Measurement of the Serum Lipoprotein Lipase Concentration Is Useful for Studying Triglyceride Metabolism: Comparison With Postheparin Plasma

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The catalytically inactive form of lipoprotein lipase (LPL) is detectable at high levels in serum, although its physiologic role remains unknown. The aim of this study was to elucidate the clinical significance of serum LPL compared with postheparin LPL or the net increment (Δ) of LPL (postheparin − preheparin LPL). We measured the LPL mass before and 15 minutes after the injection of heparin in 164 subjects with hyperlipidemia. LPL mass was measured by a sensitive sandwich enzyme-linked immunosorbent assay (ELISA). Serum LPL was one fifth of the postheparin LPL concentration. There was a weak correlation between the serum LPL and postheparin LPL concentrations ($r = .225$, $P \leq .005$). The Δ LPL concentration was strongly related to the postheparin LPL concentration ($r = .965$, $P \leq .0001$), but not to the preheparin LPL mass, suggesting that the weak correlation between serum LPL and postheparin LPL levels was attributable to contamination of postheparin plasma by pre-existing LPL (preheparin LPL). Both serum and postheparin LPL were significantly lower in diabetic patients and in subjects with high levels of triglyceride or low levels of high-density lipoprotein (HDL). Serum LPL was correlated negatively with triglyceride, remnants, and insulin resistance and was positively correlated with HDL cholesterol and low-density lipoprotein (LDL) size. Postheparin LPL was strongly correlated with HDL cholesterol, but not with other parameters, as was serum LPL. Δ LPL mass did not show a closer association with triglyceride metabolism than postheparin LPL or preheparin LPL. In conclusion, serum LPL measurement is simple and seems to be useful for studying triglyceride metabolism.

LIPOPROTEIN LIPASE (LPL) is a key enzyme in triglyceride-rich lipoprotein metabolism that has lipolytic activity and promotes hepatic uptake of lipoprotein particles.[1-4] A high level of LPL mass, the catalytically inactive form, is detectable in nonheparinized serum,[5] although its physiologic role remains unknown. There have been several reports that the serum LPL mass shows a significant association with the plasma triglyceride or high-density lipoprotein (HDL) cholesterol concentrations.[6-8] Shirai and colleagues[6] demonstrated that the preheparin plasma LPL mass is decreased in hypertriglyceridemic[6] or diabetic subjects[9] and is increased by the administration of bezafibrate[10] or insulin,[9] which are known to stimulate LPL synthesis. There have been some reports that serum LPL concentration is very low or not detectable in patients with LPL deficiency.[11,12] These data suggest that although serum LPL is catalytically inactive, its mass reflects the level of systemic LPL biosynthesis. However, it remains unclear whether serum LPL mass is closely associated with lipoprotein metabolism in a way similar to postheparin plasma LPL.

Measurement of the postheparin LPL activity or mass is a standard method for evaluating this enzyme, but it is not performed as a routine clinical examination, because it requires heparin injection and a fairly long waiting time (10 to 15 minutes) before harvesting the postheparin plasma sample. Therefore, it is attractive to explore the clinical significance of the serum LPL concentration. In the present study, we investigated the relationship between serum LPL and various parameters associated with the action of this enzyme and compared the results with data on postheprin LPL.

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Submitted July 29, 2003; accepted October 7, 2003.

Supported in part by a grant-in-aid (No. 14571109) from the Japanese Ministry of Education, Science, and Culture.

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0026-0495/04/5304-0034$30.00/0

doi:10.1016/j.metabol.2003.10.021

MATERIALS AND METHODS

Subjects

We enrolled 164 hypercholesterolemic subjects with a total cholesterol level greater than 220 mg/dL detected by routine medical checkups, including type 2 diabetic patients. All subjects were essentially healthy and were not suffering from liver, kidney, or heart disease, infections, or cancer. They were treated with diet plus a statin for at least 3 months (n = 84) or else were treated by diet alone without any lipid-lowering agent (n = 80). The statins used for treatment consisted of atorvastatin (n = 31, 10 mg/d), pravastatin (n = 27, 10 to 20 mg), simvastatin (n = 15, 10 mg/d), and fluvastatin (n = 11, 20 mg/d). In preliminary experiments, we found that statins did not alter the LPL mass in serum or postheparin plasma. Forty patients with type 2 diabetes included in this study were treated with diet alone or with diet plus sulfonylureas, α-glucosidase inhibitors, or metformin. Patients treated with insulin or thiazolidinediones were excluded. Blood samples were collected at least 3 months after starting treatment with diet or statin therapy.

