Apolipoprotein A and B (Sf 100-400) metabolism during bezafibrate therapy in hypertriglyceridemic subjects.

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Research Article

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Apolipoprotein A and B (S_f 100–400) Metabolism During Bezafibrate Therapy in Hypertriglyceridemic Subjects

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Abstract. This study describes the effects of bezafibrate, an analogue of clofibrate, on the plasma lipid and lipoprotein profiles of 11 hypertriglyceridemic subjects and on their metabolism of apolipoproteins A-I, A-II, and B. The major action of the drug was to lower plasma triglyceride (by 58%; $P < 0.01$). This was accompanied by a reduction in the level of very low density lipoprotein apoprotein B (Svedberg units of flotation [S_f] 60–400), whose mean residence time in the plasma fell threefold (from 3.4 to 1.0 h). Synthesis of the B protein in this fraction was not significantly altered, so the drug acts to accelerate the transit of very low density lipoprotein particles down the delipidation cascade. The metabolism of very low density lipoprotein remnant apoprotein B (S_f 12–100) changed little in response to treatment, although we detected a 30% increment ($P < 0.05$) in the plasma concentration of this fraction. The mean residence time of these remnant particles in the plasma did not correlate with that of S_f 100–400 very low density lipoprotein apoprotein B, nor was this parameter altered by the drug. The most consistent and significant perturbation seen in the S_f 0–12 fraction (low density lipoprotein) was a reduction in the fractional catabolism of its apoprotein B moiety (26%; $P < 0.05$). In those subjects who were grossly hypertriglyceridemic and who responded well to treatment, the level of this protein rose substantially owing to a combined increase in its synthesis and a reduction in its catabolism. In the group as a whole, high density lipoprotein cholesterol rose 13% ($P < 0.02$), and detailed examination showed that this was associated with a small but significant increment in the plasma concentration of the high density lipoprotein subfraction 2. High density lipoprotein subfraction 3 also rose on the average, but this was not a consistent feature in all patients. The plasma concentrations and turnovers of the A proteins (A-I and A-II) were not significantly altered by bezafibrate therapy.

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Introduction

Recognition of the risk association between atherosclerosis and hyperlipidemia has led over the last two decades to the development of a number of agents designed to lower plasma cholesterol and triglyceride. Initially their efficacy was gauged simply on their hypolipemic effect, but increasing awareness of the complexity of lipoprotein metabolism has highlighted the need for a much more critical assessment of their mechanisms of action. We need to know how they affect the entire plasma lipoprotein spectrum and in particular we must determine their influence on those apoproteins that regulate lipid metabolism. Clofibrate was one of the earliest compounds exploited clinically to lower plasma lipid levels (1). It effectively reduces plasma triglyceride (2, 3) but unfortunately also increases the lithogenic index of bile and predisposes to gallstone formation (4, 5, 6). In addition, when it was used as a lipid lowering agent in two large clinical trials (2, 7), it had no effect on the rate of death from coronary artery disease and in fact may even have predisposed overall to an increase in mortality in the drug-treated subjects (7). Recent modifications to the structure of clofibrate have produced derivatives with increased potency. One of these, bezafibrate (2,4,2-[4-chlorobenzamido]-ethyl-phenoxy-2-methylpropionic acid), is at least as effective as clofibrate when administered at a dose of 200 mg three times a day (8, 9) and does not alter bile lithogenicity (10). It is completely absorbed and rapidly eliminated, 94% appearing

in the urine in 24 h. It has multiple effects on lipoprotein metabolism, which appear to derive primarily from its influence on certain key enzymes. It enhances the activities of lipoprotein lipase and hepatic triglyceride lipase and hence promotes triglyceride clearance from the plasma (11). In addition, part of its triglyceride lowering effect might derive from inhibition of triglyceride synthesis secondary to limitation of the availability of fatty acid substrate (12). The hypocholesterolemic action of bezafibrate may follow in part from its apparent ability to suppress 3-hydroxy-3-methylglutaryl Coenzyme A reductase activity, although so far this effect has only been demonstrated in rats (13). The implications of these changes in lipoprotein metabolic pathways are not yet elucidated and form the basis of the present study.

A number of clinical trails have shown that the second generation clofibrate analogues like bezafibrate can reduce plasma triglyceride levels substantially (by ~40–50%). The change in plasma cholesterol is usually less dramatic and in fact seems to be determined by the subject's initial plasma lipid profile. In normotriglyceridemic, hypercholesterolemic subjects, plasma cholesterol (and low density lipoprotein [LDL]) falls, whereas treatment of hypertriglyceridemic individuals often results in a rise in circulating LDL levels (14). These paradoxical responses seem to reflect the balance of influence that the drug exerts on lipase-directed very low density lipoprotein to LDL conversion (VLDL → LDL) on the one hand and its effects on LDL catabolism on the other. In a previous study (15) we described how the drug promotes LDL apoprotein catabolism in Type II hyperlipoproteinemic patients. It lowered plasma triglyceride and cholesterol by 30 and 10%, respectively, by reducing circulating VLDL and LDL levels. The decrement in LDL appeared to result from an increase in the fractional clearance rate of the lipoprotein via the receptor pathway. LDL apoprotein synthesis did not fall, so we concluded that where the drug produces a reduction in the level of this lipoprotein it is due to promotion of the activity of high affinity receptors for LDL on cell membranes.

In general terms the effects of the clofibrate analogues on high density lipoprotein (HDL) metabolism are modest. Early studies failed to show any change in this lipoprotein in response to therapy. However, more recently careful examination has revealed small but consistent increments in this fraction (9, 14, 16). The greatest changes are seen in hypertriglyceridemic subjects whose initial HDL levels are low and who respond well to treatment. Since HDL apparently has a protective function that counteracts the detrimental influences of VLDL and LDL on atherogenesis, the overall merits of the drug must be assessed from its effects on these two lipoprotein families. The present work examines the impact of bezafibrate on apolipoprotein A and apolipoprotein B (apoB),[1] the major proteins found in these fractions.

1. *Abbreviations used in this paper:* apoA-I, apoA-II, and apoB, apolipoproteins A-I, A-II and B, respectively; S_f, Svedberg unit of flotation, negative sedimentation coefficient at $d = 1.063$ kg/liter and 26°C.

Methods

Subjects. 11 hyperlipoproteinemic subjects gave their informed consent for this study. Their lipid and lipoprotein profiles are shown in Table I. One was Type III, eight were Type IV, and two were Type V hyperlipoproteinemic according to the phenotypic classification system of Fredrickson et al. (17). Apart from their hyperlipidemia, all were healthy. None showed evidence of hepatic, renal, endocrine, or hematologic dysfunction, nor was the hypertriglyceridemia of the Type V subjects secondary to alcohol abuse. For the duration of the study the subjects refrained from alcohol and received no drug therapy except where specified.

Two studies that required administration of radioiodinated lipoproteins were performed, one on five subjects and the other on six. Potassium iodide tablets (60 mg three times a day) were prescribed for 3 d before and 1 mo after administration of the label to block thyroidal sequestration of radioiodide. The protocols conformed to the requirements of the Ethical Committee of Glasgow Royal Infirmary.

Study I: HDL metabolism. This study examines the metabolism of apolipoproteins A-I and A-II (apoA-I and apoA-II) before and during bezafibrate (200 mg three times a day) administration to one Type III and four Type IV hyperlipoproteinemic subjects. The experiment was conducted on an outpatient basis during which time the subjects ate their regular diet. This arrangement provides steady state conditions necessary for apolipoprotein kinetic analysis, as determined by repeated measurements of plasma cholesterol, and apoA-I and A-II. The coefficients of variation for each of these parameters were <10, 10.6, and 14.2%, respectively. The subjects' body weights showed minimal day-to-day variation. The study was done in two phases. In the initial control phase, 2 wk long, measurements were made of plasma lipid and lipoprotein levels (18), the concentrations of apoA-I and A-II (by electroimmunoassay) (19), HDL subfraction distribution and composition (by rate zonal ultracentrifugation) (20), and apoA-I and A-II turnover. Kinetic analysis was performed as follows. HDL ($1.063 < d < 1.21$ kg/liter) was prepared by sequential ultracentrifugation (21) of 30 ml of plasma obtained from each subject after a 14-h fast. The isolated lipoprotein was washed once at its upper density limit, dialyzed extensively against 0.15 M NaCl/0.01 M Tris, pH 7.0, 0.01% Na_2 EDTA, and radioiodinated with ^{125}I (supplied as carrier-free NaI by Amersham International, Amersham, England) by a modification (22) of the iodine monochloride procedure of McFarlane (23). Unbound radioiodide was removed by exhaustive dialysis against the above buffer. More than 97% of the radioactivity in the final preparation was precipitable by 10% trichloracetic acid and <4% extracted into chloroform/methanol (2:1, vol/vol). Labeling efficiency was ~60% and the ICl concentration was adjusted so that <1 mol of iodine bound to each mole of HDL apoprotein, assuming uniform labeling and a mean molecular weight for the protein of 25,000. Bezafibrate therapy did not affect the labeling characteristics of the lipoprotein. Approximately 50 μCi of the preparation was sterilized by filtration (0.22 μm filters, Amicon Corp., Lexington, MA) and injected into the bloodstream of the donor. Plasma samples were then withdrawn at 10 min and thereafter daily, after an overnight fast, for 14 d. The isotope dilution that occurred in the first 10 min gave a measurement of the subjects' plasma volume. HDL ($1.063 < d < 1.21$ kg/liter) was reisolated from 10 ml of each sample without washing, dialyzed against 0.15 M NaCl/0.01 M Tris, pH 7.0, 0.01% Na_2 EDTA, and delipidated by the addition of an equal volume of 1,1,3,3-tetramethylurea (24). The HDL apoproteins, solubilized in the tetramethylurea, were then applied to a 7.5% cylindrical preparative urea/polyacrylamide gel electrophoresis gel (1.0 × 8.0 cm) prepared as described by Kane (24). Electrophoresis was performed for 16 h at 2 mA/gel to separate the constituent apoproteins,

