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## Comparison of the effect of post-heparin and pre-heparin lipoprotein lipase and hepatic triglyceride lipase on remnant lipoprotein metabolism

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### ABSTRACT

**Background:** A comparison of post-heparin and pre-heparin plasma lipoprotein lipase (LPL) and hepatic triglyceride lipase (HTGL) on the metabolism of remnant lipoproteins (RLPs) has not been reported yet.

**Methods:** Healthy volunteers were injected with heparin for LPL and HTGL determination in the fasting (8:00) and postprandial (20:00) plasma on the same day. Plasma total cholesterol (TC), triglycerides (TG), LDL-C, HDL-C, small dense LDL (sdLDL)-C, remnant lipoprotein (RLP)-C, RLP-TG, the RLP-TG/RLP-C ratio, adiponectin and apoCIII were measured.

**Results:** LPL activity and concentration in the post-heparin plasma exhibited a significant inverse correlation with TG, RLP-C, RLP-TG, and RLP particle size estimated as RLP-TG/RLP-C ratio and sdLDL-C, and positively correlated with HDL-C. HTGL was only inversely correlated with HDL-C. LPL concentration in the pre-heparin plasma was also inversely correlated with the RLP-TG/RLP-C ratio and other lipoprotein parameters. Adiponectin was inversely correlated with RLP-TG/RLP-C ratio and apoC III was positively correlated with RLP-TG/RLP-C ratio, but not correlated with LPL activity.

**Conclusion:** LPL activity and concentration were inversely and significantly correlated with the particle size of RLP in both the post-heparin and pre-heparin plasma. Those results suggest that LPL concentration in pre-heparin plasma can take the place of LPL activity in the post-heparin plasma.

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### 1. Introduction

Remnant lipoprotein (RLP) metabolism is known to be regulated by lipoprotein lipase (LPL) and hepatic triglyceride lipase (HTGL) [1–3]. LPL plays a central role in triglyceride-rich lipoproteins (TRLs) metabolism by catalyzing the hydrolysis of triglycerides (TG) in chylomicrons (CMs) and very low-density lipoprotein (VLDL) particles and is a useful biomarker in diagnosing Type I hyperlipidemia [4] and also prediction of cardiovascular diseases [5]. HTGL has been recognized to play a role in catalyzing the hydrolysis of the smaller remnants into LDL [6]. We previously reported that postprandial RLPs in a 4 h period after a fat load are significantly larger in particle size compared to the fasting state, with a TG increase in RLPs [7]. The interaction of those lipase activities with the associated lipoproteins and RLP particle size in the fasting and postprandial plasma was the focus of this investigation.

The accumulation of RLPs of large particle size after an oral fat load is mainly due to the delayed metabolism of VLDL by LPL [7,8]. LPL and

HTGL activities and concentrations, both with and without a heparin injection, were pursued in order to elucidate the plasma TRL metabolism, especially in terms of the effect on the RLP particle size, estimated by RLP-TG/RLP-C ratio compared with HPLC assay by Okazaki et al. [9]. If there is a close similarity between the RLP particle size in pre- and post-heparin plasma, it may be possible to eliminate the heparin injection that is commonly used to measure LPL and HTGL activities and concentrations for clinical diagnostic purposes. Non-heparinized plasma (pre-heparin plasma) is known to contain a considerably large amount of LPL, but the activity of TG hydrolysis is very low or non-detectable. Watson et al. [10] tried to measure this low level of LPL activity by increasing the serum volume and prolonging the incubation time, but they still did not find a meaningful association between the pre-heparin LPL activity and the lipoprotein concentrations, suggesting that the plasma LPL concentration does not reflect a significant role in lipid metabolism, at least via its lipolytic activity.

However, recent studies have revealed that catalytically inactive LPL in pre-heparin plasma can act as a ligand for lipoprotein receptors and glucosaminoglycans in the liver [11–15]. Thus, catalytically inactive LPL might participate in lipoprotein metabolism via its ligand function rather than its lipolytic function. Because it is

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catalytically inactive, the measurement of pre-heparin LPL concentration has not received much attention as a diagnostic marker in clinical laboratories, despite the extensive studies reported by Shirai and et al. [16–23] and others [24,25].

## 2. Materials and methods

### 2.1. Study subjects

The study in relatively healthy young volunteers (some cases were overweight or obese) in a male ( $n = 36$ ) and female ( $n = 40$ ) population (Caucasian 45, Asian 10, Hispanic 9, African American 7, others 5) with a median age of 24 and BMI of 24 at the University of California, Davis (Table 1). Inclusion criteria included age from 18 to 40 y and BMI of 18–35 kg/m<sup>2</sup> with a self-report of stable body weight during the prior 6 months. Exclusion criteria included evidence of diabetes, renal disease, or hepatic disease; fasting serum TG concentrations greater than 400 mg/dl; hypertension ( $> 140/90$  mm Hg); and history of surgery for weight loss. Individuals who smoked, reported exercise of  $> 3.5$  h/week at a level more vigorous than walking, or reported having used thyroid, lipid-lowering, glucose-lowering, antihypertensive, antidepressant, or weight-loss medications were also excluded. Seventy six volunteers were injected with heparin for the LPL and HTGL activity assays. The University of California at Davis Institutional Review Board approved the experimental protocol and the subjects provided written informed consent to participate in the study. Baseline blood samples of fructose and glucose study by Stanhope et al. [26] were provided for this study and all the parameter analysis were performed at Gunma University.

