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Lipoprotein lipase does not increase significantly in the postprandial plasma

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ABSTRACT

Background: Previous reports have shown that lipoprotein lipase (LPL) activity significantly increases in the postprandial plasma associated with the increase of TG-rich lipoproteins. Therefore, we have reexamined those relationships using newly developed LPL assay with the different kinds of food intake.
Methods: Standard meal ($n = 81$), 50 g of fat ($n = 54$), 75 g of glucose ($n = 25$) and cookie (25 g fat and 75 g carbohydrate fat) ($n = 28$) were administered in generally healthy volunteers. Plasma LPL, HTGL and TC, TG, LDL-C, HDL-C, RLP-C and RLP-TG were determined at subsequent withdrawal after the food intake.
Results: Plasma TG, RLP-C and RLP-TG were significantly increased at 8 PM (2 h after dinner of standard meal) compared with 8 AM before breakfast within the same day. Also those parameters were significantly increased in 2–6 h after fat load. However, the concentrations and activities of LPL and HTGL did not significantly increase in association with an increase in the TG and remnant lipoproteins. Also LPL concentration did not significantly increase after glucose and "cookie test" within 4 h.
Conclusion: No significant increase of LPL activity was found at CM and VLDL overload after different kinds of food intake when reexamined by newly developed assay for LPL activity and concentration.

1. Introduction

Lipoprotein lipase (LPL) is a key enzyme for the hydrolysis of TG-rich lipoproteins which are known to increase significantly in diabetes, metabolic syndrome and cardiovascular diseases. As plasma triglycerides (TG) are known to be a surrogate for TG-rich lipoproteins and are present in chylomicrons (CM), very low density lipoproteins (VLDL) and their remnants. TRL and their remnants are significantly increased in the postprandial plasma and are known to predict the risk of coronary heart disease (CHD) and type 2 diabetes [1,2], independent of the total cholesterol, LDL or HDL cholesterol level. In particular, the habit of eating in diabetes patients and CHD patients is known to be deeply associated with the increase of remnant lipoproteins. Recently, plasma non-fasting TG levels have received attention as a significant risk factor for CHD events [3–5]. Zilversmit [6] first proposed that the postprandial CM is the most common risk factor for atherogenesis in the absence of familial hyperlipoproteinemia. The hypothesis of postprandial CM and CM remnants thus came to be widely accepted as a major cause of common atherogenesis, because it was well established that CM are significantly increased in the intestine after food intake, especially fat intake, and a large amount flows into the blood stream through the thoracic duct. Therefore, CM and CM remnants have been believed to be the major lipoproteins in the postprandial hyperlipidemia until recently. However, Nakajima et al. [7] and Schneeman et al. [8] showed that the major lipoproteins increased in the postprandial plasma are apoB100-carrying VLDL remnants, but not apoB48-carrying CM remnants. The

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mechanism of the postprandial increase in the remnants is strongly dependent on LPL activity, but it remains controversial whether the circulating LPL activity and concentration in plasma increase in association with the increase of TG-rich lipoproteins after food intake.

The metabolism of TG-rich lipoproteins in human plasma provides an energy-rich substrate for either immediate use in tissues or storage as adipose tissue. However, in this process, potentially atherogenic lipoprotein particles (remnants) are also generated by the function of LPL activity. Lipolysis of triglycerides from CM and VLDL is catalyzed mainly by LPL. Karpe [9] hypothesized that there is a competition for the lipolysis induced by LPL between CM and VLDL after fat intake. They reported that LPL hydrolyzes CM more efficiently than VLDL. LPL binds to the vascular endothelium because of it affinity for heparan sulfate and GPI-HBP1 [10], and low levels of LPL are released into the circulation after the hydrolysis of CM and VLDL [11,12]. The binding of LPL to the endothelium is thought to be weakened by the local fatty acid accumulation that results from the lipolysis and release into the circulation after food intake. This has been demonstrated in individuals in whom the lipolytic system was overloaded by an infusion of triglyceride emulsions [12,13] and also in cell culture experiments [14]. An excess of free fatty acids (FFA) released from TG-rich lipoproteins after food intake has therefore been proposed to exert feed-back control lipolysis and thus affect the release of LPL into the circulation. We investigated the factors that govern the release of LPL into the circulation and whether evidence for the proposed fatty acid feed-back control or similar system was obtainable under physiological conditions in humans. We studied this by examining the postprandial LPL activity and concentration in the fasting and postprandial plasma together with the associated lipid and lipoprotein concentrations in individuals subjected to a standard meal and other artificial food intake, such as fat load and sugar intake. As plasma LPL is known to be an important diagnostic marker for lipid metabolism in diabetes and cardiovascular diseases, those food loading tests like OGTT as a diagnostic tool may be necessary in non-heparin plasma.

The biggest reason for the controversy over whether LPL activity increases or not after fat intake is due to the fact that it is not possible to determine LPL activity time-dependently within a short period of time such as 1 or 2 h intervals after heparin administration. The effect of heparin on the release of LPL from the endothelium remains over a period of a few hours [15]. Therefore, the previous studies on the release of LPL from endothelium after food intake were conducted in the non-heparin plasma using a radiochemical LPL activity assay [11–13]. Because there was no clinically useful, sensitive and specific LPL immunoassay around 1990, a low LPL concentration in non-heparin plasma was difficult to be determined precisely. In this study, we selected volunteers who had a wide range of basal plasma TG concentrations but generally healthy. This provided an opportunity to identify the associations between the plasma LPL activity and concentration with the diagnostic markers for the metabolism of TG-rich lipoproteins. In particular, we paid attention to the relationship between LPL activity and the formation of large RLP particles released into circulation after different kind of food intake, using a recently developed highly sensitive and specific LPL-ELISA and activity assay in non- and post-heparin LPL plasma.

2. Materials and methods

2.1. Study subjects

The fasting and postprandial comparative study recruited relatively healthy young volunteers (some cases were overweight or obese) in a male ($n = 41$) and female ($n = 40$) population (Caucasian 45, Asian 10, Hispanic 9, African American 7, others 10) with a median age of 24 and BMI of 24 at the University of California, Davis (Tables 1 and 2). The inclusion criteria were an age from 18 to 40 y and BMI of 18–35 kg/m^2, along 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 >400 mg/dL and hypertension (>140/90 mm Hg). Eighty one volunteers were injected with heparin (50 unit/kg) 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 the fructose and glucose levels reported by Stanhope et al. [16] were provided for this study, and all of the parameter analyses were performed at Gunma University. Fasting blood samples were collected before breakfast at 08:00 h and the postprandial blood samples were collected after dinner at 20:00 h on the same day. During the day, standardized meals [17] were provided at breakfast, lunch and dinner to all of the volunteers.

