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Triglyceride content in remnant lipoproteins is significantly increased after food intake and is associated with plasma lipoprotein lipase

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ABSTRACT

Background: Previous large population studies reported that non-fasting plasma triglyceride (TG) reflect a higher risk for cardiovascular disease than TG in the fasting plasma. This is suggestive of the presence of higher concentration of remnant lipoproteins (RLP) in postprandial plasma.
Methods: TG and RLP-TG together with other lipids, lipoproteins and lipoprotein lipase (LPL) in both fasting and postprandial plasma were determined in generally healthy volunteers and in patients with coronary artery disease (CAD) after consuming a fat load or a more typical moderate meal.
Results: RLP-TG/TG ratio (concentration) and RLP-TG/RLP-C ratio (particle size) were significantly increased in the postprandial plasma of both healthy controls and CAD patients compared with those in fasting plasma. LPL/RLP-TG ratio demonstrated the interaction correlation between RLP concentration and LPL activity The increased RLP-TG after fat consumption contributed to approximately 90% of the increased plasma TG, while approximately 60% after a typical meal. Plasma LPL in postprandial plasma was not significantly altered after either type of meal.
Conclusions: Concentrations of RLP-TG found in the TG along with its particle size are significantly increased in postprandial plasma compared with fasting plasma. Therefore, non-fasting TG determination better reflects the presence of higher RLP concentrations in plasma.

1. Introduction

Zilversmit first proposed the postprandial increase of TG to be the most common form of hyperlipidemia which is associated with increased remnant lipoproteins (RLP) as a risk factor for cardiovascular disease (CVD) [1]. More recently, Nordestgaard et al. [2], Bansal et al. [3] and Iso et al. [4] reported that the triglycerides (TG) measured in non-fasting samples were more sensitive than the conventional measurements of the fasting TG concentrations in predicting the risk of cardiovascular events (the Copenhagen Heart Study, Women's Health Study and the Japanese population study). Also, the Framingham Offspring Study previously reported by us [5] showed that the fasting TG concentration was not an independent cardiovascular risk factor, while RLP-cholesterol (RLP-C) was an independent risk factor in the fasting plasma in women. Therefore, it is necessary to investigate the difference between fasting and postprandial TG and RLP: why does the postprandial TG indicate greater risk than the fasting TG concentration? It has been shown that postprandial TG is associated with increased remnants of apoB-48 carrying chylomicrons (CM) of intestinal origin and apoB-100 carrying very low density lipoproteins (VLDL) of hepatic origin in the postprandial state [6]. Postprandial TG and RLP are known to attain their highest concentrations 3–6 h after food intake [7–9]. Therefore, we analyzed the RLP-TG/TG ratio (concentration) and RLP-TG/RLP-C ratio (particle size) [10] associated with the lipoprotein lipase (LPL) activity and concentration in both the fasting and postprandial plasma. However, the definition of RLP has been unclear for many years, in part due to the variety of methods used to measure RLP. The most common definition of RLP proposed several decades ago is based

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on the intermediate density lipoproteins (IDL) isolated using an ultracentrifugation method [11]. As the most important characteristic of RLP has been shown to be the TG-rich lipoproteins that are increased after food intake [1], IDL cannot be utilized as indicative RLP, since IDL does not significantly increase following food intake [12–14]. There are other methods, such as electrophoresis, NMR and HPLC, that are used to identify RLP by charge, particle size [15] and the calculated remnant cholesterol [16], respectively, but none isolate a substantial RLP particle.

We reported the isolation method of RLP by immunoaffinity gels and its diagnostic utility in serum and plasma as RLP-C and RLP-TG over the decades [17–20]. Therefore, we were able to perform a direct comparison between the increased total TG and isolated RLP-TG in the fasting and postprandial plasma. We have already reported that the increase of RLP-TG (postprandial RLP-TG minus fasting RLP-TG) comprises approximately 80% of the increased TG (postprandial TG minus fasting TG) when a fat rich meal or high fat cream is ingested [21].

In this study, we report plasma concentrations of TG and RLP-TG in the fasting and postprandial plasma in normal controls as well as patients with CVD who consumed typical moderate meals in the course of daily life and also the results with studies using a fat load. In order to investigate the characteristics of increased TG in the postprandial plasma, the measurement of the TG in RLP (RLP-TG), rather than RLP-C, was the primary focus of this study. As we demonstrated that the majority of plasma LPL circulating in plasma is bound to RLP [22], we compared the RLP-TG/TG, RLP particle size and LPL concentration in healthy controls and CAD patients. Also we newly calculated the LPL/RLP-TG ratio as Felts et al. [23] demonstrated as LPL/TG for the interaction correlation between remnant concentration and LPL activity. Finally, we discussed the mechanism of the increase in RLP-TG and delayed clearance of RLP that occurs after food intake, which is closely associated with LPL activity and concentration on endothelial cells and release into circulation.

2. Materials and methods

2.1. Fifty four Japanese volunteers who were without evidence of CVD, diabetes or other chronic disease (30 males and 24 females, aged 28–63 y) were recruited for the oral fat load test at the Gunma University School of Health Sciences (Table 1)

The study was approved by the Ethics Committee of Gunma University School of Medicine and all of the volunteers provided written informed consent to participate in this study. Briefly, after an overnight 12 h fast, the subjects ingested 17 g/m^2 (body surface area) of fat emulsion (OFTT cream, Jomo Foods) [7]. Blood samples were taken before and 60, 120, 240 and 360 min after the oral fat load.

