B decay curve in E_4 subjects (Fig. 2 *d*).

Discussion

The polymorphism in the gene coding for apo E is the most important inherited trait modulating plasma cholesterol levels in the normal population (3). This study was designed to investigate the perturbations in apo B metabolism associated with homozygosity for the three common phenotypes, E_2, E_3, and E_4. Subjects were located during the screening of several thousand individuals for coronary risk factors. A decision was taken to select volunteers from those whose total plasma cholesterol levels on initial presentation were in a range (5.0–6.2 mmol/liter) that straddled the mean population value. By this approach we hoped to minimize any effect that gross differences in plasma lipid levels might have on apo B kinetics. Mean (±1 SD) cholesterol levels in subjects homozygous for apo E_2, apo E_3, and apo E_4 in the whole screened population were 5.28±0.71, 5.51±0.72, and 5.93±1.18 mmol/liter, respectively. There was no significant difference in plasma choles-

Table IV. Apo B Metabolism in E_2, E_3, and E_4 Normolipidemic Subjects

a. $VLDL_1$	Synthesis	Plasma pool	Direct catabolism	Transfer to $VLDL_2$
	mg/d	*mg*	*pools/d*	
$E_{2/2}$				
1	140	37	1.24	2.54
2	396	54	2.57	4.76
3	374	85	0.00	4.39
4	338	50	1.92	4.84
Median	356*‡	52	1.58*‡	4.58
$E_{3/3}$				
5	625	67	4.17	5.16
6	522	52	5.75	4.49
7	1,288	97	10.79	2.48
8	1,120	106	6.91	3.66
9	819	47	14.73	2.71
Median	819	67	6.91	3.66
$E_{4/4}$				
10	894	107	5.36	2.99
11	474	105	2.53	1.98
12	515	45	7.56	3.88
13	294	12	16.80	7.68
14	146	16	0.00	9.12
Median	474	45	5.36	3.88

c. IDL	Direct synthesis	Flux from $VLDL_2$	Plasma pool	Direct catabolism	Transfer to LDL
	mg/d		*mg*	*pools/d*	
$E_{2/2}$					
1	0	455	253	1.55	0.24
2	0	370	330	0.70	0.42
3	0	510	467	0.77	0.32
4	0	334	309	0.73	0.34
Median	0	413	320	0.75	0.33‡*
$E_{3/3}$					
5	0	621	241	0.76	1.80
6	0	495	408	0.18	1.02
7	0	517	358	0.26	1.17
8	0	385	351	0.33	0.76
9	0	189	174	0.37	0.68
Median	0	495	351	0.33	1.02
$E_{4/4}$					
10	0	593	442	0.41	0.94
11	0	387	272	0.33	1.10
12	0	305	324	0.01	0.93
13	0	235	171	0.17	1.25
14	0	370	209	0.12	1.65
Median	0	370	272	0.17	1.10

b. $VLDL_2$	Direct synthesis	Flux from $VLDL_1$	Plasma pool	Direct catabolism	Transfer to IDL and LDL
	mg/d		*mg*	*pools/d*	
$E_{2/2}$					
1	373	94	224	0.06	2.03
2	238	257	237	0.42	1.67
3	333	374	215	0.84	2.46
4	234	242	214	0.65	1.56
Median	284	250	220	0.54	1.85
$E_{3/3}$					
5	301	346	160	0.18	3.88
6	761	229	231	2.14	2.14
7	500	241	199	0.92	2.81
8	280	388	218	1.15	1.93
9	203	127	69	2.14	2.74
Median	301	241	199	1.15	2.74
$E_{4/4}$					
10	273	320	249	0.00	2.38
11	180	208	170	0.01	2.28
12	480	175	120	2.89	2.54
13	173	92	57	0.46	4.14
14	332	146	79	1.15	4.90
Median	273	175	120	0.46	2.54

d. LDL	Direct§ synthesis	Flux from IDL and $VLDL_2$	Plasma pool	LDL FCR
	mg/d		*mg*	*pools/d*
$E_{2/2}$				
1	10	61	507	0.14
2	14	165	460	0.39
3	12	164	650	0.27
4	2	106	450	0.24
Median	11*‡	135‡	484‡	0.26
$E_{3/3}$				
5	0	435	1,433	0.30
6	233	418	2,505	0.26
7	10	460	2,044	0.23
8	153	301	1,620	0.28
9	183	119	1,160	0.26
Median	153	418	1,620	0.26
$E_{4/4}$				
10	83	415	2,490	0.20
11	69	299	1,750	0.21
12	58	305	2,588	0.14
13	41	214	1,274	0.20
14	65	360	1,770	0.24
Median	65	304	1,770	0.20‡

