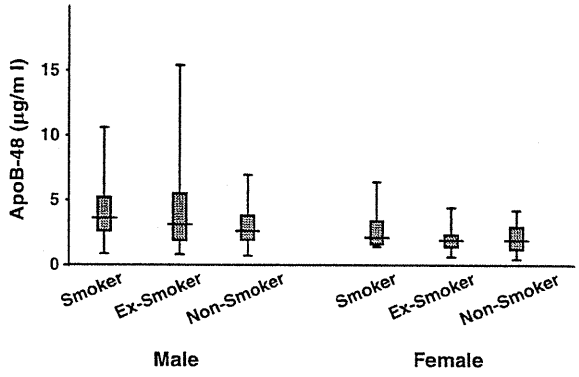

Fig. 5. Interrelationship between serum ApoB-48 levels and smoking status in males and females, respectively. Boxes represent interquartile ranges (IQRs); bold horizontal lines are median; whiskers are lowest and highest values within smokers, ex-smokers and non-smokers. Two-way ANOVA was performed to test the effect of smoking and gender status on apoB-48. Significantly different from baseline, $p < 0.05$.

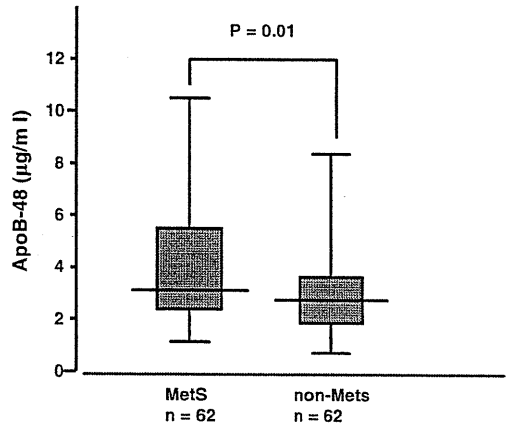

Fig. 6. Comparison of serum ApoB-48 levels between patients with the metabolic syndrome including those with only one risk component and normal subjects. Boxes represent interquartile ranges (IQRs); bold horizontal lines are median; whiskers are lowest and highest values within patients with metabolic syndrome and normal subjects.

reported the apoB-48 concentrations in a variety of patients with lipid disorders [8–11]. ApoB-48 is contained in CMs which are synthesized and secreted from the small intestines as well as in CM remnants. Abnormalities in CM remnant metabolism are related to coronary artery disease [12] and postprandial hyperlipidemia [13,14], and apoB-48 concentration is the one of the indices of abnormality in CM-remnant metabolism. In the current study, we have evaluated the basal performance of a recently developed CLEIA for measuring serum apoB-48, using the Lumipulse *f* automated immunoassay analyzer. The results reported here confirmed the reproducibility, analysis sensitivity, and linearity of the CLEIA. The measurement of apoB-48 by CLEIA was performed easily and conveniently with enough precision, exactness, and correlativity. Our data indicated that samples need to be kept refrigerated at 4 °C for short-term storage and that freezing at −20 °C is preferable in case of prolonged storage.

We analyzed the correlations between serum apoB-48 concentrations and serum lipid values as well as lifestyle-related factors. Serum apoB-48 concentration was correlated with RemL-C concentration, because RemL-C includes cholesterol in the lipoproteins containing apoB-48 (CM remnants) and in those containing apoB-100 (VLDL remnants or IDL). Mets is known to be a strong risk factor for cardiovascular diseases. Serum apoB-48 concentration was higher in male subjects than in female subjects as reported previously [5]. In the current study, we have demonstrated for the first time that serum apoB-48 value was higher in subjects who smoked. Regarding the effect of smoking and gender on serum apoB-48 concentrations, it was speculated that the significant difference between smokers and non-smokers was not due to the smoking habit itself, but to gender. Therefore, the necessity to set a standard value of serum apoB-48 in each sex was suggested. We also found that serum apoB-48 concentrations are increased in subjects with the metabolic syndrome.

It has been shown that Mets facilitates the progression of atherosclerosis [15–17]. Mets and pre-Mets subjects have higher concentrations of apoB-48 than healthy subjects. Therefore, it was suggested that a high apoB-48 value suggests the accumulation of CMs and CM-R in serum, and may be linked to atherosclerosis including cardiovascular diseases.