2.2. The comparison study in fasting and postprandial plasma was performed on samples obtained from relatively healthy young (40 males and 39 females) subjects (Caucasian 45, Asian 10, Hispanic 9, African American 7, others 8) recruited at the University of California, Davis, CA (Table 2)

Some of the subjects were overweight or obese with a median age of 24 y and a median BMI of 24 kg/m^2. Inclusion criteria included an age from 18 to 40 y and BMI of 18–35 kg/m^2 with a self-report of stable body weight during the prior 6 months. All seventy nine 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 all of the subjects provided written informed consent to participate in the study. Baseline blood samples from this study of the metabolic effects of dietary sugars were used and were obtained by the method of Stanhope et al. [24] and all of the parameter analyses were performed at Gunma University. The fasting blood samples were collected before breakfast at between 08:00 and 09:00 h and the postprandial blood samples were collected several hours after the dinner meal between 20:00 and 22:00 h on the same day. During the day, standardized meals [25] were provided as breakfast, lunch and dinner to all of the subjects.

2.3. A case control study of RLP-C and RLP-TG concentrations in patients with angiographically determined coronary artery disease (CAD) and healthy controls was performed

This is a part of the study previously reported by Leary et al. [26] to establish US reference ranges for the serum RLP-C concentrations. Men and women >17 y were recruited in 4 different parts of the USA (Austin, TX; Brighton, MA; Miami, FL; and Phoenix, AZ). These subjects were free of symptoms and any signs of CAD and also of any endocrine or metabolic disorders that might affect lipid metabolism. However, the lipid and lipoprotein concentrations per se were not part of the inclusion or exclusion criteria. None of the subjects were taking any medication expected to alter lipid or lipoprotein metabolism. Each subject was asked to participate in 2 visits: 1 after 12 h of fasting and 1 at a random time in relation to the last meal (either fasting or non-fasting). The random sample was considered to be in the postprandial state for this study. Serum concentration of RLP-C, RLP-TG, total cholesterol (TC), TG, LDL-C and HDL-C were measured at each visit. To evaluate the relative CAD risk of RLP-C and RLP-TG in predicting CAD, 203 adult patients with >20% stenosis of at least one coronary artery at the time of coronary angiography were recruited from nine medical centers in the USA and one medical center in Canada. Control subjects (n = 477) with similar ages to the CAD patients were recruited from the same 4 US centers that participated in the reference range study. Subjects with serum TG blank values >50 mg/dl (because of the heparin used during angiography before specimen collection), who did not complete both visits, or who did not have a fasting specimen collected were

Table 1
The changes of plasma lipids, lipoproteins and LPL concentration after fat load; oral fat load in healthy Japanese controls.

	0 h Median (25%–75% tile)	2 h Median (25%–75% tile)	4 h Median (25%–75% tile)	6 h Median (25%–75% tile)
TC (mg/dl)	225 (184–250)	230 (180–260)	230 (180–250)	230 (180–260)
TG (mg/dl)	113 (66–160)	140 (110−220)*	180 (140–380)*	160 (80–300)*
HDL-C (mg/dl)	67 (45–80)	70 (40–80)	70 (40–80)	70 (40–80)
LDL-C (mg/dl)	128 (105–150)	130 (100–150)	130 (100–140)	130 (100–150)
RLP-C (mg/dl)	5.6 (3.9–6.9)	6.3 (4.9–8.4)*	8.1 (5.4–13.8)*	7.5 (4.7–20)*
RLP-TG (mg/dl)	13.8 (5.5–29.3)	37.7 (35.4–68.4)*	86.2 (38.4–224.4)*	77.7 (16.3–142.4)*
RLP-TG/RLP-C	2.1 (1.3–4.9)	7.2 (5.7–8.5)*	11.6 (6.4–15.9)*	5.6 (3.4–11.4)*
RLP-TG/TG	0.11 (0.08–0.19)	0.32 (0.27–0.34)*	0.47 (0.31–0.59)*	0.36 (0.19–0.55)*
apoB-100 (mg/dl)	111.9 (88.8–162.4)	126.4 (87.7–173)	126.2 (95–160.2)	126.6 (101–168.3)
apoB-48 (μg/ml)	6 (3.1–10.3)	10.8 (6.3–14)*	13 (6.4–18.3)*	9.3 (3.8–22.8)*
LPL (ng/ml)	23.7 (19.4–25.7)	23 (18.8–28.0)	20.6 (18.0–23.0)	22.2 (16.9–25.9)
LPL/RLP-TG	1.92 (0.9–4.4)	0.5 (0.38–0.75)*	0.48 (0.22–0.8)*	0.65 (0.13–1.32)

* means $P < 0.05$.

Table 2
Characteristics of lipids, lipoproteins and lipases in the fasting and postprandial state in post-heparin plasma; effect of standard meal assessments in Americans.

