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 ($7.4 \pm 5.9 \mu\text{g/mL}$) was significantly higher than in those with hyperthyroidism ($5.1 \pm 3.5 \mu\text{g/mL}$; $p < 0.01$) and normal subjects ($4.7 \pm 3.7 \mu\text{g/mL}$; $p < 0.01$), 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

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metabolism, including synthesis, transport, and degradation, especially cholesterol metabolism in hepatic cells^{1, 2}. Overt hypothyroidism (OH) is characterized by hypercholesterolemia³. Furthermore, about 4-14% of hypercholesterolemic patients were reported to be in the hypothyroid state⁴. Hypercholesterolemia and high serum LDL-cholesterol (LDL-C) levels are strongly correlated with the development of atherosclerotic cardiovascular diseases (CVD)⁵. Thus, high

LDL-C is a major therapeutic target in the treatment of dyslipidemia⁶. 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 prevention¹¹.

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 abnormalities¹³⁻¹⁷. On the other hand, in patients with hyperthyroidism, TC and LDL-C were reduced while those of TG and HDL-C were unchanged¹⁸.

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-100²⁷. 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 assay²⁸⁻³⁰. Recently, we have established a chemiluminescent enzyme immunoassay (CLEIA) to measure serum apoB-48 concentrations³¹.

Very few studies have so far investigated the cor-

relation 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 Minami-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 μ IU/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 reported²⁹, 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 ($\mu\text{g/mL}$)		
	<i>n</i>	<i>r</i>	<i>p</i>
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 ($7.4 \pm 5.9 \mu\text{g/mL}$) than in subjects with normal thyroid function ($4.7 \pm 3.7 \mu\text{g/mL}$) ($p < 0.01$) or patients with hyperthyroidism ($5.1 \pm 3.5 \mu\text{g/mL}$) ($p < 0.01$), 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.

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 $\mu\text{IU/mL}$) 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 signifi-

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

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

* $p<0.05$, **NS

cant 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 electrophoresis³². 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 analysis³³; 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-48³⁰. 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 metabolism²⁸⁻³¹. 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 ($p < 0.05$).

that gender affects serum apoB-48³⁴⁾.

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 CVD¹⁻⁵⁾. 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 products³⁵⁾. 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

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.

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' treatment⁴¹⁾; 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 CVD¹³⁻¹⁷⁾; however some studies have shown that SH was not associated with the risk of CVD⁴²⁾. 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 (<i>n</i> = 34)	Subclinical hypothyroidism (SH) (<i>n</i> = 31)	<i>p</i>
ApoB-48 ($\mu\text{g/mL}$)	2.7 ± 1.4	5.3 ± 3.8	< 0.01
TSH ($\mu\text{IU/mL}$)	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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