Fasting blood samples were collected at 08:00 h before breakfast and postprandial blood samples were collected at 20:00 h after dinner on the same day. During the day, standardized meals were provided as breakfast, lunch and dinner to all of the volunteers. The energy content of the meals was based on each subject's energy requirements as determined by the Mifflin equation [27].

### 2.2. Determination of lipids and lipoproteins as well as lipase activities and concentrations

The plasma samples for the measurement of TC, TG, HDL-C, LDL-C, RLP-C, RLP-TG and sdLDL-C were withdrawn before (pre-heparin plasma) and after heparin injection (post-heparin plasma) and kept frozen at  $-80^{\circ}\text{C}$  until analysis. As LPL and HTGL activities were not detectable in the pre-heparin plasma, all of the lipase activities in this study were determined in the post-heparin plasma by the method of Imamura [28]. Briefly, the post-heparin plasma was withdrawn 15 min after the intravenous injection of 50 units of heparin/kg body weight for the assay of LPL, HTGL activity and concentration. The LPL and HTGL activity assays were an automated kinetic colorimetric method in post-heparin plasma by using a natural long-chain fatty acid, 1,2-diglyceride as substrate for assaying HL activity and with the presence of apoC-II for assaying LPL activity. LPL activity was calculated after subtracted HTGL activity from total lipase activity after adding apo

CII. LPL and HTGL hydrolyzed the clear substrate solution to produce a 2-monoglyceride, which in turn releases glycerol by the action of a 2-monoglyceride lipase. The glycerol produced is then assayed by a sequence of enzymatic actions that produce a violet quinone monoimine dye. The accuracy and stability of this activity assay were reported previously by Imamura et al. [28]. The pre-heparin LPL and HTGL concentrations were determined by the highly sensitive and specific ELISA kit (IBL, Fujioka, Japan) recently developed by Miyashita et al. [29]. The TC and TG concentrations were determined enzymatically. The LDL-C and HDL-C concentrations were measured using a homogenous method (Kyowa Medex, Tokyo). Glucose and insulin were determined by PolyChem (Polymedco, NY). RLP-C and RLP-TG were determined by an immunoseparation method (JIMRO II, Otsuka, Tokyo) [30]. Small dense LDL-C was determined by the method of Ito et al. [31].

### 2.3. Statistical analysis

Data were analyzed with Dr. SPSS II (SPSS). The data are presented as median values with 25th and 75th percentile values, rather than as mean values with standard deviation. The statistical significance of difference was determined by Mann–Whitney *U*-test. Pearson's correlation coefficients ( $r = \text{value}$ ) were determined and single linear regression analysis was performed to detect associations between variables.  $P < 0.05$  was considered statistically significant.

## 3. Results

### 3.1. Post-heparin plasma lipids, lipoproteins, LPL and HTGL activity and concentration analyses in the fasting and postprandial states.

Table 1 indicates the demographic data on 76 volunteers recruited at UC Davis, CA. Table 2 indicates that the mean total cholesterol (TC), LDL-C and HDL-C levels were within a normal range in the fasting and postprandial plasma. TG, RLP-C, and RLP-TG were significantly elevated in the postprandial plasma, but TC, LDL-C, HDL-C and sdLDL-C did not change. RLP-TG/RLP-C ratio in the postprandial plasma increased 2.6 fold compared to the fasting plasma both in pre-heparin and post-heparin plasma. LPL activity was significantly reduced in the postprandial plasma, but LPL concentration was not. HTGL activity and concentration were unchanged in the postprandial plasma. Both LPL and HTGL activities were not detected in pre-heparin plasma. HTGL concentration was detected negligible level compared to LPL concentration in pre-heparin plasma.

### 3.2. A single linear regression analysis of these parameters in the post-heparin and pre-heparin plasma.

A). TG correlations with the post-heparin and pre-heparin LPL and HTGL activities and concentrations in the fasting and postprandial plasma.

In post-heparin plasma (Table 3), TG concentration was inversely and significantly correlated with LPL activity and concentration in both the fasting and postprandial plasma, respectively, while no correlation was found with HTGL activity or concentration in either the fasting or postprandial plasma.

In pre-heparin plasma (Table 4), TG concentration was inversely correlated with LPL concentration in both the fasting and significantly correlated in postprandial plasma, while no correlation was found with HTGL concentration in either the fasting or postprandial plasma.

B). RLP-C correlations with the post-heparin and pre-heparin LPL and HTGL activities and concentrations in the fasting and postprandial plasma

In post-heparin plasma (Table 3), RLP-C was inversely and significantly correlated with the LPL activity and concentration in the fasting and postprandial plasma respectively, while no correlation was found with HTGL activity in either.

**Table 1**  
Clinical characteristics of 76 volunteers.

	Median	(25thtile–75thtile)
Age (y)	24	(22–30)
Gender male/female	36/40	
Body weight (kg)	73	(61–82)
BMI	24	(22–27)
Abdominal circumference (cm)	77	(67–81)
Blood pressure		
Systolic (mm Hg)	117	(110–130)
Diastolic (mm Hg)	72	(67–76)

**Table 2**

Post-heparin and pre-heparin plasma lipids, lipoproteins, LPL and HTGL activities and concentrations in the fasting and postprandial states.