The mechanisms by which atherosclerosis is enhanced by CM remnants have been investigated in vitro [18]. The particle size of CM remnants is smaller than CMs and these remnants can infiltrate into the vascular sub-endothelial space. Moreover, CM remnants are taken up by macrophages without being oxidized unlike LDL. This causes the formation of foamy macrophages. Furthermore, CM remnants enhance the expression of plasminogen activator inhibitor type I (PAI-1), apoptosis and dysfunction of endothelial cells. CM remnants also accelerate the proliferation of vascular smooth muscle cells and inflammation of vessels. The injury of vascular endothelial cell is also enhanced by an increase in small dense LDL-C and a decrease in HDL-C [19], which are accompanied in patients with postprandial hyperlipidemia.

In patients with atherosclerotic cardiovascular diseases, the increment of serum TG value after meals can be amplified by a delayed catabolism of CM remnants or an increased synthesis of CMs even if fasting serum TG value is normal. A correlation between postprandial hyperlipidemia and cardiovascular diseases has been suggested [1]. Therefore, it is important to determine the number of CM remnants at fasting and after meal for prevention of atherosclerosis.

The presence or absence of postprandial hyperlipidemia cannot be diagnosed easily by the concentrations of fasting TG. Furthermore, the amount of fat in the oral fat tolerance test varies among researchers. A method measuring the concentration of remnants by immune-affinity chromatography technique has been used, and recently a direct method for measuring CM remnants and VLDL remnants using surfactants has been developed and widely used [20]. We were able to quantitatively measure RLP-C [21]. Although, RLP-C measures CM remnants and VLDL remnants, RLP fraction contains large CMs, VLDL and HDL with apoE. Therefore, it has been difficult to distinguish between CM remnants and VLDL remnant by previous techniques [22]. In the current study, we have established and evaluated the CLEIA of apoB-48, which is included in CMs and CM- remnants, each having one apoB-48 molecule per particle.

Taken together, the apoB-48 CLEIA may allow us to evaluate the synthesis and catabolism of exogenous lipoproteins such as CMs and their remnants. A clinical study has demonstrated that the concentration of fasting apoB-48 concentration is closely correlated with the presence of postprandial hyperlipidemia [23]. Therefore, it is necessary to examine and establish the importance of measuring the concentration of fasting serum apoB-48 as a convenient tool for diagnosis and treatment of postprandial hyperlipidemia.

6. Conclusion

We performed the basal examination of apoB-48 by Lumipulse *f* automated immunoassay analyzer. The results were satisfactory enough to develop a clinical application. The concentration of fasting serum apoB-48 is a good marker of exogenous lipoproteins (CMs and CM remnants), and may contribute to the susceptibility of atherosclerotic cardiovascular diseases.

Supplementary materials related to this article can be found online at doi:10.1016/j.cca.2011.09.013.

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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-

