number of chylomicrons (CMs) and CM-R in the serum [8]. Fasting apo B-48 levels ranged from 0 to 25 μg/mL (the mean ± SD value was 5·2 ± 3·8 μg/mL) and were significantly higher in hyperlipidaemic patients with supposed accumulation of CMs and CM-R [8] as well as in patients with metabolic syndrome (MetS) [9] than in healthy subjects.

Several clinical studies have suggested a correlation between serum apo B-48 levels and atherosclerosis [10,11]. In our recent study, high levels of fasting apo B-48 significantly correlated with intima-media thickness (IMT) in subjects with normal but relatively high TG levels (100 < TG≤150 mg/dL) [12]. Emerging evidence from *in vivo* [13–15] and *in vitro* studies [5,16] suggests that CM-R might have atherogenic features and may be responsible for the initiation of atherogenesis. However, one report suggested that there was no significant correlation between fasting apo B-48 levels and CAD [17], emphasizing also that no consensus existed as to whether high levels of fasting apo B-48 were correlated with the prevalence of CAD. Moreover, it remained uncertain whether the prevalence of CAD in subjects with high levels of CAD would increase or not in combination with other metabolic disorders such as insulin resistance of MetS.

In this study, we attempted to investigate whether fasting serum levels of apo B-48 correlated with the prevalence of CAD and whether these correlations were stronger than other metabolic parameters recognized as coronary risk factors.

Subjects and methods

Subjects

A consecutive series of patients with suspected CAD were hospitalized in Osaka University Hospital and National Hospital Organization Kure Medical Center from January 2002 to December 2003. Patients who needed emergency care, had acute coronary syndrome or were already being treated with lipid-lowering drugs were eliminated. As a result, 189 subjects (120 men and 69 women) undergoing quantitative coronary angiography (CAG) were enrolled in this study. Height and weight were measured and (BMI, kg/m^2) was calculated. During hospitalization, all patients adhered to a standard diet which contained 25 kcal/kg standard body weight (BMI = 22 kg/m^2) per day (patients with hypertension took a sodium-restricted diet with the same calorie intake), and their blood pressure (BP) was measured in a supine position. The presence of hypertension was assessed by systolic BP ≥ 135 mmHg and/or diastolic BP ≥ 85 mmHg (based on the Guideline for the Management of Hypertension from the Japanese Society of Hypertension) or by intake of anti-hypertensive drugs (Ca blockers were mainly used, beta-blockers were used in only two patients in both CAD and non-CAD groups and no woman used contraceptives or received hormone replacement therapy). Angiographically significant coronary stenosis was defined as 75% or more luminal diameter stenosis by CAG. Those who had significant stenosis in the left anterior descending artery, left circumflex artery and/or right coronary artery were treated as CAD patients (n = 96, 71 men and 25 women). Age-, sex- and BMI-matched subjects who did not have significant stenosis were regarded as non-CAD subjects (n = 67, 49 men and 18 women).

Laboratory measurements and diagnosis of coronary risk factors

Immediately after blood samples were collected in the morning of CAG after an overnight fast, serum and plasma were separated by centrifugation (2000 g, 15 min, 4 °C) and stored at −80 °C until measurement. Serum total cholesterol (TC) and TG levels were determined by enzymatic methods, serum LDL-C and HDL-C levels by the direct method (Sekisui Medical Co., Ltd., Tokyo, Japan) and plasma adiponectin levels by ELISA (Otsuka Pharmaceuticals, Tokyo, Japan). The presence of dyslipidaemia was assessed by LDL-C ≥ 140 mg/dL, TG ≥ 150 mg/dL and/or HDL-C < 40 mg/dL [18]. Fasting plasma glucose (FPG) was measured by the enzymatic method, and HbA1c by ion-exchange high performance liquid chromatography (HPLC) (Sekisui Medical Co.). The presence of high fasting glucose was assessed by FPG ≥ 126 mg/dL (Japan Diabetes Society) or by intake of anti-diabetic drugs. The presence of MetS was diagnosed according to the criteria of the Japanese Society of Internal Medicine [19] and National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III), USA [20]. Serum apo B-48 levels were determined by the chemiluminescent enzyme immunoassay system (Fujirebio Inc., Tokyo, Japan) [21] which was modified from the sandwich ELISA system [8]. All samples were treated in accordance with the Helsinki Declaration.

Statistical analyses

Apo B-48, BMI and adiponectin levels were normalized by logarithmic transformation. The statistical significance of differences in TC, TG, HDL-C, LDL-C, systolic BP, diastolic BP, FPG, HbA1c, Log-apo B-48 and Log-adiponectin between CAD and non-CAD subjects was determined by Mann–Whitney U-test, by frequency of smoking, as well as by prevalence of dyslipidaemia, hypertension and high fasting glucose. The prevalence of Mets was then compared by chi-square test. The correlations between metabolic parameters and CAD were analysed by Pearson's correlation coefficients, and stepwise multiple logistic regression analysis was used to determine independent predictors of CAD. Age, sex, Log-BMI, smoking, TC, LDL-CADL-C, TG, systolic BP, diastolic BP, FPG, HbA1c, Log-apo B-48 and Log-adiponectin were included as explanatory variables in the

method. Receiver-operating characteristic (ROC) curves were used to examine the apo B-48 values for categorizing subjects on the basis of the presence of CAD, and the cut-off value was identified.

We compared the effect of different metabolic parameters of MetS (TG, HDL-C, HbA1c or plasma adiponectin; classified as low and high) on CAD prevalence in patients with low or high levels of apo B-48. The cut-off value of apo B-48 level in CAD patients was 4·34 μg/mL. We divided subjects (n = 163) into two groups according to their apo B-48 levels: low (≤ 4·34 μg/mL) and high (> 4·34 μg/mL). Both groups were also divided into low (< 150 mg/dL) or high TG levels (≥ 150 mg/dL), high (≥ 40 mg/dL) or low HDL-C levels (< 40 mg/dL), low (< 5·8%) or high HbA1c levels (≥ 5·8%) and high (≥ 4·0 μg/mL) or low plasma adiponectin levels (< 4·0 μg/mL) [22]. The statistical significance of differences in the prevalence rates of CAD was determined among these four groups by chi-square test. The data were analysed with JMP8 software (SAS Institute, Cary, NC, USA). All statistical significance were accepted at $P < 0·05$.

Results

Comparison of clinical profiles between coronary artery disease patients and non- coronary artery disease subjects

Serum FPG and HbA1c levels were significantly higher, whereas serum levels of HDL-C and adiponectin were significantly lower in patients with CAD (n = 96) than in age-, sex- and BMI-matched non-CAD subjects (n = 67) (Table 1). Fasting apo B-48 and TG levels were significantly higher in CAD patients than in non-CAD subjects ($P < 0·0001$), and the statistical significance of difference was the highest for these parameters (Table 1). Fasting serum apo B-48 levels ranged from 0 to 13 μg/mL in non-CAD subjects, and from 0 to 19 μg/mL in patients with CAD (Fig. 1). The fasting apo B-48 concentration in a large majority of CAD patients and non-CAD subjects was 10 μg/mL or less, but the peak and average of fasting apo B-48 levels were higher in patients with CAD than in non-CAD subjects (Fig. 1 and Table 1). The ROC curve analysis showed that the AUC-ROC value was 0·79, and the cut-off value of apo B-48 was identified as 4·34 (overall sensitivity, 0·82; 1-specificity, 0·33; predictive positive value, 79; predictive negative value, 61).

Correlations between the existence of CAD and metabolic parameters

The correlations between the existence of CAD and metabolic parameters related to coronary risk were analysed by logistic regression analysis in these subjects (Table 2). By Pearson's correlation analysis, significant correlations with the existence of CAD were observed in smoking, HDL-C, TG, FPG, HbA1c, Log-apo B-48 and Log-adiponectin levels. Multiple regression analysis indicated that only log-apo B-48 was a significant determinant of the existence of CAD ($P < 0·0001$) among the various metabolic parameters related to coronary risk (Table 2).

Prevalence of coronary artery disease in subjects with high Apo B-48 levels and other metabolic parameters of abnormal levels

The clustering of metabolic parameters is a high risk state for CAD. We compared the prevalence of CAD in patients with low (≤ 4·34 μg/mL) and high (> 4·34 μg/mL) levels of apo B-48 when their metabolic parameters of MetS (TG, HDL-C, HbA1c or plasma adiponectin levels) were in high risk status (detailed in *Subjects and Methods*). CAD was significantly more prevalent in subjects with high levels of apo B-48 than in subjects with low levels of apo B-48, irrespective of TG, HDL-C, HbA1c or plasma adiponectin levels (in Fig. 2). The prevalence of CAD was significantly higher in subjects with high levels of apo B-48 and high TG, low HDL-C, high HbA1c or low plasma adiponectin levels, compared with that in subjects with low levels of apo B-48 and normal TG, HDL-C, HbA1c or plasma adiponectin levels.

Discussion

This study demonstrated that fasting levels of apo B-48 were higher in patients with CAD than in those with non-CAD, and that high levels of fasting apo B-48 were definitely correlated with the prevalence of CAD among other metabolic biomarkers related to coronary risk. The combination of high fasting apo B-48 levels and other metabolic disorders represented a stronger risk state for CAD.