Table 1
Comparison of pre-heparin LPL concentration between fasting and postprandial plasma (mean ± SD).

	Fasting ($n = 79$)	Postprandial ($n = 80$)	p value
TC (mg/dL)	165.8 ± 29.8	167.7 ± 27.9	n.s.
TG (mg/dL)	79.8 ± 41.6	128.2 ± 84.6	$p < 0.001$
HDL-C (mg/dL)	51.4 ± 13.2	50.1 ± 13.2	n.s.
LDL-C (mg/dL)	92.8 ± 27.0	93.2 ± 24.3	n.s.
sd-LDL (mg/dL)	26.5 ± 12.3	29.2 ± 13.2	n.s.
RLP-C (mg/dL)	4.2 ± 2.4	6.4 ± 4.5	$p < 0.001$
RLP-TG (mg/dL)	11.8 ± 15.1	52.1 ± 46.3	$p < 0.001$
RLP-TG/RLP-C	2.7 ± 1.6	8.1 ± 3.7	$p < 0.001$
LPL (ng/mL)	48.2 ± 7.6	43.5 ± 8.3	$p < 0.001$

Fifty four volunteers for the oral fat load test without any cardiovascular disease, diabetes or other distinctly evident conditions (male 30 and female 24, aged 28–63 y) were recruited at the Graduate School of Health Sciences at Gunma University. Briefly, after 12 h fasting, the subjects ingested the equivalent of 17 g/m^2 body surface area of fat emulsion (OFTT cream, Jomo Foods) [18]. Blood samples were taken before and 60, 120, 240 and 360 min after the oral fat load.

Twenty five healthy males aged 50 to 79 without any specific diseases or drug treatment were recruited for the glucose and sugar test (OGTT) and the subjects provided written informed consent to participate in the study at Hidaka Hospital, Takasaki. The volunteers were treated with 50 g of glucose and 50 g of sucrose, respectively, with water as a control. Blood was withdrawn before and 15, 30, 60, 240 min after the treatment.

Twenty seven healthy volunteers (male 19 and female 8, aged 53 ± 11, BMI 24.6 ± 2.5) were recruited for the cookie test (25 g fat and 75 g carbohydrate) [19] and blood was withdrawn at 0, 1, 2 and 4 h. The study was approved by the Ethical Committees of Kyoto Medical Center and the volunteers provided written informed consent to participate in this study.

(We have performed fat load test in 84 patients with CHD with the same manner of fifty four volunteers above at Showa University of Medical School, but the results are going to be reported at a separate manuscript).

Table 2
Comparison of post-heparin LPL and HTGL activity and concentration between fasting and postprandial plasma (mean ± SD).

	Fasting ($n = 76$)	Postprandial ($n = 81$)	p value
TC (mg/dL)	162.0 ± 29.8	163.8 ± 27.1	n.s.
TG (mg/dL)	58.1 ± 38.1	89.8 ± 70.4	$p = 0.001$
HDL-C (mg/dL)	50.0 ± 12.9	48.6 ± 12.8	n.s.
LDL-C (mg/dL)	91.3 ± 26.9	92.3 ± 24.0	n.s.
sd-LDL (mg/dL)	26.0 ± 12.5	28.6 ± 13.1	n.s.
RLP-C (mg/dL)	3.9 ± 2.0	5.7 ± 3.6	$p < 0.001$
RLP-TG (mg/dL)	6.9 ± 11.5	26.0 ± 35.0	$p < 0.001$
RLP-TG/RLP-C	1.6 ± 1.2	3.9 ± 2.6	$p < 0.001$
LPL (U/L)	79.6 ± 19.0	69.0 ± 16.1	$p < 0.001$
LPL (ng/mL)	216.6 ± 55.0	209.9 ± 45.1	n.s.
HTGL (U/L)	224.0 ± 92.8	202.9 ± 82.1	n.s.
HTGL (ng/mL)	166.7 ± 121.7	194.3 ± 116.2	n.s.

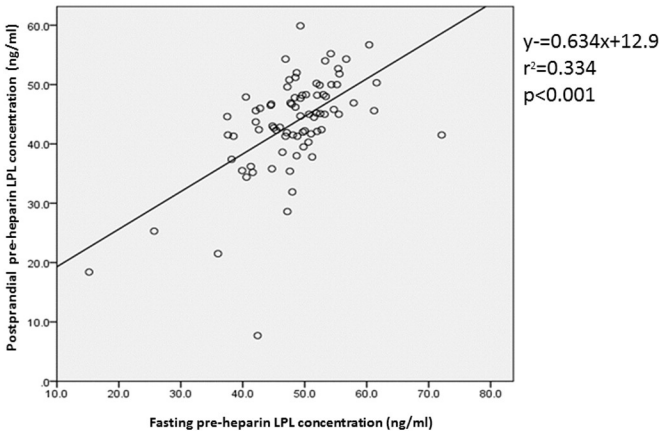

Fig. 1. Correlation between fasting and postprandial LPL concentration in pre-heparin plasma.

2.2. Measurements of lipids, lipoproteins and LPL along with the HTGL activity and concentration

The plasma samples used for the measurement of TC, TG, HDL-C, LDL-C, RLP-C, RLP-TG and sdLDL-C were withdrawn before and after heparin infusion and kept frozen at −80 °C until analysis. As the LPL and HTGL activities were undetectable in the pre-heparin plasma, all the lipase activities in this study were determined in the post-heparin plasma. The post-heparin plasma was withdrawn 15 min after intravenous injection of 50 units of heparin/kg body weight (BW) for the assay of LPL along with the HTGL activity and concentration. The pre-heparin LPL and HTGL mass were determined with a highly sensitive and specific ELISA [20,21]. The LPL and HTGL activities were determined with the automated assay [22]. Plasma samples were kept frozen at −80 °C until analysis. The TC and TG concentrations were determined enzymatically. The LDL-C and HDL-C concentrations were measured using a homogenous method (Kyowa Medex). Glucose and insulin were determined using PolyChem (Polymedco). RLP-C and RLP-TG were determined with an immunoseparation method (JIMRO II, Otsuka) [23,24]. Small dense LDL-C was determined by the method of Ito et al. (Denka-Seiken) [25].