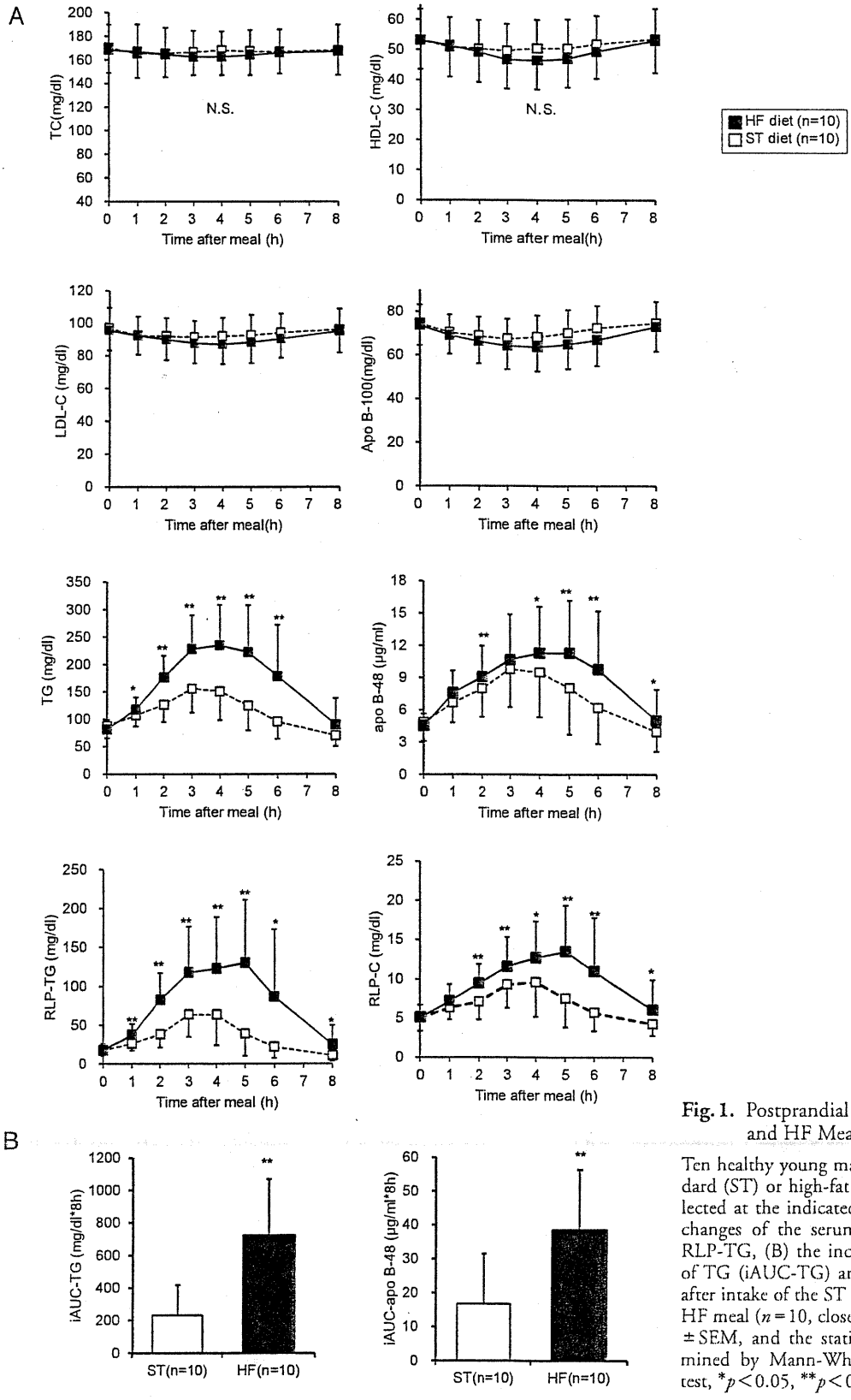

Fig. 1. Postprandial Lipid Profiles after the ST and HF Meals.

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.

