Dyslipoproteinemia and an Inhibitor of Lipolytic Enzymes in Weber-Christian Disease

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Hyperlipidemia of initially Type V and finally of Type III was observed in a patient with Weber-Christian disease. The lipoprotein lipase (LpL) and hepatic triglyceride lipase (HTGL) activities of the post-heparin plasma were low, but detectable. Both lipase activities were higher when assayed with 20 μ l of post-heparin plasma than with more than 30 μ l, indicating the presence of some inhibitory factor in the plasma. This plasma inhibited purified LpL and HTGL from human post-heparin plasma. Zonal ultracentrifugation studies showed that the inhibitor of hepatic lipase was associated with the middle fraction of intermediate lipoprotein and low density lipoproteins ($1.020 < d < 1.040$). These results are consistent with the idea that dyslipoproteinemia in this patient was partially due to dysfunction of the catabolic system caused with an inhibitor of lipolytic enzymes.

Key words: Weber-Christian disease, Type III hyperlipidemia, Inhibitor of lipoprotein lipase, Inhibitor of hepatic lipase

Hyperlipidemia is occasionally observed in Weber-Christian disease (1) but its cause is not clarified. We met a patient with Weber-Christian disease accompanied with lipoprotein abnormalities showing initially an increase in chylomicrons and very low density lipoproteins (VLDL, $d < 1.006$) and finally high levels of intermediate density lipoproteins (IDL, $1.006 < d < 1.020$). One of the causes of hyperlipidemia is a dysfunction of the lipoprotein catabolic system. In a case of hypertriglyceridemia, lipoprotein lipase deficiency or apolipoprotein CII deficiency are pointed out (2, 3). Breckenridge et al. report that hepatic triglyceride lipase deficiency is associated with hyperlipidemia characterized by triglyceride-rich low density lipoproteins ($1.019 < d < 1.063$) (LDL), and high density lipoproteins 2 ($1.063 < d < 1.125$) (HDL₂), and the presence of β -VLDL (4). In this paper, we tried to define

the cause of hyperlipidemia in Weber-Christian disease from the aspect of catabolic system dysfunction. The post-heparin plasma of this patient showed low activities of both LpL and HTGL activities, and an inhibitory effect against both LpL and HTGL. The property of this inhibitory factor was examined and its influence on the formation of dyslipoproteinemia was discussed.

MATERIALS AND METHODS

Subject

A.K., a 28-year-old man had suffered from multiple tender nodules in subcutaneous fat, fever, arthralgia and myalgia for 6 years. Skin biopsy revealed panniculitis adiposus. A diagnosis of Weber-Christian disease was made and a large dose of corticosteroid was administered. As a result the fever subsided and the subcutaneous nodules dis-

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appeared intermittently. He gradually lost the subcutaneous adipose tissue of the face and extremities. The patient was 160 cm tall, and during treatment his weight decreased from 62 to 45 kg. He had also suffered from a lung abscess due to salmonella sepsis during the previous 4 years. He had hepatomegaly and liver function tests showed elevated levels of transaminase, GOT, GPT, alkaline phosphatase and low choline esterase. Icterus occurred. Glucose intolerance was not observed.

Leukopenia and thrombopenia had appeared 6 months previously and severe hypertriglyceridemia developed although prednisolone (15 mg/day) was administered samely as before. No xanthoma was observed. There was no family history of hyperlipidemia.

Clinical chemistry

Routine laboratory tests gave the following results: red blood cells 3,030,000/mm³, white blood cells 1100/mm³, platelets 54000/mm³, no abnormal young cells. The maximum total cholesterol level (T.ch) was 317 mg/dl and that of triglyceride (TG) was 1275 mg/dl and these levels decreased to 182 and 279, respectively, during low food intake caused by deteriorated liver function. The results of liver function tests were as follows: GOT 360U (0-45), GPT 370U (0-45), Al-p 569U (<100), choline esterase 4.45U (3.5-7.5), total bilirubin 4.5 mg/dl, indirect bilirubin 1.5 mg/dl. The levels of urea nitrogen and creatinine were normal and urinalysis showed a trace of protein, no blood cells, and no abnormal sediment. Coagulation tests showed decrease in the fibrinogen level, increase in the level of fibrin degradative product (40 µg/ml) and a prolonged prothrombin time (37.8 sec), indicating disseminated intravascular coagulopathy occurred. Several bone marrow punctures were dry taps. Tests for rheumatoid arthritis and anti-nuclear antibody gave negative results. The levels of IgG, IgM, and IgA were within normal ranges. The erythrocyte sedimentation rate was high, but was 6 mm/hr in the last 3 months.