2.3. The sandwich LPL-ELISA procedure

The LPL plasma concentration was measured using a novel LPL-ELISA developed at Immuno-Biological Laboratories (IBL) [26]. This is a solid phase sandwich ELISA using 2 kinds of highly specific monoclonal antibodies against human recombinant LPL. The assay used two different monoclonal antibodies against human recombinant LPL for the sandwich ELISA, which has the capacity to detect both monomeric and dimeric LPL. Tetra methyl benzidine (TMB) was used as the coloring agent (Chromogen). The strength of the coloring observed is proportional to the quantity of human recombinant LPL. Briefly, 100 μL of plasma or standard LPL diluted >100-fold was incubated with a solid phase antibody (57A5) for 60 min at 37 °C using a plate lid. After washing the plate with phosphate buffer, another antibody (88B8) labeled with horse radish peroxidase was added and incubated for 30 min at 4 °C with the plate lid. After washing, chromogen was added and incubated for 30 min at room temperature. The plate was read at 450 nm against a reagent blank within 30 min of the addition of 1 mol/L H_2SO_4 solution to stop the reaction. The measurement range of the assay was shown to be 0.02–1.5 ng/mL. The coefficient of variation was <10% in both the intra- and inter-assays.

2.4. LPL activity assay

The LPL and HTGL activities were determined by an automated activity assay [22] for measuring the increase in absorbance at 546 nm by means of a quinoneine dye. Reaction mixture-1 (R-1) contained dioleoylglycerol solubilized with lauryldimethylaminobetaine, monoacylglycerol-specific lipase, glycerolkinase, glycerol-3-phosphate oxidase, peroxidase, ascorbic acid oxidase and apolipoprotein C-II (apoC-II). R-2 contained Tris-HCl (pH 8.7) and 4-aminoantipyrine. Automated assay of the lipase activity was performed with an automatic clinical analyzer (H7700P). In the assay for HTGL and LPL activity, 160 μL of R-1 was incubated at 37 °C with 2 μL of plasma sample for 5 min, then 80 μL of R-2 was added. LPL activity was calculated from the total lipase activity by subtracting the HL activity measured under the same conditions without apoC-II.

2.5. Statistical analysis

Data were analyzed with Dr. SPSS II. Quantitative variables are reported as the mean ± SD values. The statistical significance of difference was determined with the Mann-Whitney *U* test. The correlation between variables is presented as Pearson's correlation coefficient (r = value). $p < 0.05$ was considered statistically significant.

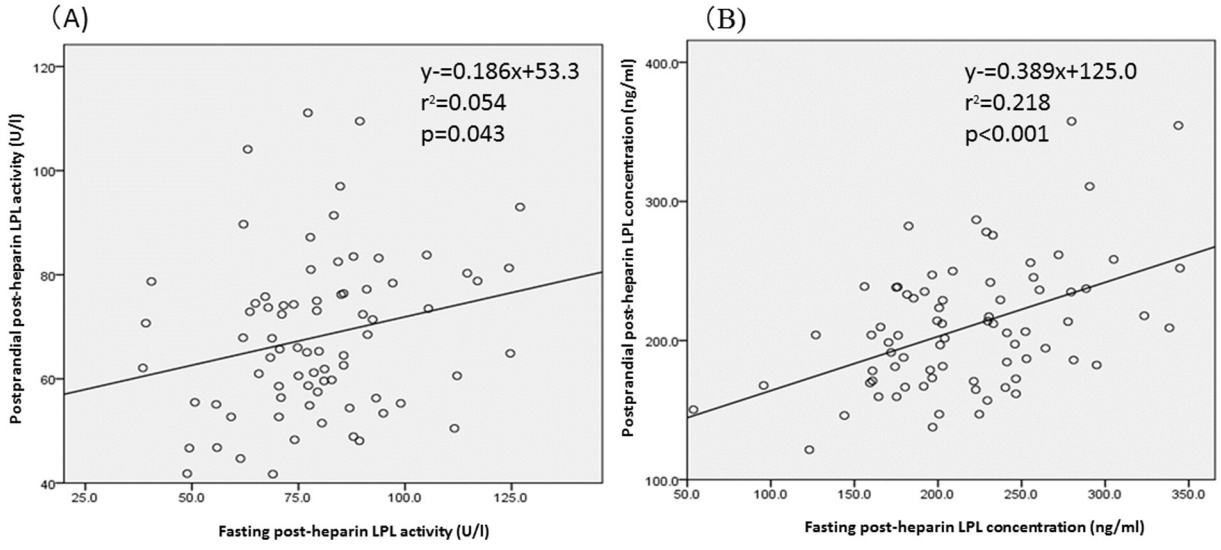

Fig. 2. Correlation between fasting and postprandial LPL activity (A) and fasting and postprandial LPL concentration (B) in post-heparin plasma.

