Metabolic Relationships among the Plasma Lipoproteins

RECIPROCAL CHANGES IN THE CONCENTRATIONS OF VERY LOW AND LOW DENSITY LIPOPROTEINS IN MAN

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ABSTRACT The changes in other plasma lipoproteins which accompany alterations in very low density lipoproteins (VLDL) were studied in 31 normal and hyperlipidemic men and women who underwent weight reduction, carbohydrate induction, or clofibrate treatment. Plasma lipids and individual lipoprotein cholesterol concentrations were measured serially during control and treatment periods. Low density lipoprotein (LDL) protein was determined by radial immunodiffusion. Oppositely directed changes in VLDL and LDL were found with each of the three metabolic perturbations. Changes in high density lipoprotein (HDL) cholesterol generally paralleled those in LDL but were less consistent. Two patients with type III hyperlipoproteinemia failed to demonstrate reciprocal increases in LDL despite more than 40% reduction in plasma glycerides or VLDL with weight reduction or clofibrate therapy. After clofibrate therapy, LDL increased in proportion to the absolute decrease in VLDL cholesterol during treatment. LDL protein changed relatively less than did LDL cholesterol. The mechanism for the interdependency of plasma VLDL and LDL concentrations over the long term is not known and may be the result of altered rates of interconversion of these lipoproteins, or to feedback inhibition by VLDL of LDL production and release.

INTRODUCTION

The regular occurrence of simultaneous or sequential changes in concentrations of different plasma lipoprotein classes may reflect biochemical relationships between individual lipoprotein species and thus provide insight into their metabolic regulation. Such relationships are of particular interest for very low density (VLDL)[1] and low density lipoproteins (LDL), since the existence of a precursor-product relationship between the two has been suggested. Shared apoprotein antigens (1, 2) and the appearance of radioiodinated, injected VLDL protein in LDL (3, 4), have led to the hypothesis that some, if not all, of the plasma LDL arises from VLDL catabolism (3, 4).

Parallel changes in VLDL and LDL occur with advancing age (5) or nicotinic acid therapy (6). By contrast, patients have shown *reciprocal* changes in VLDL and LDL or high density lipoprotein (HDL) in a variety of circumstances. To examine these relationships systematically, we have studied lipoprotein concentration and composition in man during changes in VLDL concentration induced by weight loss, carbohydrate induction, and clofibrate treatment.

METHODS

Subjects were referred to the M.I.T. Clinical Research Center for diagnosis and treatment of hyperlipidemia. Two normal young women (patients 12 and 31) were included in the carbohydrate induction experiments. Table I summarizes the salient clinical and laboratory data.

Inpatient studies were performed on the ward of the Clinical Research Center and were generally of 6 months duration. Inpatients were fed liquid formula diets supplemented with minerals and vitamins (7). Basal formula diets consisted of 10% of calories as milk or soy protein, 50% of calories as carbohydrate (Dextri-maltose or Cerelose), and 40% fat calories. The latter were added as lard

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[1] *Abbreviations used in this paper:* C/P, cholesterol-protein ratio; HDL, high density lipoprotein; LDL, low density lipoprotein; P/S, polyunsaturated/saturated fat ratio.