Lipoprotein analysis

Serum lipoprotein was fractionated by ultracentrifugation in a Beckman type 42 LpTi rotor according to the method of Bronzert (5). Serum itself (d = 1.006), and serum adjusted to a density of 1,063

with potassium bromide were centrifuged at 41,000 rpm (222,000 g) for 3 hours. The lipid contents of the bottom fractions of the preparations, and of the original whole serum were determined. The lipid content of the bottom fraction of d = 1.063 was that of high density lipoproteins (HDL). The lipid contents of low density lipoproteins (LDL, 1.006 mg/dl < d < 1.063 mg/dl) was calculated by subtracting the contents in d = 1.063 bottom fraction from that in d = 1.006 bottom fraction. Similarly the lipid in the very low density lipoprotein (VLDL) fraction was calculated by subtracting the contents in d = 1.006 mg/dl bottom fraction from that in original serum. The separation of VLDL, LDL and HDL was confirmed using dye. Zonal ultracentrifugal separation of serum was performed in a Beckman Ti14 rotor by the method of Patch et al. (6). A density gradient was formed with NaBr solution of d = 1.000 and d = 1.300 using a gradient pump (Beckman).

Determination of apolipoproteins

Plasma apolipoproteins were determined quantitatively by the single radial immunodiffusion methods. Assay kits for apolipoproteins AI, AII, B, CII and E were provided by Daiichi Chemical Company (Tokyo, Japan). Apolipoprotein E subclasses of VLDL was determined by isoelectric focusing by the method of Falko et al. (7).

Lipid and chemical analysis

Total cholesterol was assayed enzymatically with cholesterol esterase, cholesterol oxidase and peroxidase (8). Triglyceride was measured enzymatically with lipoprotein lipase, glycerol dehydrogenase and diaphorase (9). Phospholipids were determined as phospholipids containing choline. All phospholipids containing choline were assayed enzymatically with phospholipase D and choline oxidase (10). Protein was measured by the method of Lowry et al. (11).

Post-heparin lipolytic activity

The lipase activities in post-heparin plasma were determined as follows. Ten units of heparin per kg body weight was given intravenously and venous blood sample was obtained 10 min later. The plasma was stored at -20°C until assayed. Hepatic lipase activity was taken as the activity remaining after

inactivation in the LpL with 1 M NaCl (12). Lipoprotein lipase activity was taken as the difference between the total activity and remaining activity after inactivation with 1 M NaCl. The reaction mixture contained 337 nmoles of tri [^{14}C] oleoyl glycerol, 0.01% Triton X-100 and 2% fatty acid free-bovine serum albumin and post-heparin plasma (usually 20 μl) in 250 μl of 100 mM Tris HCl buffer, pH 8.4. Apolipoprotein CII (2 μg), an activator for lipoprotein lipase, was added to the reaction mixture for measurement of total lipolytic activity. After incubation for 30 min at 37°C, the reaction was stopped by addition of 2.25 ml of chloroform/methanol/heptane (10:9:7) and 1 ml of 0.1 N NaOH (13). [^{14}C] Oleic acid was extracted into the upper phase and its radioactivity was counted.

Purifications of lipoprotein lipase and hepatic triglyceride lipase

Lipoprotein lipase and hepatic triglyceride lipase were purified from human post-heparin plasma by affinity chromatography on heparin-Sepharose (14) as described by Greten and Walter (15) and Baginsky and Brown (16). The fraction eluted with 0.7 M NaCl was used as hepatic triglyceride lipase, and that eluted with 1.5 M NaCl as lipoprotein lipase. These fractions were stored in 50% glycerol at -70°C. The enzyme preparation showed the characteristic properties of lipoprotein lipase (13, 15, 16).