* Differences between groups were assessed by the Mann-Whitney test. Significantly different from E_3 group, ‡ $P < 0.02$. § Direct synthesis in LDL was calculated as the difference between the total absolute catabolic rate (observed mass × overall FCR) and the input from VLDL and IDL.

terol and triglyceride in the three groups of this study (Table I), although the E_2 homozygotes displayed the characteristic dyslipidemia associated with this phenotype, i.e., cholesterol-enriched VLDL and low LDL levels. Apo B levels in this group were low (Table II) despite the similarity in total plasma cholesterol in E_2 and E_3 subjects owing to the higher cholesterol to

apo B ratios in $VLDL_1$ and $VLDL_2$ (Table III) and higher concentrations of $VLDL_2$ and IDL particles in the circulation (Table II) of the former group.

Kinetics studies using tracers of radioiodinated $VLDL_1$ and $VLDL_2$ revealed marked differences in the metabolic behavior of apo B in E_2 and E_3 homozygotes and more subtle distinctions between the E_3 and E_4 groups. For comparative purposes, E_3 is taken as the norm since it represents the commonest variant in the population. The decay curves obtained for the four lipoprotein fractions had the general features observed in previous investigations (13, 18). Only a minor modification to the model (compartment 12, Fig. 1) was required to accommodate $VLDL_1$ apo B kinetics in the E_2 group. This model developed for normal and hypertriglyceridemic subjects has been used successfully to explain apo B kinetics in other genetically determined dyslipidemic conditions such as homozygous familial hypercholesterolemia (FH) and hepatic lipase deficiency (13, 26).

Apo B metabolism in E_3 homozygotes. In E_3 subjects most apo B entered the system at the level of $VLDL_1$. This material had two fates, catabolism and transfer to $VLDL_2$ by delipidation. Direct removal of $VLDL_1$ apo B from the circulation has been noted in virtually all subjects examined with this technique. The pathway was previously found to be present in normal and homozygous FH subjects (13) and its activity was not affected by a chemical modification of the $VLDL_1$ tracer designed to prevent the interaction of apo B with LDL receptors (27). These findings appear to eliminate the LDL receptor as the agent responsible for this catabolic route and at present the mechanism of this process is unknown. Previous work indicates that lipoprotein lipase is the agent responsible for the $VLDL_1$ to $VLDL_2$ conversion. Tracer studies show delayed catabolism of large VLDL in lipoprotein lipase–deficient subjects (28), whereas $VLDL_1$ transfer to $VLDL_2$ occurs at a normal rate in hepatic lipase (26) and LDL receptor (13) deficiency. When the enzyme is deficient (i.e. in type I hyperlipidemia [29]) or inhibited in its action (30), large triglyceride-rich VLDL accumulate. Apo B entering $VLDL_2$ by direct synthesis and by lipolysis of $VLDL_1$ in E_3 subjects was rapidly and efficiently converted to IDL and LDL (Table IV *b*); a trivial amount entered the $VLDL_2$ remnant pool (compartment 6, Table V *c*). About half of the apo B passing through the delipidation cascade from $VLDL_2$ to LDL was lost by direct catabolism probably via LDL receptors. These removal mechanisms are inhibited in homozygous FH (13) and blocked by chemical modification of apo B (27). LDL apo B was divided into two metabolically distinct pools to account for the observation that apo B derived from $VLDL_2$ appeared and was cleared more rapidly than material derived from $VLDL_1$ (13, 18).