High fasting serum apo B-48 levels in patients with coronary artery disease compared with non-coronary artery disease subjects

The fasting apo B-48 concentration in a large majority of CAD patients and non-CAD subjects was 10 μg/mL or less, and the peak and average of fasting apo B-48 levels were higher in patients with CAD than in non-CAD subjects (Fig. 1 and Table 1). In CAD patients, MetS components, such as dyslipidaemia, hypertension, high fasting glucose and low adiponectin levels, were more clustered than in non-CAD subjects (Table 1), implying that CAD patients tended to have a pathophysiological background of MetS. The presence of insulin resistance leads to a deterioration of postprandial remnant metabolism [23]. Impaired clearance of lipoproteins is related to the accumulation of CM-R in the postprandial serum and the increase in fasting apo B-48 concentrations [24]. In this study, a high prevalence of CAD was observed in patients with high levels of apo B-48 (Fig. 2). This may indicate that the

Table 1 Clinical Profiles of the Non-CAD subjects and the patients with CAD

	non-CAD (n = 67)	CAD (n = 96)
Age (years)	62·7 ± 10·8	65·1 ± 9·9
Sex[†] (m vs. w)	49 vs. 18	71 vs. 25
Smoking (%)	48·2	60·4
BMI (kg/m^2)	24·1 ± 3·6	24·4 ± 2·8
Prevalence of Dyslipidaemia[‡] (%)	40·2	66·7
TC (mg/dL)	197·6 ± 37·1	199·5 ± 36·5
TG (mg/dL)	121·4 ± 37·1	163·1 ± 83·3**
HDL-C (mg/dL)	49·5 ± 13·3	43·8 ± 13·2*
LDL-C (mg/dL)	125·1 ± 34·3	125·5 ± 34·3
Prevalence of Hypertension[§] (%)	64·1	78·2
Systolic BP (mmHg)	130·0 ± 17·2	130·0 ± 22·9
Diastolic BP (mmHg)	74·6 ± 10·6	75·4 ± 12·2
Prevalence of drug-treated patients (%)	53·1	68·3
Prevalence of High fasting glucose[¶] (%)	19·3	40·0
FPG (mg/dL)	100·5 ± 25·1	116·7 ± 42·4*
HbA1c (%)	5·4 ± 1·0	6·3 ± 1·7*
Fasting apo B-48 μg/mL	3·9 ± 2·4	6·9 ± 2·6**
Adiponectin μg/mL	7·8 ± 4·3	6·4 ± 4·2*
Prevalence of the metabolic syndrome		
In Japanese criteria (%)	17·2	29·2*
In NCEP-ATPIII criteria (%)	22·6	53·1*

BMI, body mass index; BP, blood pressure; FPG, Fasting plasma glucose; TC, total cholesterol; TG, triglyceride; CAD, coronary artery disease.
[†]Number of men vs. women.
[‡]Ratio of subjects with TG ≥ 150 mg/dL and/or HDL-C < 40 mg/dL.
[§]Ratio of subjects with systolic BP ≥ 130 mmHg or diastolic BP ≥ 85 mmHg.
[¶]Ratio of subjects with FPG ≥ 126 mg/dL). The statistical significance of differences in TC, TG, HDL-C, LDL-C, systolic BP, diastolic BP, FPG, HbA1c, fasting apo B-48 and adiponectin were determined by Mann–Whitney's U-test, those in the prevalence of smoking, dyslipidaemia, hypertension, high FPG and the Mets were determined by the chi-square test. Significance was established at $P < 0·05$. *$P < 0·01$, **$P < 0·0001$.

atherogenicity of CM-R might be prolonged throughout the day in CAD patients with a pathophysiological background of MetS. We also found that a small number of CAD patients and non-CAD subjects had high fasting apo B-48 levels (> 10 μg/mL) (Fig. 1). In our former study, we found subjects with high apo B-48 levels (> 10 μg/mL) among patients with any type of hyperlipidaemia [8]. In that study, high fasting apo B-48 levels (> 10 μg/mL) were mainly observed in patients with type I, III, IV and V hyperlipidaemia [8], probably because of the characteristics of a genetic polymorphism (impaired lipoprotein lipase (LPL) activity, the existence of apo E2/E2 phenotype or apo A5) or the existence of PH. Although we did not diagnose the type of hyperlipidaemia in subjects with high fasting apo B-48 levels (> 10 μg/mL), high levels of fasting apo B-48 in CAD patients might be partly a result of impaired lipoprotein metabolism caused by a genetic disorder of apoproteins, enzymes and receptors, or the existence of MetS.

High fasting apo B-48 levels and atherosclerosis

A number of studies have suggested that CM-R had highly atherogenic properties, but there is still no consensus as to whether CM-R accumulation correlates with the development of atherosclerotic cardiovascular diseases. By multiple regression analysis, it was determined that log-apo B-48 was the only significant determinant of the existence of CAD ($P < 0·0001$) among other metabolic parameters related to coronary risk (Table 2).

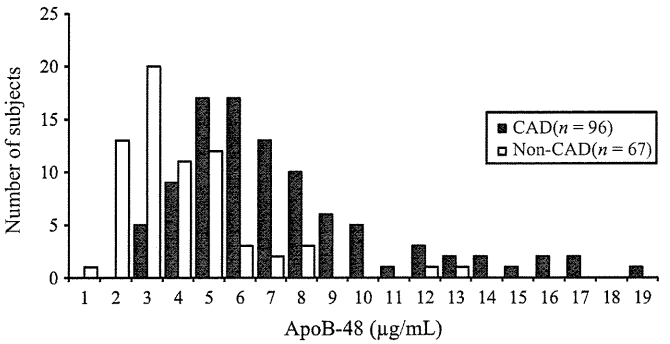

Figure 1 Distribution of Fasting Serum Apo B-48 Levels in non coronary artery disease (Non-CAD) Subjects and Patients with CAD. Fasting serum concentrations of apo B-48 in non-CAD subjects (open squares, n = 96) and patients with CAD (closed squares, n = 67). Serum apo B-48 level=1 represents concentrations between 0·0 and 1·0 μg/mL.

Table 2 Univariate and multivariate analyses of correlations between the existence of coronary artery disease and various metabolic parameters

	Univariate *P* value	Multivariate *P* value
Age	0·1581	–
Sex	0·3698	–
Log-BMI	0·4645	–
Smoking	0·0492	–
TC	0·7440	–
LDL-C	0·8508	–
HDL-C	0·0085	0·3721
Triglyceride	0·0017	0·1098
Systolic BP	0·9747	–
Diastolic BP	0·6757	–
FPG	0·0081	0·6110
HbA1c	0·0008	0·3036
Log-apo B-48	< 0·0001	< 0·0001
Log-APN	0·0239	0·6039

BMI, body mass index; TC, total cholesterol; BP, blood pressure; FPG, fasting plasma glucose; HbA1c, haemoglobin A1c. APN, adiponectin. Univariate analysis was assessed using Pearson's correlation analysis. Multivariate analysis was assessed using stepwise multiple regression analysis.

Moreover, as shown in Fig. 2, the prevalence of CAD was significantly higher in subjects with high levels of apo B-48 than in subjects with low levels of apo B-48, irrespective of other coronary risk factors such as high TG, low HDL-C, high HbA1c or low plasma adiponectin levels. These results clearly show that the frequency of coronary stenosis was significantly and strongly correlated with fasting apo B-48 level, suggesting that the accumulation of CM-R was the strongest risk factor for CAD in these study subjects. Whereas the cut-off value of apo B-48 was 4·34 as determined by ROC curve analysis, the specificity was unfortunately low (1-specificity; 0·33) perhaps because of some degree of coronary stenosis in subjects who were diagnosed as non-CHD subjects. Therefore, high levels of apo B-48 might correlate with patients with more severe CAD than with patients with less severe CAD or non-CAD subjects.

Clustering of high apo B-48 level and other coronary risk factors

The prevalence of CAD gradually increased with higher levels of apo B-48 in association with high TG, low HDL-C, high HbA1c or low plasma adiponectin levels (Fig. 2). Whereas high TG, low HDL-C, high HbA1c and low plasma adiponectin levels are independent coronary risk factors, these metabolic disorders correlated with the accumulation of CM-R. The accumulation of CM-R was associated with insulin resistance and prevalence of type II diabetes mellitus [23]. Plasma adiponectin and leptin concentrations were inversely and directly associated with plasma apo B-48, whereas plasma apo B-48 level was significantly and positively associated with plasma insulin, HOMA and visceral fat areas [25]. The combination of high levels of apo B-48 and other metabolic disorders related to coronary risk may synergistically increase atherogenicity. However, high levels of fasting apo B-48 independently enhanced the prevalence of CAD, irrespective of TG, HDL-C, HbA1c or plasma adiponectin levels. This indicates that a high level of apo B-48, namely the accumulation of CM-R, was the strongest risk status for the prevalence of CAD of all metabolic disorders, as shown in Table 2. These results suggest that without the measurement of fasting apo B-48 level, we may underestimate the CAD risk by the assessment of metabolic disorders using TG, HDL-C, HbA1c or plasma adiponectin levels. For the assessment of CAD risk in subjects with MetS or subjects with little coronary risk, measuring apo B-48 levels remains useful. Subjects with high apo B-48 levels should be assessed carefully by a variety of pharmacological and physiological approaches [26]. Both atorvastatin and fenofibrate have been shown to improve the postprandial increase of CM-R markedly [27,28]. We have also recently reported that ezetimibe, an intestinal cholesterol transporter inhibitor, improves PH in patients with type IIb hyperlipidaemia [29] by reducing the intestinal production of CMs [30]. As fasting and postprandial levels of apo B-48 decrease by these physiological and pharmaceutical interventions [26–29], the