which were detected by immersion of the gel for 5 min in a 0.01% acetic acid solution of the magnesium salt of 8-anilino-1-naphthalene-sulfonic acid (25). The proteins were visualized under ultraviolet illumination and apoA-I and A-II were identified by comparison with standards of the purified proteins run in parallel gels. The appropriate bands were excised and their contained apoprotein was electroeluted into dialysis bags (26). In control experiments, the purity of each isolated protein was checked by immunodiffusion against monospecific antisera (27). More than 90% of the radioactivity added as ^{125}I-apoA-I or ^{125}I-apoA-II to an unlabeled HDL apoprotein mixture was recovered in the dialysis bag eluate of the appropriate gel band. The specific activities of the A apoproteins in each timed sample were determined by gamma spectrometry and protein estimation (28). Decay curves were constructed and fractional catabolic rates were obtained by the procedure of Matthews (29). Absolute clearance rates were then calculated as the product of fractional clearance and apoprotein pool size (apoprotein concentration of milligrams per deciliter multiplied by the plasma volume in deciliters) expressed per kilogram of body weight.

After completion of the control phase, bezafibrate was prescribed at a dose of 200 mg three times a day for 1 mo and the study was then repeated during bezafibrate administration.

Study II: VLDL apoB metabolism. Here we examined the effects of bezafibrate on the metabolism of apolipoprotein B in its transit from VLDL through an intermediate fraction to LDL. These three fractions were isolated by a modification of the cumulative flotation ultracentrifugation procedure of Lindgren et al. (30). The gradient employed was that originally described for the separation of LDL subfractions. Centrifugation times were adjusted to permit separation of fractions of Svedberg units of flotation (S_f) 100–400 (VLDL), 12–100, and 0–12 (LDL). The centrifugal force exerted during each of these sequential spins was 10.82×10^6, 92.88×10^6, and 120.6×10^6 *g* min, respectively. Plasma, isolated after a 14-h fast, was adjusted to a density of 1.118 kg/liter by the addition of 341 mg of NaCl to a 2.0-ml aliquot. This was placed over a 0.5-ml cushion of $d = 1.182$ kg/liter NaBr solution in a cellulose nitrate tube for the Beckman SW 40 rotor (Beckman Instruments Inc., Fullerton, CA) and overlayered with a discontinuous gradient from 1.0988 to 1.0588 kg/liter (30). The rotor was subjected to centrifugation at 37,000 rpm for 66 min and allowed to decelerate without braking. The top 1.0-ml fraction, containing VLDL, was removed with a Pasteur pipette and replaced by 1.0 ml of $d = 1.0588$ g/ml solution. Centrifugation was repeated at 27,000 rpm for 17 h 36 min before removal of the intermediate fraction in 0.5 ml and then at 28,000 rpm for 21 h 12 min to isolated LDL in the upper 1.0 ml of the tube. All centrifugations were performed at 23°C. Chylomicrons, if present, were removed in a preliminary centrifugation at 10,000 rpm for 30 min in a Beckman 40.3 rotor (Beckman Instruments Inc.) (23°C). Recoveries of cholesterol were 98% of those obtained in $d < 1.063$ kg/liter lipoproteins prepared by standard procedures (21).

In the control phase of this study measurement was made of plasma lipid and lipoprotein levels (18) and of apolipoprotein B kinetics in the lipoproteins of S_f 100–400, 12–100, and 0–12 of six hypertriglyceridemic subjects (four Type IV and two Type V). To ensure constancy of dietary intake during both phases of the study, each subject was interviewed by a dietitian who prescribed and monitored his intake. Individual diets were formulated to be isocaloric with regular intake and provided 20% of the kilocalories as protein, 40% as carbohydrate, and 40% as fat. This regimen was followed for at least 1 wk before each phase of the study and throughout the turnover periods.

Blood was collected after a 14-h fast and the S_f 100–400 lipoprotein was isolated from 24 ml of plasma. The resultant 12-ml fraction (containing ~0.05–5.0 mg protein) was concentrated if necessary by pressure filtration (XM 100 filters; Amicon Corp.) to a protein content of 1 mg/ml. It was then labeled as described previously (31). Labeling efficiency was 5–10%, so an ICl/protein molar ratio of 20/1 (assuming an average protein molecular weight in VLDL of 300,000) resulted in the incorporation of ~1 mol of iodine per mole protein. Analysis of the labeled tracer showed that 20–30% of the radioactivity was extractable into 2:1 (vol/vol) chloroform methanol. Of the remainder, 40% was present in tetramethylurea precipitable protein. After labeling was done, the VLDL was dialyzed overnight against 0.15 M NaCl/0.01% Na_2EDTA/0.01 M Tris (pH 7.0), sterilized by membrane filtration (0.45 μm filters; Amicon Corp.), and injected at a dose of 20–40 μCi into the bloodstream of the donor. All injections were made at 8:00 AM and the elapsed time between collection of the plasma for sample preparation and administration of the tracer was 24 h. Plasma samples were collected frequently over the first 72 h and then daily for 14 d. On the day of injection, the subject received at regular intervals 10 small meals of equal kilocalorie content which included his daily allowance of carbohydrate and protein, but <6 g of fat (i.e., he was hypocaloric for this day). This maneuver maintained the plasma VLDL apoB concentration at a value that did not show any tendency either to rise or to fall and whose coefficient of variation was <15%.

The three lipoprotein fractions were prepared from each timed blood sample and the specific activity of their contained apoB was determined by a modification (31) of the method of Kane et al. (32). When performed at 37°C, this precipitation procedure sediments protein with the amino acid composition and sodium dodecyl sulfate gel electrophoretic behavior of apoB (31, 32); specifically, little apolipoprotein E appears in the precipitate. In subjects (like DH) whose plasma VLDL concentration was low, an approximately equivalent amount of unlabeled carrier VLDL was added to the isolated VLDL fraction before tetramethylurea precipitation. The patients' VLDL apoB concentration was then determined by difference (i.e., total protein − carrier). The pool size of apoB in the flotation ranges 100–400, 12–100, and 0–12 was calculated as the mean tetramethylurea-precipitable protein recovered in each fraction obtained from all fasting blood samples. The total apoB radioactivity in each fraction was thus determined at each time point and expressed as a fraction of the apoB activity present in the 10-min blood sample. Throughout the turnover period, variation in the plasma apoB concentration in the S_f 100–400, 12–100, and 0–12 fractions did not exceed 17, 15, and 12% of the mean value, suggesting that steady state conditions persisted. Plasma volume was taken to be 4% of the subjects' body weight. This gave values within 10% of the estimate obtained from the isotope dilution that occurred over the first 10 min after tracer injection.

ApoB kinetic analysis. The data obtained as described above were analyzed by a computer based multicompartmental modeling program similar to Simulated Analysis and Modeling (SAAM) (33) and NONLIN (34). The algorithm employed in this study was built around subroutines[2]

2. Three subroutines were employed in the simulation and parameter estimation program. The first was D02BBF, which integrates a system of first-order ordinary differential equations over a range with suitable initial conditions by use of a Runge-Kutta-Merson method. Given a set of parameters, this generated a solution based on the mathematical model provided by the user in the form of a series of differential equations. The second subroutine, E04JAF was an easy-to-use quasi-Newton algorithm for finding the minimum value of a function. This adjusted the parameters iteratively until a minimum weighted sum of

commercially available from the Numerical Algorithms Group (Cambridge, England). The routines were incorporated into a simple Fortran program which permitted data input, model specification, and output of the final parameter values with their associated errors. The total apoB radioactivity present in the three flotation intervals was calculated by multiplying the measured specific activity and the appropriate plasma apoB pool size.