Apo B metabolism in E_2 homozygotes. The metabolism of $VLDL_1$ apo B differed in E_2 compared with E_3 subjects in three aspects. First, the decay curve was biexponential probably as a result of the presence of chylomicron remnants. It has been shown that subjects homozygous or heterozygous for the apo E_2 phenotype clear chylomicrons slowly and therefore remnants are likely to be present even after a 12-h fast (9, 10). Remnant accumulation is a hallmark of type III hyperlipidemia, and we noted that the $VLDL_1$ apo B curves observed in the present E_2 group were qualitatively similar to those found previously in type III subjects (18). The slowly metabolized species in the $VLDL_1$ fraction in the present study was estimated at 10% of the apo B mass but direct quantitation of B48 vs. B100 in the tracer (a measure of chylomicron remnant content) was not performed. Secondly, the rate of $VLDL_1$ apo B synthesis was significantly reduced in comparison to the E_3 subjects. The reason for this is not clear although it is possible that impaired delivery of chylomicron lipid to the liver in E_2 homozygotes causes that organ subsequently to secrete less triglyceride-rich VLDL. Thirdly, there was less direct catabolism of $VLDL_1$ apo B. This pathway accounted for 25% of $VLDL_1$ clearance compared with 65% in E_3 subjects (Table IV *a*). As mentioned above this unknown mechanism of apo B catabolism operates in most normal and hyperlipidemic subjects. Its decreased activity in E_2 homozygotes indicates that functional apo E may be required for it to proceed efficiently and raises the possibility that a receptor may be involved. It is unlikely that the classical LDL receptor has a substantial role in this regard since the pathway works as normal in FH (13). There is evidence that the recently discovered LDL receptor–related protein has apo E–binding properties (31) and is a candidate agent for mediating chylomicron remnant removal. If very large triglyceride-rich VLDL were catabolized in a similar fashion to chylomicrons, then this would explain the decreased removal in E_2 homozygotes. Indeed, in vitro apo E_2 shows impairment in binding to the LDL receptor–related protein (32).

Apo B synthesis into $VLDL_2$ whether de novo or from $VLDL_1$ occurred at the same rate in E_2 and E_3 subjects (Table IV *b*). More apo B passing through the delipidation chain in E_2 homozygotes was diverted to remnants and the expansion of this presumably cholesteryl ester–rich population of particles in $VLDL_2$ to 13% of total apo B mass (compared to $< 2\%$ in E_3 subjects, Table V *c*) explains the abnormal composition of this lipoprotein fraction in the E_2 group (Table II). Surprisingly the catabolic rate of $VLDL_2$ remnants [L(9, 6) and L(0, 6), Table V] was not consistently reduced in our E_2 subjects. IDL formation and clearance occurred at approximately the same rate in E_2 and E_3 subjects. However, the metabolic fate of apo B differed greatly between them with a 66% reduction in the rate of LDL formation in the former. This difference was the principal cause of reduced LDL levels in E_2 homozygotes. The data in Tables IV and V indicate that in E_2 subjects the impaired IDL to LDL conversion is balanced by increased catabolism of the intermediate fraction: an observation that suggests that functional apo E is not required for effective IDL clearance. Direct removal of IDL probably occurs via LDL receptors since it is inhibited by 1,2-cyclohexanedione modification of the lipoprotein (27) and is depressed in homozygous FH (13). In fact, there was no inhibition of $VLDL_2$, IDL or LDL clearance in our E_2 subjects suggesting either that it is apo B that acts as the ligand for receptors in these lipoproteins fractions (as is found in vitro [33, 34]) or that an increase in receptor activity more than compensates for the reduced affinity of apo E_2 for the receptor. The reason why direct IDL catabolism is favored over conversion to LDL in E_2 homozygotes is unknown. The phenomenon was recorded previously in our study of the effects of bezafibrate on apo B metabolism in type III hyperlipidemic subjects (35). These were apo E_2 homozygotes with elevated lipid levels that were corrected by bezafibrate therapy. The mean on-therapy concentrations of plasma cholesterol, triglyceride, VLDL, LDL, and HDL cholesterol were 5.87, 2.12, 1.63, 2.79, and 1.46 mmol/liter, respectively; values close to those seen in our normolipemic E_2 group (Table I). Apo B kinetics in the type III

Table V. Computed Masses and Rate Constants in E_2, E_3, and E_4 Subjects

a. $VLDL_1$	L(0,1)*	L(2,1)	L(0,12)	L(6,12)	M(1)	M(12)	U(1)‡	U(12)
		d^{-1}			mg			
$E_{2/2}$								
1	1.60	3.23	0.69	0.33	27	10	130	10
2	2.78	5.36	0.00	0.95	48	6	390	6
3	0.00	4.61	0.19	0.00	81	4	373	1
4	1.94	5.27	0.00	1.78	46	4	331	7
Median	1.77‖	4.94	0.10	0.64	47	5	352‖	6.5
$E_{3/3}$								
5	4.17	5.16	—	—	67	—	625	—
6	5.75	4.49	—	—	51	—	522	—
7	10.79	2.48	—	—	97	—	1288	—
8	6.91	3.66	—	—	106	—	1120	—
9	14.73	2.71	—	—	47	—	819	—
Median	6.91	3.66	—	—	67	—	819	—
$E_{4/4}$								
10	5.36	2.99	—	—	107	—	894	—
11	2.53	1.98	—	—	105	—	474	—
12	7.56	3.88	—	—	45	—	515	—
13	16.80	7.68	—	—	12	—	294	—
14	0.00	9.12	—	—	16	—	146	—
Median	5.36	3.88	—	—	45	—	474	—