Measurement of LPL

Serum LPL mass and postheparin LPL mass were measured by the sensitive sandwich enzyme-linked immunosorbent assay (ELISA). ELISA, originally described by Kobayashi et al,[13] is commercially available as a test kit (LPL-ELISA Daiichi; Daiichi Chemical, Tokyo, Japan) that contains polyclonal and monoclonal antibodies against bovine milk LPL. The lower limit of detection was 0.5 ng/mL, while the interassay coefficient of variation (CV) was 3% and the intra-assay CV was 6%. Postheparin plasma samples were taken 15 minutes after heparin injection (30 U/kg). Changes of LPL mass before and after heparin injection were assessed, and there was an excellent correlation between LPL mass and activity in postheparin plasma as reported elsewhere.[13]

Table 1. General Characteristics of Total Subjects

	Male (n = 96)	Female (n = 68)	*P* Value
Age (yr)	52 ± 12	56 ± 7	<.02
Body mass index	24.6 ± 3.8	23.7 ± 3.6	NS
Preheparin LPL mass (ng/mL)	48 ± 21	67 ± 21	<.0001
Postheparin LPL mass (ng/mL)	242 ± 82	280 ± 98	<.01
Δ-LPL mass (ng/mL)	197 ± 81	212 ± 93	NS
Triglyceride (mg/dL)	182 ± 122	114 ± 54	<.0001
HDL-C (mg/dL)	54 ± 12	61 ± 10	<.0005
Total-C (mg/dL)	218 ± 44	222 ± 39	NS
LDL-C (mg/dL)	145 ± 48	153 ± 39	NS
Statin user* (n (%))	54 (56)	30 (44)	NS
Diabetes (n (%))	32 (33)	9 (13)	<.005

NOTE. Data represent mean ± SD.

Abbreviations: NS, not significant ($P > .05$); C, cholesterol; Δ-LPL mass, postheparin LPL minus preheparin LPL.

*Atorvastatin 10 mg (n = 31); pravastatin 10 to 20 mg (n = 27); simvastatin 5 to 10 mg (n = 15); fluvastatin 20 to 40 mg (n = 11).

Other Measurements

All measurements, including LPL mass, were performed in the morning after an overnight fast. Very–low-density lipoprotein (VLDL) (density <1.006 g/mL) was separated by ultracentrifugation using a Hitachi CP-65G (Hitachi, Tokyo, Japan) with an RP 55T-708 rotor (Hitachi). The average LDL particle diameter was measured by gradient (2% to 16%) denaturing polyacrylamide gel electrophoresis according to the method of Krauss et al.[14] Remnant-like particle (RLP) cholesterol was measured with an affinity column containing anti-apolipoprotein (apo) A1 and B100 monoclonal antibodies.[15] Immunoreactive insulin (IRI) was measured by radioimmunoassay using a commercially available test kit (Shionogi Pharmaceutical, Osaka, Japan). Insulin resistance was estimated by the homeostasis model assessment-insulin resistance (HOMA-IR) as a simple calculation based on fasting insulin and glucose levels (insulin (μU/mL) × glucose (mg/100 mL)/405).[16] Plasma triglyceride, cholesterol, and free fatty acid levels were measured by enzymatic methods. HDL cholesterol was measured in the plasma after polyanion precipitation of apo B-containing lipoproteins. LDL cholesterol was measured directly by the homogenous method, as described previously.[17] Plasma apo AI, B, CII, CIII, and E levels were determined by immunoturbidometric assay (Daiichi Pure Chemicals).

Statistics

Statistical significance was estimated by Student's unpaired *t* test. Correlation coefficients between 2 variables were determined by Pearson's simple linear regression analysis. Stepwise multiple regression analysis was performed to assess the independent association of the valuables. $P < .05$ was considered significant.

