Atherogenic postprandial remnant lipoproteins—a causal lipoproteins for the initiation of obesity and atherosclerosis

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Abstract: Oxidized low density lipoprotein (Ox-LDL) and chylomicron (CM) remnants were proposed previously as the most atherogenic lipoproteins which initiate and progress the coronary atherosclerosis as the causal lipoproteins. However, there are still controversies on these hypothesizes. Therefore, we propose a new hypothesis on postprandial remnant lipoproteins (RLP) which contain hepatic origin apoB100 and intestinal origin apoB48 in VLDL fraction. Plasma RLP-cholesterol (RLP-C) and RLP-triglyceride (RLP-TG) increased significantly after fat load associated with the increase of TG. Approximately above 80% of the increased TG after fat load is composed of TG in RLP. Postprandial RLP contained significantly greater apoB100 lipoprotein particles than apoB48 particles. Also most LPL in non-heparin plasma was found in RLP as RLP-LPL complex and found in circulation and incorporated in peripheral issues primarily for energy delivery as a ligand for VLDL receptor. Plasma LPL concentration and activity don't increase in plasma after fat intake, therefore it may cause the insufficient hydrolysis of RLP. As the results, we observed the significant increase of RLP-TG concentration with large particle size as mostly VLDL remnants in postprandial plasma. Therefore, LPL concentration was inversely correlated with RLP-C and RLP-TG concentration in postprandial plasma. Increased postprandial TG associated with increased RLP having striking similarity with Ox-LDL has been shown as the risk of cardiovascular disease by large epidemiological studies. We have shown that VLDL remnants, not CM remnants, are the major atherogenic lipoproteins increased in postprandial plasma associated with insufficient LPL activity as the possible causal factor for the initiation of atherosclerosis. Therefore, overproduced RLP in postprandial plasma should be the initial target to be controlled as the initiator of atherosclerosis by choosing the kind of food intake and appropriate exercise, without using therapeutic drugs.

Keywords: Postprandial lipoproteins; remnant lipoproteins (RLP); VLDL (remnants); chylomicron (CM) remnants; lipoprotein lipase (LPL)

Introduction

The Framingham Heart Study first proposed the concept of "risk factors" for cardiovascular disease (CVD) (1). The term "risk factors" have been used for the diagnosis and the term "causal factors" used for the therapy. "Risk" can be defined for diagnostic purpose by the results based on the prospective studies, while "causal" factor for therapeutic purpose of CVD may be particularly difficult to define. With the many emerging methodological improvements, it has been shown that the postprandial remnant lipoproteins (RLP) are the risk factor as the diagnostic purpose and also a therapeutic target related to the causal factors of CVD.

Table 1 The similarity of proatherogenic and proinflammatory properties of oxidized LDL (Ox-LDL) and remnant lipoproteins (RLP) in *in vitro* studies (4)

Ox-LDL and RLP supported macrophage foam cell formation
Ox-LDL and RLP were chemotactic for monocytes, T cells and tissue macrophages
Ox-LDL and RLP enhance the expression of adhesion molecules on monocyte and endothelium
Ox-LDL and RLP were mitogenic for smooth muscle cells and macrophages
Ox-LDL and RLP altered inflammatory gene expression in vascular cells through a redox-sensitive mechanism
Ox-LDL and RLP induced tissue factor, prothrombogenic molecules expression and platelet aggregation
Ox-LDL and RLP impaired EDR by altering NO activity
Ox-LDL and RLP were cytotoxic and could induced apoptosis in endothelial and smooth muscle cells

Therefore, we defined "risk" as the target of diagnosis for the prevention and "cause" as the target for the therapy in this review.

Oxidized low density lipoprotein (Ox-LDL) has been proposed as the major atherosclerosis-causal lipoproteins by Steinberg *et al.* (2). However, Ox-LDL in plasma was found to be less than 0.01% of LDL even in patients with CVD, indicating that it is at too low a concentration to be a risk factor of CVD when compared with RLP (3). We presented an evidence that RLP containing both apoB100 and apoB48 carrying particles has striking similarity with Ox-LDL and are likely the causal lipoproteins for the initiation and progression of atherosclerosis (*Table 1*). All these bioactive properties of Ox-LDL and RLP were observed under very similar concentrations by *in vitro* studies (3). Therefore, very low concentration of Ox-LDL in plasma can't present the bioactive properties comparable to RLP concentration in plasma. Also, the plasma concentration of RLP is similar with the effective concentration of bioactive properties shown by *in vitro* studies.

This review article intends to review RLP as causal lipoproteins for the initiation of atherosclerosis and compare RLP to Ox-LDL and chylomicron (CM) remnants which was proposed as postprandial atherogenic lipoproteins by Zilversmit *et al.* (5).

RLP increases and decreases significantly in plasma after fat intake at a dynamic phase of lipoprotein metabolism and both *in vitro* and *in vivo* studies showed RLP has similar bioactive properties with Ox-LDL (3). On the other hand, LDL doesn't significantly change after fat intake, and LDL itself has no significant atherogenic properties from in vitro studies. All the atherogenic properties of LDL were shown by Ox-LDL which was derived from the oxidation modification of LDL (2). Therefore, actively fluctuating markers such as blood sugar and RLP with bioactive properties, instead of LDL, could be the major cause of initiation of diseases in diabetes and atherosclerotic diseases.