	Post-heparin plasma	Fasting (8:00)		Postprandial (20:00)		p	Pre-heparin plasma	Fasting (8:00)		Postprandial (20:00)		p
		Median	(25%tile–75%tile)	Median	(25%tile–75%tile)			Median	(25%tile–75%tile)	Median	(25%tile–75%tile)	
t2.5	TC (mg/dl)	160	(142–181)	159	(146–183)	NS	TC (mg/dl)	164	(144–187)	165	(149–187)	NS
t2.6	TG (mg/dl)	47	(31–74)	73	(45–105)	0.001	TG (mg/dl)	74	(49–100)	108	(73–150)	<0.001
t2.7	Hdl-C (mg/dl)	47	(42–59)	47	(41–58)	NS	Hdl-C (mg/dl)	48	(44–61)	48	(43–61)	NS
t2.8	Ldl-C (mg/dl)	90	(71–107)	91	(73–107)	NS	Ldl-C (mg/dl)	90	(73–108)	92	(73–107)	NS
t2.9	sdldl-C (mg/dl)	21.4	(17.2–30.9)	24.3	(19.4–34.6)	NS	sdldl-C (mg/dl)	22.3	(17.6–32.5)	25.1	(19.4–35.3)	NS
t2.10	RLP-C (mg/dl)	3.5	(2.5–4.8)	4.6	(3.5–6.7)	<0.001	RLP-C (mg/dl)	3.4	(2.7–5.0)	5.5	(3.8–7.5)	<0.001
t2.11	RLP-TG (mg/dl)	4.2	(2.4–6.8)	14.5	(8.1–27.0)	<0.001	RLP-TG (mg/dl)	8.7	(5.3–14.3)	38.3	(23.6–66.4)	<0.001
t2.12	RLP-TG/RLP-C	1.4	(0.7–2.0)	3.6	(1.9–5.2)	<0.001	RLP-TG/RLP-C	2.6	(1.6–3.5)	8.2	(5.4–11.3)	<0.001
t2.13	LPL (U/l)	78.9	(68.7–89.3)	65.9	(57.5–77.5)	<0.001	LPL (U/l)	–	–	–	–	–
t2.14	LPL (ng/ml)	206	(179–248)	205	(173–237)	NS	LPL (ng/ml)	27.9	(21.0–34.9)	32.0	(25.7–42.5)	0.003
t2.15	HTGL (U/l)	222	(170–287)	202	(141–252)	NS	HTGL (U/l)	–	–	–	–	–
t2.16	HTGL (ng/ml)	146	(90–195)	166	(107–238)	NS	HTGL (ng/ml)	0.6	(0.3–1.5)	0.7	(0.4–1.7)	NS
t2.17	Adiponectin (μg/ml)	7.7	(6.2–10.5)	7.5	(6.1–10.8)	NS	Adiponectin (μg/ml)	8.7	(6.9–12.3)	8.4	(7.0–11.5)	NS
t2.18	ApoCIII (mg/dl)	8.3	(6.9–9.9)	8.0	(6.6–9.3)	NS	ApoCIII (mg/dl)	7.7	(6.9–9.2)	7.1	(6.2–9.0)	0.050

In pre-heparin plasma (Table 4), RLP-C correlations with LPL and HTGL concentration were inversely correlated with LPL concentration in both the fasting and postprandial plasma, respectively, while no correlation was found with HTGL concentration in either.

C). RLP-TG correlations with the post-heparin and pre-heparin plasma LPL and HTGL activities and concentrations in the fasting and postprandial plasma

In post-heparin plasma (Table 3), RLP-TG was inversely and significantly correlated with LPL activity and concentration in both the fasting and postprandial plasma, respectively, and positively correlated with HTGL concentration in the postprandial plasma (not correlated in the fasting plasma, but positively correlated in the postprandial plasma). The positive correlation between RLP-TG and HTGL activities means that HTGL does not hydrolyze RLP-TG.

In pre-heparin plasma (Table 4), RLP-TG was inversely and significantly correlated with LPL concentration in both the fasting and postprandial plasma. The correlation with RLPs and HTGL was not able to calculate because of the negligible concentration of HTGL in pre-heparin plasma. Those results indicated that HTGL did not play a significant role in RLP metabolism in both pre- and post-heparin plasma.

D). sdLDL-C correlations with the post-heparin and pre-heparin plasma LPL and HTGL activities and concentration in the fasting and postprandial plasma

In post-heparin plasma (Table 3), sdLDL-C was inversely and significantly correlated with LPL activity and concentration in the fasting, but not in the postprandial plasma, while no correlation was found with HTGL activity in either the fasting or postprandial plasma. Furthermore, sdLDL-C with TG, RLP-C and RLP-TG were positively and significantly correlated with both in the fasting and postprandial plasma.

In pre-heparin plasma (Table 4), sdLDL-C was inversely and significantly correlated with LPL concentration in both the fasting and postprandial plasma, while no correlation was found with HTGL activity in either the fasting or postprandial state. Furthermore, sdLDL-C was positively and significantly correlated with TG, RLP-C and RLP-TG in both the fasting and in the postprandial state, respectively.

E). HDL-C correlations with the post-heparin and pre-heparin plasma LPL and HTGL activities and concentrations in the fasting and postprandial plasma

In post-heparin plasma (Table 3), HDL-C was positively and significantly correlated with LPL activity and concentration in the fasting and postprandial plasma and inversely and significantly correlated with HTGL activity and concentration in both the fasting and postprandial plasma. In pre-heparin plasma (Table 4), HDL-C was positively and significantly correlated with the LPL concentration in the fasting and postprandial plasma and there was no correlation with HTGL in the fasting and postprandial plasma because of the negligible concentration of HTGL in pre-heparin plasma.

