conditions of CM overproduction.

In a previous publication, our group found that CD36KO mice have an increased TG response to acute fat loads in both plasma and lymph[11]. In the current study, we found that CD36KO showed a higher TG concentration than WT mice even in a high fat loading state in intestinal lymph (**Fig. 3**); this might suggest the hypothesis that CD36KO mice would have a larger CM than WT mice. However, since the CM fraction in our HPLC method was included in the void volume, we could not determine the specific size of individual particles in this fraction, which is considered a whole group, and therefore, we were not able to confirm whether there was really a difference in particle size between these two groups.

The reduced absorption of long-chain FAs observed in this study was in part associated with an inhibitory effect on FATP4 in CD36KO mice as well as the reduction of both FATP4 and FABP2 intestinal expression in WT mice. FATP4 is the only FATP expressed in the intestines[21], is located in the ER of the intestinal cells and has demonstrated acyl-CoA synthetase activity, which decreases the intracellular concentration of FAs, and would indirectly increase FA uptake when the extracellular concentration is high enough, as in the postprandial state[22]. FATP4 has also been associated with obesity and the insulin-resistant state[23]. Labonté *et al.*[24] reported a reduction of the FATP4 amount in the intestines of both WT mice receiving ezetimibe and NPC1L1 knockout mice compared to WT controls. Although we did not measure the amount of FATP4 protein by Western blotting, we found a decrease in the mRNA content in both treated groups, suggesting inhibition of the regulation of FATP4 at the transcriptional level, which would lead to a decreased amount of FATP4. Taken together, these findings suggest a close relationship between the presence of active NPC1L1 and the uptake, intracellular transport and esterification of long-chain FAs.

In the current study, we also found that WT mice fed a western diet under ezetimibe treatment showed a reduced expression of DGAT1 and DGAT2, two proteins involved in TG synthesis, located in the ER[25], as well as a decreased expression of SCD1, which is an important lipogenic factor associated with dietary saturated fat-related obesity[26]. SCD1 has been reported to colocalize and interact with DGAT2[27], suggesting a mechanism of the incorporation of endogenously synthesized FAs into TG. Therefore, ezetimibe might also decrease PHTG in WT mice fed a western diet by reducing the formation of TG in intestinal cells.

Interestingly, in CD36KO mice, ezetimibe administration inhibited only FATP4 expression in the steps prior to CM assembly to reduce PHTG, but not FABP2, nor any of the proteins involved in TG production, as in WT mice, which might suggest that FATP4 could play an essential role in FA metabolism in the CD36KO model, different from WT mice, which also supports the idea that intestinal lipid metabolism in CD36KO mice is different from in WT mice.

On the other hand, we found that ezetimibe administration reduced ApoB mRNA in both treated groups, and moreover, ezetimibe decreased the mRNA levels of apobec1 in CD36KO mice and Apobec1 complementary factor (ACF) in WT mice. Whether ezetimibe decreased ApoB48 mass in lymph only by inhibiting the transcription or by enhancing the post-transcriptional degradation of ApoB is not known yet, and further examination will be required to gain a better understanding of intestinal ApoB metabolism.

Apobec1 is the catalytic subunit of the ApoB editing complex; in the absence of apobec1, there is no ApoB mRNA edition; apobec1 KO mice lack ApoB48, and the only ApoB found in this model is ApoB100[28]. In our study, ezetimibe decreased apobec1 mRNA significantly in CD36KO mice; however, we did not find any traces of ApoB100 in the intestinal lymph collected; therefore, we presume that ApoB mRNA edition was not so low as to make the enterocytes produce ApoB100-containing lipoproteins, but decreased enough to reduce the production of ApoB48 which, in addition to the presence of low TG as a substrate, led to reduced CM production.

Apobec1 complementary factor (ACF), the RNA-binding subunit of the editing complex, interacts with both apobec1 and ApoB mRNA, positioning the ApoB mRNA structure in the optimal configuration to expose the C residue to apobec1, and it has been proposed to be responsible for the specificity of the reaction[29], and a stabilizer for apobec1[30]. It has been proposed that ACF plays a pivotal role independent of apobec1, since attempts to generate ACF KO mice were not successful beyond the blastocyst state, and siRNA knockdown of ACF in rat and human cells induced an increase in apoptosis. In heterozygote ACF KO mice, ACF protein was found to be decreased in the small intestines; however, intestinal ApoB mRNA edition was not compromised[31]. From this evidence, we could not draw the conclusion that the lowering effect of ezetimibe on the expression of ACF would be actually relevant to ApoB mRNA edition and the production of CM in WT mice.

We have summarized in **Fig. 6** the possible

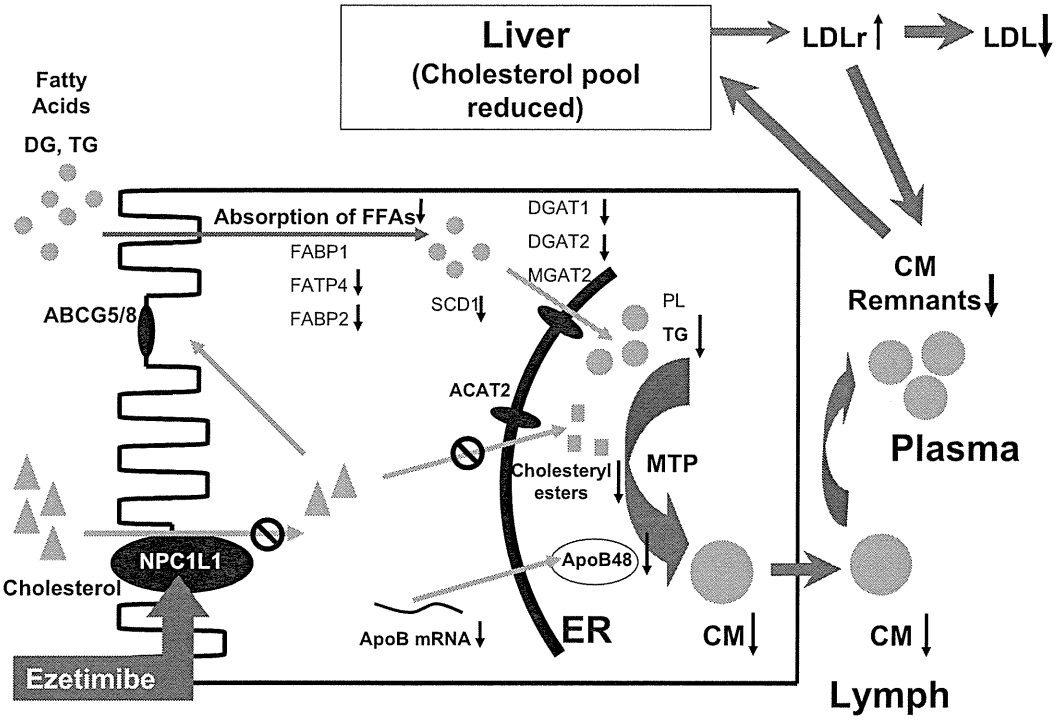


**Fig. 6.** Possible Mechanisms for the Inhibitory Effect of Ezetimibe on Postprandial Hypertriglyceridemia.

Administration of ezetimibe alone reduces PHTG by inhibiting cholesterol absorption and the expression of genes involved in the uptake, intracellular trafficking and metabolism of long-chain FAs (FATP4 in both WT and CD36KO mice and FABP2 in WT mice only), as well as by decreasing the formation of TG (SCD1, DGAT1 and DGAT2 in WT mice) and the expression of apoB (both WT and CD36KO mice), necessary for the production of ApoB48-containing lipoproteins in the small intestine. Furthermore, reduced cholesterol influx to the liver may lead to the up-regulation of hepatic LDL receptor, resulting in the enhanced catabolism of LDL and CM remnants.

mechanisms for the inhibitory effect of ezetimibe treatment on postprandial hypertriglyceridemia. The administration of ezetimibe alone reduces PHTG by inhibiting cholesterol absorption and the expression of genes involved in the uptake, intracellular trafficking and the metabolism of long-chain FAs (FATP4 in both WT and CD36KO mice and FABP2 in WT mice only), as well as by decreasing the formation of TG (SCD1, DGAT1 and DGAT2 in WT mice) and the expression of ApoB (both WT and CD36KO mice) necessary for the production of ApoB48-containing lipoproteins in the small intestine. Furthermore, reduced cholesterol influx to the liver may lead to the up-regulation of hepatic LDL receptor, resulting in the enhanced catabolism of LDL and CM remnants.

In conclusion, ezetimibe alone reduces PHTG in mouse models of MetS by inhibiting cholesterol absorption and uptake, intracellular trafficking and the metabolism of long-chain FAs, as well as decreasing the formation of TG and the expression of apoB, necessary for the production of apoB48-containing lipoproteins in the small intestine. Thus, ezetimibe strongly attenuates the intestinal production of CM, resulting in the inhibition of PHTG, which may eventually lead to the reduction of atherosclerosis in both animal models and humans.

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### References

1) Third Report of the National Cholesterol Education Program (NCEP): Expert Panel on Detection, Evaluation and Treatment of High Blood cholesterol in Adults (Adult treatment panel Ⅲ). Final Report. Circulation, 2002;

106: 3163-3223
2) Nordestgaard B, Benn M, Schnohr P, Tybjærg-Hansen A: Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. JAMA, 2007; 298: 299-308
3) Freiberg JJ, Tybjaerg-Hansen A, Jensen JS, Nordestgaard BG: Nonfasting triglycerides and risk of ischemic stroke in the general population. JAMA, 2008; 300: 2142-2152
4) Huff MW: Dietary cholesterol, cholesterol absorption, postprandial lipemia and aterosclerosis. Can J Clin Pharmacol, 2003; 10 (Suppl A): 26A-32A
5) Zilversmit DB: Atherogenesis: A postprandial phenomenon. Circulation, 1979; 60: 473-485
6) Fujioka Y, Ishikawa Y: Remnant lipoproteins as strong key particles to atherogenesis. J Atheroscler Thromb, 2009; 16: 145-154
7) Tanaka A: Postprandial hypertriglyceridemia and atherosclerosis. J Atheroscler Thromb, 2004; 11: 322-329
8) Su X, Abumrad NA: Cellular fatty acid uptake: A pathway under construction. Trends Endocrinol Metab, 2009; 20: 72-77
9) Yamashita S, Hirano K, Kuwasako T, Janabi M, Toyama Y, Ishigami M, Sakai N: Physiological and pathological roles of a multi-ligand receptor CD36 in atherogenesis; insights from CD36-deficient patients. Mol Cell Biochem, 2007; 299: 19-22
10) Goudriaan JR, den Boer MA, Rensen PC, Febbraio M, Kuipers F, Romijn JA, Havekes LM, Voshol PJ: CD36 deficiency in mice impairs lipoprotein lipase-mediated triglycerides clearance. J Lipid Res, 2005; 46: 2175-2181
11) Masuda D, Hirano K, Oku H, Sandoval JC, Kawase R, Yuasa-Kawase M, Yamashita Y, Takada M, Tsubakio-Yamamoto K, Tochino Y, Koseki M, Matsuura F, Nishida M, Kawamoto T, Ishigami M, Hori M, Shimomura I, Yamashita S: Chylomicron remnants are increased in the postprandial state in CD36 deficiency. J Lipid Res, 2009; 50: 999-1011
12) Ge L, Wang J, Qi W, Miao HH, Cao J, Qu YX, Li BL, Song BL: The cholesterol absorption inhibitor ezetimibe acts by blocking the sterol-induced internalization of NPC1L1. Cell Metab, 2008; 7: 508-519
13) Stein E, Stender S, Mata P, Sager P, Ponsonnet D, Melani L, Lipka L, Suresh R, MacCcubin D, Veltri E: Achieving lipoprotein goals in patients at high risk with severe hypercholesterolemia: Efficacy and safety of ezetimibe co-administered with atorvastatin. Am Heart J, 2004; 148: 447-455
14) McKenney JM, Farnier M, Lo KW, Bays HE, Perevozkaya I, Carlson G, Davies MJ, Mitchel YB, Gumbiner B: Safety and efficacy of long-term co-administration of fenofibrate and ezetimibe in patients with mixed hyperlipidemia. J Am Coll Cardiol, 2006; 47: 1584-1587
15) Hajer GR, Dallinga-Thie GM, van Vark-van der Zee LC, Visseren FL: The effect of statin alone or in combination with ezetimibe on postprandial lipoprotein composition in obese metabolic syndrome patients. Atherosclerosis, 2009; 202: 216-224
16) Masuda D, Nakagawa-Toyama Y, Nakatani K, Inagaki M, Tsubakio-Yamamoto K, Sandoval JC, Ohama T, Nishida M, Ishigami M, Yamashita S: Ezetimibe improves postprandial hyperlipidaemia in patients with type IIb hyperlipidaemia. Eur J Clin Invest, 2009; 39: 689-698
17) Moore KJ, J El Khoury J, Medeiros LA, Terada K, Geula C, Luster AD, Freeman MW: A CD36-initiated signaling cascade mediates inflammatory effects of beta-amyloid. J Biol Chem, 2002; 277: 47373-47379
18) Okazaki M, Usui S, Fukui A, Kubota I, Tomoike H: Component analysis of HPLC profiles of unique lipoprotein subclass cholesterols for detection of coronary artery disease. Clin Chem, 2006; 52: 2049-2053
19) Bollman, JL, Cain JC, Grindlay JH: Techniques for the collection of lymph from the liver, small intestine, or thoracic duct of the rat. J Lab Clin Med, 1949; 33: 1349-1352
20) Altmann SW, Davis HR, Zhu LJ, Yao X, Hoos LM, Tezloff G, Iyer SPN, Maguire M, Golovko A, Zeng M, Wang L, Murgolo N, Graziano MP: Niemann-Pick C1 Like 1 protein is critical for intestinal cholesterol absorption. Science, 2004; 303: 1201-1204
21) Stahl A: A current review of fatty acid transport proteins (SLC27). Pflugers Arch, 2004; 447: 722-727
22) Milger K, Herrmann T, Becker C, Gotthardt D, Zickwolf J, Ehehait R, Watkins P, Stremmel W, Füllekrug J: Cellular uptake of fatty acids driven by the ER-localized acyl-CoA synthetase FATP4. J Cell Sci, 2006; 119: 4678-4688
23) Fisher RM, Gertow K: Fatty acid transport proteins and insulin resistance. Curr Opin Lipidol, 2005; 16: 173-178
24) Labonté ED, Camarota LM, Rojas JC, Jandacek RJ, Gilham DE, Davies JP, Ioannou YA, Tso P, Hui DY, Howles PN: Reduced absorption of saturated fatty acids and resistance to diet induced obesity and diabetes by ezetimibe-treated and Npc1l1-/- mice. Am J Physiol Gastrointest Liver Physiol, 2008; 295: G776-G783
25) Yen CLE, Stone SJ, koliwad S, Harris C, Farese RV: DGAT enzymes and triacylglycerol biosynthesis. J Lipid Res, 2008; 49: 2283-2301
26) Sampath H, Miyazaki M, Dobrzyn A, Ntambi JM: Stearoyl-CoA desaturase-1 mediates the pro-lipogenic effects of dietary saturated fat. J Biol Chem, 2007; 282: 2483-2493
27) Man WC, Miyazaki M, Chu K, Ntambi J: Colocalization of SCD1 and DGAT2: implying preference for endogenous monounsaturated fatty acids in triglyceride synthesis. J Lipid Res, 2006; 47: 1928-1939
28) Hirano K, Young SG, Farese RV Jr, Ng J, Sande E, Warburton C, Powell-Braxton LM, Davidson NO: Targeted disruption of the mouse apobec-1 gene abolishes apolipoprotein B mRNA editing and eliminates apolipoprotein B48. J Biol Chem, 1996; 271: 9887-9990
29) Maris C, Masse J, Chester A, Navaratnam N, Allain FHT: NMR structure of the apoB mRNA stem-loop and its interaction with the C to U editing APOBEC1 complementary factor. RNA, 2005; 11: 173-186
30) Chester A, Weinreb V, Carter CW, Navaratnam N: Optimization of apolipoprotein B mRNA editing by APOBEC1 apoenzyme and the role of its auxiliary factor, ACF. RNA, 2004; 10: 1399-1411
31) Blanc V, Henderson JO, Newberry EP, Kennedy S, Luo J, Davidson NO: Targeted deletion of the murine apobec-1 complementation factor (acf) gene results in embryonic lethality. Mol Cell Biol, 2005; 25: 7260-7269