Zilversmit *et al.* (5) first proposed the hypothesis that postprandial lipoproteins are the major risk of atherosclerosis in patients besides familial hypercholesterolemia. He proposed that CM remnants were the major "risk factor" of atherosclerosis in postprandial plasma while did not emphasize the role of VLDL remnants. We sought to elucidate the characteristics of postprandial remnant lipoproteins by using the isolation method of RLP, especially after fat load (6). Namely, RLP is not simply a risk factor of atherosclerosis, but was shown to be likely a causal factor for both initiation and progression of atherosclerosis, based on its striking similarity in bioactive properties with Ox-LDL (*Table 1*) (3). The epidemiological and clinical investigations have shown the atherogenic characteristics of lipoproteins. In contrast, we investigated the characteristics of RLP in postprandial plasma using the immuno-separation method and analyzed the composition and biological and biochemical properties of lipoproteins (6). Although Zilversmit *et al.* proposed CM remnants as the major atherogenic remnants after fat intake, Havel and we identified that VLDL remnants are the major postprandial remnant lipoproteins which was shown by the ratio of apoB100 and apoB48 in RLP and their particle sizes (4,6-8). Therefore, we reconsidered the difference of therapeutic and diagnostic target in the context of LDL and VLDL remnants. LDL has been the target of CVD therapy, while VLDL remnants may be the target of CVD prevention rather than the therapy of CVD. VLDL remnants are the initial metabolites of postprandial lipoproteins induced by the excessive fat intake, specifically composed of long chain fatty acids in CM and VLDL, and
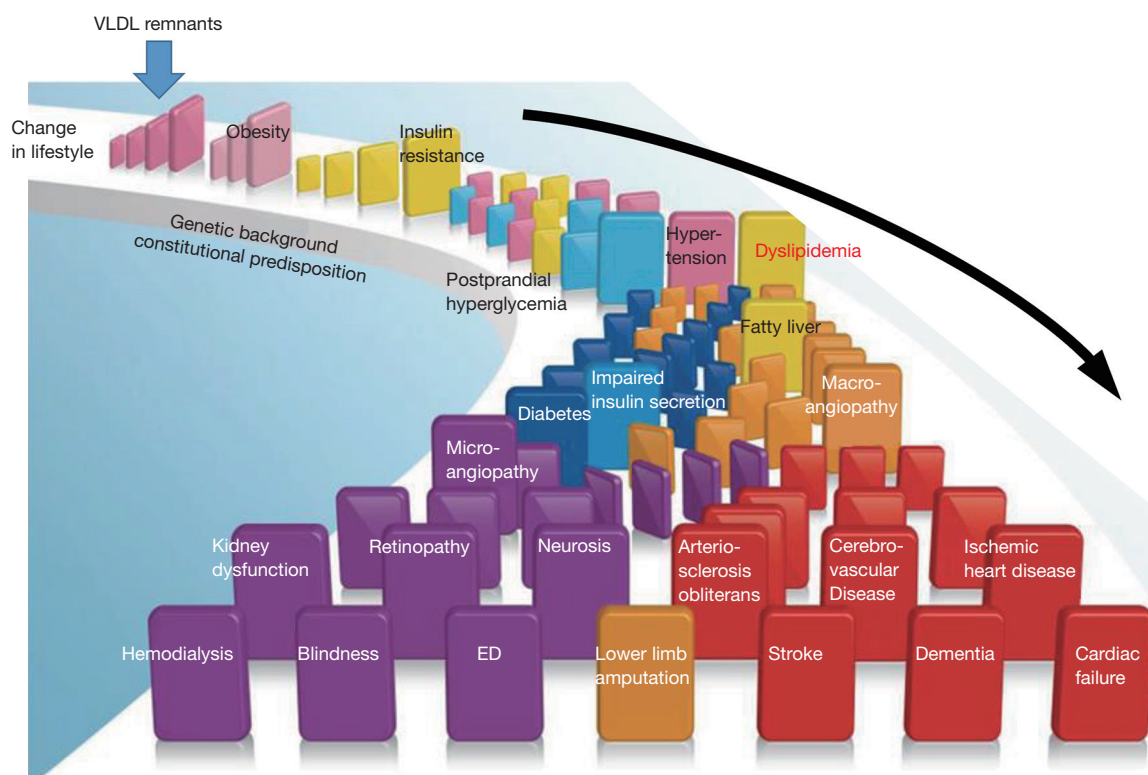
Figure 1 The flow of metabolic domino. Currently accepted position of postprandial dyslipidemia is after insulin resistance. Therefore, postprandial remnant lipoproteins (RLP) are positioned at the result of insulin resistance. However, our hypothesis is that VLDL remnants position before obesity and insulin resistance. By changing the life style, we can stop the increase of VLDL remnants. VLDL remnants could be a most appropriate target for the prevention of insulin resistance and associated atherosclerotic diseases.

is key to the initiation of lipoprotein metabolism (*Figure 1*). The initial step of lipoprotein metabolism can be controlled by taking less fat and more exercise which can reduce VLDL remnants in plasma (9). This step succeeds to the obesity and its associated insulin resistance. LDL is the final metabolite of postprandial lipoproteins and can be controlled if VLDL remnants are not excessively formed, secreted into circulation and accumulated in adipose tissue. However, if excessive VLDL remnants keep remaining in circulation, plasma LDL will increase as a result. Therefore, LDL fits better as the secondary target for therapy, but not for prevention of atherosclerosis.