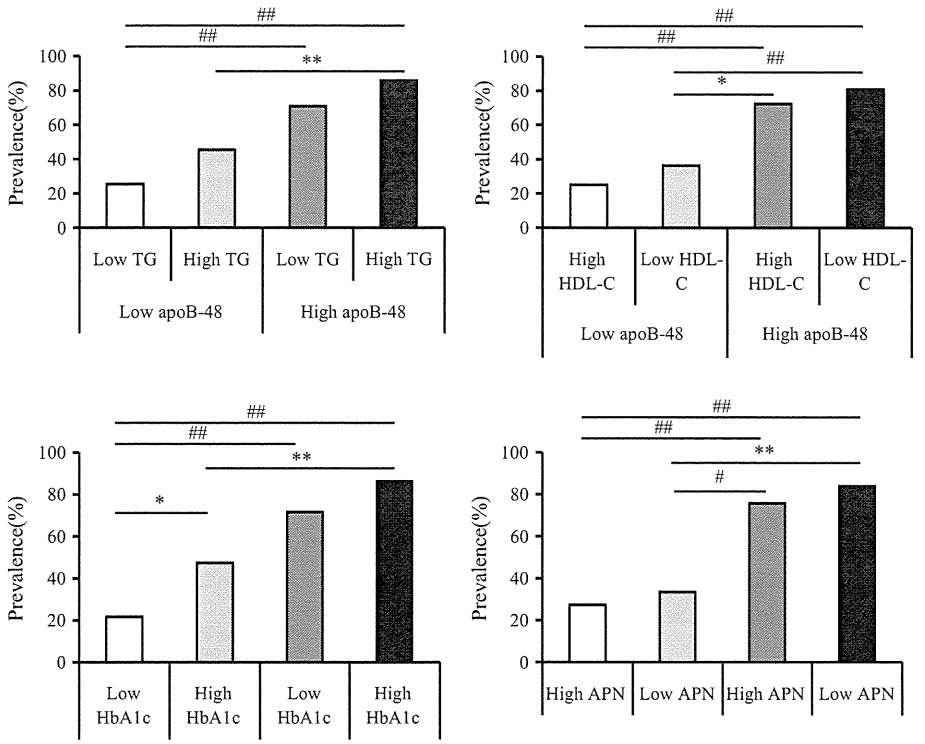

Figure 2 Prevalence Rate of coronary artery disease (CAD) in Subjects with a Combination of High Apo B-48 Levels and Other Coronary Risk Factors. We compared the effect of different metabolic parameters of metabolic syndrome (TG, HDL-C, HbA1c or plasma adiponectin; classified as low and high) on CAD prevalence in patients with low or high apo B-48 levels. We divided all subjects (n = 163) into low (≤ 4·34 μg/mL) and high (> 4·34 μg/mL) apo B-48 levels; these two groups were also divided according to low (< 150 mg/dL) or high (≥ 150 mg/dL) TG levels, high (≥ 40 mg/dL) or low (< 40 mg/dL) HDL-C levels, low (< 5·8%) or high (≥ 5·8%) HbA1c levels or high (≥ 4·0μg/mL) or low (< 4·0 μg/mL) plasma adiponectin levels. The prevalence rates of coronary artery disease were determined in each group, and the statistical significance of differences among these four groups was verified by chi-square test. $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, $^{\#}P < 0{\cdot}001$ and $^{\#\#}P < 0{\cdot}0001$

measurement of apo B-48 levels may be useful for managing CAD risk in subjects with MetS or PH.

Limitation of the study

In this study, the subjects were collected from outpatients who came to the cardiovascular department and were susceptible to having CAD. These subjects had already been treated with anti-diabetic drugs or anti-hypertension drugs, and the total number of patients was relatively small compared with that in other related studies.

Conclusion

In conclusion, fasting serum apo B-48 levels are significantly correlated with the existence of CAD and other metabolic disorders. The measurement of fasting apo B-48 is useful for detecting and managing CAD risk in subjects with MetS or low coronary risk.

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Original Article

Thyroid Function Influences Serum Apolipoprotein B-48 Levels in Patients with Thyroid Disease

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Aim: **Apolipoprotein B-48 (apoB-48) is a major apolipoprotein of intestine-derived chylomicrons (CM) and CM remnants (CMR). Clinically overt hypothyroidism (OH) has been associated with premature and accelerated coronary atherosclerosis. To clarify the clinical significance of apoB-48 measurement in patients with thyroid disease, we investigated the correlations between the serum apoB-48 level and thyroid hormones.**

Methods: **From outpatients of Osaka University Hospital, patients with OH, subjects with subclinical hypothyroidism (SH) and subjects with normal thyroid function were collected and analyzed by measuring serum TSH, FT4 and FT3 levels. Serum apoB-48 levels were measured by a chemiluminescence enzyme immunoassay and the correlations with thyroid hormone levels or lipid profiles were assessed. These levels were compared among subjects with OH, SH and healthy controls.**

Results: **Serum apoB-48 level was correlated with TSH, total cholesterol (TC) and triglycerides (TG), but negatively with FT4 and FT3 level. LDL-C and HDL-C levels were not correlated with serum apoB-48 levels. Serum apoB-48 in patients with OH (7.4±5.9 μg/mL) was significantly higher than in those with hyperthyroidism (5.1±3.5 μg/mL; $p<0.01$) and normal subjects (4.7±3.7 μg/mL; $p<0.01$), but decreased after levo-thyroxine replacement. ApoB-48, TG and TSH were significantly higher in SH subjects than normal subjects, suggesting that serum apoB-48 level depends on the thyroid function status, similar to TC, LDL-C and TG.**

Conclusion: **Increased serum apoB-48 concentrations and CMR may contribute to the increased risk of atherosclerosis and premature coronary artery disease in the hypothyroid state.**

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Key words; Apolipoprotein B-48 (apoB-48), Hypothyroidism (OH), Chylomicrons (CM), Chylomicron remnants (CMR), Subclinical hypothyroidism (SH)

Introduction

Thyroid hormones influence all aspects of lipid metabolism, including synthesis, transport, and degradation, especially cholesterol metabolism in hepatic cells[1, 2]. Overt hypothyroidism (OH) is characterized by hypercholesterolemia[3]. Furthermore, about 4-14% of hypercholesterolemic patients were reported to be in the hypothyroid state[4]. Hypercholesterolemia and high serum LDL-cholesterol (LDL-C) levels are strongly correlated with the development of atherosclerotic cardiovascular diseases (CVD)[5]. Thus, high

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LDL-C is a major therapeutic target in the treatment of dyslipidemia[6]. While there is controversy regarding serum triglyceride (TG) concentrations in patients with hypothyroidism[7, 8], serum LDL-C and TG-rich lipoproteins (TRL), including chylomicrons (CM), very-low-density lipoproteins (VLDL), and their remnant lipoproteins, were reported to be increased in patients with hypothyroidism[9, 10]. Therefore, increased LDL-C and remnant lipoproteins in patients with OH are clinically important from the point of CVD prevention[11].

Subclinical hypothyroidism (SH) is defined as the clinical status of elevated serum thyroid-stimulating hormone (TSH) in the presence of normal free thyroxine (FT4) and free triiodothyronine (FT3)[12]. SH as mild thyroid failure has clinical importance because of its high prevalence, the risk of progression to OH and consequences including neurobehavioral, cardiac and lipid abnormalities[13-17]. On the other hand, in patients with hyperthyroidism, TC and LDL-C were reduced while those of TG and HDL-C were unchanged[18].

It has been reported that postprandial hyperlipidemia is an independent risk factor for atherosclerotic cardiovascular diseases[19, 20], which is due to the postprandial increase of TRL and their hydrolyzed products, remnants. TRL derived from the small intestines in the postprandial state are CM, and CMR are the hydrolyzed products of CM by lipoprotein lipase (LPL)[21-24]. CMR are taken up by monocyte-derived macrophages by many kinds of receptors and lead to foam cell formation. Many basic studies have suggested that accumulated CMR particles may promote atherogenicity in the arterial wall. Indeed, elevated intestinally derived remnant lipoproteins have been associated with an increased risk for cardiovascular diseases[25, 26]. CM and CMR have a characteristic apolipoprotein B-48 (apoB-48), each having one apoB-48 molecule per particle. In contrast, VLDL and their remnants (intermediate-density-lipoproteins, IDL), or VLDL remnants (VLDL-R)) contain one apolipoprotein B-100 (apoB-100) molecule per particle. CMR contain apoB-48, but not apoB-100[27]. Both CM and CMR contain one molecule of apoB-48 per particle, and it is assumed that the measurements of serum apoB-48 concentration can evaluate the synthesis and metabolism of CMR. We previously developed a novel enzyme-linked immunosorbent assay (ELISA) to measure serum apoB-48 concentration, using a microplate assay[28-30]. Recently, we have established a chemiluminescent enzyme immunoassay (CLEIA) to measure serum apoB-48 concentrations[31].