TABLE I

Initial Clinical, and Laboratory Data in the 27 Study Patients

Patient No.	Age	Sex	Diagnosis	Lipoprotein phenotype (22)	Glycerides	Total cholesterol	VLDL cholesterol	LDL cholesterol	LDL protein	HDL cholesterol
					mg/100 ml					
Weight reduction										
1	54	M	Obesity	IV	1109	302	186	97	75	19
2	37	M	Obesity	IV	923	231	186	75	75	20
3	48	M	Obesity, xanthelasma	III	636	299	210	77	40	12
4	61	M	Obesity, CAD, MI	IV	392	157	72	72	68	13
5	53	M	Obesity, gout	IV	357	186	85	72	64	29
6	49	M	Obesity, hypertension	IV	258	224	82	108	90	34
Carbohydrate induction										
7*	60	M	CAD, angina, obesity	IV	625	267	122	123	114	22
8*	49	M	CAD, angina pectoris	II	423	524	107	379	212	38
9*	50	F	CAD, angina, obesity	IV	310	229	91	110	98	28
10*	35	M	CAD, MI, sudden death	II	204	275	24	223	137	28
11*	35	M	CAD, MI, sudden death	II	177	265	13	214	106	38
12*	19	F	Normal	Normal	44	110	9	80	30	21
13*	19	F	Normal	Normal	23	115	26	104	73	25
Clofibrate treatment										
14	30	M	Asymptomatic	V	2922	329	280	37	43	13
15*	56	F	Obesity, CAD, angina	IV	2662	432	375	37	41	22
16*	35	M	Abdominal pain, eruptive xanthoma	V	2241	325	277	33	38	15
17	31	M	Asymptomatic	V	1372	525	477	35	41	13
18*	48	M	CAD, angina, cerebral ischemia	III	394	425	200	185	72	40
19	44	M	CAD, sudden death	IV	380	232	70	138	100	24
20*	52	M	Possible CAD	III	291	309	154	123	62	32
21	50	M	Asymptomatic	II‡	286	293	54	207	116	32
22	50	M	Asymptomatic	II‡	278	295	48	218	132	29
23	44	M	Asymptomatic	II‡	277	277	44	207	135	26
24	49	M	Peripheral vascular disease	II‡	160	384	22	316	147	46
25	36	F	CAD	II	144	234	15	193	112	26
26	18	F	Asymptomatic	II	50	333	13	287	130	33
27	39	F	Xanthomatosis	II	47	426	12	370	177	44

Coronary artery disease (CAD), either myocardial infarction (MI), or angina pectoris was present in 11 of the 27 patients.

* Inpatient studies.

‡ Type II with hyperprebetalipoproteinemia on a free diet.

(lard formula). The approximate cholesterol content of the lard formula was 75 mg/1000 cal. Carbohydrate induction was produced by feeding a formula with 10% protein and 90% carbohydrate (Dextri-maltose) calories (fat-free formula). Patients were weighed daily on a metabolic scale and caloric intake was adjusted to maintain constant body weight.

Outpatients were given a thorough medical examination and interviewed by a dietitian. Laboratory investigations were repeated at 2- to 8-wk intervals. Outpatients were instructed in diet selection and preparation and met with the dietitian regularly. The diets included a basic diet (40% mixed fat with a polyunsaturated/saturated fat [P/S] ratio of about 1.5, 25–40% carbohydrate, and 15–20% protein), weight-reducing diets with a similar caloric distribution, and a low cholesterol (200–300 mg/day), high P/S (2.0–3.0) diet for the type II patients. Appropriate dietary therapy always preceded drug therapy which was started or changed only when stable lipid and lipoprotein values had been obtained on three consecutive occasions. Clofibrate was given by mouth at a dose of 1 g twice daily.

Patients were bled after a 12–16 hr overnight fast; blood was collected in tubes containing disodium ethylenediaminetetraacetate (EDTA), 1 mg/ml of blood. Plasma was separated by low speed centrifugation at 4°C. Lipoprotein electrophoresis of whole plasma and of the supernates and infranates after ultracentrifugation at density 1.006 g/ml was performed on each sample to establish the lipoprotein phenotype and to confirm the ultracentrifugal separation of VLDL from HDL and LDL before lipoprotein quantitation. The diagnosis of type II hyperlipoproteinemia was made on the basis of a consistent elevation of LDL-cholesterol concentration in untreated patients. Patients with hyperprebetalipoproteinemia and hyperbetalipoproteinemia ("type II B") are specified in Table I.

Cholesterol and glycerides were measured by an Auto-Analyzer (8–10). The interassay coefficients of variation for replicate samples were 3.4%, n = 29 and 8.2%, n = 30 for cholesterol and glycerides, respectively. HDL cholesterol was measured directly in the supernate of plasma after precipitation of LDL and VLDL with dextran sulfate and calcium chloride (10). The sum of LDL and HDL-cholesterol concentrations was measured in the density 1.006 infranatant fraction after ultracentrifugation of plasma. LDL and VLDL cholesterol were then calculated by difference (10):

[LDL cholesterol] = [Density 1.006 infranate cholesterol] − [HDL cholesterol],

and

[VLDL cholesterol] = [Total plasma cholesterol] − [density 1.006 infranate cholesterol].

A comparison of direct VLDL cholesterol measurement in density 1.006 supernates and the indirect method showed close agreement ($r = 0.943$, $n = 72$).