Tributyryn hydrolyzing activity

To determine the hydrolyzing activity of monomeric substrate by hepatic lipase and lipoprotein lipase, [^{14}C] tributyrin was used as a substrate, and the assay procedures were performed according to the method described by previous report (21).

Gel filtration of plasma

Gel filtration of plasma was performed on an Ultrogel ACA 22 column (2.5 cm $\times$ 30 cm). Material was eluted with 0.9% NaCl in 10 mM Tris-HCl buffer, pH 7.4, containing 1 mM EDTA and 0.01 NaN_3 at a flow rate of 10 ml/hr, and fractions of 2.5 ml were collected.

RESULTS

Profiles of lipoprotein lipids and apolipoproteins of the patient

The lipoprotein fractionations when the patient's serum triglyceride level was 729 mg/dl and the total cholesterol level was 278 mg/dl are shown in Table 1. In the lipoprotein fraction of $d < 1.006$ mg/ml, the triglyceride level was markedly elevated, and the total cholesterol and phospholipid level were also increased. The ratio of the triglyceride to the total cholesterol content was high. Agarose electrophoresis of serum after 12 hr starvation showed the presence of lipid at the origin, indicating the presence of chylomicrons. In the fraction of $1.006 < d < 1.063$, the triglyceride, cholesterol and phospholipid levels were increased, the level of triglyceride being especially high indicating the presence of in-

Table 1. Lipid Concentrations in Various Lipoprotein Fractionations

Density	Date	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	Phospholipids (mg/dl)
—1.006 (mg/dl)	Aug. 20	120 (23 $\pm$ 10)*	612 (80 $\pm$ 45)	262 (45 $\pm$ 20)
	Nov. 13	36	42	13
1.006—1.063	Aug. 20	136 (111 $\pm$ 29)	111 (26 $\pm$ 8)	114 (78 $\pm$ 17)
	Nov. 13	119	221	224
1.063—1.21	Aug. 22	22 (45 $\pm$ 10)	6 (16 $\pm$ 4)	47 (89 $\pm$ 15)
	Nov. 13	28	16	45
Total	Aug. 20	278	729	423
	Nov. 13	183	279	282

*Normal ranges were obtained from 49 healthy men without hyperlipidemia, and the values are shown by mean $\pm$ standard deviation (SD).

Table 2. Apolipoproteins in Patient's Serum

Apolipoprotein	Concentration (mg/dl)	
	Patient	Control
Apo A-I	24	125±25*
A-II	18	28.2±6.4
B	95	72.3±16.5
C-II	4.4	3.8±1.7
E	12.5	3.8±0.9

The serum sample from the patient was obtained on Nov. 13 as for Table 1. The value of control was obtained from normal healthy 120 persons.

* = mean±standard deviation.

intermediate density lipoproteins ($1.006 < d < 1.019$).

The general condition of the patient gradually deteriorated. After 2 months of low food intake, his triglyceride level had decreased to 279 mg/dl and his total cholesterol level to 183 mg/dl, as shown in Table 1. In the fraction of $d < 1.006$ mg/ml, the triglyceride level was low, but that of total cholesterol was still high, indicating the presence of cholesterol-rich particles. In the fraction of $1.006 < d < 1.063$ mg/ml, the triglyceride level was high compared to that of total cholesterol. In the fraction of $1.063 < d < 1.21$ mg/ml, the total cholesterol level was still low.

Results of zonal ultracentrifugal analysis at a later date are shown in Fig. 1 comparing with those of a normal subject and of Type III patient with apolipoprotein E phenotype 2/2. The high total cholesterol level in the intermediate fraction of $1.006 \text{ mg/ml} < d < 1.012 \text{ mg/ml}$ was eluted like that of the Type III patient rather than the Type IV patient, indicating the presence of IDL. These results indicate that the phenotype of this hyperlipidemia was initially Type V and later Type III. The apolipoprotein levels on the second examination are shown in Table 2. There was no deficiency of apolipoprotein C-II (4.4 mg/dl). The apolipoprotein A-I and A-II levels were low, and the apolipoprotein E level was high. Isoelectric focusing electrophoresis of apo VLDL show the apolipoprotein E isoform 3/4.