Fluctuation of plasma lipids and lipoproteins after food intake and the role of lipoprotein lipase (LPL)
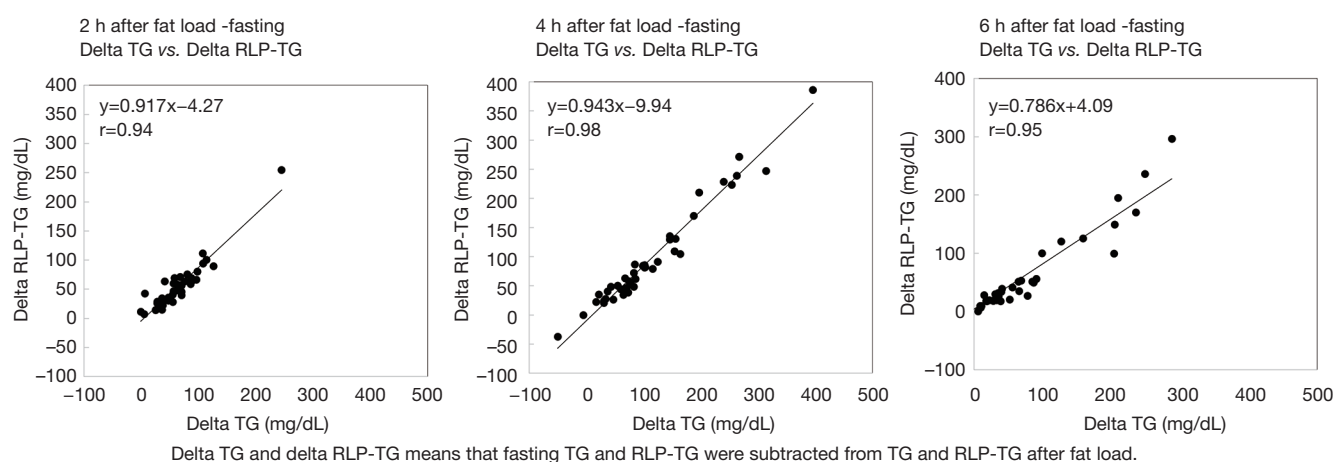
As non-fasting plasma TG concentration is now recognized as a risk for cardiovascular diseases (10-12),

TG concentration in postprandial plasma rather than in fasting plasma, gained more attention to investigate the role of TG on atherosclerosis and CVD. We reported that non-fasting TG was more significantly correlated with RLP (RLP-C and RLP-TG) compared to fasting TG (8,13). Approximately 80% or more of the increased TG (postprandial TG – fasting TG) after fat intake consists of RLP-TG (*Table 2*, *Figure 2*). The postprandial RLP showed their main peak at particle size of VLDL, but not of large CM (*Figure 3*). Interestingly, the particle size of apoB48 carrying RLP was shown to be very similar with apoB100 carrying RLP in 4 hours after fat load in a typical hyperlipidemic patient (*Figure 4*). These results may indicate the possibility that CM apoB48 remnants are incorporated into liver after fat load and reconstructed into VLDL apoB48 in liver and secreted with VLDL apoB100 from liver and form VLDL remnants in circulation with the same pathway. Plasma RLP as well as TG increase significantly during most of the day except in early morning.

Table 2 The changes of plasma lipids, lipoproteins and lipoprotein lipase (LPL) concentration after fat load (14) (n=54)

Parameter	0 h	2 h	4 h	6 h
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)*
apo B100 (mg/dL)	111.9 (88.8–162.4)	126.4 (87.7–173)	126.2 (95–160.2)	126.6 (101–168.3)
Apo B-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)

Data are shown as median (25%tile – 75%tile). 0 h *vs.* 2 h, 4 h, and 6 h by Dunn test, *$P<0.05$.

2 h after fat load -fasting
Delta TG *vs.* Delta RLP-TG

$y=0.917x–4.27$
$r=0.94$

4 h after fat load -fasting
Delta TG *vs.* Delta RLP-TG

$y=0.943x–9.94$
$r=0.98$

6 h after fat load -fasting
Delta TG *vs.* Delta RLP-TG

$y=0.786x+4.09$
$r=0.95$

Delta TG and delta RLP-TG means that fasting TG and RLP-TG were subtracted from TG and RLP-TG after fat load.

Figure 2 The changes of plasma delta TG and delta RLP-TG concentration in 2, 4, and 6 h after fat load (15).

These increases depend significantly on the kinds of food taken. The typical foods in Japan did not significantly increase TG and RLP concentration in plasma during a given day compared to fat-rich foods in Western countries (16-18).

Schneeman *et al.* (19) reported the postprandial increase of apoB-48 and apoB-100 carrying lipoprotein particles in TG-rich lipoproteins (TRL). The increase of apoB-48 in TRL showed a 3.5-fold in concentration compared to a 1.6-fold increase in apoB-100 in TRL. However, apoB-100 increased in TRL occupied more than 80% in lipoprotein particles, indicating the rich of VLDL remnants. We also reported that apoB-100 particles in RLP after fat intake significantly more increased than apoB-48 particles in RLP in the postprandial plasma (6,8,20) (*Figure 5*).

