Effect of gemfibrozil on levels of lipoprotein[a] in Type II hyperlipoproteinemic subjects

Peter H. Jones, Henry J. Pownall, Wolfgang Patsch, J. Alan Herd, John A. Farmer, Charlotte Payton-Ross, Kay T. Kimball, Antonio M. Gotto, and Joel D. Morrisett[1]

Department of Medicine, Baylor College of Medicine, The Methodist Hospital, 6565 Fannin Street, Houston, TX 77030

Abstract Plasma lipoprotein[a] (Lp[a]) levels are highly correlated with angiographically demonstrable coronary heart disease, and elevated Lp[a] is an independent risk factor for atherosclerosis. Previous studies have provided evidence that the levels of Lp[a] and triglyceride are related, suggesting that Lp[a] might be altered by gemfibrozil, a drug well known for its efficacy in reducing plasma triglycerides. Accordingly, 18 type IIa and 16 type IIb hyperlipoproteinemic males aged 35–58 were treated for 3 months with 600 mg of gemfibrozil twice daily. The efficacy of the drug in altering lipid and lipoprotein levels was different in the two type groups. In type IIa and IIb subjects the respective changes in median levels were: total cholesterol, -7.5 and -8.5%; triglycerides, -35.6 and -54.4%; HDL-cholesterol, +9.0 and +11.0%; and Lp[a], -17.2 and +6.1%. Before and after gemfibrozil treatment, 7 type IIa and 10 type IIb subjects were given a 100 g/2 m^2 oral-fat load; triglycerides and Lp[a] were measured post-prandially at 0, 2, 4, 6, 8, and 10 h. The differences between before- and after-gemfibrozil post-prandial curve integrated areas (PPCIA) were compared for triglycerides and Lp[a]. The changes in median PPCIA for triglycerides in types IIa and IIb were -54% and -53%, and for Lp[a] were -8% and +8%, respectively. These results indicate *i)* that the levels of Lp[a] are about 2 times higher in type IIa than IIb subjects, and *ii)* that although gemfibrozil elicits a rather uniform decrease in fasting and post-prandial triglyceride levels in type IIa and IIb patients, the drug causes heterogeneous changes in Lp[a], suggesting that different metabolic mechanisms may be dominant in subjects showing opposing effects.–**Jones, P. H., H. J. Pownall, W. Patsch, J. A. Herd, J. A. Farmer, C. Payton-Ross, K. T. Kimball, A. M. Gotto, and J. D. Morrisett.** Effect of gemfibrozil on levels of lipoprotein[a] in Type II hyperlipoproteinemic subjects. *J. Lipid Res.* 1996. **37:** 1298–1308.

Supplementary key words lipoproteins • triglyceride • postprandial lipemia • apolipoproteins • fibrates

A number of different drugs developed for reducing plasma levels of cholesterol and triglyceride and/or the major lipoproteins transporting these lipids have also been tested for their capacity to reduce Lp[a] (1, 2). Atorvastatin, a new HMG-CoA reductase inhibitor, has recently been evaluated in patients with primary hypercholesterolemia. When the drug was administered at dosages of 5, 20, and 80 mg/day, LDL-cholesterol was markedly reduced by 29, 44, and 61%, but much less striking reductions of 4.0, 7.9, and 14.2% were observed for Lp[a] (3). The earlier generation reductase inhibitors are not very effective in lowering Lp[a] levels, (4–8), and in some cases even elevate them (9). One notable exception to the above generalization is a study in which lovastatin decreased Lp[a] by 39% in hyperlipidemic kidney graft recipients (10).

Nicotinic acid has long been used to treat hypercholesterolemic patients who are able to tolerate efficacious dosages. However, the effect of low dose niacin (1.5 g/day) on plasma Lp[a] levels appears to be minimal (0%) (11). Nevertheless, a higher dose (1 g four times daily) lowers Lp[a] by 38% (12). Neomycin is effective alone, and even more so in combination with niacin (13). In type II hyperlipoproteinemic subjects given 2 g neomycin/day, a 24% reduction in Lp[a] was observed. When neomycin (2 g/day) was administered with niacin (3 g/day), Lp[a] was reduced by 45%. Significantly, those patients with the initially highest Lp[a] levels experienced the greatest reduction in Lp[a] with this combination therapy. In hypercholesterolemic patients with angiographically documented coronary artery disease, cholestyramine (3 g/day) and niacin (up to 6 g/day) for 1 year produced a 29% reduction in Lp[a] (14). In patients with primary hypercholesterolemia receiving niceritrol, a derivative of nicotinic acid, (1.5 g/day) for 12 weeks, the drug was not effective in lowering Lp[a] if those subjects had an initial mean level of 10.6 mg/dl, but the drug produced a 34% decrease in a subgroup

Abbreviations: total-C, total cholesterol; TG, triglyceride; VLDL-C, very low density lipoprotein cholesterol calculated by dividing the triglyceride value by 5; HDL-C, high density lipoprotein cholesterol; Lp[a], lipoprotein[a]; Lp[a]-P, Lp[a] protein (includes apo[a]) and apoB-100); Lp[a]-T, Lp[a] total mass estimated by dividing Lp[a]-P by 0.33; Lp[a]-C, Lp[a] cholesterol estimated by multiplying Lp[a]-P by 1.05; LDL-C, low density lipoprotein calculated from the equation LDL-C = (Total-C)-(VLDL-C)-(HDL-C)-(Lp[a]-C); PPCIA, post-prandial curve integrated area.

[1]To whom correspondence should be addressed.

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whose mean initial level was 19.2 mg/dl (15). Niceritrol has also been found to be effective in lowering Lp[a] in patients with end-stage renal disease, a condition in which levels are typically elevated. Acipimox, another analog of niacin, is better tolerated but less efficacious than the parent drug (16).

