Effect of Levo-thyroxine Replacement on Postprandial Hyperlipidemia in Patients with Subclinical Hypothyroidism

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ABSTRACT

Objective: To investigate the effect of levo-thyroxine (L-T4) replacement on the postprandial lipid metabolism in subclinical hypothyroidism (SH), we performed an oral fat-loading test in patients with SH before and after L-T4 replacement.

Patients: 9 patients with SH and 10 euthyroid controls were recruited. Patients with SH received L-T4 for 3 months to achieve euthyroidism.

Measurements: Serum lipid profiles, including remnant-like particle cholesterol (RLP-C) levels in both fasting and postprandial states before and after L-T4 replacement.

Results: At baseline in the fasting state, the serum RLP-C levels in patients with SH were significantly higher than those in the euthyroid controls; however, the levels of total cholesterol, triglyceride, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and lipoprotein (a) were not. After L-T4 replacement, serum RLP-C levels significantly reduced, but changes in other lipid profiles were not significant. After fat loading, patients with SH had significantly higher areas under the curve (AUC) of RLP-C than the controls (P = 0.04). The euthyroid patients treated with L-T4 showed a significant decrease in the AUC of RLP-C (P = 0.02) as well as TG (P = 0.03).

Conclusions: The disturbed postprandial RLP-C metabolism is observed in patients with SH, which can be corrected by L-T4 replacement therapy.

INTRODUCTION

Subclinical hypothyroidism (SH) is defined as an elevated level of serum thyroid-stimulating hormone (thyrotropin, TSH) in the presence of normal levels of free thyroid hormone (free thyroxine [T4] and free...
some studies have reported that SH was prevalent in 4.3% [1] and 9.0% [2] of the general population, and 16% of elderly women [3].

With regard to the fasting lipid profile, patients with SH show significantly more elevated levels of total cholesterol (T-C) and low-density lipoprotein cholesterol (LDL-C) than euthyroid subjects [4,5]. Previously, we reported that the fasting remnant lipoprotein levels [6] and non-high-density lipoprotein cholesterol (non-HDL-C) levels of patients with SH are significantly more elevated [7] than those of euthyroid controls.

We are virtually in the postprandial state for the major part of our daily lives. The risk of the occurrence of postprandial hyperlipidemia would not be detected if the evaluation is carried out only in the fasting state. Therefore, postprandial hyperlipidemia should be determined; postprandial hyperlipidemia is closely related to coronary heart disease (CHD) suggesting an independent risk factor [8,9] even in the impaired metabolism of postprandial lipoproteins [10,11]. In order to evaluate atherosclerotic risk of postprandial hyperlipidemia, the measurement of remnant-like particle cholesterol (RLP-C) levels, which reflect remnant lipoproteins, is important and necessary [12]. Increased levels of remnant lipoprotein are related to the presence or development of CHD [13], type 2 diabetes [14], insulin resistance [15], and growth hormone (GH) deficiency [16]. Previously, we reported that patients with overt hypothyroidism have disturbed metabolism of remnant lipoproteins, including RLP-C, not only in the fasting state but also in the postprandial state [17]. However, postprandial hyperlipidemia in patients with SH has not been sufficiently examined.

The aims of this study were to clarify whether postprandial hyperlipidemia is present in patients with SH, and to evaluate the effect of thyroid hormone replacement on the postprandial lipid metabolism. This was done by measuring the serum triglyceride (TG) and RLP-C levels in the fasting as well as postprandial states in patients with SH before and after levo-thyroxine (L-T4) replacement therapy.

**PATIENTS and METHODS**

**Patients**
We recruited 9 patients with SH, (age, 57 ± 9 years; sex, 7 women, 2 men; and body mass index, 23.0 ± 2.5 kg/m²; mean ± SD) who had been referred to Kuma Hospital or Osaka Medical College Hospital. All the patients were diagnosed as suffering from SH because SH was continuously detected for at least 3 months before enrollment in this study. The causes of SH included Hashimoto thyroiditis (n = 7) and radiiodine therapy (administered 5 and 7 years ago, n = 2) for hyperthyroidism. None of the patients had a history of CHD, acute illness, pregnancy, or disorders affecting lipid metabolism (e.g., diabetes mellitus, renal failure, nephrotic syndrome, or pancreatitis). These patients were compared with 10 euthyroid subjects with similar profiles, i.e., age (51 ± 13 years), gender (7 women, 3 men), and body mass index (22.1 ± 3.7 kg/m²). All subjects provided informed consent to participate in this study; this study was approved by the Institutional Ethics Committees of the respective institutions.