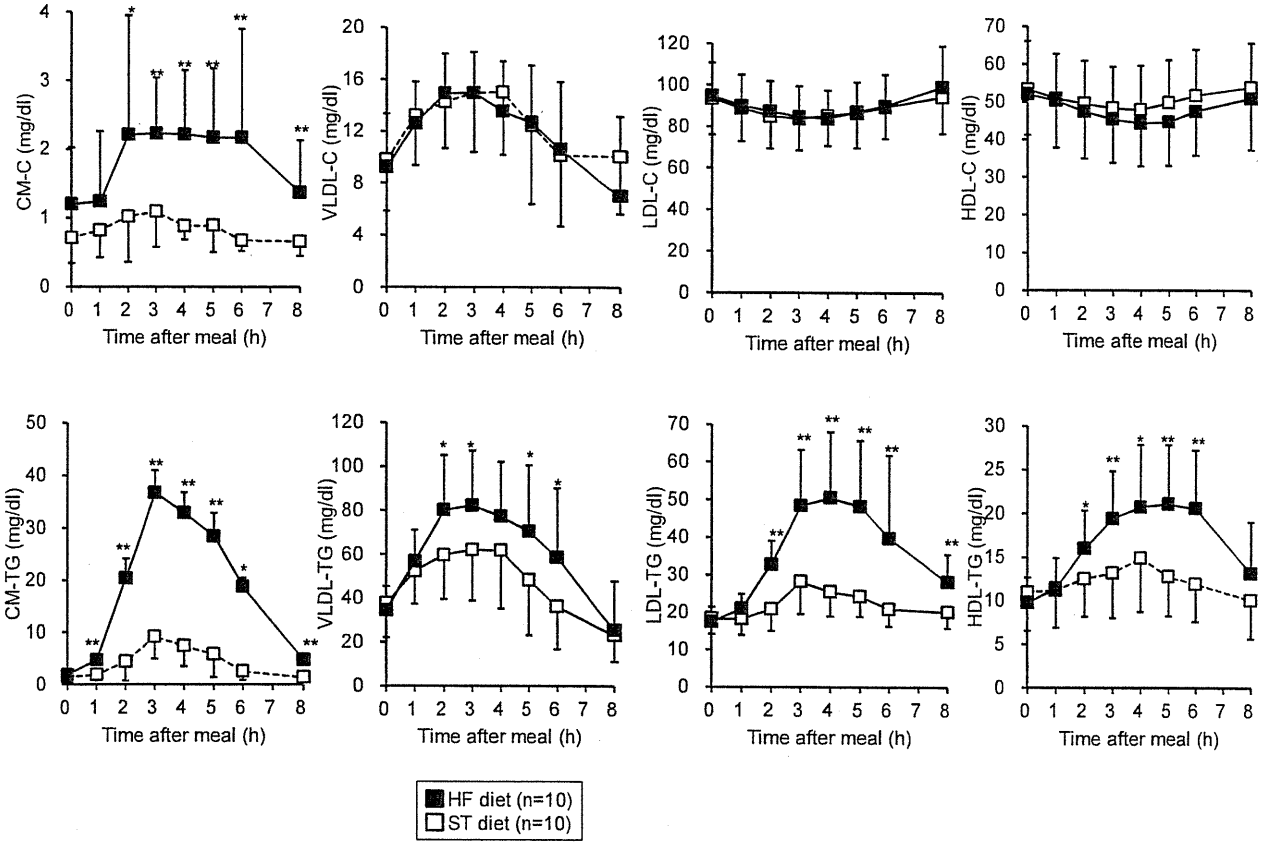

Fig. 2. Postprandial Lipoprotein Profiles after the ST and HF Meals.

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.
*$p=0.0046$

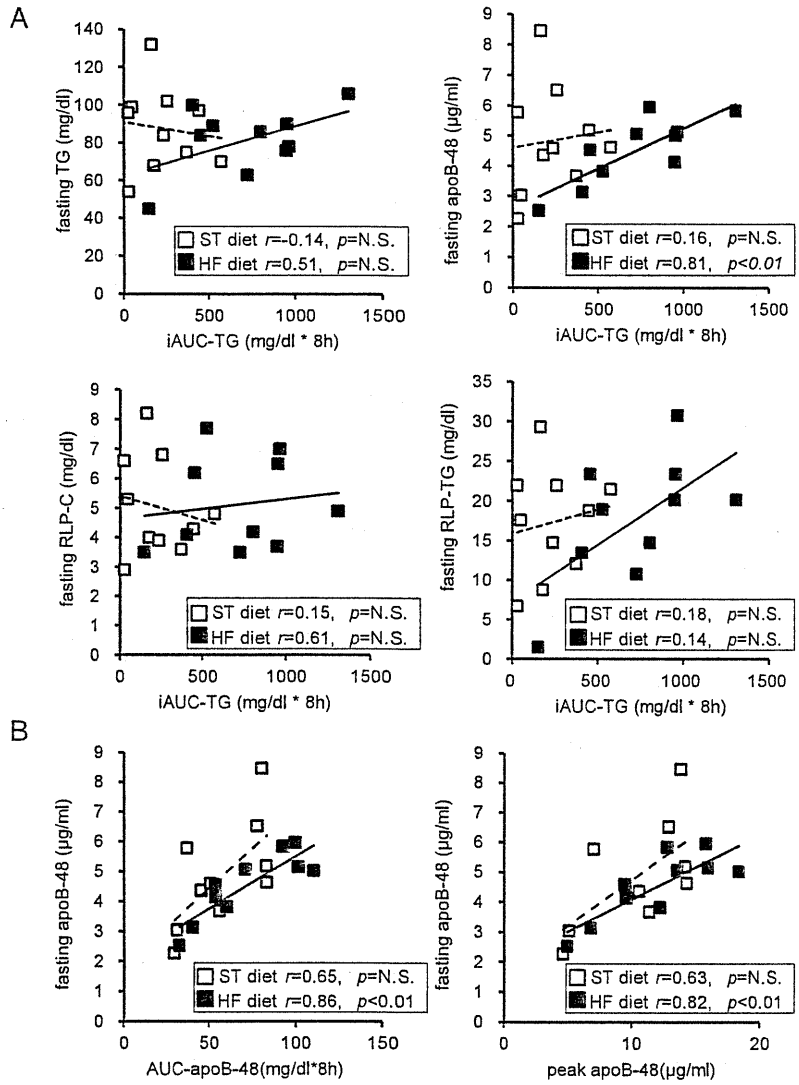

Fig. 3. Correlations between the iAUC-TG and Fasting Lipid Parameters.

(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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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 $n=164$	Group N-1 TG < 100 $n=58$	Group N-2 100 ≤ TG < 150 $n=53$	Group H 150 ≤ TG $n=53$
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).

