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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 $P < 0.05$.