MEDDOCS Open Access Publisher

ISSN: 2637-4927

Annals of Biotechnology

Open Access | Hypothesis

ApoB48 of Chylomicrons (CM) after Fat Load are Incorporated into the Liver and Reconstituted to VLDL ApoB48 in Humans

Yoshiharu Tokita[1,2]*; Katsuyuki Nakajima[3]*; Akira Tanaka[4]

[1]Kagawa Nutrition University, Tokyo, Japan.

[2]Faculty of Medical Technology and Clinical Engineering, Gunma University of Health and Welfare, Maebashi, Gunma, Japan.

[3]Department of Clinical Laboratory Medicine, Gunma University, Graduate School of Medicine, Maebashi, Japan.

[4]Kichijoji Futaba Professional and Vocational College of Culinary Nutrition, Tokyo, Japan.

***Corresponding Author(s): Yoshiharu Tokita[1] & Katsuyuki Nakajima[2]**

[1]Kagawa Nutrition University, Tokyo, Japan.

[2]Department of Clinical Laboratory Medicine, Gunma University, Graduate School of Medicine, Maebashi, Japan.

Tel: +81-27-253-0294; Email: tokitay@shoken-gakuen.ac.jp & katsuyuki@nakajima-associates.com

Received: Apr 26, 2023

Accepted: May 12, 2023

Published Online: May, 20, 2023

Journal: Annals of Biotechnology

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Keywords: Remnant lipoproteins (RLP); Chylomicrons (CM); Very low density lipoproteins (VLDL 1, VLDL 2). apoB48; apoB100; Postprandial plasma; Fat-rich meal intake

Abstract

The majority of Remnant Lipoproteins (RLP) in the postprandial plasma have been believed to be chylomicron (CM) remnants (exogenous remnants; RLP-apoB48) derived from the intestine, not VLDL remnants (endogenous remnants; RLP-apoB100) derived from the liver. However, we propose that the majority of CM remnants are first incorporated into the liver by endocytosis via the LDL receptor and LDL-receptor-related protein (LRP) and reused for the reconstitution of VLDL as VLDL apoB48 formation. Therefore, most of the postprandial remnants with apoB48 in the postprandial plasma are not the escaped CM remnants from the hepatic capture along with lipids and other apolipoproteins. Subsequently, apoB48 is reconstituted in VLDL as VLDL apoB48 through the recycling endosome and Microsomal Transfer Protein (MTP) itinerary, which is similar to that of VLDL apoB100 formation in the liver, and it is secreted into the circulation as VLDL apoB48. Because these particles are newly reconstituted in the liver as a portion of VLDL, we propose that both RLP-apoB100 and RLP-apoB48 are endogenous VLDL remnants secreted from the liver after fat-rich meal intake. Furthermore, we predict the presence of a metabolic pathway for the formation of VLDL apoB48 functionally active for several hours after fat-rich meal intake along with the processing of VLDL apoB100 in the liver in humans.

Introduction

Plasma apoB48 is a component of intestinal chylomicrons (CM) which are metabolized to CM remnants by lipoprotein lipase (LPL) at the endothelium, and incorporated into the liver with a very short half-life after a fat-rich meal [1]. The particle size of CM remnants is significantly smaller than CM and is of similar size to VLDL and its remnants. Therefore, the CM remnants with apoB48 have been thought of different origin from VLDL remnants with apoB100, which are produced and secreted from the liver. Both of these remnant lipoproteins (RLP) in the postprandial plasma are simultaneously isolated and determined by means of an immuno-separation method (RLP-C and RLP-TG assay) [2,3]. Therefore, we have long believed that the

Cite this article: Tokita Y, Nakajima K, Akira T. ApoB48 of Chylomicrons (CM) after Fat Load are Incorporated into the Liver and Reconstituted to VLDL ApoB48 in Humans. Ann Biotechnol. 2023; 6(1): 1027.

RLP in the postprandial plasma contain both CM remnants (exogenous remnants; RLP-apoB48) and VLDL remnants (endogenous remnants; RLP-apoB100) of different origin site, i.e. were produced and secreted from the intestine and liver, respectively. However, as we found there to be a well synchronized fluctuation of apoB48 and RLP-C, RLP-TG in the postprandial plasma after a fat load **(Table 1)** [4,5], we suspected that the apoB48 in the postprandial plasma detected as RLP–apoB48 may be derived from the liver along with RLP-apoB100. RLP-apoB48 in plasma may be composed of newly reformed VLDL derived from CM remnants in the liver, which is not directly secreted from the intestine or the CM remnants that have escaped hepatic capture. In an effort to understand the characteristics of remnants, we have paid attention to the postprandial remnants reported by others as well as ourselves to clarify if there really are two kinds of RLP with different origins in plasma, as is generally accepted.