b. $VLDL_2$	L(4,2)	L(6,2)	L(9,6)	L(0,6)	L(0,4)	L(8,4)	L(11,4)	L(9,4)	L(0,5)	L(7,5)	L(10,5)
						d^{-1}					
$E_{2/2}$											
1	8.40	0.67	0.03	0.36	0.00	0.70	0.00	0.30	0.00	3.77	0.00
2	8.40	0.33	0.13	0.30	0.00	1.23	0.14	0.02	10.97	17.38	0.00
3	8.40	0.65	0.68	0.00	1.99	1.70	0.14	0.00	0.00	7.75	0.00
4	7.48	0.24	0.40	0.00	0.18	1.04	0.00	0.30	10.14	11.13	0.00
Median	8.40	0.49‖	0.27	0.15	0.09	1.14‖	0.07	0.01	5.07	9.44‖	0.00
$E_{3/3}$											
5	6.22	0.03	0.56	0.00	0.00	5.76	0.00	0.90	0.56	5.45	0.00
6	7.16	0.02	0.41	0.00	1.92	2.22	0.00	0.60	2.72	2.42	0.00
7	6.96	0.13	0.76	0.00	0.00	3.14	0.68	0.00	1.87	3.23	0.00
8	8.92	0.09	0.31	0.32	1.63	2.19	0.08	0.00	1.24	2.37	0.39
9	7.03	0.10	0.00	0.90	0.33	10.35	0.00	1.26	3.65	1.56	0.00
Median	7.03	0.02	0.41	0.00	0.33	3.14	0.00	0.60	1.87	2.37	0.00
$E_{4/4}$											
10	8.40	0.03	0.38	0.00	0.00	2.44	0.00	0.00	0.00	3.54	0.00
11	3.89	0.01	0.00	0.28	0.00	1.84	0.00	0.44	0.00	7.51	0.00
12	8.64	0.40	0.34	0.39	1.94	0.87	0.06	0.00	7.12	7.87	0.00
13	8.59	0.07	0.26	0.14	0.78	1.64	0.02	0.28	0.02	14.40	0.00
14	8.10	0.04	0.00	0.52	2.56	0.51	0.47	0.58	0.00	13.26	0.00
Median	8.40	0.04	0.26	0.28	0.78	1.64§	0.02	0.28	0.00	7.87‖	0.00

c. $VLDL_2$	M(2)	M(4)	M(6)	M(5)	U(5)
			mg		
$E_{2/2}$					
1	10	81	35	99	373
2	29	177	23	8	238
3	41	91	41	43	333
4	31	153	18	11	234
Median	30	122	29‖	27	284
$E_{3/3}$					
5	56	52	3	50	301
6	32	48	3	148	761
7	34	62	6	98	500
8	43	99	6	70	280
9	18	11	2	39	203
Median	34	52	3	70	301
$E_{4/4}$					
10	38	172	3	77	273
11	53	91	2	24	180
12	19	58	10	32	480
13	10	32	2	12	173
14	18	35	2	25	332
Median	18	58	2	25	273

Table V. (Continued)

d. IDL	L(10,7)	L(0,7)	L(0,8)	L(11,8)	L(0,9)	M(7)	M(8)	M(9)
			d^{-1}				*mg*	
$E_{2/2}$								
1	1.89	0.34	4.97	0.42	0.34	167	10	75
2	0.06	1.27	1.24	0.00	0.16	110	176	44
3	0.77	0.60	0.91	0.04	0.43	242	161	64
4	0.26	0.54	3.23	0.60	0.46	150	41	118
Median	0.52‖	0.57§	2.24	0.23§	0.39	159	101	69
$E_{3/3}$								
5	0.00	4.09	1.65	2.04	0.52	66	81	94
6	0.00	1.39	0.80	1.10	0.32	256	56	95
7	0.00	1.58	0.61	0.70	0.37	199	148	11
8	0.00	1.44	0.49	0.44	0.38	114	231	5
9	0.00	0.80	1.21	1.39	0.24	76	42	56
Median	0.00	1.44	0.80	1.10	0.37	114	81	56
$E_{4/4}$								
10	0.00	1.53	0.69	0.55	0.19	178	260	5
11	0.00	2.03	0.58	1.41	0.39	89	84	103
12	0.00	1.11	0.00	0.63	0.20	225	81	18
13	0.00	2.55	0.37	0.66	0.20	70	52	49
14	0.00	2.69	0.30	0.74	0.29	123	17	69
Median	0.00	2.03	0.37	0.66	0.20	123	81	49