The model shown in Fig. 1 represents the simplest mamillary system that provided acceptable (i.e., unique and consistent [35]) solutions for the parameters. When this system was used, the first order rate constants describing S_f 100–400, 12–100, and 0–12 apoB kinetics were obtained and these, together with the apoprotein pool sizes, permitted calculation (where appropriate) of steady state transfer rates of apoB between compartments. Overall fractional clearance rates for S_f 100–400 and 12–100 apoB were calculated from the relationships shown in Fig. 1. The analyses for Study I were repeated after 4 wk of bezafibrate therapy.

Statistical analyses. Data were compared by the pair difference *t* test or by the Wilcoxon rank sign test.

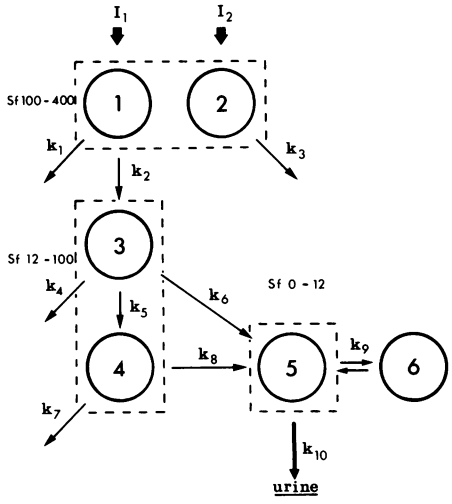

Figure 1. Metabolic model used in apoB kinetic analysis.

Results

All subjects showed a hypotriglyceridemic response to bezafibrate therapy (Table I). The reduction in the two Type V subjects was 53 and 80% (PK and VS, respectively), and the mean decrement in the nine others was 58% ($P < 0.01$). Overall, plasma cholesterol did not change significantly, but its distribution among the major lipoprotein fractions was altered. The level of the lipid in VLDL fell consistently (50%; $P < 0.001$) in proportion to the reduction in plasma triglyceride. The change in LDL cholesterol, on the other hand, was variable. In those subjects (e.g., AK, PK, VS) in whom it was initially subnormal and whose triglyceride levels were grossly elevated (i.e., >500 mg/dl), it rose. The other subjects, whose plasma triglyceride was moderately elevated and whose LDL cholesterol was within our reference range, failed to show any significant change. This is a characteristic response of hypertriglyceridemic individuals to clofibrate analogue therapy. The cholesterol content of the plasma HDL fraction rose by 13% ($P < 0.02$), but there was no obvious correlation by simple regression analysis between this change in any individual and his hypotriglyceridemic response.

Study I: effects of bezafibrate on HDL metabolism. In the group of eleven subjects, HDL cholesterol rose significantly ($P < 0.02$; Table I) as a result of bezafibrate therapy. However, there was no change in plasma apoA-I and A-II concentrations (Table II) in the subjects examined in Study I. Determination of the HDL subfraction distribution in this group revealed that HDL_2 ($1.063 < d < 1.21$ kg/liter), which was typically low (36), rose in response to therapy ($P < 0.05$; Table II). HDL_3 ($1.125 < d < 1.21$ kg/liter), the major subfraction, showed a similar tendency but here the increment was not statistically significant. The mean compositions of the individual HDL subfractions were unaffected by treatment (Table III). The effects of the drug on HDL apoA-I and A-II kinetics are shown in Table IV. The drug did not perturb either synthesis or catabolism of these apoproteins within the limitations of the experimental procedure which was used.

Study II: effects of bezafibrate on apoB metabolism. The action of bezafibrate seems to be directed against the metabolism of triglyceride-rich particles as evidenced by the substantial reduction of plasma triglyceride that followed treatment. We therefore studied the details of this effect by examining the transit of apoB through the S_f 100–400, 12–100, and 0–12 metabolic cascade. We chose to label VLDL (S_f 100–400) prepared by the cumulative flotation procedure of Lindgren et al. (30) rather than the commonly selected $d < 1.006$ kg/liter fraction since the latter includes particles of intermediate density and with different metabolic characteristics (37). By our separation procedure, they were included in the fraction of S_f 12–100.

Development of the kinetic model. Typical delay curves describing the conversion of S_f 100–400 to 12–100 apoB in a normal, a Type IV, and a Type V subject are shown in Fig. 2. In the normal subject, S_f 100–400 apoB was cleared monoexponentially and appeared virtually quantitatively in the S_f 12–100 flotation range (i.e., k_1 was 0.006 h^{-1} and k_2 was 0.68 h^{-1}; Fig. 1). Thereafter, the decay of radioactivity from the latter was clearly biexponential. A similar pattern was noted in the Type IV subject although his fractional VLDL apoB clearance was slower ($k_1 = 0.0$ h^{-1}; $k_2 = 0.13$ h^{-1}). Again, all VLDL apoB reached the intermediate density particle in this patient. In contrast to the above, the Type V subject cleared

squares was achieved. The radioactivity data for VLDL, IDL, and LDL apoB were weighted in the least squares fitting procedure according to their estimated errors (33). This allowed calculation of the variance of the parameters, which were finally obtained (33, 34) using the third subroutine, F01ADF, a matrix inversion procedure. A comparison was made between the parameters and their variances calculated for a set of VLDL apoB → LDL apoB decay curves analyzed by SAAM (31) and by the present algorithm. The parameter estimates were within 10% of each other and the variances, were within 20%.

Table I. Effects of Bezafibrate on the Plasma Lipids and Lipoproteins of Hypertriglyceridemic Subjects

Subject (sex)	Hyperlipoproteinemic phenotype	Age	Height	Body weight		Plasma triglyceride		Plasma cholesterol		VLDL cholesterol		LDL cholesterol		HDL cholesterol	
				Control	Drug	Control	Drug	Control	Drug	Control	Drug	Control	Drug	Control	Drug
				$n = 14$		$n = 5$		$n = 5$		$n = 5$		$n = 5$		$n = 5$	
		yr	*cm*	*kg*	*kg*	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*
Study I (HDL)															
RB (M)	IV	29	183	80.8±0.5*	79.2±0.7	381±94	153±33	250±17	204±3	59±16	19±6	142±9	129±18	48.4±5.8	55.3±14.3
MC (M)	IV	52	168	65.3±0.4	67.0±0.4	463±108	195±35	263±16	279±14	81±17	39±7	143±19	190±15	42.6±4.3	52.6±3.9
JF (M)	III	57	186	84.7±0.6	85.0±0.7	295±18	174±34	261±14	205±12	107±7	70±12	110±2	82±2	43.7±1.2	54.2±2.9
AK (M)	IV	61	180	78.8±0.1	81.9±0.2	577±118	350±64	225±11	236±15	97±24	66±11	85±22	139±9	38.7±8.3	34.8±5.5
JMcD (M)	IV	58	177	61.2±0.1	65.3±0.3	290±121	141±24	236±23	225±8	39±18	23±10	136±6	143±22	61.9±3.5	61.1±1.0
Study II (VLDL apoB)															
DH (F)	IV	57	162	57.2±0.4	57.3±0.67	485±74	305±15	311±12	241±17	110±22	65±2	159±12	127±13	42.2±2.0	50.0±3.1
JR (M)	IV	52	183	78.9±0.5	78.6±0.6	1,215±203	251±34	330±10	281±16	171±39	50±10	118±27	182±19	36.4±1.9	48.0±2.7
RS (M)	IV	64	176	68.8±1.0	66.2±0.8	756±194	328±21	270±23	298±13	120±39	61±5	113±44	144±9	37.2±2.3	51.5±4.3
IS (M)	IV	46	179	70.0±0.4	70.1±0.4	470±75	161±30	201±12	178±12	58±5	30±7	120±23	109±9	38.3±3.5	39.1±2.7
PK (M)	V	48	181	81.4±0.8	81.6±0.7	2,091±533	1,099±211	393±15	417±11	294±13	201±16	72±2	197±2	28.3±1.2	19.0±4.6
VS (M)	V	56	185	87.6±0.6	87.6±0.6	4,682±1,372	412±34	669±124	231±13	619±128	75±27	34±7	98±9	25.5±3.9	48.4±1.6
Mean±SD						548±272	229±77	261±38	239±38	94±38	47±19	125±21	138±31	43.3±3.5	49.6±8.1
Pair difference *t* test vs. control						$P < 0.01$		NS‡		$P < 0.001$		NS		$P < 0.02$	

* Mean±1 SD. ‡ Not significant.

Table II. Study I: Effects of Bezafibrate on Plasma HDL Apoprotein and Subfraction Concentrations

	Plasma apoA-I		Plasma apoA-II		Plasma HDL_2		Plasma HDL_3	
	Control	Drug	Control	Drug				
Subject	$n = 5$		$n = 5$		Control	Drug	Control	Drug
	mg/dl	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*
RB	149±10*	141±9	39±4	36±2	14.7	20.2	277	367
MC	141±4	137±10	49±3	36±3	8.0	19.5	309	355
JF	132±14	131±8	40±4	39±3	16.9	20.1	259	383
AK	132±4	142±11	34±3	35±5	15.0	26.9	352	355
JMcD	127±11	135±11	45±2	48±2	14.5	15.3	416	396
Mean±1 SD	136±8	137±4	43±4	39±5	13.8±3.0	20.4±3.7	323±56	371±16
Pair difference *t* test vs. control	NS‡		NS		$P < 0.05$		NS	

* Mean±1 SD. ‡ Not significant.

