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Hepatic Triglyceride and Lipoprotein Lipase Activities of Post-heparin Plasma in Normals and Hypertriglyceridemics

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ABSTRACT

Post-heparin plasma hepatic triglyceride lipase (H-TGL) and lipoprotein lipase (LPL) activities were 8-34 and 3.5-21 (range) µmol/ml/hr respectively in males with normal serum lipid concentrations. In females the corresponding values were 4-25 and 4-16 µmol/ml/hr. No significant differences were observed between ages or between the two sexes.

Male patients with hypertriglyceridaemia had similar activities of H-TGL but significantly lower values for LPL activities than the control males.

Significant linear correlations were found between serum triglyceride concentrations and LPL activities both for males (coefficient of correlation = r = -0.64) and for females (r = -0.62).

INTRODUCTION

Several studies (1-3) have demonstrated that the serum triglyceride (S-TG) concentrations in man during fasting conditions to a great part are determined by the removal process of TG rich S-lipoproteins - very low density lipoproteins (VLDL) and chylomicrons - from the blood stream. This removal process is thought to be mediated essentially by lipoprotein lipase (4). In man the activity of this enzyme has been demonstrated in adipose (5-6) and muscle tissue (7). Although the most common way to determine this enzyme activity in man has been in post-heparin plasma (4). However, recently post-heparin lipolytic activity (PHLA) has been shown to contain at least two triglyceride lipase activi-

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ties*. One probably originating from liver and the other from extrahepatic tissues (8). A method for isolation from post-heparin plasma, these two triglyceride lipase activities and for quantitative determination of the two enzyme activities separately has previously been described (9). This method includes affinity chromatography where the enzymes are partly purified to avoid interference from serum lipoproteins and apo-lipoproteins. The present report demonstrates a clinical application of this method in which human subjects with normal serum lipid concentrations and male patients with hypertriglyceridaemia have been investigated.

METHODS

Subjects and experimental procedure

The subjects were 42 males between 20 and 81 years and 38 females between 12 and 78 years of age. In both these groups all subjects had normal S-TG <2.48 mmol/l (220 mg/100 ml) and cholesterol <7.8 mmol/l (300 mg/100 ml) concentrations. The subjects were recruited either from the laboratory personel or from volunteers in the community. The latter subjects participated in a pilot study done prior to a health screening survey. All were free living and apparently healthy. They had normal fasting blood sugar and urine analyses. None were on prescription except for digoxin medications. The male patients with hypertriglyceridaemia were those referred to the Lipid Research Clinic or the metabolic unit at the Veterans Administration Hospital, San Diego. No patients with diabetes mellitus, hypothyroidism, liver or renal disease were included in the study.

The procedure was performed in the morning after over-night fast. Firstly the subjects were weighed and a short medical history was recorded. A scalp vein needle was introduced into an anticubital vein and a venous blood sample of 15 ml was taken into EDTA glas tubes. Immediately thereafter 60 IU per kg body weight of heparin (Riker laboratories, 5000 IU/ml) was injected intravenously through the scalp vein needle followed by about 10 ml of saline wash. Fifteen minutes after the heparin injection the post-heparin sample was withdrawn (30 ml of blood). All blood samples were kept on ice for 1 to 2 hours before centrifugation.

Analytical methods

Blood samples were centrifuged at 5000 g x min and the blood plasma was recovered into capped vials. The preheparin samples were kept for 1 to 36 hours at 4°C before serum lipid and lipoprotein analyses were done. Post-heparin plasma samples were frozen in 6 ml aliquots at -70°C for not more than one month

*In this paper these two lipase activities are called hepatic triglyceride lipase (H-TGL) activity and lipoprotein lipase (LPL) activity.

Table 1. Weight/height index, serum triglyceride (TG) and cholesterol concentrations, beta- and alfa-lipoprotein cholesterol concentrations and hepatic triglyceride (H-TGL) and lipoprotein lipase (LPL) activities in normotriglyceridaemic females.