Very few studies have so far investigated the correlation between fasting serum apoB-48 levels and the development of atherosclerosis among subjects with hypothyroidism and hyperthyroidism. In the current study, we measured the serum apoB-48 concentration in patients with hyperthyroidism and hypothyroidism and evaluated the correlations between serum apoB-48 and thyroid hormones.

Materials and Methods

Subjects

Outpatients ($n=376$) at Osaka University Hospital and healthy subjects ($n=34$) at Minani-Osaka Hospital were enrolled in the current study. We measured thyroid hormone, thyroid stimulating hormone (TSH), free thyroxine (FT4) and free triiodothyronine (FT3). The following diagnosis was made using the standard values, which were decided by Osaka University Hospital. Patients with hyperthyroidism ($n=128$; male 33, female 95; age 14-78) were diagnosed by FT3 >3.4 pg/mL and FT4 >1.6 ng/dL, while those with OH ($n=147$; male 59, female 88; age 13-88) were diagnosed by FT3 <2.0 pg/mL and FT4 <0.9 ng/dL. Subjects with SH ($n=31$; male 15, female 16; age 18-80) were diagnosed by TSH >3.8 μIU/ml and normal FT4, and the rest ($n=104$) were diagnosed as normal. Normal subjects ($n=104$; male 50, female 54; age 22-79) were outpatients at Osaka University Hospital with euthyroid ($n=70$) and healthy subjects ($n=34$) who underwent a medical examination at Minami-Osaka Hospital. None of the patients had disorders which affect lipid metabolism, including diabetes mellitus, renal failure, nephrotic syndrome, or pancreatitis, and patients with dyslipidemia (TC ≥300 mg/dL or TG ≥300 mg/dL) were excluded. This study was approved by the Ethics Committee of Osaka University Hospital, and all participants gave written informed consent.

Laboratory Measurements

Blood samples were collected after overnight fasting. The basal performance of a recently developed CLEIA for apoB-48 measurement kit (Fujirebio Inc., Tokyo, Japan) has been reported[29], and the assay was carried out on a Lumipulse *f* fully automated immunoassay analyzer (Fujirebio Inc.). Choletest CHO (Sekisui Medical Ltd., Tokyo, Japan) was used for the measurement of total cholesterol (T-CHO); Choletest TG (Sekisui Medical Ltd.) for triglycerides; Choletest LDL (Sekisui Medical Ltd.) for LDL-cholesterol; and Choletest N HDL (Sekisui Medical Ltd.) for HDL-cholesterol, respectively. BM2250 fully automated chemical analyzer (Nihondenshi Ltd., Tokyo, Japan)

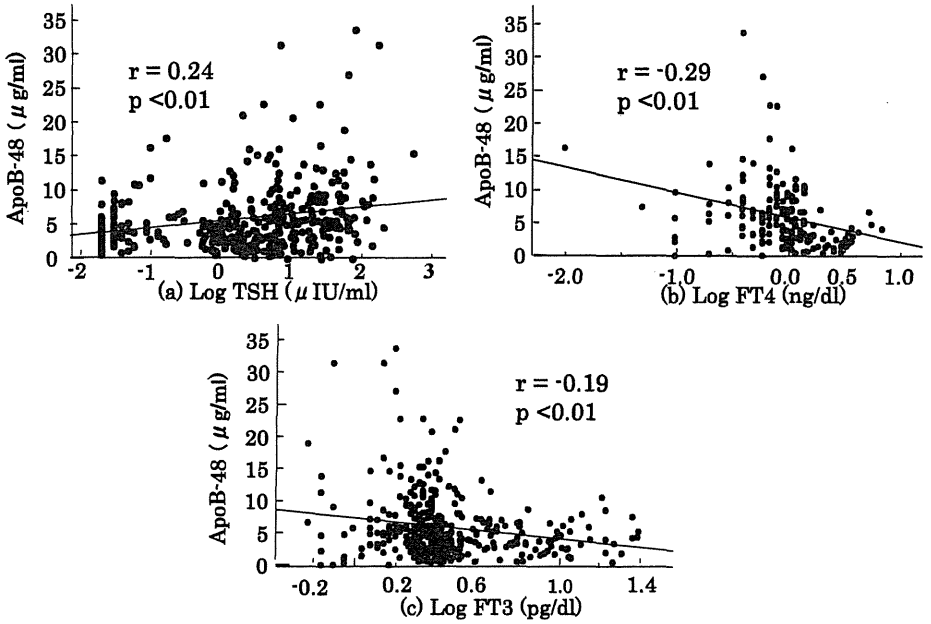

Fig. 1. Correlations between serum levels of apoB-48 and thyroid hormones.

In all subjects, including patients with hyperthyroidism ($n=128$), and subjects with overt hypothyroidism (OH, $n=147$), subclinical hypothyroidism (SH, $n=31$) and normal thyroid function ($n=104$), we analyzed the correlations between thyroid hormone and apoB-48 levels. (a) serum apoB-48 and TSH ($n=375$), (b) serum apoB-48 and FT4 ($n=405$), and (c) serum apoB-48 and FT3 ($n=393$). Correlations were assessed by Spearman's rank correlation coefficients.

was used for automated measurements. TSH, FT4 and FT3 were measured by enzyme immunoassays with the AIA-1800 analyzer (TOSOH Co., Ltd., Tokyo, Japan). All samples were treated in accordance with the Helsinki Declaration.

Statistical Analyses

Statistical analyses were performed using Stat Flex V5.0 statistical software. Correlation coefficients were assessed by Spearman's rank correlation coefficient. ANOVA and Dunnett's test was used to compare statistical differences between groups. Statistical significance was established at $p<0.01$ or $p<0.05$.

Results

In 376 patients with thyroid disorders and 34 healthy controls, the correlations between serum apoB-48, TSH and thyroid hormones (FT4, FT3) and lipid levels (TC, TG, LDL-C, HDL-C) were analyzed. As shown in **Fig. 1**, serum apoB-48 was positively correlated with the TSH and negatively with FT4 and FT3.

Serum apoB-48 was positively correlated with TC and TG; however there was no significant correlation between serum apoB-48 and LDL-C or HDL-C

Table 1. Correlations between apoB-48 and other related parameters

	ApoB-48 (μg/mL)		
	n	*r*	*p*
TC (mg/dL)	299	0.619	<0.01
LDL-C (mg/dL)	156	0.065	0.430
HDL-C (mg/dL)	186	−0.036	0.089
TG (mg/dL)	276	0.653	<0.01

(**Table 1**). These data suggested that serum apoB-48 had a significant correlation with hypothyroidism.

In order to further investigate the relationship between serum apoB-48 and thyroid function, serum apoB-48 was determined in subjects with normal thyroid function ($n=104$), patients with OH ($n=147$) and in those with hyperthyroidism ($n=128$). As shown in **Fig. 2**, serum apoB-48 was significantly higher in patients with OH (7.4±5.9 μg/mL) than in subjects with normal thyroid function (4.7±3.7 μg/mL) ($p<0.01$) or patients with hyperthyroidism (5.1±3.5 μg/mL) ($p<0.01$), respectively. There was no significant difference in serum apoB-48 concentrations between subjects with normal thyroid function and patients

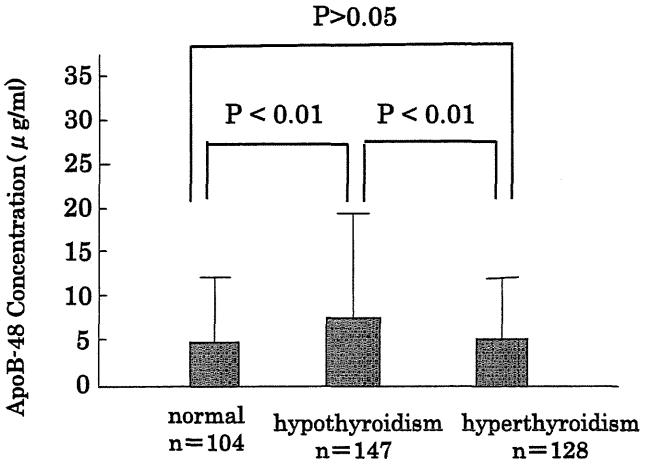

Fig. 2. Relationship between serum apoB-48 in subjects with normal thyroid function and patients with overt hypothyroidism and hyperthyroidism.

Serum apoB-48 was compared among subjects with normal thyroid function ($n=104$), and patients with hypothyroidism ($n=147$) and hyperthyroidism ($n=128$). Statistical significance was assessed by ANOVA and Dunnett's test.

Table 2. Comparison of lipid profiles between normal control subjects and patients with OH and hyperthyroidism

	N	Mean ± SD	p	
TC (mg/dL)				
Normal	86	204.0 ± 43.9	**	*
Hypothyroidism	100	211.9 ± 44.4	*	
Hyperthyroidism	90	163.2 ± 34.2		
TG (mg/dL)				
Normal	77	89.0 ± 42.4	*	**
Hypothyroidism	93	121.5 ± 57.6	*	
Hyperthyroidism	83	97.7 ± 57.3		
HDL-C (mg/dL)				
Normal	55	62.4 ± 14.7	*	*
Hypothyroidism	68	59.8 ± 24.6	*	
Hyperthyroidism	45	56.5 ± 15.7		
LDL-C (mg/dL)				
Normal	43	145.9 ± 40.1	*	*
Hypothyroidism	60	121.0 ± 40.2	*	
Hyperthyroidism	42	93.8 ± 26.6		

*$p<0.05$, **NS

with hyperthyroidism. Serum TC and TG were higher in patients with OH than in other groups, but serum LDL-C and HDL-C were higher in subjects with normal thyroid function (**Table 2**).