VLDL apoB biexponentially from his plasma, although, once more, the transfer of VLDL apoB from pool 1 into S_f 12–100 particles was complete ($k_1 = 0.007\ h^{-1}$; $k_2 = 0.10\ h^{-1}$). These features of VLDL apoB metabolism led to the formulation of the model shown in Fig. 1. Two VLDL apoB pools were required to explain the biexponential nature of the decay curve of this protein in grossly hypertriglyceridemic subjects, since, as discussed in previous publications (31, 38, 39), VLDL appears to be constrained as a result of its size to the plasma compartment. Pool 1 contains the bulk of S_f 100–400 VLDL apoB in most subjects. Pool 2 constitutes a minor VLDL component in the Type IV group but acquired substantial proportions in the two Type V's (Tables V and VI). It represents a slowly cleared apoB pool in this density range and may be attributable to the presence of chylomicron remnants whose metabolic fate differs from that of VLDL. The fitted model did not require this compartment to feed lipoproteins of a higher density, nor did it interconnect with pool 1. Each of these VLDL pools therefore had independent inputs. In the kinetic analysis it was necessary to distribute the mass of VLDL apoB between them. We did this, on the assumption that the B protein in each pool was labeled to the same specific activity, by curve peeling the biexponential VLDL apoB decay and extrapolating to zero time. The derived intercepts then gave the distribution between the pools. Radioactive apoB in compartment 1 was either lost directly from the plasma or transferred into compartment 3. The model (Fig. 1) restricts intermediate lipoproteins to the plasma compartment, in accordance with the proposals of others (38, 39). The biexponential decay of apoB radioactivity in the S_f 12–100 flotation interval is therefore modeled as two pools (pools 3 and 4). Although other permutations are possible, the simplest arrangement involves the direct delipidation cascade shown in the model. This scheme resembles the chain delipidation system used by others to describe the catabolism of triglyceride-rich lipoproteins (38, 39, 40). The rate constants k_1, k_4, and k_7 therefore delineate potential sites of output along the length of the cascade. It is possible to calculate a

Table III. Study I: Effects of Bezafibrate on HDL Subfraction Compositions (Grams/100 Grams)

	Free cholesterol		Cholesteryl esters		Triglyceride		Phospholipid		Protein	
Lipoprotein	Control	Drug	Control	Drug	Control	Drug	Control	Drug	Control	Drug
HDL_2 ($n = 5$)	4.9±2.8*	3.6±0.8	16.4±5.5	15.8±6.0	6.2±2.6	5.1±3.1	28.7±5.6	29.0±5.1	43.7±4.8	45.3±3.4
HDL_3 ($n = 5$)	1.8±0.4	2.1±0.7	12.1±2.4	12.7±3.1	2.9±2.2	3.6±3.4	29.4±4.4	26.0±3.0	53.7±3.6	54.7±3.4

* Mean±1 SD.

Table IV. Study I: Effects of Bezafibrate on HDL Apoprotein Metabolism

	apoA-I kinetics				apoA-II kinetics			
	Fractional catabolic rate		Absolute catabolic rate*		Fractional catabolic rate		Absolute catabolic rate	
Subject	Control	Drug	Control	Drug	Control	Drug	Control	Drug
	pools/d	*pools/d*	*mg/kg per d*	*mg/kg per d*	*pools/d*	*pools/d*	*mg/kg per d*	*mg/kg per d*
RB	0.375	0.424	17.8	19.6	0.291	0.200	3.62	2.38
MC	0.264	0.308	15.9	15.9	0.150	0.269	3.14	3.65
JF	0.268	0.242	10.9	10.3	0.155	0.229	1.91	2.91
AK	0.280	0.264	11.1	11.2	0.284	0.247	2.90	2.58
JMcD	0.255	0.209	10.9	11.5	0.221	0.243	3.35	4.68
Mean±1 SD	0.288±0.044	0.289±0.075	13.3±2.9	13.7±3.5	0.22±0.060	0.238±0.022	2.98±0.59	3.24±0.84
Pair difference *t* test vs. control	NS‡		NS		NS		NS	

* The product of calculated fractional catabolic rate and apoprotein pool size divided by body weight (in kilograms). ‡ Not significant.

fractional catabolic and flux rate for the initially labeled pool of S_f 100–400 VLDL apoB. However, determination of the true fractional and absolute catabolic rate of S_f 12–100 intermediate lipoproteins would require that the radioactive apoB transferred from S_f 100–400 to the 12–100 flotation range achieved chemical equilibrium (40) with all lipoproteins present in this range. Studies by Packard et al. indicate that this is not the case and that these lipoproteins derived from S_f 100–400 VLDL have a decay rate distinct from that observed when the whole S_f 12–100 fraction is labeled (37). Therefore, it is possible to calculate a fractional clearance for the S_f 12–100 remnants of large triglyceride-rich particles but not flux rates for the total mass of apoB within this flotation range. The fall in S_f 12–100 apoB radioactivity over the first 50 h was associated with a rise in the activity of LDL apoB, although this transit (Fig. 3) from intermediate to S_f 0–12 lipoproteins was incomplete, most apoB leaving the S_f 12–100 range directly. There was no requirement in the formulation of the computer model for S_f 100–400 apoB to be transferred into LDL. LDL apoB kinetics were described conventionally as a two pool system, the vascular compartment (pool 5) exchanging with an extravascular one. Capillary transfer rates between pools 5 and 6 were limited to maintain 72% of LDL apoB radioactivity in the plasma compartment in accordance with values obtained from studies of LDL kinetics (41, 42). The fractional catabolic rate of LDL apoB is represented by k_{10} in Fig. 1.

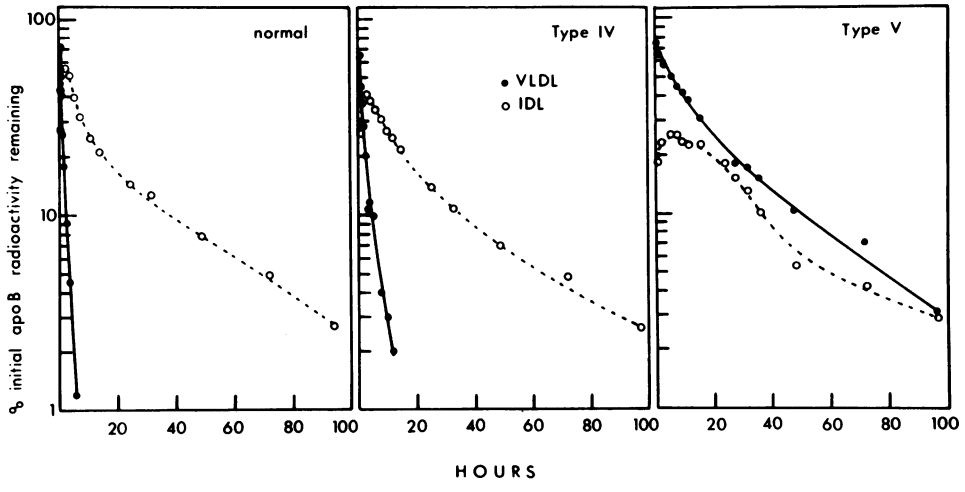

Figure 2. Transit of apoB from VLDL (S_f 100–400) to intermediate lipoproteins (S_f 12–60) in a normal, a Type IV (IS) and a Type V subject (VS). Plasma lipid and lipoprotein values for the normal subject were: total cholesterol, 215 mg/dl; total triglyceride, 168 mg/dl; VLDL cholesterol 27 mg/dl; LDL cholesterol, 143 mg/dl; and HDL cholesterol, 83 mg/dl. JR was the Type IV subject and VS the Type V.