Lipoprotein lipase and hepatic triglyceride lipase in post-heparin plasma

The LpL and HTGL activities in post-heparin plasma were very low, then they were measured

Fig. 1. Zonal ultracentrifugal analysis of plasma of the patient with Weber-Christian Disease.

Normal plasma (20 ml), Type III patient plasma (TG = 729 mg/dl, t.ch = 429 mg/dl) (7 ml), and the patient's plasma (TG = 279, T.ch = 182) (20 ml) were subjected to zonal ultracentrifugation in Beckman Ti 42 Rotor. The density gradient was formed with NaBr of $d = 1.0$ and $d = 1.3$, using a Beckman Gradient Maker by the method of Patsch et al. (6).

Fig. 2. Effects of sera from patients with various hyperlipidemic diseases on purified LpL (A) and purified HTGL (B). Incubations were performed as described under Materials and Methods. Serum lipid levels were as follows; In Type III patient serum, triglyceride = 729 mg/dl, total cholesterol = 429 mg/dl and in Type IV patient's serum triglyceride 704 mg/dl, total cholesterol = 329 mg/dl, in patient's serum triglyceride = 279 mg/dl, total cholesterol 176 mg/dl.

using 30 μ l of post-heparin plasma. But, these activities were detected when measured using less than 20 μ l of the post-heparin plasma, as shown in Table 3. The activities were higher when assayed with 5 μ l of the post-heparin plasma than with 10 or 20 μ l. Thus the low activities of both LpL and HTGL were not due to enzyme deficiency, but to an inhibitor in post-heparin plasma.

Effect of serum on LpL and HTGL activities

There are several reports describing inhibitory factors against lypolytic enzyme activities in the

Table 3. LpL and HTGL Activities in Post-heparin Plasma

Plasma volume in assay mixture	LpL activity (nmol/hr)		HTGL activity (nmol/hr)	
	Patient	Control	Patient	Control
100	0	120±34	0	190±25
75	2	110±32	0	182±24
50	0	100±34	3	164±22
40	6	92±32	5	152±20
30	8	80±24	10	128±17
20	19.9	68±24	16	110±14
10	31	40±12	24	72±10
6	25	22±6	12	42±6

Incubations were performed as described under Materials and Methods. Post-heparin plasma was obtained at mild liver dysfunction (ChE=4.45 U (normal range = 3.5-7.5)).

plasma (4, 17, 18). To evaluate the specificity of the inhibitory factor in the patient's plasma, we examined the effects of various hyperlipidemic sera on the activities of purified LpL and HTGL.

As shown in Fig. 2, the patient's serum (TG=279 mg/dl, T.ch=182 mg/dl) cause dose-dependent decreases in both LpL and HTGL activity. A volume of 100 μ l of control serum (TG=120 mg/dl, t.ch=176 mg/dl) caused about 20% decrease. Volumes of 100 μ l of Type III (TG=729 mg/dl, T.ch=429 mg/dl) and Type IV (TG=704 mg/dl, T.ch 329 mg/dl) sera decreased the LpL and HTGL activities about 50% to 60%, and 50 μ l decreased the activities to 25% to 45%. These inhibition rates were far less than those by the patient's serum.

Characteristics of the inhibitor against LpL and HTGL

The properties of inhibitory factor were examined using purified HTGL. As shown in Table 4, the inhibitory activity was decreased by heating the post-heparin plasma at 80°C for 30 min. The activity was not affected by dialysis in tubing permeable to molecules of less than 18000 daltons, and was not lost on treatment with trypsin (100 μ g/ml) for 20 min at 37°C. However, freezing and thawing decreased the inhibitory activity slightly.

Localization of the inhibitor of HTGL

On an Ultrogel ACA 22 column chromatography of the patient's plasma the inhibitory activity against HTGL was eluted associating with the lipoprotein

Fig. 3. Ultrogel ACA 22 gel filtration profile of the patient's plasma and the inhibitor of HTGL.

