Effects of bezafibrate on apolipoprotein B metabolism in Type III hyperlipoproteinemic subjects

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Abstract This study was designed to investigate the response of Type III hyperlipoproteinemic subjects to bezafibrate therapy. The metabolism of apolipoprotein B was examined in four lipoprotein subclasses of S_f 60–400 (large very low density lipoprotein (VLDL)), S_f 20–60 (small VLDL), S_f 12–20 (intermediate density lipoprotein (IDL)), and S_f 0–12 (low density lipoprotein (LDL)) before and during bezafibrate therapy. Treatment reduced the plasma concentration of VLDL and raised high density lipoprotein (HDL) cholesterol. There was no net change in LDL cholesterol or its associated apolipoprotein B. The decrease in plasma VLDL derived mainly from an inhibition of synthesis of both large and small subfractions which reduced the number of particles in the circulation without normalizing their lipid composition. Catabolism of the larger VLDL also increased, presumably as a result of lipoprotein lipase activation. Although the plasma concentration of LDL was unchanged, both its synthesis and catabolism were perturbed. Its fractional catabolic rate fell by 50%, but the impact that this would have had on its steady state level in the circulation was apparently blunted by a decrease in its synthesis from S_f 12–20 IDL. In the control phase of the study, most IDL apolipoprotein B was converted to LDL. Bezafibrate therapy channelled this material towards direct catabolism. —**Packard, C. J., R. J. Clegg, M. H. Dominiczak, A. R. Lorimer, and J. Shepherd.** Effects of bezafibrate on apolipoprotein B metabolism in Type III hyperlipoproteinemic subjects. *J. Lipid Res.* 1986. **27:** 930–938.

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Under normal circumstances, apolipoprotein (apo) B-containing chylomicrons and very low density lipoproteins (VLDL), secreted as triglyceride-rich particles by the intestine and liver, enter a metabolic cascade in which they lose their triacylglycerol by lipolysis and at the same time gain cholesteryl ester and additional apolipoprotein E by transfer from high density lipoproteins (HDL) in the circulation (1–3). The resulting remnants appear to have two metabolic fates. Some VLDL and apparently all chylomicrons are removed irreversibly from the plasma by receptor-mediated pathways (4–6). The remainder of the VLDL, possibly that component secreted as particles of small diameter, are transformed (7–9) in or close to the plasma compartment to low density lipoproteins (LDL).

The above metabolic pathway is defective in Type III hyperlipoproteinemic individuals who accumulate remnants of both chylomicrons and VLDL in the bloodstream (10). This defect arises from the presence of an underlying genetic lesion that affects the primary structure of apoE and diminishes its ability to interact with the receptors that bind apoE-containing lipoproteins (11, 12). The frequency of this mutation (13) in the population (approximately 1%) indicates that it alone is not responsible for the clinical expression of the condition which affects only about 1 in 5000 individuals. Consequently, it has been postulated that other environmental or genetic factors may combine with the E protein defect to unmask the disease (10). Whatever these other factors are, they seem particularly sensitive to therapy with chlorophenoxyisobutyric acid (CPIB) derivatives which invariably produce beneficial reductions of plasma lipid levels (10), often to within the normal range. Obviously the therapy itself cannot eradicate the genetic defect and so even in patients successfully treated with such drugs there remains a compositional anomaly in VLDL (14).

This study was designed to examine the influence of the second generation clofibrate analogue, bezafibrate, on the metabolism of apolipoprotein B in Type III hyperlipoproteinemic subjects. We hoped to establish where bezafibrate had its effect on apoB turnover, with the expectation that this knowledge might give some insight into those factors responsible for triggering clinical expression of the disease in individuals homozygous for the apoE defect. In previous publications (9, 15), we followed the

Abbreviations: S_f, negative sedimentation coefficient at d = 1.63 $kg \cdot l^{-1}$ and 26°C; VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; apoB, apolipoprotein B; apoE, apolipoprotein E; CPIB, chlorophenoxyisobutyric acid.

divergent metabolic pathways traced by large and small VLDL by separating this fraction into subclasses using a cumulative flotation ultracentrifugation procedure and measuring the rate of conversion of each of these into intermediate density lipoprotein (IDL, S_f 12–20) and LDL (S_f 0–12). Here we adopt this experimental protocol to examine the influence of bezafibrate on the expression of Type III hyperlipoproteinemia.