RESULTS

Table 1 shows serum and postheparin LPL mass and the plasma lipid profile of 164 hyperlipidemic subjects treated with diet alone or diet plus statins. Half of the subjects were given statins. Serum LPL and postheparin LPL mass were significantly lower in males than in females, and serum LPL mass was about one fifth of the postheparin LPL mass. A significant gender difference was not observed in the case of Δ-LPL mass (postheparin LPL − serum LPL). The serum triglyceride level was significantly higher, and HDL cholesterol was lower in males than in females. When the serum and postheparin LPL mass values were adjusted for the serum triglyceride level, the gender difference of LPL mass was no longer significant (data not shown).

Fig 1 demonstrates the correlation between serum LPL and postheparin LPL concentration in all subjects. There was a weak correlation between these variables ($y = 0.059X + 40.6$, $r = .225$, $P = .0038$). The Δ-LPL concentration was substantially related to the postheparin LPL concentration ($y = 0.941X - 40.6$, $r = .965$, $P < .0001$), but not to the preheparin LPL mass ($r = .12$, not significant [NS]).

Table 2 shows serum LPL, postheparin LPL mass, and the serum lipid profile in subjects with or without type 2 diabetes. The diabetic patients had hyperglycemia with a normal insulin level, so they had a higher HOMA-IR level. Serum triglyceride, total cholesterol, LDL cholesterol, and HDL cholesterol levels were comparable between diabetic and nondiabetic subjects, except that diabetics had higher free fatty acid levels. Serum LPL mass was significantly lower in diabetic patients than in nondiabetic subjects. Postheparin LPL and Δ-LPL mass was also significantly lower in diabetics than nondiabetics. The diabetic patients were treated with diet alone or oral hypoglycemic agents, but we did not observe significant differences of

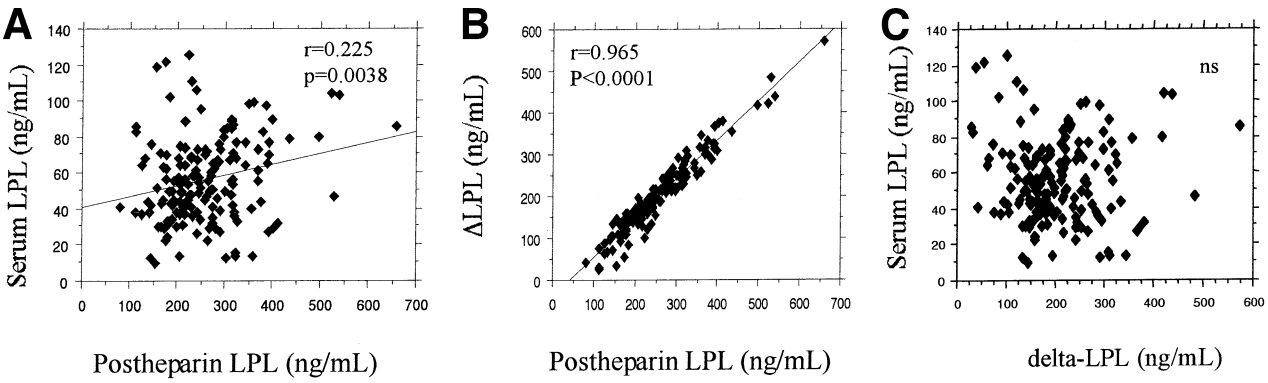

Fig 1. (A) Correlation between serum LPL and postheparin LPL mass. The equation of the linear regression line is y = 0.059X + 40.6, *r* = .225, *P* = .0038. (B) Correlation between Δ-LDL and postheparin LPL mass. The equation of the linear regression line is y = 0.941X − 40.6, *r* = .965, *P* < .0001. (C) There was no correlation between serum LPL and Δ-LPL mass. Δ-LPL = postheparin LPL mass − serum LPL mass.