### 3.3. Correlations of RLP-TG/RLP-C ratio with LPL and HTGL activities and concentrations in the post-heparin and pre-heparin plasma LPL

In post-heparin plasma, the RLP-TG/RLP-C ratio was inversely and significantly correlated with LPL activity and concentration in the fasting and postprandial plasma, respectively (Fig. 1), while no correlation was found with HTGL activity and concentration in either the fasting and postprandial plasma activity, but correlated positively in postprandial HTGL concentration (Fig. 2). Positive correlation means that HTGL does not play a role to hydrolyze RLP.

In pre-heparin plasma, the RLP-TG/RLP-C ratio was inversely and significantly correlated with LPL concentration in the fasting and postprandial plasma (Fig. 3) as correlated with LPL activity. Neither HTGL activity nor concentration was detected in pre-heparin plasma.

### 3.4. Correlations of RLP-TG/RLP-C ratio with the adiponectin and apoC III in the post-heparin plasma

In post-heparin plasma, the RLP-TG/RLP-C ratio was inversely and significantly correlated with adiponectin in both the fasting and postprandial plasma (Table 3). In pre-heparin plasma, the RLP-TG/RLP-C ratio was inversely and significantly correlated with adiponectin in both the fasting and postprandial plasma (Table 4; Fig. 4).

RLP-TG/RLP-C ratio in post-heparin plasma was positively and significantly correlated with apoCIII in the fasting and postprandial plasma (Table 3). RLP-TG/RLP-C ratio in pre-heparin plasma was also positively and significantly correlated with apoCIII in the fasting, but not significantly in the postprandial plasma (Table 4).

### 3.5. Correlations of LPL activity and concentration with the adiponectin and apoCIII in the post-heparin plasma

In post-heparin plasma, LPL activity was not correlated with adiponectin in either the fasting or postprandial plasma (Table 3). However, in pre-heparin plasma, LPL concentration was positively and significantly correlated with adiponectin in the fasting and postprandial plasma (Table 4; Fig. 5).

In post-heparin plasma, LPL activity was not significantly correlated with apoCIII in both the fasting or postprandial plasma (Table 3). Also in pre-heparin plasma, LPL concentration was also not correlated with apoCIII in both fasting and postprandial plasma (Table 4), indicating no direct inhibitory effect of apoCIII on LPL activity.

## 4. Discussion

We reported previously that postprandial RLPs increased significantly after food intake and exhibited a 4.4 fold increase of RLP-TG/RLP-C

**Table 3**

A single regression analysis of these parameters in post-heparin fasting and postprandial plasma.

	TG	HDL-C	sdLDL-C	RLP-C	RLP-TG	RLP-TG/RLP-C	LPL (activity)	LPL (concentration)	HTGL (activity)	HTGL (concentration)	Adiponectin	ApoCIII
<i>Fasting (8:00)</i>												
TG												
HDL-C	−0.360**											
sdLDL-C	0.657***	−0.287*										
RLP-C	0.455***	−0.245*	0.512***									
RLP-TG	0.877***	−0.440***	0.496***	0.477***								
RLP-TG/RLP-C	0.734***	−0.341*	0.260*	−0.012	0.836***							
LPL (activity)	−0.566***	0.198	−0.262*	−0.313**	−0.513***	−0.408***						
LPL (concentration)	−0.432***	0.399***	−0.146	−0.171	−0.473***	−0.422***	0.526***					
HTGL (activity)	−0.295*	−0.213	−0.131	0.015	−0.166	−0.187	0.282*	0.231*				
HTGL (concentration)	−0.209	−0.316**	−0.094	0.083	−0.062	−0.107	0.178	0.059	0.906***			
Adiponectin	−0.331**	0.445***	−0.325**	−0.343**	−0.421***	−0.234*	−0.005	0.225	0.005	−0.066		
ApoCIII	0.325**	0.170	0.282*	0.209	0.244*	0.214	0.054	0.258*	0.043	0.037	−0.178	
<i>Postprandial (20:00)</i>												
TG												
HDL-C	−0.587***											
sdLDL-C	0.691***	−0.416***										
RLP-C	0.619***	−0.471***	0.573***									
RLP-TG	0.905***	−0.616***	0.514***	0.651***								
RLP-TG/RLP-C	0.780***	−0.504***	0.348**	0.248*	0.870***							
LPL (activity)	−0.328**	0.217	−0.093	−0.215	−0.251*	−0.204						
LPL (concentration)	−0.144	0.242*	0.038	0.031	−0.229*	−0.308**	0.418***					
HTGL (activity)	−0.075	−0.241*	−0.061	0.161	0.094	0.008	0.445***	0.220				
HTGL (concentration)	0.199	−0.382**	0.032	0.349**	0.396***	0.269*	0.205	0.102	0.851***			
Adiponectin	−0.475***	0.410***	−0.385**	−0.396***	−0.432***	−0.293*	0.036	0.008	0.022	−0.024		
ApoCIII	0.555***	−0.064	0.448***	0.428***	0.464***	0.340**	0.009	0.412***	0.124	0.247*	−0.251*	

\*\*\*  $p < 0.001$ .\*\*  $p < 0.01$ .\*  $p < 0.05$ .



**Table 4**

A single regression analysis of these parameters in pre-heparin fasting and postprandial plasma.