	Fasting (n = 79) Median (25% tile–75% tile)	Postprandial (n = 79) Median (25% tile–75% tile)	p
TC (mg/dl)	160 (141–182)	160 (145–184)	n.s.
TG (mg/dl)	47 (31–74)	73 (44–106)	p < 0.001
HDL-C (mg/dl)	47 (41–59)	47 (40–58)	n.s.
LDL-C (mg/dl)	90 (70–107)	91 (73–108)	n.s.
sd-LDL (mg/dl)	21.4 (16.9–31.0)	24.4 (18.7–36)	n.s.
RLP-C (mg/dl)	3.5 (2.5–4.8)	4.7 (3.5–6.8)	p < 0.001
RLP-TG (mg/dl)	4.2 (2.3–7.3)	14.6 (8.0–28.5)	p < 0.001
RLP-TG/RLP-C	1.4 (0.7–2.0)	3.6 (1.9–5.3)	p < 0.001
RLP-TG/TG	0.09 (0.06–0.11)	0.23 (0.15–0.30)	p < 0.001
LPL (U/l)	79 (68.5–89.3)	66 (57.0–78.6)	p < 0.001
LPL (ng/ml)	206.4 (176.9–250.9)	205.5 (175.7–237.8)	n.s.
LPL (U/l)/RLP-TG	19.6 (10.4–34.0)	4.4 (2.2–8.9)	p < 0.001
LPL (ng/ml)/RLP-TG	56.5 (26.9–98.5)	12.5 (7.2–28.0)	p < 0.001

excluded from the data analysis. The final population included 151 CAD patients and 301 controls based on the inclusion and exclusion criteria. All clinical protocols were approved by the Institutional Review Board, and informed consent forms were signed by all of the participating subjects. All samples were sent to Pacific Biometrics, Inc., a commercial specialty lipid laboratory in Seattle, WA accredited by the College of American Pathologists, for analysis of the lipids and lipoproteins.

2.4. Measurements of lipids and lipoproteins and LPL and HTGL activity/concentration

The plasma samples for the measurement of TC, TG, HDL-C, LDL-C, RLP-C, RLP-TG and sdLDL-C were obtained before and after heparin infusion and kept frozen at −80 °C until analysis. As LPL and HTGL activities were not detectable in the pre-heparin plasma, all the lipase activities in this study were determined in post-heparin plasma. The post-heparin plasma sample was collected 15 min after the intravenous injection of 50 units of heparin/kg body weight (BW) for the assay of the LPL/HTGL activity and concentration. Pre-heparin LPL and HTGL concentration were determined with a highly sensitive and specific ELISA [27,28]. LPL and HTGL activity was analyzed by the Imamura method [29]. TC and TG concentration were determined enzymatically. LDL-C and HDL-C concentrations were measured using a homogenous method (Kyowa Medex). Glucose and insulin were determined by PolyChem (Polymedco). RLP-C and RLP-TG were determined using an immunoseparation method (JIMRO II) [18]. Small dense LDL-C was determined by the method of Ito et al. (Denka-Seiken) [30].

2.5. Statistics

All values were expressed as the median and the 25th and 75th percentiles. Data were analyzed with Stat Flex version 5.0 for Windows (Artec). The statistical significance of differences was determined by Wilcoxon test for two-group comparison and Kruskal-Wallis test and then multiple comparison tests for non-parametric analysis and Dunn test for multi-group comparisons. The comparison between the healthy control group and CAD group conducted by Mann-Whitney's *U* test. The correlation between variables is presented as Pearson's correlation coefficient (r value). A $p < 0.05$ was considered statistically significant.

3. Results

3.1. Effect of oral fat load on lipids, lipoproteins and the LPL concentration in plasma

Serum TG concentrations increased 1.6-fold 4 h after the fat load, while RLP-TG increased 6.2-fold, as shown in Table 1. Therefore, the RLP-TG/TG ratio increased significantly from 0.11 (0 h) to 0.47 in a 4 h period (4.3-fold). The increase in RLP-C in the postprandial plasma was 1.5 fold and the RLP-C/TG ratio was 5.0% in the fasting plasma and was decreased slightly at 4 h (4.5%) in the postprandial plasma. The RLP-TG/RLP-C ratio, which reflects the RLP particle size [10] was increased 5.5-fold 4 h after the fat load, which means that the RLP particles became significantly larger (i.e., more TG-rich) than the fasting RLP particles. Fig. 1 shows the changes in the RLP-TG/TG ratio from before the fat load ($y = 0.230x - 8.1$) to 4 h after the fat load ($y = 0.677x - 33.8$), indicating a more than two fold increase of RLP-TG in the total TG. Fig. 2 shows the changes of the plasma delta TG concentration (the postprandial TG minus the fasting TG) and delta RLP-TG (the postprandial RLP-TG minus the fasting RLP-TG) at 2, 4 and 6 h after the fat load. Significantly higher RLP-TG content was found in the delta TG concentration, approximately 90% of which is comprised of the increased TG (delta TG) after the fat load and which kept increasing for 2 to 6 h in the postprandial plasma. However, the LPL concentration did not increase in association with the increase in TG and RLP-TG after the fat load (Table 1). Therefore, the LPL/RLP-TG ratio was highest before the fat load and decreased to approximately 1/4 of that after the fat load. Those results show that the large RLP particles after a fat load carry significantly low LPL concentration ($p < 0.001$) compared with the small RLP particles in the fasting plasma.