Table 2. Comparison of Metabolic Parameters Between Diabetic and Nondiabetic Subjects

	Type 2 DM	Non DM	*P* Value
No. (M:F)	41 (32:9)	124 (64:59)	
Age	57 ± 11	52 ± 10	<.0001
Body mass index	23.5 ± 3.0	24.5 ± 4.0	NS
Hemoglobin A_{1c} (%)	8.5 ± 2.4	5.2 ± 0.4	<.0001
Glucose (mg/dL)	165 ± 64	96 ± 9	<.0001
IRI (μU/mL)	5.1 ± 1	4.9 ± 1	NS
HOMA-IR	2.0 ± 1.4	1.2 ± 0.9	<.0001
Triglyceride (mg/dL)	158 ± 86	152 ± 111	NS
Free fatty acid (μmol/L)	775 ± 380	464 ± 213	<.0001
HDL-C (mg/dL)	56 ± 16	57 ± 13	NS
LDL-C (mg/dL)	145 ± 55	150 ± 41	NS
Preheparin LPL (ng/mL)	44 ± 21	60 ± 23	<.0005
Postheparin LPL (ng/mL)	222 ± 78	270 ± 91	<.005
Δ-LPL mass (ng/mL)	177 ± 81	212 ± 87	<.05

Abbreviations: IRI, immunoreactive insulin; DM, diabetes mellitus; NS, not significant; HOMA-IR, homeostasis model assessment-insulin resistance.

serum LPL or postheparin LPL between the treatments (data not shown).

Table 3 shows serum and postheparin LPL concentrations in subjects with or without hypertriglyceridemia (triglyceride >150 mg/dL) or low HDL cholesterolemia (HDL cholesterol <40 mg/dL). Serum LPL mass in subjects with hypertriglyceridemia, low HDL cholesterol, or both was significantly lower than in subjects with normal levels of triglyceride and HDL cholesterol. Subjects with hypertriglyceridemia or low HDL cholesterol levels had a lower postheparin LPL mass than the subjects with normotriglyceridemia and normal HDL cholesterol level. There were no significant differences of Δ-LPL between the subjects with or without hypertriglyceridemia or low HDL cholesterol.

There was a significant inverse correlation between serum LPL mass and triglyceride, but the association between postheparin LPL and triglyceride did not attain statistical significance, as indicated in Table 4. However, because serum triglyceride showed a skewed distribution, logarithmically transformed triglyceride data were used to re-examine the correlation with serum LPL or postheparin LPL. Fig 2 shows a significant correlation between serum LPL and log triglyceride ($r = -.310$, $P < .0001$) (Fig 2A), as well as between postheparin LPL and log triglyceride ($r = .240$, $P = .0019$) (Fig 2B). Stepwise multiple regression analysis revealed that the significant associations of log triglyceride with serum LPL and

Table 4. Correlations of Serum LPL and Postheparin Plasma LPL Concentrations With Various Parameters

	Preheparin LPL Mass	*P*	Postheparin LPL Mass	*P*	Delta-LPL Mass	*P*
Age	−0.105	NS	−0.020	NS	−0.109	NS
Body mass index	−0.285	<.0005	0.021	NS	0.086	NS
Total-C	−0.060	NS	−0.085	NS	0.075	NS
Triglyceride	−0.303	<.0001	−0.064	NS	0.005	NS
HDL-C	0.337	<.0001	0.452	<.0001	0.384	<.0001
LDL-C	−0.065	NS	−0.015	NS	−0.024	NS
RLP-C	−0.185	<.02	−0.027	NS	0.015	NS
VLDL-TG	−0.267	<.001	−0.013	NS	0.052	NS
VLDL-C	−0.082	NS	−0.001	NS	0.020	NS
Apo AI	0.283	<.0005	0.440	<.0001	0.377	<.0001
Apo B	−0.078	NS	−0.069	NS	−0.045	NS
Apo CII	−0.267	<.001	−0.011	NS	0.042	NS
Apo CIII	−0.258	<.001	−0.047	NS	0.099	NS
Apo E	−0.174	<.05	0.159	<.05	0.209	<.01
Mean LDL diameter	0.181	<.02	0.081	NS	0.035	NS
Free fatty acid	−0.379	<.0001	−0.040	NS	0.125	NS
Glucose	−0.231	<.005	−0.094	NS	−0.048	NS
Hemoglobin A_{1c}	−0.187	<.005	−0.176	<.05	−0.148	NS
IRI	−0.165	NS	−0.031	NS	0.0025	NS
HOMA-IR	−0.233	<.01	−0.064	NS	−0.019	NS

NOTE. n = 165.
Abbreviation: NS, not significant.

postheparin LPL were independent of each other (*F* values were 12.7 and 5.6, respectively).