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# Serum apolipoprotein B-48 levels are correlated with carotid intima-media thickness in subjects with normal serum triglyceride levels

Kazuhiro Nakatani[a,1], Taizo Sugimoto[b,2], Daisaku Masuda[a,*], Rieko Okano[c,3], Tomoko Oya[c,3], Yasumasa Monden[c,3], Taiji Yamashita[a,1], Ryota Kawase[a,1], Hajime Nakaoka[a,1], Miwako Inagaki[a,1], Miyako Yuasa-Kawase[a,1], Kazumi Tsubakio-Yamamoto[a,1], Tohru Ohama[a,1], Makoto Nishida[a,d,1,4], Masato Ishigami[a,e,1,5], Issei Komuro[a,1], Shizuya Yamashita[a,1]

[a] *Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan*
[b] *Sugimoto Clinic, 2-21-18 Momodani, Ikuno, Osaka 544-0034, Japan*
[c] *Osaka Police Hospital Dock, 15-15 Ishigatsuji, Tennouji, Osaka 543-0031, Japan*
[d] *Health Care Center, Osaka University, 1-7 Machikaneyama, Toyonaka, Osaka 560-0043, Japan*
[e] *Division of Health Sciences, Osaka University Graduate School of Medicine, 1-7 Yamadaoka, Suita, Osaka 565-0871, Japan*

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ABSTRACT

*Background:* Postprandial hyperlipidemia (PPHL) is an independent risk factor for coronary heart disease (CHD) which is based on the accumulation of chylomicrons (CM) and CM remnants containing apolipoprotein B-48 (apoB-48). Since atherosclerotic cardiovascular diseases are frequently observed even in subjects with normal serum triglyceride (TG) level, the correlation between fasting apoB-48 containing lipoproteins and carotid intima-media thickness (IMT) was analyzed in subjects with normal TG levels.

*Methods:* From subjects who took their annual health check at the Osaka Police Hospital ($n = 245$, male), one-hundred and sixty-four male subjects were selected to take part in this study; the excluding factors were: systolic blood pressure ≥140 mmHg, intake of antihypertensive or antihyperlipidemic drugs, or age >65 years. The association between biochemical markers and IMT was analyzed and independent predictors of max-IMT were determined by multiple regression analysis in all subjects and in groups N-1 (TG < 100 mg/dl, $n = 58$), N-2 (100 ≤ TG < 150 mg/dl, $n = 53$) and H (150 ≤ TG mg/dl, $n = 53$), respectively.

*Results:* Fasting total cholesterol, LDL–cholesterol, HDL–cholesterol, apoB-100 and ln RemL–C (remnant lipoprotein–cholesterol) levels were not correlated with max-IMT, but ln TG and ln apoB-48 were significantly correlated with max-IMT in all subjects. Ln apoB-48 and apoB-48/TG ratio were significantly correlated with max-IMT in group N-2. By multiple regression analysis, age and ln apoB-48 were independent variables associated with max-IMT in group N-2.

*Conclusion:* Serum apoB-48 level might be a good marker for the detection of early atherosclerosis in middle-aged subjects with normal-range levels of blood pressure and TG.



## 1. Introduction

Hypercholesterolemia, including high serum LDL–cholesterol (LDL–C) level, is strongly correlated to the development of atherosclerotic cardiovascular diseases [1]. Statins significantly decrease LDL–C levels and the morbidity of atherosclerotic cardiovascular diseases; however, they cannot completely prevent the occurrence of these diseases yet [2]. Epidemiologic studies have revealed that fasting hypertriglyceridemia is also associated with atherosclerosis, independent of other coronary risk factors such as high LDL–C level [3,4]. A case-control study showed that fasting and non-fasting TG levels were also superior among patients with coronary heart disease (CHD) as compared with control subjects [5]. A Japanese prospective study demonstrated that not only fast-

*Abbreviations:* BMI, body mass index; apoB-48, apolipoprotein B-48; PPHL, postprandial hyperlipidemia; CM, chylomicrons; CMR, chylomicron remnants; RemL–C, remnant lipoprotein–cholesterol; TG, triglycerides; LDL, low-density lipoprotein; HDL, high-density lipoprotein; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; HOMA-IR, homeostasis model assessment as an index of insulin resistance; IRI, immuno-reactive insulin; IMT, intima-media thickness.

* Corresponding author. Tel.: +81 6 6879 3633; fax: +81 6 6879 3645.
*E-mail address:* masuda@imed2.med.osaka-u.ac.jp (D. Masuda).
[1] Tel.: +81 6 6879 3633; fax: +81 6 6879 3645.
[2] Tel.: +81 6 6716 4910; fax: +81 6 6715 1724.
[3] Tel.: +81 6 6775 3131; fax: +81 6 6775 3121.
[4] Tel.: +81 6 6850 6015; fax: +81 6 6850 6040.
[5] Tel.: +81 6 6879 2588; fax: +81 6 6879 2499.

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ing but also non-fasting TG levels were significantly correlated with CHD morbidity [6]. In this study, the authors also showed that an increase in TG levels was significantly correlated with an increase in CHD morbidity even though TG levels remained below 150 mg/dl, a level which has been recognized as borderline of high risk status for atherosclerotic cardiovascular diseases on the basis of Framingham Study [7]. Therefore, we need to evaluate the emerging risk of atherosclerotic cardiovascular diseases even in subjects with normotriglyceridemia (TG < 150 mg/dl).

Postprandial hyperlipidemia (PPHL) is caused by the impaired metabolism of lipoproteins, which is mainly characterized by a postprandial accumulation of intestine-derived lipoproteins, chylomicrons (CM) and their hydrolyzed lipoproteins, chylomicron remnants (CM-R). In subjects with normal lipoprotein metabolism, CM and CM-R are promptly hydrolyzed, diminished in size and cleared from the circulation by the liver within a few hours after a meal. PPHL does not indicate the postprandial increase of lipids and lipoproteins which are promptly cleared from the circulation in subjects with normal lipoprotein metabolism. However, in patients with PPHL, CM-R continue to accumulate for over 6–8 h after a meal, penetrating into the vessels to form foam cells. Many recent studies have proved that PPHL is an independent risk factor for the development of CHD and atherosclerosis of carotid arteries [8–10]. Many basic studies have suggested that accumulated CM-R particles may promote atherogenicity in the arterial wall [11]. An oral fat loading (OFL) test is sometimes used to assess PPHL levels; however, this it is not a suitable testing option for routine clinical use because it requires a lot of time (6–8 h). Further, consensus has not yet been reached regarding the indication and the interpretation of data from this test. We developed a novel enzyme-linked immuno-sorbent assay (ELISA) to measure serum levels of apolipoprotein B-48 (apoB-48) [12]. Since one apoB-48 molecule is included in one CM and CM-R particle up to the clearance by the liver, serum apoB-48 level represents the number of both CM and CM-R particles and is suitable for the quantitative evaluation of postprandial changes. In patients with suspected accumulation of CM and CM-R, serum apoB-48 levels are significantly higher at the fasting state and increased after OFL in normolipidemic subjects [12]. High levels of fasting serum apoB-48 suggest the existence of PPHL, without performing an OFL test [13], and are reportedly related to the development of atherosclerotic cardiovascular diseases [14–17]. These results suggest that fasting apoB-48 level is a good marker for the evaluation of atherogenic risk in patients with hypertriglyceridemia. However, very few studies have so far investigated the correlation between fasting serum apoB-48 levels and the development of atherosclerosis among subjects with normal fasting TG levels.

In the current study, we have investigated the correlations between profiles of apoB-48-containing lipoproteins and the progression of atherosclerosis in subjects with normal TG levels. For the evaluation of atherosclerosis progression, intima-media thickness (IMT) of carotid arteries was measured using a diagnostic ultrasound, which was shown to be significantly correlated with the development and prognosis of CHD and cerebrovascular diseases [18,19].

## 2. Subjects and methods

### 2.1. Subjects

A consecutive series of subjects ($n$ = 245, male) who came to Osaka Police Hospital for the annual health checkup were picked up serially. One-hundred and sixty-four male subjects were finally enrolled by the following exclusion criteria: systolic blood pressure ≥140 mmHg, age over 65 years and intake of any drugs affecting lipid metabolism and blood pressure. This study was approved by the Ethical Committee of Osaka Police Hospital, and all participants gave their written informed consent.

### 2.2. Biochemical analyses

Height, weight, and waist circumference were measured in the standing position. Systolic and diastolic blood pressures were measured at rest in the sitting position. Blood samples were collected after an overnight fast, followed by an immediate separation of serum and plasma. Total cholesterol (TC), triglycerides (TG), HDL-C, fasting plasma glucose (FPG) and uric acid (UA) levels were measured by enzymatic methods, LDL-C levels by direct method, and serum apoB levels by immunoturbidity method, respectively (Sekisui Medical Co., Ltd., Tokyo, Japan). Hemoglobin A1c (HbA1c) levels were determined by high performance liquid chromatography (HPLC) method and immunoreactive insulin (IRI) levels by the immunoturbidity method (SRL Inc., Tokyo, Japan). Serum apoB-48 levels were measured by the chemiluminescent enzyme immunoassay (CLEIA) using anti-human apoB-48 monoclonal antibodies, which we developed previously with minor modification (Fujirebio Inc., Tokyo, Japan). Remnant lipoprotein-cholesterol (RemL-C) levels were measured by the homogenous assay (Kyowa Medex, Tokyo, Japan) [12]. ApoB-100 levels were calculated by subtracting the value of apoB-48 from the value of serum apoB. Plasma adiponectin levels were determined by the human adiponectin ELISA kit (Otsuka Pharmaceuticals, Tokyo, Japan). Subjects were divided into 3 groups by serum TG level: group N-1 ($n$ = 58), TG < 100 mg/dl; Group N-2 ($n$ = 53), 100 ≤ TG < 150 mg/dl and Group H ($n$ = 53), 150 ≤ TG mg/dl.