Fig. 3 shows the effect of levo-thyroxine (L-T4) replacement on serum apoB-48 in patients with OH. In 13 patients with OH, serum apoB-48 was significantly decreased after one or two months of L-T4 replacement ($p<0.05$). Furthermore, **Fig. 4** presents the correlation coefficients between the reduction in TSH and the reduction in apoB-48. The reduction in apoB-48 significantly correlated with the reduction in TSH ($r=0.156$, $p<0.05$) and with the increase in FT4 ($r=-0.452$, $p<0.01$) and FT3 ($r=-0.330$, $p<0.01$).

We investigated the difference in serum apoB-48 between subjects with normal thyroid function and SH. Subjects with SH ($n=31$) were collected by the diagnosis of both elevated serum TSH (≥5 μIU/mL) and within the normal range of FT4 and FT3. In these patients and 34 subjects with normal thyroid function, serum apoB-48, TSH, FT4, FT3, TC and TG were measured and compared. Normal subjects included those with euthyroid ($n=70$) in **Table 2**, and patients with euthyroid who had been treated with L-T4 were excluded from this analysis. Serum apoB-48 ($p<0.01$), TG ($p<0.01$) and TSH ($p<0.0001$) were significantly higher in SH subjects than in subjects with normal thyroid function, while no significant differences were noted in TC, HDL-C, FT4 and FT3. Serum LDL was lower in SH subjects (**Table 3**).

Discussion

The accumulation of remnant lipoproteins is very important as well as LDL for the development of atherosclerotic plaque[25, 26]. Previously, the accumulation of remnant lipoproteins was shown to be related to the development of atherosclerosis in patients with acute myocardial infarction assessed by polyacrylamide gel electrophoresis[32]. For a more quantitative analysis of remnant lipoproteins, the measurement of cholesterol in remnant-like lipoprotein particles (RLP-C and RemL-C) has been developed and used for statistical analysis[33]; however, remnant lipoproteins consist of two different lipoprotein particles, chylomicron remnants and VLDL remnants (or IDL). No assessment system can distinguish these two lipoproteins. Thus, for the first time, we developed an ELISA system for the measurement of apoB-48[30]. Since one apo B-48 is present in CM and CMR per particle, the measurement of apoB-48 is critically useful for the selective and dynamic evaluation of CM and CMR metabolism[28-31]. The accumulation of CMR is considered to be related to the development of atherosclerotic plaque, so serum apoB-48 measurement may provide important information for assessment of the global cardiovascular risk. In a previous study, we reported

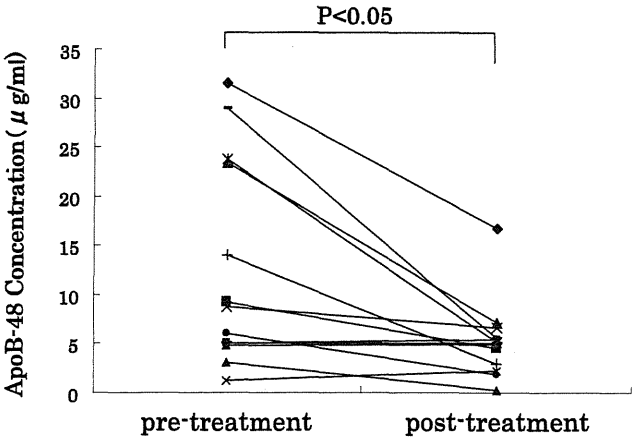

Fig. 3. Effect of L-T4 replacement on serum apoB-48 levels.

ApoB-48 was measured before and after L-T4 treatment. After L-T4 replacement, serum apoB-48 decreased in 13 patients with OH ($p < 0.05$).

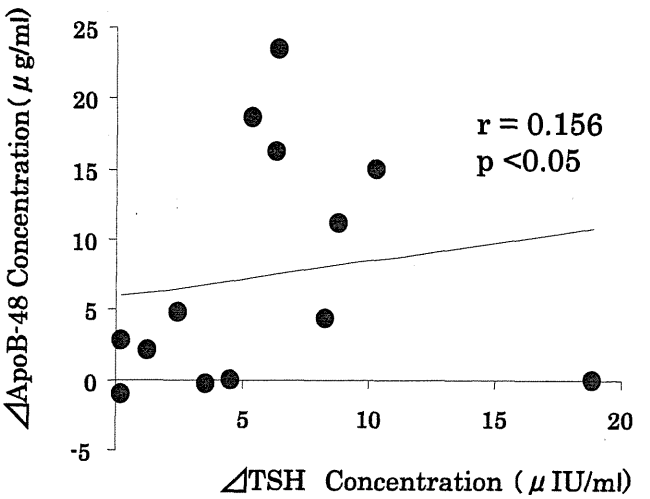

Fig. 4. Relationship between treatment-induced reductions in TSH and ApoB-48 in patients with OH.

The relationship between treatment-induced reduction in TSH and apoB-48 in 13 patients with OH was investigated.

that gender affects serum apoB-48[34].

The increased LDL-C observed in patients with OH is clinically important because it has been associated with an increased risk for the development of CVD[1-5]. Thyroid hormone binds with a thyroid hormone nuclear receptor, and the hormone-receptor complex induces SREBP-2 (sterol regulatory element-binding protein-2) and increases protein products[35]. Since SREBP-2 upregulates the expression of LDL receptor and increases the cholesterol contents in hepatocytes, in a state of hypothyroidism, decreased SREBP-2 downregulates the expression of LDL receptor in hepatocytes, resulting in increases in serum TC, LDL-C and IDL-C. At the same time, when thyroid hormone is decreased markedly, the activities of hepatic triglyceride lipase (HTGL) and plasma cholesteryl ester transfer protein (CETP) are reduced and serum TG increases because of the increase in TG-rich lipoproteins (CM and VLDL)[2]. In previous studies, the reduced activities of LPL and HTGL impaired the metabolism of remnant lipoproteins[36, 37] and the accumulation of remnant lipoprotein particles[25, 26]. In the current study, serum apoB-48 was higher in patients with OH than in other groups, positively correlated with TSH and negatively with FT4 and FT3. These data suggested that lower thyroid hormone was associated with the accumulation of apoB-48-containing lipoproteins (CM and CMR) because the activities of LPL and HTGL were impaired in patients with hypothyroidism. Moreover, since many studies reported that there were significant changes in LDL-C after L-T4 replacement in patients with OH[7, 38-40], we investigated changes in serum apoB-48 after L-T4 replacement in patients with OH. In the current study we found that L-T4 replacement decreased apoB-48, suggesting that L-T4 replacement therapy may have enhanced the activity of HTGL and LPL, leading to the accelerated metabolism of CM. Furthermore, there was a positive correlation between the reduction in TSH and apoB-48, suggesting that L-T4 replacement therapy improves lipid abnormalities as well as thyroid function. Ito *et al.* also reported that serum TC, non-HDL-C, LDL-C, apoB and RLP-C were markedly decreased after 3 months' treatment[41]; therefore, it can be speculated that L-T4 replacement therapy might be effective to improve the impaired metabolism of CMR, as well as VLDL or LDL.

Many studies have emerged that SH is a strong indicator for the development of atherogenesis and increased risk of CVD[13-17]; however some studies have shown that SH was not associated with the risk of CVD[42]. In order to elucidate the possible contribution of CMR in subjects with SH, we compared serum apoB-48 in these subjects with normal subjects and found that serum apoB-48 and TG were significantly higher. It was supposed that this was due to the decreased activities of LPL and HTGL in subjects with SH, resulting in the increase of TG-rich lipoproteins, VLDL or CM. Taken together, SH caused high serum apoB-48 because of the accumulation of CMR.

In conclusion, in subjects with OH and SH, high apoB-48 obviously suggested that the impairment of thyroid function accelerates the accumulation of CMR and may enhance atherogenicity, which

Table 3. Characteristics of patients with subclinical hypothyroidism and subjects with normal thyroid function

	Normal (n=34)	Subclinical hypothyroidism (SH) (n=31)	p
ApoB-48 (μg/mL)	2.7±1.4	5.3±3.8	<0.01
TSH (μIU/mL)	1.6±0.8	7.0±1.9	<0.0001
FT4 (ng/dL)	1.2±0.2	1.2±0.1	0.66
FT3 (pg/dL)	2.6±0.3	2.5±0.3	0.13
TC (mg/dL)	214.9±32.1	200.7±41.8	0.06
TG (mg/dL)	72.8±24.5	110.8±52.5	<0.01
LDL-C (mg/dL)	143.1±32.0	103.7±15.4	<0.01
HDL-C (mg/dL)	63.3±15.4	56.9±12.3	0.18

might be ameliorated by the administration of L-T4.

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Conflict of Interest

No.