The patient's plasma (TG=279, T.ch=182, 4 ml) was applied to a column of Ultrogel ACA 22 (2.0 $\times$ 30 cm) and material was eluted with 0.9% NaCl, 10 mM Tris-HCl buffer, pH 7.4. Twenty μ l of each fraction was incubated with HTG (10 μ g) and triolein hydrolyzing activity was determined as described under Materials and Methods.

fraction (Fig. 3). This fraction also inhibited purified LpL activity (data not shown). Zonal ultracentrifugal analysis showed that the inhibitor was associated with fractions between IDL and LDL (Fig. 4). Addition all effect of various other lipoproteins to lipase reaction mixtures were shown in Table V. VLDL and LDL from patients and type III hyperlipidemia (apoE2/2) had scarcely affected LpL and HTGL activities. But, patient's IDL fraction inhibited LpL and HTGL activities, and IDL from type III did not inhibited.

Fig. 4. Zonal ultracentrifugation of plasma of the patient with Weber-Christian disease, and the inhibitor of hepatic triglyceride lipase activity.

Volumes of 20 ml of normal plasma (Fig. 4A) and the patient's plasma (Fig. 4B) were subjected to zonal ultracentrifugation in a Beckman Ti 42 rotor as for Fig. 1. Fifty μ l of each fraction was incubated with purified HTGL (5 μ g) and triolein hydrolyzing activity were determined as described under Materials and Methods.

Effects of inhibitor on tributyrin and triolein hydrolyzing activities of LpL and HTGL

In the presence of inhibitor (20 μ g), triolein hydrolyzing activities of LpL and HTGL were decreased as shown in Table VI, but tributyrin hydrolyzing activities of LpL and HTGL were not decreased.

DISCUSSION

This report describes a case of Weber Christian disease associated with hyperlipoproteinemia. The plasma was rich in VLDL and triglyceride-rich LDL, and poor in HDL. IDL was present even after a period of low food intake.

The administration of large dose of glucocorticoid is well known to cause hyperlipidemia (24). In this case, the dose of prednisolone (15mg/day) was not so large, and severe hyperlipidemia occurred during the same maintenance dose of prednisolone. Therefore, it is suggested that the effect of gluco-

Table 4. Effect of various treatment on inhibitory factor against LpL and HTGL activities

Treatment of inhibitory factor	LpL activity	HTGL activity
None	12	5
Heating (80°C, 30 min)	60	56
Dialysis	12	5
Freezing and thawing	24	28
Trypsin treatment (1000 μ g/ml, 30 min)	6	2
no inhibitory factor	100%	100%

Inhibitory factor (20 μ g) obtained from tube No. 22 in Fig. 4, was treated as shown above and was mixed with purified LpL (8 μ g) or HTGL (10 μ g), and tri[14 C] oleylglycerol, 2% bovine serum albumin and 0.1 M Tris-HCl, pH 8.3, with 2 μ g of apolipoprotein C-II for measurement of LpL. Incubations were for 60 min at 37°C.

corticoid is not a main cause of hyperlipidemia in this patient.

It is known that several factors such as lipolytic enzymes and apolipoproteins, are involved in catabolic system of VLDL and IDL. In this patient, apolipoprotein CII was present and apolipoprotein E isoform was not 2/2, and both LpL and HTGL activities were detectable when measured using small amount of post-heparin plasma. But, lipolytic activity was barely detectable when measured using a large amount of post-heparin plasma, indicating the presence of inhibitor against both lipases. This inhibitory effect of serum might partly be by substrate dilution, but the inhibitory effect of serum from this Weber Christian disease patient was far more than those by sera of Type III and Type IV patients, which had even higher triglyceride contents than that of the patient as shown in Fig. 2.

Brunzell et al. report the presence of an inhibitor of LpL in the plasma of cases of familial chylomicronemia (17). Therefore, hypertriglyceridemia observed in this case with Weber-Christian disease (at Aug. 20 in Table 1) might be due to LpL dysfunction caused by the presence of the inhibitor.