Fibric acid derivatives have also been studied for their potential to lower Lp[a]. In type IIa, IIb, and IV subjects treated with sustained-release bezafibrate for 42 weeks, there were reductions of 30, 23, and 24% in Lp[a] levels (17), respectively. A somewhat smaller reduction was observed in a group of patients with type IIa or IIb hyperlipoproteinemia where bezafibrate with retarded release of the active drug (400 mg/day) caused a 15% decrease in Lp[a] levels within 3 months (18). Conflicting results have been reported about the effects of gemfibrozil on Lp[a] levels. When used to treat type II or type IV patients with elevated cholesterol (>7.7 mmol/l) and/or triglyceride (>2.5 mmol/l), the drug caused no significant changes in Lp[a] (19). In the Helsinki Heart study, a group of subjects receiving gemfibrozil experienced a 27% reduction in serum apoB, but virtually no change in Lp[a] (20). When gemfibrozil was administered to cynomolgus monkeys in weekly escalating doses of 50, 125, and 200 mg/kg per day, the drug caused dose-dependent reductions in plasma Lp[a] of 16.3, 39.5, and 63.8% (21), respectively, an effect reversible within 3 weeks. In a recent multi-drug study, the effects of three different fibrates on Lp[a] levels were compared in type IV hyperlipoproteinemics (22). Bezafibrate, gemfibrozil, and fenofibrate raised Lp[a] levels by 53, 28, and 44%, respectively. In a small study of 12 subjects with mean age 70 years, with hypercholesterolemia, and with Lp[a] levels >30 mg/dl, gemfibrozil treatment combined with a Mediterranean diet lowered the median Lp[a] level from 36.5 to 8.4 mg/dl (23). Finally, in a group of 27 nondiabetic patients aged 37–68 with hypercholesterolemia and hypertriglyceridemia, the mean Lp[a] level was decreased from ~24 to ~18 mg/dl (25%) by a 12-week treatment with gemfibrozil (24).

In the present study, the effect of gemfibrozil on Lp[a] levels has been examined in type IIa and type IIb patients not only in the fasted state, but post-prandially as well. A primary objective of the study was to determine hyperlipidemic conditions under which the drug might be effective, and also to identify patient subpopulations in which the drug might be more efficacious than others.

METHODS

Subject selection

Study subjects were males between 18 and 65 years of age who had fasting total cholesterol levels of >200 mg/dl on at least two separate visits to the Lipid Metabolism and Atherosclerosis Clinic of The Methodist Hospital. A single exception was subject 4 whose cholesterol level decreased to 194 mg/dl after screening but before the study was begun. Criteria for exclusion from the study included: active liver disease, biliary cirrhosis, or biliary dysfunction causing elevation of serum transaminase more than 20% above normal levels; pre-existing gall bladder disease such as cholelithiasis or cholecystitis; treated hyperthyroidism, nephrotic syndrome, or dislipoproteinemias; secondary hypercholesterolemia due to obstructive liver disease; chemical substance abuse including alcoholic beverages (more than 4 oz of alcohol/day); treatment with a lipid-lowering drug and/or procedure (e.g., apheresis) without a full month cessation for metabolic re-equilibration; treatment with other drugs that might affect lipid levels or interact with gemfibrozil such as immunosuppressive or anti-coagulant agents or neomycin; severe obesity (>30% over ideal body weight); unstable angiopectoris; myocardial infarction within the previous 4 months. Subjects qualifying for the study were thoroughly instructed in the details of the protocol which had been approved by the human research review boards of Baylor College of Medicine and The Methodist Hospital. Upon signing a statement of informed consent, 40 prospective subjects were entered into the study; 6 of these did not complete the study. Type IIa and IIb patients were distinguished by fasting triglyceride levels below and above 250 mg/dl, respectively.

Study design

After the study subjects were identified in the screening phase, a baseline phase of 8 weeks was begun. During this phase, the NIH National Cholesterol Education Program Step One Diet was initiated with each subject, and its effects were determined by comparing the total plasma lipid, lipoprotein, and apoprotein profiles of each subject at the beginning and end of this period. Immediately before the treatment phase was begun, a standardized fat load experiment lasting 10 h was conducted (25). The test meal consisted of 350 ml of heavy whipping cream (39.5% fat), 2 tablespoons of chocolate-flavored syrup, 1 tablespoon of granulated sugar, and 1 tablespoon of instant nonfat dry milk. Of the 1,362 calories, 2.8% were derived from protein (9.5 g), 14% from carbohydrate (48 g), and 8.6% from fat (130 g); cholesterol content was 480 mg, and the polyunsaturated/saturated fat ratio was 0.059. Ingredients were mixed but not blended so as to obtain a readily drinkable liquid. The test meal was administered per 2 m^2 of body surface to adjust adequately for major variations in blood volume. After a 14-h fast subsequent to a light dinner, the study was begun by drawing a 50-ml blood sample. Immediately after this postabsorptive sample was drawn (0 h), the test

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meal was ingested within 5 min by the study subject. Subsequent blood samples (10 ml) were drawn at 2, 4, 6, 8, and 10 h. During this 10-h period only consumption of water or 2 cups of unsweetened and uncreamed coffee or tea was allowed.

This experiment was followed by a 12-week regimen of gemfibrozil (Lopid, 600 mg twice daily). The study was terminated with a second fat-load experiment. During the 12-week treatment phase, each subject was evaluated at 4-week intervals to determine physical and clinical status and toleration of the medication. Medication compliance was judged from interviewing subjects and counting remaining tablets in the medication bottle that was refilled with a known number of tablets at each visit.

Analytical methods

Lipid and lipoprotein measurements (**Table 1** and **Table 2**) were performed on plasma samples obtained by 4°C centrifugation of venous blood collected after a 12-h fast into Vacuutainer™ tubes containing EDTA. Total plasma cholesterol (26) and triglycerides (27) were measured enzymatically (Boehringer Mannheim Diagnostics, Indianapolis, IN). High density lipoprotein cholesterol (HDL-C) was determined by measuring cholesterol in the supernatant liquid after precipitation of the plasma with $MgCl_2$ and dextran sulfate (28). Low density lipoprotein cholesterol (LDL-C) levels were calculated according to Friedewald, Levy, and Fredrickson (29) with correction for Lp[a] cholesterol determined by multiplying Lp[a] protein by 1.05. LDL-C could not be accurately estimated in subjects with plasma triglycerides >400 mg/dl. Levels were determined with an enzyme-linked immunoassay (ELISA) using goat polyclonal anti-apo[a] as the trapping antibody and rabbit polyclonal anti[a] as the detecting antibody (30). Lp[a] protein levels reported here in mg/dl represent triplicate determinations of the total protein moiety of Lp[a], including apoprotein[a] and apoprotein B-100. Purified Lp[a] was used as a primary standard for calibrating plasma samples containing low (4–5 mg/dl), medium (8–11 mg/dl), or high (18–22 mg/dl) levels of Lp[a] protein as secondary standards. The working range for the assay is 1.3–7.0 μg/ml. Total Lp[a] mass cannot be accurately calculated from the protein concentration due to variations in apo[a] isoform molecular weight and lipoprotein lipid content among different individuals. However, multiplying the protein content by 3.0 provides a reasonable estimate of total Lp[a]. The bio-