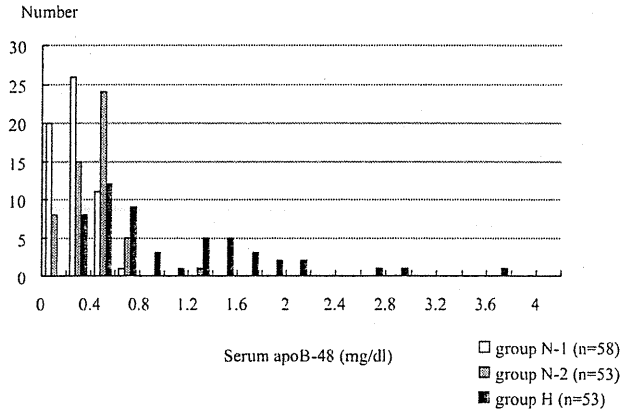

Fig. 1. Distribution of fasting serum apoB-48 levels. Geometric means were 0.24 mg/dl in group N-1, 0.41 mg/dl in group N-2 and 0.69 mg/dl in group H. The distribution of apoB-48 levels was significantly shifted to higher values; the data was normalized by logarithmic transformation for further statistical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

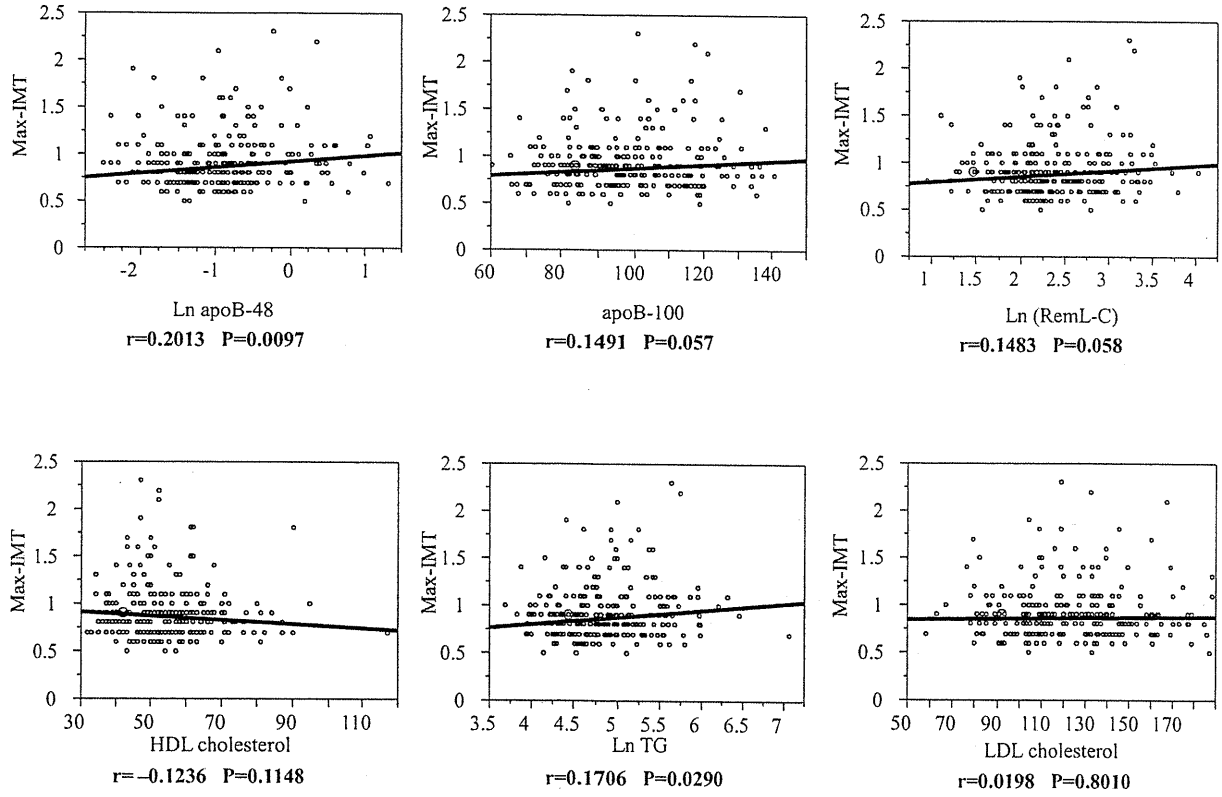

Fig. 2. Correlations between max-IMT and fasting lipid profiles. Because the distribution of apoB-48, TG and RemL-C was skewed to the left, the data were normalized by logarithmic transformation for statistical analysis. The fasting serum concentrations of TC, LDL-C, HDL-C, apoB-100 and ln RemL-C were 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.