Table V. Kinetic Parameters of apoB Metabolism: Effects of Bezafibrate Therapy

Subject	Synthesis rates			Rate constants								
	I_1	I_2	Total	k_1	k_2	k_3	k_4	k_5	k_6	k_7	k_8	k_{10}
	mg/kg per d	*mg/kg per d*	*mg/kg per d*	h^{-1}	h^{-1}	h^{-1}	h^{-1}	h^{-1}	h^{-1}	h^{-1}	h^{-1}	h^{-1}
DH*	3.9	0.0	19.0	0.0	0.31	0.0	0.046	0.035	0.019	0.022	0.0	0.0142
DH‡	5.5	0.0	23.5	0.0	2.87	0.0	0.055	0.012	0.006	0.003	0.014	0.0129
JR*	8.9	0.03	16.3	0.0	0.13	0.012	0.11	0.075	0.0	0.012	0.011	0.0152
JR‡	11.3	0.03	19.0	0.007	0.50	0.013	0.12	0.07	0.004	0.0067	0.011	0.0121
RS*	16.3	0.0	31.8	0.43	0.46	0.0	0.17	0.15	0.0	0.01	0.016	0.0177
RS‡	9.7	0.0	25.7	0.076	0.93	0.0	0.11	0.06	0.024	0.003	0.015	0.0158
IS*	13.2	0.0	29.3	0.043	0.26	0.0	0.33	0.10	0.05	0.008	0.02	0.0167
IS‡	10.3	0.0	15.1	0.0	0.92	0.0	0.064	0.036	0.0	0.0	0.023	0.0164
PK*	10.1	0.4	23.3	0.039	0.011	0.05	0.079	0.17	0.047	0.022	0.0	0.014
PK‡	9.2	0.1	13.0	0.0	0.10	0.015	0.062	0.20	0.0	0.006	0.007	0.0071
VS*	13.1	3.6	19.5	0.007	0.10	0.027	0.041	0.01	0.026	0.008	0.004	0.041
VS‡	11.5	0.0	17.2	0.13	0.21	0.0	0.0	0.05	0.0	0.03	0.02	0.0222

The rate constants are those defined in Fig. 1. * Control phase. ‡ Bezafibrate treatment phase.

In order to assess the reproducibility of the above procedures we have examined the process of apoB transfer between VLDL, intermediate particles, and LDL in three healthy male subjects with similar plasma lipoprotein profiles. Their mean (∓1 SD) lipoprotein lipid values were: plasma triglyceride, 180±13 mg/dl; plasma cholesterol, 218±5 mg/dl; VLDL cholesterol, 30±3.0 mg/dl; and LDL cholesterol, 135±7 mg/dl. The apoB fractional clearance parameters that we obtained were: for VLDL, 12.5±2.9 pools/d; for S_f 12–100 VLDL remnants, 1.30±0.26 pools/d; and for LDL, 0.35±0.071

Table VI. Study II: Effects of Bezafibrate on VLDL (S_f 100–400) apoB Kinetics

Subject	VLDL apoB concentration						VLDL apoB fractional catabolic rate		VLDL synthetic rate*	
	Control			Drug						
	Pool 1	Pool 2	Total	Pool 1	Pool 2	Total	Control	Drug	Control	Drug
	mg/dl	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*	*pools/d*	*pools/d*	*mg/kg per d*	*mg/kg per d*
DH	1.3	0.0	1.3	0.2	0	0.2‡	7.5	68.9	3.9	5.5
JR	6.6	0.3	6.9	2.3	0.3	2.6	3.2	11.5	8.9	11.3
RS	1.9	0.0	1.9	1.0	0.0	1.0	21.4	24.2	16.3	9.7
IS	4.6	0.0	4.6	1.2	0.0	1.2	7.2	22.1	13.2	10.5
PK	20.9	0.9	21.8	9.3	0.6	9.9	1.2	2.3	10.5	9.3
VS	12.7	13.7	26.4	3.5	0.0	3.5	1.6	8.2	16.7	11.5
Mean±SD			10.5±10.8			3.1±3.6	7.0±7.5	22.9±24.0	11.6±4.9	9.6±2.2
Wilcoxon rank sign test			$P < 0.05$				$P < 0.05$		NS§	

* The product of calculated fractional catabolic rate and apoprotein pool size divided by body weight (in kilograms). This parameter, the absolute catabolic rate, equals the synthetic rate of the protein under steady state conditions. ‡ The low level of apoB in this fraction precluded its accurate determination. The value quoted is an approximation. § Not significant.

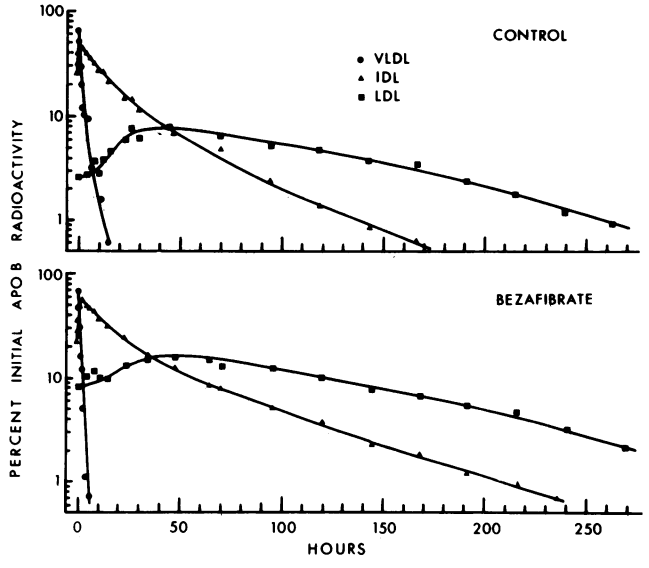

Figure 3. Effects of bezafibrate on apoB metabolism in lipoproteins of S_f 100–400, 12–100, and 0–12 hypertriglyceridemic subject IS. ^{125}I-VLDL (S_f 100–400) was injected intravenously and the transit of radioactivity in apoB was followed into IDL (S_f 12–100) and LDL (S_f 0–12) during control and treatment phases.

pools/d. Finally, the computer derived individual rate constants based on the model shown in Fig. 1 were: k_1, 0.0027±0.0025 h^{-1}; k_2, 0.52±0.12 h^{-1}; k_3, 0 h^{-1}; k_4, 0.144±0.052 h^{-1}; k_5, 0.053±0.021 h^{-1}; k_6, 0.007±0.007 h^{-1}; k_7, 0.005±0.006 h^{-1}; k_8, 0.015±0.007 h^{-1}.

Packard et al. give a more detailed description of the situation in normal subjects (37).

Effects of bezafibrate therapy on apoB metabolism. The above model produced the fractional clearance rates and apoB fluxes shown in Tables V, VI, VII, and VIII. In the control phase, S_f 100–400 VLDL apoB had a fractional clearance rate (7.0 pools/d) substantially slower than the value found in normal individuals (see reference 37). The absolute clearance rate of VLDL apoB in the hypertriglyceridemics was 11.6 mg/kg per d. On the average, one-third of this was removed directly and the remainder appeared in the S_f 12–100 flotation range. Bezafibrate administration lowered the mean plasma S_f 100–400 VLDL apoB concentration to 30% of its initial value primarily by promoting its catabolism (Fig. 4). The mean residence time of the protein fell from 3.4 to 1.0 h, whereas its rate of synthesis remained constant. The biexponential decay of S_f 100–400 VLDL apoB which was seen in the Type V subjects in the control phase could be resolved to virtually a single exponential during bezafibrate therapy (see the profile for Type V subject VS in Fig. 4).

In contrast to the S_f 100–400 VLDL apoB pool size which reflected mainly plasma triglyceride levels, the apoB concentration in the intermediate S_f 12–100 fraction varied less from subject to subject. In the control situation the mean residence

Table VII. Study II: Effects of Bezafibrate on S_f 12–100 Apolipoprotein B Kinetics

	S_f 12–100 apoB concentration		S_f 12–100 apoB fractional catabolic rate	
Subject	Control	Drug	Control	Drug
	mg/dl	*mg/dl*	*pools/d*	*pools/d*
DH	30.9	49.0	0.92	1.01
JR	31.5	32.3	1.01	0.86
RS	28.0	45.3	1.12	1.06
IS	21.0	24.2	2.45	1.57
PK	40.0	52.3	0.82	0.40
VS	28.5	30.2	1.07	0.94
Mean±1 SD	30.0±6.2	38.9±11.5	1.23±0.55	0.98±0.38
Pair difference *t* test vs. control	$P < 0.05$		NS*	

* Not significant.

time of S_f 12–100 lipoproteins derived from S_f 100–400 VLDL was 19.5 h. Drug treatment did not change the fractional clearance rate of these remnant lipoproteins (Table VII). Overall, there was a 30% increase in the plasma S_f 12–100 apoB pool size ($P < 0.05$) but for the reasons discussed above this rise could not be clearly attributed to altered synthesis or catabolism. In fact, it would be necessary to label and study this fraction directly to obtain this information. In the present group of hypertriglyceridemic subjects, the majority (70%) of apoB in S_f 12–100 remnant particles was cleared directly from the plasma without appearing in LDL. Bezafibrate administration did not change this situation. The LDL pool in the plasma tended to rise during therapy, particularly in those individuals whose plasma LDL apoB concentration was initially very low. They responded with a substantial reduction in fractional LDL apoB clearance which, for the group as a whole, fell significantly (by 27%; $P < 0.05$) during bezafibrate treatment. Since, unlike apoB in S_f 12–100 lipoproteins, the clearance of B protein from LDL does not appear to vary according to its source (i.e., whether derived originally from the S_f 100–400 or 12–100 flotation range) the fractional catabolic rates quoted in Table VIII are representative of the whole LDL pool and can therefore be used, with the plasma LDL apoB concentration, to calculate the synthetic rate of this protein, which did not change (Table VIII) during bezafibrate therapy.