TABLE 1. Levels and changes of plasma lipids and lipoproteins in Type IIa and IIb hyperlipoproteinemic subjects

Subjects	Pre-Gemfibrozil	Post-Gemfibrozil	Change	% Change	*P*
	mg/dl				
Type IIa (n = 18)					
Lp[a]-protein	11.1 ± 12.8	9.6 ± 9.7	-14.5	-11.7	
	7.0	**5.8**	-0.74	-17.2	0.04
TG	152 ± 61	93 ± 39	-59	-35.0	
	140	**86**	**-49**	**-35.6**	**<0.0001**
Total-C	252 ± 53	232 ± 52	-20	-7.3	
	238	**229**	**-23**	**-7.5**	**0.008**
LDL-C	163 ± 51	153 ± 51	-10	-5.3	
	156	**150**	**-12**	**-7.8**	**0.12**
HDL-C	41 ± 10	46 ± 13	4.7	+11.4	
	42	**43**	**3**	**9**	**0.01**
Type IIb (n = 16)					
Lp[a]-protein	5.0 ± 5.9	5.5 ± 7.1	+0.5	+70.4	
	1.8	2.2	+0.05	+6.1	0.33
TG	364 ± 115	147 ± 51	-216	-55.1	
	339	**141**	**-180**	**-54.4**	**<0.0001**
Total-C	273 ± 37	251 ± 36	-22	-6.8	
	270	245	-26	**-8.5**	**0.02**
LDL-C	155 ± 34	172 ± 34	17	17.5	
	153	175	**9**	**6.8**	**0.17**
HDL-C	38 ± 10	42 ± 8	4	13.2	
	34	**40**	**5**	**11.0**	**0.03**

Values are given for **median** and mean ± SD levels (mg/dl) and changes of plasma lipids and lipoproteins in Type IIa and IIb hyperlipoproteinemic subjects after a 12-week course of gemfibrozil (600 mg b.i.d.).

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logical variation was estimated from five measurements made on a normolipidemic adult female over a 5-month period (mean ± SD, 34.2 ± 2.1; C.V. = 6.1%), and from five measurements on a normolipidemic male made over 5 weeks (mean ± SD, 20.5 ± 1.8; C.V. = 8.8%). The intra- and inter-assay coefficients of variation were 4.0% and 9.0%, respectively. For normal Lp[a] protein levels in the range of 1–10 mg/dl, the contribution of plasminogen at physiological concentrations (200 mg/dl) to Lp[a] levels was negligible. Apo[a] phenotyping was performed using the 11 polymorph system described by Gaubatz et al. (31).

For in vitro lipolysis experiments, aliquots of triglyceride-rich plasma were diluted with 0.23 M Tris, 0.39 M NaCl, pH 8.5, containing BSA (92.5 mg/dl). To this substrate solution (0.4 ml) was added purified bovine milk lipase (25 µl) and purified human apoC-II (15 µl) (32). The mixture was incubated in a shaking water bath at 37°C for 30 min. Aliquots of the reaction mixture (25 µl) were analyzed for free fatty acid using a WAKO analysis kit (Biochemical Diagnostics, Inc.) according to the prescribed procedure. Separate aliquots were taken for triplicate determination of Lp[a] concentrations using ELISA methodology (32) (**Table 3**).

Statistical methods

The effects of gemfibrozil therapy on fasting lipid and lipoprotein levels were evaluated using paired *t*-tests (33). Log or rank transformations of triglyceride and Lp[a] levels were used to meet the assumptions of the *t*-test. The association of plasma Lp[a] with other lipids were assessed with Pearson's correlation or, when appropriate, Spearman's correlation analysis. The effects of gemfibrozil therapy on post-prandial triglyceride and Lp[a] levels were assessed by comparing the post-prandial curve integrated areas (PPCIA) before and after drug administration. Curve integrated area, defined by plotting triglyceride or Lp[a] versus time over

TABLE 2. Effect of gemfibrozil on post-prandial Lp[a] and triglyceride curve integrated areas in Type IIa and IIb hyperlipoproteinemic subjects

Subject No.	Apo[a] Phenotype	Lp[a] PPCIA Pre	Lp[a] PPCIA Post	Change	% Change	TG PPCIA Pre	TG PPCIA Post	Change	% Change
		mg protein/dl				mg/dl			
Type IIa									
7	2,-	188.8	146.4	-42.3	-22	6762	3724	-3038	-45
8	7,1	4.2	3.5	-0.7	-16	3610	1647	-1963	-54
9	7,9	10.8	5.6	-5.2	-48	5790	1304	-4486	-77
11	8,-	41.6	58.6	17.0	41	2492	1258	-1234	-50
16	ND	140.4	128.5	-11.9	-8	8321	1288	-7033	-85
17	6,7	225.8	218.2	-7.6	-3	2887	2529	-358	-12
18	5,9	38.5	61.5	23.0	60	3518	1106	-2412	-69
Mean		92.9	88.9	-3.9	0	4769	1837	-2932	-56
Median		41.6	61.5	-5.2	-8	3610	1304	-2412	-54
P					0.64				0.013
Type IIb									
1	9,-	11.8	16.1	4.3	36	9456	4163	-5293	-56
2	5,-	30.6	19.4	-11.2	-37	5556	1816	-3740	-67
5	4,-	8.4	10.0	1.6	19	7706	3821	-3885	-50
6	8,-	17.6	10.0	-7.6	-43	7216	2651	-4565	-63
7	7,-	6.5	3.8	-2.7	-42	5281	2274	-3007	-57
8	10,-	14.8	16.1	1.3	9	3224	3954	730	23
10	ND	270.5	287.8	17.3	6	3936	3268	-668	-17
11	ND	4.8	35.1	30.3	634	8255	2414	-5841	-71
14	8,11	77.1	116.2	39.2	51	5520	2883	-2637	-48
15	3,-	4.2	4.2	0	0	2910	3992	1082	37
Mean		44.6	51.9	7.3	63	5906	3124	-2782	-37
Median		13.3	16.1	1.4	8	5538	3076	-3374	-53
P					0.20				0.005

TG, triglyceride; PPCIA, post-prandial curve integrated areas, ND, not determined.