It has been generally believed when CM and VLDL particles are hydrolyzed by LPL and converted to smaller particles such as intermediate density lipoproteins (IDL) identified as typical remnant lipoproteins. However, several studies, including our own (21-23) reported that RLP is predominantly composed of large size VLDL mostly derived from VLDL1, which remained comparatively large
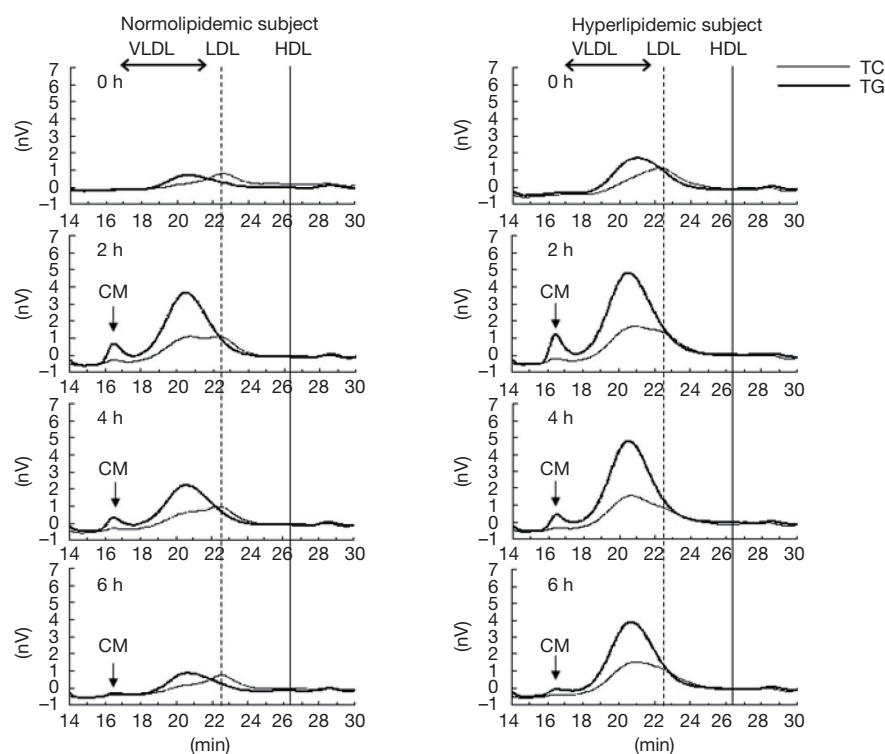
Figure 3 HPLC profiles of remnant lipoproteins (RLP) after fat load (0, 2, 4, 6 h) monitored by TC and TG in a normal control and a hyperlipidemic patient. Major lipoprotein fractions in RLP after fat load are found in VLDL particle size both in normal control and hyperlipidemia patients (13). Only a small peak of CM remnant particle sizes were observed in both cases after fat load.
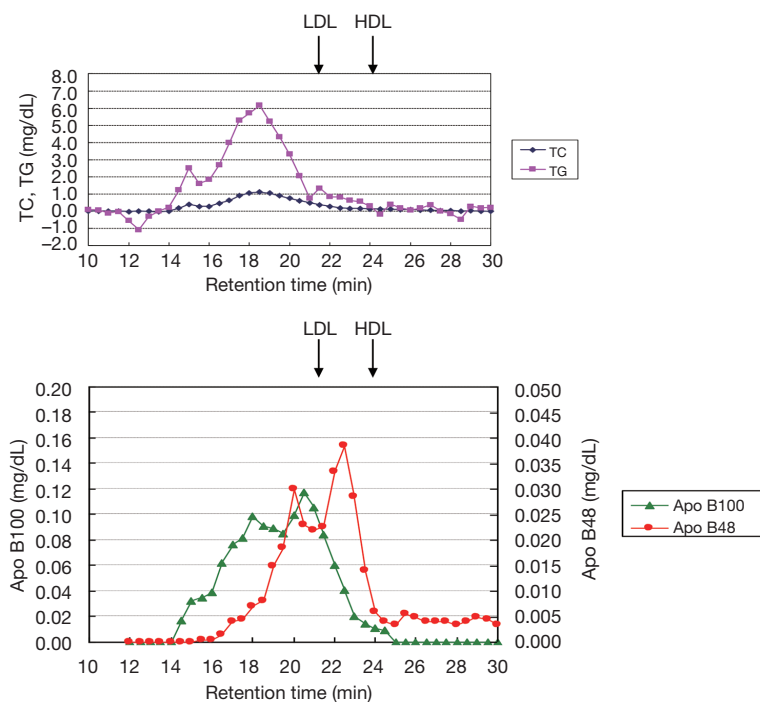

Figure 4 Cholesterol, TG, apo B48 and apo B100 analysis of remnant lipoproteins (RLP) at 4 h after fat load in plasma of a typical type II hyperlipidemic patient by HPLC analysis (4).
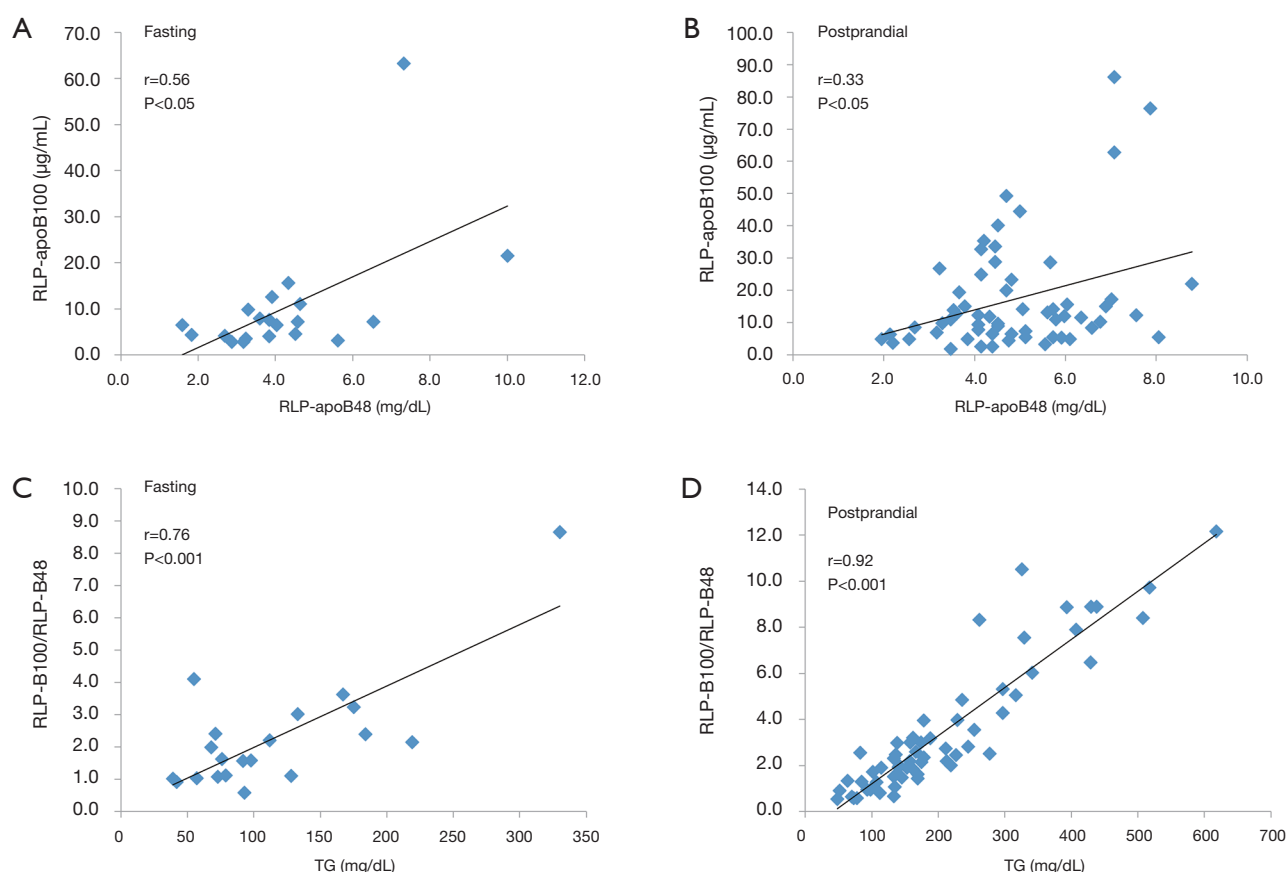
Figure 5 Comparison between RLP-apoB100 and RLP-apoB48 concentration and its ratio in RLP in the fasting and postprandial plasma (8). RLP, remnant lipoproteins.