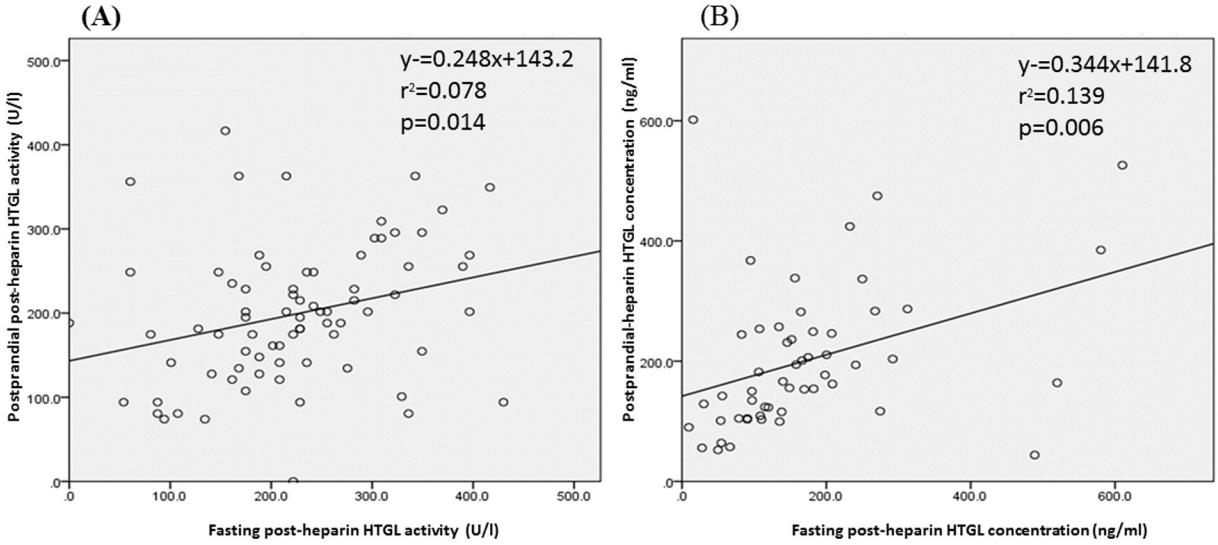

Fig. 3. Correlation between fasting and postprandial HTGL activity (A) and fasting and postprandial HTGL concentration (B) in post-heparin plasma.

3. Results

3.1. Effect of a standard meal on the LPL and HTGL concentrations and activities as well as plasma lipids and lipoproteins in the pre-heparin and post-heparin plasma

The fasting as well as postprandial plasma levels of lipids and lipoproteins in the pre-heparin and post-heparin plasma in 81 volunteers are shown in Tables 1 and 2. The mean total cholesterol (TC), LDL-C, sdLDL-C and HDL-C levels were within the normal range and did not change between the fasting (8 AM) and postprandial (8 PM) plasma. TG, RLP-C, RLP-TG and the RLP-TG/RLP-C ratio were significantly elevated in the postprandial state in both the pre-heparin and post-heparin plasma. As the LPL and HTGL activities were undetectable in the non-heparin plasma, only the LPL concentration was determined (Table 1), while the LPL and HTGL activities and concentrations were both determined in the post-heparin plasma (Table 2).

The LPL activity and concentration were found to be slightly decreased in the postprandial plasma compared with the fasting plasma, with significantly elevated TG and remnant lipoproteins. However, the HTGL activity and concentration were unchanged in the postprandial plasma (Table 2).

As shown in Fig. 1, the fasting pre-heparin LPL concentration and postprandial pre-heparin LPL concentration were positively correlated ($r^2 = 0.334$, $p < 0.001$), and the fasting LPL level was slightly, but not significantly higher in the postprandial plasma than in the pre-heparin plasma, although TG-rich lipoproteins were significantly elevated in the postprandial plasma.

Fig. 2 shows the weak but positive correlation between the fasting post-heparin LPL activity and postprandial post-heparin LPL activity (Fig. 2A) ($r^2 = 0.054$, $p = 0.043$), while the fasting post-heparin LPL concentration was more strongly correlated with the postprandial post-heparin LPL concentration ($r^2 = 0.218$, $p < 0.001$) (Fig. 2B).

The HTGL activity (Fig. 3A) and concentration (Fig. 3B) were also correlated between the fasting and postprandial post-heparin plasma ($r^2 = 0.078$, $p = 0.014$: $r^2 = 0.139$, $p = 0.006$). Those results showed that there were no significant increase of LPL and HTGL activity and concentration in the postprandial plasma compare to the fasting plasma.

3.2. Effect of oral fat load on the LPL concentration in the pre-heparin plasma of healthy volunteers

The serum TG concentrations increased 2-fold 4 h after an oral fat load, while RLP-TG increased 7-fold, as shown in Table 3. Therefore, the RLP-TG/total TG ratio increased significantly from 12% (0 h) to 46% in 4 h (3.8-fold). The increase in RLP-C in the postprandial plasma was <2 fold and the RLP-C/total TG ratio was only 5,1% in the fasting state, and decreased slightly at 4 h (4.4%) in the postprandial state. The RLP-TG/RLP-C ratio, which is indicative of the RLP particle size [27], increased 4.6-fold 4 h after the oral fat load, which means that the RLP particles released into circulation after hydrolysis of TG-rich lipoproteins on endothelium were significantly larger (i.e. were more TG-rich) than the fasting RLP particles. Table 3 also shows that >80% of the increased TG (ΔTG) after an oral fat load was derived from the increased RLP-TG (ΔRLP-TG) and kept increasing in for 2 to 6 h in the postprandial state. However, the LPL concentration did not increase in association with the increase in TG and remnant lipoproteins (Fig. 4).

Table 3
The changes of plasma lipids, lipoproteins and LPL concentration after fat load (mean ± SD).

	0 h	2 h	4 h	6 h
TC (mg/dL)	207.7 ± 29.4	210.1 ± 34.2	209.5 ± 31.4	209.1 ± 29.3
TG (mg/dL)	99.8 ± 55.9	161.6 ± 74.1*	208.2 ± 115.1*	157.4 ± 111.8*
HDL-C (mg/dL)	67.0 ± 17.1	66.5 ± 17.4	64.6 ± 17.0	65.0 ± 16.5
LDL-C (mg/dL)	123.6 ± 27.2	122.8 ± 29.2	120.0 ± 27.1	121.4 ± 26.3
RLP-C (mg/dL)	5.1 ± 2.4	7.4 ± 3.1*	9.1 ± 4.8	8.1 ± 5.8*
RLP-TG (mg/dL)	14.8 ± 15.1	67.3 ± 43.9*	107.1 ± 84.6*	64.1 ± 71.1*
RLP-TG/RLP-C	2.6 ± 1.6	8.9 ± 3.3*	11.0 ± 4.7*	6.9 ± 3.4*
LPL (ng/mL)	62.3 ± 45.7	65.5 ± 37.5	66.9 ± 42.6	81.2 ± 53.1

0 h vs 2 h, 4 h, and 6 h by *U* test.
* $p < 0.05$.