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TABLE 3. Effect of lipolysis on immunoassayable Lp[a] protein in hypertriglyceridemic plasma

Plasma	Triglyceride	Fatty Acid			Lp[a]		
		Before	After	% Change	Before	After	% Change
	mgdl	*μEq/l*			*mg protein/dl*		
1	822	1070	5480	69	13.54	15.20	+12
2	377	672	3720	48	2.03	2.20	+8

the 10-h post-prandial period, was calculated for each subject using the trapezoidal rule (34). Data are reported as means ± standard deviations. All statistical tests were two-tailed. Actual *P*-values are reported for all tests; however, the interpretation of statistical significance is based on the family-wise error rate ≤0.05 for each group of related hypotheses. The statistical analyses were performed using STATA Release 4.0, 1995 (STATA Corp., College Station, TX). Histograms and x–y plots were done with Prism Version 2.0 (Graph Pad Software, Inc., San Diego, CA).

RESULTS

To determine whether triglyceride levels have a significant effect on immunoassayable Lp[a] in hypertriglyceridemic plasma, samples from two individuals were subjected to in vitro lipolysis stimulated by the addition of exogenous bovine milk lipase and human apoC-II. Sixty-nine percent lipolysis of plasma sample #1 was attended by a 12% increase in immunoassayable Lp[a], and a second sample that had undergone 48% lipolysis displayed an 8% increase in Lp[a] immunoreactivity (Table 3).

Fasting Lp[a] protein levels of the 34 type II subjects ranged from <1 to 54 mg/dl (open bars, **Fig. 1**). Eleven subjects (32%) had levels ≥10 mg/dl, equivalent to 30 mg/dl total Lp[a], a widely accepted threshold for cardiovascular risk. Among these subjects at risk, the majority (7/11, 64%) were type IIa and the minority (4/11, 36%) type IIb. Among the subjects with Lp[a] protein <10 mg/dl, approximately equal numbers were type IIa (11/23, 48%) and IIb (12/23, 52%). This tendency of type IIa subjects toward higher Lp[a] levels is reflected in their median value of 7.0 mg/dl, about 4 times higher than the level for IIb subjects (Table 1).

Apo[a] isoforms were detectable for 29 of the 34 type II subjects. Nineteen displayed a single band and 10 showed double bands. A histogram of the dominant isoform from heterozygotes and the only isoform from homozygates reveals a bimodal distribution (**Fig. 2**). One mode is centered on isoform 4 (M_r ~553 kD) and the second on isoform 8 (M_r ~742 kD). In this group of subjects no association was observed between apo[a] isoform size and fasting Lp[a] concentration or type IIa/IIb hyperlipoproteinemia.

The effect of gemfibrozil on fasting Lp[a] in each subject is depicted in Fig. 1. Among the 34 subjects, Lp[a] levels decreased in 21 (62%); 13 of these 21 (62%) were type IIa. Lp[a] levels rose in 13 subjects (38%), the majority of whom were type IIb (62%). These subgroup changes are reflected in the composite data presented in Table 1. The median Lp[a] level decreased significantly in type IIa subjects (-17%, $P = 0.04$), and slightly increased in IIb subjects (+6%), although this latter change was not significant. Earlier studies have suggested an association of Lp[a] and triglyceride levels (12, 13, 16). As gemfibrozil produced large, highly significant fasting triglyceride changes in the IIa (-35.6%) and IIb (-54.4%) subjects, it was desirable to determine whether these changes were associated with changes in Lp[a]. A weak negative association was observed by linear regression analysis ($r = -0.24$), but was not significant ($P = 0.18$).

Previous reports (24) have indicated the greater utility of post-prandial triglyceride over fasting triglyceride values for assessing their clearance rates and cardiovascular risk. Thus the effect of gemfibrozil on post-prandial triglyceride and Lp[a] levels was studied in 7 of the type IIa and 10 of the type IIb subjects. Plasma concentrations of triglycerides and Lp[a] during the 10-h post-prandial period were plotted versus time. Most of the triglyceride versus time plots increased monotonically until a maximum was reached at 5–6 h, then decreased over the remaining 5–4 h. In contrast, Lp[a] versus time plots did not exhibit this bell-shaped pattern, especially the post-gemfibrozil curves which were frequently flat (**Fig. 3**). Areas under the post-prandial triglyceride and Lp[a] curves (PPCIA) were computed and used for quantitative comparison of the post-prandial responses of the 17 subjects (**Fig. 4**). Among the 7 type IIa subjects, all showed significant decrease in triglyceride PPCIA (median = -54%, $P = 0.013$) (Table 2). Five of these (71%) exhibited minimal to moderate decrease in Lp(a) PPCIA, but these changes were almost offset by rather large increases in 2 subjects (median = -8%, $P = 0.64$). In the 10 type IIb subjects, 8 had moderate to large decreases in triglyceride PPCIA and 2 had increases (median = -53%, $P = 0.005$). These subjects had widely varying changes in Lp[a] PPCIA; one even increased 634%. The median change was small and not