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Original Article

Apolipoprotein B-48 to Triglyceride Ratio Is a Novel and Useful Marker for Detection of Type III Hyperlipidemia after Antihyperlipidemic Intervention

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Aim: Remnant lipoproteins are atherogenic and are accumulated in patients with type III hyperlipidemia (HL). Although type III HL is diagnosed by phenotyping apolipoprotein (apo) E, this procedure is time-consuming and inconvenient for routine clinical use. Clinical indices for screening type III HL in untreated HL patients have been proposed; however, in clinical settings, HL patients are promptly treated with lipid-lowering agents without diagnosing the underlying cause. We investigated whether existing clinical indices for screening type III HL as well as the apo B-48/triglyceride (TG) ratio, which was suggested to be related to the accumulation of small chylomicron (CM) remnants, are useful after the initiation of lipid-lowering therapies.

Methods: In 25 normolipidemic subjects and 191 treated HL patients (type I, $n=6$; IIa, 62; IIb, 66; III, 12; IV, 22; and V, 23) from Osaka University Hospital and related hospitals, fasting low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), TG, and apolipoproteins were measured and clinical indices were evaluated statistically.

Results: Apo B-48 levels were significantly higher in patients with type I, III, and V HL, and TG levels were significantly higher in patients with type I and V HL. The apo B-48/TG ratio was significantly higher only in patients with type III HL compared with other types of HL ($p<0.001$), and was statistically significant among the other clinical indices (AUC-ROC value, 0.895; cut-off value, 0.110).

Conclusion: The apo B-48/TG ratio is a novel and useful marker for detecting type III HL even after the initiation of lipid-lowering interventions.

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Key words; Apolipoprotein B-48, Type III hyperlipidemia, Chylomicron remnants, Coronary heart disease

Introduction

Type III hyperlipidemia (HL) is a rare familial disorder characterized by a combined elevation in serum total cholesterol (TC) and triglycerides (TG), based on the marked accumulation of remnant lipoproteins, including chylomicron (CM) and VLDL remnants[1]. Many recent studies have proved that oxidized LDL and remnant lipoproteins have atherogenic features[2,3] and a strong correlation with the morbidity of coronary heart disease (CHD)[4-7]; therefore, type III HL should be diagnosed and treated as soon as possible.

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Type Ⅲ HL can be definitively diagnosed by phenotyping (genotyping) apo E (mainly apo E2/E2) using Western blotting or isoelectric focusing, and by demonstrating the presence of a broad β pattern (consisting of β-VLDL, IDL or VLDL remnants) on agarose gel electrophoresis or polyacrylamide gel disc electrophoresis (PAGE)[8-11]; however, these analyses are time-consuming and inconvenient for routine clinical use. Moreover, in clinical settings, HL patients are promptly treated with diet and exercise therapy as well as lipid-lowering agents without diagnosing the underlying cause.

Five groups of investigators have identified clinical indices for screening type Ⅲ HL. The apo E/apo B ratio was significantly greater in patients with type Ⅲ HL than in those with other types of HL[12]. The apo B/TC ratio was significantly lower in patients with dysbetalipoproteinemia whose apo E genotype was E2/E2 than in those with mixed HL[13]. A simple algorithm was developed for the diagnosis of type Ⅲ HL on the basis of a TC/apo B ratio of >6.2 and a TG/apo B ratio of <10.0, which were not detected in patients with other types of HL[14]. The non-HDL-C/apo B ratio was significantly greater in patients with type Ⅲ HL and an apo E genotype of E2/E2 than in those with combined HL other than type Ⅲ HL or hypothyroidism[15]. In Japanese patients with type Ⅲ HL (apo E2/2 phenotype), the apo E/C-Ⅲ index was considerably higher than in patients with other types of HL[16]. As these studies were well designed and had high sensitivity and specificity for screening untreated type Ⅲ HL, these clinical criteria might be distorted and their specificity and sensitivity might decrease after lipid-lowering intervention. Drug withdrawal for the diagnosis of type Ⅲ HL may increase the risk of cardiovascular events; therefore, practical and simple criteria for screening type Ⅲ HL after lipid-lowering intervention must be established.

A novel sandwich enzyme-linked immunosorbent assay (ELISA)[17] and chemiluminescent enzyme immunoassay (CLEIA) system[18] to measure serum apo B-48 were developed. Fasting apo B-48 levels are correlated with postprandial TG increase[19] and are significantly higher in patients with accumulated CM and CM remnants[17] and metabolic syndrome (MetS)[20]. High fasting apo B-48 levels are correlated with premature carotid artery stenosis[21, 22], and postprandial increases in TG and apo B-48 are correlated with CHD[23]. Emerging evidence has suggested that CM remnants are responsible for the initiation and development of atherosclerotic plaques *in vitro* and *in vivo*[2]. In our previous study, we observed high fasting serum TG and apo B-48 in patients with type Ⅲ HL and the apo B-48 to TG (B-48/TG) ratio was significantly higher only in patients with type Ⅲ HL than in those with other types of HL, none of whom had received any lipid-lowering interventions[17]. It has been suggested that a high apo B-48/TG ratio is related to the accumulation of small CM remnants, which contain one molecule of apo B-48 and smaller amounts of TG than those of the larger CMs or CM remnants. Small CM remnants may be accumulated after therapy because of a genetic disorder of apoE, and a high apoB-48/TG ratio might be useful for detecting type Ⅲ HL even after the initiation of lipid-lowering interventions.

Thus, the aim of the present study was to evaluate whether the apo B-48/TG ratio is useful for detecting type Ⅲ HL even after the initiation of lipid-lowering interventions.

Subjects and Methods

Subjects and Patients

Subjects with NL and patients with HL were identified from candidates registered in the central registration database for evaluating the clinical usefulness of apo B-48 in patients with dyslipidemia, diabetes mellitus, MetS, and CHD. These candidates consisted of cardiovascular medicine outpatients at Osaka University Hospital (n=151) and another university hospital in Japan (n=65). Fasting TC and TG levels at the time of diagnosis, before any lipid-lowering interventions for dyslipidemia were administered, were examined for all subjects (n=216). HL was diagnosed using criteria based on the Japan Atherosclerosis Society (JAS) guidelines for the diagnosis and prevention of atherosclerotic cardiovascular diseases among Japanese individuals[24]. First, 25 subjects were considered to have NL as they had low TC and TG levels (TC <220 mg/dL and TG <150 mg/dL). The remaining 192 subjects were diagnosed with HL as they had high TC and TG levels (TC ≥220 mg/dL and/or TG ≥150 mg/dL) and had not received any lipid-lowering agents. The HL phenotype was determined based on the criteria proposed by the Research Committee for Primary Hyperlipidemia of the Ministry of Health and Welfare of Japan[24]. The diagnosis of type Ⅲ HL (n=12) with the apo E2/2 phenotype was performed based on the standard criteria previously reported[25]. All patients with HL were already undergoing treatment with oral agents for hyperlipidemia, including statins, fibrates, probucol, nicotinic acid, and anion-exchange resins. This study was approved by the Ethics Committee of Osaka University Hospital and the other university hospital; informed consent was obtained from all participants in this study.

Table 1. Clinical and biochemical profiles of patients with hyperlipidemia

	NL	Type I	Type IIa	Type IIb	Type III	Type IV	Type V
Male/Female	12/13	4/2	44/18	31/35	5/7	6/16	3/20
Medications							
Statin (%)		0	67.7	54.5	8.3	27.3	13.0
Fibrate (%)		33.3	18.2	18.2	100	63.6	39.1
Probucol (%)		0	3.0	3.0	0	9.1	0
Nicotinic acid (%)		16.7	6.1	6.1	0	13.6	4.3
Colestimide (%)		0	1.5	1.5	0	0	0
Ezetimibe (%)		0	6.1	6.1	0	0	0
Age (years)	67±15	44±20***	66±10	64±10	66±15	64±9	54±13***
BMI (kg/m^2)	24.0±4.6	23.4±5.0	23.3±3.6	24.0±3.6	25.4±2.9	24.5±3.9	25.4±3.8
BP (mmHg)							
Systolic BP	144±18	114±13*	139±22	139±12	139±22	126±18	126±10*
Diastolic BP	84±13	69±8	81±12	79±12	80±11	82±9	79±10
TC (mg/dL)	195±31	227±59	226±49*	210±47	209±63	192±34	237±65*
HDL-C (mg/dL)	59±14	31±14***	67±16*	56±13	60±12	43±15***	41±11***
LDL-C (mg/dL)	118±23	76±40	137±44	122±25	85±47	115±31	111±47
TG (mg/dL)	95±44	1096±610***	103±62	156±71	213±122	190±120	571±440***
ApoB (mg/dL)	91±19	79±34	107±27	107±25	84±48	105±24	120±32**
ApoE (mg/dL)	4.9±1.1	10.5±2.8***	4.9±1.5	4.9±1.3	10.3±3.4***	6.3±4.9	8.6±4.9***
ApoCIII (mg/dL)	8.2±2.4	21.9±5.3***	10.3±3.0	11.1±4.0	13.4±5.4	12.1±8.1	20.1±12.8***
nonHDL-C (mg/dL)	136±27	195±63*	159±49	144±28	148±67	142±47	186±76***
ApoB48 (μg/mL)							
Median	2.6	21.4**	2.5	3.2	21.2***	3.0	16.2***
(25-75th percentile)	(2.0-3.6)	(18.1-33.6)	(1.7-4.1)	(2.3-6.6)	(11.6-33.9)	(1.8-9.8)	(7.4-28.2)
ApoB48/TG ratio							
Median	0.032§	0.026§	0.029§	0.026§	0.126***	0.022§	0.036§
(25-75th percentile)	(0.021-0.045)	(0.016-0.035)	(0.020-0.040)	(0.019-0.037)	(0.055-0.194)	(0.151-0.030)	(0.021-0.053)

All data, except for nonparametric variables (apoB-48 and apoB-48/TG ratio), are expressed as the means ± S.D. or frequencies . ApoB-48 and apoB-48/TG ratio are expressed as medians, 25th percentile and 75th percentile. Normally distributed variables were analyzed by one-way ANOVA with Dunnett's multiple comparison test and the nonparametric variables (apoB-48 and apoB-48/TG ratio) were analyzed by the Kruskal-Wallis test with Steel's test between NL and other types of HL. *$p<0.05$ vs NL, **$p<0.01$ vs NL, ***$p<0.001$ vs NL. Differences of the apoB-48/TG ratio between patients with type III HL and subjects with NL or other types of HL were analyzed using Wilcoxon's rank sum test. §$p<0.001$ vs type III HL.