LDL-protein concentration was determined by radial immunodiffusion of plasma in agarose gel containing antiserum against human LDL prepared in rabbits. The concentration of LDL protein in unknown samples was obtained by comparison of the areas of the precipitin rings with those of a series of LDL standards for which the protein concentrations were known (11). The cholesterol-protein ratio (C/P) was calculated as an index of LDL composition as reported elsewhere (11). The interassay coefficient of variation for LDL protein was about 10%. Data were stored in an IBM 360-75 computer. Statistical and display programs were provided by S. S. David and R. Warren of M.I.T.'s Charles Stark Draper Laboratory.

Mean values for control and treatment periods of individual patients were compared by Student's *t* test and by the Wilcoxon sign-rank test for paired data. The results were similar and *t* test results were used, except in the case of HDL cholesterol, for which both statistics are given. For HDL-cholesterol values, the sign-rank test results are believed to be more accurate, since the data are grossly nonparametric in nature. Per cent change was calculated from the expression: (initial value) − (final value) ÷ (initial value) × 100.

Subjects were excluded from the study if alcohol intake appeared to be a factor in the genesis of their hyperlipidemia. Weight reduction patients were included only when there was a 40% or greater fall in plasma glyceride concentration.

RESULTS

Weight reduction. Six outpatients successfully lost weight and decreased their plasma glyceride concentration by 40% or more with therapeutic caloric re-

TABLE II

Lipoprotein and Lipid Values in the Patients who Demonstrated a Change of 40% or More in Plasma Glycerides from Control (C) to Treatment (T) Periods

Patient No.	Glyceride		VLDL cholesterol		LDL cholesterol		LDL protein		LDL-C/P ratio		HDL cholesterol		Number of observations	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T
	mg/100 ml		*mg/100 ml*		*mg/100 ml*		*mg/100 ml*				*mg/100 ml*			
Weight reduction, (n = 6)														
1	1109	243	186	60	97	166	75	116	1.23	1.42	19	33	1	1
2	923	173	186	73	75	150	75	94	0.99	1.58	20	46	1	1
3*	636*	201*	210*	81*	77*	82*	40*	41*	1.89*	1.99*	12*	24*	1	1
4	392	142	72	28	72	125	68	72	1.05	1.72	13	25	1	1
5	308	102	75	31	77	138	61	75	1.24	1.83	29	31	1	1
6	258	81	82	16	108	149	90	93	1.19	1.59	34	35	1	1
	$P < 0.05$		$P < 0.02$		$P < 0.001$		$P < 0.05$		$P < 0.01$		$P < 0.05$*			
Carbohydrate induction, (n = 7)														
7	635	2266	125	289	140	71	117	86	1.20	0.82	23	15	7	3
8	589	820	119	155	240	143	157	119	1.54	1.19	19	19	6	7
9	395	644	81	102	106	65	80	58	1.36	1.15	20	16	10	4
10	207	519	36	86	147	74	87	68	1.59	1.07	18	12	9	7
11	131	366	21	67	127	110	72	80	1.78	1.34	29	12	11	4
12	36	110	9	20	77	57	38	43	2.05	1.36	25	21	4	4
13	97	140	22	24	104	82	61	67	1.53	1.22	30	24	4	4
	NS		$0.05 < P < 0.1$		$P < 0.01$		NS		$P < 0.001$		$P < 0.02$			
Clofibrate treatment, (n = 8)														
14	2916	1048	288	184	33	93	44	93	0.75	0.98	12	13	4	3
15	982	258	156	37	78	182	53	88	1.47	1.83	19	25	10	14
16	1253	346	171	53	34	124	33	78	1.01	1.59	12	19	8	13
17	2159	303	500	88	39	149	48	93	0.81	1.49	12	25	2	5
18*	524*	272*	173*	91*	121*	136*	48*	52*	2.49*	2.67*	17*	18*	12	9
19	644	269	103	50	136	222	102	144	1.32	1.54	26	28	3	2
20*	514*	169*	191*	44*	152*	135*	60*	58*	2.54*	2.31*	21*	33*	4	10
25	170	136	45	12	149	189	90	105	1.68	1.79	32	29	7	6
	$P < 0.05$		$P < 0.05$		$P < 0.001$		$P < 0.001$		$P < 0.02$		$P = 0.05$‡			

* Data from type III patients were not included in determining the significance (*P*) of the difference between C and T periods by Student's *t* test for paired data.

‡ Not significantly different by Student's *t* test; significantly different with the indicated probability by the Wilcoxon sign-rank test for nonparametric data.