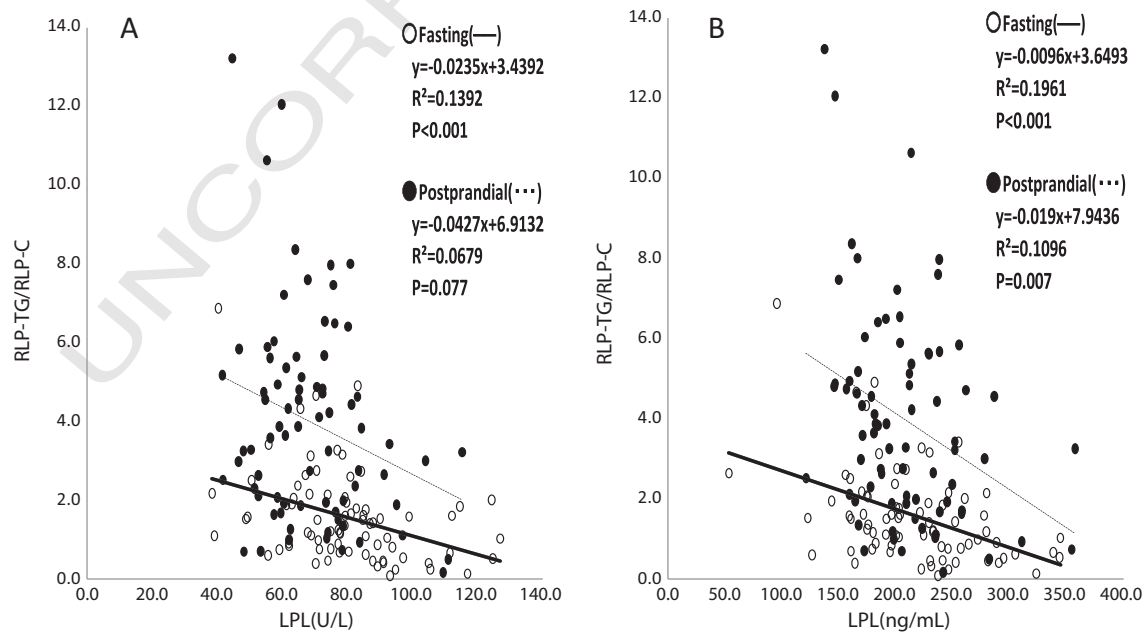
	TG	HDL-C	sdLDL-C	RLP-C	RLP-TG	RLP-TG/RLP-C	LPL (concentration)	HTGL (concentration)	Adiponectin	ApoCIII
<b>Fasting (8:00)</b>										
TG										
HDL-C	−0.343**									
sdLDL-C	0.628***	−0.299**								
RLP-C	0.426***	−0.212	0.551***							
RLP-TG	0.859***	−0.339**	0.538***	0.519***						
RLP-TG/RLP-C	0.689***	−0.239*	0.200	−0.095	0.751***					
LPL (concentration)	−0.141	0.379**	−0.020	−0.103	−0.175	−0.156				
HTGL (concentration)	−	−	−	−	−	−	−			
Adiponectin	−0.385**	0.477***	−0.344**	−0.338**	−0.445***	−0.324**	0.387**	−		
ApoCIII	0.449**	0.143	0.383**	0.218	0.436***	0.362**	0.085	−		−0.166
<b>Postprandial (20:00)</b>										
TG										
HDL-C	−0.559***									
sdLDL-C	0.609***	−0.381**								
RLP-C	0.703***	−0.408***	0.609***							
RLP-TG	0.884***	−0.550***	0.420***	0.639***						
RLP-TG/RLP-C	0.524***	−0.327**	−0.001	−0.019	0.721***					
LPL (concentration)	−0.552***	0.543***	−0.284*	−0.390**	−0.597***	−0.435***				
HTGL (concentration)	−	−	−	−	−	−	−			
Adiponectin	−0.511***	0.436***	−0.423***	−0.409***	−0.465***	−0.218	0.352**	−		
ApoCIII	0.652***	−0.065	0.574***	0.532***	0.496***	0.136	−0.152	−		−0.294**

\*\*\*  $p < 0.001$ .\*\*  $p < 0.01$ .\*  $p < 0.05$ .

