DYSLIPIDEMIA is related to the pathogenesis of atherosclerosis. The relationship between cardiovascular disease (CVD) and hypercholesterolemia, increased low-density lipoprotein cholesterol (LDL-C), or decreased high-density lipoprotein cholesterol (HDL-C) has been well documented.

Dietary fats are transported as chylomicrons, which are macromolecules synthesized exclusively by the intestine. Chylomicrons when first secreted are triglyceride (TG) rich; however, once in circulation, they rapidly undergo hydrolysis to produce cholesterol-enriched remnants. It is the TG-depleted remnants that are considered to be atherogenic, because they are able to penetrate arterial tissue and become trapped within the sub-endothelial space. Indeed, chylomicron remnants can induce substantial macrophage lipid loading, a hallmark feature of early atherogenesis [1, 2].

Several tests are used to assess TG-rich lipoprotein kinetics. Elevated fasting plasma TG level and post-prandial response have been widely used as markers for the metabolism of chylomicrons. Alternatively, chylomicron remnant metabolism is often studied after an oral fat load containing esterified vitamin A [3]. Chylomicrons and chylomicron remnants have a characteristic apolipoprotein, apolipoprotein B (ApoB), and ApoB-48 decreased significantly after L-T4 replacement. The serum levels of triglycerides (TG), HDL-C, low-density lipoprotein cholesterol (LDL-C), apolipoprotein A1 (ApoA-1), and Lp(a) did not change significantly. In all 36 patients, the reduction in the ApoB-48 levels correlated significantly with the reduction in TSH levels (r = 0.39, P<0.05). This study showed clearly that L-T4 replacement might reduce serum levels of ApoB-48 in both OH and SH patients. Such altered serum levels of ApoB-48 in patients with OH and SH may be related to the disturbed metabolism of chylomicron remnants in patients with hypothyroidism.

**Key words:** Apolipoprotein B-48, Chylomicron remnant, Hypothyroidism, L-thyroxine, Lipoprotein
lipid profile that includes greater serum concentrations of LDL-C [9, 10] and apolipoprotein(a) (Lp(a)) [11]. With regard to the TG-rich lipoprotein in hypothyroidism, we previously reported the disturbed metabolism of remnant lipoprotein in overt hypothyroidism (OH) [12] and subclinical hypothyroidism (SH) [13].

In the present study, we measured the serum concentrations of ApoB-48 in patients with OH and SH before and after T4 replacement to investigate the effect of thyroid hormone replacement on ApoB-48 concentration, a good indicator of chylomicron remnants derived from the intestine, in patients with hypothyroidism.

**Materials and Methods**

**Patients**

We recruited 18 patients with OH (mean age, 54±9 years; mean body mass index, 24.8±4.8 kg/m²) and 18 patients with SH (mean age, 58±7 years; mean body mass index, 21.8±3 kg/m²) who had been referred to Kuma Hospital in Kobe, Japan. OH was diagnosed on the basis of elevated serum TSH levels and lowered free thyroxine (free T4) levels. The causes of OH included Hashimoto thyroiditis (n = 17) and radioiodine therapy (n = 1) for hyperthyroidism. SH was diagnosed on the basis of elevated serum TSH levels (≥6 μIU/mL) and free thyroid hormone levels (free T4 and free T3) within the normal range. The onset of SH in each patient was well established, as the patients had been followed for several months, beginning when the increase in TSH level was detected. The causes of SH included Hashimoto thyroiditis (n = 17) and radioiodine therapy (n = 1) for hyperthyroidism. None of the patients had a history of coronary heart disease, acute illness, or disorders that affect lipid metabolism (e.g., diabetes mellitus, renal failure, nephrotic syndrome, or pancreatitis). None of the patients were on a lipid-lowering agent during the study period. All patients gave their informed consent for participation in the study, which was approved by the Institutional Ethics Committee.

**Study protocol**

After both the patients with OH and the patients with SH fasted overnight, blood samples were drawn to determine the serum lipid concentrations and thyroid function test results at the baseline. L-T4 replacement was then initiated (25 or 50 μg/day) in the patients. All patients were advised to maintain their dietary habits during the study period. To normalize the serum TSH levels, the L-T4 dosage was adjusted according to the serum free T4 and TSH concentrations measured at 4-week intervals after L-T4 replacement was initiated. The mean final dose of L-thyroxine required to normalize the serum TSH levels was 99 ± 29μg/day in the OH patients and 60 ± 13μg/day in the SH patients. In the patients treated with L-T4, the lipid profiles were evaluated after 3 months.