3.2. Effect of a standard meal on TG and RLP-TG in healthy subjects

Fasting and postprandial plasma concentrations of lipids and lipoproteins as well as the LPL and HTGL activity and concentration in the post-heparin plasma in 79 volunteers are shown in Table 2. The median TC, LDL-C, sdLDL-C and HDL-C concentrations were within the normal

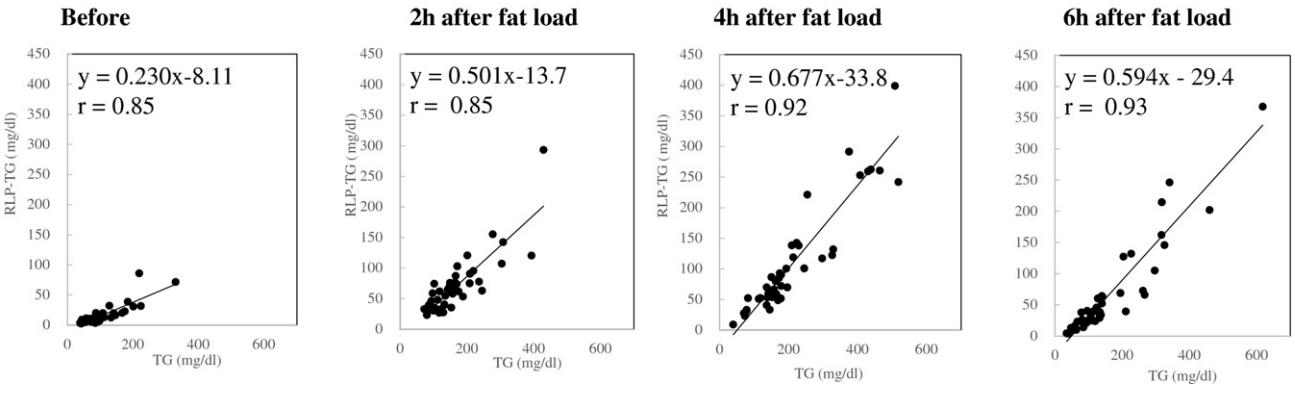

Fig. 1. The changes of plasma TG and RLP-TG concentration and ratio before and 2, 4, and 6 h after fat load.

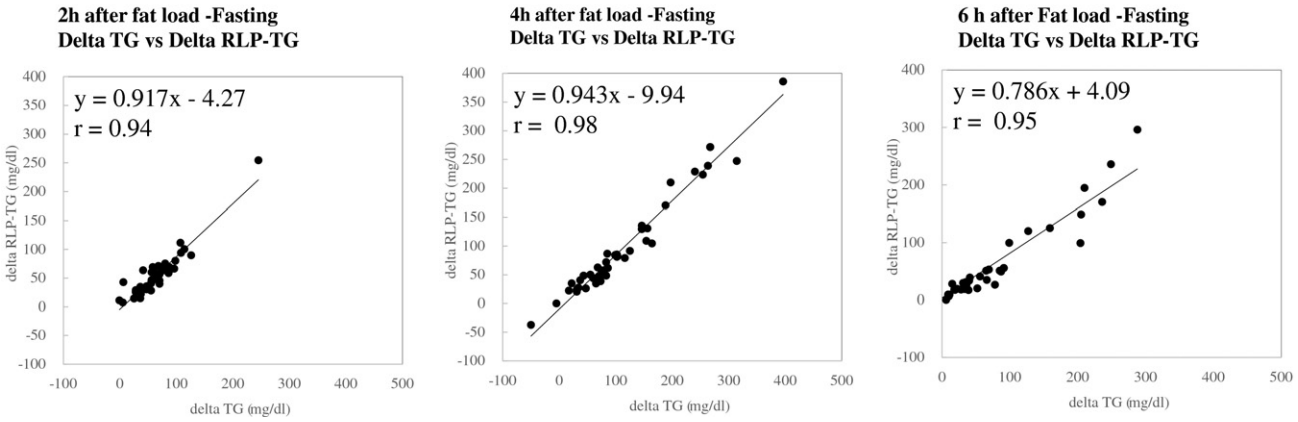

Fig. 2. The changes (delta) of plasma TG and delta RLP-TG concentration in 2 h, 4 h, and 6 h after fat load.

range and were unchanged in the fasting (8 AM) and postprandial (8 PM) plasma. TG, RLP-C, RLP-TG, RLP-TG/RLP-C and RLP-TG/TG were significantly increased in the postprandial state in the post-heparin plasma. LPL activity and concentration were slightly decreased in postprandial plasma compared with fasting plasma followed by significantly increased TG and RLP-TG (Table 2). Therefore, LPL/RLP-TG ratio was significantly higher in fasting than in postprandial plasma and decreased to less than 1/4 of that in postprandial plasma. Those results show that the large RLP particles in postprandial plasma carry significantly low amount of LPL ($p < 0.001$) compared with the small RLP particles in the fasting plasma. Those results showed the same phenomena after fat load. HTGL activity and concentration were unchanged in the postprandial plasma (data not shown).

A significantly higher RLP-TG/TG ratio ($p < 0.001$) was observed in the postprandial plasma ($y = 0.501x - 12.3$) than in the fasting plasma ($y = 0.281x - 10.7$) (Fig. 3) and showed the difference of correlation between TG and RLP-TG in fasting ($r = 0.77$) and postprandial plasma ($r = 0.93$). The increase of RLP-TG (delta RLP-TG) contributed to approximately 60% of the TG increase (delta TG) after the standard meal and these were highly correlated ($r = 0.92$; $p < 0.001$; Fig. 3). These results show that postprandial TG contained a significantly higher concentration of RLP-TG than was measured in the fasting plasma samples.