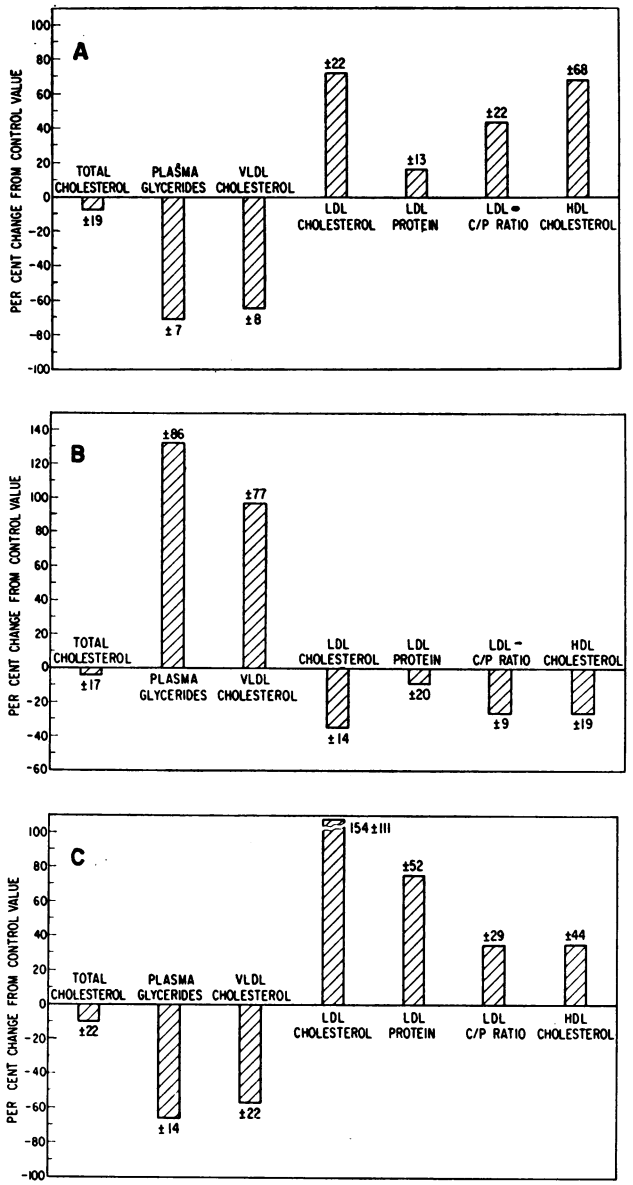

FIGURE 1 Per cent change ±SD from control periods in plasma lipids, lipoprotein cholesterol, and protein concentrations for: *A*, weight reduction; *B*, carbohydrate induction; and *C*, clofibrate treatment.

striction (mean change from initial body weight was −12.0%, range −7.5 to −18.1%). Single values at maximum and minimum weights were compared.

Lipid and lipoprotein changes for these six patients are shown in Table II and Fig. 1. In the type IV patients, LDL cholesterol and C/P ratio rose by 72.4 and 43.1%, respectively. The rise in LDL protein was slight (18.2±13%, $P < 0.05$). There was a 68% mean rise in HDL-cholesterol concentration although individual response was highly variable ($P < 0.05$).

Patient 3 with type III hyperlipoproteinemia was unique, since his LDL cholesterol and LDL protein failed to change with weight reduction, even though the decrease in plasma glycerides, weight, and VLDL cholesterol were comparable with those of the type IV patients. LDL-C/P in patient 3 was high initially and rose only slightly during weight reduction.

Carbohydrate induction. The values for mean plasma lipid and lipoprotein concentrations during control and carbohydrate induction periods are shown in Table II and Fig. 1. Mean body weight did not change from control to induction periods (range 98.8–101.8% of control). Plasma glycerides and VLDL cholesterol rose 134 and 96.4%, respectively. Total cholesterol fell slightly. In each subject, cholesterol and LDL-C/P ratio fell ($P < 0.01$ and < 0.001, respectively). There was no consistent change in LDL-protein concentration (mean −9.0% from control mean, $P > 0.1$). HDL cholesterol fell 26% ($P < 0.02$).