in particle size when given the excessive supply of CM and VLDL after fat load. Also we recently noticed that the presence of postprandial RLP in plasma at blood sampling may exhibit the large particle size RLP, which is different from the remnant definition of smaller particle size. Interestingly, LPL didn't increase in circulation after fat intake reported by Ishiyama et al. (15,24). The significant increase of RLP particle size manifested as increased RLP-TG which resulted in less LPL per postprandial RLP. Although Karpe et al. (25) reported that LPL activity increased after fat load associated with the increase of TG, the increase of LPL concentration and activity after fat-rich meal was not evident in our study (15). It is possible that the LPL activity assay used by Karpe et al. lacked the sensitivity and/or accuracy compared with our method (22,24). Although insulin is reported to increase LPL in plasma (26), plasma LPL concentration didn't change after glucose, carbohydrate load or combined food intake of fat and glucose (cookie test) (24,27).

Increased formation of remnants by cholesteryl ester transfer protein (CETP) in postprandial plasma

Fielding et al. (28) and Guerin et al. (29) reported that plasma CETP activity significantly increased almost in parallel with the increase of TG concentration after fat load. The increase in RLP concentrations (both RLP-C and RLP-TG) in plasma was positively correlated with CETP activity in patients with proteinuria (30). All these findings indicate that CETP activity is closely correlated with remnant lipoprotein formation in plasma.

Patients with CETP deficiency show a phenotype of low LDL-C with high HDL-C and particularly with apoE-rich HDL particles. ApoE-rich large HDL provides cholesteryl ester (CE) and apoE to CM or VLDL during lipolysis, accelerating formation of RLP in the postprandial phase. Inazu et al. (31) investigated CETP deficiency in one homozygote and three heterozygotes of apoE2 and

controls with an apoE3/3 phenotype and reported the role of CETP in postprandial lipoprotein metabolism. After fat load, TG, RLP-TG and apoB48 concentration expressed as area under the curve (AUC) increased significantly lower in 2, 4, 6 h in heterozygous CETP deficient subjects compared to the controls. Moreover, the homozygous deficient subjects showed a significantly lower AUC for the increase of TG, RLP-TG and apoB-48 concentration after fat load. However, HPLC profiles in homozygous deficient subjects showed that RLP-C increased after fat load was not composed of VLDL size, but of large HDL size, namely, apoE-rich HDL. In heterozygous deficient subjects, a bimodal distribution of the RLP particle size increased after fat load was found to be from VLDL and large HDL. The subjects with CETP deficiency clearly showed the low responder to TRL and reduced remnant lipoprotein formation after a fat-load than controls.

Okamoto *et al.* (32) reported the role of CETP for RLP formation using CETP inhibitor (JTT-705) by *in vitro* assays. The results clearly showed that CETP enhanced the formation of RLP by transferring CE from HDL to TRL. CETP inhibitor was shown to inhibit RLP formation in the in vitro assay system. Therefore, the inhibition of RLP formation by CETP inhibitor is through a different mechanism from statins and fibrates. We believe that the most applicable use of CETP inhibitor is to suppress the postprandial increase of VLDL remnants, rather than to increase of the cholesterol efflux capacity by HDL.

TG in RLP (RLP-TG) is the marker for the postprandial remnants, while cholesterol in RLP (RLP-C) is for the marker for fasting remnants in plasma

RLP-C has been shown as an independent risk factor for cardiovascular diseases reported during last two decades as summarized in (3,6,33). Most of the reports described the RLP-C concentration as an independent CVD risk in the fasting plasma. We further established a sensitive RLP-TG assay to determine the normal range of RLP-TG in the fasting and postprandial plasma of Japanese population (34). The measurement of TG and cholesterol in RLP provided a RLP-TG/RLP-C ratio which was highly correlated with the RLP particle size exhibited by the HPLC profiles reported by Okazaki *et al.* (35). The RLP-TG/ RLP-C ratio exhibited various RLP particle sizes in lipid disorders; Type III exhibited a significantly low RLP-TG/RLP-C ratio which indicated the higher cholesterol and comparatively lower TG

in RLP (reflecting IDL fraction) (36). However, the RLP-TG/RLP-C ratio was significantly high in the postprandial plasma, indicating the increased TG content in RLP (reflecting large VLDL fraction) (*Table 2*). By calculating the RLP-TG/RLP-C ratio, it is feasible to predict the size of RLP particle which is comparable with the HPLC analysis and can distinguish the kind of abnormal lipoproteins such as LpX (37).

