Metabolism of Triglyceride in Hyperlipidemia

AKIO NOMA AND HAJIME ORIMO

IT HAS been considered that the serum triglyceride level is mainly regulated by the intestinal absorption, mobilization of free fatty acids from adipose tissue, hepatic synthesis and removal from the general circulation. Hyperlipidemias are caused by the impairment of these regulatory mechanisms. In an attempt to clarify the role of the removal of triglyceride from general circulation in the etiology of hyperlipidemia, we have studied the changes in the plasma lipoprotein lipase and monoglyceride hydrolase activities in the patients with hyperlipidemia. According to *Greten et al.*¹, it can be considered that lipoprotein lipase activity is the summation of the activities of the different enzymes, namely, triglyceride lipase (TGL), diglyceride lipase (DEL), monoglyceride hydrolase (MGH) and so on.

MATERIALS AND METHODS

Experimental 1:

The subjects for the study was the hyperlipidemic patients with various disorders. The patients were considered hyperlipidemic, when either the serum total cholesterol was above 230 mg/dl or serum triglyceride, above 180 mg/dl. Serum lipoproteins were fractionated by cellulose acetate and polyacrylamide gel electrophoresis, and the patients were classified according to the criteria of WHO. Blood samples were taken 10 min after the i.v. injection of 10 u/kg b.w. of heparin and plasma was obtained by the centrifugation. The plasma lipoprotein

lipase activity was measured by the following method (Table I). Plasma was incubated with ³H-glyceryl trioleate, emulsified with Triton X-100 in Tris-HCl buffer, pH8.5, at 27°C for 60 min. After the incubation, lipids were extracted and separated into tri-, di-, monoglyceride and free glycerol fractions, and the radioactivities in each fraction were determined. LPL activity was calculated by the sum of the released FFA from the whole procedure and TGL activity was calculated by the release of FFA from triglyceride.

MGH activity was measured by the method shown in Table II. Plasma was incubated with ³H-glyceryl monooleate, emulsified with Triton

TABLE I MEASUREMENT OF LIPOPROTEIN LIPASE ACTIVITY

<i>Incubation Mixture:</i>	
5 μmoles ³ H-glyceryl trioleate (final conc. 2.5 mM)	
0.05 ml 1% Triton X-100	
0.15 ml 1% FFA-poor bovine serum albumin	
0.15 ml plasma	
1.45 ml 0.2M Tris-HCl buffer, pH 8.5	
+0.2 ml postheparin plasma	
<i>Incubation at 27°C for 60 min</i>	
<i>Extraction of Lipids:</i>	
3 vol. of hexane-ether-ethanol (1 : 1 : 1, by vol.)	
<i>TLC:</i>	
Kieselgel G plate	
Developing solvent; hexane-ether-methanol-acetic acid (90 : 20 : 3 : 2, by vol.)	
<i>Determination of radioactivities in TG, DG, MG and glycerol fractions</i>	
<i>Calculation of Free Fatty Acids:</i>	
$1/3 \times (3 \times \text{glycerol} + 2 \times \text{monoglyceride} + 1 \times \text{diglyceride})$	

Key Words:

Hyperlipidemia
Lipoprotein lipase
Monoglyceride hydrolase
Liver origin

Tokyo Metropolitan Geriatric Hospital, Tokyo

Gist of this paper was presented at Symposium on "Circulatory Disease and Hyperlipidemias" at the 38th Annual Meeting of the Japanese Circulation Society in Yamaguchi, April 7, 1974.

Fig.1. LPL activity in Hyperlipidemias.

Relationship between LPL and MGH Activities

Fig.2. Relationship between LPL and MGH activities.

Relationship between Serum TG and LPL Activity

Fig.3. Relationship between serum TG and LPL activity.

TABLE II MEASUREMENT OF MONOGLYCERIDE HYDROLASE ACTIVITY

Incubation Mixture:

$\left\{ \begin{array}{l} 4 \mu\text{moles } ^3\text{H-glycerol monooleate (final conc. 4 mM)} \\ 0.9\text{ml } 0.04\% \text{ Triton X-100 in } 0.2\text{M Tris-HCl buffer,} \\ \text{pH } 9.0 \\ +0.1\text{ml post-heparin plasma} \end{array} \right.$

Incubation at 37°C for 60 min

Extraction of Lipid:

3 vol. of hexane-ether-ethanol (1 : 1 : 1, by vol.)

Determination of radioactivities in the upper phase (MG) and in the lower phase (free glycerol).

X-100 in Tris-HCl buffer, pH 9.0, at 37°C for 60 min. Lipids were extracted and fractionated into monoglyceride and free glycerol fractions. MGH activity was calculated by the radioactivities in these fractions.

Experimental 2:

Male Wistar rats, 200 g b.w., were injected i.v. with 200 u/kg heparin and blood samples were taken 10 min after the injection. Liver, epididymal adipose tissue, heart and kidney were

obtained immediately after the collection of blood samples, and then slices were obtained from each of these tissues. Approximately 1 g of these slices were incubated with or without 0.4 u/ml of heparin in Krebs-Ringer phosphate buffer, pH 7.0, at 37°C for 60 min, and MGH activities in the medium were measured.