### 2.3. Ultrasound measurements

The IMT of carotid arteries was determined using ultrasonography in the supine position. High-resolution B-mode ultrasound images were obtained (Toshiba Nemio, Toshiba Corp., Tokyo, Japan) with a 12 MHz linear array transducer. Three arterial wall segments in each carotid artery were imaged from a fixed lateral transducer angle at the far wall. All segments, including both sides of common carotid artery, the carotid bifurcation, and the internal carotid artery, were scanned. The thickest part of the IMT was recorded as max-IMT, and the IMT of the far wall was measured at 3 continuous sites at a 1.0-cm interval proximal to the thickest part of IMT in each side and then averaged to obtain mean-IMT. The mean-IMT value and greater max-IMT value obtained from scans of the right and left carotid arteries in each subject were used for statistical analyses.

### 2.4. Statistical analysis

Values were expressed as mean ± SD. ApoB-48 levels were normalized by logarithmic transformation. Between-group comparisons of the means and median were performed by Tukey's HSD test among group N-1, group N-2 and group H. The correlations between metabolic parameters and mean-/max-IMT were calculated by Pearson's correlation coefficients. Stepwise multiple regression analysis was used to determine independent predictors of max-IMT measurement with $P$ value-to-enter set at 0.20. Age, sBP, dBP, total cholesterol, ln TG, LDL-C, HDL-C, apoB-48, apoB-100, ln RemL-C, FPG, HbA1c, ln HOMA-IR, and IRI were included as explanatory variables in the method. Data were analyzed with JMP8 software (SAS Institute, Cary, NC). All statistical significance was accepted at $P$ < 0.05.

**Table 1**
Clinical profiles of subjects investigated.

| | Total<br>$n=164$ | Group N-1<br>TG < 100<br>$n=58$ | Group N-2<br>100 ≤ TG < 150<br>$n=53$ | Group H<br>150 ≤ TG<br>$n=53$ |
|---|---|---|---|---|
| Age (year) | 52 ± 6 | 53 ± 6 | 52 ± 6 | 52 ± 7 |
| BMI (kg/m2) | 24.7 ± 3.0 | 23.4 ± 2.3 | 24.6 ± 2.5 | 26.1 ± 3.4$^{\#}$ |
| Waist circ. (cm) | 87 ± 8 | 83 ± 6 | 88 ± 7$^{**}$ | 91 ± 8$^{\#}$ |
| sBP (mmHg) | 120 ± 12 | 117 ± 12 | 120 ± 11 | 123 ± 12 |
| dBP (mmHg) | 82 ± 9 | 79 ± 9 | 82 ± 9 | 84 ± 9 |
| TC (mg/dl) | 208 ± 30 | 201 ± 27 | 211 ± 30 | 213 ± 32 |
| HDL-C (mg/dl) | 54 ± 13 | 60 ± 14 | 56 ± 11 | 47 ± 8$^{\#\#}$ |
| LDL-C (mg/dl) | 124 ± 28 | 123 ± 24 | 129 ± 26 | 119 ± 33 |
| TG (mg/dl) | 152 ± 120 | 77 ± 15 | 122 ± 15$^{*}$ | 264 ± 156$^{\#\#}$ |
| apoB-48 (mg/dl) | 0.57 ± 0.55 | 0.28 ± 0.14 | 0.42 ± 0.19 | 1.03 ± 0.74$^{\#\#}$ |
| apoB-100 (mg/dl) | 97.8 ± 17.3 | 89.6 ± 15.1 | 100.8 ± 14.6$^{*}$ | 103.7 ± 18.8 |
| RemL-C (mg/dl) | 12.2 ± 8.3 | 7.0 ± 5.0 | 9.5 ± 2.1 | 20.4 ± 8.9$^{\#\#}$ |
| FPG (mg/dl) | 96 ± 14 | 96 ± 13 | 98 ± 15 | 95 ± 15 |
| HbA1c (%) | 5.1 ± 0.5 | 5.1 ± 0.5 | 5.2 ± 0.4 | 5.1 ± 0.6 |
| HOMA-IR | 1.3 ± 0.9 | 1.0 ± 0.5 | 1.3 ± 0.8 | 1.6 ± 1.1 |
| IRI (μU/ml) | 5.2 ± 2.9 | 4.0 ± 1.9 | 5.1 ± 3.0 | 6.5 ± 3.4$^{\#}$ |
| Adiponectin (μg/ml) | 5.4 ± 3.1 | 6.4 ± 3.9 | 5.2 ± 2.5 | 4.5 ± 2.3 |

From male subjects who took their annual health checkup at Osaka Police Hospital, one-hundred and sixty-four male subjects (aged 52 ± 6 years) were divided into 3 groups by serum TG level; group N-1 ($n=58$), TG < 100 mg/dl; group N-2 ($n=53$), 100 ≤ TG < 150 mg/dl; group H ($n=53$), 150 ≤ TG mg/dl, respectively. Values are the mean ± SD; between-group comparisons of the means and median were performed by Tukey's HSD test among group N-1, group N-2 and group H.

$^{*}$ $P<0.05$ (group N-2 compared with group N-1).
$^{**}$ $P<0.005$ (group N-2 compared with group N-1).
$^{\#}$ $P<0.05$ (group H compared with group N-2).
$^{\#\#}$ $P<0.005$ (group H compared with group N-2).

## 3. Results

### 3.1. Clinical profiles

Table 1 shows the clinical profiles of all patients ($n=164$), group N-1 ($n=58$, TG < 100 mg/dl), group N-2 ($n=53$, 100 ≤ TG < 150 mg/dl) and group H ($n=53$, 150 ≤ TG mg/dl). The subjects were 52 ± 6 years-old (mean ± SD), and apoB-48 level was 0.57 ± 0.55 mg/dl. Waist circumference, TG and apoB-100 levels in group N-2 were significantly higher than those of group N-1. BMI, waist circumference, TG, apoB-48, apoB-100 and RemL-C levels in group H were significantly higher, and HDL-C levels were significantly lower in group H than in group N-2. Mean- and max-IMT were measured in all subjects, and between-group comparisons of the means and median were performed by Tukey's HSD test among total subjects, group N-1, group N-2 and group H. There was no significant difference in mean-IMT (total subjects, 0.80 ± 0.18 mm; group N-1, 0.75 ± 0.13 mm; group N-2, 0.79 ± 0.17 mm; and group H, 0.84 ± 0.23 mm, respectively) and in max-IMT (total subjects, 0.87 ± 0.23 mm; group N-1, 0.81 ± 0.16 mm; group N-2, 0.86 ± 0.22 mm; and group H, 0.93 ± 0.29 mm, respectively).

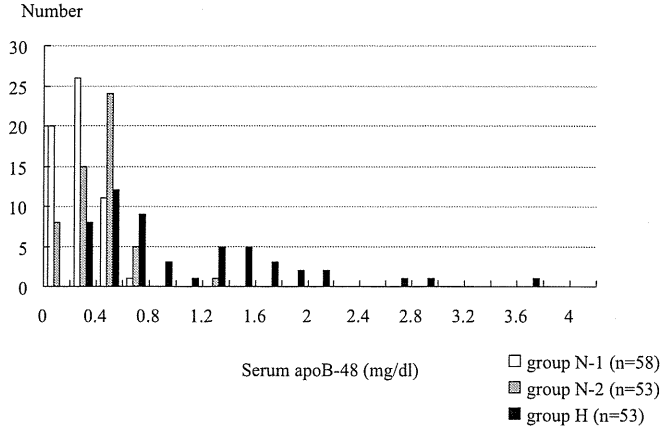


**Fig. 1.** Distribution of fasting serum apoB-48 levels. Geometric means were 0.24 mg/dl in group N-1, 0.41 mg/dl in group N-2 and 0.69 mg/dl in group H. The distribution of apoB-48 levels was significantly shifted to higher values; the data was normalized by logarithmic transformation for further statistical analysis.

### 3.2. Distribution of apoB-48 in each TG group

For the analysis of the correlation between apoB-48 levels and IMT, the distribution of apoB-48 levels was compared among groups N-1, N-2 and H (Fig. 1). The distribution of apoB-48 levels in group H was significantly shifted to higher values as compared with group N-1 and group N-2. ApoB-48 levels in group N-2 were also shifted to higher values compared with group N-1. In order to compare the apoB-48 levels in these TG groups, we normalized the apoB-48 levels by logarithmic transformation for further statistical analysis.

### 3.3. Correlation analysis in all subjects with max-IMT

Coronary risk factors such as TC, LDL-C, HDL-C, apoB-100 and ln RemL-C levels showed no significant correlations with mean- and max-IMT as assessed by Pearson's correlation coefficients in total subjects. To the contrary, ln TG and ln apoB-48 levels were significantly correlated with max-IMT (Fig. 2), and not significantly correlated with mean-IMT levels.

### 3.4. Correlation analysis in each TG group with max-IMT

The correlation of fasting apoB-100 levels and ln apoB-48 levels was analyzed with max-IMT in groups N-1, N-2 and H, respectively (Fig. 3). ApoB-100 levels were not significantly correlated with max-IMT in each TG group. Ln apoB-48 levels were significantly correlated with max-IMT only in group N-2.

### 3.5. Correlation analysis of apoB-48/TG ratio in each TG group with max-IMT

The significant correlation between ln apoB-48 levels and max-IMT means that the increase in apoB-48-containing lipoproteins might promote atherosclerosis in the carotid artery. The correla-

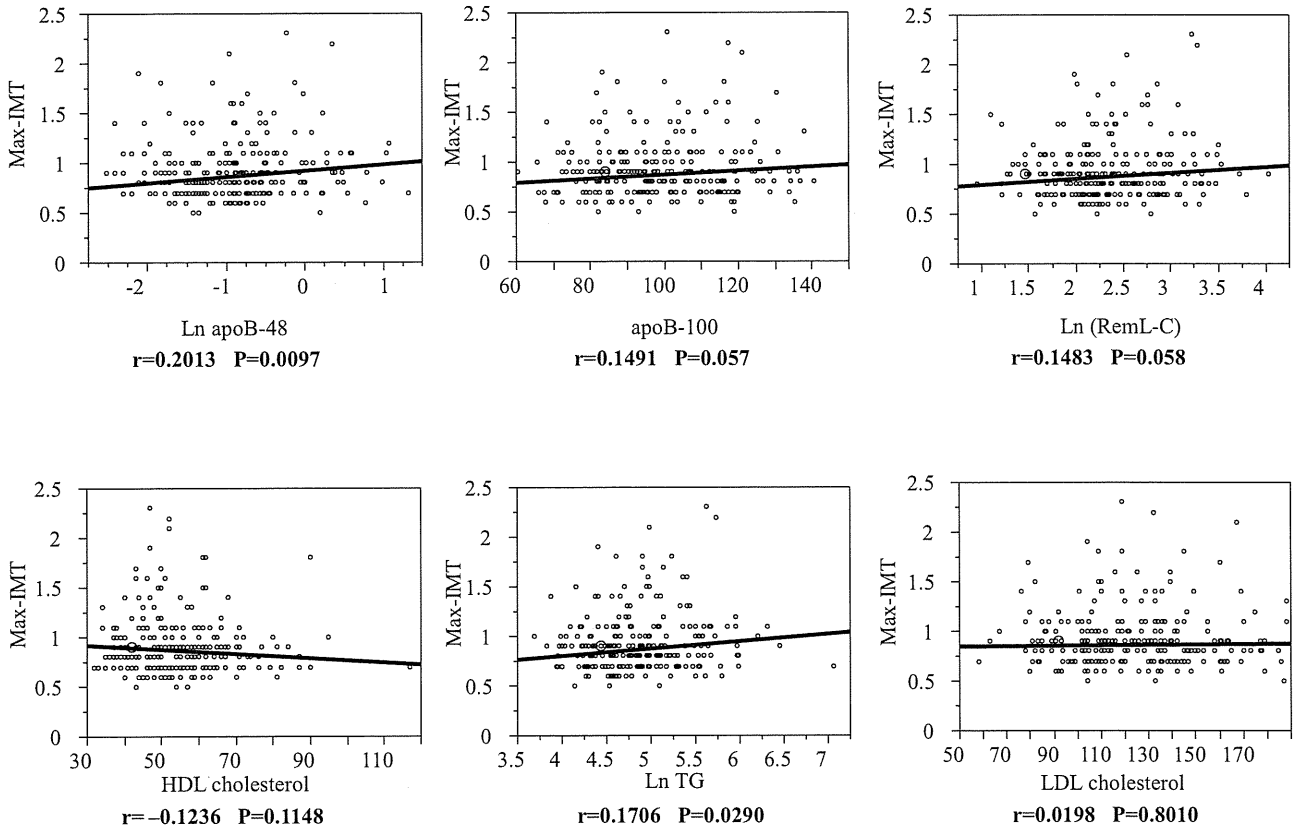


**Fig. 2.** Correlations between max-IMT and fasting lipid profiles. Because the distribution of apoB-48, TG and RemL-C was skewed to the left, the data were normalized by logarithmic transformation for statistical analysis. The fasting serum concentrations of TC, LDL-C, HDL-C, apoB-100 and ln RemL-C were not significantly correlated with max-IMT, but ln TG and ln apoB-48 were significantly correlated with max-IMT. The correlations were calculated by Pearson's correlation coefficients, and statistical significance was accepted at $P < 0.05$.

tions with max-IMT of fasting apoB-48/TG ratio, which refers to the number of CM-R lipoprotein particles, were evaluated and shown to be significant in group N-2, but not in N-1 and H (Fig. 4).

