Serum Leptin Levels and Bioelectrical Impedance Assessment of Body Composition in Patients with Graves’ Disease and Hypothyroidism

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Abstract. We investigated whether thyroid status modulates serum leptin concentrations and body composition as determined by bioelectric impedance analysis (BIA). The percent body fat mass (%FM) in male Graves’ disease was significantly lower than that in age- and sex- matched normal subjects, at the levels of 11.4 ± 6.4% (mean ± SD) vs 19.9 ± 9.2% for men (n=12, P < 0.05) but not for women (22.6 ± 7.6% vs 24.9 ± 13.1%, n=28). In contrast, in female hypothyroidism (n=11) %FM was significantly higher than that in normal subjects (32.9 ± 11.5%, P < 0.01). Among other body composition parameters, the percentage of body water (%BW), and lean body mass (LBM) were significantly lower in hypothyroid patients, and the ECM (extracellular mass)/BCM (body cell mass) ratio was significantly (P < 0.0001) increased in Graves’ disease which was the result of marked depletion of BCM with concomitant expansion of ECM. The serum leptin levels were significantly decreased in male Graves’ patients (2.3 ± 0.7 ng/ml, P < 0.05), whereas in female Graves’ patients (8.8 ± 5.9 ng/ml) and patients with hypothyroidism (9.5 ± 7.6 ng/ml), the levels were not different from those of normal controls matched for BMI or %FM. There was a positive correlation between serum leptin levels and %FM in female Graves’ patients (r=0.635, P=0.001) and in hypothyroid patients (r=0.801, P=0.014) but not in male Graves patients. There was no significant relationship between serum leptin levels and thyroid hormones, TRAb, or TSAb. In euthyroid obese subjects there was a positive relationship between serum leptin levels and serum TSH levels (r=0.37, P<0.01). These results suggest that hyperthyroidism is characterized by the decreased fat mass and serum leptin levels in men, but female patients appear to be resistant to the effect of thyroid hormones. Together with previous reports, thyroid status has a minor role in the regulation of serum leptin levels.

Key words: Leptin, Body composition, Graves’ disease, Hypothyroidism

THE ob gene is an adipocyte-specific gene that encodes leptin, a protein that regulates body weight and energy expenditure [1–3]. In humans, obesity is associated with the increased expression of ob gene mRNA as well as plasma leptin levels, and there is a positive correlation between circulating leptin concentrations and body fat mass [4, 5]. However, the mechanism by which obesity increases leptin production has not been clarified. Recent in vivo and in vitro studies have shown that insulin [7–10], glucocorticoids [11, 12], and estrogens [13] are positive regulators in leptin production, whereas β3- adrenergic stimulators [14] or androgens [15] negatively regulate leptin mRNA expression.

As to thyroid hormones, alterations in thyroid hormone levels are frequently associated with changes in body weight, and are closely related to energy expenditure [16]. Nevertheless, the effect of
thyroid hormones on leptin production and serum leptin concentrations in thyroid disorders are inconsistent in many in vitro and in vivo studies. In in-vitro studies, Yoshida et al. [17] demonstrated that thyroid hormones stimulate the expression of leptin mRNA and secretion of leptin in 3T3-L1 adipocytes. On the other hand, in in vivo studies, Fain et al. [18] observed increased leptin mRNA expression in hypothyroid rats and a reduction in expression in response to T3 treatment. In contrast, T3 administration did not change the serum leptin levels in humans [19]. Thus, there are many conflicting results on serum leptin levels in thyroid disorders [20-26].

To assess the chronic effect of thyroid hormones on serum leptin levels, we measured serum leptin levels in untreated patients with Graves’ disease and hypothyroidism, and correlated them with body composition determined by bioelectric impedance method.

Methods

Subjects

Forty patients with untreated Graves’ disease (12 men and 28 women; mean age, 42.0±11.3 and 40.9±11.3 years, respectively), and 11 hypothyroidism due to Hashimoto’s disease (all women; mean age, 40.7±12.5 years) were included in this study. Graves’ disease was diagnosed with the clinical features, the elevated thyroid hormone levels, and the positive TSH receptor antibody (TRAb) with suppressed TSH levels (<0.1 μU/ml). The diagnosis of hypothyroidism was based on clinical symptoms and signs, elevated serum TSH (>4.0 μU/ml), and reduced serum free thyroxine (<0.95 ng/dl). The anti-microsomal and anti-thyroglobulin antibodies were positive in all hypothyroid patients. One hundred and two normal subjects (49 men and 53 women; mean age, 42.2±13.8 and 41.7±12.5 years, respectively) served as controls with matching age and sex. Blood samples were collected between 13:00-15:00 h in our outpatient clinic, and the serum was frozen at −80°C until analysis.