Subjects Age ranges years	(n)	Weight/ Height index*	Serum TG concentration mmol/l**	Serum cholesterol concentration mmol/l*	Beta-lipoprotein cholesterol estimate mmol/l*	Alfa-lipoprotein cholesterol estimate mmol/l*	H-TGL activity umol/ ml/hr**	LPL activity umol/ ml/hr**
<30	(9)	0.84±0.05	0.69 (0.63-0.75)	4.3±0.3	2.2±0.2	1.7±0.2	16.2 (14.5-18.1)	7.4 (6.6- 8.4)
31-45	(13)	0.90±0.04	0.82 (0.75-0.92)	5.0±0.3	2.8±0.3	1.6±0.2	12.9 (11.6-14.3)	7.4 (6.6- 8.4)
46-60	(5)	0.98±0.04	1.29 (1.05-1.58)	5.9±0.5	3.7±0.3	1.8±0.3	9.1 (7.2-11.4)	6.1 (5.4- 7.0)
>60	(11)	0.98±0.03	0.98 (0.92-1.06)	6.1±0.2	3.9±0.2	1.8±0.1	14.8 (13.4-16.4)	9.2 (8.3-10.2)
Total	(38)	0.91±0.02	0.88 (0.84-0.94)	5.3±0.2	3.2±0.2	1.7±0.1	13.5 (12.7-14.5)	7.7 (7.3- 8.2)

*=Mean value ± standard error of the mean are given. **=Antilogarithm of mean value and within brackets antilogarithm of mean value ± standard error of the mean are given. n=number of subjects within each group.

before determination of H-TGL and LPL activities were done. Serum lipids and lipoproteins were determined according to the standardized methods routinely used at the Lipid Research Clinic Laboratory (10).

Isolation of H-TGL and LPL activities from postheparin plasma were done with affinity chromatography on sepharose covalently linked with heparin (11) according to a method previously described in detail (9). Aliquots of the enzyme fractions recovered from the chromatography were incubated with a labelled triglyceride emulsion (8). Labelled free fatty acids released during incubation for 30 minutes were measured (12). Lipase activity was expressed as umoles fatty acids released per ml of post-heparin plasma per hour of incubation. During the whole investigation a frozen control post-heparin plasma sample was run to check for the interday variations of the procedures. Statistical analyses were done according to Snedecor (13).

RESULTS

Postheparin triglyceride lipase activity in normal subjects.

Results obtained in normal subjects, males and females, are presented in tables 1 and 2 respectively.

The females were not obese and the average S-TG concentration was 0.88 mmol/l with no significant trend of either increase or decrease with age. S- cholesterol concentration (both total and in beta-lipoproteins) showed the increase with age which has been demonstrated several times before. Mean value of the whole group was 5.3 ± 0.2 mmol/l for total and 3.2 ± 0.2 mmol/l for beta-lipoprotein cholesterol concentration.

H-TGL activities of the younger females (<30 years) were on the average higher than for the groups of older people. However this difference was not significant. No significant change with age was demonstrated for LPL activity. Mean values of H-TGL and LPL activities for normal females were 13.5 and 7.7 umoles/ml/hr respectively.

The normal male subjects were slightly heavier than the females. Similar concentrations of S-TG and total and beta-cholesterol were found for males and females. Alfa-lipoprotein cholesterol concentrations were slightly lower in the males compared to the females. H-TGL and LPL activities did not change with age in the normal males. The mean values were 19.6 and 6.6 umol/ml/hr

Table 2. Weight/height index, serum triglyceride (TG) and cholesterol concentrations, beta- and alfa-lipoprotein cholesterol concentrations and hepatic triglyceride (H-TGL) and lipoprotein lipase (LPL) activities in normotriglyceridaemic males.

Subjects Age ranges years	(n)	Weight/ Height index*	Serum TG concen-tration mmol/l**	Serum chol-esterol concentra-tion mmol/l*	Beta-lipo-protein choleste-rol esti-mate mmol/l*	Alfa-lipo-protein choleste-rol esti-mate mmol/l*	H-TGL activity umol/ ml/hr**	LPL activity umol/ ml/hr**
<30	(11)	0.87±0.02	0.95 (0.88-1.04)	4.5±0.2	2.6±0.4	1.4±0.1	19.4 (17.8-21.2)	6.3 (5.7-6.8)
31-45	(9)	1.03±0.05	1.03 (0.87-1.21)	5.0±0.2	3.4±0.3	1.3±0.1	19.2 (16.9-21.8)	6.4 (5.5-7.3)
46-60	(9)	1.03±0.03	1.03 (0.88-1.21)	5.5±0.5	3.7±0.5	1.3±0.2	20.9 (18.3-23.8)	6.2 (5.6-6-9)
>60	(13)	1.03±0.03	1.02 (0.92-1.13)	5.2±0.2	3.2±0.2	1.5±0.1	17.8 (16.4-19.5)	7.3 (6.5-8.2)
Total	(42)	0.99±0.02	0.99 (0.95-1.07)	5.0±0.2	3.3±0.2	1.4±0.1	19.6 (18.6-20.4)	6.6 (6.2-6.9)

*=Mean value ± standard error of the mean are given. **=Antilogarithm of mean value and within brackets antilogarithm of mean value ± standard error of the mean are given. n=number of subjects within each group.