Breckenridge et al. report abnormalities of lipoprotein associated with familial deficiency of hepatic lipase (4). Their patient shows an abnormally high proportion of triglyceride in LDL and the presence of β -VLDL. Goldberg et al. (19) report that injection of anti-HTGL caused an increase in

Table 5. Effect of various lipoprotein fractions on LpL and HTGL activity

Added lipoproteins	Remaining Activity	
	LpL	HTGL
No addition	100%	100%
The patient's		
VLDL	84	92
IDL	24	21
LDL	88	84

Type III patient's (apoE2/2)		
VLDL	78	94
IDL	76	84
LDL	85	88

The added amount of all lipoprotein's were 30 μ g of total cholesterol. The reaction conditions were the same as described in Table IV.

triglyceride-rich intermediate lipoprotein particles. These evidences indicate that clearance of IDL requires HTGL activity, and the elevated IDL in our patient might be explained by HTGL dysfunction caused by the inhibitor. To clarify the precise mechanism of the inhibitory factor, the inhibitor was purified. The inhibitor was associated with the IDL fraction (Fig. 4). The specificity of this inhibitory factor was compared with IDL from type III (apoE 2/2) patient (Table 5). IDL from the patient had stronger inhibitory effect than those from Type III patient.

Next, the properties of the inhibitory factor in this patient were compared with those in previous reports. An inhibitor of lipolytic activity in post-heparin plasma is reported to be associated with lipoproteins ($d < 1.25$ g/ml) in a hepatic lipase deficient patient (4). Kubo et al. (18) report that

apolipoprotein A-I and apolipoprotein A-II inhibit HTGL. In the serum of our patients, the apolipoprotein A-I and apolipoprotein A-II levels were low, and the IDL fraction did not contain apolipoprotein A-I or apolipoprotein A-II, indicating that the inhibitor was not apolipoprotein A-I or apolipoprotein A-II. The effects of various treatments shown in Table 4 indicated that the inhibitor might be some protein that was insensitive to trypsin. The relation between the presence of disseminated intravascular coagulopathy and the inhibitor is obscure.

The change of the dyslipoproteinemic pattern from Type V to Type III looks curious. At the stage of type V, IDL were supposed to be included in addition to chylomicrons and VLDL, because LDL ($1.006 < d < 1.063$) was rich in triglyceride. At that time, productions of chylomicrons and VLDL were sufficient because the patient's general condition was good and food intake was sufficient. But after about one month of low food intake, VLDL production decreased, and accumulation of IDL were supposed to become prominent.

The inhibitions of both LpL and HTGL by this factor suggest that common functional units of LpL and HTGL was susceptible to the inhibitor. To evaluate the mechanism of inhibitory effect, the effects of inhibitor on triolein and tributyrin hydrolyzing activities were studied on the base of hypothesis that LpL and HTGL enzyme molecules are composed of catalytic site and lipid interface recognition site (20, 21, 22). Namely, monomeric substrate tributyrin could be hydrolyzed by catalytic site alone, but triolein hydrolysis requires both catalytic site and lipid interface recognition site (21, 22). Therefore, if the inhibitor works on lipid interface recognition site, tributyrin hydrolysis could not be disturbed, but triolein hydrolysis could be im-

Table 6. Effect of inhibitory factor on tributyrin hydrolyzing activity and triolein hydrolyzing activity of LpL and HTGL.

Inhibitor	LpL		HTGL	
	triolein hydrolysis	tributyrin hydrolysis	triolein hydrolysis	tributyrin hydrolysis
0	100%	100%	100%	100%
20 μ g	12%	98%	5%	102%

Assay conditions were the same as in Table IV.

paired. As shown in Table VI, the inhibitor decreased only triolein hydrolyzing activities of both lipases, but not tributyrin hydrolyzing activities of both lipases. These results suggest that the inhibitor may work on the lipid interface recognition site of LpL and HTGL, but not catalytic site. These similar effects on LpL and HTGL may indicate that LpL and HTGL molecule, especially the region of lipid interface recognition site may be very similar as suggested by LpL and HTGL amino acid sequence analysis (23). The properties of the inhibitor is still not defined. The origin of this inhibitor may be related to some immunoglobulin, which is supposed to relate to paniculitis, but no precise data was not available yet.

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