e. LDL	L(0,10)	L(0,11)	M(10)	M(11)
	d^{-1}			*mg*
$E_{2/2}$				
1	0.14	0.15	391	30
2	0.41	0.29	341	88
3	0.32	0.13	447	154
4	0.28	0.17	296	145
Median	0.30	0.16	365	117‖
$E_{3/3}$				
5	0.42	0.21	640	793
6	0.30	0.15	1,206	417
7	0.28	0.16	1,124	920
8	0.32	0.23	606	485
9	0.28	0.25	219	231
Median	0.30	0.21	606	485
$E_{4/4}$				
10	0.20	0.18	1,136	782
11	0.22	0.20	819	592
12	0.14	0.15	1,830	353
13	0.21	0.17	828	199
14	0.24	0.22	1,406	132
Median	0.21¶	0.18	1,136	353

* Rate constants, L, k (destination, source) and masses, M( ). ‡ U( ) represents de novo synthesis of apo B into a compartment. § Significantly different from E_3 group, $P < 0.05$, ‖ $P < 0.02$, ¶ $P < 0.01$. Difference between groups was assessed by the Mann-Whitney test.

Table VI. Comparison of Apo B Turnover in Normolipemic E_2 Homozygotes and Type III Hyperlipidemic Subjects before and during Bezafibrate Therapy

	$VLDL_1$			$VLDL_2$			IDL			LDL		
Subjects	Total production	Plasma concn.	FCR	Total production	Plasma concn.	FCR	Total production	Plasma concn.	FCR	Total production	Plasma concn.	FCR
	mg/d	*mg/dl*	*pool/d*	*mg/d*	*mg/dl*	*pools/d*	*mg/d*	*mg/dl*	*pools/d*	*mg/d*	*mg/dl*	*pools/d*
Type III* ($n = 6$)	885	15.9	2.0	1125	30.8	1.2	630	13.7	1.3	439	21	0.21
Type III on bezafibrate	385	2.9	4.5	783	14.6	1.7	568	14.0	1.2	138	17	0.33
$E_{2/2}$ normolipemic	356	2.6	6.2	534	8.6	2.3	413	13.1	1.1	135	21	0.26

* Data on type III hyperlipidemic subjects were taken from reference 35.

subjects before and during bezafibrate treatment are presented in Table VI. There is a remarkable similarity between treated type III and normolipemic E_2 subjects in both the apo B content of lipoprotein fractions and metabolic behavior indicating that bezafibrate removes the hyperlipidemia component from the type III pattern but does not correct the abnormality owing to E_2 genetic variant. Interestingly, before drug therapy the type III patients converted 76% of IDL to LDL (similar to the E_3 subjects in Table IV), whereas on treatment this fell to 26%, i.e., close to the value seen in normolipemic E_2 subjects. Thus bezafibrate had two effects; it reduced VLDL synthesis correcting the hyperlipidaemia (but not the dysbetalipoproteinemia) and altered the nature of the IDL to LDL metabolic link from that seen in E_3 to that seen in E_2 subjects.

There was no increase in the fractional clearance rate of LDL apo B in E_2 homozygotes in the present study (Table IV *d*). This contrasts with an earlier preliminary report by Gregg et al. (12) in which a small increase in LDL FCR was observed in two normolipemic E_2 homozygotes. However, further work by the same authors in an apo E–deficient patient demonstrated a normal catabolic rate for autologous LDL apo B (36, 37). The low LDL apo B level observed in that condition was due to reduced synthesis at 20% of normal and in general LDL kinetics in apo E deficiency were similar to those reported here for E_2 subjects (Table IV *d*).