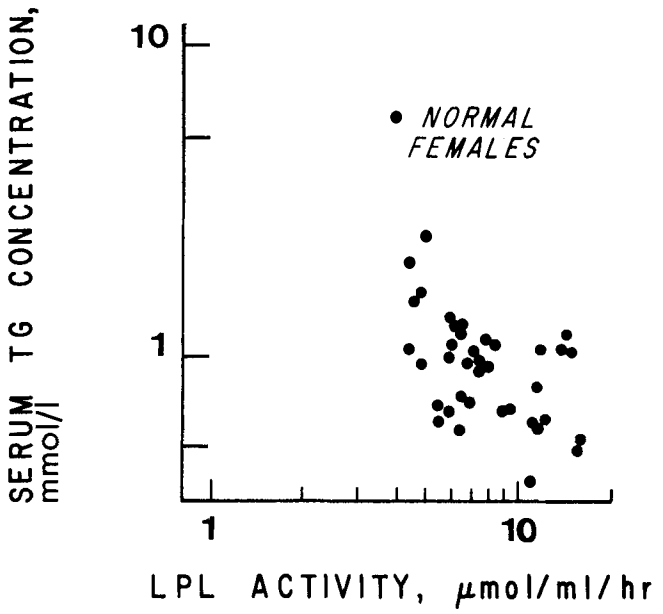

Fig.1. Relationship between post-heparin lipoprotein lipase (LPL) activity and serum triglyceride (TG) concentration in healthy females with "normal" serum lipid values. Correlation analysis of logarithmic values of both parameters resulted in r=-0.62, p<0.001.

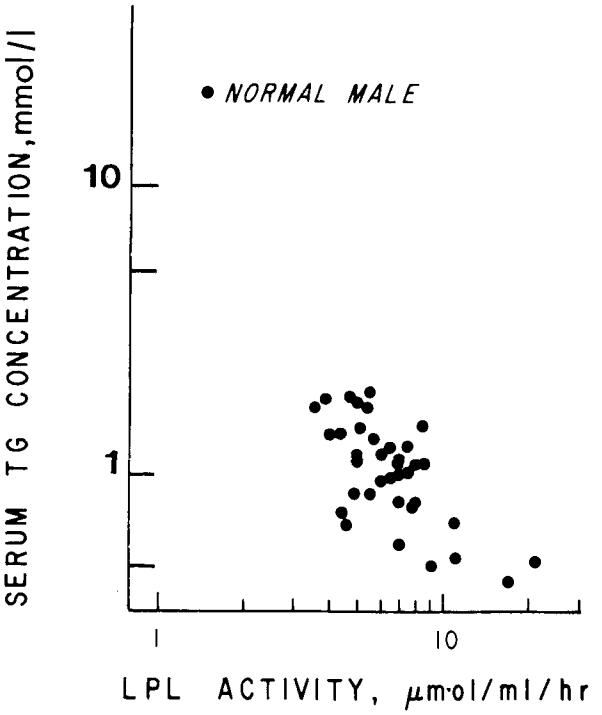

Fig. 2. Relationship between post-heparin lipoprotein lipase (LPL) activity and serum triglyceride (TG) concentration in healthy males with "normal" serum lipid values. Correlation analysis of logarthmic values of both parameters resulted in r=-0.64, p<0.001.

respectively. These values for H-TGL activity are significantly higher for the males than for the females, while no significant difference of LPL activity was found between the sexes.

Postheparin triglyceride lipase activities in patients with hypertriglyceridemia

Only male patients with hypertriglyceridemia were investigated. Results for a comparison between normal and hypertriglyceridemicmales are summarized in table 3 . Naturally serum TG and cholesterol concentrations were higher in the hypertriglyceridemics compared to the controls. Furthermore the hypertriglyceridemics were heavier and had significantly lower alfa-lipoprotein cholesterol concentrations. H-TGL activity was on the average significantly higher in the patients with elevated S-TG concentrations than the controls, showing mean values of 22.8 compared to 19.6 umol/ml/hr for the controls. LPL activities on the other hand were significantly ($p < 0.001$) lower in the patients with hypertriglyceridemia compared to the controls. The mean values were 4.7 for hypertriglyceridemics compared to 6.6 umol/ml/hr for the male controls.