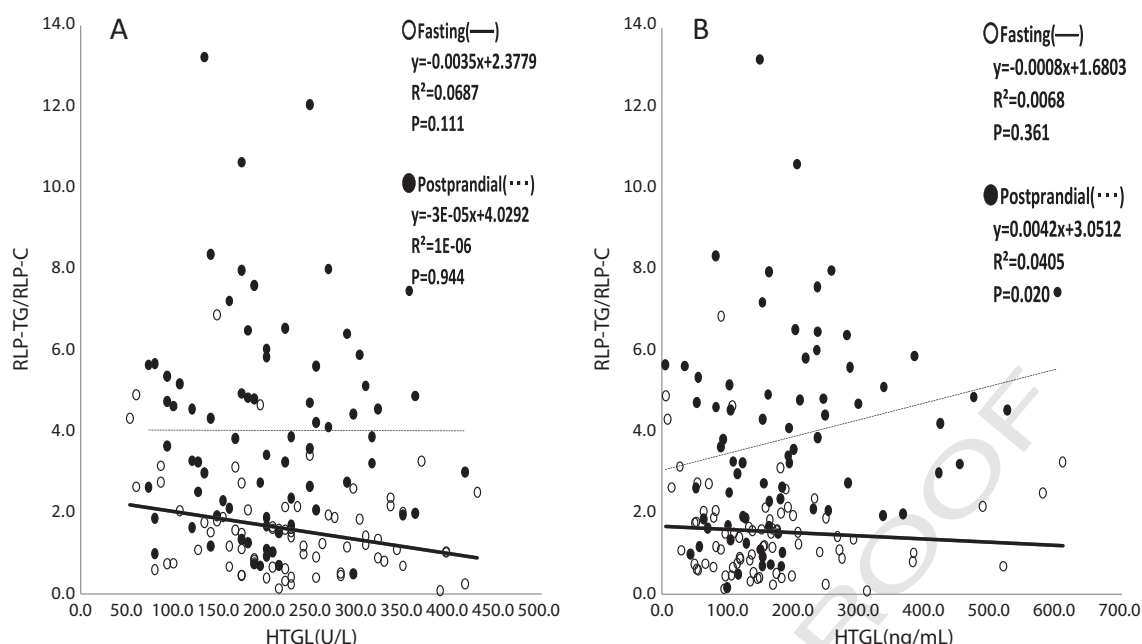
ratio in a 4 h period after fat intake reflecting the RLP particle size, along with a specific increase in RLP-TG, which is mainly composed of VLDL remnants [7,8]. The VLDL remnants, which were not fully metabolized by lipases and remained in the plasma after food intake, have significantly large TG-rich particles. To the best of our knowledge, this is the first report on a relationship between the particle size of remnant lipoproteins and the associated LPL and HTGL activities and concentrations in the fasting and postprandial plasma. We have found that the pre-heparin plasma LPL concentration displays a high degree of similarity with post-heparin plasma LPL activity in terms of TRL metabolism, particularly judging from the comparative study of RLP particle size. The RLP particle size may be the most appropriate marker to elucidate

the effect of lipase activities. The slope of the relationship between the RLP particle size and different variables in the postprandial plasma was more significant than those in the fasting plasma.

We confirmed that the plasma TG, RLP-C, RLP-TG and sdLDL-C concentrations were inversely correlated with LPL activities and concentration in the post-heparin plasma, as previously reported [3]. However, in this study population, LPL and HTGL activities and concentrations and associated lipoproteins in the post-heparin plasma were not necessarily consistent between in the fasting and postprandial plasma. This is probably because lipase activity gradually decreases at room temperature, which makes it difficult to use effectively for general clinical examination purposes. Further, it is still a matter of debate as to how much



**Fig. 1.** Correlations of RLP-TG/RLP-C with the LPL activity and concentration in the post-heparin plasma in 76 volunteers. Plasma RLP-TG/RLP-C ratio was inversely correlated with LPL activity (A) and concentration (B) in the post-heparin plasma in the fasting and postprandial state. Seventy-six plots reflect the total in each volunteer in the fasting (○) and postprandial (●) plasma.

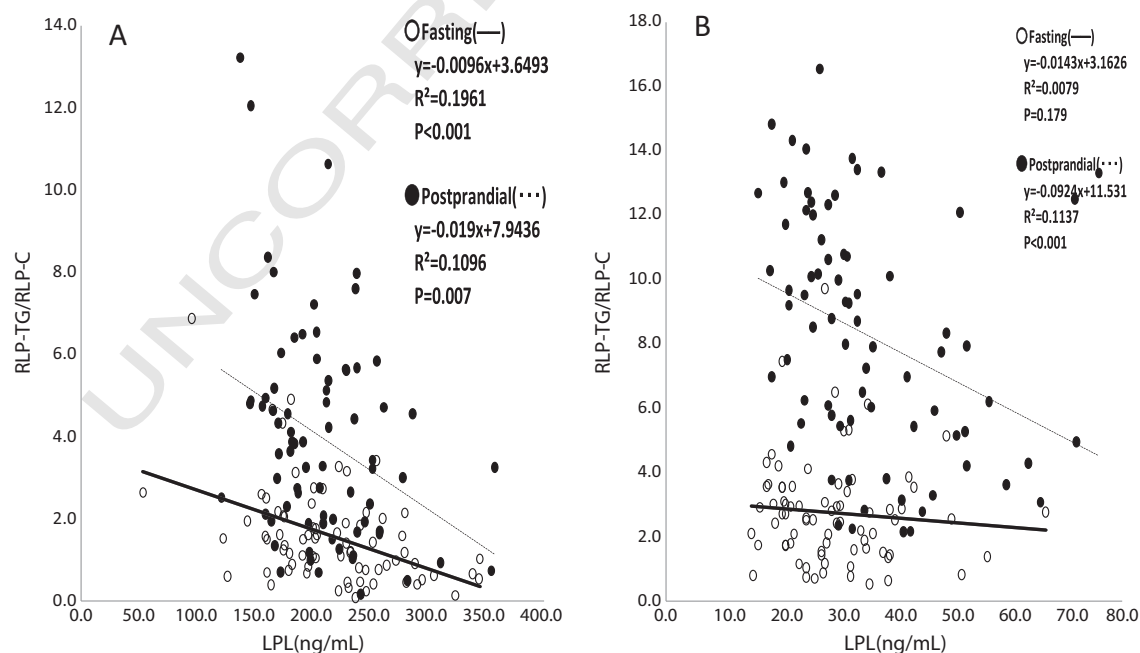


**Fig. 2.** Correlation of RLP-TG/RLP-C ratio between HTGL activity and concentration in the post-heparin plasma in 76 volunteers. Plasma RLP-TG/RLP-C ratio was not correlated with HTGL activity (A) and concentration (B) in post-heparin plasma in the fasting and postprandial state. Although a significantly positive correlation was found between RLP-TG/RLP-C ratio and HTGL concentration in postprandial state, it means that HTGL does not play a role to hydrolyze RLP. Seventy-six plots reflect the total in each volunteer in the fasting (o) and postprandial (•) plasma.