3.3. Case control study of RLP-C and RLP-TG concentration in patients with angiographically determined CAD and healthy control subjects

Table 3 shows that RLP-C, RLP-TG, RLP-TG/RLP-C and RLP-TG/TG were all significantly increased in postprandial (random) plasma compared with fasting plasma in both healthy control subjects and CAD patients. A significant difference in RLP-C ($p < 0.05$) was observed between the controls and CAD patients in fasting plasma, while no significant difference in RLP-TG was found between the controls and CAD patients in either fasting or postprandial (random) plasma.

Fig. 4 shows that a significantly higher RLP-TG/TG ratio ($p < 0.001$) was observed in postprandial (random) plasma ($y = 0.404x - 23.6$) than in fasting plasma ($y = 0.282x - 13.9$) in the controls. The increase in RLP-TG (delta RLP-TG) contributed to approximately 60% of the TG increase (delta TG) after the regular meal and the correlation between these two increased ($r = 0.85$; $p < 0.001$). However, a similar RLP-TG/TG ratio was observed in postprandial (random) plasma ($y = 0.481x - 37.7$) and fasting plasma ($y = 0.422x - 35.7$) in CAD patients (Fig. 5). The differences in the TG and RLP-TG correlation in fasting and postprandial (random) plasma was significant in CAD patients ($p < 0.001$). The increase in RLP-TG (delta RLP-TG) contributed to approximately 60% of the TG increase (delta TG) and these two increase was highly correlated ($r = 0.83$; $p < 0.01$). Fig. 6 shows that the RLP-TG/TG ratio was significantly higher ($p < 0.001$) in postprandial (random) plasma than fasting plasma in both the normal controls and CAD patients.

4. Discussion

We reported that RLP is approximately 1/3 comprised of VLDL ($d < 1.006$) in the fasting plasma and its ratio changes depending on the physiological conditions [31]. Although our previous studies were mostly focused on the cholesterol content in RLP as RLP-C, a major RLP component is RLP-TG, not RLP-C. The plasma TG concentration is known to reflect the total VLDL as a surrogate marker in both the fasting

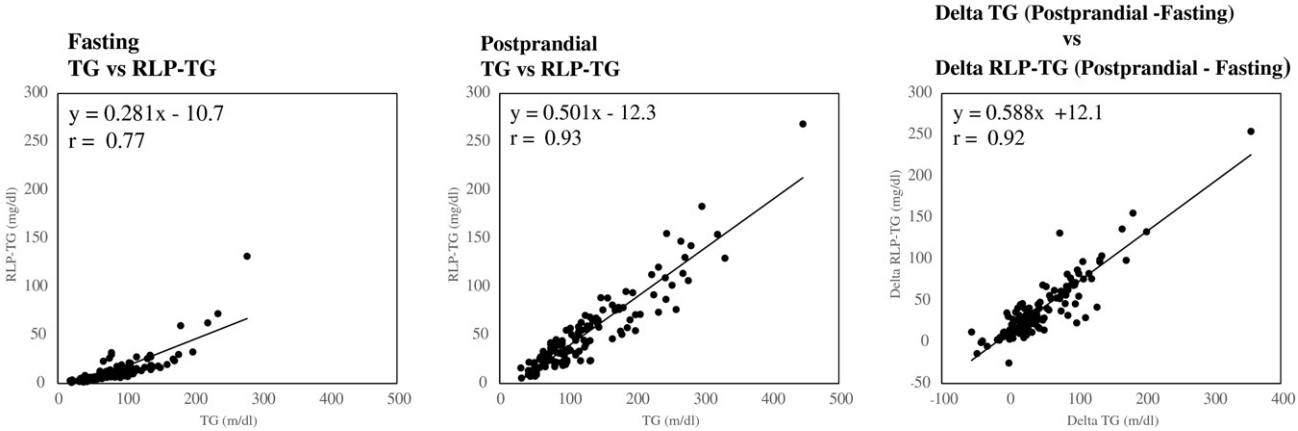

Fig. 3. The correlation between plasma TG and RLP-TG concentration in the fasting and postprandial plasma and between the delta TG and delta RLP-TG.

Table 3
Plasma lipid and lipoprotein concentration in the healthy controls and the cases with coronary artery disease; comparison between CAD cases vs. controls.