### 3.6. Stepwise multiple regression analysis between max-IMT and biochemical parameters

By multiple regression analysis, the correlations between max-IMT and age, blood pressure, lipid profiles and glucose-related parameters were assessed. Age, systolic blood pressure (sBP), diastolic blood pressure (dBP), TC, ln TG, LDL-C, HDL-C, apoB-48, apoB-100, ln RemL-C, FPG, HbA1c, ln HOMA-IR, and IRI were independent variables. Among these parameters, age, sBP and ln apoB-48 were independent variables associated with max-IMT level in all subjects (Table 2). In group N-2, age and ln apoB-48 were independent variables associated with max-IMT, but sBP was not. HbA1c was an independent variable associated with max-IMT in group N-1.

## 4. Discussion

A positive correlation between fasting serum apoB-48 levels and IMT was observed in patients with hypertriglyceridemia or diabetes mellitus [14,17]. Significantly high TG level (TG >150 mg/dl) is correlated to an impaired metabolism of TG-rich lipoproteins in endogenous (VLDL and LDL) and exogenous (CM and CM-R) lipoprotein pathways, which are strongly related to the development of atherosclerosis and the morbidity of cardiovascular diseases. In the current study, our results showed that fasting serum apoB-48 levels are correlated with max-IMT in subjects with relatively high, but normal TG level (from 100 to 150 mg/dl).

### 4.1. Contribution of increased CM-R to atherosclerosis

A postprandial increase in remnants has been considered as atherogenic since Zilversmit proposed his postprandial hyperlipidemia concept over 30 years ago [20]. Several studies indicate that apoB-48-containing lipoproteins have various kinds of atherogenic

**Table 2**
Stepwise multiple regression analysis of max-IMT in relation to age, blood pressure, lipid profiles, and glucose-related parameters.

| | All subjects | | Group (N-1) | | Group (N-2) | | Group (H) | |
|---|---|---|---|---|---|---|---|---|
| | *F* value | *P* value | *F* value | *P* value | *F* value | *P* value | *F* value | *P* value |
| Age | 18.889 | <0.0001 | Not remain | | 5.51 | 0.023 | 12.603 | 0.0009 |
| sBP | 6.467 | 0.0120 | Not remain | | Not remain | | 8.249 | 0.0060 |
| ln apoB-48 | 5.542 | 0.0198 | Not remain | | 5.106 | 0.0283 | Not remain | |
| HbA1c | 2.541 | 0.1129 | 6.123 | 0.0164 | 2.098 | 0.1538 | Not remain | |

Stepwise multiple regression analysis was used to determine independent predictors of max-IMT measurement with *P* value-to-enter and *P* value-to-retain set at 0.20. Age, sBP, dBP, TC, ln TG, LDL-C, HDL-C, apoB-48, apoB-100, ln RemL-C, FPG, HbA1c, ln HOMA-IR, and IRI were included as explanatory variables in the method.

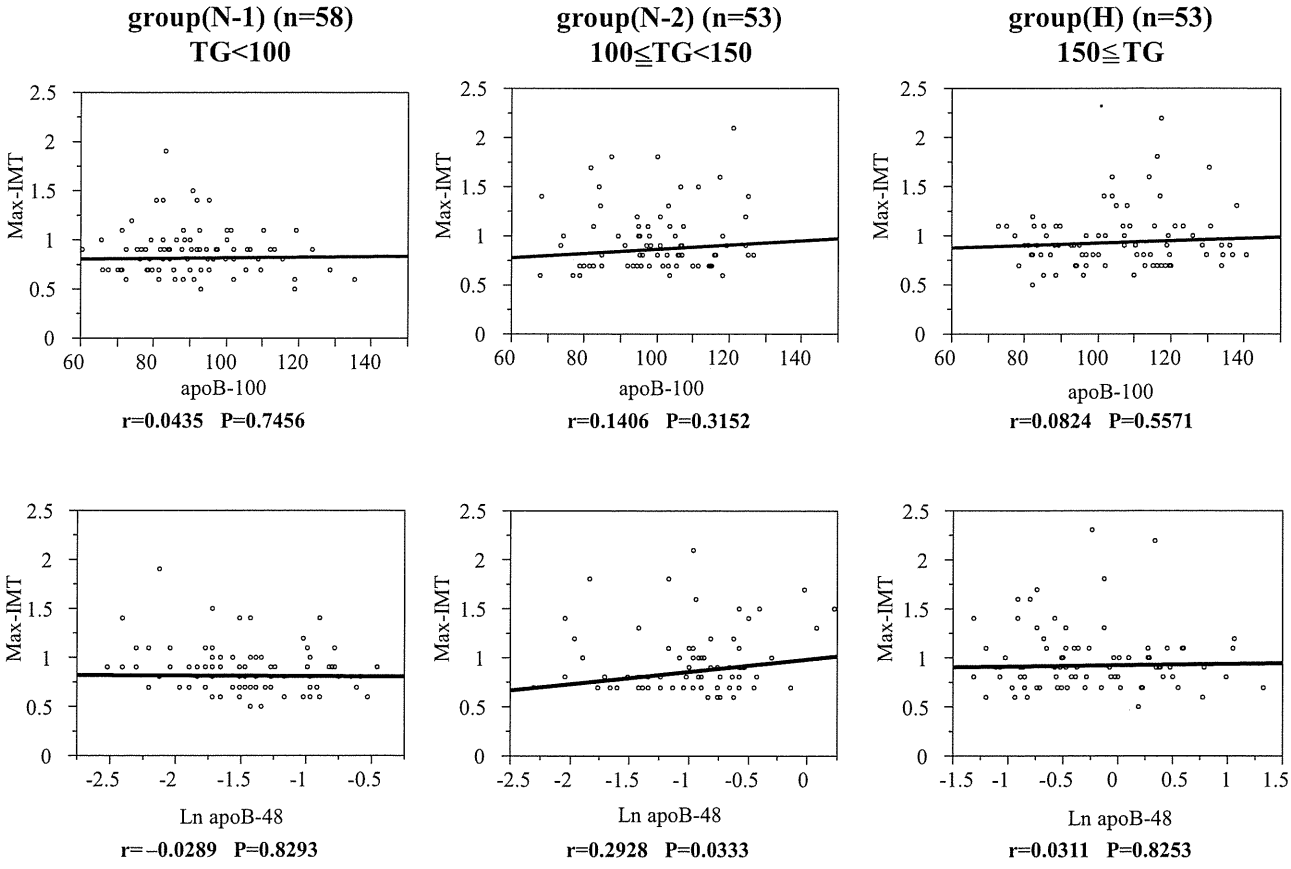


**Fig. 3.** Correlations between fasting apoB-100 levels or fasting Ln apoB-48 levels and max-IMT. There was no significant correlation between fasting apoB-100 levels and max-IMT in each TG group. Although the correlations between fasting apoB-48 levels and max-IMT in group N-1 and group H were not significant, there were a significant correlation between fasting apoB-48 levels and max-IMT in group N-2 as assessed by Pearson's correlation coefficients ($P < 0.05$).

features [11]. ApoB-48 was identified *in vivo* in human atherosclerotic plaques from femoral and carotid endarterectomy samples [21]. CM-R were shown to cause foam cell formation of mouse peritoneal and human monocyte-derived macrophages *in vitro* by both LDL-receptor-dependent and -independent mechanisms [11,22], stimulate MCP-1 expression in cultured vascular smooth muscle cells (VSMCs) [23], induce early growth response factor-1 (Egr-1) and proinflammatory cytokines, such as interleukin-2 (IL-2) and interferon-γ (IFN-γ) in VSMCs [24], increase the production of plasminogen activator inhibitor-1 (PAI-1) in endothelial cells via the MAPK pathway and redox system [25] and enhance endothelial cell apoptosis [26]. We found that fasting apoB-48 level was an independent risk factor for coronary stenosis assessed by coronary angiography (OR of apoB-48; 6.4, 95% CI; 3.64–1.79) (Masuda et al., unpublished observation). The increase in carotid IMT is significantly correlated with CHD and stroke [27]. Only a few studies have

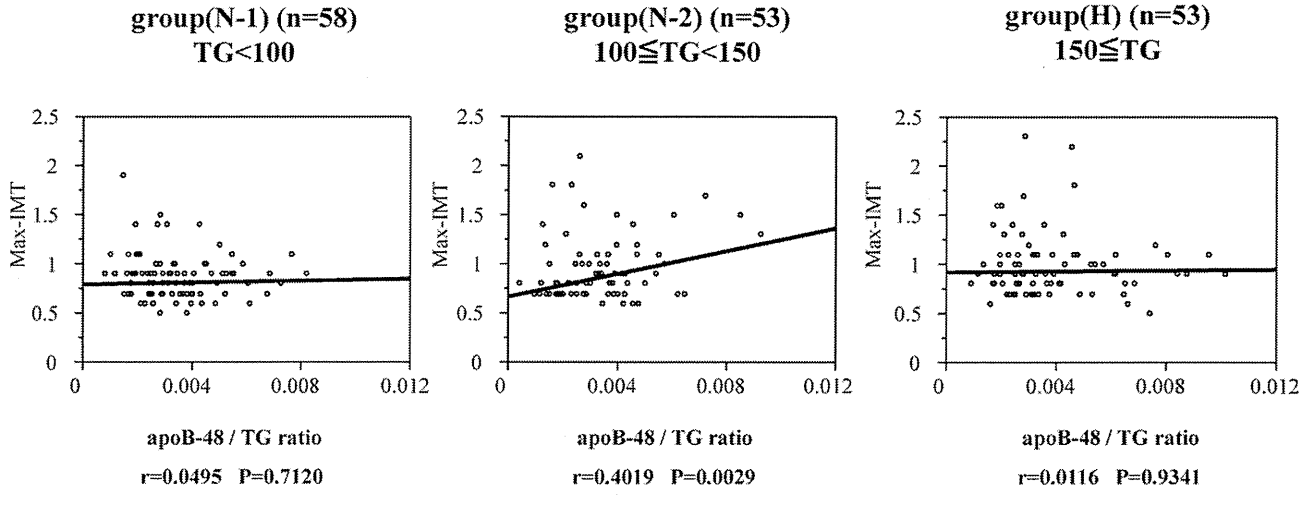


**Fig. 4.** Correlations between apoB-48/TG ratio and max-IMT.
The correlation between apoB-48/TG ratio and max-IMT was not significant in group N-1 and group H, but there was a significant correlation between apoB-48/TG ratio and max-IMT in group N-2 as assessed by Pearson's correlation coefficients ($P < 0.05$).

shown that there was a highly significant, independent correlation between the postprandial TG response and IMT [10], and that the presence of carotid plaque was associated with fasting apoB-48 and TG levels in age- and gender-adjusted analysis in type 2 diabetic patients [17]. As shown in the current study, the increase in apoB-48-containing lipoproteins, mainly CM-R, had a significant relationship with max-IMT. ApoB-48 level was also shown to be an independent variable of max-IMT in group N-2 (Fig. 3 and Table 2) which may significantly affect the development of systemic atherosclerosis associated with CHD and stroke. Ln apoB-48 level was associated with max-IMT, but LDL-C or apoB-100 levels were not correlated (Table 1 and Figs. 3 and 4). Tanimura et al. [17] also showed that the presence of carotid plaque was associated with high fasting apoB-48 levels but not with fasting TG levels in subjects with normal LDL-C (<140 mg/dl) levels. It was speculated that the impaired clearance and the accumulation of CM-R might be linked to carotid IMT and the development of atherosclerotic cardiovascular diseases, independent of the impaired clearance of VLDL and LDL.

### 4.2. CM-R particle size and atherogenic status

The size of CM produced by the small intestines is too large to penetrate the arterial wall; however, through the hydrolysis of TG by lipoprotein lipase (LPL) CM-R can become small enough to penetrate the arterial wall, be retained in the subendothelial space and affect the development of atherosclerotic plaques [11]. As shown in our former study, the size of CM-R changes from that of CM to that of HDL in the postprandial state [28]. Interestingly, in the current study, there was a strong correlation between apoB-48/TG ratio and max-IMT in group N-2 (Fig. 4). The high ratio of apoB-48/TG indicates that the number of apoB-48-containing lipoprotein particles increased while the number of TG components of these lipoproteins decreased, suggesting that the number of small-sized CM-R increased. The correlation between ln apoB-48 level and max-IMT in group N-2 whose TG levels were small indicates that the increase of small-sized CM-R was associated with the development of carotid atherosclerosis. Thus, serum apoB-48 level might be a good marker for the detection of early atherosclerosis in middle-aged, normotensive subjects with normal TG level.