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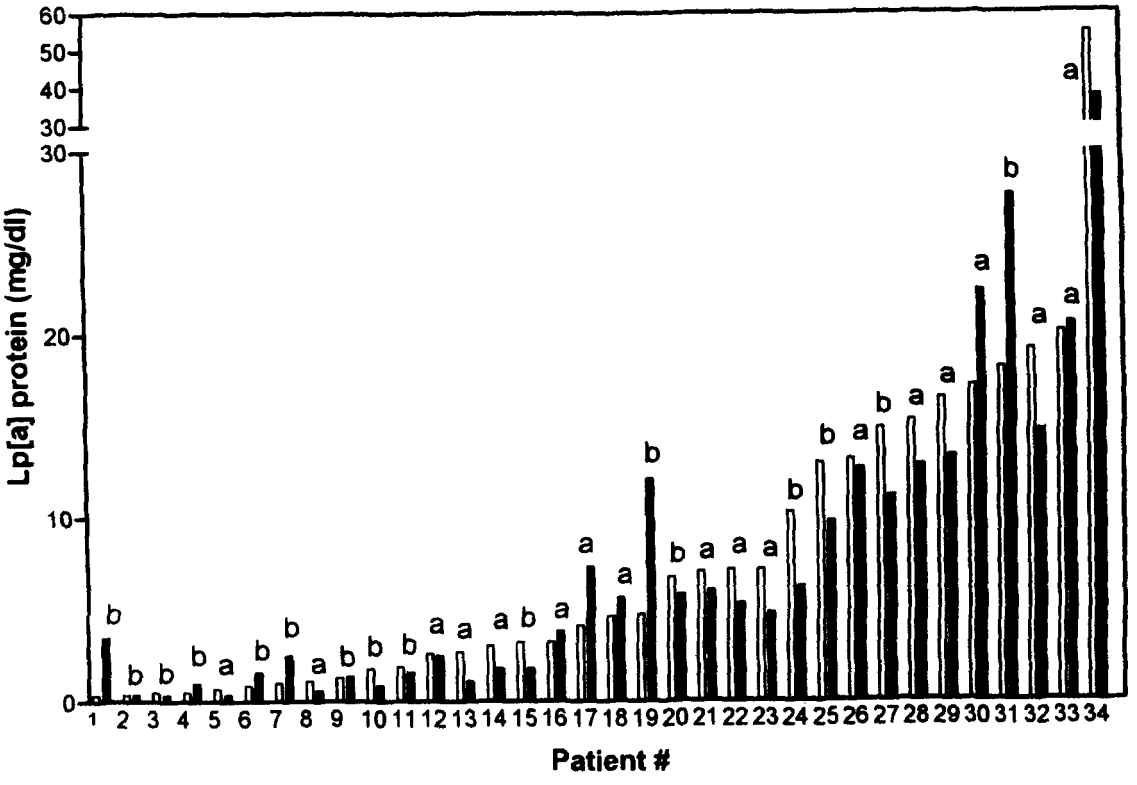

Fig. 1. Histogram indicating the fasting Lp[a] levels of 18 type IIa and 16 type IIb subjects before treatment (open bars) and after 12 weeks of gemfibrozil therapy (solid bars).

significant (+8%, $P = 0.20$). Spearman correlation analysis revealed a positive association between triglyceride PPCIA and Lp[a] PPCIA, but it was not significant ($r = 0.31$, $P = 0.23$).

For these 17 subjects from whom both fasting and post-prandial data were obtained in response to gemfibrozil (Fig. 4), the association of fasting Lp[a] changes with PPCIA changes was extremely high ($r = 0.98$, $P < 0.0001$). Hence, it appears that single point measurements may be adequate for assessing the effect of gemfibrozil on Lp[a]. For some lipids and lipoproteins, their susceptibility to lowering by selected drugs is directly related to their pretreatment plasma concentration (13, 15). However, for Lp[a], this relationship appears to be inverted. Pre-gemfibrozil fasting Lp[a] levels are negatively correlated with changes in fasting Lp[a] caused by the drug ($r = -0.39$, $P = 0.024$) (**Fig. 5**). A similar correlation may also exist between baseline Lp[a] levels and Lp[a] PPCIA ($r = 0.32$, $P = 0.21$) but did not reach significance in this study, probably due to insufficient sample size.

DISCUSSION

Fibric acid derivatives comprise a family of hypolipidemic drugs that effectively lower plasma triglyceride levels. The drug is thought to activate lipoprotein lipase thereby enhancing the lipolysis of triglyceride-rich particles (35). The effects of fibrates on cholesterol levels are variable, depending on initial plasma triglyceride levels. In subjects with highly elevated triglycerides, LDL particles are typically small and dense, probably resulting from extensive facilitated transfer of cholesteryl ester from LDL to VLDL and triglyceride from VLDL

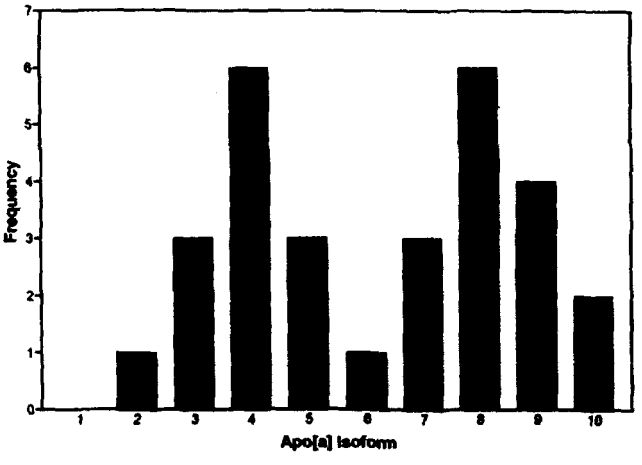

Fig. 2. Histogram indicating the relative abundance of the only apo[a] isoform in homozygotes and predominant isoform in heterozygotes among 29 type II subjects in whom the isoform could be determined by immunoblotting.