Fig 3 shows the significant correlations ($P < .0001$) between HDL cholesterol and serum LPL ($r = .337$) or postheparin LPL ($r = .464$). The correlation coefficient (*r* value) was stronger for postheparin LPL than serum LPL, but stepwise multiple regression analysis revealed that these significant associations of HDL cholesterol with serum LPL and postheparin LPL were independent of each other (*F* values were 12.3 and 34.6, respectively).

Table 4 shows the simple correlations of serum LPL, postheparin LPL, or Δ-LPL with various parameters in all of the subjects. Serum LPL was negatively correlated with body mass index, serum triglyceride, apo B, CII, CIII, and E, VLDL triglyceride, VLDL cholesterol, and RLP cholesterol, while it was positively correlated with HDL cholesterol, apo A1, and LDL size. In contrast, postheparin LPL did not have a signif-

Table 3. Comparison of Metabolic Parameters Between Dyslipidemic Subjects

	Normo TG With Normo HDL-C	Hyper TG	Low HDL-C	Hyper TG With Low HDL-C
No. (M:F)	107 (49:58)	42 (34:8)	6 (5:1)	9 (8:1)
TG (mg/dL)	102 ± 30	262 ± 117*	113 ± 21	302 ± 143*
HDL-C (mg/L)	61 ± 13	52 ± 7*	38 ± 1*	37 ± 2*
Preheparin LPL mass (ng/mL)	61 ± 22	49 ± 25*	42 ± 25†	40 ± 13*
Postheparin LPL mass (ng/mL)	275 ± 93	239 ± 70†	185 ± 56†	217 ± 93
Δ-LPL mass (ng/mL)	214 ± 87	190 ± 81	144 ± 70	177 ± 103

Abbreviations: TG, triglyceride; hyper TG (>150 mg/dL); low HDL-C (<40 mg/dL). *$P < .01$, †$P < .05$ *v* normo TG with normo HDL-C.

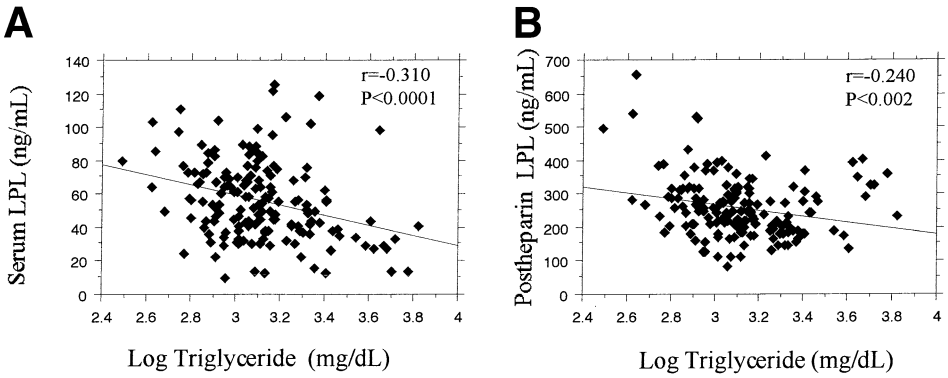

Fig 2. (A) Correlation between serum LPL mass and logarithmically transformed triglyceride levels. (B) Correlation between postheparin LPL mass and logarithmically transformed triglyceride levels.

icant association with serum triglyceride, apoproteins, VLDLs, RLP cholesterol, or LDL size, but there was a stronger inverse association with HDL cholesterol and apo A1. Serum LPL was significantly correlated with free fatty acid, fasting plasma glucose, hemoglobin A_{1c}, and HOMA-IR, whereas postheparin LPL was not. Δ-LPL had similar associations with the various parameters as those found for postheparin LPL.