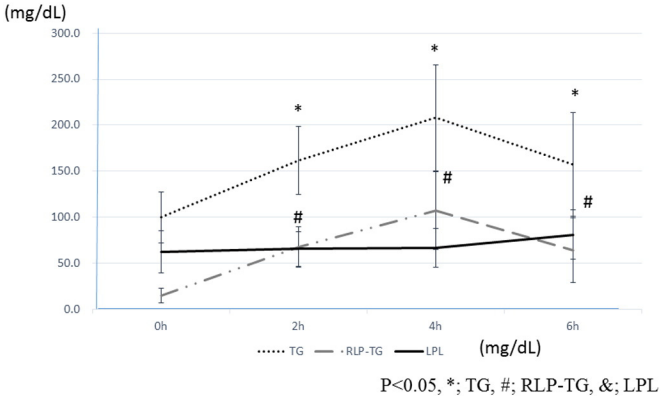

Fig. 4. The changes of plasma TG, RLP-TG and LPL at 0h, 2h, 4h and 6h after fat load.

3.3. Effect of oral glucose load on LPL activity and concentration

Two kinds of carbohydrate (glucose and sucrose) were administered to the volunteers, with water as the control (Table 4). Blood glucose and insulin (IRI) levels were significantly increased in 30 min, but the LPL concentration did not change (Fig. 5). LPL activity was undetectable 30 and 60 min after carbohydrate intake in the pre-heparin plasma. The TG and remnant lipoprotein levels were unchanged after the glucose and sugar treatment.

3.4. Effect of an oral fat and sugar combination load on the LPL concentration

The "cookie" contained both fat and sugar. Therefore, the blood sugar and TG levels increased significantly after treatment in all cases (Table 5). However, the LPL concentration did not change significantly in the pre-heparin plasma.

4. Discussion

Recently LPL has been paid more attention to the clinical significance of measuring this parameter in non-heparin plasma in more common metabolic disorders such as diabetes and metabolic syndrome than of rare LPL deficiency [28]. Studies have shown that low LPL activity and concentration has significant relationships with elevated serum lipids and lipoproteins, visceral fat area, insulin resistance, and even the development of coronary atherosclerosis [28]. Furthermore, a prospective study has demonstrated that low LPL concentration predicts future coronary events in prenon-heparin plasma in non-heparin plasma-heparin plasma [29]

The present study investigated the regulation of the plasma LPL activity and concentration after food intake in generally healthy volunteers. Although we found the similar results in patients with CHD and diabetes, we have focused on the results of healthy subjects under the physiological condition. In particular, the aim of this study was to reexamine whether the LPL activity and concentration increases along with the increase in TG-rich lipoproteins after food intake using a newly developed LPL assay methods. The experimental results showed a substantial increase in plasma triglycerides after food intake. Previous studies have reported that LPL activity in the non-heparin plasma significantly increased after an oral fat load and the increase in LPL activity occurred in parallel with the postprandial FFA response derived directly from chylomicrons [13,30]. Two parameters that might be causally related are the rise in plasma triglycerides [11], since LPL is known to bind to TG-rich lipoproteins [31–33], and recirculation in the plasma of the unesterified fatty acids derived from hydrolysis of the lipoprotein triglycerides [12], since there is evidence that fatty acids can dissociate LPL from its binding sites [13,14]. FFA was the parameter that showed the strongest association with LPL. Furthermore, an 18:2 FFA correlated with LPL, whereas there was no significant correlation between the plasma triglycerides and LPL. This indicates that the magnitude of the response was similar between LPL and the 18:2 FFA, but not between LPL and plasma triglycerides [13,30]. In the other previous study, healthy young men were subjected to an intravenous infusion of a triglyceride emulsion (Intralipid®) at a rate exceeding the normal removal capacity for triglycerides [13]. In this overload situation, a substantial increase in plasma FFA occurred in some, but not all subjects, and was closely with a rise in the plasma LPL activity. The study by Saxena et al. [14] reported that oleic acid dissociated LPL from cultured endothelial cells. The same group studied the effect of different fatty acid (FA) species on the release of LPL from endothelial cells [34]. Also oral fat load by OFTT cream showed the similar increase of FFA in Japanese population [18,35]. Furthermore, the mixed meal used in the studies contained a substantial amount of glucose, which led to postprandial hyperinsulinemia significantly in diabetes patients. However, even though insulin is strongly antilipolytic [36,37], the total FFA level was undiminished, which presupposes FFA input into the plasma compartment from sources other than adipose tissue. Nevertheless, insulin may also exert a direct effect on plasma LPL activity. Eckel et al. [38] demonstrated that plasma LPL activity increased after an oral glucose load in the non-heparin plasma of diabetes patients. However, we were unable to detect any increase in either the LPL activity or concentration after glucose and sucrose load in healthy volunteers as well as in diabetes patients (data not shown). Cookie test which contained both fat and carbohydrate did not also show the increase of LPL concentration after intake associated with the increase of TG-rich lipoproteins (Table 5).

Beisiegel et al. [39] and others presented data suggesting that LPL serves as a ligand for the recognition of chylomicron remnants by hepatic receptors. Hence, it may be that LPL is released from its endothelial binding by high local FFA concentration induced by active lipolysis and subsequently binds to lipoprotein remnants (RLP-LPL complex) [33], serving as a ligand that the particles are ready to be taken up by the liver through the receptors such as LRP1. These results suggested that an increase of LPL is associated with an increase of remnant lipoproteins. However, we recently reported that the amount of LPL present per RLP particle was inversely correlated with the RLP-TG or RLP-LPL/RLP-TG ratio (reflecting particle size) [26,33]. This means that RLP with large particle size may be the result of insufficient hydrolysis of TG-rich lipoproteins by LPL on the endothelium after fat intake.

Table 4
The changes of plasma lipids, lipoproteins, insulin and LPL concentration after glucose ant sucrose treatment (mean ± SD).