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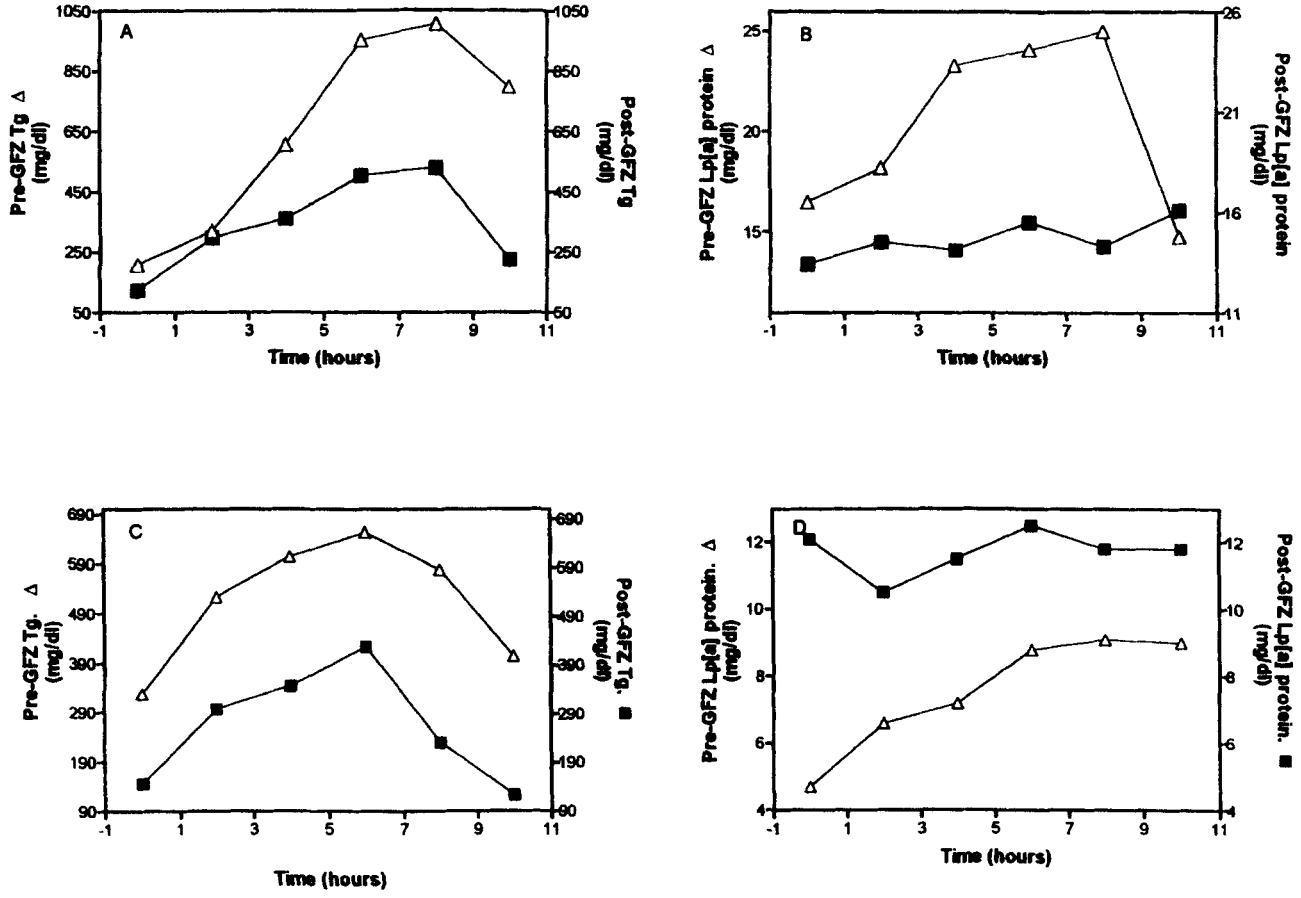

Fig. 3. Post prandial curves for two type II subjects in whom gemfibrozil treatment induced different responses. In subject IIa-7, the PPCIA decreased for triglycerides (A) and for Lp[a] (B). In subject IIb-14, PPCIA decreased for triglycerides (C), but increased for Lp[a]. Pre-treatment values are indicated by open triangles and post-treatment values by closed squares.

to LDL; hepatic lipase-catalyzed lipolysis of in-exchanging triglycerides produces a particle with a reduced hydrophobic core volume and reduced lipid–protein ratio. By reducing the concentration of triglyceride-rich lipoproteins, fibrates suppress the formation of small, dense LDL and decrease their flux through receptor-independent pathways (36). Production of normal LDL is then increased with consequent redirection of LDL catabolism through the receptor-mediated pathway. By contrast, in hypercholesterolemic subjects with normal triglyceride levels, initially elevated LDL levels are reduced by fibrates. Earlier studies suggested that gemfibrozil suppresses hepatic cholesterol synthesis thereby up-regulating LDL receptors; but more recent data support the view that the drug, by restoring normal LDL composition and size, also restores its affinity for the LDL receptor and normalizes its flux through that pathway (37).

In our type IIa subjects with a rather normal median triglyceride level of 140 mg/dl, gemfibrozil caused a 36% reduction to 86 mg/dl. This was attended by a very moderate 7.5% decrease in total cholesterol, attributable mostly to reduction in VLDL. In type IIb subjects, gemfibrozil decreased mean triglycerides from 339 to 141 (-54.4%). In this case, plasma cholesterol was reduced a moderate 8%, not reflective of the substantial decrease in VLDL-cholesterol (-61%) and partially compensating increase in median LDL-cholesterol (6.8%) and HDL-cholesterol (11.0%). This elevation of LDL-cholesterol in the hypertriglyceridemic type IIb subjects is consistent with the view that gemfibrozil, by reducing triglyceride levels, is normalizing small, dense LDL to larger, less dense LDL. The elevation in HDL is consistent with an improved lipolytic process by which HDL_3 is converted to HDL_2 (24).

Although the mean baseline LDL-cholesterol concentrations in type IIa (163 mg/dl) and type IIb (155 mg/dl) were quite similar, the mean Lp[a] cholesterol level of the type IIa subjects (~11.1 mg/dl) was more than twice that of type IIb subjects (5.0 mg/dl). Hiraga et al. (38) have observed a similar inverse correlation between Lp[a] and triglyceride concentrations in type II subjects. Seventeen of their type IIa subjects with a mean triglyceride of 123 had a mean total Lp[a] of 24.8 mg/dl;