METHODS

Subjects

Six Type III hyperlipoproteinemic subjects gave their informed consent to the study. Their lipid and lipoprotein profiles are shown in **Table 1** and demonstrate the characteristic features of the condition, including a VLDL cholesterol/total plasma triglyceride ratio of greater than 0.7 (mol/mol) and the presence of lipoproteins with globulin-like electrophoretic mobility in the VLDL density interval (10). Isoelectric focusing of the VLDL apolipoproteins from all subjects showed that they were homozygous for the defective (E2) isoform of apolipoprotein E (11). None of the subjects had clinical or biochemical evidence of hepatic, renal, or endocrine disease. The study conformed to the requirements of the Ethical Committee of Glasgow Royal Infirmary.

Experimental protocol

Each subject was examined on two occasions. In the control phase of the study, serial measurements were made of plasma lipid and lipoprotein levels (16) and of the kinetics of apoB transport from VLDL to LDL. These analyses were repeated following 8 weeks of bezafibrate therapy (200 mg three times daily). The separation procedure that was employed was a modification of the cumulative flotation ultracentrifugation technique of Lindgren, Jensen, and Hatch (17). Briefly, 2.0 ml of plasma, from which chylomicrons had been removed in a preliminary centrifugation (9), was adjusted to a density of 1.118 $kg \cdot l^{-1}$ by the addition of NaCl. The specimen was then placed over a 0.5-ml cushion of d 1.182 $kg \cdot l^{-1}$ NaBr solution in a Beckman SW40 rotor tube (Beckman Instruments Inc., Palo Alto, CA) and overlayered with a discontinuous salt gradient from 1.0988 to 1.0588 $kg \cdot l^{-1}$ (17). The rotor and its contents were subjected to centrifugation at 39,000 rpm for 98 min (23°C) and allowed to decelerate without braking. Large VLDL (S_f 60–400) in the top 1.0 ml of the tube were removed with a Pasteur pipette and the gradient was reconstituted by careful overlayering of 1.0 ml of d 1.0588 $kg \cdot l^{-1}$ solution. Three further consecutive centrifugations were used to isolate VLDL of S_f 20–60 (18,500 rpm, 15 hr, 41 min), IDL of S_f 12–20 (39,000 rpm, 2 hr, 34 min), and LDL of S_f 0–12 (30,000 rpm, 21 hr, 10 min). The small VLDL and IDL were harvested in 0.5 ml while LDL was recovered in a 1.0-ml aliquot.

The lipoprotein fractions were subsequently treated with 1,1,3,3, tetramethylurea as described elsewhere (18) to permit the determination of apoB specific activity in each fraction; and the mean amount of apoB present in each fraction that had been isolated from fasting blood specimens was used to calculate the plasma concentration of apoB in that density interval (15).

TABLE 1. Effects of bezafibrate on plasma lipids and lipoproteins in Type III hyperlipoproteinemic subjects

Subject	Treatment	Sex	Age	Body Weight	Plasma Triglyceride	Plasma Cholesterol	Cholesterol in VLDL	Cholesterol in LDL	Cholesterol in HDL	VLDL Chol / Plasma TG
			yr	*kg*			*mmol · l⁻¹*			
1	Control	M	35	98.0	4.35 ± 1.64	7.35 ± 1.17	3.90 ± 1.77	2.45 ± 0.15	1.00 ± 0.08	0.90
1	Bezafibrate			96.8	2.11 ± 0.55	5.45 ± 0.29	1.55 ± 0.39	2.83 ± 0.34	1.10 ± 0.09	0.73
2	Control	M	52	77.0	5.14 ± 1.02	9.63 ± 0.49	4.40 ± 0.81	4.05 ± 0.80	1.18 ± 0.05	0.85
2	Bezafibrate			76.4	1.93 ± 0.55	5.58 ± 0.43	1.40 ± 0.35	2.90 ± 0.25	1.28 ± 0.20	0.72
3	Control	M	68	74.0	3.68 ± 0.54	7.60 ± 0.67	3.63 ± 0.62	2.99 ± 0.30	0.99 ± 0.06	1.00
3	Bezafibrate			75.5	1.91 ± 0.65	6.48 ± 0.46	1.66 ± 0.53	3.40 ± 0.79	1.42 ± 0.12	0.87
4	Control	F	47	85.0	8.47 ± 1.11	15.98 ± 0.75	10.66 ± 1.10	3.98 ± 0.83	1.72 ± 0.03	1.25
4	Bezafibrate			87.7	2.09 ± 0.61	6.86 ± 0.14	2.03 ± 0.23	2.60 ± 0.27	2.24 ± 0.18	1.00
5	Control	M	33	71.9	10.38 ± 3.00	11.43 ± 0.66	8.14 ± 1.07	2.25 ± 0.76	0.89 ± 0.08	0.78
5	Bezafibrate			70.0	2.79 ± 0.48	5.42 ± 0.33	1.89 ± 0.19	2.25 ± 0.37	1.28 ± 0.09	0.68
6	Control	M	48	80.5	8.13 ± 0.23	14.80 ± 0.45	8.40 ± 0.07	5.05 ± 0.38	1.05 ± 0.03	1.00
6	Bezafibrate			83.4	1.86 ± 0.14	5.45 ± 0.15	1.25 ± 0.31	2.73 ± 0.37	1.45 ± 0.32	0.67
Mean ± SD	Control				6.69 ± 2.44	11.13 ± 3.32	6.52 ± 2.68	3.46 ± 0.99	1.14 ± 0.27	0.97
	Bezafibrate				2.12 ± 0.32	5.87 ± 0.58	1.63 ± 0.27	2.79 ± 0.35	1.46 ± 0.37	0.77
Paired *t* test	*P*				<0.005	<0.005	<0.005	NS	<0.005	