**Study protocol**
Blood samples were drawn at baseline to determine serum lipid and thyroid function levels after a 12-h overnight fast. After initiating L-T4 (50 or 50 µg/d) replacement therapy in patients with SH, the serum levels of free T₄, free T₃, and TSH were determined at 4-week intervals to adjust the T₄ dosage to achieve euthyroidism. All patients were advised to maintain their dietary habits during the study period. The mean final dose of L-T₄ that was required to normalize serum TSH levels was 63.9 ± 13.2 µg/d. The lipid profiles of patients treated with L-T₄ were re-evaluated 3 months after the initiation of treatment. We administered a dose of 160 g of OFTT cream (Jomo Food Industry, Gunma, Japan) containing 35% fat without sugar, 2.5% protein, 7.4% carbohydrates, and 0.3% minerals with a total energy content of 547 kcal to 3 groups of subjects, namely, the controls, untreated patients with SH, and euthyroid patients treated with L-T₄. To prevent dehydration, the patients were allowed to consume a small amount of water during the study period. The blood samples were drawn before and 2, 4, 6, and 8 h after the fat loading. Data were analyzed as areas under the curve (AUC). The AUCs were calculated by adding the areas under the graph between each pair of consecutive observations.

**Laboratory determinations of thyroid function and lipids, including RLP-C**
The serum levels of free T₄ (reference range, 9.0–20.6 pmol/l), free T₃ (2.6–5.7 pmol/l), and TSH (0.3–5.0 mU/l) were measured by performing chemiluminescent immunoassay (ARCHITECT i2000; Ab-
The serum levels of T-C, TG, and high-density lipoprotein cholesterol (HDL-C) were measured by performing enzyme assays. The LDL-C level was calculated by Friedewald formula [LDL-C = T-C - (HDL-C + TG/5)]. Serum TG levels higher than 4.52 mmol/l can lead to an incorrect calculation of LDL-C; however, no patient or normal control demonstrated such levels. The serum lipoprotein (a) [Lp (a)] concentration was measured using a latex agglutination assay (Daichi Pure Chemicals Co., Ltd., Tokyo, Japan). The RLP-C was prepared using an immunoseparation technique (Japan Immunoresearch Laboratories, Takasaki, Japan) [18].

**Statistical analysis**

Grouped data were expressed as mean ± SD. An unpaired t test was used to compare the values obtained for the patients and controls. Treatment effects (pre- vs. post-L-T4 replacement) were analyzed by a paired t test. The correlations between fasting levels and areas under the postprandial curves of TG and RLP-C were determined by Pearson’s correlation analysis. Statistics were calculated with SPSS version 15.0 for windows (SPSS Inc, Chicago, IL). A P-value < 0.05 was considered statistically significant.

**RESULTS**

The clinical characteristics of the study participants in the fasting state are shown in Table 1. At baseline, the body mass indices of all the groups were similar; the mean value in controls was 22.1 ± 3.7 kg/m², compared with 23.0 ± 2.5 kg/m² in the patients with SH (P = 0.51). The serum TSH levels in patients with SH were higher than those in the controls. The free T4 levels in patients with SH were significantly lower than those in the controls, and there was no significant difference in the free T3 levels between the 2 groups.

After L-T4 replacement therapy, the serum levels of TSH, free T4, and free T3 were all within the normal ranges. During the treatment period, the body mass index did not change significantly and refer to Table 1.

**Lipoprotein Profile**

At baseline in the fasting state, patients with SH had significantly higher serum RLP-C levels (P = 0.03) than the euthyroid controls, whereas no differences were noted in the serum levels of T-C (P = 0.23), TG (P = 0.08), HDL-C (P = 0.29), LDL-C (P = 0.14), and Lp (a) (P = 0.88). After L-T4 replacement therapy in the patients with SH, serum levels of T-C and TG decreased, but the change was not significant. Further, no significant change was observed in the levels of HDL-C, LDL-C, and Lp (a). However, serum RLP-C levels were significantly reduced (from 0.17 ± 0.08 mmol/l to 0.13 ± 0.05 mmol/l; P = 0.01).