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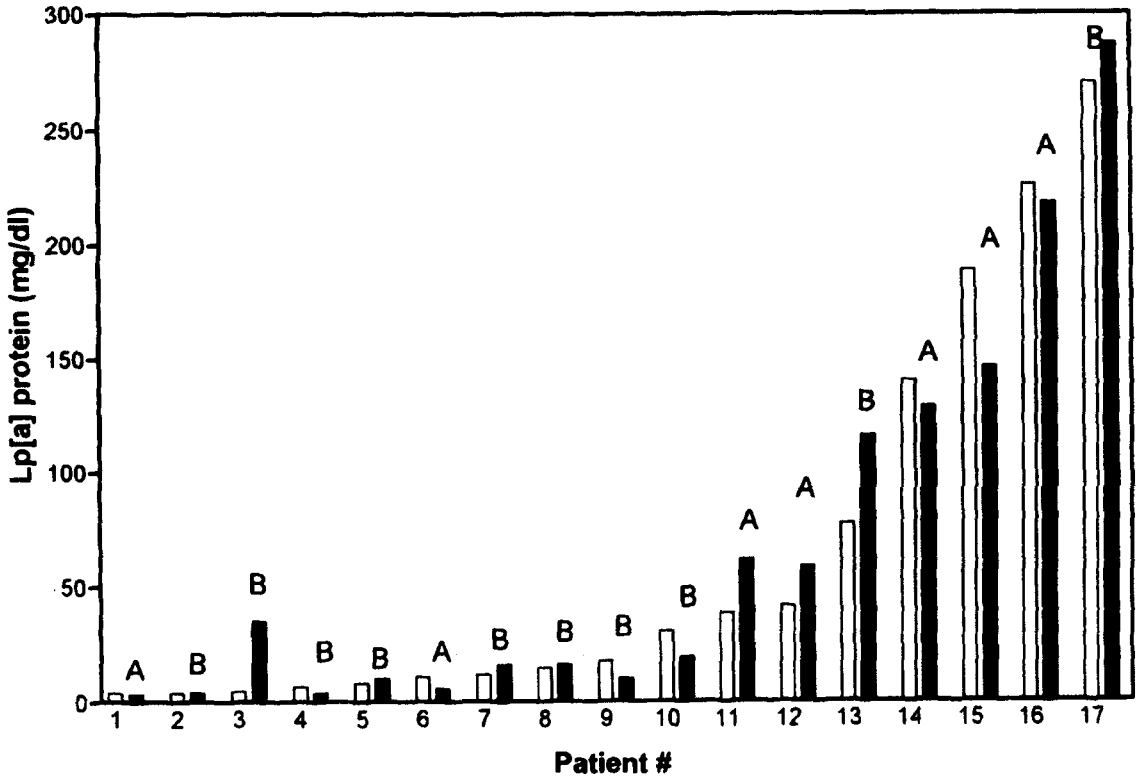

Fig. 4. Hist gram indicating the post-prandial curve integrated areas for Lp[a] in 7 type IIA and 10 type IIB subjects before treatment (open bars) and after 12 weeks of gemfibrozil therapy (solid bars).

11 type IIb subjects with a mean triglyceride of 341 had a mean Lp[a] of 19.0 mg/dl. In a comparison of plasma Lp[a] levels in post-myocardial infarction subjects, Kostner (36) found that a slightly smaller percentage of hypertriglyceridemic type IIb subjects had Lp[a] levels >30 mg/dl (48.3%) than those with Lp[a] below this level (51.7%). For normotriglyceridemic type IIa subjects, 80.5% had Lp[a] >30 mg/dl while 19.5% had Lp[a] below this level. This negative trend toward correlation between Lp[a] and triglyceride levels has also been observed in individuals with other types of hyperlipoproteinemia. Bartens et al. (39) studied the distribution of Lp[a] in a group of patients with either type I, type IV, type V hyperlipoproteinemia or diabetes mellitus type II. These severely hypertriglyceridemic patients had a mean Lp[a] of 13 mg/dl compared to 22 mg/dl in a group of normotriglyceridemic controls. The subjects in the lowest triglyceride quintile had the highest median Lp[a] level; a significant negative correlation between triglyceride and Lp[a] plasma concentrations was observed ($r = 0.69$, $P = 0.03$).

This negative relationship between fasting triglyceride and Lp[a] levels may be due, in part, to reduced availability of apoB-100, an apolipoprotein shared by Lp[a] and VLDL. Increased hepatic synthesis of triglyceride requires commensurate amounts of apoB-100 for the production of VLDL. This process may reduce the pool of apoB-100 thereby decreasing the production of Lp[a]. Another mechanism invokes a concept espoused by McConathy et al. (40) based on their observation that Lp[a] undergoes significant noncovalent interactions with triglyceride-rich lipoproteins, thereby forming metastable complexes. As remnants produced by the lipolysis of triglyceride-rich lipoproteins are taken up by hepatic receptors, it may be possible that Lp[a] complexed to these remnant particles are also taken up with comparable efficiency. Hence, elevated levels of VLDL and their lipolytic remnants could significantly enhance the removal of Lp[a], causing a net decrease in its plasma concentration. Both of these mechanisms are consistent with the well known capacity of estrogen to elevate triglyceride-rich lipoproteins (41) and decrease Lp[a] (42).

The possibility of noncovalent binding of Lp[a] to triglyceride-rich lipoproteins raises two important questions. First, does the binding of Lp[a] to VLDL alter the catalytic efficiency of lipoprotein lipase toward the VLDL triglyceride? McConathy et al. (40) have reported that Lp[a] causes only about 13% inhibition of VLDL lipolysis by lipoprotein lipase in vitro. Second, does the binding of Lp[a] to VLDL alter the immunoreactivity of the apo[a] protein thereby compromising Lp[a] assay

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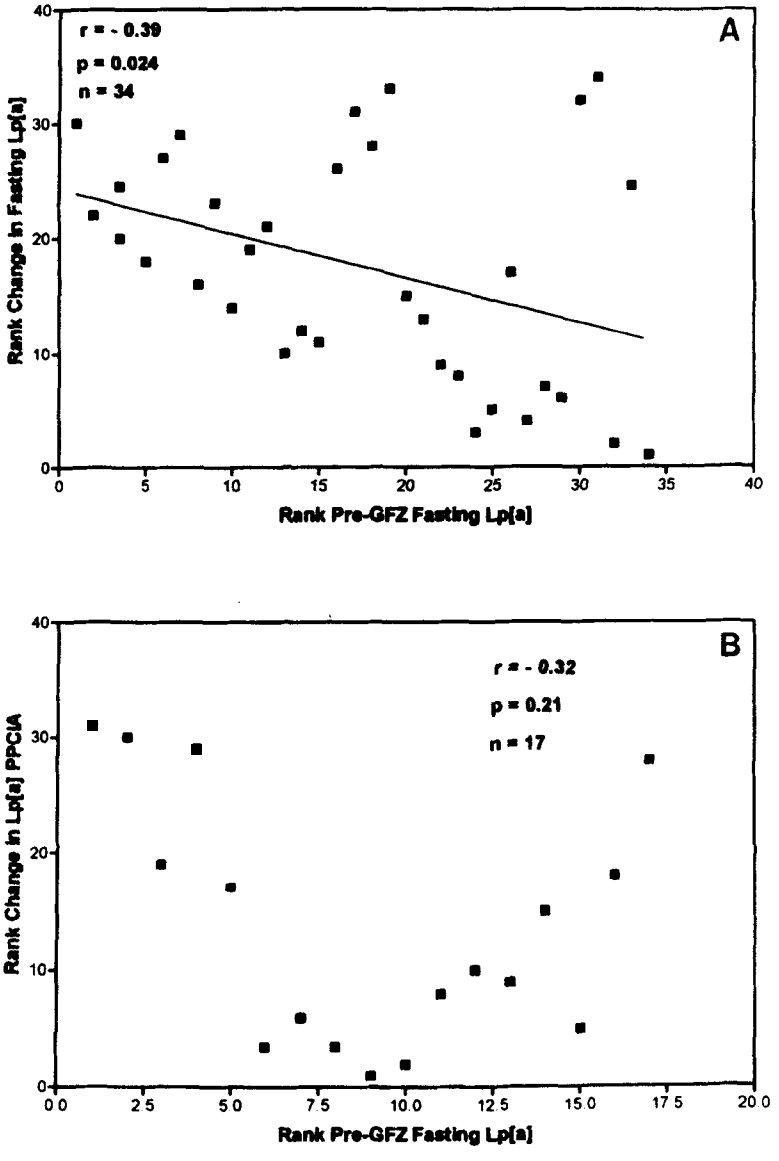