Study design

Fifty-ml venous blood specimens were collected from each participant in the fasting state in order to prepare VLDL of S_f 60–400 and S_f 20–60 by the procedure outlined above. The larger VLDL was labeled with ^{125}I and the smaller with ^{131}I using a modification (19) of the Macfarlane ICl method (20). The labeled tracers were sterilized by membrane filtration (0.45 μm Amicon filters, Amicon Corp., Bedford, MA) prior to reinjection into the bloodstream of the donor. The total preparation time of the tracers was less than 48 hr, and reversal of the isotopes did not affect the results that were obtained. The tracers were administered at 8:00 AM, and throughout the first day of the study the subjects received a hypocaloric fat-restricted diet which contained their normal intake of carbohydrate and protein. This approach was adopted to minimize chylomicron production. Following injection of the tracers, blood was collected at frequent intervals over the first 72 hr and then daily for 14 days. ApoB was isolated from large and small VLDL, from IDL, and from LDL at each time point in order to trace the metabolic fate of the labeled particles. For 3 days prior to the injection and for 1 month thereafter the subjects were prescribed 60-mg KI tablets three times daily in order to prevent thyroidal sequestration of radioiodide.

ApoB kinetic analysis

The apoB specific activity decay curves obtained as described above were analyzed by a computer-based multicompartmental modeling system as outlined earlier (9, 15). The model that we used (**Fig. 1**) is a subset of a version developed to determine the metabolic behavior of VLDL subfractions (9). In this model, VLDL comprises two compartments (1 and 4, Fig. 1) that allow for the bi-exponential nature of the lipoprotein's decay curve. Compartment 1 contains material that is rapidly catabolized; in compartment 4 it is slowly metabolized. The latter material, which is abundant in the present Type III hyperlipoproteinemic subjects, probably contains VLDL remnants. The passage of radioactivity through the denser lipoprotein classes occurs via two channels. The flux through compartments 1 to 3 is rapid and leads ultimately to LDL synthesis. The second channel (compartments 4, 5, and 6) transports material slowly and makes a much smaller contribution to LDL production. The present report concentrates on the metabolism of the small VLDL (S_f 20–60) and its conversion through IDL to LDL.

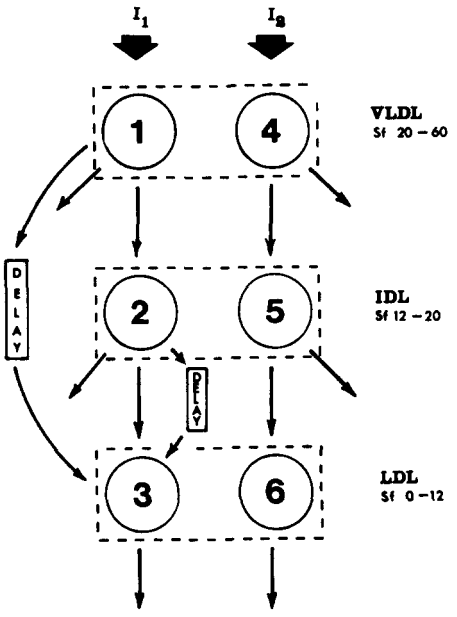

Fig. 1. Multicompartmental model describing the kinetics of small VLDL, IDL, and LDL. The rate constants in Table 4 are of the form $k_{x,y}$, where x is the source component and y is the destination of the metabolic flow. When the material is lost to the system (i.e., directly catabolized), y = 0. The delay loops introduced between compartments 1 and 3 and 2 and 3 are designated compartments 7 and 9, respectively, in Table 4.