Measurements

In all subjects, height and weight were measured, body mass index (BMI) was calculated, and blood pressure was measured in a sitting position in the morning. Blood samples were collected in the morning after an overnight fast. Sera were separated immediately by low-speed centrifugation (3,000×g) for 10 min at 4℃ and stored at −80℃ until used. Serum TC and TG were determined by an enzymatic method; serum LDL-C and HDL-C levels by a direct method using Cholestest N HDL (Sekisui Medical Co Ltd, Tokyo, Japan) and Cholestest LDL (Sekisui Medical Co Ltd, Tokyo, Japan); and serum apo B, apo E, and apo C-III levels by an immunoturbidity method (Sekisui Medical Co Ltd). Serum apo B-48 levels were determined using our own CLEIA system (Fuji Rebio Inc., Tokyo, Japan)[18]. A broad β pattern of serum lipoproteins was determined by PAGE, and the presence of the apo E phenotype was determined by isoelectric focusing gel electrophoresis (JOKOH Co., Tokyo, Japan)[26]. All samples were treated in accordance with the Helsinki Declaration[27]. Non-HDL-C levels were calculated by subtracting HDL-C values from TC values. Clinical indices for screening type III HL (apo E/apo B ratio, apo B/ TC ratio, TC/apo B ratio, TG/apo B ratio, non-HDL-C/apo B ratio, and apo E/C-III ratio) were calculated[12-16] in addition to the apo B-48/TG ratio[17].

Statistical Analysis

Biochemical parameters concerning lipid and lipoprotein profiles without apo B-48 were normally

Table 2. Clinical and lipid profiles in patients with type Ⅲ hyperlipidemia

Case	Gender	Age (years)	ApoE phenotype	Lipid-lowering drug	TC (mg/dL)	TG (mg/dL)	ApoB-48 (μg/mL)	ApoB (mg/dL)	ApoE (mg/dL)
1	M	65	E2/E2	Fenofibrate	155	254	8.9	49	8.8
2	M	59	E2/E2	Fenofibrate	193	120	13.2	59	10.9
3	M	78	E2/E2	Bezafibrate	173	221	25.7	66	9.8
4	F	80	E2/E2	Bezafibrate	194	123	16.7	61	9.0
5	F	64	E2/E2	Bezafibrate	216	195	32.5	86	13.6
6	F	85	E2/E2	Fenofibrate	138	62	11.1	38	7.3
7	M	82	E2/E2	Fenofibrate	232	208	41.2	87	11.5
8	M	66	E2/E2	Fenofibrate + Pravastatin	224	179	37.8	81	17.8
9	M	45	E2/E2	Fenofibrate	245	286	29.8	86	12.8
10	M	55	E2/E2	Bezafibrate	235	268	10.0	124	5.1
11	M	36	E2/E2	Bezafibrate	372	581	13.2	216	8.5
12	F	75	E2/E2	Bezafibrate	145	152	34.4	52	9.2

Case	ApoCⅢ (mg/dL)	non-HDL-C (mg/dL)	ApoB48/TG	ApoE/apoB	apoB//TC	TC/apoB	TG/apoB	non-HDL-C/ apoB	apoE/ apoCⅢ
1	0.9	104	0.035	0.18	0.32	3.16	5.18	2.12	0.88
2	14.8	113	0.110	0.18	0.31	3.26	2.03	1.91	0.74
3	9.3	120	0.116	0.15	0.38	2.62	3.35	1.82	1.05
4	10.3	121	0.135	0.15	0.31	3.19	2.02	1.99	0.87
5	9.3	168	0.167	0.16	0.40	2.51	2.26	1.95	1.46
6	3.8	59	0.179	0.17	0.28	3.63	1.63	1.55	0.89
7	13.2	170	0.198	0.13	0.37	2.67	2.39	1.97	0.87
8	9.9	167	0.211	0.22	0.36	2.76	2.21	2.06	1.80
9	19.0	175	0.104	0.15	0.35	2.84	3.32	2.03	0.67
10	21.6	166	0.039	0.04	0.55	1.83	2.08	1.34	0.24
11	24.1	323	0.025	0.04	0.58	1.72	2.48	1.50	0.35
12	12.0	92	0.293	0.18	0.39	2.60	2.25	1.77	0.77

distributed variables, and apo B-48 levels were non-parametric variables[17, 21]; these levels are expressed as the mean ± SD. The overall difference in biochemical parameters, apoB-48 and the apoB-48/TG ratio was compared between subjects with NL and patients with any type of HL, one-way ANOVA with Dunnett's multiple comparison tests were used for the difference in biochemical parameters and the Kruskal-Wallis and Steel's tests were used for that in apo B-48 and the apo B-48/TG ratio. Moreover, differences in the apo B-48/TG ratio between patients with type Ⅲ HL and subjects with NL as well as patients with other types of HL were analyzed by Wilcoxon's rank sum test. Multiple comparisons in clinical indices for screening type Ⅲ HL[12-16] between subjects with NL and patients with different types of HL were analyzed by Dunnett's multiple comparison test or Steel's test (apo B-48/TG ratio) and those between patients with type Ⅲ HL and other types of HL were also analyzed by Dunnett's multiple comparison test or Steel's test. Statistical significance was declared if the one-sided p value was < 0.05. Receiver-operating characteristic (ROC) curves were used to examine the value of the apo B-48/TG ratio useful for categorizing subjects on the basis of the presence of type Ⅲ HL. Data were analyzed by linear discriminant analysis, and the error rate for subjects with NL and subjects with other types of HL was evaluated relative to patients with type Ⅲ HL. Statistical analyses were performed using JMP 9 software (SAS Institute, Cary, NC).

Results

Clinical Profiles of Patients with HL after Anti-Hyperlipidemic Intervention

The clinical profiles and biochemical markers of lipid and lipoprotein metabolism were compared among subjects with NL and patients with type I, Ⅱa, Ⅱb, Ⅲ, Ⅳ, and Ⅴ HL (**Table 1**). Patients with HL were already receiving treatment with lipid-lowering drugs.

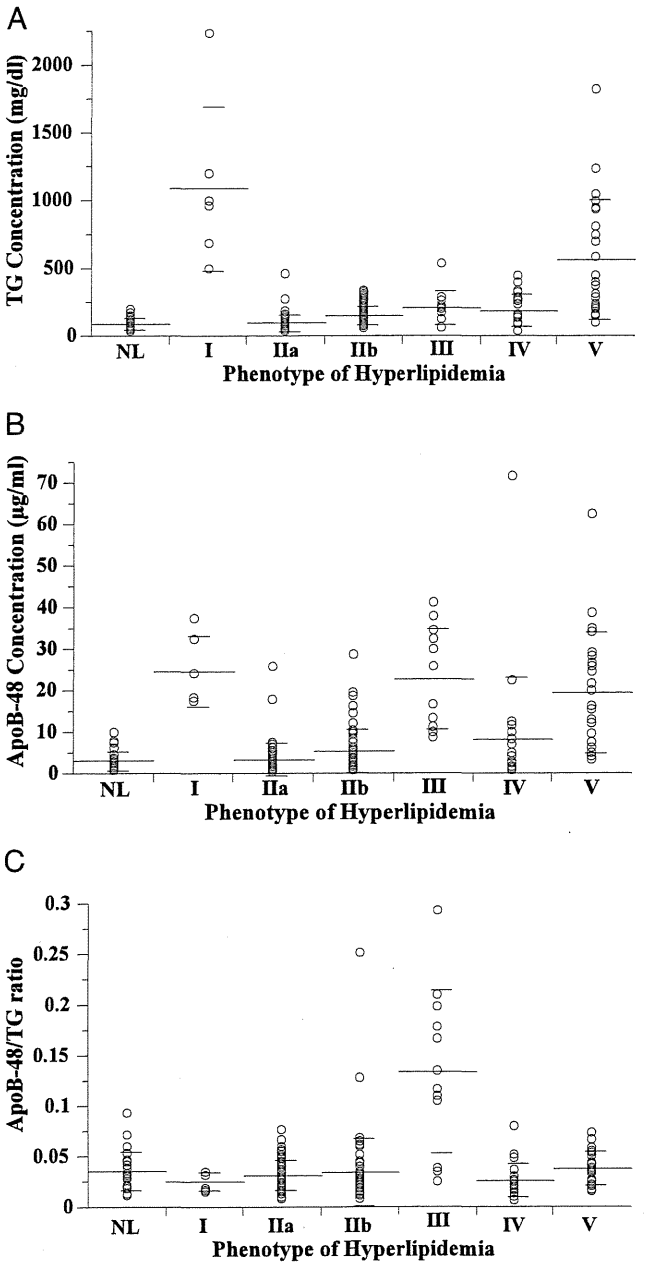

Fig. 1. Fasting serum apo B-48 levels in various types of hyperlipidemia after antihyperlipidemic treatments.