	Water (n = 6)		Glucose (n = 9)		Sucrose (n = 10)	
	Before	120 min	Before	120 min	Before	120 m
TC (mg/dL)	219.5 ± 38.5	211.3 ± 35.5	200.3 ± 12.5	191.6 ± 12.8	187.8 ± 37.4	187.6 ± 43.5
TG (mg/dL)	184.0 ± 123.6	134.8 ± 48.1	91.7 ± 35.5	95.3 ± 38.9	120.1 ± 75.6	121.1 ± 77.8
LDL-C (mg/dL)	135.0 ± 39.9	133.6 ± 36.6	119.6 ± 16.7	114.1 ± 16.5	104.9 ± 32.2	105.9 ± 35.3
RLP-C (mg/dL)	5.7 ± 2.9	7.7 ± 5.3	5.1 ± 2.1	5.5 ± 3.1	6.3 ± 2.9	6.5 ± 3.9
RLP-TG (mg/dL)	23.7 ± 16.7	23.8 ± 13.7	16.3 ± 3.4	18.1 ± 6.4	24.4 ± 16.6	24.6 ± 17.1
IRI (μU/mL)	6.3 ± 1.9	4.4 ± 2.3	5.9 ± 2.8	12.8 ± 10.3	6.2 ± 2.7	5.6 ± 2.8
LPL (ng/mL)	67.1 ± 17.7	72.0 ± 19.5	61.4 ± 22.7	67.1 ± 30.7	69.5 ± 19.5	83.2 ± 17.7

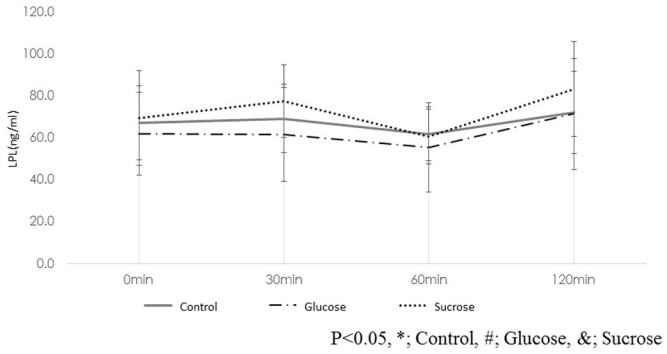

Fig. 5. The changes of plasma LPL concentration at 0, 30, 60, 120 min after glucose, sucrose and water treatment.

Therefore, large remnant particles expressed as high RLP-TG/RLP-C ratio [27] were significantly increased in postprandial plasma. This may not be caused by the increased formation of RLP particles at the endothelium or increased VLDL formation in the liver, but because of insufficient capacity of LPL hydrolysis activity at endothelium against the overloaded CM and VLDL exposure after a fat intake. In particular, as Karpe [9] proposed, LPL hydrolyzes CM more efficiently than VLDL after fat intake, large VLDL remnants rather than CM remnants increase prevalently in postprandial plasma [7,8]. Therefore, the apoB48 particles in RLP is reportedly smaller than that of the apoB100 particle [40], which means that apoB48 carrying particles are preferably hydrolyzed by LPL. Therefore, when the LPL activity is not sufficient to hydrolyze a large amount of CM or VLDL on endothelium, the RLP particle size becomes significantly increased along with the high RLP-TG/RLP-C ratio and less amount of LPL concentration. We reported that RLP particle size in type 2 diabetes and metabolic syndrome was significantly larger than in controls and was inversely correlated with the circulating LPL concentration, indicating the pro-atherogenic characteristics of remnants [41]. The delayed clearance of RLP in plasma may largely depend on the amount of LPL on RLP, which is the ligand for the receptors of liver and other tissues and organs. However, in case of LPL deficiency, CM and VLDL particles are not metabolized to smaller atherogenic remnants and increase in plasma without cardiovascular disease, but often causes acute pancreatitis [42].

In sharp contrast to the findings for LPL, we found that there was no change in the HTGL activity or concentration between fasting and after the intake of a standard meal, although HTGL has been believed to play a role in remnant metabolism. Peterson et al. [13] reported no change in HTGL activity after an infusion of lipid emulsions. These findings indicate that the plasma HTGL remains in a fairly static equilibrium with the level of HTGL at the vascular binding sites, and that shifts in metabolic conditions do not rapidly change either HTGL activity or its interaction with these vascular sites. The experimental conditions used in the present study most probably did not result in high local FFA concentrations near the HTGL binding site, due to the lipoprotein specificity of HTGL, which essentially does not include large TG-rich lipoproteins [38].

In contrast with the previous studies of fat and glucose load [13,30,38,43–46], we did not detect any significant increase in the LPL concentration after oral fat and sugar intake. The previous results were mostly obtained by using a radiochemical LPL activity assay method in the non-heparin plasma, which detected a very low LPL activity level compared with the post-heparin LPL activity level. The LPL activity assay [22] we used for the non-heparin plasma did not detect any increased LPL activity after a fat and glucose load, unlike shown by Peterson et al. [13], Karpe et al. [30] and Eckel et al. [38]. Although the method we used for the LPL activity assay did not detect LPL activity in the non-heparin plasma [22,33], the LPL concentration was 50 ng/mL or higher, which is approximately 1/5 that of the post-heparin LPL concentration. The LPL activity reported in the non-heparin plasma in previous reports was <1/100 of the post-heparin LPL activity. This is the other discrepancy between the previous reports and our own results.

Watson et al. [47] subsequently reported an improved radiochemical method for the selective measurement of post-heparin lipase activity which was adapted to lipoprotein lipase and hepatic lipase in the pre-heparin plasma. The assay sensitivity was increased approximately four-fold by doubling both the volume of the plasma used and the volume of lipolytic products taken for liquid scintillation counting, and was further improved by increasing the incubation period by 50% to 90 min. Using the improved assay, it was reported that the pre-heparin activities of hepatic lipase, but not lipoprotein lipase, appear to be a useful measure of the physiological function of "whole body" enzyme activity in cross-sectional and metabolic studies in which heparinization is not possible. The pre-heparin LPL activity, may reflect displacement of the enzyme by FFA and the subsequent binding to the remnants of TG-rich lipoproteins. However, the LPL bound to remnants is known to be inactive [33], so only a very small portion of the LPL detected in the non-heparin plasma may accurately reflect the activity. For example in the previous report [30], LPL activity in patients with myocardial infarction (MI) was shown to be significantly higher than in the control group before and after a fat load. However, the recent studies have shown that the plasma LPL activity and concentration in MI patients are significantly lower in both the non- and post-heparin plasma [28,29]. Therefore, the LPL activity increased after food intake in previous reports may reflect an increased activity of other lipases in plasma, using the radiochemical method.

However, as the TG and remnant lipoprotein levels vary greatly within a day of food intake [48], the LPL concentration in the non-heparin plasma which remains unchanged over a certain period time may serve as a usefully stable diagnostic marker of TG-rich lipoprotein metabolism that would enable to determine at any time point in either the fasting or postprandial plasma in diabetes and cardiovascular patients.