RESULTS

Type III hyperlipoproteinemia responded well to pharmacologic intervention with bezafibrate (Table 1). Plasma triglyceride fell substantially (68%, $P < 0.005$) during therapy and this fall was accompanied by a reduction in the cholesterol content of the circulating VLDL fraction. This accounted for most of the decrement in total plasma cholesterol. In fact, LDL cholesterol was not significantly changed overall, and HDL cholesterol rose during therapy (by 28%, $P < 0.005$). It is noteworthy that, contrary to experience with other hyperlipidemic subjects (21–23), the Type III patients with an initially low plasma LDL cholesterol did not show the expected increment in that parameter upon correction of their hypertriglyceridemia. However, those three individuals (subjects 2, 4, and 6) with the highest initial LDL cholesterol values responded by lowering the level of this circulating lipoprotein fraction. Type III hyperlipoproteinemia is characterized by a high VLDL cholesterol/plasma triglyceride ratio (24) which usually exceeds 0.7 mol/mol. Treatment of the condition with bezafibrate reduced the ratio (from a mean of 0.97 in the control phase to 0.77 during therapy) but did not normalize it. So, while the drug corrected plasma triglyceride and cholesterol levels, the compositional abnormality in VLDL persisted (Table 1).

The impact of therapy on VLDL was examined by following the metabolism of large and small VLDL particles. The changes induced in apoB metabolism in large (S_f 60–400) VLDL are presented in **Table 2.** On average, the plasma concentration of apoB in this fraction fell by 81% ($P < 0.005$). This resulted from a combination of inhibited synthesis and accelerated catabolism of the particle. The dieaway curve of large VLDL apoB (not shown) was

TABLE 2. Effects of bezafibrate on VLDL apoB (S_f 60–400) metabolism

Subject	Treatment	VLDL ApoB Plasma Concentration	$k_{1,0}$[a]	$k_{2,0}$[a]	Fractional Catabolic Rate[b]	Synthetic Rate
		$mg \cdot dl^{-1}$		d^{-1}		$mg \cdot kg^{-1} \cdot d^{-1}$
1	Control	12.7	2.4	0.57	1.9	9.8
1	Bezafibrate	4.0	4.2	0.74	3.6	5.7
2	Control	8.8	4.6	0.59	3.7	13.0
2	Bezafibrate	3.1	4.6	0.83	4.7	5.8
3	Control	8.1	2.6	0.47	2.2	7.0
3	Bezafibrate	2.0	7.6	1.07	6.6	5.3
4	Control	21.2	2.4	0.34	1.6	13.7
4	Bezafibrate	2.0	5.9	0.62	5.3	4.2
5	Control	18.5	1.1	0.52	0.98	7.1
5	Bezafibrate	4.2	2.2	0.79	2.1	3.6
6	Control	23.5	2.1	0.37	1.5	14.2
6	Bezafibrate	2.0	5.5	1.06	4.6	3.6
Mean ± SD	Control	15.5 ± 5.9	2.5 ± 1.0	0.48 ± 0.09	2.0 ± 0.9	10.8 ± 3.0
	Bezafibrate	2.9 ± 0.9	5.0 ± 1.7	0.85 ± 0.16	4.5 ± 1.4	4.7 ± 0.9
P (paired t test)		<0.005	<0.01	<0.005	<0.005	<0.005

[a]The parameters $k_{1,0}$ and $k_{2,0}$ refer to the decay constants for two unlinked compartments which together describe the metabolism of S_f 60–400 apolipoprotein B. The decay curves of this fraction were biexponential; $k_{1,0}$ and $k_{2,0}$ refer to the rapid and slow catabolic phases, respectively. The distribution of mass between the two compartments was derived from the intercepts of both exponentials with the zero time axis.

[b]Fractional catabolic rate represents the sum of the fluxes from both compartments in S_f 60–400 VLDL divided by the plasma pool of apolipoprotein B in this fraction.

characteristically biexponential. The rate constants k_1 and k_2 (Table 2) represent, respectively, catabolism of the initial and terminal components of the system. Both increased during treatment with the drug so that the overall fractional clearance rate from this compartment was more than doubled. However, this alone did not account for the dramatic reduction in the pool of large VLDL apoB and calculation of the flux rate of this protein indicated that its synthesis had diminished to about half of the control value.

ApoB metabolism in the S_f 20–60 small VLDL fraction was examined at the same time as that of large VLDL. A typical specific activity decay curve is shown in **Fig. 2** and the derived kinetic parameters are presented in **Table 3** and **Table 4.** The percentage fall in apoB in the small VLDL fraction (50%) was substantially less than that recorded for the larger species. This reduction on the whole came from a diminution of synthesis. Changes in catabolism were variable and not significantly altered.