Fasting TG levels (A), apo B-48 levels (B) and apo B-48/TG ratio (C) were determined for 25 subjects with NL, and type I ($n=6$), type IIa ($n=62$), type IIb ($n=66$), type III ($n=12$), type IV ($n=22$), and type V ($n=23$) HL patients. Data are expressed as a scatter plot using the mean ± SD.

Patients with type I HL were treated with a low-fat diet, fibrates and nicotinic acids. Patients with type IIa and IIb HL were mainly treated with statins, and patients with type III, IV, and V HL were mainly treated with fibrates (**Table 1**). Clinical profiles of 12 patients with type III HL are shown in **Table 2**; these patients had already been treated with fibrates (fenofibrate or bezafibrate) and statins. TC levels were significantly higher among patients with type IIa and V HL than among subjects with NL after lipid-lowering treatments; however, there was no significant difference in LDL-C levels between these groups. TG levels were much higher among patients with type I and V HL than in subjects with NL, but not among patients with type III HL (**Table 1**, **2** and **Fig. 1**). Apo B levels were significantly higher among patients with type V HL than among subjects with NL, but not among patients with type IIa or IIb HL. Apo E levels were significantly higher among patients with type I, III, and V HL, and apo C-III levels were significantly higher among patients with type I and V HL than subjects with NL (**Table 1**).

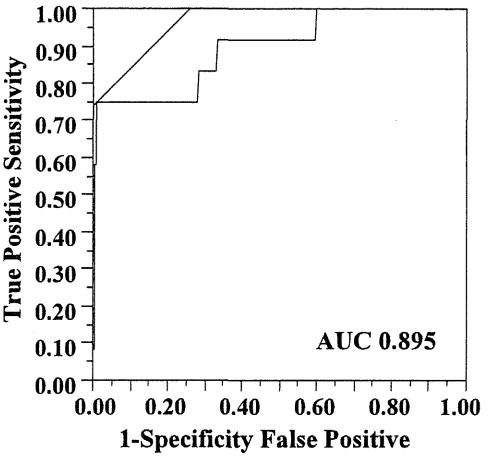

Fig. 2. ROC curve for Apo B48/TG ratio.

The receiver-operating characteristic (ROC) curve illustrating the utility of the apo B48/TG ratio in the diagnosis of type III HL. AUC: area under the curve.

ApoB-48 Levels and Apo B-48/TG Ratios

As shown in **Table 1** and **Fig. 1**, there was no significant difference in TG levels between patients with type III HL and subjects with NL; however, apo B-48 levels among patients with type I, III, and V HL were significantly higher than among subjects with NL ($p<0.001$) (**Table 1** and **Fig. 1**). The apo B-48/TG ratio was also significantly higher among patients with type III HL than among subjects with NL ($p<0.001$, assessed by Steel's multiple comparisons test) and patients with other types of HL ($p<0.001$, assessed by Wilcoxon's rank sum test). When discriminating patients with type III HL from patients with other types of HL, the apo B48/TG ratio yielded an AUC-ROC value of 0.895 (**Fig. 2**), indicating that the apo B-48/TG ratio maintained high accuracy for detecting

Table 3. Clinical indexes for screening type Ⅲ hyperlipidemia

	NL (n=25)	type Ⅰ (n=6)	type Ⅱa (n=62)	type Ⅱb (n=66)	type Ⅲ (n=12)	type Ⅳ (n=22)	type Ⅴ (n=23)
apoE/apoB[12]	0.05±0.01†	0.15±0.07***	0.05±0.02†	0.05±0.01†	0.15±0.05***	0.06±0.04†	0.07±0.04†
apoB/TC[13]	0.47±0.06†	0.37±0.16**	0.47±0.06	0.51±0.05*,†	0.38±0.09***	0.55±0.07***,†	0.51±0.06†
TC/apoB[14]	2.17±0.27§	3.75±3.17***,§	2.15±0.27§	1.88±0.45†	2.76±0.57*	1.78±0.46†	1.89±0.48†
TG/apoB[14]	1.02±0.36	20.99±24.3***,†	0.97±0.48	1.42±0.56	2.49±0.94	1.75±1.19	5.17±4.66**
non-HDL-C/apoB[15]	1.49±0.07	3.33±3.07***,†	1.48±0.11	1.03±0.64*,§	1.83±0.25	1.36±0.32	1.55±0.47
apoE/apoCⅢ[16]	0.30±0.04†	0.36±0.00†	0.40±0.03***,†	0.52±0.04***,†	0.62±0.01***	0.70±0.04***	0.97±0.25***
apoB48/TG ratio	0.036±0.019†	0.026±0.010†	0.032±0.016†	0.035±0.034†	0.137±0.08***	0.026±0.017†	0.038±0.017†
Median	0.032†	0.026†	0.029†	0.026†	0.126***	0.022†	0.036†
(25-75th percentile)	(0.021-0.045)	(0.016-0.035)	(0.020-0.040)	(0.019-0.037)	(0.055-0.194)	(0.151-0.030)	(0.021-0.053)

All data are expressed as the means±S.D. In addition, apoB-48/TG ratios are expressed as medians, 25th percentile and 75th percentile. Multiple comparisons between NL and any types of HL were analyzed by Dunnett's multiple comparison test or Steel's test (apoB-48/TG ratio). $^{*}p<0.05$ vs NL, $^{**}p<0.01$ vs NL, $^{***}p<0.001$ vs NL.
Multiple comparisons between type Ⅲ HL and NL or any types of HL were analyzed by Dunnett's multiple comparison test or Steel's test (apoB-48/TG ratio). $^{\S}p<0.05$ vs type Ⅲ HL, $^{\dagger}p<0.001$ vs type Ⅲ HL

type Ⅲ HL even after lipid-lowering interventions. The cut-off value of the apo B48/TG ratio was identified as 0.110; linear discriminant analysis revealed a 2.8% error rate for all types of HL and NL vs. type Ⅲ HL.

Apo B48/TG Ratio and Other Proposed Clinical Indices for Screening of Type Ⅲ HL

Many researchers have proposed clinical indices for screening type Ⅲ HL[12-16]; we evaluated whether these clinical indices and the apo B-48/TG ratio remained useful for screening type Ⅲ HL after the initiation of lipid-lowering interventions (**Table 3**). Analyses were performed with Dunnett's or Steel's multiple comparison tests; statistical significance of differences was observed between patients with type Ⅲ HL and those with other types of HL when we adopted the apo E/apo B, apo B/TC, TC/apo B, apo E/apo C-Ⅲ, and apo B-48/TG ratios. However, when the apo E/apo B, and TC/apo B ratios were adopted, a statistically significant difference was also observed between patients with type Ⅰ HL and those with other types of HL. When the apo B/TC ratio was adopted, a statistically significant difference was also observed between patients with type Ⅰ, Ⅱa, Ⅲ, and Ⅳ HL and those with other types of HL. Only when the apo B-48/TG ratio was adopted was a statistically significant difference observed only between patients with type Ⅲ HL and those with other types of HL.

Discussion

The accumulation of "atherogenic" remnants in patients with type Ⅲ HL is strongly correlated with the development of atherosclerotic cardiovascular diseases[6,7]. Nutritional or pharmacological interventions are administrated to these patients as soon as possible without investigating the etiology of dyslipidemia, so the assessment of HL type in patients with dyslipidemia on the basis of their biochemical data, such as TC and TG levels, is very difficult. In this study, we assessed a simple marker for screening type Ⅲ HL,the apoB-48/TG ratio, which is suitable for use even after initiation of lipid-lowering interventions.

Clinical Profiles of Type Ⅲ HL after Initiation of Lipid-Lowering Interventions

Appropriate intervention with lipid-lowering drugs reduces levels of lipids and apolipoproteins to within normal limits in some patients with HL (**Table 1**). TG and apo B-48 levels were decreased after administration of lipid-lowering drugs to patients with type Ⅰ, Ⅲ, Ⅳ, and Ⅴ HL compared with those before administration of these drugs, but not in patients with type Ⅱa and Ⅱb HL (17 and **Table 1**). Fibrate administration decreased TG levels in patients with type Ⅲ HL to the levels observed in subjects with NL, but it did not achieve a sufficient TG decrease in patients with type Ⅰ and Ⅴ HL (**Fig. 1A**). On the other hand, apo B-48 levels remained significantly high in patients with type Ⅲ HL as well as type Ⅰ and Ⅴ HL after the administration of fibrates. We suspected that these differences in lipid-lowering effects between type Ⅰ, Ⅲ, and Ⅴ HL may be due to increased deterioration of LPL activity by fibrates or nicotinic acids. Since fibrates enhance LPL-mediated catabolism of VLDL-TG and CM-TG, the TG content of remnant lipoproteins might have decreased and their particle sizes diminished. In patients with type Ⅲ HL, increased