In summary, the present study examined the plasma LPL activity and concentration using newly developed ELISA in response to an oral fat and sugar intake in generally healthy subjects covering a wide range of plasma TG levels. No significant increase in the LPL activity and concentration took place in parallel with the accumulation of TG-rich lipoproteins, unlike the findings previously reported. Therefore, no increase of LPL activity on endothelium at hydrolysis after food intake may enhance the formation of large RLP particles and delayed clearance of RLP associated with small amount of LPL on RLP as a ligand to receptors.

Table 5
The changes of plasma lipids, lipoproteins, blood sugar and LPL concentration after cookie load (0 h vs 1 h, 2 h and 4 h by *U* test) (mean ± SD).

	0 h	1 h	2 h	4 h
TC (mg/dL)	218 ± 63.3	212.7 ± 58.4	210.0 ± 59.5	214.4 ± 61.9
TG (mg/dL)	154.2 ± 139.3	181.0 ± 125.3	233.3 ± 135.3*	240.2 ± 160.5*
HDL-C (mg/dL)	56.0 ± 13.7	54.4 ± 12.4	52.7 ± 12.1	53.0 ± 11.9
LDL-C (mg/dL)	132.7 ± 57.2	128.7 ± 53.2	125.3 ± 53.2	127.3 ± 52.9
BS (mg/dL)	116.4 ± 22.8	172.8 ± 59.9*	170.7 ± 62.2*	125.5 ± 43.5
LPL (ng/dL)	69.7 ± 39.9	57.2 ± 19.3	61.8 ± 21.4	66.8 ± 28.3

* $p < 0.05$.