DISCUSSION

Measurement of LPL activity in postheparin plasma is a standard procedure for the assay of LPL in vivo. However, this activity rapidly decreases at room temperature,[1] which makes it difficult to use for general clinical examination. Furthermore, there are various methods for the measurement of LPL activity, and a standard method has not yet been established. Therefore, the normal/abnormal range has not been decided. Measurement of LPL mass in postheparin plasma overcomes these problems. However, this mass method also needs heparin injection. It is still a matter of debate as to how much heparin should be injected and when the postheparin sample should be taken to obtain the maximum value of LPL mass or activity in postheparin plasma. Heparin injection occasionally promotes bleeding, which might be dangerous for patients with a peptic ulcer or proliferative diabetic retinopathy. Thus, postheparin LPL mass determination also has problems that interfere with becoming a widely used examination.

Nonheparinized serum contains a considerable LPL mass, but the activity of triglyceride hydrolysis is very low. Watson et al[18] tried to measure this low LPL activity by increasing the serum volume and prolonging the incubation time, but they did not find a meaningful association between preheparin LPL activity and lipoprotein concentrations, suggesting that serum LPL does not play a significant role in lipid metabolism through its lipolytic activity. Recent studies have revealed that catalytically inactive LDL can act as a ligand for lipoprotein receptors or glucosaminoglycans in the liver.[2,3,19] Thus, catalytically inactive serum LPL might participate in lipoprotein metabolism via its ligand function. There have been several studies indicating a significant correlation between serum LPL and lipoprotein concentrations.[6-10] Tornvall et al[8] reported that serum LPL mass showed an inverse correlation with triglyceride and a positive correlation with HDL cholesterol in patients with coronary heart disease (CHD), whereas they failed to observe these correlations in control subjects. Watanabe et al[6] and Saito et al[7] did find a significant correlation between serum LPL mass and triglyceride or HDL cholesterol in non-CHD subjects. However, because few studies have involved the measurement of pre- and postheparin LPL mass simultaneously, it is poorly understood how strongly the serum LPL mass is associated with lipoprotein metabolism when compared with postheparin LPL, and whether measurement of serum LPL can be used as a substitute for the postheparin LPL assay. The aim of the present study was to address these issues.

Serum LPL mass and postheparin LPL mass were significantly lower in males than females, which was a finding in agreement with previous reports.[6,7] However, this difference of the LPL mass disappeared when adjusted for triglyceride, sug-

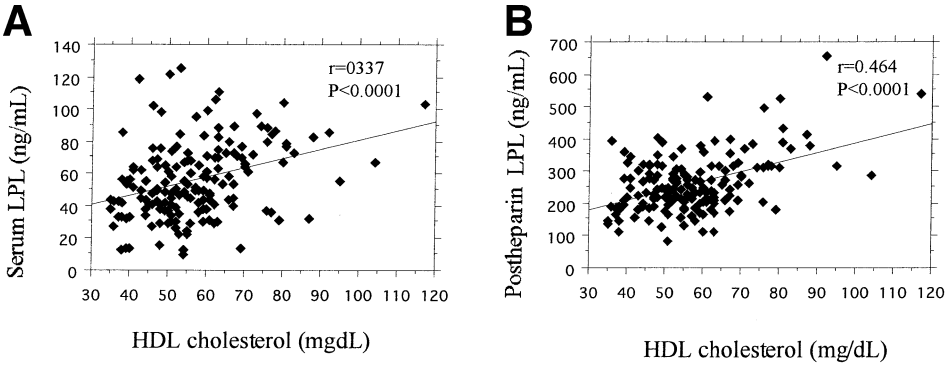

Fig 3. (A) Correlation between serum LPL mass and HDL cholesterol levels. (B) Correlation between postheparin LPL mass and HDL cholesterol levels.

gesting that LPL is closely associated with triglyceride metabolism in both sexes.

The LPL gene has an insulin-sensitive element that promotes LPL biosynthesis,[20] therefore, LPL production is expected to be reduced in type 2 diabetic patients who are characterized by inadequate insulin action. Insulin resistance, as estimated by the HOMA index, was significantly associated with serum LPL, but not with postheparin plasma LPL, suggesting that serum LPL more sensitively reflects insulin-mediated LPL synthesis. Shirai et al[21] demonstrated that troglitazone, an enhancer of insulin action, or insulin injection significantly increase the serum LPL mass. Further studies will be needed to determine whether insulin-mediated LPL production is more clearly detected by assay of serum than postheparin plasma.