Early studies of CM and VLDL metabolism

Nestel et al [6,7] and others [8-10] have reported the importance of the liver for the clearance of CM in hepatectomized dogs and rats in the 1960s and '70s. The hepatectomized animals in these studies displayed significantly delayed clearance of CM remnants from the circulation **(Figure 1)**. When similarly isotope-labeled CM were infused into normal dogs, the liver removed almost all of the infused CM cholesterol radioactivity with a half-life of 10 min, but only a smaller and variable fraction of the triglyceride fatty acid radioactivity was detected. However, in the hepatectomized dogs in which the liver was either completely or partially excluded from the circulation, radioactivity was removed considerably more slowly in the cholesterol fraction than in the triglyceride fatty acid and phospholipid fractions [7] **(Figure 1)**. TG and phospholipids are metabolized by Lipoprotein Lipase (LPL) in extrahepatic tissue, but cholesterol and apolipoproteins are removed by the liver via endocytosis of CM remnants. Therefore, apoB48 is incorporated into the liver and metabolized at VLDL production. (As apoB48 was not discovered until 1980, we have compared RLP-C with cholesterol in plasma)

We examined the relationship between CM remnants and VLDL apoB100 after fat load in healthy and unhealthy humans. We found a close parallelism between apoB 48 and RLP-C in the postprandial plasma in 4 h and 6 h after a fat load, both in healthy- and unhealthy- volunteers (unpublished data) **(Figure 2)**.

Johanson et al. [11] employed an 8-h meal tolerance test (919 kcal, 51 g fat) in which lipoproteins were separated by a density gradient ultracentrifugation method [12,13]. They separated the TG-rich lipoproteins such as CM apoB48 and CM apoB100, VLDL1 apoB100 and VLDL1 apoB-48, and VLDL2 apoB100 and VLDL2 apoB48. ApoB-48 and apoB-100 were found in all of the fractions, which were separated by Svedberg flotation (Sf) rate ultracentrifugation (CM >sf 400, VLDL1 (sf 60-400) and VLDL 2 (sf 20-60)) [11]. As shown in the AUC analysis after a fat rich meal, the CM apoB48 and CM apoB100 concentrations were very low, while the VLDL 1 apoB48 and VLDL1 apoB100 concentrations were parallel and the highest after meal intake. A similar trend was observed for VLDL2 after a fat-rich meal intake, which is a smaller VLDL fraction with a much higher apoB48 concentration than CM apoB48. These results suggest that apoB48 is mostly present in the VLDL fraction after fat-rich meal intake, while a small amount of CM apoB48 remains in the circulation for an 8 hour period. These highly synchronized apoB48 and apoB100 fluctuations suggest that VLDL apoB48 and VLDL apoB100 may be formed simultaneously in the liver after the intake of a fat-rich meal intake. Johanson el al. [11] also reported that elevated VLDL1- TRL in the postprandial state is a potentially atherogenic trait before any changes in fasting lipid parameters, body composition or lifestyle are detectable, and may well contribute to an excessive risk for future coronary events. These clinical results are very similar to those reported for RLP-C as a risk factor for atherosclerosis [14,15].

Kinetics of apoB48 and apoB100 after a fat-rich meal

Schaefer [16] observed that the plateau for TRL apoB48 approached that of VLDL apoB-100. They investigated TRL apoB-48 and apoB-100 kinetics within VLDL-, IDL-, and LDL-containing lipoproteins. Primed-constant infusion of deuterated leucine was used for subjects maintained in a constantly fed state. Multicompartmental modeling was performed to assess the difference in the kinetic parameters between apoB48 and apoB100. The overall results of human stable isotope kinetics studies reported in the 1990s indicated that the average residence time of CM apoB-48 and VLDL apoB-100 in plasma was similar [4-5 h]. However, VLDL apoB-100 production was approximately 10-fold higher than CM apoB-48 in the fed state.

Recently Björnson E et al. [17] published a kinetic study of apoB48 and apoB100 after a fat-rich meal **(Figure 3)**. They showed human apoB48 metabolism using a new, integrated non-steady-state model of apoB48 and apoB100 kinetics. Mass spectrometric techniques were used to determine the mass and trace enrichment of apoB48 in the CM, VLDL1 and VLDL2 density intervals. An integrated non-steady-state multi-compartmental model was constructed to describe the metabolism of apoB48- and apoB100-containing lipoproteins following a fat-rich meal, as well as during prolonged fasting. The kinetic model analyzes the metabolism of apoB48 in CM, VLDL1 and VLDL2. It predicted a low level of basal apoB48 secretion and, during fat absorption, an increment in apoB48 release into not only CM, but also directly into VLDL1 and VLDL2. ApoB48 particles with a long residence time were present in VLDL, and in subjects with high plasma triglycerides, these lipoproteins contributed to the apoB48 measured under fasting conditions. The fractional catabolic rates for apoB48 in VLDL1 and VLDL2 were substantially lower than for apoB48 in CM. They displayed very similar kinetic results for plasma apoB48 VLDL 1 and 2 along with apoB100 VLDL1 and 2 after a fat-rich meal, as Johanson el al. [11] reported. However, we realized that Björnson et al. [17] still defined VLDL1 apoB48 as the origin of CM remnants because of the presence of apoB48 in the lipoprotein fraction.