Discussion

Bezafibrate effectively lowers plasma triglyceride. In our group of subjects the concentration of this lipid fell 58%, similar to the reductions achieved in other studies (9, 14). Since apoB remains an integral component of triglyceride-rich particles throughout their lifetime in the plasma, it constitutes a tracer of their metabolic fate. This report describes the metabolism

Table VIII. Study II: Effects of Bezafibrate on LDL (S_f 0–12) apoB Kinetics

Subject	LDL apoB concentration		LDL apoB fractional catabolic rate		LDL apoB absolute catabolic rate	
	Control	Drug	Control	Drug	Control	Drug
	mg/dl	*mg/dl*	*pools/d*	*pools/d*	*mg/kg per d*	*mg/kg per d*
DH	72	64	0.34	0.31	9.8	7.9
JR	43	90	0.37	0.29	6.2	10.5
RS	89	86	0.43	0.38	15.1	13.0
IS	76	54	0.40	0.39	12.2	8.5
PK	30	109	0.34	0.17	4.0	7.4
VS	19	54	0.97	0.53	7.4	11.5
Mean±1 SD	55±28	76±22	0.47±0.25	0.35±0.12	9.1±4.1	9.8±2.2
Wilcoxon rank sign test	NS*		$P < 0.05$		NS	

* Not significant.

of apoB in VLDL particles of S_f 100–400 and delineates its transfer to the intermediate delipidation products (S_f 12–100) and LDL (S_f 0–12). Our choice of lipoprotein density cuts was based on the experience of others (43, 44) who concluded that S_f 20–100 VLDL has a metabolic behavior more akin to that of an intermediate density lipoprotein than to that of a triglyceride-rich particle. Indeed, after heparin-induced in vivo lipolysis, VLDL of S_f 100–400 rapidly disappears from the plasma while S_f 12–100 particles accumulate and subsequently are catabolized more slowly (43). This finding and the kinetic studies of Reardon et al. (44, 45) indicate that the intermediate density particles are under metabolic control separate from that of large triglyceride-rich VLDL, justifying the selection of the three spectral densities that we chose.

The triglyceride lowering action of the drug was associated with a threefold reduction in the mean residence time of S_f 100–400 VLDL apoB in the circulation (Table VI; Fig. 4). Overall, synthesis of the protein was not affected. Therefore, the fall in the VLDL apoB pool size derived from an improvement in the efficiency of the delipidation pathway. Examination of the VLDL apoB clearance curves in Type V subject VS before and during drug treatment (Fig. 4) suggested that, in addition to accelerating the transit of VLDL down the delipidation cascade, the drug also influenced the distribution of particles in the S_f 100–400 range. Initially, substantial amounts of material were found in VLDL apoB pool 2 (Table VI) which led to the biexponential appearance of the VLDL apoB clearance curve (Fig. 4). On the drug, catabolism of VLDL apoB was monoexponential, and the requirement for pool 2 in the computer model was eliminated. In an earlier study we

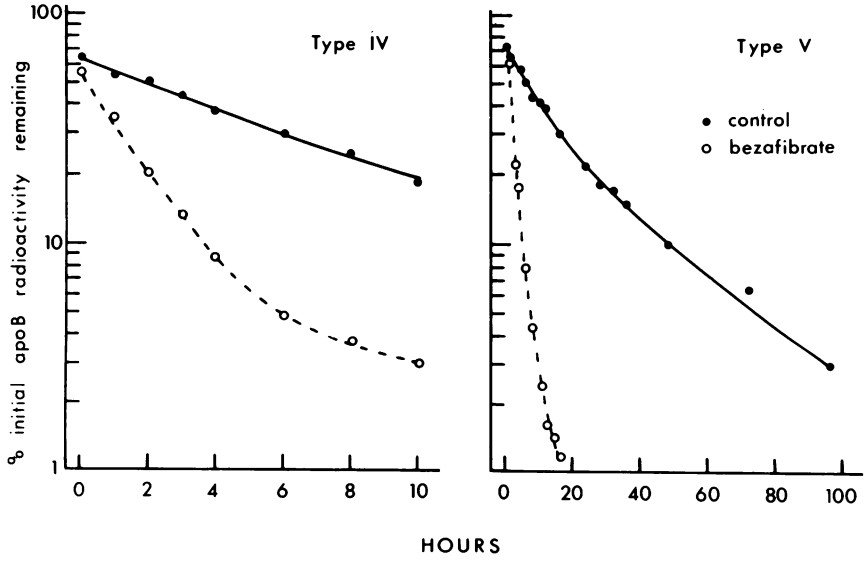

Figure 4. Effects of bezafibrate therapy on VLDL apoB catabolism in Type IV and Type V subjects JR and VS.

investigated the nature of $d < 1.006$ kg/liter VLDL apoB catabolism in Type V hyperlipoproteinemic subjects (31). The initial rapid VLDL apoB decay that we observed was thought to represent true VLDL catabolism for two reasons. First, its disappearance was associated with the rise in LDL apoB radioactivity; and second, it was removed from the plasma at the same rate as simultaneously injected normal or Type IV VLDL. The slowly metabolized species appeared to have no counterpart in normal or Type IV VLDL but was seen in Type III S_f 100–400 VLDL where chylomicron remnants are known to accumulate (see reference 37). We therefore concluded that it represented chylomicrons or their remnants in these subjects. If this is so, one interpretation of the result seen in Fig. 4 is that bezafibrate, by enhancing the efficiency of the normal VLDL delipidation pathway, prevents accumulation of the slowly metabolized chylomicrons.

The decreased residence time of apoB in VLDL may be the result of a number of possible drug-induced changes. For example, it may derive from an alteration in the composition of newly synthesised VLDL. The association between apoB and triglyceride synthesis in VLDL is loose (40). Therefore, since chlorophenoxyisobutyric acid derivative treatment is known to suppress plasma free fatty acid levels (12), it is possible that it may limit the availability of substrate for hepatic triglyceride production, leading to secretion of VLDL with reduced triglyceride content. The time taken for such a particle to be converted to intermediate lipoproteins would therefore be shortened. However, Barter et al. (46) have shown that during treatment with clofibrate there is a reduced dependency on circulating fatty acids as substrate for triglyceride synthesis. The liver, in response to the drug, can increase *de novo* fatty acid production (47), thereby potentially maintaining hepatic triglyceride output. In fact, Sodhi et al. (47) have evidence from triglyceride turnover studies to suggest that, in humans, plasma triglyceride levels fall in response to clofibrate as a result of an increase in catabolism rather than a reduction in synthesis. These data agree with the alternative hypothesis that the chlorophenoxyisobutyric acid derivatives act by an effect on plasma lipoprotein lipase. Vessby et al. (48) have found that bezafibrate increases plasma lipoprotein lipase activity apparently as a result of stimulation of the muscle enzyme rather than its counterpart in adipose tissue (49). In their subjects the rise in skeletal muscle lipase activity correlated well with the improvement they observed in the plasma clearance of an exogenously supplied lipid emulsion. Hence, it may be argued on the basis of these data that the reduction in VLDL apoB residence time in the plasma results from direct stimulation of the catabolic mechanism (i.e., lipoprotein lipase). This idea agrees with the recent finding that the activity of this enzyme is a major determinant of VLDL apoB clearance (50, 51).

The model used to analyze the transit of apoB from S_f 100–400 through S_f 12–100 to 0–12 gave a satisfactory fit to data obtained during both phases of the study and to decays observed for these lipoprotein classes in normals subjects (see reference 37). The results differ significantly from those of previous studies using separations based on fixed-angle ultracentrifugation for reasons discussed in reference 37. Specifically, the clearance rate of intermediate lipoproteins of S_f 12–100 is slower than that seen in earlier investigations and the extent of transit into S_f 0–12 is much less than would be predicted. Our intention was to attempt to separate, metabolically distinct entities by use of the swinging bucket rotor flotation technique. The finding that the observed catabolic rates of S_f 100–400 VLDL and intermediate lipoprotein apoB show little interrelationship suggested that this maneuver may have been successful. Whereas bezafibrate therapy increased threefold the fractional clearance rate of S_f 100–400 VLDL apoB (Table VI), that of remnant lipoproteins in the S_f 12–100 range was unaffected (Table VII). Nor was there any difference between the calculated fractional clearance rates of S_f 12–100 remnants in normal and Type IV subjects (1.30±0.26 and 1.23±0.55 pools/d, respectively; Table VII). S_f 100–400 VLDL apoB clearance as noted above is probably determined by the activity of lipoprotein lipase, but the fractional catabolic rate of apoB in intermediate lipoproteins has been shown to correlate neither with the activity of this enzyme nor with that of hepatic triglyceride lipase (44). Therefore, whereas these enzymes may play a role in the conversion of S_f 12–100 remnants to LDL (54, 55) they seem not to be rate limiting. Preliminary observations from this laboratory (56) suggest that although in normal subjects chemical modification of the arginyl residues on S_f 100–400 lipoproteins does not alter their conversion to S_f 12–100 particles, it does delay subsequent catabolism of the latter to LDL. We interpreted this to mean that the rate determining step in the transformation of intermediate density lipoproteins (IDL) to LDL may involve the operation of a cell membrane receptor like the LDL receptor. This premise is in accordance with the recent finding that familial hypercholesterolemic subjects, whose high affinity LDL receptors are defective or absent, have supranormal plasma intermediate lipoprotein levels (57).