Fig. 5. Plots indicating the association of fasting Lp[a] levels before gemfibrozil treatment with (A) change in fasting Lp[a] levels and (B) change in post-prandial curve integrated areas after treatment.

measurements? We addressed this latter question in an experiment in which two hypertriglyceridemic plasma samples were subjected to in vitro lipolysis. For lipolysis proceeding to the extent of 69%, a 12% increase in immunoreactivity was observed; for 48% lipolysis, an 8% increase was seen. Hence, as determined by our enzyme-linked immunoassay, significant changes in plasma Lp[a] levels occurring during post-prandial lipemia cannot be attributed mainly to reduced immunoreactivity of the apo[a] protein in a VLDL/Lp[a] complex, unlike the effect of hypertriglyceridemia on the electroimmunoassay results reported by Walek et al. (43).

Gemfibrozil caused an almost homogeneous decrease in fasting triglyceride levels in our type II subjects. Among 16 type IIa individuals, 16 (89%) had decreased fasting triglycerides (mean change = -41%). Among 16 type IIb subjects, 100% had decreased fasting triglycerides (mean change = -55%). Comparable changes were also seen in post-prandial triglycerides quantified as the integrated area under the curve (Table 2). All 7 of the type IIa subjects studied post-prandially had decreased triglyceride areas (mean change = -56%). Of 10 type IIb subjects, 8 (80%) had decreased areas (mean change = -54%).

In contrast to rather homogeneous changes in fasting triglyceride, fasting Lp[a] changes were heterogeneous in response to gemfibrozil. Among the type IIa patients, 13 (72%) had decreased fasting Lp[a] (-26%), and 5 (28%) had increased levels (+31%); for all 18 subjects the median change was -17.2% which was statistically significant (P = 0.04). A very similar distribution of changes was seen in the Lp[a] curve-integrated areas of type IIa

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subjects studied post-prandially; 5 (71%) showed a decrease (-19%) and 2 (29%) showed an increase (+50%) but the magnitude of these individual changes was such that the median composite change was only -8%, which was not significant ($P = 0.64$).

In the IIb group, 9 (56%) showed a decrease (-29%) in fasting Lp[a] and the other 7 (44%) an increase (+184.6%), giving a median change of +6.1% that was not significant ($P = 0.33$). Among the IIb subjects studied post-prandially, 30% had a decreased Lp[a] curve area (-40%), 60% had an increased area (+126%), and 10% had no change, giving a median change for the entire group of +8%, which was not significant ($P = 0.20$).

The heterogeneous perturbations induced by gemfibrozil in fasting and post-prandial Lp[a] in type IIa and IIb patients suggest that different metabolic mechanisms may be controlling Lp[a] levels when they change in opposite directions. For individuals in whom Lp[a] levels rise after gemfibrozil therapy, the decreased availability of triglyceride for VLDL synthesis may concomitantly lower the demand for apoB-100 normally drawn from a pool that may also supply this apoprotein for Lp[a] assembly, resulting in Lp[a] elevation. An additional or alternative mechanism could involve reduced formation of Lp[a]/VLDL complexes (described above). When VLDL concentrations are decreased, fewer Lp[a]/VLDL complexes should be formed, and fewer complexes cleared by remnant receptors, thereby raising Lp[a] levels. In the post-prandial state, the situation may be complicated by the presence of multiple triglyceride-rich lipoprotein species (e.g., chylomicrons and VLDL subfractions) that could bind Lp[a] with differing affinities and undergo clearance at different rates. The capacity of gemfibrozil to lower Lp[a] in some of the type IIa and IIb subjects may also involve a direct effect of the drug on hepatic synthesis or secretion of Lp[a] components. Ramharack and co-workers (21) have shown that in cynomolgus monkeys given higher than human doses, Lp[a] levels progressively decrease with increasing dosage. Notably, the basal levels of Lp[a] in these monkeys are also generally higher than those in humans.

Of greatest concern are those type II subjects who have Lp[a] levels at or approaching the cardiovascular risk threshold of ~25 mg/dl. Ten type IIa subjects and five type IIb subjects had baseline Lp[a] protein levels >6.7 mg/dl (~20 mg/dl total Lp[a]). Gemfibrozil lowered the mean Lp[a] protein level in 80% (8/10) of these type IIa subjects by 23.2%. Gemfibrozil also reduced the mean protein Lp[a] level in 80% (4/5) of the at-risk type IIb subjects by 25.9%. Thus, type II subjects with clinically important, elevated Lp[a] seem to be a dominant subpopulation (~80%) in whom gemfibrozil lowers Lp[a] by about 25%. Similar results have been obtained by Ramires et al. (24) in a group of hyperlipidemic patients with basal Lp[a] levels >24 mg/dl.

This study indicates that in type II subjects, the fasting Lp[a] levels of normotriglyceridemics are about two times higher than those of hypertriglyceridemics; gemfibrozil treatment significantly decreases fasting Lp[a] in normotriglyceridemic but not in hypertrigly-ceridemic type II subjects; in 80% of type II subjects with clinically significant fasting Lp[a] (i.e., >20 mg/dl), gemfibrozil reduces Lp[a] by ~25%; post-prandial Lp[a] changes induced with gemfibrozil are heterogeneous with respect to lipoprotein phenotype, however, when Lp[a] is reduced by the drug in the fasting state, it is also reduced in the post-prandial state.

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