Postprandial remnant lipoproteins; CM remnants and VLDL remnants

We previously reported the characteristics and clinical significance of VLDL remnants (i.e. RLP) in the postprandial plasma [4,5]. The RLP level is strongly associated with the habits of daily life such as the kind of foods taken and frequency and strength of exercise [18], including those that lead to deleterious conditions such as metabolic syndrome. However, the key plasma factor which critically initiates and bridges the "fat-rich meal and lack of exercise" life habits and "obesity and insulin resistance" has not been established. We hypothesized that RLP may be the bridge between life style factors and metabolic disorders and that these RLP are originally based on the VLDL in the liver that is derived from the CM remnants formed after the intake of a fat-rich meal in order to distribute energy to the

peripheral tissues. Since it has been shown that the formation of VLDL remnants (RLP-C and RLP-TG) after food consumption can be controlled by appropriate food intake and exercise [19], atherosclerotic disease may be most effectively prevented by changing the life style so as to reduce VLDL remnant formation. As VLDL remnants precede the manifestation of insulin resistance in the metabolic domino process [18], VLDL remnants are a better target for disease prevention than LDL-C, which cannot be easily reduced by a modulation of exercise or food intake. We believe that when elevated VLDL remnant levels in the plasma persist, insulin resistance is induced associated with visceral obesity and this accelerates the initiation and progression of various atherosclerotic diseases. Therefore, it may be more important to determine the origin of VLDL remnants than any of the other lipoproteins, as they constitute the bridge between life style factors and metabolic disorders, so serve as an excellent target for preventative measures.

We have reconsidered the role of CM remnants with apoB48 that are derived from a fat-rich meal and have been commonly believed to be the major postprandial remnants since Zilversmit first proposed this characterization [20]. CM remnant particles are recognized by the liver and rapidly cleared from plasma. This process is believed to occur in two steps. (i) An initial sequestration of remnant particles on hepatic cell surface proteoglycans, and (ii) receptor-mediated endocytosis of remnants by hepatic parenchymal cells. The initial binding to proteoglycans may be facilitated by lipoprotein lipase and hepatic lipase, each of which possesses both lipid- and heparin-binding domains. The subsequent endocytic process may be mediated by LDL receptors and/or LRP [21]. Both of these receptors have a high affinity for apoE, a major apolipoprotein component of remnant particles. CM is hydrolyzed by the LPL in capillary endothelial cells, and most CM remnants are incorporated into the liver a short time after entering into the blood circulation. The CM remnant half-life after intravenous injection is reported to be 5-13 min in normal controls [6-8]. Grundy et al. [9] reported that in 21 patients with normal TG levels, the CM clearance rate was extremely rapid (t1/2 for chylomicron-TG=4.5±2.9 (SD) min). In 30 patients with lipemia, this clearance was generally prolonged (t1/2=23±5.5 min). These results correlate with the delay in postprandial remnant clearance found in cardiovascular diseases [22,23] **(Figure 2)**. However, hepatectomized dogs and rats display significantly delayed CM remnant clearance from the circulation after fat or CM ingestion [6-10]. This means the liver plays the major role in clearing CM remnants bearing apoB48 from the circulation by endocytosis. The postprandial plasma apoB48 increases in parallel with TG 4-6 h after fat intake, the same timing as the RLP-C and RLP-TG increase **(Table 1)**. We reported previously that the RLP particle size is larger than non-RLP VLDL [24], because RLP significantly increases the TG content after fat intake. The HPLC profiles of RLP after a fat load (0, 4h, and 6 h) monitored by cholesterol and TG in a normal control and hyperlipidemic patient exhibited a major peak of VLDL sized particles, not CM [4,5] **(Figure 4)**, as confirmed by ultracentrifugation [11]. Also, the plasma TG, RLP-TG and ApoB48 levels after a fat-load test displayed significantly related fluctuations of the parameters in cases of normal controls and CEPT deficiency.

An approximate 10-fold concentration of VLDL remnants (RLP-apoB100), not CM remnants (RLP-apoB48), was shown to be associated with increased plasma TG after a fat load [5]. HPLC analysis also showed that the particle size of postprandial RLP-apoB48 is similar to that of RLP apoB100 [25]. These results support the notion that the apoB48 incorporated into the liver as CM remnants is reconstituted as a portion of VLDL to form VLDL apoB48 after fat intake by an unknown mechanism, but which may be a physiological pathway that is similar to VLDL apoB100 formation. If these increased apoB48 particles are CM apoB48 particles that have escaped hepatic capture, the profile of related fluctuations after fat intake would not be so well synchronized with the VLDL apoB100 particles.