**Laboratory determinations**

The levels of total cholesterol (TC), HDL-C, and TG were measured by enzyme assays. The non-HDL-C levels were calculated as [TC – HDL-C]. The LDL-C levels were calculated by the Friedewald formula. The serum Lp(a) concentration was measured using a latex agglutination assay (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan). Apolipoprotein B (ApoB), Apolipoprotein E (ApoE), and apolipoprotein A1 (ApoA-1) were measured by immunoturbidimetry. The remnant-like particle cholesterol (RLP-C) was prepared using an immunoseparation technique (Japan Immunoresearch Laboratories, Takasaki, Japan) [14]. Apolipoprotein B-48 (ApoB-48) was measured by the ELISA method using a monoclonal antibody [15]. The intraassay coefficient of variation (CV) for serum ApoB-48 ranged from 2.3-4.4 %. The normal ranges were 3.06 - 6.14μg/mL for ApoB-48. Serum concentrations of TSH, FT4, and FT3 were measured with a chemiluminescent immunoassay (ARCHITECT i2000; Abbott Japan, Tokyo, Japan). The normal ranges were 0.3 - 5.0 μIU/mL for TSH, 0.7-1.6 ng/dL for FT4, and 1.7 - 3.7 pg/mL for FT3.

**Statistical analysis**

Grouped data were expressed as means±SD. Treatment effects (pre- versus post-L-thyroxine- T4 (L-T4) replacement) were analyzed using a paired t-test for normal distribution and using the Wilcoxon signed rank test for nonparametric distribution. Significance was defined as a corresponding P value of less than 0.05 (two-sided). Pearson’s correlation coefficient test was used to assess the correlation between the reductions in TSH, ApoB-48, and other variables.

**Results**

The characteristics and lipid profiles of patients with OH and SH before and after L-T4 replacement
Apolipoprotein B-48 in hypothyroidism

The serum Lp(a) levels were unchanged. In patients with SH, the serum levels of TC, non-HDL-C, RLP-C, and ApoB decreased significantly after L-T4 replacement. The serum levels of TG, HDL-C, LDL-C, ApoA-1, and Lp(a) did not change significantly.

In both the patients with OH and those with SH, the levels of serum ApoB-48 were significantly decreased after L-T4 replacement ($P < 0.005$, $P < 0.05$, respectively) (Table 1). Fig. 1 shows the changes in the serum concentrations of ApoB-48 before and after L-T4 replace-

<table>
<thead>
<tr>
<th>Table 1</th>
<th>The characteristics and lipid profiles in patients with overt and subclinical hypothyroidism before and after L-T4 replacement therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overt hypothyroidism (n=18)</td>
<td>Subclinical hypothyroidism (n=18)</td>
</tr>
<tr>
<td></td>
<td>Before</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.8±4.8</td>
</tr>
<tr>
<td>TSH (μIU/mL)</td>
<td>78.7±26</td>
</tr>
<tr>
<td>FT4 (ng/dL)</td>
<td>0.33±0.19</td>
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<tr>
<td>FT3 (pg/mL)</td>
<td>1.68±0.55</td>
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<tr>
<td>TC (mg/dL)</td>
<td>267±57</td>
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<tr>
<td>TG (mg/dL)</td>
<td>124±45</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>77±17</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>166±48</td>
</tr>
<tr>
<td>Non-HDL-C (mg/dL)</td>
<td>190±52</td>
</tr>
<tr>
<td>ApoB (mg/dL)</td>
<td>121±36</td>
</tr>
<tr>
<td>ApoA-1 (mg/dL)</td>
<td>170±24</td>
</tr>
<tr>
<td>Lp(a) (mg/dL)</td>
<td>16±15</td>
</tr>
<tr>
<td>RLP-C (mg/dL)</td>
<td>6.1±2.1</td>
</tr>
<tr>
<td>ApoB-48 (μg/mL)</td>
<td>22.6±21.3</td>
</tr>
</tbody>
</table>

Values are the mean ± SD. Replacement effects of L-T4 were analyzed by paired $t$ test and by *Wilcoxon signed rank test for nonparametric distribution. $P < 0.05$ vs. before L-T4 replacement therapy.

Fig. 1 Individual changes in the serum levels of apolipoprotein B-48 before and after L-thyroxine replacement in patients with overt hypothyroidism and subclinical hypothyroidism. Open squares represent means.
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In addition, the reduction in ApoB-48 levels correlated significantly with the reduction in TSH levels and the elevation in FT4 levels. This change was accompanied by reductions in serum levels of TC, non-HDL-C, RLP-C, and ApoB, and no change in Lp(a). These accompanied data were compatible with those of our previous report [16].

Recently, Mugii et al. evaluated the correlations between serum ApoB-48 and thyroid hormone in patients with various thyroid states, and reported that serum ApoB-48 concentrations were significantly higher in OH and SH subjects compared to those in normal subjects [17]. They also evaluated the effect of L-T4 replacement on the serum ApoB-48 concentration in OH patients. However, there were differences between our study and theirs in the treatment periods of L-T4 and the number of patients. The treatment periods in the present study was three months, while their treatment period was only one month. The present study included 18 patients, while Mugii et al. evaluated 5 patients, and only indicated the data from two patients. Moreover, they did not indicate the effect of L-T4 replacement on the serum ApoB-48 concentration in SH patients.

In regard to metabolism of chylomicrons in hypothyroidism, Abrams et al. reported no abnormality in

Fig. 2  Relation between treatment-induced reductions in levels of thyroid-stimulating hormone (TSH) (A) and free T4 (FT4) (B) and apolipoprotein B-48 in patients with hypothyroidism
Closed circles represent overt hypothyroid patients. Open circles represent subclinical hypothyroid patients.