Treatment of hyperlipidemic subjects with bezafibrate or other clofibrate analogues produces a change in LDL which, though variable in its size and magnitude, is predictable on the basis of the subject's initial lipid levels (14). As noted earlier, the response seen in normotriglyceridemic hypercholesterolemic subjects is a fall in LDL. This seems to derive from an increase in receptor-mediated catabolism of the lipoprotein apoprotein (15). However, if the drug is administered to hypertriglyceridemic subjects, whose LDL cholesterol levels are often subnormal, the plasma concentration of this lipoprotein rises. A response of this kind was observed in several of the subjects examined here (Table I). Kinetic analysis showed (Table VIII) that the drug suppressed significantly the fractional clearance rate of LDL for the group as a whole, but the effect was most marked in the grossly hypertriglyceridemic individuals. They, in addition, exhibited a substantial increase in LDL synthesis, so their LDL apoB pool size expanded two- to threefold. Whether this change in LDL fractional clearance is

a primary phenomenon or is secondary to the drug-induced perturbations of VLDL metabolism is not clear. Certainly, it is difficult to envisage how therapy could directly induce contrary responses (i.e., stimulate LDL catabolism in one group while suppressing it in another) in different subjects. It is tempting to speculate that its action on LDL in hypertriglyceridemic individuals is secondary to its effect on VLDL metabolism. Indeed, Sigurdsson et al. (58) proposed that high VLDL levels predisposed to LDL hypercatabolism. By extrapolation, the bezafibrate-induced reduction in plasma VLDL might be expected to reduce the rate of LDL clearance, as we in fact observed.

Epidemiologic (59) and metabolic (60) studies have linked the metabolism of triglyceride-rich particles with that of HDL. Consequently, it is to be expected that a lowering of VLDL levels might perturb HDL. Bezafibrate raised HDL cholesterol (by 13%; $P < 0.02$) in the 11 hypertriglyceridemic subjects that we examined (Table I). We probed the mechanism behind this effect in five of these (Study I). They did not show a rise in the levels of the major HDL apoproteins, A-I and A-II (Table II), nor did the drug affect the turnover of these proteins (Table IV). However, we did observe a small but significant rise in HDL_2 which may have derived from drug induced activation of lipoprotein lipase. During the process of lipolysis, this enzyme apparently releases surface components of VLDL (protein, free cholesterol, and phospholipid) into the HDL density range where they combine with resident particles to produce the larger, less dense HDL_2 (61, 62). However, the increment in HDL_2 was modest relative to the marked fall in VLDL and may have been blunted through the drug's reported stimulatory effect on hepatic lipase (11). According to the proposal of Nikkila et al. (63), this enzyme may function to shuttle the components of HDL_2 back into the HDL_3 density range.

Acknowledgments

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References

1. Oliver, M. F. 1962. Reduction of serum lipid and uric acid levels by an orally active androsterone. *Lancet.* I:1321–1323.

2. Coronary Drug Project. 1975. Clofibrate and niacin in coronary heart disease. *JAMA (J. Am. Med. Assoc.).* 231:360–381.

3. Wolfe, B. M., J. P. Kane, R. J. Havel, and H. P. Brewster. 1973. Mechanism of the hypolipidemic effect of clofibrate in post-absorptive man. *J. Clin. Invest.* 52:2146–2159.

4. Pertsemledis, D., D. Panveliwalla, and E. H. Ahrens. 1974. Effects of clofibrate and of an estrogen-progestin combination on fasting biliary lipids and cholic acid kinetics in man. *Gastroenterology.* 66:565–573.

5. Grundy, S. M., and H. Y. I. Mok. 1977. Colestipol, clofibrate and phytosterols in combined therapy of hyperlipidemia. *J. Lab. Clin. Med.* 89:354–366.

6. The Coronary Drug Project Group. 1977. Gallbladder disease as a side effect of drugs influencing lipid metabolism. *N. Engl. J. Med.* 296:1188–1190.

7. Committee of Principal Investigators. 1978. A cooperative trial in the primary prevention of ischaemic heart disease using clofibrate. *Br. Heart J.* 40:1069–1118.

8. Stork, H., and P. D. Lang. 1975. Some characteristics of a new hypolipidemic agent—BM 15.075. *In* Lipids, Lipoproteins and Drugs. D. Kritchevsky, R. Paoletti, and W. L. Holmes, editors. Plenum Press, New York. 485–489.

9. Olsson, A. G., and P. D. Lang. 1978. One-year study of the effect of bezafibrate on serum lipoprotein concentrations in hyperlipoproteinemia. *Atherosclerosis.* 31:429–433.

10. Schlierf, G., H. Fischer, A. Roche, A. Stiehl, P. Oster, B Schellenberg, and J. Vollmar. 1979. Effect of bezafibrate on bile composition in healthy volunteers and patients with hyperlipoproteinemia. *In* Lipoproteins and Coronary Heart Disease. H. Greten, P. D. Lang, and G. Schettler, editors. Gerhard Witzstrock Publishing House, New York. 172–173.

11. Klose, G., J. Behrendt, J. Vollmar, and H. Greten. 1979. Effect of bezafibrate on the activity of lipoprotein lipase and hepatic triglyceride hydrolase in healthy volunteers. *In* Lipoproteins and Coronary Heart Disease. H. Greten, P. D. Lang and G. Schettler, editors. Gerhard Witzstrock Publishing House, New York. 182–184.

12. Kissebah, A. H., P. W. Adams, P. Harrigan, and V. Wynne. 1974. The mechanism of action of clofibrate and tetranicotinylfructose (Bradilan) on the kinetics of plasma free fatty acid and triglyceride transport in Type IV and Type V hypertriglyceridaemia. *Eur. J. Clin. Invest.* 4:163–174.

13. Berndt, J., R. Gaumert, and J. Still. 1978. Mode of action of the lipid lowering agents clofibrate and BM 15.075 on cholesterol biosynthesis in rat liver. *Atherosclerosis.* 30:147–152.

14. Olsson, A. G., S. Rossner, G. Walldius, L. A. Carlson, and P. D. Lang. 1977. Effects of BM 15.075 on lipoprotein concentrations in different types of hyperlipoproteinemia. *Atherosclerosis.* 27:279–287.

15. Stewart, J. M., C. J. Packard, A. R. Lorimer, D. E. Boag, and J. Shepherd. 1982. Effects of bezafibrate on receptor-mediated and receptor independent low density lipoprotein catabolism in type II hyperlipoproteinemic subjects. *Atherosclerosis.* 44:355–365.

16. Hunninghake, D. B., C. Bell, and L. Olsson. 1981. Effect of colestipol and clofibrate, singly and in combination, on plasma lipid and lipoproteins in type IIb hyperlipoproteinemia. *Metab. Clin. Exp.* 30:610–615.

17. Fredrickson, D. S., R. I. Levy, and R. S. Lees. 1967. Fat transport in lipoproteins—an integrated approach to mechanisms and disorders. *New Engl. J. Med.* 276:34–44, 94–103, 148–156, 215–225, 273–281.

18. Lipid Research Clinics Program, Manual of Laboratory Operations. Vol. 1. 1975. Department of Health, Education, and Welfare Publications, (NIH) 75-268. Government Printing Office, Washington, DC.

19. Shepherd, J., C. J. Packard, J. R. Patsch, A. M. Gotto, and O. D. Taunton. 1978. Metabolism of apolipoproteins AI and AII and its influence on the high density lipoprotein subfraction distribution in males and females. *Eur. J. Clin. Invest.* 8:115–120.

20. Packard, C. J., J. M. Stewart, J. L. H. C. Third, H. G. Morgan,

T. D. V. Lawrie, and J. Shepherd. 1980. Effects of nicotinic acid therapy on high density lipoprotein metabolism in Type II and Type IV hyperlipoproteinaemia. *Biochim. Biophys. Acta.* 618:53–62.

21. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* 34:1345–1353.

22. Bilheimer, D. W., S. Eisenberg, and R. I. Levy. 1972. Metabolism of verylow density lipoproteins. I. Preliminary in vitro and in vivo observations. *Biochim. Biophys. Acta.* 260:212–221.

23. McFarlane, A. S. 1958. Efficient trace-labeling of proteins with iodine. *Nature (Lond.).* 182:53.

24. Kane, J. P. 1973. A rapid electrophoretic technique for identification of subunit species of apoproteins in serum lipoproteins. *Anal. Biochem.* 53:350–364.

25. Nerenberg, S. T., C. Granger, and L. DeMarco. 1971. Rapid fluorescent staining of non-denatured protein bands in agar and polyacrylamide gels. *Anal. Biochem.* 43:564–574.