Currently accepted concepts regarding CM remnants in the postprandial plasma

Although CM remnants are cleared from the blood circulation with a very short half-life, it takes considerable time to form CM in the intestine from digested long-chain fatty acid triglycerides (LCT) after fat intake. Because of the hydrophobicity of LCT, the formation of CM is necessary in order to distribute LCT into the circulation via the lymph ducts. Therefore, the increase of apoB48 in the postprandial plasma may be delayed for 2-4 h after oral fat intake, which is very similar in timing to the increase in VLDL apoB100 and their remnants in the circulation. However, certain fatty acids, such as medium chain triglycerides (MCT) and diacylglycerol (DG), do not significantly increase the plasma TG or RLP levels (including apoB48), because MCT and DG barely form CM in the intestine, as they are absorbed in the intestine and smoothly incorporated into the liver via the portal vein because of their hydrophilicity [26,27].

These results suggest that the CM remnants that are originally formed in the intestine are increased in the circulation 2-4 hours after oral fat intake, a timing which coincides with the increase in VLDL apoB100. These CM remnants have been considered to be lipoproteins that have escaped hepatic capture. Another factor is the presence of small sized apoB48 particles in the postprandial plasma. These particles are usually identified as small CM remnants. Therefore, VLDL or IDL sized apoB48 particles are believed to be hydrolyzed CM remnants. These particles fluctuate similarly in the circulation in parallel with VLDL apoB100 particles after fat intake, having escaped hepatic capture. These concepts are generally well accepted and are ones we have long believed. However, the relationship between small CM remnants and VLDL1 apoB48 and VLDL2 apoB48, has yet to be well elucidated [28-30].

Mechanism underlying the formation of VLDL apoB48 remnants

VLDL apoB48 is originally proposed because of the high concordance of the postprandial increase and decrease of apoB100 in VLDL and RLP throughout the course of a large number of fat loading tests. It is well known that apoB48 in humans is not synthesized in the liver because of the lack of APOBEC-1, unlike the case in mice and rats [31,32]. Therefore, the apoB48 protein synthesis in the liver that occurs after fat intake in these animals does not occur in humans. The endocytosis of CM remnants through receptors such as LRP in the liver may be associated with the formation of VLDL apoB48. The mechanism for the uptake of CM remnants into non-degrading intracellular itineraries for re-secretion (transcytosis, retro-endocytosis) as VLDL is largely unknown [33], but we speculate the presence of apoB48 recycling endosome pathway after early or late endosome process which functions during several hours after fat-rich meal intake. It has become clear that most of the CM remnants with apoB48 are very rapidly incorporated into the liver from the circulation along with lipids and other apolipoproteins via the LDL receptor and LRP. Véniant et al. [34] reported that

LRP is most important for the clearance of apo-B48–containing lipoproteins, but plays no significant role in the clearance of apo-B100–containing lipoproteins. Subsequently, apoB48 may not be degraded in the liver, but rather, used for the reconstitution in VLDL as VLDL apoB48 through the recycling endosome pathway utilizing Microsomal Transport Protein (MTP) [35]. This is similar to the process of VLDL apoB100 formation, and it is secreted into the circulation as VLDL1, 2 apoB48 and their remnants [11]. As CM is only scantly synthesized in the intestine by MCT and DG, VLDL apoB48 may not be evidently produced or increased in the circulation after those fat intake [26, 27]. These results suggest that apoB48 and apoE are the ligands of the CM remnants used for the endocytosis in the liver, a process mediated mainly by LRP [35]. Therefore, VLDL apoB48 is reconstituted in the liver and fluctuates in parallel with TG and VLDL apoB100 (i.e. RLP) in the postprandial plasma for the formation of VLDL remnants **(Figure 5)**. These lipoprotein profiles after fat intake display an unexpected concordance. Therefore, most CM remnants are incorporated into the liver and reappear in the plasma in a small pool of soluble lipoproteins as VLDL apoB48 in the form of VLDL1 and VLDL 2 [11]. Although the precise mechanism underlying this metabolic pathway for the formation of VLDL apoB48 is currently unknown, most of the apoB48 particles in the postprandial plasma are found in the VLDL1 and 2 fractions, which are produced in the liver, and they were identified as VLDL by ultracentrifugation separation and their particle size was determined by HPLC.