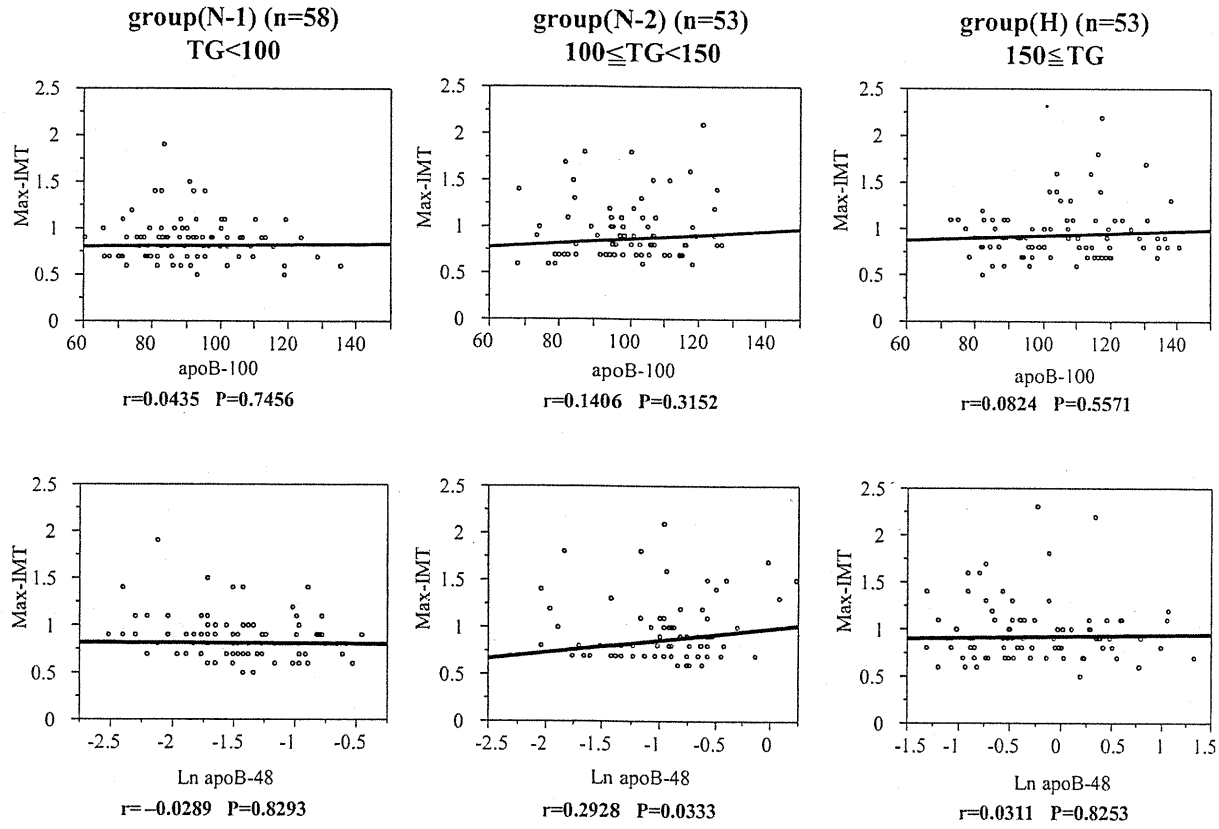

Fig. 3. Correlations between fasting apoB-100 levels or fasting Ln apoB-48 levels and max-IMT. There was no significant correlation between fasting apoB-100 levels and max-IMT in each TG group. Although the correlations between fasting apoB-48 levels and max-IMT in group N-1 and group H were not significant, 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

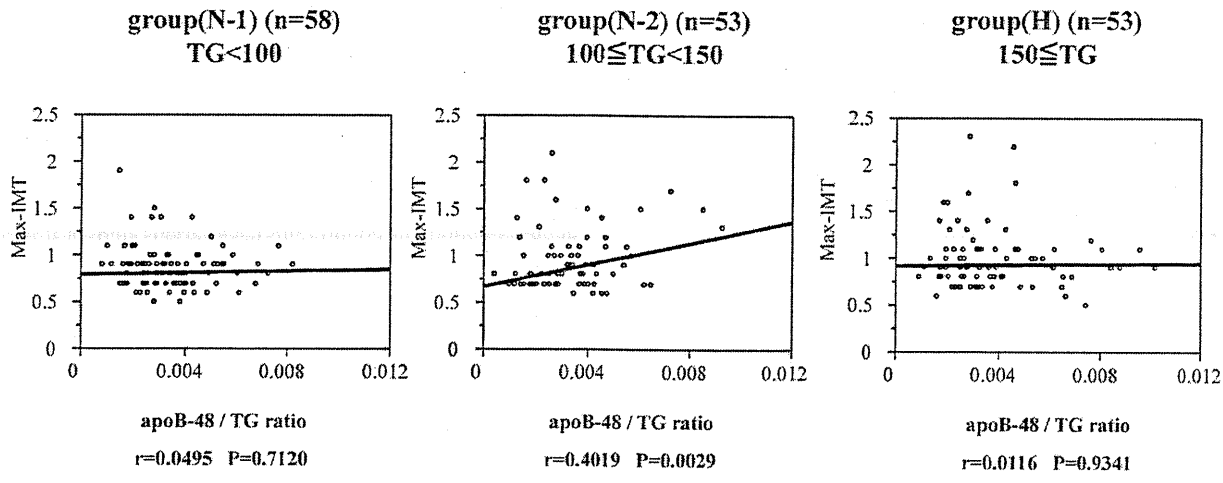

Fig. 4. Correlations between apoB-48/TG ratio and max-IMT.
The correlation between apoB-48/TG ratio and max-IMT was not significant in group N-1 and group H, but there was a significant correlation between apoB-48/TG ratio and max-IMT in group N-2 as assessed by Pearson's correlation coefficients ($P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