	Control (n = 302)		CAD (n = 151)		p			
	A. Fasting Median (25% tile–75% tile)	B. Random Median (25% tile–75% tile)	C. Fasting Median (25% tile–75% tile)	D. Random Median (25% tile–75% tile)	A vs. B	C vs. D	A vs. C	B vs. D
Age	53 (62–69)	50 (58–72)	66 (59–70)	65 (57–70)	n.s.	n.s.	p = 0.022	p = 0.022
Gender (M/F)	205/75				135/37			
Body mass index (kg/m2)	24 (26–29)	26 (27–29)	28 (25–30)	28 (25–31)	n.s.	n.s.	p = 0.006	p = 0.006
Systolic BP (mm Hg)	117 (127–138)	118 (129–131)	140 (126–150)	140 (126–154)	n.s.	n.s.	p < 0.001	p < 0.001
Diastolic BP (mm Hg)	70 (78–84)	70 (73–83)	80 (70–85)	80 (70–88)	n.s.	n.s.	n.s.	n.s.
TC (mg/dl)	187 (215–238)	199 (264–279)	199 (173–227)	206 (178–231)	n.s.	n.s.	p = 0.036	n.s.
TG (mg/dl)	92 (119–178)	134 (153–208)	134 (91–207)	175 (122–257)	p = 0.004	p < 0.001	n.s.	p < 0.001
HDL-C (mg/dl)	38 (44–53)	40 (50–54)	39 (34–47)	38 (32–47)	n.s.	n.s.	p < 0.001	p = 0.001
LDL-C (mg/dl)	122 (146–170)	132 (190–200)	132 (114–155)	129 (111–153)	n.s.	n.s.	p = 0.009	p = 0.004
RLP-C (mg/dl)	6.5 (5.1–8.5)	8.1 (6.2–11.1)	7.1 (5.7–10.2)	9.3 (7.2–12.8)	p < 0.001	p < 0.001	p < 0.05	p < 0.01
RLP-TG (mg/dl)	19.0 (11.7–33.2)	48.4 (25.4–64.4)	18.4 (12.3–35.7)	42.8 (20.6–75.6)	p < 0.001	p < 0.001	n.s.	n.s.
RLP-TG/RLP-C	2.9 (2.0–3.6)	6 (2.4–4.7)	2.6 (2.1–3.8)	4.6 (2.7–6.3)	p < 0.001	p < 0.001	n.s.	p = 0.001
RLP-TG/TG	0.16 (0.13–0.20)	0.21 (0.16–0.28)	0.15 (0.12–0.2)	0.24 (0.17–0.33)	p < 0.001	p < 0.001	n.s.	p = 0.012
Glucose (mg/dl)	86 (93–100)	82 (88–99)	105 (95–131)	101 (90–139)	n.s.	n.s.	p < 0.001	p < 0.001
Insulin (μU/ml)	7 (9–13)	7 (13−21)	13 (9–23)	11 (9–13)	n.s.	n.s.	p < 0.001	n.s.

and postprandial states [32]. Furthermore, one fifth of the total TG is used clinically as VLDL-C concentration. However, VLDL-C and TG have not been recognized as a well-established CHD independent risk factor because of the many inconsistent results obtained among various clinical studies, particularly in the fasting plasma.

The postprandial TG increase has long known as to RLP increase in plasma [1]. However, increase of RLP in the postprandial plasma has not been clearly shown by ultracentrifugation separation (IDL) or other methods [13–15]. Using RLP isolation method, we reported the differences between increased ratio of TG or VLDL and of RLP in both the fasting and postprandial plasma [21]. Although the cholesterol content in RLP (RLP-C) is commonly found to be <10% of TC even in the postprandial plasma, TG in remnant lipoproteins (RLP-TG) is found to be >20% in the fasting plasma TG and as much as 50% in the postprandial plasma TG under various conditions. We have shown in this study that significantly higher RLP-TG is contained in the postprandial plasma than fasting plasma when the TG concentration is adjusted as the RLP-TG/TG ratio (concentration) in healthy controls and CAD cases (Fig. 6). These results show clearly that the amount and ratio of RLP in the postprandial TG increased significantly compared with the fasting plasma TG. In particular, the increase in the postprandial delta RLP-TG concentration contributed to approximately 50–60% of the increase in the postprandial delta TG after typical moderate meal intake. However, >80% of the increased delta TG was comprised of delta RLP-TG after fat load or fat rich meal. These results clearly show that the kind of foods as contained in a fat rich meal greatly enhance the formation of RLP in the postprandial plasma compared with a typical moderate meal. Marcoux et al. [33], Ooi et al. [9] and Nakajima et al. [34] previously reported similar results in small number of Caucasian and Japanese healthy volunteers, in whom approximately 60–80% of the delta RLP-TG in delta TGs were found 3–6 h after a fat rich meal. The rest of the increased TG consisted of increased non-RLP-VLDL-TG, LDL-TG and HDL-TG in the postprandial plasma.

The postprandial RLP contained both apoB-48 and apoB-100 carrying particles [21,35,36]. The increase in the number of RLP apoB-100 particles (VLDL remnants) in fact was greater than that of apoB-48 containing lipoproteins (CM remnants) in the postprandial state. Of note, the accumulation of large TRL apoB-100 particles seems to be a particular characteristic of hypertriglyceridemic patients with coronary heart disease (CHD) compared with healthy hypertriglyceridemic subjects, suggesting a link between the accumulation of large VLDL and the development of atherosclerosis [37].