Although RLP-C increased after fat load, the changes in the RLP-C/total TG or RLP-C/total TC ratio in the postprandial plasma were small and not significantly different from the fasting RLP-C/TC ratio. This is because RLP-C in the total TG (RLP-C/TG ratio) was approximately 5% in the fasting and rather decrease to 4% in the postprandial plasma in normal controls. Only RLP-C/TG ratio above 10% in the fasting plasma is now used as a diagnostic marker for type III hyperlipidemia (36,38). In contrast to RLP-C/TG, the RLP-TG/TG ratio was around 10% in the fasting plasma and more than 40% in the postprandial plasma in healthy subjects (*Table 2*). We preferred to determine the postprandial increase of RLP as the RLP-TG/TG ratio after fat load (15,24,34). The postprandial RLP-TG/TG ratio increased 3 fold in 2 h and RLP-TG increased approximately 50% of the total TG. The most of the increased TG was represented by the increased RLP-TG after fat load (*Table 2*).

The particle size shown by the RLP-TG/RLP-C ratio reveals the time dependently increased TG in RLP in the postprandial plasma. RLP-TG/RLP-C ratio increased more than 5-fold in 4 h, while RLP-C increased 1.5-fold (*Table 2*). Therefore, we think that RLP-TG reflects more dynamic physiological feature of the postprandial lipoprotein metabolism than RLP-C for the purpose of direct comparison with total TG metabolism. A higher RLP-TG/TG ratio is associated with higher RLP-C in plasma with an increased risk for CHD (39,40).

RLP-LPL complex is the new characteristics of remnant lipoproteins

The characteristics of RLP was investigated since 1993 by using an immunoaffinity gel separation method we developed and investigated the bioactive properties and clinical significance of plasma remnant lipoproteins (3,8,41,42). We found that the most of lipoprotein lipase (LPL) presented in RLP fraction in non-heparin plasma which we have isolated and indicated as remnant lipoproteins (43). The characteristics of RLP-LPL are summarized as follows: (I) Approximately 80% of LPL

Table 3 The plasma lipids, lipoproteins and LPL concentrations in pre-heparin and post-heparin plasma of 29 healthy volunteers (43)

| Parameter	Pre-heparin	Post-heparin	Pre-heparin – Post-heparin	P value
TC (mg/dL)	174±33	169±32	−5±8	NS
TG (mg/dL)	139±78	103±58	−36±24	P<0.01
LDL-C (mg/dL)	102±28	100±27	−2±5	NS
HDL-C (mg/dL)	49±13	47±12	−2±3	NS
RLP-C (mg/dL)	7.2±4.6	6.7±4.1	−0.5±1.2	NS
RLP-TG (mg/dL)	45±367	26±24	−19.1±18.3	P<0.01
RLP-TG/RLP-C	6±1.7	3.5±1.4	−58%±13%	P<0.01
Plasma LPL (ng/mL)	81±27	438±95	357±89	P<0.01
RLP–LPL (ng/mL)	62±26	117±30	64±23	P<0.01
RLP-LPL/plasma LPL (%)	77±11	27±5	NC	P<0.01
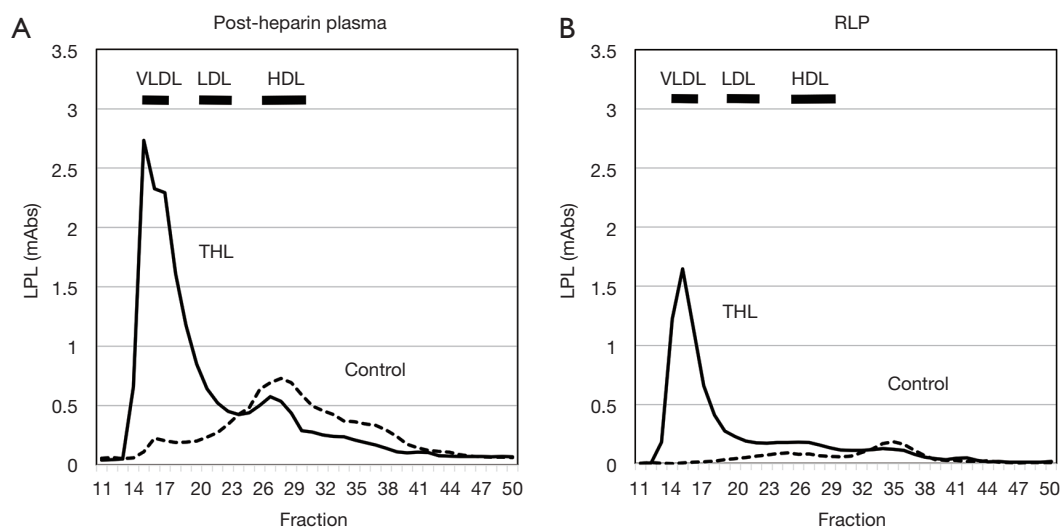
Figure 6 Effect of an LPL inhibitor (tetrahydrolipstatin: THL) on the gel filtration profiles of LPL in the post-heparin plasma (A) and RLP (B). (A) A large amount of the LPL dimers was found in the VLDL elution range, with minor peaks in the LDL and HDL elution range in the presence of THL. However, in the absence of THL, a small amount of the LPL dimers was found in the VLDL elution range, with major peaks in the HDL elution rang. (B) The RLP, in the presence of THL, a large amount of the LPL dimer was found in the VLDL elution range with minor LDL and HDL elution range peaks (43).