As shown in Table 3, the serum LPL concentration was significantly decreased in subjects with hypertriglyceridemia and/or low HDL cholesterol levels. A significant correlation between serum LPL mass and the serum triglyceride or HDL cholesterol levels (shown in Figs 2 and 3) supports the data in Table 3, and suggest that serum LPL is significantly associated with triglyceride metabolism. Postheparin LPL mass was also decreased in subjects with hypertriglyceridemia or low HDL cholesterol levels. However, the correlation of postheparin LPL with triglyceride level was weaker than that of serum LPL mass. There have been a number of studies showing a significant correlation between the plasma triglyceride level and postheparin LPL activity or mass, but the correlations were relatively weak[8,18] and did not far exceed the correlation between serum LPL mass and triglyceride reported elsewhere.[6,7] Tornvall et al[8] reported that postheparin LPL mass was not correlated with VLDL triglyceride in CHD patients, but preheparin LPL mass was. Taken together, it seems that serum LPL concentration is more closely associated with triglyceride than postheparin LPL.

Postheparin LPL was significantly associated with HDL cholesterol levels, showing a stronger correlation coefficient than that of serum LPL. It has consistently been reported that postheparin LPL has a closer correlation with HDL cholesterol than with triglyceride.[8,22] The functionally active LPL released after heparin injection may play a more important role in the production and maturation of HDL than inactive serum LPL. Nevertheless, serum LPL mass was correlated with HDL cholesterol levels, independently of the postheparin LPL mass, suggesting that serum LPL also reflects lipolysis of triglyceride-rich lipoproteins to generate HDL.

Serum LPL, but not postheparin LPL, showed an inverse correlation with RLP cholesterol and a positive correlation with LDL size, which are newly recognized risk factors for CHD.[23,24] LDL size[14,23] and RLP cholesterol[15,24] both have a substantial association with serum triglyceride. Thus, the stronger association of triglyceride with serum LPL than with postheparin LPL could explain why serum LPL had a significant association with RLP cholesterol or LDL size, but postheparin LPL did not. A low serum LPL mass may become a new CHD risk factor for integrating the atherogenic lipoprotein profile, such as small dense LDL, increased remnants, and low HDL cholesterol. Indeed, it has been reported that patients with CHD have lower serum LPL levels than normal controls.[25,26]

We assumed that heparin-releasable LPL (Δ-LPL mass: postheparin LPL − preheparin LPL) has more clinical significance than postheparin LPL or preheparin LPL. Contrary to our expectation, however, Δ-LPL mass was only related to postheparin LPL, but did not show a more meaningful association with triglyceride metabolism than postheparin LPL or preheparin LPL. The weak correlation between postheparin LPL and preheparin LPL may be attributable to contamination of postheparin plasma by preexisting LPL (preheparin LPL).

In conclusion, serum LPL mass may reflect systemic LPL biosynthesis. Measurement of the serum LPL concentration is a simple and useful method for investigating LPL-mediated lipoprotein metabolism. Because a low serum LPL level seems to be closely associated with "the metabolic syndrome," the next step will be investigation of the use of serum LPL to predict coronary heart disease in large populations.

REFERENCES

1. Nilsson-Ehle P, Garfinkel AS, Schotz MC: Lipolytic enzymes and plasma lipoprotein metabolism. Annu Rev Biochem 49:667-693, 1980

2. Olivecrona G, Olivecrona T: Triglyceride lipases and atherosclerosis. Curr Opin Lipidol 6:291-305, 1995

3. Goldberg IJ: Lipoprotein lipase and lipolysis: Central roles in lipoprotein metabolism and atherogenesis. J Lipid Res 37:693-707, 1996

4. Merkel M, Eckel RH, Goldberg IJ: Lipoprotein lipase: Genetics, lipid uptake, and regulation. J Lipid Res 43:1997-2006, 2002

5. Vilella E, Joven J, Fernandez M, et al: Lipoprotein lipase in human plasma is mainly inactive and associated with cholesterol-rich lipoproteins. J Lipid Res 34:1555-1564, 1993

6. Watanabe H, Miyashita Y, Murano T, et al: Preheparin serum lipoprotein lipase mass level: The effects of age, gender, and types of hyperlipidemias. Atherosclerosis 145:45-50, 1999