RESULTS AND DISCUSSION

As shown in Table III, plasma LPL, TGL and MGH activities in the aged normal subjects were

TABLE III POST-HEPARIN LIPOLYTIC ACTIVITIES IN HYPERLIPEMIAS

Group	No.	Age	LPL	TGL	MGH
<i>mμmoles/min/ml plasma</i>					
Young control	24	28.9	82.6 ± 12.8	43.9 ± 5.5	380.8 ± 86.5
Aged control	33	65.0	70.9 ± 13.1 (<i>p</i> < 0.01*)	36.2 ± 6.6 (<i>p</i> < 0.01*)	281.6 ± 91.4 (<i>p</i> < 0.01*)
Type IIa	44	66.7	48.4 ± 19.6 (<i>p</i> < 0.01**)	24.5 ± 10.2 (<i>p</i> < 0.01**)	206.5 ± 95.8 (<i>p</i> < 0.01**)
Type IIb	20	62.6	43.2 ± 14.1 (<i>p</i> < 0.01**)	21.6 ± 7.2 (<i>p</i> < 0.01**)	226.8 ± 96.2 (NS**)
Type IV	27	69.1	38.8 ± 17.2 (<i>p</i> < 0.01**)	19.7 ± 8.5 (<i>p</i> < 0.01**)	204.6 ± 97.0 (<i>p</i> < 0.01**)
Type III	1	72	26.2	13.0	271.6
Type V	1	21	21.3	12.8	267.4

* vs. Young control, ** vs. Aged control

TABLE IV HEPARIN-INDUCED MGH ACTIVITY IN RAT TISSUES

<i>Heparin not injected rats</i>					
Tissue	No.		Heparin (-)	Heparin (+)	Significance
Plasma	5	0.09 ± 0.03*			
Adipose tissue	5		0.54 ± 0.15	0.64 ± 0.17	NS
Heart	5		0.59 ± 0.32	0.70 ± 0.13	NS
Kidney	5		4.37 ± 0.27	5.62 ± 0.74	<i>p</i> < 0.01
Liver	5		10.93 ± 2.13	16.61 ± 1.92	<i>p</i> < 0.01
<i>Heparin injected rats</i>					
Tissue	No.		Heparin (-)	Heparin (+)	Significance
Plasma	5	1.12 ± 0.06			
Adipose tissue	5		0.55 ± 0.25	0.45 ± 0.06	NS
Heart	5		0.47 ± 0.19	0.73 ± 0.30	NS
Kidney	5		4.40 ± 1.19	3.75 ± 1.23	NS
Liver	5	12.52 ± 4.49	11.66 ± 2.91	11.66 ± 2.91	NS

* mean ± S.D.

μmoles/min/g wet wt. tissue or ml plasma

significantly lower than those in the young normal subjects. Furthermore, these enzyme activities in the hyperlipidemic patients were significantly less than those in the aged normal subjects. Contrary to the previous findings, plasma LPL activity in Type IIa hyperlipidemia was significantly lower than that in the normal subjects. The reason for this discrepancy is not clear. However, it is possible that the cholesterol

concentration in the plasma may influence on the assay results, since according to Fielding² cholesterol or low-density lipoprotein is known to inhibit the LPL activity. LPL activities in the patients with various types of hyperlipidemia with ischemic ST changes were significantly decreased than those of the hyperlipidemic patients without ischemic ST changes. With regard to MGH activity, no such difference was

found between hyperlipidemic patients with ischemic ST change and those without. There was no correlation between LPL or MGH activities and the degree of obesity. As shown in Fig. 2, there was a high correlation between plasma LPL and MGH activities in these patients. There was a negative correlation between plasma LPL activity and serum triglyceride content in these patients (Fig. 3). This correlation becomes much more marked when the patients with Type IIa hyperlipidemia was excluded. Our present results are inconsistent with those of Huttunen and Nikkilä³ who could not find any correlation between plasma LPL and triglyceride, when they used artificial substrate for the enzyme assay. With regard to the plasma MGH activity and triglyceride, there was no correlation between these two factors.

Recently, it was reported by LaRosa *et al.*⁴ that post-heparin lipolytic activity was mainly of liver origin. However, scarcely any reports are available on the origin of post-heparin MGH activity and its characteristics. In rats, in which heparin was previously injected, the release of MGH into the medium after the incubation of various tissues with or without heparin was not significantly different from each other. On the other hand, in control rats, the release of MGH into the medium after the incubation of kidney and liver with heparin was significantly greater than those without heparin. These results suggest

that post-heparin MGH is mainly of liver and kidney origins, and furthermore because of its heavier weight, liver may be more important than the kidney as a site of production of post-heparin MGH.

Subsequently, the characteristics of post-heparin MGH and pre-heparin MGH were compared. The pH optimum of post-heparin MGH was 9.5–10.0, whereas that of pre-heparin MGH was 9.0. Although pre-heparin MGH was markedly activated by the addition of low concentration of sodium deoxycholate, post-heparin MGH was inhibited by the same agent. These results suggest that pre- and post-heparin MGH may be different from each other.

REFERENCE

1. GRETEN, H., LEVY, R. I. & FREDRICKSON, D. S.: Evidence for separate monoglyceride hydrolase and triglyceride lipase in post-heparin human plasma. *J. Lipid Res.* 10: 326–330, 1969.
2. FIELDING, C. J.: Human lipoprotein lipase. Inhibition of activity by cholesterol. *Biochim. Biophys. Acta* 218: 221–226, 1970.
3. HUTTUNEN, J. K. & NIKKILÄ, E. A.: Postheparin plasma lipase in human subjects. Studies with chylomicrons, very-low density lipoproteins and a fat emulsion. *Europ. J. Clin. Invest.* 3: 483–490, 1973.
4. LAROSA, J. C., LEVY, R. I., WINDMUELLER, H. G. & FREDRICKSON, D. S.: Comparison of the triglyceride lipase of liver, adipose tissue, and postheparin plasma. *J. Lipid Res.* 13: 356–363, 1972.