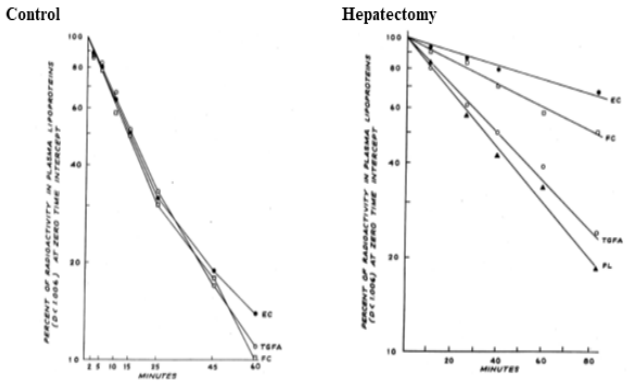
Conclusions

It has long been a question for us why the fluctuations of apoB48 in RLP after fat intake are always synchronized with apoB100 in RLP (RLP-C) in the fat loading studies over the course of many years in spite of the different origin and secretion sites. Therefore, we hypothesized that the CM remnant apoB48 after fat intake in humans is reconstituted or remodeled in the liver as VLDL apoB48 along with VLDL apoB100 formation by a specific pathway that is actively functional for a period of several hours after fat-rich meal intake, unlike the case in mice and rats which can synthesize apoB48 in the liver. In order to prove this hypothesis, it is necessary to clarify the new pathway underlying the incorporation of apoB48 into the VLDL particles in the liver after fat intake. The interaction with MTP and the presence of recycling endosome needs to be investigated to properly elucidate the role of the apoB48 particle formation pathway in the liver. If the new pathway of VLDL apoB48 formation is indeed demonstrated, the remnant lipoproteins isolated by an immuno-separation method as “RLP apoB48” would be defined as VLDL remnants, not CM remnants, because those particles are newly reconstituted in the liver as a portion of VLDL, not CM remnant particles that have escaped incorporation into the liver. Therefore, we propose that RLP-apoB100 and RLP-apoB48 are both origin of endogenous VLDL remnants produced in the liver after fat-rich meal intake. The majority of CM remnants after fat intake are metabolized in the liver, reconstituted as VLDL and VLDL remnants and then provided to the heart, muscles, adipocytes, endothelium, etc. as an energy source in the form of lipoproteins, similar to the role of blood sugar **(Figure 6)**. However, VLDL remnants play the major role as the initiator of the metabolic domino effect in cardiovascular diseases when supplied continuously in excess [18,19,36].

Table 1: Serum levels of lipids, lipoproteins and its ratio after oral fat load in healthy Japanese male volunteers.

	0hr	4hr	6hr
TC (mg/dL)	208±7	209±6	208±5
TG (mg/dL)	101±15	210±31**	165±33*
HDL-C (mg/dL)	70±6	68±6	68±8
LDL-C (mg/dL)	121±8	118±8*	119±7
RLP-C (mg/dL)	4.8±0.5	8.2±1.1**	8.0±1.6*
RLP-TG (mg/dL)	13±2	100±20**	69±20**
Apo B (mg/dL)	100±5	100±5	100±6
Apo B-48 (μg/mL)	0.53±0.04	0.99±0.14**	0.92±0.14**
RLP-TG/TG	0.13±0.04	0.46±0.14	0.36±0.13
RLP-TG/RLP-C	2.8±0.4	12.3±1.5**	7.8±0.9**

The statistical significance of difference was determined with the Mann-Whitney U test. $^{*}p<0.05$, $^{**}p<0.01$.



P.J Nestel et al. Metabolism of constituent lipids of dog chylomicrons. Journal of Clinical Investigation. 1963; 42: 1313-21.

Figure 1: Metabolism of the constituent lipids of canine chylomicrons. When isotope labeled chylomicrons were infused in normal dogs, the liver removed almost all the infused chylomicron cholesterol radioactivity with half-life of 10 min, but only a smaller and variable fraction of the triglyceride fatty acid radioactivity. However, in hepatectomized dogs in which the liver was either completely or partially excluded from the circulation, the radioactivity was removed considerably more slowly in the cholesterol fractions than in the triglyceride fatty acid or phospholipid fractions.