The transfer of apolipoprotein B from small VLDL through IDL to LDL was examined using a multicompartmental computer model (Fig. 1). This allows for the direct transit of apoB from VLDL→IDL→LDL and also permits conversion of VLDL or IDL to LDL to take place in an extravascular compartment as signified by the delay loops in the model. These features have been described previously in earlier models proposed by ourselves (25) and others (26). In most subjects, the conversion of small VLDL to IDL was substantial during both phases of the study. Treatment with the drug did not change the proportions of apoB that were distributed into the various VLDL catabolic routes.

The input of apoB into IDL could be accounted for in its entirety by transfer from VLDL, and this remained true for most individuals, even during bezafibrate therapy (but c.f. subject 4, Table 3). In addition, overall catabolism of the protein from this fraction was unchanged by the drug. So, bezafibrate appears to have little effect either on the plasma concentration or on the overall fractional catabolism of IDL apoB, at least in these Type III hyperlipoproteinemic individuals. However, the proportion of this protein which reached LDL via its major synthetic pathway (Table 4, $(k_{2,3} + k_{2,9})/(k_{2,0} + k_{2,3} + k_{2,9})$) fell from 76% of the total IDL apoB flux to 26% during therapy ($P < 0.02$). This was balanced by an increase in the proportion of IDL apoB that was catabolized directly.

The diminished transfer of apoB from IDL into LDL helps explain why the plasma LDL pool is hardly affected by bezafibrate despite a 100% drug-induced diminution in its catabolic rate. It should be noted that in some individuals (subjects 4, 5, and 6, Table 3) the flux of B protein from VLDL into LDL did not completely account for the amount of the latter which was synthesized, suggesting that direct input of apoB into LDL was occurring during bezafibrate therapy.

DISCUSSION

Of all the hyperlipidemias, Type III is one of the easiest to treat. The affected individual usually responds well to diet and/or drug therapy so that his lipid levels normalize. This happened in our group of six subjects. However, the composition and plasma concentration of their circulating

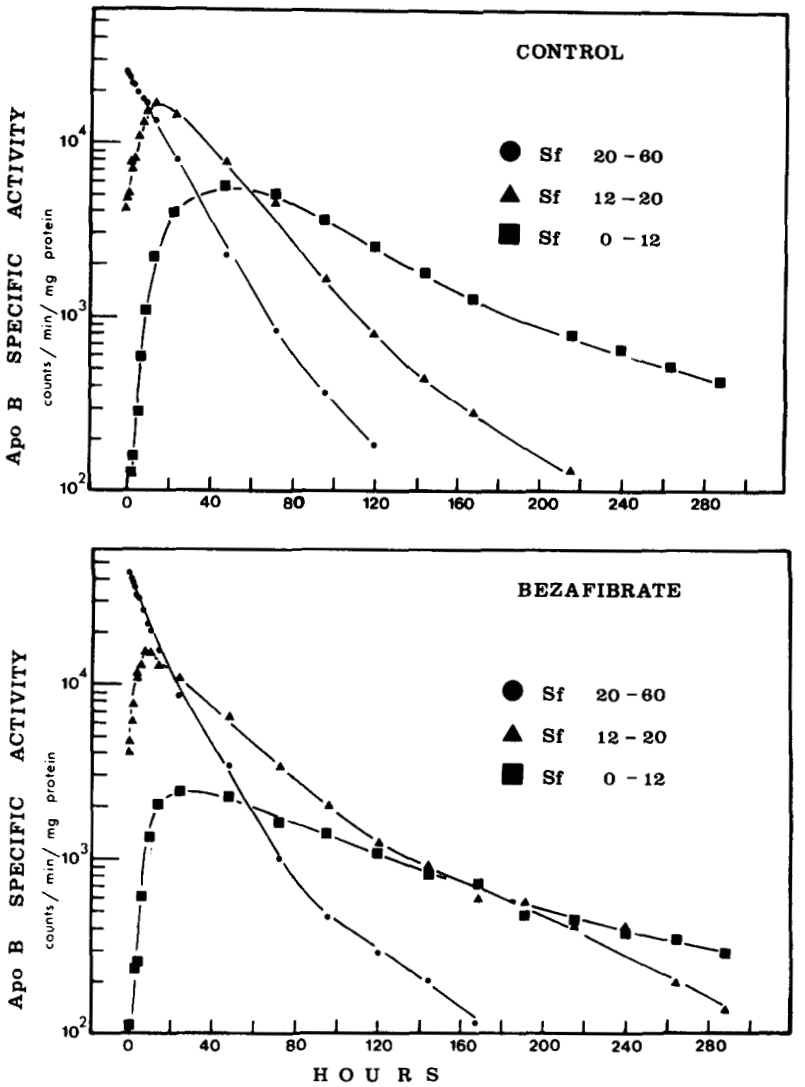

Fig. 2. Apolipoprotein B specific activity decay curves of small VLDL, IDL, and LDL in subject 6. Panel A refers to the control phase and panel B to the bezafibrate treatment.