### 4.3. Other metabolic phenotypes and apoB-48 levels

In subjects with high TG level (group H), there is a strong risk factor for the development of atherosclerosis. BMI, waist circumference, TG, apoB-48, RemL-C and IRI levels were significantly higher and HDL-C levels were significantly lower in group H than in group N-2 (Table 1), indicating that subjects in group H were capable of accumulating abdominal visceral fat which strongly affects insulin resistance or adipocytokine dysregulation. However, there was no significant correlation between ln apoB-48 level and max-IMT (Fig. 3). This discrepancy might be due to the clearance of CM and CM-R in subjects with abdominal visceral fat accumulation. The existence of insulin resistance deteriorates the lipoprotein metabolism of apoB-48-containing lipoproteins as well as apoB-100 -containing lipoproteins, which has been mainly explained by the impaired activity of LPL [29]. In these patients, low LPL activity causes an accumulation of large-sized CM-R or VLDL-R, resulting in an increase in TG and apoB-48. It was suggested that this buildup in large-sized lipoproteins was not precisely correlated with the enhancement of atherogenicity. There was no significant difference in LDL-C and apoB-100 levels among each TG group (Table 1), and fasting TG levels were mainly related to the accumulation of CM-R, less related to insulin resistance or apoB-100-containing lipoprotein metabolism. We could not find a positive correlation between RemL-C and max-IMT in our study subjects. The increase in CM-R did not properly reflect the increase in remnant lipoprotein cholesterol (RemL-C) levels which were shown to consist of CM-R and VLDL remnants. These CM-R and VLDL remnants have different origins, and their serum concentrations may vary depending upon the impairment of different pathways of lipoprotein metabolism. Above all, in the current study, there was a significantly positive correlation between apoB-48 level and max-IMT in group N-2, which was mainly associated with the increase in small-sized CM-R.

### 4.4. Limitation of the study

We aimed at evaluating the effectiveness of apoB-48 measurement in healthy subjects for the prediction of asymptomatic carotid atherosclerotic change. We recruited only men and focused on subjects coming to the Osaka Police Hospital, and therefore these factors are supposed to be the baseline of the bias.

## 5. Conclusion

In conclusion, these data suggest that the accumulation of CM-R might be an independent risk factor for the development of atherosclerosis among subjects with TG levels between 100 mg/dl and 150 mg/dl. The measurement of fasting apoB-48 level is very useful for the detection of early onset of atherosclerotic plaques.

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## Disclosure

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## References

[1] Kannel WB, Castelli WP, Gordon T, McNamara PM. Serum cholesterol, lipoproteins, and the risk of coronary heart disease. The Framingham study. Ann Intern Med 1971;74(1):1–12.
[2] LaRosa JC, Grundy SM, Waters DD, et al. Treating to New Targets (TNT) Investigators. Intensive lipid lowering with atorvastatin in patients with stable coronary disease. N Engl J Med 2005;352(14):1425–35.
[3] Albrink MJ, Man EB. Serum triglycerides in coronary artery disease. AMA Arch Intern Med 1959;103(1):4–8.
[4] Austin MA. Plasma triglyceride and coronary heart disease. Arterioscler Thromb 1991;11(1):2–14.
[5] Eberly LE, Stamler J, Neaton JD. Relation of triglyceride levels, fasting and nonfasting, to fatal and nonfatal coronary heart disease. Arch Intern Med 2003;163(9):1077–83.
[6] Iso H, Naito Y, Sato S, et al. Serum triglycerides and risk of coronary heart disease among Japanese men and women. Am J Epidemiol 2001;153(5):490–9.
[7] Castelli WP. Lipids, risk factors and ischaemic heart disease. Atherosclerosis 1996;124(Suppl.):S1–9.
[8] Mori Y, Itoh Y, Komiya H, et al. Association between postprandial remnant-like particle triglyceride (RLP-TG) levels and carotid intima-media thickness (IMT)

in Japanese patients with type 2 diabetes: assessment by meal tolerance tests (MTT). Endocrine 2005;28(2):157–63.
[9] Patsch JR, Miesenböck G, Hopferwieser T, et al. Relation of triglyceride metabolism and coronary artery disease. Studies in the postprandial state. Arterioscler Thromb 1992;12(11):1336–45.
[10] Ryu JE, Howard G, Craven TE, et al. Postprandial triglyceridemia and carotid atherosclerosis in middle-aged subjects. Stroke 1992;23(6):823–8.
[11] Fujioka Y, Ishikawa Y. Remnant lipoproteins as strong key particles to atherogenesis. J Atheroscler Thromb 2009;16(3):145–54.
[12] Sakai N, Uchida Y, Ohashi K, et al. Measurement of fasting serum apoB-48 levels in normolipidemic and hyperlipidemic subjects by ELISA. J Lipid Res 2003;44(6):1256–62.
[13] Smith D, Watts GF, Dane-Stewart C, et al. Post-prandial chylomicron response may be predicted by a single measurement of plasma apolipoprotein B48 in the fasting state. Eur J Clin Invest 1999;29(3):204–9.
[14] Karpe F, de Faire U, Mercuri M, et al. Magnitude of alimentary lipemia is related to intima-media thickness of the common carotid artery in middle-aged men. Atherosclerosis 1998;141(2):307–14.
[15] Mero N, Malmström R, Steiner G, et al. Postprandial metabolism of apolipoprotein B-48- and B-100-containing particles in type 2 diabetes mellitus: relations to angiographically verified severity of coronary artery disease. Atherosclerosis 2000;150(1):167–77.
[16] Meyer E, Westerveld HT, de Ruyter-Meijstek FC, et al. Abnormal postprandial apolipoprotein B-48 and triglyceride responses in normolipidemic women with greater than 70% stenotic coronary artery disease: a case-control study. Atherosclerosis 1996;124(2):221–35.
[17] Tanimura K, Nakajima Y, Nagao M, et al. Association of serum apolipoprotein B48 level with the presence of carotid plaque in type 2 diabetes mellitus. Diabetes Res Clin Pract 2008;81(3):338–44.
[18] Bots ML, Hoes AW, Koudstaal PJ, et al. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. Circulation 1997;96(5):1432–7.
[19] Chambless LE, Heiss G, Folsom AR, et al. Association of coronary heart disease incidence with carotid arterial wall thickness and major risk factors: the Atherosclerosis Risk in Communities (ARIC) Study, 1987–1993. Am J Epidemiol 1997;146(6):483–94.
[20] Zilversmit DB. Atherogenesis: a postprandial phenomenon. Circulation 1979;60(3):473–85.
[21] Pal S, Semorine K, Watts GF, et al. Identification of lipoproteins of intestinal origin in human atherosclerotic plaque. Clin Chem Lab Med 2003;41(6):792–5.
[22] Fujioka Y, Cooper AD, Fong LG. Multiple processes are involved in the uptake of chylomicron remnants by mouse peritoneal macrophages. J Lipid Res 1998;39(12):2339–49.
[23] Domoto K, Taniguchi T, Takaishi H, et al. Chylomicron remnants induce monocyte chemoattractant protein-1 expression via p38 MAPK activation in vascular smooth muscle cells. Atherosclerosis 2003;171(2):193–200.
[24] Takahashi Y, Fujioka Y, Takahashi T, et al. Chylomicron remnants regulate early growth response factor-1 in vascular smooth muscle cells. Life Sci 2005;77(6):670–82.
[25] Morimoto S, Fujioka Y, Hosoai H, et al. The renin-angiotensin system is involved in the production of plasminogen activator inhibitor type 1 by cultured endothelial cells in response to chylomicron remnants. Hypertens Res 2003;26(4):315–23.
[26] Kawasaki S, Taniguchi T, Fujioka Y, et al. Chylomicron remnant induces apoptosis in vascular endothelial cells. Ann N Y Acad Sci 2000;902:336–41.
[27] Simon A, Gariepy J, Chironi G, et al. Intima-media thickness: a new tool for diagnosis and treatment of cardiovascular risk. J Hypertens 2002;20(2):159–69.
[28] Masuda D, Nakagawa-Toyama Y, Nakatani K, et al. Ezetimibe improves postprandial hyperlipidaemia in patients with type IIb hyperlipidaemia. Euro J Clin Invest 2009;39(8):689–98.
[29] Syvänne M, Taskinen MR. Lipids and lipoproteins as coronary risk factors in non-insulin-dependent diabetes mellitus. Lancet 1997;350:20–3.

Original Article

# Fasting Serum Apolipoprotein B-48 Can be a Marker of Postprandial Hyperlipidemia

Daisaku Masuda[1], Naohiko Sakai[2], Taizo Sugimoto[3], Rika Kitazume-Taneike[1], Taiji Yamashita[1], Ryota Kawase[1], Hajime Nakaoka[1], Miwako Inagaki[1], Kazuhiro Nakatani[1], Miyako Yuasa-Kawase[1], Kazumi Tsubakio-Yamamoto[1], Tohru Ohama[1,4], Yumiko Nakagawa-Toyama[1,4], Makoto Nishida[1,4], Masato Ishigami[1,5], Yoshiro Masuda[6], Akifumi Matsuyama[7], Issei Komuro[1] and Shizuya Yamashita[1]

[1]Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Osaka, Japan
[2]Sakai Clinic, Osaka, Japan
[3]Sugimoto Clinic, Osaka, Japan
[4]Health Care Center, Osaka University, Osaka, Japan
[5]Department of Biomedical Informatics, Division of Health Sciences, Osaka University Graduate School of Medicine, Osaka, Japan
[6]Clinical Research Department, Pharmaceutical Division, Japan Tobacco Inc., Tokyo, Japan
[7]Department of Somatic Stem Cell Therapy, Institute of Biomedical Research and Innovation, Foundation for Biomedical Research and Innovation, Hyogo, Japan

***Aim***: Postprandial hyperlipidemia (PH) is thought to be caused by the impaired postprandial metabolism of triglycerides (TG)-rich lipoproteins in both endogenous and exogenous pathways; however, there is no consensus. It is difficult to estimate the presence of PH without performing a time-consuming oral fat loading (OFL) test, so postprandial lipoprotein metabolism was analyzed by measuring the postprandial levels of apolipoprotein (apo) B-48 and apo B-100, and the correlation between postprandial TG increase and fasting apoB-48 levels was assessed to establish a good marker of PH without performing an OFL test.
***Methods***: Ten male normolipidemic subjects were loaded with a high-fat (HF, 1045 kcal) or standard (ST, 566 kcal) meal, and the lipids, apolipoproteins and lipoprotein profiles were analyzed after each meal.
***Results***: TG, apo B-48, remnant-like particles (RLP)-cholesterol and RLP-TG levels were increased and their levels were significantly higher after intake of the HF meal than the ST meal; however, there was no postprandial increase in apo B-100 and LDL-C levels. Postprandial increases in TG levels of CM, VLDL, LDL and HDL were significantly higher after intake of the HF meal than the ST meal. Fasting apo B-48 levels were strongly correlated with the incremental area under the curve of TG after intake of the HF meal, but not the ST meal.
***Conclusion***: Postprandial TG increase was mainly due to increased CM and CM-R, but not VLDL. Measurement of fasting serum apo B-48 may be a simple and useful method for assessment of the existence of PH.

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**Key words;** Apolipoproteins, Atherosclerosis, Chylomicrons, Postprandial hyperlipidemia, Remnants

## Introduction

Several epidemiological studies have recently demonstrated that both fasting and non-fasting hypertriglyceridemia are closely related to the development of atherosclerosis[1,2]. Non-fasting hypertriglyceridemia is partially associated with postprandial hyperlipidemia (PH) in patients with dyslipidemia, which is characterized by the postprandial accumulation of excess TG-rich lipoproteins (TRLs) and their partially hydrolyzed product, remnants or remnant lipoprotein particles. The atherogenicity of postprandial accumu-

Address for correspondence: Daisaku Masuda, Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan
E-mail: masuda@imed2.med.osaka-u.ac.jp
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lation of TRLs and their remnants was predicted by Zilversmit over 30 years ago[3], and has been demonstrated in numerous subsequent studies[4-6]. For the quantitative evaluation of remnant lipoprotein particles, two methods for measuring remnant lipoprotein cholesterol levels have been developed. Remnant-like particle cholesterol (RLP-C) is determined by measuring cholesterol concentrations in remnant-like particles (RLP), the unbound fraction of serum via immunoaffinity columns attaching monoclonal antibodies against apo B-100 and apo A-I[7]. Remnant lipoprotein cholesterol (RemL-C) is assessed by directly measuring the cholesterol level in a mixture of CM remnants and VLDL remnants[8]. These serum remnant lipoprotein cholesterol levels are a very useful marker related to atherosclerosis because they correlate with the morbidity of coronary heart disease (CHD)[9, 10].

In the postprandial state, serum TG levels increase rapidly around 3-4 hours after the meal because of the prompt production of TRLs. TRLs and their remnants are heterogeneous and originate from two different organs, that is, the small intestines (CM and CM remnants) and liver (VLDL and VLDL remnants), respectively; however, it is unclear whether the increase in TRLs is mainly due to the increase in CM or VLDL in the postprandial state and whether the postprandial increase in remnant lipoprotein particles is due to the increase in CM-R or VLDL-R. For quantitative analysis of postprandial lipoprotein profiles, the development of new methods for analyzing fasting serum levels and postprandial changes in the levels of CM-R and VLDL-R separately and stably has long been awaited.