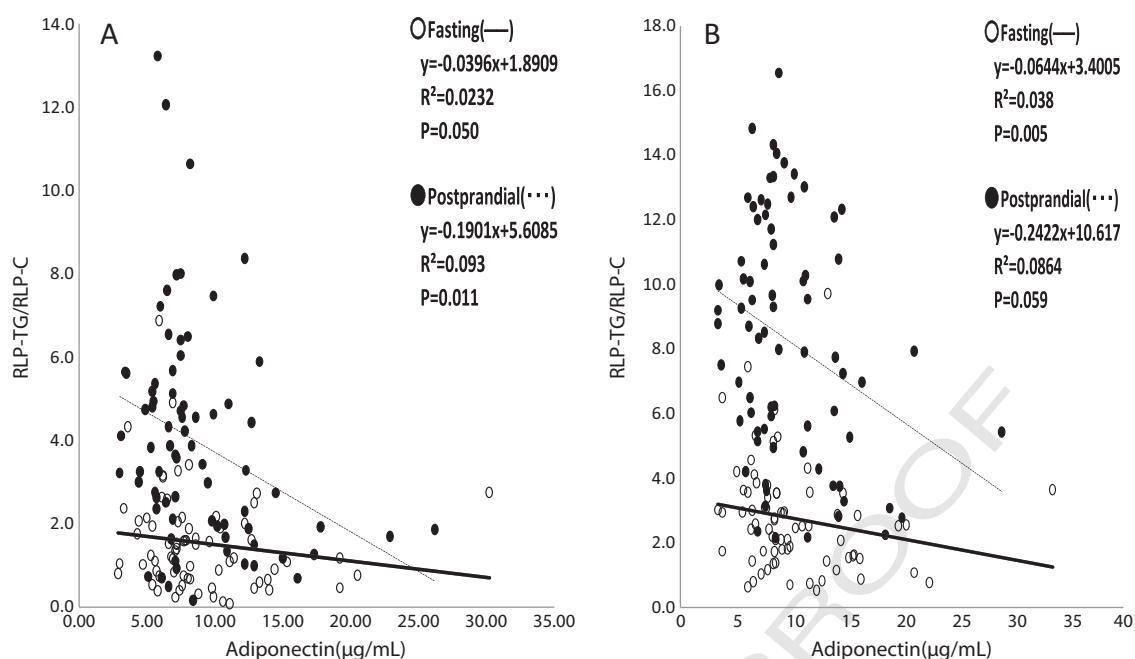
heparin should be injected and precisely when the post-heparin sample should be taken in order to obtain the maximum value of LPL and HTGL activities or concentrations in the post-heparin plasma. For example, RLP-TG and RLP-TG/RLP-C ratios were almost in parallel with the LPL activity and concentration in the post-heparin fasting and postprandial plasma, but RLP-C was only in parallel with LPL activity and concentration in the fasting plasma, not the postprandial plasma.

HDL-C and sdLDL-C displayed a similar trend. The highest similarity was found between the post-heparin LPL activity in the fasting plasma and the pre-heparin LPL concentration in the postprandial plasma.

Unexpectedly, HTGL activity in post-heparin plasma was not correlated with TG, RLP-C, RLP-TG and sdLDL-C both in the fasting and postprandial plasma. HTGL has been generally believed to have a role in hydrolyzing small VLDL remnants or IDL in order to form



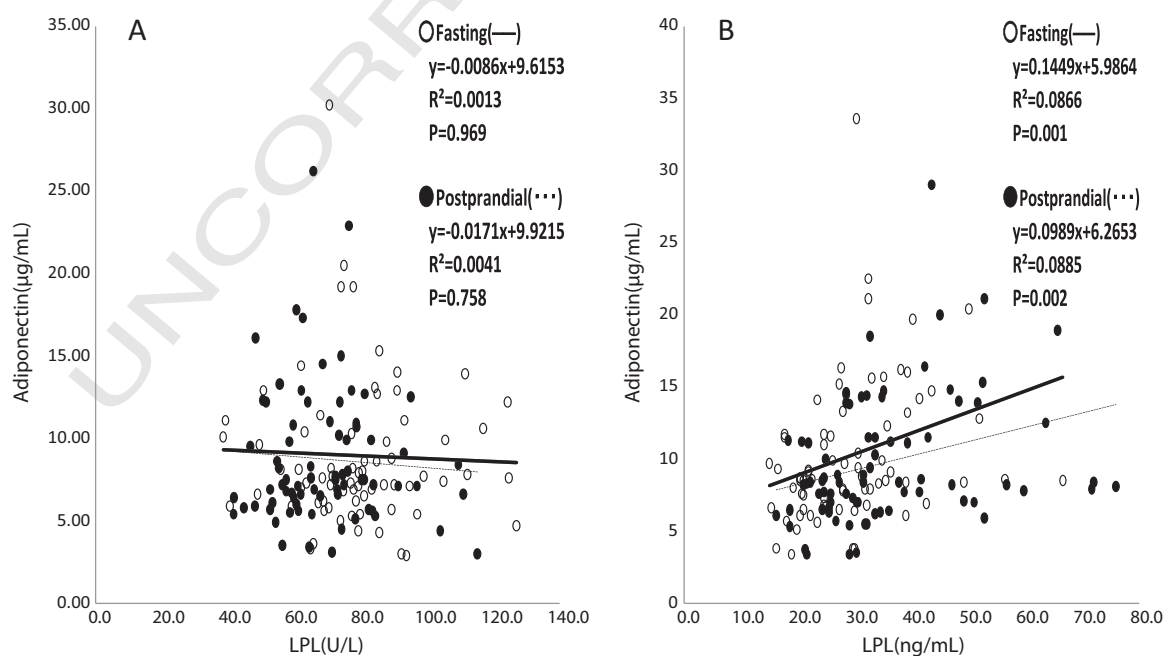
**Fig. 3.** Correlation of RLP-TG/RLP-C ratio with LPL concentration in the post-heparin (A) and pre-heparin plasma (B) in 76 volunteers. Plasma RLP-TG/RLP-C ratio was inversely correlated with LPL concentration in post-heparin plasma in both fasting and postprandial state (A) and also inversely correlated in pre-heparin plasma in both the fasting and postprandial state (B). Post-heparin LPL concentration was approximately 10 fold higher in post-heparin plasma than in pre-heparin plasma. Seventy six plots reflect the total in each volunteer in the fasting (o) and postprandial (•) plasma.