Apo B metabolism in E_4 homozygotes. Apo B synthesis in $VLDL_1$ was lower in E_4 than E_3 subjects (Table IV *a*) but the difference was not significant. The circulating mass of apo B and clearance rate of the fraction were similar in the two groups. Likewise, $VLDL_2$ apo B synthesis, pool size, and overall catabolic rate were similar in E_3 and E_4 subjects as was the turnover of IDL (Table IV). However, calculation of the amount of apo B undergoing direct catabolism from $VLDL_2$ and IDL compared to that channeled down the delipidation cascade revealed that E_4 homozygotes exhibited a relative decrease in direct removal and, per milligram of apo B entering $VLDL_2$, directed more towards LDL production (Table IV). Thus, despite the fact the E_4 subjects synthesized apo B in VLDL ($VLDL_1$ plus $VLDL_2$) at only two-thirds of the rate seen in E_3 homozygotes (Table IV *a* and *b*) they had apo B levels that were at least as high as those seen in the latter group (Table II). LDL levels in E_4 subjects were further increased by the low FCR seen for apo B in this flotation interval (Tables IV *d* and V *e*). This was due to a decrease in the rate of apo B degradation from the larger of the two LDL compartments (compartment 10, Table V *e*) with the result that both LDL pools were cleared at the same rate. This is consistent with a reduced receptor-mediated LDL clearance in E_4 subjects. In fact, the concentration and distribution of apo B–containing lipoproteins in E_4 homozygotes appears to be the result of suppressed receptor-mediated catabolism of $VLDL_2$, IDL, and LDL. It is likely that the low synthetic rate for apo B observed in our E_4 group was a result of patient selection. E_4 homozygotes with an apo B synthetic rate similar to that seen in the E_3 group (800 mg/d) would be predicted to have a plasma apo B level of about 150 mg/dl and a plasma cholesterol in excess of 7.0 mmol/liter.

Influence of apo E on apo B metabolism. It is clear from the above that variation in the apo E gene has a profound impact on apo B metabolism throughout the S_f 0–400 lipoprotein spectrum. This information can be integrated with other studies on cholesterol metabolism (38) and chylomicron kinetics (9, 10) to generate an overall picture of the influence of the polymorphism which refines and in places corrects the model previously suggested by Davignon et al. (3). Cholesterol absorption from the gut is reportedly higher in E_4 vs. E_3 vs. E_2 subjects. This together with differential clearance rate of chylomicron remnants ($E_4 > E_3 > E_2$; references 9 and 10) will affect the delivery of intestinal (including dietary) cholesterol to the liver. The result is a predicted decrease in the hepatic sterol pool in E_2 vs. E_3 and E_4 subjects which gives rise to the observed graded difference ($E_2 > E_3 > E_4$) in the rates of cholesterol and bile acid production (38, 39).

According to current concepts changes in the liver cholesterol pool will alter hepatic LDL receptor activity. The latter is predicted to be higher in E_2 compared to E_3 subjects and they in turn will be higher than E_4 subjects. Since we have found previously that LDL receptors play a role in $VLDL_2$, IDL, and LDL metabolism (13, 27), this mechanism explains why E_2 homozygotes exhibit more and E_4 homozygotes less direct catabolism of $VLDL_2$ and IDL than E_3 subjects. In this scenario LDL removal should be increased in E_2 homozygotes but this was not observed in our group (Table IV *d*) or in an apo E–deficient patient (37) possibly because the small amount of LDL that is present is abnormal, having a conformation of apo B that fails to recognize receptors efficiently. Evidence to support this hypothesis comes from studies of the kinetic behavior of LDL from apo E–deficient or E_2 homozygous subjects in normals; it is cleared more slowly than autologous LDL (12, 37). In contrast, normal LDL given to an apo E–deficient patient is cleared rapidly as would be the case if LDL receptors were up-regulated (37). The redirection of the metabolic fate of IDL from conversion to LDL to direct catabolism in E_2 homozygotes may be further influenced by a requirement for functional apo E for lipolysis to proceed efficiently. In vitro studies suggest that VLDL from E_2 subjects is not a good substrate for lipase and lipolysis of this lipoprotein does not lead to the formation of LDL (40). Addition of apo E_3 enhances the reaction and LDL is formed. Retarded delipidation would provide a mechanism for the formation of $VLDL_2$ remnants and the very low conversion of IDL to LDL in our E_2 subjects. This integrated model, which is now modified and considerably strengthened by the availability of detailed apo B kinetic data, can be used to help explain why individuals with varying apo E phenotypes respond differently to diet and drug therapies (41, 42).

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