VLDL was still abnormal. This is the typical response of the Type III patient to most therapies (10) with the apparent exception of estrogen administration which has been reported to normalize VLDL composition (14).

If diet therapy fails, the drug of choice in the treatment of Type III hyperlipoproteinemia is clofibrate or one of its analogs. These agents have a number of systemic effects (27, 28) on lipid and lipoprotein metabolism, including *a*) activation of lipoprotein lipase (29, 30); *b*) suppression of free fatty acid release from adipose tissue (31, 32); *c*) inhibition of hepatic triglyceride synthesis (33); and *d*) suppression of 3-hydroxy-3-methylglutaryl coenzyme A reductase (34).

The first three actions would be expected to influence the level of VLDL in the circulation by a combination of mechanisms. Promotion of lipoprotein lipase accelerates VLDL catabolism as was noted in previous investigations (28, 35), including one from our laboratory (15) that examined the effects of bezafibrate on S_f 100–400 VLDL apoB metabolism in hypertriglyceridemic (Type IV and Type V hyperlipoproteinemic) subjects. There we noted a threefold increase in the fractional catabolic rate of this apolipoprotein. The Type III subjects in the present study also showed a marked increase in this parameter during therapy (Table 2, **Fig. 3**), indicating that in this respect the response to bezafibrate appears to be independent of lipoprotein phenotype. However, we must bear in mind that B protein transit through the S_f 100–400 density interval depends not only on the rate of triglyceride hydrolysis but also on the composition of the particle. It is reasonable to postulate that the drug might cause the liver to synthesize particles with a reduced triglyceride content which have an accelerated transit through the lipolytic cascade. There is at present a paucity of information on the nature of newly secreted lipoproteins and so we still await definitive evidence for the operation of this

TABLE 3. Effect of bezafibrate on apolipoprotein B metabolism in small VLDL, IDL, and LDL

		ApoB in Small VLDL (S_f 20–60)			ApoB in IDL (S_f 12–20)				ApoB in LDL (S_f 0–12)			
Subject	Treatment	Synthetic Rate	Plasma Concentration	Fractional Catabolic Rate	Synthesis from Small VLDL	Total Synthetic Rate	Plasma Concentration	Fractional Catabolic Rate	Synthesis from IDL + Small VLDL	Total Synthetic Rate	Plasma Concentration	Fractional Catabolic Rate
		$mg \cdot kg^{-1} \cdot d^{-1}$	$mg \cdot dl^{-1}$	$pools \cdot d^{-1}$	$mg \cdot kg^{-1} \cdot d^{-1}$		$mg \cdot dl^{-1}$	$pools \cdot d^{-1}$	$mg \cdot kg^{-1} \cdot d^{-1}$		$mg \cdot dl^{-1}$	$pools \cdot d^{-1}$
1	Control	14.7	20.4	1.80	10.0	10.0	9.9	1.80	3.40	3.40	12.3	0.64
1	Bezafibrate	10.5	15.0	1.70	6.4	6.4	10.6	1.30	1.54	1.70	16.0	0.26
2	Control	11.8	26.1	1.10	7.7	7.7	14.4	1.30	7.70	10.6	29.5	0.90
2	Bezafibrate	11.9	14.0	2.1	7.8	7.8	14.2	1.40	1.80	2.0	21.9	0.23
3	Control	14.2	25.8	1.40	6.1	6.1	13.3	1.15	4.90	4.90	17.2	0.72
3	Bezafibrate	10.6	16.7	1.60	6.0	6.0	14.2	1.05	2.90	3.00	12.9	0.58
4	Control	12.9	45.0	0.72	7.8	7.8	18.5	1.06	6.20	7.40	27.8	0.67
4	Bezafibrate	5.8	10.0	1.45	5.3	8.2	17.1	1.20	0.40	1.64	17.1	0.24
5	Control	13.8	30.5	1.13	4.5	4.5	11.4	0.98	4.90	4.90	13.9	0.74
5	Bezafibrate	7.9	17.9	1.10	3.7	3.7	13.1	0.72	0.80	1.30	12.7	0.24
6	Control	15.6	37.0	1.06	9.5	9.5	14.4	1.66	5.70	5.70	25.1	0.57
6	Bezafibrate	11.4	13.8	2.06	8.4	9.5	14.5	1.63	2.90	3.50	21.1	0.42
Mean ± SD	Control	13.8 ± 1.23	30.8 ± 8.1	1.20 ± 0.33	7.6 ± 1.9	7.6 ± 1.9	13.7 ± 2.7	1.31 ± 0.3	5.5 ± 1.3	6.2 ± 2.3	21.0 ± 6.8	0.71 ± 0.10
	Bezafibrate	9.7 ± 2.1	14.6 ± 2.5	1.66 ± 0.35	6.3 ± 1.6	6.9 ± 1.9	14.0 ± 1.9	1.2 ± 0.3	1.7 ± 0.9	2.2 ± 0.8	17.0 ± 3.6	0.33 ± 0.13
P (paired t test)		<0.01	<0.02	NS	NS	NS	NS	NS	<0.005	<0.002	NS	<0.01