Relationship between S-TG concentrations and H-TGL and LPL activities

No significant correlations were found between H-TGL activity and concentrations of any serum lipid or lipoprotein fraction.

Between S-TG concentrations and LPL activities significant correlations were found for both female and male normotriglyceridemics (figures 1 and 2). The correlation coefficients were $r = -0.62$ and $r = -0.64$. As shown in figure 3 about one third of the male patients with hypertriglyceridemia fell outside the range of LPL activity for the controls. However, for the rest of the patients there were overlap of the data.

As demonstrated in figure 4 no significant correlations occurred between the two post-heparin triglyceride lipase activities. The two enzyme activities seem to vary quite independently in both the female and male controls and also in the patients with high S-TG concentrations.

Table 3. Antropometric data, serum lipids, serum lipoproteins and post-heparin hepatic (H-TGL) and lipoprotein lipase (LPL) activities in male normo- and hypertriglyceridaemics.

	Age years*	Weight / Height index*	Serum TG concentration mmol/l**	Serum CHOL concentration mmol/l*	Beta-lipoprotein CHOL estimate mmol/l*	Alfa-lipoprotein CHOL concentration mmol/l*	H-TGL activity umol/ml/hr**	LPL activity umol/ml/hr**
Normotriglyceridaemics n=42	46.9±2.9	0.99±0.02	0.99 (0.95-1.07)	5.0±0.2	3.3±0.2	1.4±0.1	19.6 (18.6-20.4)	6.6 (6.2-6.9)
Hypertriglyceridaemics n=33	48.2±1.7	1.13±0.02	6.07 (5.14-7.18)	6.4±1.6	3.1±0.2	0.9±0.1	22.8 (21.5-24.3)	4.7 (4.4-5.0)
t-value	0.36	4.36	15.72	4.64	-0.45	-2.23	2.39	-3.86
Statistical significance	p>0.05	p<0.001	p<0.001	p<0.001	p>0.05	p<0.05	p<0.05	p<0.001

*=Mean value ± standard error of the mean are given. **=Antilogarthm of the mean value and within brackets antilogarithm of mean value ± standard error of the mean are given. n=number of subjects within each group.

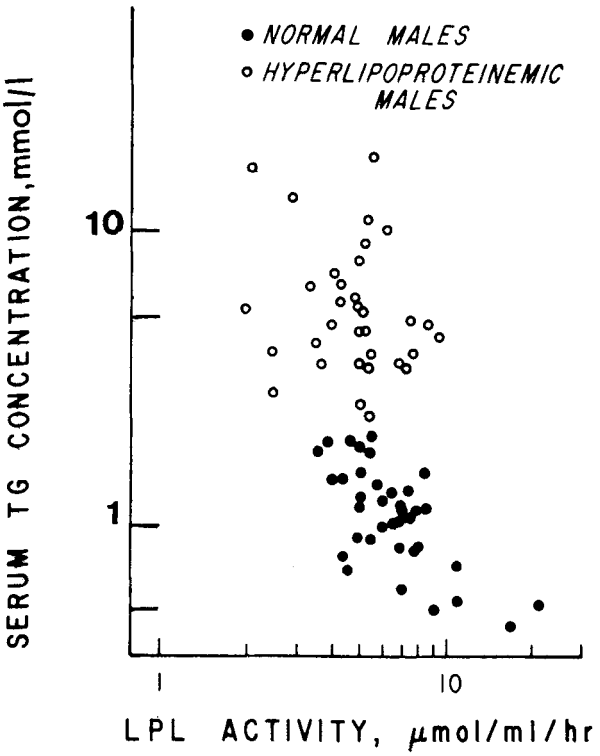

Fig. 3. Relationship between post-heparin lipoprotein lipase (LPL) activity and serum triglyceride (TG) concentration in normotriglyceridemic and hypertriglyceridemic males. Further data of the former group see under figure 2.