in non-heparin plasma was found in RLP fraction as RLP-LPL complex (*Table 3*). (II) The LPL found in RLP in the pre- and post-heparin plasma was shown to be inactive. (III) When LPL activity was inhibited by tetrahydrolipstatin (THL) in the post-heparin plasma, most LPL was found to elute at VLDL particle size, identical with RLP (*Figure 6*). After the hydrolysis of CM and VLDL by LPL, most of the LPL is dissociated from endothelium into circulation as the RLP-LPL complex with inactive forms. A small amount of LPL bound to non-RLP fraction were also found in non-heparin plasma. Majority of circulating plasma LPL containing activity are believed to be in dimeric form, but Beigneux *et al.* recently reported that active LPL is a monomeric form rather than dimeric form (44). They suspect that ANGPTL4 likely inactivates LPL by promoting the unfolding of active LPL monomers as opposed to by converting catalytically active homodimers into inactive monomers. Those results indicate
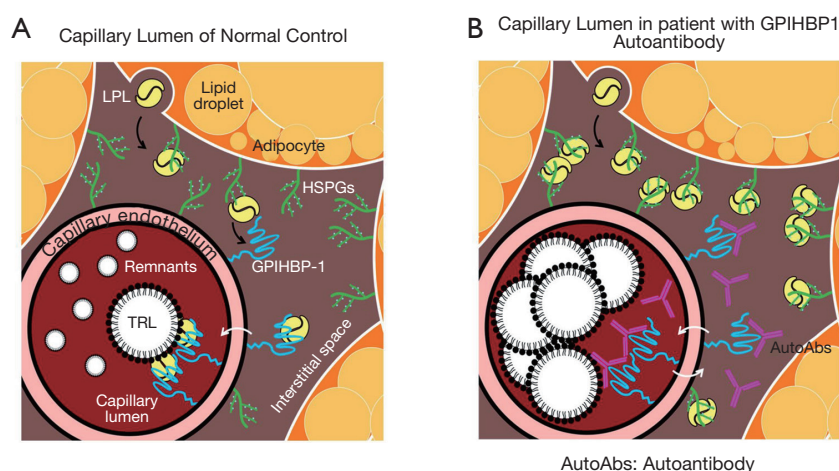
Figure 7 Normal lipolysis and defective triglyceride processing in GPIHBP1-autoantibody syndrome. (A) shows the normal intravascular processing of triglycerides in a healthy person, and (B) shows defective triglyceride processing in a patient with the GPIHBP1-autoantibody syndrome. Normally, the lipoprotein lipase that is secreted by parenchymal cells (e.g., adipocytes and myocytes) is captured by GPIHBP1 on the basolateral surface of endothelial cells. GPIHBP1 then transports lipoprotein lipase across endothelial cells to the capillary lumen, where the lipoprotein lipase hydrolyzes the triglycerides in triglyceride-rich lipoproteins (e.g. CM and VLDL). GPIHBP1 autoantibodies block the binding of lipoprotein lipase to GPIHBP1 and therefore block the transport of lipoprotein lipase to the capillary lumen, resulting in an accumulation of TG-rich lipoproteins in the plasma (hypertriglyceridemia). However, the formation of remnant lipoproteins is critically inhibited and the atherogenicity reduced but increased opportunity of acute pancreatitis (47).

the possibility that most LPL is present more than one molecule on one RLP in pre-heparin plasma.

Chylomicronemia is a typical condition lacking LPL mass or lipolytic activity (45,46). These patients can't form remnant lipoproteins, therefore, large nascent CM and VLDL remain in plasma. For example, typical RLP is not formed when LPL activity is completely blocked by autoantibodies against GPIHBP1 (47) (*Figure 7*, *Table 4*). As these very large particles don't carry LPL, these lipoproteins can't be defined as remnant lipoproteins although patients have very high CM and VLDL concentration in plasma. The scenario with TG above 10 mmol/L (880 mg/dL) have been controversial on whether it is atherogenic or non-atherogenic. It could be differentiated by the presence or absence of LPL activity in abnormally high TG patients. Many of the LPL deficient patients are exposed to high risk of acute pancreatitis (45,46), but rarely the risk of atherosclerosis due to the lack of RLP formation.

RLP-Lp(a) complex may cause the oxidized characteristics of remnant lipoproteins

Diffenderfer *et al.* (48) suggested the hypothesis that Lp(a) is bound to RLP which may cause the oxidized form of RLP, because Lp(a), specifically phospholipids bound to apo(a), is known to be oxidized significantly (49,50). Nagasawa et al recently reported that most of the non-covalent bound apo(a) in Lp(a) binds to RLP. RLP-apo (a) increased significantly in postprandial plasma although total Lp(a) concentration barely changes (51). The RLP-apo(a) complex may explain why RLP is oxidized in plasma without any oxidation *in vitro*, unlike oxidized LDL. Tsimikas *et al.* (14) reported close correlation between Ox-LDL and Lp(a) concentration in plasma, implying the presence of common oxidized molecules. In particular, this may explain why the increased postprandial RLP concentration is associated with the alterations of endothelial function. Shige *et al.* (52), Maggi *et al.* (53) and Funada *et al.* (54) performed the studies of alterations in endothelial function after fat load followed by time-dependent blood sample collection. Flow mediated dilation (FMD) of the brachial artery for the determination of endothelial function was assessed concurrently. The postprandial increase of RLP-C showed the significantly high correlation with decrease in FMD. They concluded that RLP is strongly associated with the endothelial dysfunction in postprandial hyperlipemia that occurs after fat load. These results support the hypothesis that the RLP-apo(a) complex increased after a fat load may

Table 4 Cases with GPIHBP1 autoantibodies and GPIHBP1 gene mutation (47)

ID	TG (mg/dL)	GPIHBP1 autoAbs (AU/mL)	GPIHBP1 (pg/mL)	LPL (ng/mL)	ANA	Mutation
38	2660	327	85	9.8	–	Normal range
101	1,213	70	29	4.6	+++	TG <150 mg/dL
102	6,500	2,366	4	5.8	+++	LPL 40–85 ng/mL
103	9,090	81	NT	NT	++	GPIHBP1 315–1,200 pg/mL
111	1,389	98	9	32.1	–	
157	549.0	134	156	16.9	+++	
164	4,784	196	?	NT	+++	
3	>25,000	ND	36	7.3		GPIHBP1 null[a] (homozygous)
6	6,480	ND	31	13.1		GPIHBP1-C68Y[e] (homozygous)
39	468.1	ND	11	18.5		GPIHBP1-C89F/null[b] (compound het)
11	1,524	ND	3	70.6		GPIHBP1-C89X (homozygous)
15	4,665	ND	6	11.9		GPIHBP1-C89X (homozygous)
21	3,164	ND	7	7.9		GPIHBP1-S107C[c] (homozygous)
27	842	ND	9	10		GPIHBP1-S107C[c] (homozygous)