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References

[1] R.J. Havel, Postprandial hyperlipidemia and remnant lipoproteins, Curr. Opin. Lipidol. 5 (1994) 102–109.
[2] R.J. Havel, Remnant lipoproteins as therapeutic targets, Curr. Opin. Lipidol. 11 (2000) 615–620.
[3] H. Iso, Y. Naito, S. Sato, et al., Serum triglycerides and risk of coronary heart disease among Japanese men and women, Am. J. Epidemiol. 153 (2001) 490–499.
[4] B.G. Nordestgaard, M. Benn, P. Schnohr, A. Tybjaerg-Hansen, Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women, JAMA 298 (2007) 299–308.
[5] S. Bansal, J. Buring, N. Rifai, S. Mora, F.M. Sacks, P.M. Rider, Fasting compared with nonfasting triglycerides and risk of cardiovascular events in women, JAMA 298 (2007) 309–316.
[6] D.B. Zilversmit, Atherogenesis: a postprandial phenomenon, Circulation 60 (1979) 473–485.
[7] K. Nakajima, T. Nakano, Y. Tokita, et al., The characteristics of remnant lipoproteins in the fasting and postprandial plasma, Clin. Chim. Acta 413 (2012) 1077–1086.
[8] B.O. Schneeman, L. Kotite, K.M. Todd, R.J. Havel, Relationships between the responses of triglyceride-rich lipoproteins in blood plasma containing apolipoprotein B-48 and B-100 to a fat-containing meal in normolipidemic humans, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) (2069-73.9).
[9] F. Karpe, Postprandial lipoprotein metabolism and atherosclerosis, J. Intern. Med. 246 (1999) 341–355.
[10] C.N. Goulbourne, P. Gin, A. Tatar, C., et al., The GPIHBP1-LPL complex is responsible for the margination of triglyceride-rich lipoproteins in capillaries, Cell Metab. 19 (2014) 849–860.
[11] R.H. Eckel, Lipoprotein lipase, a multifactorial enzyme relevant to common metabolic diseases, N. Engl. J. Med. 320 (1989) 1060–1068.
[12] T. Olivecrona, G. Bengtsson-Olivecrona, Lipoprotein and hepatic lipase, Curr. Opin. Lipidol. 1 (1990) 222–230.
[13] J. Peterson, B.E. Bihain, G. Bengtsson-Olivecrona, R.J. Deckelbaum, Y.A. Carpentier, T. Olivecrona, Fatty acid control of lipoprotein lipase: a link between energy metabolism and lipid transport, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 909–913.
[14] U. Saxena, L.D. Witte, I.J. Goldberg, Release of endothelial cell lipoprotein lipase by plasma lipoproteins and free fatty acids, J. Biol. Chem. 264 (1989) 4349–4355.
[15] P.A1. Kern, R.A. Martin, J. Carty, I.J. Goldberg, J.M. Ong, Identification of lipoprotein lipase immunoreactive protein in pre- and postheparin plasma from normal subjects and patients with type I hyperlipoproteinemia, J. Lipid Res. 31 (1990) 17–26.
[16] K.L. Stanhope, A.A. Bremer, V. Medici, et al., Consumption of fructose and high fructose corn syrup increase postprandial triglycerides, LDL-cholesterol, and apolipoprotein-B in young men and women, J. Clin. Endocrinol. Metab. 96 (2011) E1596–E1605.
[17] M.D. Mifflin, S.T. St Jeor, L.A. Hill, B.J. Scott, S.A. Daugherty, Y.O.A. Koh, A new predictive equation for resting energy expenditure in healthy individuals, Am. J. Clin. Nutr. 51 (1990) 241–247.
[18] A. Tanaka, N. Tomie, T. Nakano, et al., Measurement of postprandial remnant-like particles following a fat-loading test, Clin. Chim. Acta 275 (1998) 43–52.
[19] Y. Harano, T. Miyawaki, J. Nabiki, et al., Development of cookie test for the simultaneous determination of glucose intolerance, hyperinsulinemia, insulin resistance and postprandial dyslipidemia, Endocr. J. 53 (2006) 173–180.
[20] T. Machida, K. Miyashita, T. Sone, et al., Determination of serum lipoprotein lipase using a latex particle-enhanced turbidimetric immunoassay with an automated analyzer, Clin. Chim. Acta 442 (2015) 130–135.
[21] K. Miyashita, J. Kobayashi, S. Imamura, et al., A new enzyme-linked immunosorbent assay system for human hepatic triglyceride lipase, Clin. Chim. Acta 424 (2013) 201–206.
[22] S. Imamura, J. Kobayashi, K. Nakajima, et al., A novel method for measuring human lipoprotein lipase and hepatic lipase activities in postheparin plasma, J. Lipid Res. 49 (2008) 1431–1437.
[23] K. Nakajima, T. Saito, A. Tamura, et al., Cholesterol in remnant-like lipoproteins in human serum using monoclonal anti apo B-100 and anti apo A-I immunoaffinity mixed gel, Clin. Chim. Acta 223 (1993) 53–71.
[24] K. Nakajima, M. Okazaki, A. Tanaka, et al., Separation and determination of remnant-like particles in serum from diabetes patients using monoclonal antibodies to apo B-100 and apo A-I, J. Clin. Ligand Assay 19 (1996) 177–183.
[25] Y. Ito, M. Fujimara, M. Ohta, T. Hirane, Development of a homogeneous assay for quantification of small, dense LDL cholesterol, Clin. Chem. 57 (2011) 57–65.
[26] T. Shirakawa, K. Nakajima, Y. Shimomura, et al., Comparison of the effect of post-heparin and pre-heparin lipoprotein lipase and hepatic triglyceride lipase on remnant lipoprotein metabolism, Clin. Chim. Acta 440 (2014) 193–200.
[27] M. Okazaki, S. Usui, N. Tada, T. Nakano, K. Nakajima, Relation between RLP- triglyceride to RLP-cholesterol ratio and particle size distribution in cholesterol profiles by HPLC, Clin. Chim. Acta 296 (2000) 135–149.
[28] J. Kobayashi, A. Nohara, M. Kawashiri, et al., Serum lipoprotein lipase mass: clinical significance of its measurement, Clin. Chim. Acta 378 (2007) 7–12.
[29] J. Rip, M.C. Nierman, N.J. Wareham, et al., Serum lipoprotein lipase concentration and risk for future coronary artery disease: the EPIC-Norfolk prospective population study, Arterioscler. Thromb. Vasc. Biol. 26 (2006) 637–642.
[30] F. Karpe, T. Olivecrona, G. Walldius, A. Hamsten, Lipoprotein lipase in plasma after an oral fat load: relation to free fatty acids, J. Lipid Res. 33 (1992) 975–984.
[31] I.J. Goldberg, J.J. Kandel, C.B. Blum, H.N. Ginsberg, Association of plasma lipoproteins with post-heparin plasma lipase activities, J. Clin. Invest. 78 (1986) 1523–1528.
[32] A. Zambon, I. Schmidt, U. I, J.D. Brunzell, Dimeric lipoprotein lipase is bound to triglyceride-rich plasma lipoproteins, J. Lipid Res. 37 (1996) 2394–2404.
[33] K. Sato, F. Okajima, K. Miyashita, S. Imamura, et al., Most lipoprotein lipase is bound to remnant lipoproteins in plasma: a new definition for remnant lipoproteins, Clin. Chem. Acta 461 (2016) 114–125.
[34] U. Saxena, I.J. Goldberg, Interaction of lipoprotein lipase with glycosaminoglycans and apolipoprotein C-II: effects of free fatty acids, Biochim. Biophys. Acta 1043 (1990) 161–168.
[35] S. Hashimoto, K. Ootani, S. Hayashi, M. Naito, Acute effects of shortly pre- versus postprandial aerobic exercise on postprandial lipoprotein metabolism in healthy but sedentary young women, J. Atheroscler. Thromb. 18 (2011) 891–900.
[36] E.G. Ball, R.L. Jungas, Effects of insulin on fatty acids and glycerol production with and without glucose, Biochemistry 2 (1963) 383–389.
[37] J.N. Fain, V.P. Kovacev, R.O. Scow, Antilipolytic effect of insulin in fat cells of the rat, Endocrinology 78 (1966) 773–779.
[38] R.H. Eckel, I.J. Goldberg, L. Steiner, T.J. Yost, T.R. Paterniti, Plasma lipolytic activity. Relationship to postheparin lipolytic activity and evidence for metabolic regulation, Diabetes 37 (1988) 610–615.
[39] U. Beisiegel, W. Weber, G. Bengtsson-Olivecrona, Lipoprotein lipase enhances the binding of chylomicrons to low density lipoprotein receptor-related protein, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 8342–8346.
[40] T. Nakano, A. Tanaka, M. Okazaki, Y. Tokita, T. Nagamine, K. Nakajima, Particle size of apoB-48 carrying lipoproteins in remnant lipoproteins isolated from postprandial plasma, Ann. Clin. Biochem. 48 (2011) 57–64.
[41] T. Shirakawa, K. Nakajima, Yatsuzuka, et al., The role of circulating lipoprotein lipase and adiponectin on the particle size of remnant lipoproteins in patients with diabetes mellitus and metabolic syndrome, Clin. Chim. Acta 440 (2014) 123–132.
[42] T.G. Redgrave, G.F. Watts, I.J. Martins, Chylomicron remnant metabolism in familial dyslipidemias studied with a remnant-like emulsion breath test, J. Lipid Res. 42 (2001) 710–715.
[43] M.-R. Taskinen, T. Kuusi, Enzymes involved in triglyceride hydrolysis, Bailliere Clin. Endocrinol. Metab. 1 (1987) 639–666.
[44] T. Olivecrona, G. Bengtsson-Olivecrona, M. Hultin, Lipases and lipoprotein metabolism, in: J. Shepherd (Ed.), Lipoprotein and the Pathogenesis of Atherosclerosis, Elsevier, Amsterdam 1991, pp. 51–58.
[45] J. Peterson, T. Olivecrona, G. Bengtsson-Olivecrona, Distribution of lipoprotein lipase and hepatic lipase between plasma and tissues: effect of hypertriglyceridemia, Biochim. Biophys. Acta 837 (1985) 262–270.
[46] G. Bengtsson-Olivecrona, T. Olivecrona, Assay of lipoprotein lipase and hepatic lipase, in: R.E. Skinner, C.A. Converse (Eds.), Lipoprotein Analysis. A Practical Approach, Practical Approach Series, Oxford University Press, Oxford, 1992.
[47] T.D. Watson, C.E. Tan, M. McConnell, S.K. Clegg, L.F. Squires, C.J. Packard, Measurement and physiological significance of lipoprotein and hepatic lipase activities in preheparin plasma, Clin. Chem. 41 (1995) 405–412.
[48] K. Nakajima, T. Nakano, Y. Tokita, et al., The characteristics of remnant lipoproteins in the fasting and postprandial plasma, Clin. Chim. Acta 413 (2012) 1077–1086.