The Framingham Offspring Study have reported the differences in clinical significance between TG and RLP in the fasting state [5,38,39]. Namely, the fasting RLP-C concentration was shown to be significantly more predictive of cardiovascular events than the fasting TG concentration. Those studies suggest that when samples are collected in the fasting state, RLP-C determination may be needed in addition to the TG concentration. Although RLP-TG in CAD patients was not shown to be significantly increased compared with that of normal controls in Table 3, the RLP-C concentration in CAD was significantly increased compared with that of normal controls in both the fasting and postprandial plasma. This result may be influenced by the effect of heparin administration on the decrease of RLP-TG at the time of blood

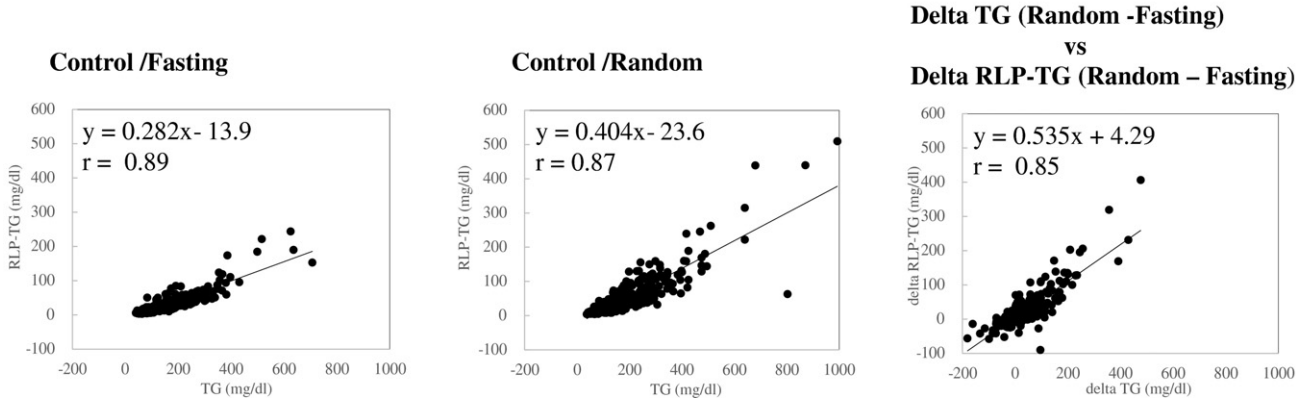

Fig. 4. The correlation in controls between plasma TG and RLP-TG in the fasting and random (postprandial) plasma and between delta TG and RLP-TG.

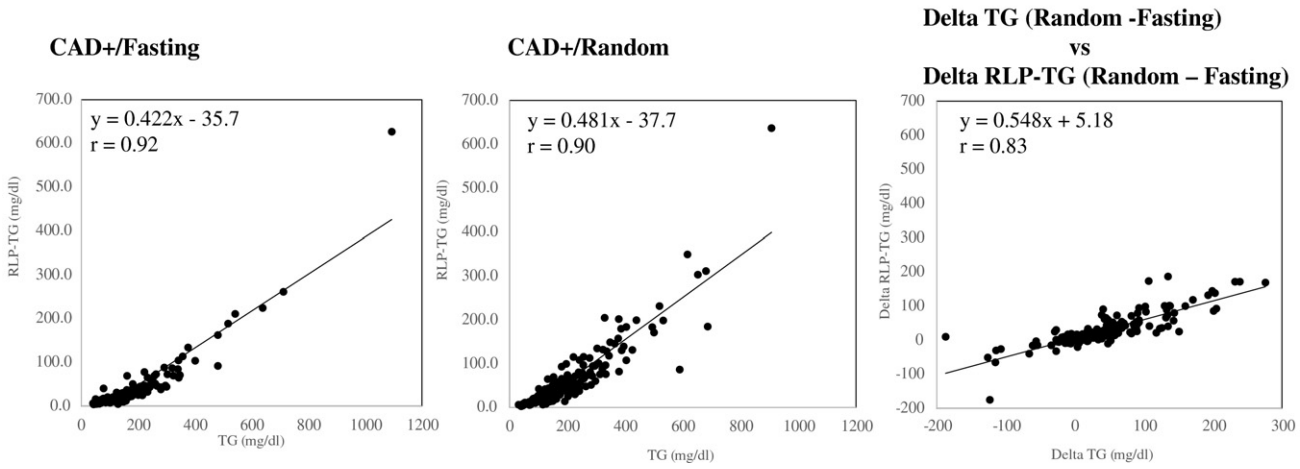

Fig. 5. The correlation in CAD patients between plasma TG and RLP-TG in the fasting and random (postprandial) plasma and between delta TG and RLP-TG.

withdrawal with angiography in CAD patients in this study, but not RLP-C, as shown by Sato et al. [22]. These results suggest that postprandial RLP with large particle size was more strongly associated with cardiovascular risk than fasting RLP with small particle size. These findings suggest the same conclusion as in recent population studies [2–4], that the TG concentration measured in fasting samples is less sensitive than the non-fasting TG in predicting the risk of cardiovascular events. Given the high correlations between the postprandial RLP-TG and postprandial total TG concentrations we demonstrated, the measurement of the postprandial TG concentration may obviate the need for measuring RLP-TG in routine clinical practice.