All cases showed very low serum LPL concentration. Those cases had no severe atherosclerosis, but some had the history of acute pancreatitis. a: Rios JJ, Shastry S, Jasso J, *et al. J Inherit Metab Dis* 2012;35:531-40. b: Charrière S, Peretti N, Bernard S, *et al. J Clin Endocrinol Metab* 2011;96:E1675-9. c: Plengpanich W, Young SG, Khovidhunkit W, *et al. J Biol Chem* 2014;289:19491-9. ND, not detected; NT, not tested.

cause endothelial dysfunction as reported by Doi *et al.* (55), possibly through the interaction with VLDL receptor on endothelial cells (56). Karpe *et al.* (57) reported previously that most of the postprandial remnant particles were cleared in adipose tissue and muscle in humans. Therefore, we recognize that the postprandial remnant lipoproteins are mostly cleared by the VLDL receptor in adipose tissue and muscle. Since Lp(a) is known to be one of the ligands for the VLDL receptor (58), the interaction of RLP-apo(a) and RLP-LPL with VLDL receptor (43) may be potential initiation of obesity and insulin resistance and associated more advanced atherosclerotic diseases.

Japan Eicosapentaenoic Acid Lipid Intervention Study (JELIS) presented the positive results of EPA in hypercholesterolemia from randomized clinical trial of cardiovascular events

Epidemiological and clinical studies clarified that the intake of long-chain n-3 fatty acids protects against coronary artery diseases and reduce the mortality (59-62). JELIS is the study of long-term use of eicosapentaenoic acid (EPA) that investigated its effect for prevention of major coronary

events in hypercholesterolaemic patients who took statins in Japan (63).

The 18,645 patients with a total cholesterol of 6.5 mmol/L or greater were recruited by physicians throughout Japan between 1996 and 1999. Patients were randomly assigned to receive either 1,800 mg of EPA daily with statin (EPA group) or statin only (controls) during a 5-year follow-up. At the mean follow-up of 4.6 years, the primary endpoint was detected in 262 (2.8%) patients in the EPA group and 324 (3.5%) in controls-a 19% relative reduction in major coronary events (P=0.011) by taking EPA. Unstable angina and non-fatal coronary events were also significantly reduced in the EPA group. In patients with a history of coronary artery disease, major coronary events were reduced by 19% by taking EPA. Therefore, EPA was shown to be a promising treatment for prevention of major coronary events, and especially non-fatal coronary events, in Japanese hypercholesterolaemic patients.

Those results revealed that EPA plus statin was significantly more effective against cardiovascular events than statin alone, probably because EPA prevents the formation of RLP raised by the excessive fat intake and lack of exercise at the initial step of lipoprotein metabolic pathway. As RLP

is known to be a precursor of sdLDL-C, it was speculated that EPA reduced plasma RLP significantly (64) and statins reduced sdLDL-C (65), and this combination of EPA and statins prevented the initiation and progression of atherosclerotic diseases. RLP is a key factor which can control the generation of sdLDL and other downstream lipoproteins known as atherogenic lipoproteins.

The flow of metabolic domino; increased postprandial VLDL remnants in plasma appear prior to the obesity and induce insulin resistance, but not the consequence of insulin resistance

The prevalent opinion on postprandial dyslipidemia places it after insulin resistance in the flow of metabolic domino, a similar position to postprandial hyperglycemia. It is believed that dyslipidemia including elevated RLP is commonly observed in patients with insulin resistance due to the decreased LPL activity (33). In line with this theory, elevated postprandial RLP is deemed as the consequence of insulin resistance. However, more recent findings point to the proposed cascade that VLDL remnants appear prior to obesity and insulin resistance in the flow of metabolic domino (*Figure 1*). Because the elevation of plasma VLDL remnants after fat-rich meal intake occurs at the initial steps in the metabolic pathway. Subsequently, the excessive VLDL remnants are delivered to peripheral tissues, in particular to visceral adipocytes and enlarged adipocytes to induce insulin resistance (66). These events occur in the "eat more and exercise less" life style. By improving life style, we are able to prevent the increase of VLDL remnants in plasma and the induction of obesity and following metabolic domino. VLDL remnants are the true residual risk factor need to be controlled. Increased sdLDL in plasma is the result of increased VLDL remnants formation (22). Therefore, sdLDL-C enhances the progression of atherosclerotic diseases, but not the initiation of atherosclerosis. VLDL remnants anchor at the very initial step of metabolic domino and are the most appropriate target to control the initiation and the progression of atherosclerotic diseases.

Conclusions

Ox-LDL and RLP share the striking similarity of pro-atherogenic and pro-inflammatory characteristics. However, Ox-LDL was not found at sufficient concentration in plasma as RLP to initiate and advance of atherosclerosis. RLP is the first product of lipoprotein metabolism circulating in plasma after fat-rich meal for energy delivery to the peripheral tissues. However, when RLP presents continuously and excessively in plasma, visceral adipocytes incorporate and accumulate it as TG and enlarge the adipocytes which cause insulin resistance. Once insulin resistance is induced, the metabolic domino fires and enhance severer dyslipidemia leading to various atherosclerotic diseases down the road. Therefore, the persistent elevation of RLP following intake of fat-rich meal is the initiator of atherosclerosis and the target to be tightly controlled in daily life.

Acknowledgments

Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/jlpm.2020.03.01). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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