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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Original Article

Relationships between Circulating Adiponectin Levels and Fat Distribution in Obese Subjects

Ken Kishida[1], Kyoung Kon Kim[2], Tohru Funahashi[1], Yuji Matsuzawa[3], Hee-Cheol Kang[4], and Iichiro Shimomura[1]

[1]Department of Metabolic Medicine, Graduate School of Medicine, Osaka University, Suita, Osaka, Japan
[2]Department of Family Medicine, Gachon University Gil Medical Center, Namdong-gu, Incheon, Korea
[3]Sumitomo Hospital, Osaka, Japan
[4]Department of Family Medicine, Yonsei University College of Medicine, Seodaemun-Gu, Seoul, Korea

Aim: Most studies have reported that circulating levels of adiponectin are negatively correlated with the body mass index (BMI), and hypoadiponectinemia is related to cardiometabolic disorders; however, not all obese subjects have hypoadiponectinemia. The present study investigated circulating adiponectin levels and fat distribution, i.e. subcutaneous fat area (SFA) and visceral fat area (VFA), in obese subjects.

Methods: Sixty-eight obese Korean subjects underwent fat distribution by computed tomography (CT) scan and laboratory tests including circulating total adiponectin levels (APN) and circulating high molecular weight adiponectin levels (HMW-APN).

Results: Log-APN and log-HMW-APN did not correlate with log-BMI either in obese males or females in this study; however, log-APN significantly and negatively correlated with log-VFA both in obese males ($r=-0.691$, $p=0.009$) and females ($r=-0.319$, $p=0.002$), and log-HMW-APN also correlated negatively with log-VFA both in obese males ($r=-0.650$, $p=0.016$) and females ($r=-0.370$, $p=0.005$). Log-VFA was a significant determinant of log-APN and log-HMW-APN in obese subjects. In contrast, neither log-APN nor log-HMW-APN was significantly correlated with log-SFA in obese males and females.

Conclusions: The present study demonstrated that APN and HMW-APN correlated with VFA, but not BMI and SFA, in obese subjects, and suggest that hypoadiponectinemia may represent dysfunction of adipose tissue in obesity.

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Key words; Adiponectin, Fat distribution, Visceral obesity

Obesity is a world-wide health problem and is a common cause of cardiovascular diseases in industrialized countries. Adiponectin, which we identified as an adipocytokine in the human adipose tissue cDNA project, had anti-atherosclerotic, anti-diabetic and anti-inflammatory properties in experimental studies. We and others demonstrated that circulating levels of adiponectin are negatively correlated with the body mass index (BMI), and hypoadiponectinemia is related to cardiometabolic disorders[1]; however, not all obese subjects have hypoadiponectinemia[1]. Some obese people show normal or even high adiponectin levels even though they are morbidly obese. Both genetic and environmental factors affect circulating adiponectin levels. Several reports have demonstrated single nucleotide polymorphisms in the adiponectin gene that influence adiponectin levels. Body fat distribution may be related to the adiponectin level. Substantial evidence suggests that intra-abdominal visceral fat accumulation rather than BMI is related to the dysfunction of adipocytes and cardiometabolic disorders in obesity[2-4]. The relationship between the circu-

Address for correspondence: Ken Kishida, Department of Metabolic Medicine, Graduate School of Medicine, Osaka University, 2-2 B-5, Yamada-oka, Suita, Osaka 565-0871, Japan
E-mail: kkishida@imed2.med.osaka-u.ac.jp
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lating adiponectin level and precise evaluation of fat distribution by computed tomography (CT) scan has not been explored in obese subjects. The present study investigated circulating adiponectin levels and fat distribution, i.e. subcutaneous fat area (SFA) and visceral fat area (VFA), in obese subjects.