The reason why RLP-TG increased in plasma after food intake is apparently associated with LPL activity and concentration. For many years, it has been believed that LPL activity in plasma increases after food intake in order to enhance the postprandial lipid metabolism of overloaded CM and VLDL, but we recently reported that LPL did not significantly increase after any kind of food intake [40]. RLP-TG increase means not only an increase in the TG concentration in RLP particle, but also an increase in the particle size, as shown by the increased RLP-TG/RLP-C ratio after food intake [41,42]. A significant increase in the RLP-TG/RLP-C ratio was always found in the postprandial plasma after a fat load (Table 1) or typical food intake (Tables 2 and 3) in both normal subjects and CAD patients. When LPL activity is not sufficient to hydrolyze overloaded CM or VLDL on the endothelium, less efficient hydrolysis may occur and enhance the formation of less metabolized RLP particles along with the higher RLP-TG/RLP-C ratio. Those RLP particles carry a significantly lower LPL per particle compared to small RLP particles, as shown by the LPL/RLP-TG ratio (Tables 1 and 2). Because unchanged number of LPL after food intake have to react with significantly increased CM and VLDL particles on the endothelium. Therefore, when the LPL activity and concentration are not enough to hydrolyze the excessive amount of TG-rich lipoproteins, large size RLP particles are formed on the endothelium and released into the circulation in the postprandial plasma.

As majority of LPL is bound to RLP in pre-heparin plasma, LPL could be a ligand for remnant receptors in liver and muscles [43–45]. Also more clearly, we can express the interaction correlation between TG content and LPL activity in RLP particles as LPL/RLP-TG ratio. For example, LPL concentration in RLP increased significantly after heparin administration and RLP-TG decreased significantly as the result of its

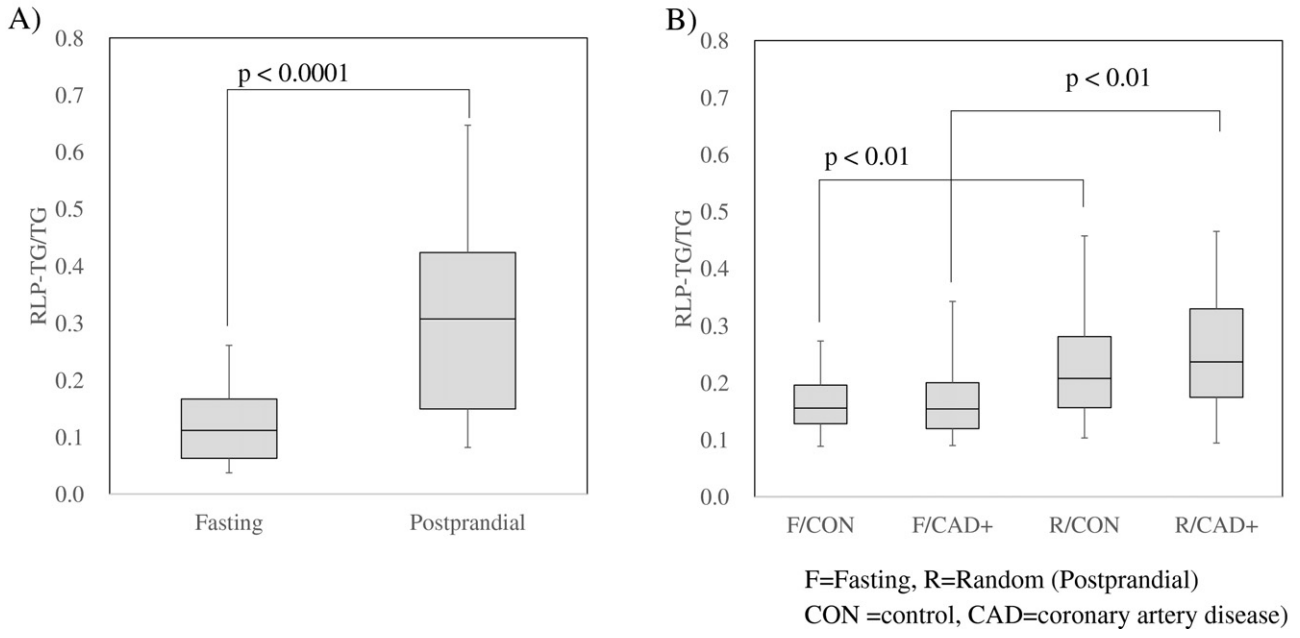

Fig. 6. RLP-TG/TG ratio in the fasting and postprandial plasma in healthy volunteers (A), CAD and controls in the fasting and random (postprandial) plasma (B).

interaction [22]. Therefore, its interaction correlation was expressed as LPL/RLP-TG ratio. As postprandial large RLP particles showed approximately 1/4 of LPL/RLPTG ratio compared to fasting small RLP particles (Tables 1 and 2), the function of LPL as a ligand for the receptor binding may become less effective for the clearance of remnants and accumulate more in plasma. Therefore, high RLP-C and RLP-TC concentration in patients with cardiovascular disease and diabetes are known to be associated with the delayed clearance of RLP [46,47].

As RLP is known to be cleared by LRP and VLDL receptors in liver and muscle, those interactions may be controlled by LPL concentration in RLP as a ligand to the receptors [48,49]. Those results suggest that the large RLP particles with small amount of LPL in the postprandial plasma is a higher risk factor for cardiovascular disease, as shown previous reports [37,47,50].

In summary, these results support the previous studies that showed the non-fasting TG concentrations to be stronger predictor for the risk of CVD than fasting TG, because of the higher concentration of a larger sized RLP particles expressed as higher RLP-TG along with a small amount of LPL in the postprandial plasma compared to the fasting plasma. Therefore, non-fasting TG measurements performed 3–6 h after food intake may be able to take the place of the direct measurement of RLP-TG for the assessment of cardiovascular disease risk.

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