As shown in Figure 1, serum TG and RLP-C levels were remarkably increased after fat loading in both the controls and the patients with untreated SH. The peak levels of the mean TG and RLP-C

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Baseline</th>
<th>P-value*</th>
<th>Patients</th>
<th>P-value †</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls (n = 10)</td>
<td>Patients (n = 9)</td>
<td></td>
<td>Patients (n = 9)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22.1 ± 3.7</td>
<td>23 ± 2.5</td>
<td>0.51</td>
<td>22.7 ± 2.3</td>
</tr>
<tr>
<td>TSH (mU/l)</td>
<td>1.96 ± 0.89</td>
<td>8.25 ± 3.21</td>
<td>0.0003</td>
<td>2.16 ± 1.75</td>
</tr>
<tr>
<td>FT4 (pmol/l)</td>
<td>15.06 ± 1.67</td>
<td>13.38 ± 1.54</td>
<td>0.03</td>
<td>18.66 ± 3.09</td>
</tr>
<tr>
<td>FT3 (pmol/l)</td>
<td>4.85 ± 0.43</td>
<td>4.7 ± 0.57</td>
<td>0.51</td>
<td>4.99 ± 0.84</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.53 ± 0.68</td>
<td>6.04 ± 1.08</td>
<td>0.23</td>
<td>5.85 ± 1.11</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>0.98 ± 0.51</td>
<td>1.5 ± 0.70</td>
<td>0.08</td>
<td>1.25 ± 0.35</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.91 ± 0.52</td>
<td>1.66 ± 0.51</td>
<td>0.29</td>
<td>1.61 ± 0.50</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>3.2 ± 0.64</td>
<td>3.72 ± 0.84</td>
<td>0.14</td>
<td>3.66 ± 0.82</td>
</tr>
<tr>
<td>Lipoprotein (a) (mg/dl)</td>
<td>16.4 ± 13.6</td>
<td>17.3 ± 12.9</td>
<td>0.88</td>
<td>16.8 ± 12.1</td>
</tr>
<tr>
<td>RLP cholesterol (mmol/l)</td>
<td>0.1 ± 0.04</td>
<td>0.17 ± 0.08</td>
<td>0.03</td>
<td>0.13 ± 0.05</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± SD.
*Replacement effects of L-thyroxine were analyzed by paired t test.
†For comparison of patients and controls at baseline.
HDLC = high-density lipoprotein; LDL = low-density lipoprotein; T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid-stimulating hormone; RLP = Remnant-like particle.

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Figure 1. Changes in serum triglyceride (a) and RLP-C (b) after fat loading. Open circles represent mean value when patients were subclinical hypothyroidism; closed dots represent mean value when euthyroid following L-thyroxine replacement. Closed squares represent mean value of the controls.

were obtained at 4 h, and the patients with SH had higher peaks and significantly higher serum TG levels at 2, 4, 6 h and RLP-C levels at all the points as compared to the controls (P < 0.05). In the euthyroid patients treated with L-T4, the levels of TG and RLP-C also peaked at 4 h; however, the increased levels consequently dropped, and the serum TG levels at 8 h and RLP-C levels at 0, 8 h had significantly decreased. The T-C, HDL-C, and LDL-C levels, however, did not change significantly (data not shown).

The grouped data for the AUCs of TG and RLP-C after fat loading in the 3 groups of the subjects, i.e., the controls, patients with untreated SH, and euthyroid patients treated with L-T4. Statistically significant differences were observed between the controls and the patients with untreated SH with regard to the AUC of RLP-C (1.14 ± 0.55 mmol·h/l vs. 1.97 ± 1.06 mmol·h/l, respectively; P = 0.04). On the other hand, the AUC of TG showed a tendency of differences between them, but it was not significant (10.72 ± 6.60 mmol·h/l vs. 17.70 ± 8.54 mmol·h/l, respectively; P = 0.06). As shown in Figure 2, eutroid patients treated with L-T4 showed a larger decrease in the AUC of TG than the controls, in the absence of a significant difference in the AUC of RLP-C.

Figure 2. Individual changes in area under TG (a) and RLP-C (b) curve 0-8 h after fat-loading test before and after L-thyroxine replacement therapy. Open squares represent mean value level; bars represent the SD.
thyroid patients treated with L-T4 showed a statistically significant decrease as compared to the patients with untreated SH with regard to the AUCs of both TG (17.70 ± 8.54 mmol·h/l vs. 12.94 ± 4.07 mmol·h/l, respectively; P = 0.03) and RLP-C (1.97 ± 1.06 mmol·h/l vs. 1.33 ± 0.54 mmol·h/l, respectively; P = 0.02). In addition, fasting serum TG and RLP-C levels positively correlated with their respective AUCs as shown in Figure 3 (TG, r = 0.92; P = 0.0005 and RLP-C, r = 0.89; P = 0.001).