For accurate analysis of fasting and postprandial changes in the levels of CM and CM-R, we previously developed a novel sandwich enzyme-linked immunosorbent assay (ELISA) system to measure serum apolipoprotein B-48 (apo B-48) concentrations[11]. Both CM and CM-R continue to possess one apo B-48 molecule at a time until they are cleared by the liver; therefore, serum apo B-48 concentrations represent the number of CM and CM-R. Fasting apo B-48 levels were distributed over a wide range (mean ± SD was 5.2 ± 3.8 μg/mL) in normolipidemic and hyperlipidemic subjects[11]. Fasting apo B-48 was significantly higher in patients with supposed accumulation of CM and CM-R[11] and in patients with metabolic syndrome (MetS)[12] compared with normolipidemic subjects. Fasting apo B-48 levels may be influenced by postprandial changes of CM and CM-R derived from the last meal; however, there have been no report on whether fasting apo B-48 is correlated with postprandial changes of CM and CM-R and whether it can be a good marker of these lipoproteins. Many clinical studies have reported the relationship between high serum apo B-48 and atherosclerosis[13, 14], and emerging evidence suggests that CM-R might be responsible for the initiation of atherogenesis in the arterial wall[6]. If the correlation between fasting and postprandial levels of apo B-48 could be clarified, it would become very easy to speculate the existence of PH by a single measurement of fasting apo B-48.

In the current study, we attempted to investigate whether apo B-48-containing lipoproteins or apo B-100-containing lipoproteins were the main lipoproteins that increased in the postprandial state and whether the fasting serum level of apo B-48 might be a simple and useful marker of PH, using a crossover study in healthy subjects loaded with an HF meal.

## Subjects and Methods

### Subjects

Ten healthy young male volunteers were enrolled and hospitalized at Kitasato University Research Center for Clinical Pharmacology. None of the subjects had obesity (body mass index, BMI ≥25), dyslipidemia (fasting serum total cholesterol (TC) ≥200 mg/dL and/or fasting serum TG ≥150 mg/dL), abnormal renal or hepatic functions, symptoms of illness, family history of premature CHD (before 60 years of age) or hypertension (systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg). None of the subjects was taking any medications known to affect carbohydrate or lipoprotein metabolism. Their mean age was 23.9 ± 3.1 years (mean ± SD) and the mean BMI was 21.3 ± 1.6 kg/m$^2$. Written informed consent was obtained from the subjects and the study design was approved by the ethics committee of the university.

### Oral Meal Loading Test

Subjects were divided into two groups (group A and group B; each, $n=5$) and these groups were matched for age and BMI. We prepared two kinds of meals. The ST meal contained 566 kcal, consisting of 20.1 g fat (32% of the total calories), 16.4 g protein (12%) and 81.2 g carbohydrate (56%), respectively, while the HF meal contained 1,045 kcal, consisting of 62.6 g fat (54%), 36.2 g protein (14%) and 80.9 g carbohydrate (32%), respectively. On day 1 and 3 of hospitalization, both groups were loaded with the ST meal and directed not to eat after supper. Group A was loaded with the HF meal in the morning on day 2 and the ST meal on day 4. For a cross-over study, group B was loaded with the ST meal on day 2 and the HF meal on day 4. Blood was collected during

fasting and 1, 2, 3, 4, 5, 6 and 8 hours after meal loading. Sera were separated immediately by low-speed centrifugation (15 minutes, 2,000 g at 4℃) and stored at −80℃ until measurements.

**Measurements**

Serum TC and TG levels were determined by enzymatic methods, serum apo B-100 levels by an immunoturbidity method, serum LDL-C and HDL-C levels by a direct method (Sekisui Medical Co., Ltd., Tokyo, Japan), and serum RLP-C and RLP-TG levels by the immunoaffinity isolation method (Jimro-II; Japanese Immunoresearch Laboratories Co., Tokyo, Japan), respectively. Serum apo B-48 levels were determined by a chemiluminescent enzyme immunoassay (CLEIA) system (Fuji Rebio Inc., Tokyo, Japan) which was modified from a sandwich ELISA system which we developed in a previous study[11]. Cholesterol and TG levels of CM, VLDL, LDL and HDL were measured by the densitometry method after being separated by electrophoresis (CholeTriCombo, Helena Laboratories, Tokyo, Japan). All samples were treated in accordance with the Helsinki Declaration. The areas under the curve (AUC) of these parameters were calculated by the trapezoidal method and the incremental AUC (iAUC) values were also calculated by ignoring the area beneath the fasting level.

**Statistical Analysis**

The statistical significance of differences between the subjects on the HF meal and ST meal was estimated by Mann-Whitney's *U* test and Wilcoxon's test. The correlation coefficients (r) and statistical significance of differences were analyzed between the lipid profiles and iAUC-TG, between fasting apo B-48 and the postprandial peak of apo B-48, and between fasting apo B-48 and AUC-apo B-48 by Spearman's rank-order correlation coefficient analysis. All statistical assessments were conducted using StatView statistical software (Hulinks Inc., Tokyo, Japan).

## Results

**Postprandial Changes of Serum Lipoprotein and Apolipoprotein Profiles**

All subjects were loaded with the ST and HF meals and postprandial changes of lipoprotein and apolipoprotein profile were analyzed. There was no significant postprandial increase in TC, LDL-C, HDL-C or apo B-100 after the intake of either meal (**Fig. 1A**). In contrast, TG, apo B-48, RLP-C and RLP-TG increased after intake of each meal in a time-dependent manner and decreased after their peak at 3 to 5 hours (**Fig. 1A**). The postprandial levels of these parameters were significantly higher after intake of the HF meal than the ST meal, and their peaks were delayed at 4 to 5 hours after intake of the HF meal (**Fig. 1A**). The iAUC-TG and iAUC-apo B-48 values, which indicated the net postprandial increase in TG and apo B-48, were about 2-fold higher after the intake of the HF meal than the ST meal (**Fig. 1B**).

**Postprandial Changes of Serum Lipoprotein Profiles**

For the analysis of postprandial changes of lipoproteins, the cholesterol and TG contents of CM, VLDL, LDL and HDL were measured before and after the intake of each meal. There were no significant increases in LDL-C and HDL-C in the postprandial state after the intake of each meal (**Fig. 2**). CM-C and CM-TG levels were significantly higher after intake of the HF meal than the ST meal. VLDL-C increased after the intake of each meal but there was no significant difference in postprandial VLDL-C levels between the ST and HF meals. VLDL-TG, LDL-TG and HDL-TG increased after the intake of each meal, and were significantly higher after intake of the HF meal than the ST meal (**Fig. 2**).

**Correlations between the Serum iAUC-TG and Fasting Lipid Levels**

We assessed the correlations between iAUC-TG levels and fasting/postprandial lipid parameters. The correlation coefficients between the iAUC-TG and fasting levels of TC, TG, HDL-C, LDL-C, apo B-48, apo B, RLP-C and RLP-TG were estimated after intake of the ST meal ($n=10$), HF diet ($n=10$) or a combination of the two meals (ST + HF meal; $n=20$) (**Table 1**). Significant correlation was observed only between the iAUC-TG and fasting serum apo B-48 level after intake of the HF meal (**Table 1** and **Fig. 3**). Between the iAUC-TG and postprandial peaks of TG, RLP-C, RLP-TG and apo B-48, the significant correlations were observed most prominently 5 hours after intake of the HF meal (TG; $r=0.950$, $p<0.0001$, RLP-C; $r=0.811$, $p<0.01$, RLP-TG; $r=0.926$, $p<0.001$, apo B-48; $r=0.775$, $p<0.01$). Moreover, the fasting apoB-48 level was significantly correlated with AUC-apo B-48 and the postprandial peak level of apo B-48 after intake of the HF meal, but not the ST meal (**Fig. 3**).

## Discussion

The current study has demonstrated for the first time that the postprandial increase in TG was mainly due to the increase in apo B-48-containing lipopro-

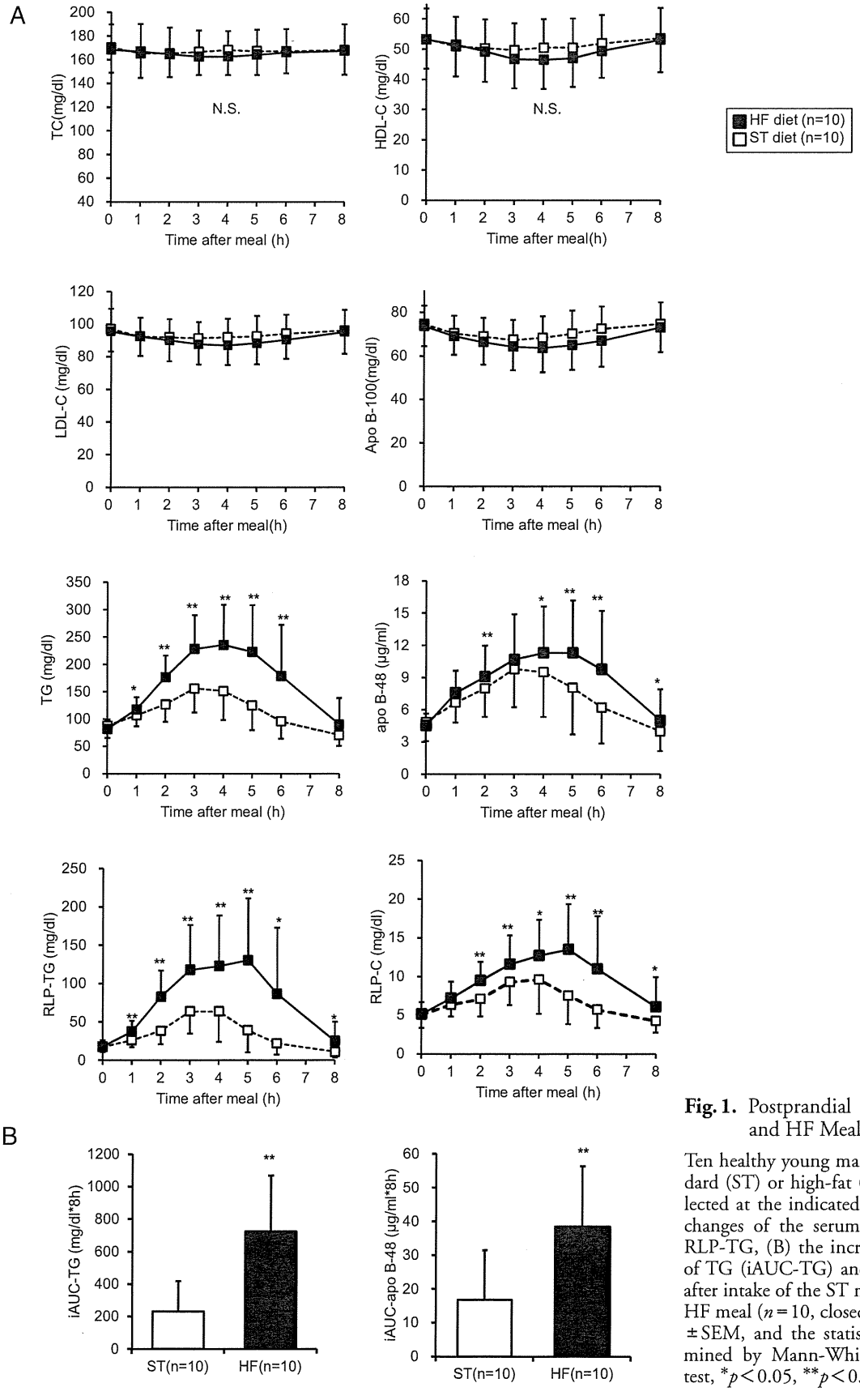


**Fig. 1.** Postprandial Lipid Profiles after the ST and HF Meals.

Ten healthy young male subjects consumed the standard (ST) or high-fat (HF) meal and blood was collected at the indicated time-points. (A) Postprandial changes of the serum TG, apo B-48, RLP-C and RLP-TG, (B) the incremental area under the curve of TG (iAUC-TG) and apo B-48 (iAUC-apo B-48) after intake of the ST meal ($n=10$, open squares) and HF meal ($n=10$, closed squares). Values are the mean ± SEM, and the statistical significances were determined by Mann-Whitney's $U$ test and Wilcoxon's test, $^{*}p<0.05$, $^{**}p<0.01$ vs. the ST diet.