Discussion

We have demonstrated that L-T4 replacement induces a reduction of fasting serum levels of ApoB-48, which is a static marker of chylomicrons and chylomicron remnants, in patients with OH and SH. In addition, the reduction in ApoB-48 levels correlated significantly with the reduction in TSH levels and the elevation in FT4 levels.

This change was accompanied by reductions in serum levels of TC, non-HDL-C, RLP-C, and ApoB, and no change in Lp(a). These accompanied data were compatible with those of our previous report [16].

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In regard to metabolism of chylomicrons in hypothyroidism, Abrams et al. reported no abnormality in
patients with hypothyroidism [18], while other investigators showed significantly decreased clearance of chylomicron remnants in hypothyroid rats [19, 20]. Weintraub et al. reported that postprandial accumulation of chylomicron remnants exists in the hypothyroid state as a way of indicating an absorption disorder of vitamin A [21]. However, such a method has some limitations, and one of them is that the metabolism of vitamin A may be disturbed in subjects with hypothyroidism [22]. Additionally, this method requires fat-loading with vitamin A and monitoring of a long time course. In this study, we measured fasting serum levels of ApoB-48, which is a more quantitative and direct method. It has been reported that a high fasting ApoB-48 level reflects high postprandial levels of chylomicrons and/or chylomicron remnants [23, 24]; therefore, we assumed that a high fasting serum level of ApoB-48 indicated the existence of postprandial hyperlipidemia associated with chylomicrons and/or chylomicron remnants. Our results are in agreement with the findings of animal studies and indirect human study in hypothyroidism. These results may be explained in part by findings in previous studies. LPL and HTGL, which are essential for the degradation of chylomicrons or chylomicron remnants, were demonstrated to significantly increase after T<sub>4</sub> replacement [10, 12]. Hepatocyte B-E receptors that are responsible for the uptake of LDL and chylomicrons were depressed in subjects with hypothyroidism, with an increase in expression in response to the administration of thyroid hormone [25, 26].

Because our subjects with hypothyroidism showed higher serum levels of ApoB-48, it is possible that chylomicron remnants play a role in the increased risk of atherosclerosis related to hypothyroidism. Proctor et al. reported intimal retention of cholesterol derived from ApoB-48-containing lipoproteins in the carotid arteries of hyperlipidemic rabbits [27]. Several clinical studies indicated that serum ApoB-48 level was associated with atherosclerosis in humans [28, 29]. Whether the serum ApoB-48 level in hypothyroidism influences atherosclerosis remains to be determined.

Both ApoB-48 and RLP-C were used as markers of TG-rich lipoprotein; however, the reduction in the ApoB-48 levels with L-T<sub>4</sub> replacement was not correlated to the reduction in RLP-C levels in the present study. One possible explanation is that the change in the cholesterol content of chylomicron remnants (reflected by RLP-C) with L-T<sub>4</sub> replacement did not differ from the change in remnant particle number (reflected by ApoB-48). Another possibility is that the reduction in the RLP-C levels reflects a reduction in the levels of hepatic lipoproteins, because RLP-C reflects a lipoprotein population that is both intestinally (chylomicron remnants) and hepatically derived (VLDL remnants) [30]. On the other hand, ApoB-48 mainly reflects chylomicrons and chylomicron remnants derived from the intestine. Indeed, several previous studies reported that there is a discrepancy in serum basal level or in treatment effect between ApoB-48 and RLP-C in patients with hyperlipidemia [5, 31]. These findings may explain the lack of correlation between the reduction effect of L-T<sub>4</sub> replacement in ApoB-48 and that in RLP-C in hypothyroid patients.

OH, with its accompanying hypercholesterolemia, is widely recognized as a risk factor for atherosclerosis and cardiovascular disease [32]. Necropsy studies confirmed an association between OH and coronary heart disease [33, 34]. On the other hand, although SH is highly prevalent, it is controversial whether SH is a risk factor for cardiovascular disease. Some previous studies [35, 36] suggested that SH indicated a risk for cardiovascular disease, but others suggested that it did not [37]. Whether there is an association between ApoB-48 levels and cardiovascular disease in patients with OH and SH remains to be determined.

There were some possible limitations in the present study. First, the study was not a placebo-controlled design. In addition, in the present study, the reduction in the ApoB-48 levels and those in the TSH were not significant in only SH patients. We may not observed a significant correlation because of our relatively small sample or short-term (3 months) treatment period, or because our SH patients had relatively mild increases in serum TSH levels (mean, 9.34 μIU/mL). Properly controlled studies are needed to demonstrate whether T<sub>4</sub> replacement therapy alters ApoB-48 levels in patients with hypothyroidism.

In summary, the present study demonstrated, probably for the first time, that L-T<sub>4</sub> replacement induces a reduction of serum concentrations of ApoB-48 in both OH and SH. Our results also suggest that L-thyroxine replacement may have beneficial effects on the metabolism of ApoB-48 in addition to the already known LDL-C and ApoB, which may be relevant for reducing the risk of cardiovascular complications in SH.
References