DISCUSSION

We found that postprandial RLP-C metabolism is disturbed and L-T4 replacement therapy reduced postprandial elevation of RLP-C levels in patients with SH. Furthermore, we observed that L-T4 replacement therapy resulted in reduced serum TG levels in the postprandial state.

Some studies suggested that SH is associated with atherogenic changes, such as those in the mean intima-media thickness [19], and increased brachial artery flow-mediated dilatation (FMD) [20]. Other studies, including a meta-analysis of 14 studies [21], have indicated that the frequency of ischemic heart diseases and the risk for CHD is higher in patients with SH than in euthyroid controls [22,23,24]. In addition, SH is an independent coronary risk factor [22,25]. On the other hand, a previous study has reported that it is not always necessary to treat patients with SH [26]. Therefore, whether SH should be treated or not is controversial.

Remnant lipoproteins, such as chylomicron and very-low-density lipoprotein (VLDL) remnants, are TG-rich lipoproteins. TG-rich lipoproteins in the plasma originate either in the liver (VLDL) or intestine (chylomicron). They are cleared rapidly from blood circulation and are present in low concentrations in the plasma. However, in the case of a disturbed metabolism, the levels of serum remnant lipoproteins increase. TG-rich lipoproteins are considered to be atherogenic because they are taken up by macrophages in the arterial walls to produce foam cells. Therefore, this type of lipid is known to be a risk factor for CHD [13]. Postprandial hyperlipidemia is closely related to CHD, because the serum levels of such atherogenic lipoproteins remain high almost throughout the day, even until several hours before breakfast. Only one study has investigated postprandial hyperlipidemia in patients with SH in regard to serum TG levels; however, no significant differences were observed between the AUC of TG after fat loading in the patients with SH and euthyroid controls [27]. The abovementioned study had some limitations in that other TG-rich lipoproteins were not measured; therefore, the data were not sufficient to carry out the evaluation in the postprandial state.

We measured RLP-C levels as well as TG levels to correctly evaluate the postprandial state. A report suggested that postprandial increase in RLP-C levels is an independent risk factor for restenosis after successful percutaneous coronary intervention; fur-
thermore, this observation was valid even if the fasting RLP-C levels were within the normal range [28]. In active acromegalic patients known to have a risk of premature atherosclerosis, the incremental area under the postprandial RLP-C curve (delta AUC of RLP-C) after the fat-loading test was significantly elevated as compared to that in the controls, but no significant differences were observed in the delta AUC of TG [29]. Therefore, we were unable to confirm the absence of postprandial hyperlipidemia by measuring only the postprandial TG levels. In addition to the TG levels, we should measure serum RLP-C levels, which is a marker of postprandial hyperlipidemia.

It is practically difficult to carry out the fat-loading test in daily clinical situations. According to a previous report, a single measurement of RLP-C in the fasting state can be adequate and useful for evaluating the daily atherogenic remnant lipoproteins [17, 30], and in our study, the fasting RLP-C levels were also closely related to the AUC of RLP-C, which is in agreement with the previous reports. Therefore, this study suggests that the fasting RLP-C level is important and can be used as a representative of postprandial RLP-C levels in patients with SH.

On the other hand, we did not observe a significant difference in other lipid profiles, including the T-C, TG, and LDL-C levels, between normal controls and patients with SH. Further, no significant effect of L-T4 replacement was observed between them. With regard to the postprandial state, no significant differences were observed in the AUC of TG levels between the normal controls and patients with SH; however, a trend of reduction was observed (P = 0.06). These results are partly because the serum TSH levels (mean, 8.3 mU/I) of the patients in our study showed a relatively small increment. In fact, a meta-analysis of the effect of L-T4 replacement on lipoproteins in patients with SH demonstrated a reduction in the T-C and LDL-C levels [31]. A study of mild SH (TSH levels, 5–10 mU/I), however, has not shown significant improvement in the fasting lipid profiles after thyroxine treatment [32]. Our results of fasting lipid profiles, except the RLP-C level, concur with those of the abovementioned report. On the other hand, as compared to the TG levels, the RLP-C levels promptly and significantly changed in not only the fasting but also postprandial states; therefore, this study indicates that serum RLP-C levels are a better indicator of postprandial hyperlipidemia than TG levels.

In summary, the present study has demonstrated, probably for the first time, that the metabolism of postprandial RLP-C levels is disturbed in patients with SH, and that L-T4 replacement therapy induces a reduction in RLP-C levels in both the fasting and postprandial states. Such decreases in RLP-C levels could be important for improving the risks of cardiovascular complications in L-T4-treated patients with SH.

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