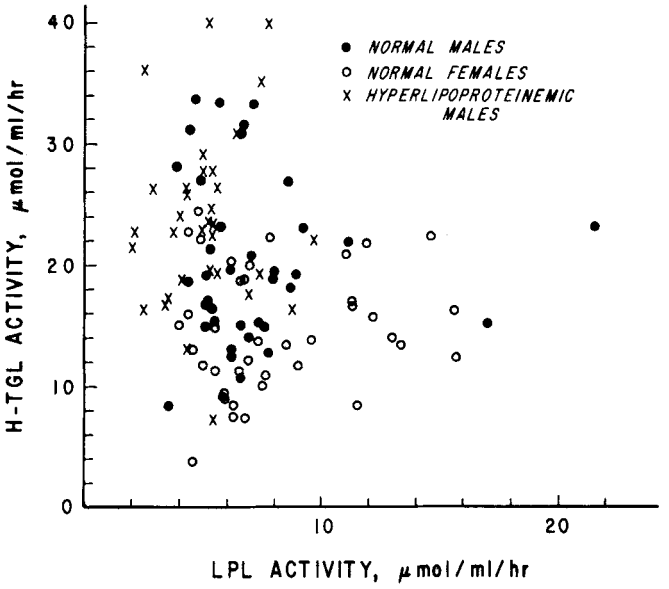

Fig. 4. Relationship between lipoprotein lipase (LPL) activity and hepatic triglyceride lipase (H-TGL) activity.

DISCUSSION

The present study reports on a clinical application of a method which quantitatively determines two different TG lipase activities in post-heparin plasma. The main advantage with the method used (9) compared to other methods described (14-16) is that no interference with plasma components like apolipoproteins or whole lipoproteins can occur. Two other methods have been described to estimate H-TGL and LPL activities in human post-heparin plasma (14-16). One of them using an antibody against H-TGL activity which is aimed to completely abolish the contribution of this enzyme to whole TG lipase activity in post-heparin plasma (15,16). The other method is based on the suggestion that H-TGL activity is not inhibited while LPL activity is completely inhibited by protamin-sulphate (14). In both these methods whole post-heparin plasma from the investigated patient is present during the assay, which means presence of the patient´s own apoproteins an lipoproteins which might serve as sustrate for the enzymes competing with the exogenous substrate added to the assay. There might also be inhibitors present in the whole post-heparin plasma (17). Thus the present method seems to have one advantage compared to previously described methods in quantitating H-TGL and LPL in human post-heparin plasma. However, the importance of this aspect is not fully evaluated since the in vitro addition of serum lipoproteins did not change the estimated activities of either H-TGL or LPL in the two methods described earlier (14,15).

The application of this method in normal males and females and in male patients with hypertriglyceridemia demonstrated no relationship at all between the two post-heparin TG activities H-TGL and LPL. This observation is an additional piece of evidence indicating that these two enzyme activities really are two different enzymes. This has recently been demonstrated in a study where the two enzyme proteins have been purified and characterized (18). H-TGL activity was higher in normal males compared to females, and also higher in normal males compared to male patients with hypertriglyceridemia. None of the patients or subjects were missing the H-TGL activity and we have found no evidence for a relationship between this enzyme activity and any physiological or pathophysiological condition. Others have reported, however, that this enzyme activity might be low in patients with hypothyroidism (14).

LPL activities in normal males and females occurred within the same ranges and there was no age dependence demonstrable. The range of activity presented here is slightly higher than described by Krauss et al (14), who used the method dependent on complete protamine-sulphate inhibition of LPL activity. The latter authors also found an age dependent LPL activity in females. A decrease of LPL activity with age in both males and females was demonstrated by Huttunen et al (19) who used the selective immunochemical method (15). These

authors also demonstrated higher absolute values than presented in this study for both H-TGL and LPL activities (19). The reason for this difference is not known but might be explained either by differences in substrate preparation or differences in the recovery of the enzyme isolation procedures. The isolation procedure used in the present paper probably gives a lower yield than the immunochemical method.

There were significant correlations between LPL activities and S-TG concentrations both for normal females and normal males. This is in agreement with two earlier studies (19,20) where different but still rather specific methods have been used to quantitate LPL activities. Earlier it has been shown that there is no correlation between endogenous S-TG production ("turnover") and S- TG concentration in males with hypertriglyceridemia (3). This finding may indicate that the level of S-TG concentration in normals generally are determined by the rates of clearance of S-TG (or by the fractional removal rate) evidence for which has been gained earlier with other methods (3,4). Since the enzyme LPL most probably plays an important role in the removal process of the S-TG the significant correlations found in this study between LPL activities and S-TG concentrations fit well with previous studies on serum TG turnover.

Earlier studies on post-heparin LPL (20) and tissue LPL activities (21) have reported significantly lower values in patients with hypertriglyceridemia. However, the range of values measured for the LPL activities in these patients overlaps that from analyses of subjects with normal serum TG concentrations. Overlapping of activities in these two groups occurred also in this report, however, this was slightly less pronounced. LPL activities were significantly lower in patients with hypertriglyceridemia. In about one third of the patients the low LPL activities might be the cause of the hypertriglyceridemia while in the rest of the patients contribution of other factors must be added to explain the cause of the hypertriglyceridemia.

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