**Fig. 4.** Correlation of RLP-TG/RLP-C ratio with adiponectin in the post-heparin (A) and pre-heparin plasma (B) in 76 volunteers. RLP-TG/RLP-C ratio was inversely correlated with adiponectin in the post-heparin and pre-heparin plasma in both the fasting and postprandial state. Seventy-six plots reflect the total in each volunteer in the fasting (○) and postprandial (●) plasma.

sdLDL [32,33]. However, our data did not show a correlation between HTGL activity and either RLPs or sdLDL-C concentration, although we found a strong inverse correlation between LPL activity and the sdLDL-C concentration in the same cases. As it is known that sdLDL is positively correlated with TG and RLPs in plasma [3,33], these data suggest that RLPs are the precursors of sdLDL and metabolized mainly in the same pathway by LPL, but not by HTGL. From these data, HTGL does not seem to play a significant role in the metabolic pathway of remnant lipoprotein metabolism, unlike the studies reported previously [6,34–38], but rather, plays a definitive role in HDL metabolism in human plasma [3].

The RLP-TG/RLP-C ratio, an estimated index of RLP particle size, which was first reported by Okazaki et al. using an HPLC analysis [9], was found to be inversely correlated with LPL activity and concentration in both the pre- and post-heparin plasma, but no correlation was found with the HTGL activity or concentration. The pre-heparin LPL concentration was also inversely correlated with RLP-TG/RLP-C ratio in the postprandial plasma in similar fashion to the post-heparin plasma LPL activity. Although the RLP-TG/RLP-C ratio in pre-heparin plasma exhibited a trend of being inversely correlated with the LPL concentration in the fasting plasma, it was not statistically significant, probably because of a low TG concentration in the fasting plasma.



**Fig. 5.** Correlation of adiponectin with LPL activity (A) and concentration (B) in the fasting and postprandial plasma in 76 volunteers. Adiponectin was not correlated with LPL activity in post-heparin plasma, but significantly and positively correlated with LPL concentration in pre-heparin plasma both in the fasting and postprandial state. Seventy six plots reflect the total in each volunteer in the fasting (○) and postprandial (●) plasma.



Adiponectin is a plasma protein expressed in adipose tissue that is known to be closely associated with LPL and cardiovascular risk [39]. Interestingly, adiponectin in pre-heparin plasma was inversely and significantly correlated with the RLP-TG/RLP-C ratio and positively correlated with LPL concentration, but adiponectin was not correlated with LPL activity in post-heparin plasma. These results indicate that LPL concentration in pre-heparin plasma is more physiologically associated with adiponectin than the maximum LPL activity or concentration dissociated from endothelium after heparin injection. Therefore, LPL activity or concentration when measured in post-heparin plasma may not reflect the physiological condition on TG-rich lipoprotein metabolism. LPL concentration in pre-heparin plasma may present more diagnostic usefulness than the measurement of LPL activity in post-heparin plasma. Apo CIII is a well-known inhibitor of LPL activity [40]. However, there was no direct correlation between LPL activity and apoCIII concentration in the fasting and postprandial plasma (Table 3). However, apoCIII was positively correlated with the RLP-TG/RLP-C ratio, as shown correlated with RLP-C and RLP-TG, respectively [41], but it does not seem to inhibit LPL activity directly in plasma. ApoCIII may exert an inhibitory effect on the receptors which are associated with the ligand function of LPL rather than inhibiting the lipolytic activity of LPL. ApoC1 seems to be more direct inhibitor of LPL in RLP [42].

In conclusion, a significantly increased particle size of RLP, estimated by the RLP-TG/RLP-C ratio, was observed in the postprandial plasma. LPL activity and concentration in post-heparin plasma were inversely correlated with the RLP particle size, but HTGL was not. These results suggest that RLPs are mainly metabolized by LPL in both the post-heparin and pre-heparin plasma. LPL was positively correlated with adiponectin levels, but not with the apoCIII levels. As RLP-TG/RLP-C ratio and other lipoproteins in TRL metabolism were shown to be inversely correlated with LPL activity (post-heparin) and concentration (pre-heparin) in the same manner, LPL concentration in the pre-heparin plasma may effectively take the place of LPL activity in the post-heparin plasma for clinical diagnosis purpose.

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