7. Saito K, Sakurabayashi I, Manabe M: Serum lipoprotein lipase in healthy subjects: Effects of gender and age, and relationships to lipid parameters. Ann Clin Biochem 35:733-738, 1998

8. Tornvall P, Olivecrona G, Karpe F, et al: Lipoprotein lipase mass and activity in plasma and their increase after heparin are separate parameters with different relations to plasma lipoproteins. Arterioscler Thromb Vasc Biol 15:1086-1093, 1995

9. Miyashita Y, Shirai K, Itoh Y, et al: Low lipoprotein lipase mass in preheparin serum of type 2 diabetes mellitus patients and its recovery with insulin therapy. Diabetes Res Clin Pract 56:181-187, 2002

10. Totsuka M, Miyashita Y, Ito Y, et al: Enhancement of preheparin serum lipoprotein lipase mass by bezafibrate administration. Atherosclerosis 153:175-179, 2000

11. Auwerx JH, Babirak SP, Fujimoto WY, et al: Defective enzyme protein in lipoprotein lipase deficiency. Eur J Clin Invest 19:433-437, 1989

12. Kern PA, Martin RA, Carty J, et al: Identification of lipoprotein lipase immunoreactive protein in pre- and postheparin plasma from normal subjects and patients with type I hyperlipoproteinemia. J Lipid Res 31:17-26, 1990

13. Kobayashi J, Hashimoto H, Fukamachi I, et al: Lipoprotein lipase mass and activity in severe hypertriglyceridemia. Clin Chim Acta 216:113-123, 1993

14. Krauss RM, Burke DJ: Identification of multiple subclasses of

plasma low density lipoproteins in normal humans. J Lipid Res 23:97-104, 1982

15. Nakajima K, Saito T, Tamura A, et al: Cholesterol in remnant-like lipoproteins in human serum using monoclonal anti apo B-100 and anti apo A-I immunoaffinity mixed gels. Clin Chim Acta 223:53-71, 1993

16. Matthews DR, Hosker JP, Rudenski AS, et al: Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412-419, 1985

17. Sakaue T, Hirano T, Yoshino G, et al: Reactions of direct LDL-cholesterol assays with pure LDL fraction and IDL: Comparison of three homogeneous methods. Clin Chim Acta 295:97-106, 2000

18. Watson TD, Tan CE, McConnell M, et al: Measurement and physiological significance of lipoprotein and hepatic lipase activities in preheparin plasma. Clin Chem 41:405-412, 1995

19. Merkel M, Kako Y, Radner H, et al: Catalytically inactive lipoprotein lipase expression in muscle of transgenic mice increases very low density lipoprotein uptake: Direct evidence that lipoprotein lipase bridging occurs in vivo. Proc Natl Acad Sci USA 95:13841-13846, 1998

20. Ong JM, Kirchgessner TG, Schotz MC, et al: Insulin increases the synthetic rate and messenger RNA level of lipoprotein lipase in isolated rat adipocytes. J Biol Chem 263:12933-12938, 1988

21. Shirai K, Itoh Y, Sasaki H, et al: The effect of insulin sensitizer, troglitazone, on lipoprotein lipase mass in preheparin serum. Diabetes Res Clin Pract 46:35-41, 1999

22. Glaser DS, Yost TJ, Eckel RH: Preheparin lipoprotein lipolytic activities: Relationship to plasma lipoproteins and postheparin lipolytic activities. J Lipid Res 33:209-214, 1992

23. Austin MA, King MC, Vranizan KM, et al: Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk. Circulation 82:495-506, 1990

24. Kugiyama K, Doi H, Takazoe K, et al: Remnant lipoprotein levels in fasting serum predict coronary events in patients with coronary artery disease. Circulation 99:2858-2860, 1999

25. Hitsumoto T, Ohsawa H, Uchi T, et al: Preheparin serum lipoprotein lipase mass is negatively related to coronary atherosclerosis. Atherosclerosis 153:391-396, 2000

26. Hitsumoto T, Yoshinaga K, Aoyagi K, et al: Association between preheparin serum lipoprotein lipase mass and acute myocardial infarction in Japanese men. J Atheroscler Thromb 9:163-169, 2002