Clofibrate treatment. 14 patients had undergone control and clofibrate treatment periods of sufficient duration to permit analysis (10–80 wk, Table II). Control and treatment weights did not differ (mean treatment weights were 98.4 to 101.7% of control). 8 of the 14 experienced decreases in mean glyceride concentration of at least 40% from control values (Table II). A representative study is shown in Fig. 2 (patient 15). The responses of the two type III patients, 18 and 20, differed from the rest of the group despite comparable changes in plasma glycerides and VLDL cholesterol. In the type III patients, LDL cholesterol, LDL protein, and LDL-C/P ratio did not change appreciably. The remaining six of the eight responders had increases in mean LDL cholesterol (154%, $P < 0.001$), mean LDL protein (74.2%, $P < 0.001$), and mean LDL-C/P ratio (39.4%, $P < 0.02$) (Fig. 1). The *per cent changes* in LDL cholesterol, LDL protein, and

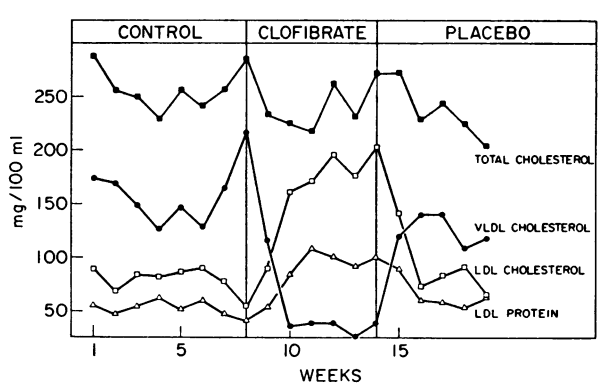

FIGURE 2 Lipoprotein cholesterol and protein concentrations during control, clofibrate, and placebo periods in patient 15.

LDL-C/P ratio correlated with the absolute change in VLDL cholesterol concentration during clofibrate treatment for all except the two type III patients (Fig. 3). The *absolute* changes in LDL cholesterol, LDL protein, and LDL-C/P also correlated significantly with the absolute change in VLDL cholesterol but to a lesser degree ($r = -0.648$, -0.572, and -0.785, respectively, all significant at $P = 0.05$). Thus the proportional changes in LDL cholesterol, LDL protein, and LDL-C/P were a function of the absolute fall in VLDL cholesterol.

DISCUSSION

These data show that reciprocal changes in the cholesterol content of plasma VLDL and LDL occur commonly, if not universally, in metabolic states which lead to substantial changes in plasma glycerides and VLDL. They define conditions during which reciprocal lipoprotein changes occur consistently, i.e., when drug treatment or dietary changes result in large absolute changes in VLDL cholesterol and plasma glycerides. Every subject with large absolute changes in VLDL cholesterol has shown this inverse relationship except those with type III hyperlipoproteinemia.

Throughout this study, changes in total plasma cholesterol were small and inconsistent despite marked changes in the relative concentrations of individual lipoprotein classes. These data re-emphasize that total plasma cholesterol, by itself, only poorly reflects changes in individual lipoprotein classes.

Reciprocal changes in LDL and VLDL have been noted by other workers in a variety of circumstances (12–14). The fall in VLDL during dietary treatment of hyperlipemia by fat and caloric restriction was accompanied by reciprocal increases in LDL cholesterol,

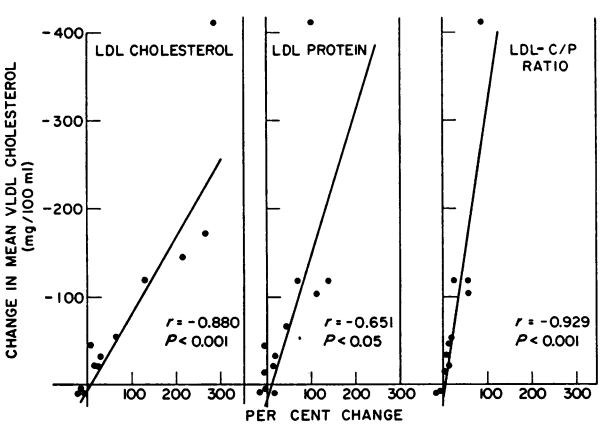

FIGURE 3 Linear regression analysis of the per cent change in LDL cholesterol, LDL protein, and LDL-C/P ratio vs. absolute change in VLDL cholesterol for the entire group of clofibrate-treated patients. Data from the two type III patients (18 and 20) are not included.