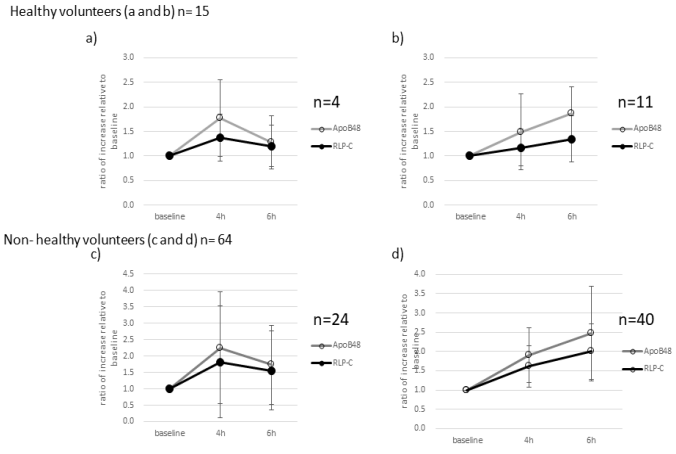
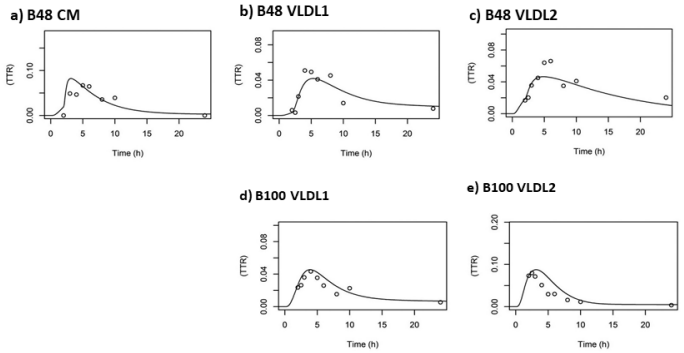
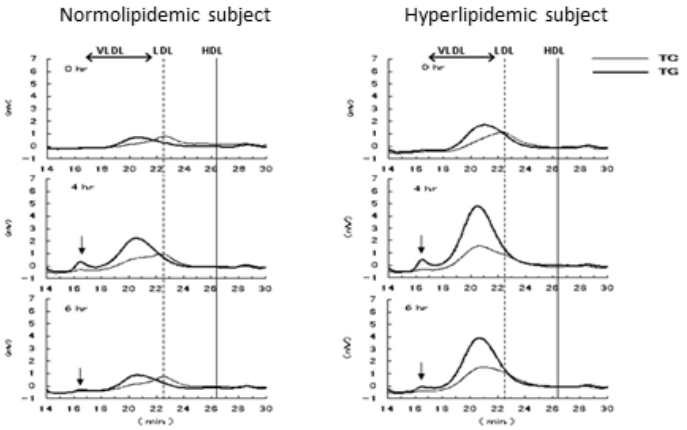


Figure 2: A close parallelism was found between ApoB 48 and RLP-C in the postprandial plasma in 4 h and 6 h after a fat load. The fluctuation of apoB48 and RLP-C in after a fat load is shown in figures **(a)** to **(d)**.



E. Bjornson *et al*. Journal of internal Medicine: 2019; 285: 562-577.

Figure 3: Kinetics of apoB48 and apoB100 after a fat-rich meal. Human apoB48 metabolism was studied using a new, integrated non-steady-state model of apoB48 and apoB100 kinetics. Mass spectrometric techniques were used to determine the mass and trace enrichment of apoB48 in the CM, VLDL1 and VLDL2 density intervals.



Nakajima K et al. Clin Chim Acta. 412 (2011)1306

Figure 4: HPLC profiles of RLP after a fat load (0, 2h, 4h, 6 h) monitored by TC and TG in a normal control and a hyperlipidemic patient. The majority of RLP was detected in the VLDL fraction, not the CM fraction.

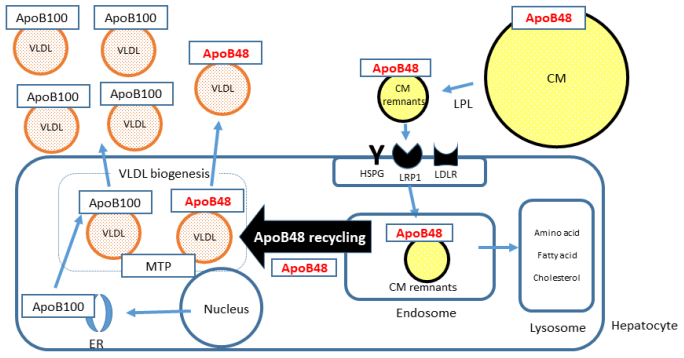


Figure 5: Mechanisms of TRL endocytosis and ApoB48 recycling. TRL endocytosis in a prototypical hepatocyte is presented. The mechanism for the uptake of CM remnants into non-degrading intracellular itineraries for re-secretion (transcytosis, retro-endocytosis). The presence of apoB48 recycling endosome pathway after early or late endosome process together with MTP for the formation of VLDL apoB48 is proposed in hepatocyte.