TABLE 4. Kinetic rate constants of apolipoprotein B metabolism in Type III subjects before and during bezafibrate therapy

		Parameter (d^{-1})											
Subject	Treatment	$k_{1,0}$	$k_{1,2}$	$k_{1,7}$	$k_{2,0}$	$k_{2,3}$	$k_{3,0}$	$k_{4,0}$	$k_{4,5}$	$k_{5,0}$	$k_{5,6}$	$k_{6,0}$	$k_{2,9}$
1	Control	0.38	1.30	0.22	1.56	0.31	0.67	0.26	0.26	0.14	0.48	0.28	0.00
1	Bezafibrate	0.62	1.30	0.12	1.22	0.05	0.26	0.48	0.24	0.55	0.00	0.00	0.19
2	Control	0.00	1.20	0.00	0.43	0.36	0.96	0.41	0.17	0.24	0.24	0.17	0.60
2	Bezafibrate	0.36	1.92	0.19	1.39	0.07	0.23	0.00	0.67	0.60	0.00	0.00	0.10
3	Control	0.41	0.96	0.24	0.48	0.49	0.72	0.53	0.12	0.48	0.00	0.00	0.24
3	Bezafibrate	0.48	0.96	0.24	0.88	0.15	0.58	0.43	0.17	0.34	0.00	0.00	0.12
4	Control	0.17	0.53	0.00	0.34	0.17	0.67	1.08	0.00	0.00	0.00	0.00	0.55
4	Bezafibrate	0.10	1.44	0.05	1.29	0.00	0.24	0.04	0.40	0.31	0.00	0.00	0.05
5	Control	0.58	0.62	0.00	0.29	0.00	0.84	0.29	0.31	0.43	0.24	0.17	0.72
5	Bezafibrate	0.55	0.53	0.05	0.65	0.05	0.24	0.00	0.00	0.00	0.00	0.00	0.05
6	Control	0.24	0.88	0.00	1.18	0.00	0.67	0.43	0.48	0.24	0.36	0.19	0.72
6	Bezafibrate	0.34	1.68	0.24	1.44	0.12	0.43	0.25	0.29	0.24	0.07	0.14	0.24
Mean ± SD	Control	0.30 ± 0.19	0.92 ± 0.28	0.08 ± 0.10	0.71 ± 0.43	0.22 ± 0.18	0.76 ± 0.11	0.50 ± 0.27	0.22 ± 0.15	0.26 ± 0.16	0.22 ± 0.18	0.14 ± 0.10	0.47 ± 0.26
	Bezafibrate	0.41 ± 0.17	1.31 ± 0.46	0.15 ± 0.08	1.15 ± 0.28	0.07 ± 0.05	0.33 ± 0.13	0.20 ± 0.20	0.30 ± 0.21	0.34 ± 0.20	0.012 ± 0.026	0.02 ± 0.05	0.13 ± 0.07
P (paired t test)		NS	NS	NS	NS	NS	<0.01	NS	NS	NS	NS	NS	NS