LDL protein, and LDL-C/P ratio in one report (14). Strisower, Adamson, and Strisower (13) reported a reciprocal rise in LDL as VLDL fell during clofibrate treatment. Our data on the effects of clofibrate therapy differ in several ways from those of Strisower and coworkers. Although the latter found an increase in LDL (S_f 0–20 lipoproteins) in patients with types IV and V hyperlipoproteinemia after clofibrate treatment, their data showed a slight LDL decrease in patients with type II hyperlipoproteinemia or type II accompanied by hyperprebetalipoproteinemia. Increases in LDL when present, were relatively smaller than those recorded here, perhaps as a result of the shorter time course of their study. The data are not entirely comparable, however, since Strisower measured total lipoprotein concentration in the analytical ultracentrifuge. whereas we measured specific lipoprotein components (i.e., LDL cholesterol and protein).

Short-term studies of plasma lipid and lipoprotein concentrations after acute metabolic perturbation have revealed sequential, similarly directed changes in VLDL and LDL (6). The time relationships have been relatively short, several hours to a few days. Our measurements were made at weekly intervals and so do not exclude the possibility that similarly directed changes in VLDL and LDL concentration occurred in the immediate postperturbation period.

An inverse relationship between HDL (alpha lipoprotein) concentration and VLDL concentration has been found after carbohydrate induction (2), intravascular lipolysis (2), in vitro incubation of VLDL with postheparin plasma (15), and clofibrate therapy (13). Our data are generally in accord with these observations; plasma HDL concentration changed inversely with VLDL in most instances. There were, however, large differences in the absolute and relative changes in HDL between patients.

The physiologic basis for these reciprocal changes in plasma VLDL and LDL-cholesterol concentrations is not clear. At least two possibilities exist: first, these changes may reflect differences in the rates of conversion of VLDL to LDL. Thus, if conversion of VLDL to LDL were accelerated, as might occur with clofibrate treatment (16), VLDL concentration would fall and LDL concentration would rise. On the other hand, plasma VLDL concentration itself might modulate the endogenous (hepatic) synthesis and secretion of LDL or its lipid components.

Numerous experimental data (1–4) and theoretical considerations, (4) which imply a precursor-product relationship between VLDL and LDL, are in support of the former possibility. Reciprocal changes might then result from changes in the rate of conversion of VLDL to LDL. The behavior of plasma LDL during carbo-

hydrate induction is difficult to fit into this scheme, however, since neither LDL-protein nor LDL-cholesterol concentrations rose, despite the probable increase in VLDL synthesis and release into plasma (17). An additional effect of carbohydrate (CHO) induction on LDL synthesis or removal has not been described, to our knowledge.

If a major portion of LDL synthesis and release depends on *de novo* synthesis from nonlipoprotein precursors, then feedback inhibition by VLDL or VLDL constituents might determine plasma LDL concentration in a reciprocal fashion. LDL-glyceride synthesis is an unlikely regulatory site since LDL glycerides rise as plasma VLDL increases (18). Hepatic cholesterol synthesis, however, is subject to feedback inhibition by chylomicron cholesterol at the hydroxymethylglutaryl-CoA reductase step (19, 20). If VLDL also have the capacity to inhibit hepatic cholesterogenesis, and if this process is rate-limiting in LDL synthesis and release, then LDL cholesterol and LDL-C/P would vary inversely with VLDL-cholesterol concentration. Moreover, the changes in LDL cholesterol and LDL-C/P would be expected to be more pronounced than those in LDL protein. Our data are in accord with such an hypothesis but do not exclude alternative possibilities.

This study confirms the failure of LDL concentration to change as VLDL falls during treatment in type III hyperlipoproteinemia (13). From the limited number of patients studied thus far, the failure of VLDL-LDL reciprocity appears to be characteristic of this disorder and may provide further evidence for a defect in VLDL catabolism in type III (21).

These data have several practical implications. During therapeutic weight loss or clofibrate administration, LDL-cholesterol concentration will often rise as VLDL falls. In some instances, hyperbetalipoproteinemia, a potentially more serious abnormality (22), may result. Two patients in our series (15 and 19) showed LDL cholesterol increases to levels above defined normal limits (22) after clofibrate. In patient 15, LDL cholesterol gradually returned to normal. The other patient (19) died suddenly after 4 months of treatment. If total plasma cholesterol falls only slightly or even rises despite a significant drop in plasma glycerides during treatment of hyperlipidemia, the validity of such therapy should be reconsidered.

In the treatment of patients with type II hyperlipoproteinemia, one can make a rough prediction concerning the response of plasma LDL concentration to clofibrate therapy. Many patients with type II hyperlipoproteinemia and increased VLDL concentrations (type II B) will have an increase in plasma LDL concentration. In such cases, adjunctive or alternative drug therapy will probably be appropriate.

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