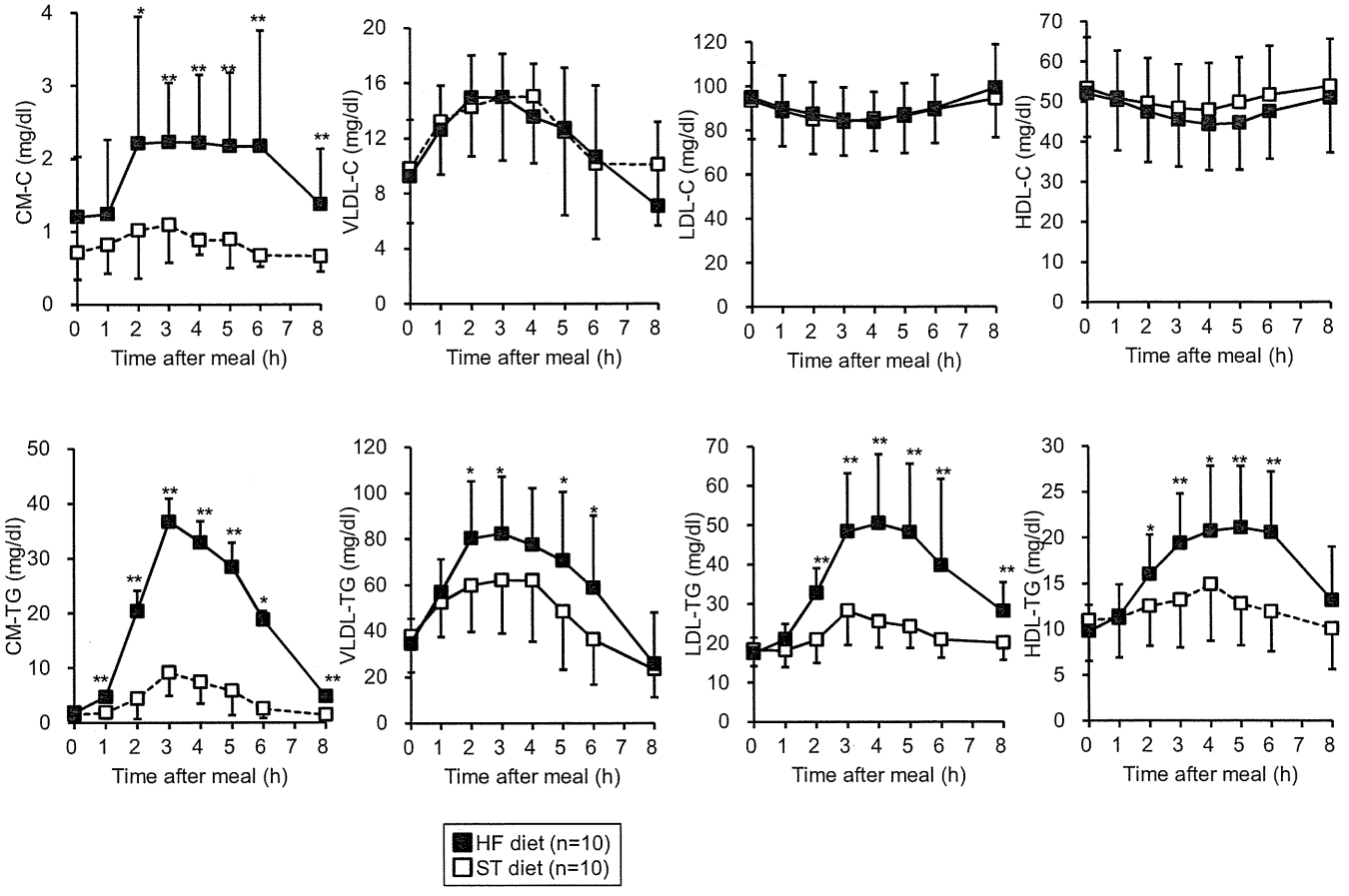


**Fig. 2.** Postprandial Lipoprotein Profiles after the ST and HF Meals.

Postprandial changes in cholesterol and TG of CM, VLDL, LDL and HDL after the intake of each meal were measured by the densitometry method after being separated by agarose gel electrophoresis (CholeTriCombo; Helena Laboratories, Tokyo, Japan). Values are the mean ± SEM, and statistical significances were determined by Mann-Whitney's *U* test and Wilcoxon's test, $^{*}p<0.05$, $^{**}p<0.01$ vs. ST.

teins, but not due to the increase in apo B-100-containing lipoproteins. Fasting serum apo B-48 levels are most strongly associated with iAUC-TG levels.

### Measurement of Serum ApoB-48

In the current study, we evaluated fasting and postprandial CM and CM-R metabolism by measuring apo B-48 concentrations using a CLEIA system, which is suitable for automatic quantitative statistical analyses in clinical settings. Retinyl palmitate (RP) and SDS-PAGE coupled with Western blotting were previously acceptable for the analysis of CM metabolism; however, these two methods are not suitable for exact quantitative analysis for the following reasons: uniform labeling of CM by RP is disrupted in the presence of CM-R[15], the quantity of apo B-48 assessed by SDS-PAGE is variable and unstable for repeated measurements[16-18] and many samples cannot be handled at the same time. The ELISA system using

**Table 1.** Correlation coefficients (r) between iAUC-TG and Various Fasting Parameters

| | ST diet ($n=10$) | HF diet ($n=10$) | ST+HF ($n=20$) |
|---|---|---|---|
| TC | 0.028 | −0.061 | −0.052 |
| TG | −0.142 | 0.505 | 0.047 |
| HDL-C | 0.286 | 0.017 | 0.081 |
| LDL-C | 0.011 | 0.111 | −0.084 |
| Apo B-48 | 0.162 | 0.809* | 0.238 |
| Apo B-100 | −0.781 | −0.455 | −0.314 |
| RLP-C | −0.175 | 0.144 | 0.035 |
| RLP-TG | 0.151 | 0.608 | 0.345 |

The incremental area the curve of TG (iAUC-TG) was calculated in both groups and correlation coefficients (r) were calculated.
*$p=0.0046$

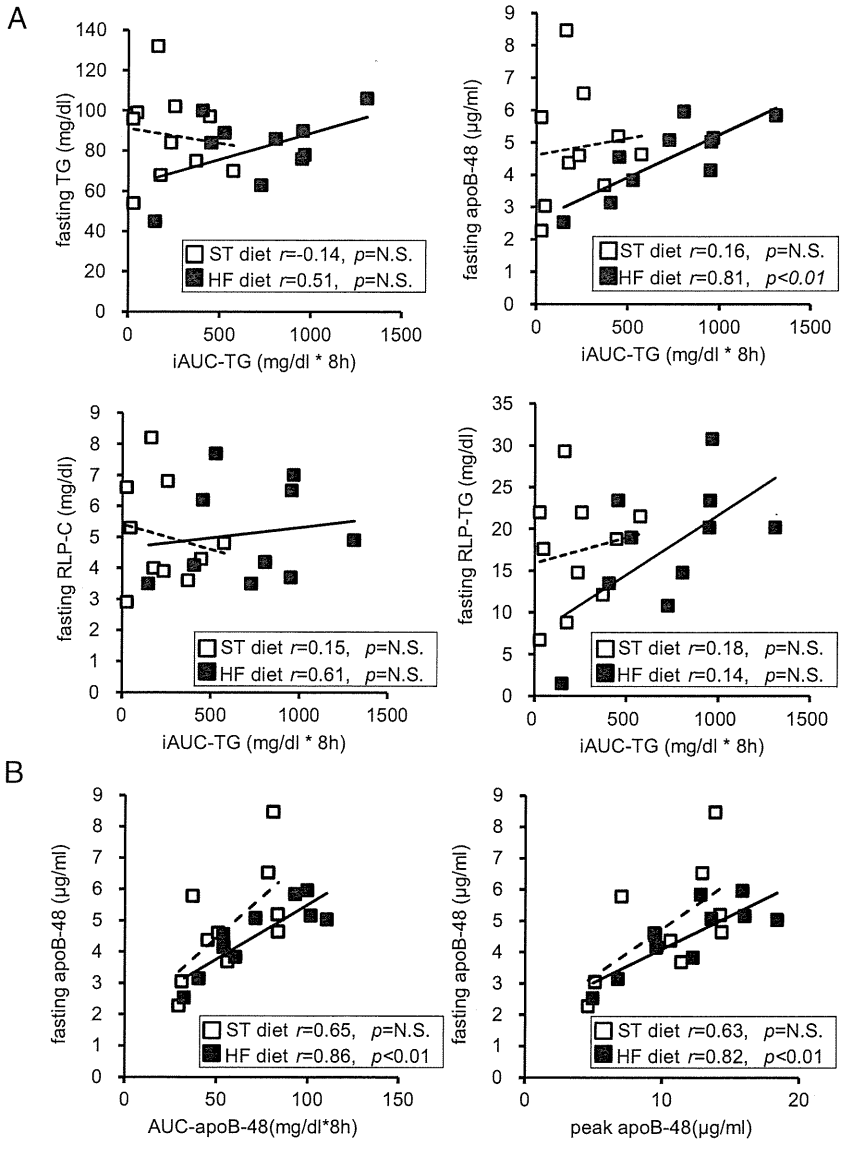


**Fig. 3.** Correlations between the iAUC-TG and Fasting Lipid Parameters.

(A) Correlations of iAUC-apo B-48 with fasting TG, apo B-48, RLP-C and RLP-TG, (B) correlations of fasting apo B-48 with AUC-apo B-48 or peak apo B-48, were determined after intake of the ST meal ($n=10$, open squares and dotted line) or the HF meal ($n=10$, closed squares and continuous line). The correlation coefficients (r) and the statistical significances of differences (p) were calculated using Spearman's rank-order correlations. Significance was assumed at $p<0.01$.

polyclonal antibody against apo B-48 has been used for stable and kinetic studies of CM/CM-R[11, 19, 20]; however, polyclonal antibodies are not reproducible for strict statistical analysis with high specificity compared with monoclonal antibodies[18, 19, 21]. Therefore, our CLEIA system, which uses monoclonal antibodies against apo B-48 molecule and could be used with an autoanalyzer (results within 2 hours), is suitable for strict statistical analyses related to apo B-48-containing lipoprotein metabolism. In the present study, we could measure apo B-48 both in the fasting and postprandial states for use in accurate statistical analysis with high quality and reproducibility.

### ApoB-48-Containing Lipoproteins But Not Apo B-100-Containing Lipoproteins Are Increased in the Postprandial State

After meal loading, serum TG levels gradually

increased because of the postprandial increase in TRLs. These TRLs might consist of both apo B-48-containing lipoproteins, which are produced in the intestine, and apo B-100-containing lipoproteins from the liver. In the present study, we focused on which lipoproteins were increased in the postprandial state. After the intake of each meal, TG and apo B-48 increased, but LDL-C and apoB-100 did not (**Fig. 1A**), which clearly indicated that apo B-48-containing lipoproteins were increased in postprandial serum, but apo B-100-containing lipoproteins were not. Karpe *et al.* showed that the percentage of VLDL in TRLs was 96-97% in the fasting state and 91-96% in the postprandial state, respectively, and suggested that both VLDL and CM particles increased in the postprandial state, but VLDL particles were mainly increased because the lipoprotein lipase (LPL)-induced hydrolysis of VLDL was halted by competitive hydrolysis of CM/CM-R[5]; however, no postprandial increase in apo B-100 and LDL-C levels indicates the absence of postprandial increase in apo B-100-containing lipoproteins and the postprandial increase in apo B-48-containing lipoproteins can decrease the VLDL/whole TRLs ratio. It was thus suggested that postprandial increase in TRLs was mainly due to the progressive accumulation of CM and CM-R, not due to that of VLDL or LDL.

Postprandial increases in TG and apo B-48 (iAUC-TG and iAUC-apoB-48) were higher after intake of the HF meal than the ST meal, indicating that intestinal absorption of a high fat meal promoted more abundant CM production from the intestine (**Fig. 1B**). Intake of the HF meal caused higher postprandial increases in CM-C and CM-TG levels (**Fig. 2**), suggesting that the proportion of fat which was contained in the meal directly affected the quantity of CM from the intestine. VLDL-TG, LDL-TG and HDL-TG levels were increased after the intake of each meal, and postprandial increases in these levels were higher after intake of the HF meal than the ST meal (**Fig. 2**). In our previous study, we demonstrated that the particle size of CM-R in patients with PH varied from large CM to small LDL, using fractionated flow-through by HPLC[22]. Since there was little postprandial increase in LDL-C and apo B-100, it was suggested that postprandial increases in VLDL-C, VLDL-TG and LDL-TG were mainly due to the increase in CM-R, which might be related to the increase in CM production after intake of the HF meal. The postprandial increase in HDL-TG was higher when subjects were loaded with the HF meal than the ST meal (**Fig. 2**), which might be due to postprandial TG exchanges between CM and HDL; the TG contained in CM are transferred to HDL in exchange for cholesteryl esters from HDL to CM by the action of plasma cholesteryl ester transfer protein (CETP).

**Fasting Serum Apo B-48 is a Good Marker of Postprandial Increases in CM and CM-R**

Previously, the oral fat loading (OFL) test or the stable isotope study was used to evaluate postprandial dynamic changes in the lipid and lipoprotein profile; however, the study subjects must tolerate overnight fasting and restraint for over 8 hours before 7 collections of blood samples after administration of the fatty meal or a stable isotope[23]. Therefore, these tests are not suitable for routine studies of the postprandial lipoprotein metabolism. In the current study, we assessed the correlation coefficients of the fasting serum apo B-48 and postprandial lipid and lipoprotein metabolism. As we have clearly shown in **Table 1**, among other lipid parameters, only the apo B-48 level was demonstrated to have a significant correlation with iAUC-TG after intake of the HF meal. This appears quite reasonable because the fasting apo B-48 indicates the particle number of residual CM-R produced by the last meal and remaining in the fasting serum. Intake of the HF meal causes higher CM production and CM-R accumulation than the ST diet. As shown in **Fig. 3**, the correlations between fasting apo B-48 and iAUC-TG, between fasting apo B-48 and AUC-apo B-48, and fasting apo B-48 and peak apo B-48, were significant after intake of the HF meal but not significant after intake of the ST meal. High levels of iAUC-TG, AUC-apo B-48 and peak apo B-48 may indicate that postprandial CM production was enhanced after meal loading and/or CM-R accumulation might have occurred due to the impaired catabolism of CM-R; therefore, increased fasting apo B-48 was significantly reflected by the postprandial increases in CM and CM-R. Recently, Sato *et al.* also reported that fasting TG and RemL-C were significantly higher and fasting apo B-48, RLP-C and RLP-TG were relatively higher in subjects with healthy, but high postprandial TG than in subjects with normal postprandial TG using TEST MEAL A[24]. In the current study, using HF and ST meals as a control, we found a significant correlation between fasting apoB-48 and postprandial increases of CM and CM-R. These results clearly suggest that fasting apo B-48 correlated with the postprandial accumulation of TRLs, mainly CM and CM-R, and fasting apo B-48 was the best explanatory variable for the impaired accumulation of TRLs and their remnants.