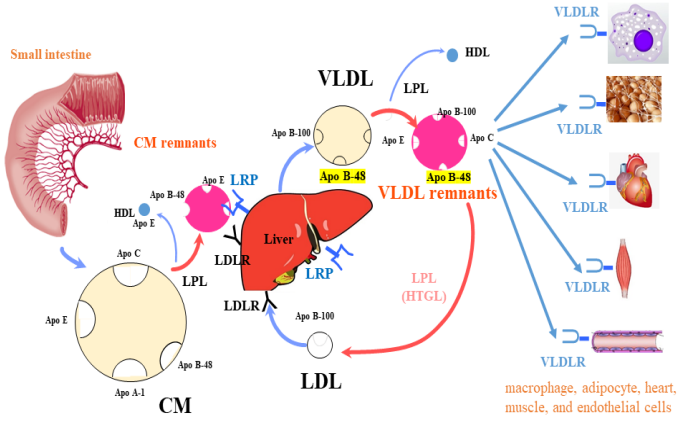


Figure 6: Metabolic pathway of postprandial lipoproteins after fat intake. The intestine secretes CM, the triglycerides of which are lipolyzed by LPL. The LPL reaction constitutes the initial process in the formation of CM remnants. CM remnants interact with the LDL receptor and LRP, and are internalized in the liver, where they are reformed into VLDL as VLDL apoB100 and VLDL apoB48. The VLDL secretion process is partly regulated by the rate of free fatty acid formation from CM remnants influx into the liver. VLDL triglycerides are lipolyzed by endothelial-bound LPL and VLDL remnant particles are formed. The final VLDL remnant composition contains apoB100, apoB48, LPL, Lp(a) and other ligands, and is modulated by the cholesterol ester transfer protein (CETP) reaction with HDL and hepatic lipase (HTGL) along with the exchange of soluble apolipoproteins such as C-I, C-II, C-III and E. The great majority of the remnants are removed from the plasma by VLDL receptor-mediated processes. The principal receptors for CM remnants are the LDL receptor and LRP in the liver, whereas the VLDL remnants are more likely to bind and be internalized by the VLDL receptor in the peripheral tissues such as the heart, adipocytes, muscle, brain and endothelium.

Conflict of Interest

All of authors have no conflict of interest.

References

1. Nakajima K, Nagamine T, Fujita MQ, Tanaka A, Schaefer E. Apolipoprotein B-48: A Unique Marker of Chylomicron Metabolism. Advances in Clinical Chemistry, Burlington: Academic Press. 2014; 64: 117-177.

2. Nakajima K, Saito T, Tamura A, Suzuki M, Nakano T, 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 1993; 223: 53-71.

3. Nakajima K, Okazaki M, Tanaka A, Pullinger CR, Wang T, 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. 1996; 19: 177-183.

4. Nakajima K, Nakano T, Tokita Y, Nagamine T, Inazu A, et al. Postprandial lipoprotein metabolism: VLDL vs chylomicrons. Clin Chim Acta. 2011; 412: 1306-1318

5. Nakajima K , Nakano T , Tokita Y, Nagamine T, Yatsuzuka S, et al. The characteristics of remnant lipoproteins in the fasting and postprandial plasma. Clin Chim Acta. 2012; 413: 1077-1086.

6. Nestel PJ, Havel RJ, Bezman A. Metabolism of constituent lipids of dog chylomicrons. J Clin Invest. 1963; 42: 1313-1321.

7. Nestel P. Relationship between plasma triglycerides and removal of chylomicrons. J Clin Invest. 1964; 43: 943-949.

8. Redgrave T. Formation Cholesteryl Ester-Rich Particulate Lipid during Metabolism of Chylomicrons. J Clin Invest. 1970; 49: 465-471.

9. Grundy SM, Mok HY. Chylomicron clearance in normal and hyperlipidemic man. Metabolism. 1976; 25: 1225-1239.

10. Redgrave T. G, Small D. M. Phospholipid of Chylomicrons to the High Density Lipoprotein Fraction during the Catabolism of Chylomicrons in the Rat. J Clin Invest. 1979; 64:162-171.

11. Johanson EH, Jansson PA, Gustafson B, Lonn L, Snitt U, et al. Early alterations in the postprandial VLDL1 apoB-100 and apoB-48 metabolism in men with strong heredity for type 2 diabetes. J Intern Med. 2004; 255: 273-279.

12. Karpe F, Steiner G, Uffelman K, Olivecrona T, Hamsten A. Postprandial lipoproteins and progression of coronary atherosclerosis. Atherosclerosis. 1994; 106: 83-97.

13. Mero N, Syvanne M, Rosseneu M, Labeur C, Hilden H, et al. Comparison of three fatty meals in healthy normolipidaemic men: high postprandial retinyl ester response to soybean oil. Eur J Clin Invest. 1998; 28: 407-415.

14. Twickler TB, Dallinga-Thie GM, Cohn JS, Chapman MJ. Elevated remnant-like particle cholesterol concentration: a characteristic feature of the atherogenic lipoprotein phenotype. Circulation 2004; 109: 1918-1925.

15. Nakajima K, Nakano T, Tanaka A. Article title: The oxidative modification hypothesis of atherosclerosis: the comparison of atherogenic effects on oxidized LDL and remnant lipoproteins in plasma. Clin Chim Acta. 2006; 367: 36-47.