The present study included 68 obese Korean subjects (male/female: 13/55), who visited the hospital and were not taken on medications for diabetes, hypertension or dyslipidemia. This present study defined obesity as BMI ≥25 kg/m^2. **Table 1** summarizes the profiles of all subjects. All subjects were briefed on the research procedures, and written consent for participation was obtained from each subject. The registration number of the trial at the UMIN is 000002997. Height, weight and waist circumference were measured in a standing position, and blood pressures were measured with a standard mercury sphygmomanometer after the subjects had rested in a sitting position for at least 5 minutes. BMI was calculated using the formula [weight (kg)/height (m)^2]. To measure abdominal fat, after obtaining a single tomographic slice at the L4-L5 level by CT scanning, SFA and VFA were determined using a Hounsfield unit (HU) range for adipose tissue of -190 to -30 HU. After an overnight fast, venous blood samples were collected for measurements of glucose, total cholesterol, triglyceride high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol, and adiponectin in blood while the subject was in a sitting position. Circulating total adiponectin levels (APN) and circulating high molecular weight adiponectin levels (HMW-APN) were measured using a sandwich enzyme-linked immunosorbent assay system[1, 5]. Since BMI, VFA, SFA, APN and HMW-APN showed non-Gaussian skewed distribution, they were log-transformed before analysis. Data are presented as the mean ± SD (range). A p value <0.05 was considered significant. Pearson's correlation coefficient was used to examine the relationship between APN, HMW-APN, and fat distribution. Stepwise multiple regression analysis was conducted to identify the parameters that significantly contributed to log-APN and log-HMW-APN. Parameters with an F value >4.0 were subsequently entered into the regression analysis as independent variables. All statistical analyses were performed with StatView-J 5.0 (HULINKS, Inc., Tokyo).

Interestingly, log-APN and log-HMW-APN did not correlate with log-BMI either in obese males ($p=0.109$, $p=0.064$, respectively) or females ($p=0.064$, $p=0.055$, respectively) (**Fig. 1A** and **1B**) in this study; however, log-APN significantly and negatively correlated with log-VFA both in obese males ($r=-0.691$, $p=0.009$) and females ($r=-0.319$, $p=0.002$) (**Fig. 1C**), and log-HMW-APN also correlated negatively with log-VFA both in obese males ($r=-0.650$, $p=0.016$) and females ($r=-0.370$, $p=0.005$) (**Fig. 1D**). In contrast, neither log-APN nor log-HMW-APN was significantly correlated with log-SFA in obese males ($p=0.051$, $p=0.068$, respectively) and females ($p=0.070$, $p=0.133$, respectively) (**Fig. 1E** and **1F**). In obese males, log-APN and log-HMW-APN did not correlate with each metabolic parameter, such as systolic and diastolic blood pressures, total cholesterol, triglyceride, high density lipoprotein-cholesterol (HDL-C) and glucose (**Table 2**). In obese females, log-APN and log-HMW-APN correlated negatively with HDL-C only for the above metabolic parameters ($p<0.001$, $p<0.001$, respectively). Stepwise multiple regression analysis identified HDL-C and VFA as significant determinants of log-APN and log-HMW-APN (**Table 2**).

We have previously shown that APN is negatively correlated with BMI in the general population[1]. Fur-

Table 1. Clinical characteristics of all subjects

Number (males/females)	68 (13/55)
Age (year)	33.4 ± 8.4 (20-58)
Body mass index, BMI (kg/m^2)	
all	29.4 ± 3.0 (25.2-40.1)
males	28.9 ± 1.5 (26.4-31.0)
females	29.5 ± 3.2 (25.2-40.1)
BMI ≥25-<30 (%)	58.8
BMI ≥30-<35 (%)	36.8
BMI ≥35 (%)	4.4
Subcutaneous fat area (cm^2)	291.0 ± 76.9 (99.0-454.9)
Visceral fat area (cm^2)	99.3 ± 36.1 (40.7-218.7)
Systolic blood pressure (mmHg)	125.8 ± 13.4 (98-160)
Diastolic blood pressure (mmHg)	82.4 ± 11.9 (52-110)
Total cholesterol (mg/dL)	181.7 ± 32.2 (118-258)
Triglyceride (mg/dL)	138.9 ± 104.2 (49-622)
HDL-cholesterol (mg/dL)	52.4 ± 11.4 (31-89)
Glucose (mg/dL)	90.6 ± 11.4 (73-134)
Circulating total adiponectin (μg/mL)	
males	5.6 ± 2.6 (1.9-10.3)
females	7.4 ± 4.0 (2.2-24.9)
Circulating HMW-adiponectin (μg/mL)	
males	3.8 ± 2.5 (0.8-9.3)
females	6.0 ± 4.2 (0.7-23.5)
Smoking status	
Current smoker	$n=16$
Ex-smoker	$n=3$
Non-smoker	$n=49$

Data are the mean ± SD (range).
HDL, high density lipoprotein; HMW, high molecular weight