26. Stevens, R. E. 1975. High resolution preparative SDS polyacrylamide gel electrophoresis: fluorescent visualization and electrophoretic elution—concentration of protein bands. *Anal. Biochem.* 65:369–379.

27. Ouchterlony, O. 1952. Antigen-antibody reactions in gels. Part 6: types of reaction in coordinated systems of diffusion. *Acta Pathol. Microbiol. Scand.* 32:231–247.

28. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265–275.

29. Matthews, C. M. E. 1957. The theory of tracer experiments with ^{131}I labeled plasma proteins. *Phys. Med. Biol.* 2:36–54.

30. Lindgren, F. J., L. C. Jensen, and F. T. Hatch. 1972. The isolation and quantitative analysis of serum lipoproteins. *In* Blood Lipids and Lipoproteins: Quantitation, Composition and Metabolism. G. J. Nelson, editor. John Wiley & Sons, New York. 181–274.

31. Packard, C. J., J. Shepherd, S. Joerns, A. M. Gotto, and O. D. Taunton. 1980. Apolipoprotein B metabolism Type IV and Type V hyperlipoproteinemic subjects. *Metab. Clin. Exp.* 29:213–222.

32. Kane, J. P., T. Sata, R. L. Hamilton, and R. J. Havel. 1975. Apoprotein composition of very low density lipoproteins of human serum. *J. Clin. Invest.* 56:1622–1634.

33. Berman, M., and M. F. Weiss. 1974. SAAM Manual. U. S. Public Health Service Publication 1703. U. S. Government Printing Office, Washington, DC. 1–160.

34. Metzler, C. M., G. L. Elfring and A. J. McEwan. 1974. A package of computer programs for pharmacokinetic modeling. *Biometrics.* 30:569–582.

35. Berman, M. 1963. The formulation and testing of models. *Ann. NY Acad. Sci.* 108:182–194.

36. Shepherd, J., and C. J. Packard 1980. Effect of drugs on high density lipoprotein metabolism. *In* Atherosclerosis V. A. M. Gotto, L. C. Smith, and B. Allen, editors. Springer-Verlag, New York Inc., New York. 591–595.

37. Packard, C. J., A. Munro, A. R. Lorimer, A. M. Gotto, and J. Shepherd. 1984. Metabolism of apolipoprotein B in large triglyceride-rich very low density lipoproteins of normal and hypertriglyceridemic subjects *J. Clin. Invest.* 74:2178–2192.

38. Berman, M., M. Hall, R. I. Levy, S. Eisenberg, D. W. Bilheimer, R. D. Phair, and R. H. Goebel. 1978. Metabolism of apoB and apoC lipoproteins in man: kinetic studies in normal and hyperlipoproteinemic subjects. *J. Lipid Res.* 19:38–56.

39. Eaton, R. P., R. C. Allen, and D. Schade. 1982. B-apolipoprotein secretion in man: investigation by analysis of ^{75}Se-labeled amino acid incorporation into apoprotein. *In* Lipoprotein Kinetics and Modeling. M. Berman, S. M. Grundy, and B. V. Howard, editors. Academic Press, New York. 77–97.

40. Fisher, W. R. 1982. Apoprotein B kinetics in man: concepts and questions. *In* Lipoprotein Kinetics and Modeling. M. Berman, S. M. Grundy, and B. V. Howard, editors. Academic Press, New York. 43–68.

41. Packard, C. J., J. L. H. C. Third, J. Shepherd, A. R. Lorimer, H. G. Morgan, and T. D. V. Lawrie. 1976. Low density lipoprotein metabolism in a family of familial hypercholesterolemic patients. *Metab. Clin. Exp.* 25:995–1006.

42. Shepherd, J., S. Bicker, A. R. Lorimer, and C. J. Packard. 1979. Receptor mediated low density lipoprotein catabolism in man. *J. Lipid Res.* 20:999–1006.

43. Eisenberg, S., D. W. Bilheimer, R. I. Levy, and F. T. Lindgren. 1973. On the metabolic conversion of human very low density lipoprotein to low density lipoprotein. *Biochim. Biophys. Acta.* 326:361–377.

44. Reardon, M. G., and G. Steiner. 1982. The use of kinetics in investigating the metabolism of very low and intermediate density lipoproteins. *In* Lipoprotein Kinetics and Modeling. M. Berman, S. M. Grundy, and B. V. Howard, editors. Academic Press, New York. 99–112.

45. Reardon, M. F., N. H. Fidge, and P. J. Nestel. 1978. Catabolism of very low density lipoprotein B apoprotein in man. *J. Clin. Invest.* 61:850–860.

46. Barter, P. J., P. J. Nestel, and F. C. Kevin. 1972. Precursor of plasma triglyceride fatty acid in humans. Effect of glucose consumption, clofibrate administration and alcoholic fatty liver. *Metab. Clin. Exp.* 21:117–124.

47. Sodhi, H. S., B. J. Kudchodkar, and L. Horlick. 1971. Effects of chlorophenoxyisobutyrate on the metabolism of endogenous glycerides in man. *Metab. Clin. Exp.* 20:309–318.

48. Vessby, B., H. Lithell, K. Hellsing, A.-M. Ostlund-Lindqvist, I.-B. Gustaffson, J. Boberg, and H. Ledermann. 1980. Effects of bezafibrate on serum lipoprotein lipid and apolipoprotein composition, lipoprotein triglyceride removal capacity and the fatty acid composition of the plasma lipid esters. *Atherosclerosis.* 37:257–269.

49. Vessby, B., H. Lithell, and H. Ledermann. 1982. Elevated lipoprotein lipase activity in skeletal muscle tissue during treatment of hypertriglyceridemic patients with bezafibrate. *Atherosclerosis.* 44:113–118.

50. Magill, P., S. N. Rao, N. E. Miller, A. Nicoll, J. Brunzell, J. St. Hilaire, and B. Lewis. 1982. Relationships between the metabolism of high density and very low density lipoproteins in man: studies of apolipoprotein kinetics and adipose tissue lipoprotein lipase activity. *Eur. J. Clin. Invest.* 12:113–120.

51. Taylor, K. G., G. Holdsworth, and D. J. Galton. 1980. Lipoprotein lipase in adipose tissue and plasma triglyceride in patients with primary hypertriglyceridemia. *Eur. J. Clin. Invest.* 10:133–138.

52. Deleted in press.

53. Deleted in press.

54. Nicoll, A., and B. Lewis. 1980. Evaluation of the roles of lipoprotein lipase and hepatic lipase in lipoprotein metabolism. *In vivo* and *in vitro* studies in man. *Eur. J. Clin. Invest.* 10:487–495.

55. Goldberg, I. J., N.-A. Le, J. R. Paterniti, H. N. Ginsberg, F. T. Lindgren, and W. V. Brown. 1982. Lipoprotein metabolism during acute inhibition of hepatic triglyceride lipase in the Cynomolgus monkey. *J. Clin. Invest.* 70:1184–1192.

56. Boag, D. E., D. K. Bedford, C. J. Packard, and J. Shepherd. 1981. 1,2-cyclohexanedione modification of human very low density lipoprotein slows its conversion to low density lipoprotein. *Biochem. Soc. Trans.* 9:211P. (Abstr.)

57. Soutar, A. K., A. B. Myant, G. Thompson, and B. Knight. 1982. The metabolism of very low density and intermediate density lipoproteins in patients with familial hypercholesterolemia. *Atherosclerosis.* 43:217–226.

58. Sigurdsson, G., A. Nicholl, and B. Lewis. 1976. The metabolism of low density lipoprotein in endogenous hypertriglyceridemia. *Eur. J. Clin. Invest.* 6:151–158.

59. Lewis, B., A. Chait, I. D. P. Wootton, C. M. Oakley, D. M. Krikler, A. February, B. Maurer, and J. Birkhead. 1974. Frequency of risk factors for ischemic heart disease in a healthy British population with particular reference to serum lipoprotein levels. *Lancet.* II:141–146.

60. Wilson, D. E., and R. S. Lees. 1972. Metabolic relationships among the plasma lipoproteins. *J. Clin. Invest.* 51:1051–1072.

61. Patsch, J. R., A. M. Gotto, T. Olivecrona, and S. Eisenberg. 1978. Formation of high density lipoprotein$_2$-like particles during lipolysis of very low density lipoproteins *in vitro. Proc. Natl. Acad. Sci. USA.* 75:4519–4523.

62. Behr, S. R., J. R. Patsch, T. Forte, and A. Bensadoun. 1981. Plasma lipoprotein changes resulting from immunologically blocked lipolysis. *J. Lipid Res.* 22:443–451.

63. Nikkila, E. A., T. Kuusi, and M. R. Taskinen. 1982. Role of lipoprotein lipase and hepatic endothelial lipase in the metabolism of high density lipoproteins. *In* Metabolic Risk Factors in Ischemic Cardiovascular Disease. L. A. Carlson and B. Pernow, editors. Raven Press, New York. 205–216.