16. Schaefer EJ, McCollum EV. Award Lecture: lipoproteins, nutrition, and heart disease. Am. J. Clin. Nutr. 2002; 75: 191-212.

17. Björnson E, Packard CJ, Adiels M, Andersson L, Matikainen N, et al. Investigation of human apoB48 metabolism using a new, integrated non-steady-state model of apoB48 and apoB100 kinetics. J Intern Med. 2019; 285: 562-577.

18. Nakajima K, Tokita Y, Tanaka A. Hypothesis: Postprandial remnant lipoproteins are the causal factors that induce the insulin resistance associated with obesity. Clin Chim Acta. 2018; 485: 162-132.

19. Nakajima K, Tanaka A. Postprandial remnant lipoproteins as targets for the prevention of atherosclerosis. Curr Opin Endocrinol Diabetes Obes. 2018; 25:108-117.

20. Zilversmit DB. Atherogenesis: a postprandial phenomenon. Circulation 1979; 60: 473-485.

21. Chappell DA, Medh JD .Receptor-mediated mechanisms of lipoprotein remnant catabolism. Prog Lipid Res. 1998; 37: 393-422.

22. TC Ooi, Cousins M, Ooi DS, Steiner G, Uffelman KD, Nakajima K, Simo IE. Postprandial remnant- like lipoproteins in hypertriglyceridemia. J Clin Endocrinol Metab. 2001; 86: 3134-3142.

23. Ai M, Tanaka A, Shimokado K, Ohtani R, Inazu A, et al. A deficiency of cholesteryl ester transfer protein whose serum remnant-like particle-triglyceride significantly increased, but serum remnant-like particle-cholesterol did not after an oral fat load. Ann Clin Biochem. 2009; 46: 457-463.

24. Campos E, Nakajima K, Tanaka A, Havel RJ. Properties of an apolipoprotein E-enriched fraction of triglyceride-rich lipoproteins isolated from human blood plasma with a monoclonal antibody to apolipoprotein B-100. J Lipid Res. 1992; 33: 369-380.

25. Nakano T, Tanaka A, Okazaki M, Tokita Y, Nagamine T, et al. Particle size of apoB-48 carrying lipoproteins in remnant lipoproteins isolated from postprandial plasma. Ann Clin Biochem. 2011; 48: 57-64.

26. Nagao T, Watanabe H, Goto N, Onizawa K, Taguchi HN. Dietary diacylglycerol suppresses accumulation of body fat compared to triacylglycerol in men in a double-blind controlled trial. J Nutr. 2000; 130: 792-797.

27. St-Onge MP, Jones PJ. Physiological effects of medium-chain triglycerides: potential agents in the prevention of obesity. J Nutr. 2002; 132: 329-332.

28. Duvillard L, Caslake MJ, Petit JM, Vergès B, Gambert P, et al. Distinct patterns of heparin affinity chromatography VLDL1 and VLDL2 subfractions in the different dyslipidaemias. Atherosclerosis. 2008; 199: 27-33.

29. Gill JM, Brown JC, Bedford D, Wright DM, Cooney J, et al. Hepatic production of VLDL1 but not VLDL2 is related to insulin resistance in normoglycaemic middle-aged subjects. Atherosclerosis. 2004; 176: 49-56.

30. Deighan CJ, Caslake MJ, McConnell M, Boulton-Jones JM, Packard CJ. Patients with nephrotic-range proteinuria have apolipoprotein C and E deficient VLDL1. Kidney Int. 2000; 58: 1238-1246.

31. Greeve J, Altkemper I, Dieterich JH, Greten H, Windler E. Apolipoprotein B mRNA editing in 12 different mammalian species: hepatic expression is reflected in low concentrations of apoB-containing plasma lipoproteins. J Lipid Res. 1993; 34: 1367-1383.

32. Davidson NO, Anant S, MacGinnitie AJ. Apolipoprotein B messenger RNA editing: insights into the molecular regulation of post-transcriptional cytidine deamination. Curr. Opin. Lipidol. 1995; 6: 70-74.

33. Zanoni P, Velagapudi S, Yalcinkaya M, Rohrer L, von Eckardstein A. Endocytosis of lipoproteins. Atherosclerosis. 2018; 275: 273-295.

34. Véniant M, Zlot C, Walzem R, Pierotti V, Driscoll R, et al. Lipoprotein Clearance Mechanisms in LDL Receptor–Deficient "Apo-B48-only" and "Apo-B100-only" Mice. J. Clin. Invest. 1998. 102: 1559-1568.

35. Wetterau R, Lin C, Jamil H. Microsomal Triglyceride Transfer Protein. Biochim Biophys Acta. 1997; 1345: 136-150.

36. Nakajima K, Tanaka A. Postprandial remnant lipoproteins VLDL remnants as a causal factor for atherosclerosis. Clin Chim. 2018; 478: 200-215.