Many reports have suggested that not only oxi-

dized LDL, but also CM-R are associated with atherogenicity[6]. The accumulation of CM-R was associated with insulin resistance and the prevalence of type II diabetes mellitus[25]. Plasma apo B-48 was inversely correlated with plasma adiponectin and leptin levels and positively associated with plasma insulin, HOMA, and visceral, subcutaneous and total adipose tissue areas[26]. High fasting serum apoB-48 should be reduced carefully by a variety of nutritional and pharmacological approaches along with clinical interventions for the improvement of other impaired metabolic diseases and atherosclerotic cardiovascular diseases. Since PH has been established as one of the risk factors for CHD, its detection is very important for the prevention of CHD. The measurement of fasting serum apo B-48 may lead to straightforward detection of PH in a variety of patients at risk without a time-consuming meal test. Taken together, the current apo B-48 assay may have a number of applications in future studies.

## Limitations of the Current Study

In the present study, we investigated study subjects who were young (23.9 ± 3.1 years old), lean (mean BMI; 21.3 ± 1.6 kg/m$^2$) and healthy males. In further investigations, we would also examine postprandial lipoprotein metabolism in females, normolipidemic obese, aged, diabetic and hyperlipidemic subjects.

## Conclusion

In conclusion, postprandial high TG is mainly caused by the postprandial accumulation of CM and CM-R in subjects with normolipidemia after ingesting the HF meal. Fasting serum apo B-48 is a simple and useful marker of postprandial high TG and the accumulation of CM-R.

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## Disclosures

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### References

1) Hokanson JE, Austin MA: Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population based prospective studies. J Cardiovasc Risk, 1996; 3: 213-219
2) Iso H, Naito Y, Sato S, Kitamura A, Okamura T, Sankai T, Shimamoto T, Iida M, Komachi Y: Serum triglycerides and risk of coronary heart disease among Japanese men and women. Am J Epidemiol, 2001; 153: 490-499
3) Zilversmit DB; Atherogenesis: a postprandial phenomenon. Circulation, 1979; 60: 473-485
4) Havel RJ: Postprandial hyperlipidemia and remnant lipoproteins. Curr Opin Lipidol, 1994; 5: 102-119
5) Karpe F: Postprandial lipoprotein metabolism and atherosclerosis. J Intern Med, 1999; 246: 341-355
6) Fujioka Y, Ishikawa Y: Remnant lipoprotein as strong key particles to atherogenesis. J Atheroscler Thromb, 2009; 16: 145-154
7) Nakajima K, Saito T, Tamura A, Suzuki M, Nakano T, Adachi M, Tanaka A, Tada N, Nakamura H, Campos E, Havel RJ: Cholesterol in remnant-like particles in human serum using monoclonal anti apo B-100 and anti A-I immunoaffinity mixed gel. Clin Chim Acta, 1993; 223: 53-71
8) Miyauchi K, Kayahara N, Ishigami M, Kuwata H, Mori H, Sugiuchi H, Irie T, Tanaka A, Yamashita S, Yamamura T: Development of a homogenous assay to measure remnant lipoprotein cholesterol. Clin Chem, 2007; 53: 2128-2135
9) Kugiyama K, Doi H, Takazoe K, Kawano H, Soejima H, Mizuno Y, Tsunoda R, Sakamoto T, Nakano T, Nakajima K, Ogawa H, Sugiyama S, Yoshimura M, Yasue H: Remnant lipoprotein levels in fasting serum predict coronary events in patients with coronary artery disease. Circulation, 1999; 99: 2858-2860
10) Nakada Y, Kurosawa H, Tohyama J, Inoue Y, Ikewaki K: Increased remnant lipoprotein in patients with coronary artery disease; evaluation utilizing a newly developed remnant assay, remnant lipoproteins cholesterol homogenous

assay (RemL-C). J Atheroscler Thromb, 2007; 14: 56-64
11) Sakai N, Uchida Y, Ohashi K, Hibuse T, Saika Y, Tomari Y, Kihara S, Hiraoka H, Nakamura T, Ito S, Yamashita S, Matsuzawa Y: Measurement of fasting serum apoB-48 levels in normolipidemic and hyperlipidemic subjects by ELISA. J Lipid Res, 2003; 44: 1256-1262
12) Kinoshita M, Ohnishi H, Maeda T, Yoshimura N, Takeoka Y, Yasuda D, Kusano J, Mashimo Y, Saito S, Shimamoto K, Teramoto T: Increased serum apolipoprotein B48 concentration in patients with metabolic syndrome. J Atheroscler Thromb, 2009; 16: 517-522
13) Meyer E, Westerveld HT, de Ruyter-Meijstek FC, van Greevenbroek MM, Rienks R, van Rijn HJ, Erkelens DW, de Bruin TWA: Abnormal postprandial apolipoprotein B-48 and triglyceride responses in normolipidemic women with greater than 70% stenotic coronary artery disease: a case-control study. Atherosclerosis, 1996; 124: 221-235
14) Tanimura K, Nakajima Y, Nagao M, Ishizaki A, Kano T, Harada T, Okajima F, Sudo M, Tamura H, Ishii S, Sugihara H, Yamashita S, Asai A, Oikawa S: Association of serum apolipoprotein B48 level with the presence of carotid plaque in type 2 diabetes mellitus. Diabetes Res Clin Pract, 2008; 81: 338-344
15) Lemieux S, Fontani R, Uffelman KD, Lewis GF, Steiner G: Apolipoprotein B-48 and retinyl palmitate are not equivalent markers of postprandial intestinal lipoproteins. J Lipid Res, 1998; 39: 1964-1971
16) Schneeman BO, Kotite L, Todd KM, Havel RJ: Relationships between the responses of triglyceride-rich lipoproteins in blood plasma containing apolipoproteins B-48 and B-100 to a fat-containing meal in normolipidemic humans. Proc Natl Acad Sci USA, 1993; 90: 2069-2073
17) Smith D, Proctor SD, Mamo JC: Highly sensitive assay for quantitation of apolipoprotein B-48 using an antibody to human apolipoprotein B and enhanced chemiluminescence. Ann Clin Biochem, 1997; 34: 185-189
18) Jackson KG, Williams CM: Apolipoprotein B-48: comparison of fasting concentrations measured in normolipidaemic individuals using SDS-PAGE, immunoblotting and ELISA. Atherosclerosis, 2004; 176: 207-217
19) Lovegrove JA, Isherwood SG, Jackson KG, Williams CM, Gould BJ: Quantitation of apolipoprotein B-48 in triacylglycerol-rich lipoproteins by a specific enzyme-linked immunosorbent assay. Biochim Biophys Acta, 1996; 1301: 221-229
20) Lorec AM, Juhel C, Pafumi Y, Portugal H, Pauli AM, Lairon D, Defoort C: Determination of apolipoprotein B-48 in plasma by a competitive ELISA. Clin Chem, 2000; 46: 1638-1642
21) Uchida Y, Kurano Y, Ito S: Establishment of monoclonal antibody against human ApoB-48 and measurement of apoB-48 in serum by ELISA method. J Clin Lab Anal, 1998; 12: 289-292
22) Masuda D, Nakagawa-Toyama Y, Nakatani K, Inagaki M, Tsubakio-Yamamoto K, Sandoval JC, Ohama T, Nishida M, Ishigami M, Yamashita S: Ezetimibe improves postprandial hyperlipidaemia in patients with type IIb hyperlipidaemia. Eur J Clin Invest, 2009; 39: 689-698
23) Tremblay AJ, Lamarche B, Hogue JC, Coture P: Effects of ezetimibe and simvastatin on apolipoprotein B metabolism in males with mixed hyperlipidemia. J Lipid Res, 2009; 50: 1463-1471
24) Sato I, Ishikawa Y, Ishimoto A, Katsura S, Toyokawa A, Hayashi F, Kawano S, Fujioka Y, Yamashita S, Kumagai S: Significance of measuring serum concentrations of remnant lipoproteins and apolipoprotein B-48 in fasting period. J Atheroscler Thromb, 2009; 16: 12-20
25) Funada J, Sekiya M, Otani T, Watanabe K, Sato M, Akutsu H: The close relationship between postprandial remnant metabolism and insulin resistance. Atherosclerosis, 2004; 172: 151-154
26) Chan DC, Watts GF, Ng TW, Uchida Y, Sakai N, Yamashita S, Barrett PH: Adiponectin and other adipocytokines as predictors of markers of triglyceride-rich lipoprotein metabolism. Clin Chem, 2005; 51: 578-585

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# Establishment of chemiluminescence enzyme immunoassay for apolipoprotein B-48 and its clinical applications for evaluation of impaired chylomicron remnant metabolism

Hiroyuki Hanada [a], Satomi Mugii [a], Manabu Okubo [a], Ikuhiro Maeda [a], Kazuya Kuwayama [b], Yoh Hidaka [c], Rika Kitazume-Taneike [d], Taiji Yamashita [d], Ryota Kawase [d], Hajime Nakaoka [d], Miwako Inagaki [d], Miyako Yuasa-Kawase [d], Kazuhiro Nakatani [d], Kazumi Tsubakio-Yamamoto [d], Daisaku Masuda [d], Tohru Ohama [d], Akifumi Matsuyama [e], Masato Ishigami [f], Makoto Nishida [g], Issei Komuro [d], Shizuya Yamashita [d,*]

[a] Division of Laboratory for Clinical Investigation, Department of Medical Technology, Osaka University Hospital, 2-15 Yamadaoka, Suita, Osaka 565-0871, Japan
[b] Department of Clinical Laboratory, Minami Osaka General Hospital, 1-18-18 Higashikagaya, Suminoe, Osaka 559-0012, Japan
[c] Department of Laboratory Medicine, Osaka University Graduate School of Medicine, 2-2, Yamadaoka, Suita, Osaka 565-0871, Japan
[d] Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan
[e] Department of Somatic Stem Cell Therapy, Institute of Biomedical Research and Innovation, Foundation for Biomedical Research and Innovation, TRI305, 1-5-4, Minatojima-minamimachi, Chuo-ku, Kobe, Hyogo 650-0047, Japan
[f] Department of Biomedical Informatics, Division of Health Sciences, Osaka University Graduate School of Medicine, 1-7, Yamadaoka, Suita, Osaka 565-0871, Japan
[g] Health Care Center, Osaka University, 1-7 Machikaneyama, Toyonaka, Osaka 560-0043, Japan

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ABSTRACT

*Background:* Apolipoprotein B-48 (apoB-48) is a constituent of chylomicron remnants synthesized in the small intestines. The serum concentration of apoB-48 at fasting has been reported to be a marker of postprandial hyperlipidemia, a presumed risk factor for atherosclerosis.
*Methods:* We evaluated the basal performance of a recently developed chemiluminescent enzyme immunoassay (CLEIA). We also examined the correlations between serum apoB-48 concentrations and other lipid concentrations or life style patterns, including smoking and drinking. We analyzed the data of 273 clinical samples by multiple regression analysis to examine the influence of other serum lipid values, age, sex, smoking, drinking status and BMI on serum apoB-48 values.
*Results:* Within-run and between-run precision was obtained with 1.7–2.7% and 1.2–7.3%, respectively. The correlativity of enzyme-linked immunosorbent assay was correlation coefficient $r = 0.953$, and regression $y = 1.02 \times -1.59$. Serum apoB-48 concentrations were higher in males than in females, and were correlated with the status of smoking as well as with remnant-like particle-cholesterol (RLP-C) concentrations. Patients with the metabolic syndrome showed higher values of serum apoB-48 compared with control subjects.
*Conclusion:* Serum apoB-48 measurement by CLEIA was satisfactory for clinical use to assess abnormalities in the chylomicron remnant metabolism.



## 1. Introduction

In the postprandial period, we observe an increase of serum triglycerides (TG), which are mainly transported by chylomicrons (CMs) and their remnants (CM remnants). Recently, postprandial hyperlipidemia has been considered as an independent determinant of cardiovascular diseases [1,2]. Dietary fats are absorbed by the small intestines and transported as chylomicrons (CMs), which are macromolecules synthesized exclusively by the small intestines. After the excretion of CMs into the intestinal lymph and their entrance into the systemic circulation, the TG moiety of CMs is promptly hydrolyzed by lipoprotein lipase (LPL), resulting in the production of CM remnants. Thereafter, CM remnants are promptly taken up by the liver via CM-remnant receptors.

Postprandial hyperlipidemia is a state characterized by the impaired catabolism of exogenous triglyceride-rich lipoproteins (TRL), in which the number of CMs and CM remnants is increased. However, no method has so far been developed to quantitatively and accurately measure the serum concentrations of CMs and CM remnants. CMs and CM remnants have a characteristic apolipoprotein B48 (apoB-48), each with one apoB-48 molecule per particle. In contrast, very-low-density lipoproteins (VLDL) and their remnants (intermediate-density-lipoproteins,

* Corresponding author at: Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan. Tel.: +81 6 6879 3633; fax: +81 6 6879 3634.
*E-mail address:* shizu@imed2.med.osaka-u.ac.jp (S. Yamashita).

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