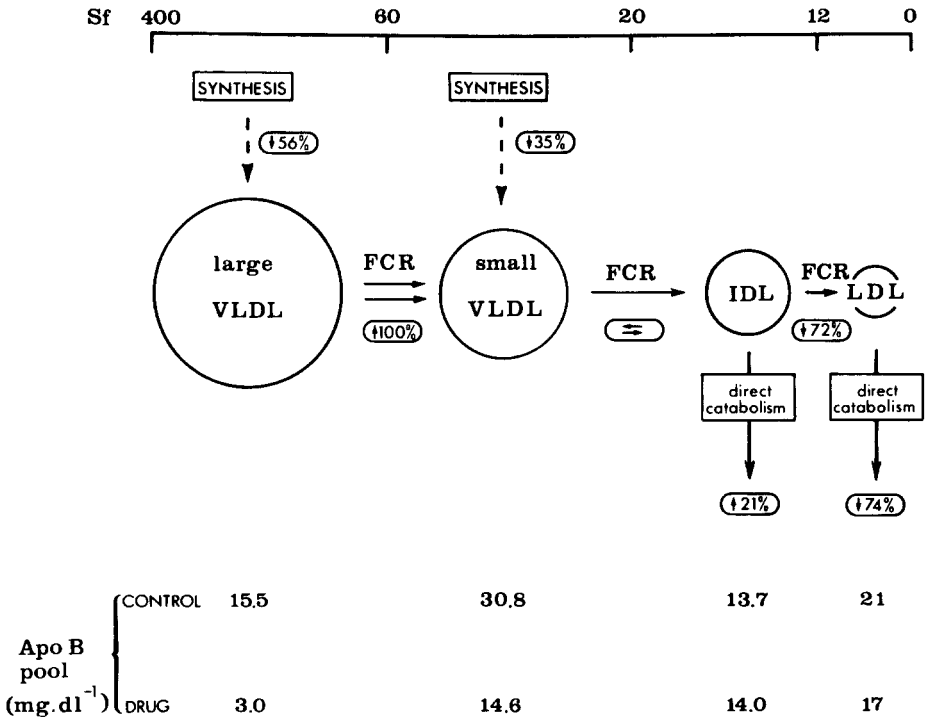

Fig. 3. Schematic representation of the effects of bezafibrate on apolipoprotein B metabolism in Type III hyperlipoproteinemia; FCR, fractional catabolic rate.

additional mechanism in man. Nevertheless, a number of investigators (36, 37) have reported a reduction of triglyceride secretion into the plasma during clofibrate therapy but there are equally strong data against this proposal (38) and at the present time the question is unresolved. On the face of it, the present study and earlier work from our laboratory (15) fail to clarify the issue. The results presented in Table 2 show clearly that there is a uniform reduction in apoB synthesis into the S_f 60–400 range during bezafibrate administration to Type III hyperlipoproteinemic subjects. However, the response of the hypertriglyceridemic patients who were examined previously (15) was variable. Only half showed a clear reduction in S_f 100–400 apoB output and overall there was no significant change. The simplest explanation is that interpatient variability is at the root of these differences. The importance of synthetic changes in the current study is emphasized in Table 3, which shows that the 50% decrement in the B protein content of the small VLDL (S_f 20–60) pool in the plasma derived primarily from reduced production.

It is perhaps surprising that the plasma LDL cholesterol level did not rise in response to therapy as one might have expected on the basis of many earlier studies of the effects of fibrates on hypertriglyceridemic subjects in general. We suspect that Type III hyperlipoproteinemic individuals may be peculiar in this regard and similar data from Falko et al. (39) support such a view. One possible explanation for this apparent lack of effect relates to the method that is commonly used to measure LDL cholesterol (6). The protocol adopted by the Lipid Research Clinics includes in the "LDL" fraction a small component of IDL that can be ignored in most individuals but not in Type III subjects. It is arguable that such patients respond to fibrate therapy with reciprocal changes in IDL and LDL which cancel each other out. However, the cumulative flotation ultracentrifugation technique which we used to isolate IDL and LDL separately shows that the plasma concentration of neither changed substantially during treatment (Table 3). However, there were subtle underlying perturbations of the kinetics of B protein metabolism in these fractions. In the basal state, our Type III hyperlipoproteinemic patients exhibited LDL hypercatabolism (Table 3) which resolved upon institution of bezafibrate therapy. A similar response was reported in two earlier studies (15, 23) of hypertriglyceridemic subjects and produced an increment in circulating LDL. We did not see such an effect in the present study, apparently because the drug simultaneously altered the metabolic fate of IDL. Prior to therapy, most of the IDL apoB was converted to LDL. Treatment redirected this flux away from LDL into other pathways responsible for direct catabolism of the intermediate density particle (Fig. 3). Other work from this laboratory suggests that receptors may be involved in this process (6).

The study reported here was not meant to address the

etiology of Type III hyperlipoproteinemia but in fact allows us to make some remarks on the subject. Reardon, Poapst, and Steiner (40) have suggested from kinetic observations of VLDL (S_f 60–400) and IDL (S_f 12–60) that direct synthesis of the latter plays a major part in its accumulation in Type III hyperlipoproteinemic patients. The data presented in Tables 2 and 3 are in agreement with their findings that the synthetic rate of small VLDL (which they included in the IDL density range) exceeded that of large triglyceride-rich VLDL. Whether such a phenomenon is a characteristic feature of the Type III condition remains to be seen. At present it seems most likely that a combination of oversynthesis and defective catabolism of the remnants is responsible for the disease. Bezafibrate ameliorates the problem by acting primarily on the former. It does little for the catabolism of small VLDL particles in these Type III subjects, which is presumably governed by the defect in the E protein.

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