Measurement

The percentage of body fat and other parameters were calculated by bioelectric impedance analysis [27] (RJL Systems Inc., Mount Clemens, MI). The percent body fat mass (%FM), lean body mass (LBM), body cell mass (BCM), and extracellular mass (ECM) were calculated by resistance and reactance by using a computer program provided by the manufacturer of the instrument. Serum leptin concentrations were measured by RIA (Linco Research, Inc., St. Charles, MO) using recombinant human leptin. The limit of detection was 0.5 ng/ml, and the intra- and inter-assay coefficient of variation were 4.1% and 6.5%, respectively. Serum TSH levels were measured with IRMA kits (TSH kit, Daiichi RI, Tokyo, Japan). FT4 were measured with commercial RIA kits (Daiichi RI, Tokyo, Japan). TSH receptor antibody (TRAb) was measured with a kit from Johnson & Johnson Clinical Diagnostics Ltd., Amersham, England. Thyroid stimulating antibodies (TSAb) were measured in Graves’ patients using a commercial kit measuring cAMP production in cultured porcine thyroid cells (Yamasa Co., Chiba, Japan). The normal ranges of TRAb and TSAb are less than 10% and less than 160%, respectively.

Statistical analysis

All data are presented as means±SD. Comparisons among groups were performed with one-way ANOVA using Scheffe’s test for multiple comparisons. To determine the independent effects of variables on serum leptin levels, simple and multiple linear regression analyses were performed after leptin values were log-transformed to increase the normality of their distribution. Paired t-test was used to compare the serum leptin levels before and after treatment. P<0.05 was considered significant. All statistical calculations were performed with Stat View 4.5 and Super ANOVA software (Abacus Concepts, Berkeley, CA).

Results

Body composition analysis

Body mass index (BMI), thyroid function data, and %FM in normal subjects and patients with Graves’ disease and hypothyroidism are shown in Table 1. Compared with age- and sex-matched
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Table 1. Clinical data in normal subjects and patients with Graves’ disease and hypothyroidism

<table>
<thead>
<tr>
<th></th>
<th>sex</th>
<th>cases</th>
<th>age (years)</th>
<th>Height (cm)</th>
<th>BW (kg)</th>
<th>BMI (kg/m²)</th>
<th>fT4 (ng/dl)</th>
<th>TSH (ng/ml)</th>
<th>TRAb (%)</th>
<th>TSAb (%)</th>
<th>% FM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>M</td>
<td>49</td>
<td>42.2±13.8</td>
<td>168.0±7.2</td>
<td>73.3±12.9</td>
<td>25.7±4.2</td>
<td>1.3±0.2</td>
<td>1.9±1.1</td>
<td>3.6±1.4</td>
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<td>19.9±9.2</td>
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<tr>
<td></td>
<td>F</td>
<td>53</td>
<td>41.7±12.5</td>
<td>156.1±5.2</td>
<td>56.3±11.7</td>
<td>24.5±6.1</td>
<td>1.2±0.3</td>
<td>2.0±0.9</td>
<td>2.9±1.9</td>
<td>-</td>
<td>24.9±13.1</td>
</tr>
<tr>
<td>Graves’ disease</td>
<td>M</td>
<td>12</td>
<td>42.0±13.0</td>
<td>171.3±7.3</td>
<td>60.6±10.0</td>
<td>20.7±3.5</td>
<td>5.4±2.4</td>
<td>&lt;0.1</td>
<td>52.0±22.0</td>
<td>433±460</td>
<td>11.4±6.4c</td>
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<tr>
<td></td>
<td>F</td>
<td>28</td>
<td>40.9±11.3</td>
<td>155.2±4.5</td>
<td>51.4±10.8</td>
<td>21.5±4.0</td>
<td>4.9±2.6</td>
<td>&lt;0.1</td>
<td>63.6±25.0</td>
<td>439±560</td>
<td>22.6±7.6</td>
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<tr>
<td>Hypothyroidism</td>
<td>F</td>
<td>11</td>
<td>40.7±12.5</td>
<td>158.2±5.6</td>
<td>65.0±13.2</td>
<td>25.5±4.8</td>
<td>0.6±0.5</td>
<td>60.2±39.4</td>
<td>2.8±4.0</td>
<td>-</td>
<td>32.9±11.5a</td>
</tr>
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</table>

These data are shown as mean±SD.
a: P<0.01 and b: P<0.05 vs normal subjects in each gender, and c: P<0.005 vs female subjects.

healthy controls, BMI was significantly (P<0.05, by ANOVA) lower in Graves’ patients for both men and women. The %FM determined by bioelectrical impedance analysis showed a significant decrease in male Graves’ patients compared to normal male subjects (11.4±6.4% vs 19.9±9.2%, P<0.05) or female Graves’ group (11.4±6.4% vs 22.6±7.6%, P<0.005). In contrast, in patients with hypothyroidism %FM was significantly increased compared to normal female subjects (32.9±11.5% vs 24.9±13.1%, P<0.01). However, %FM was not directly related to thyroid hormone (fT4) or TSH levels. As shown in Fig. 1, among other body composition parameters, lean body mass (%LB), and the percentage of body water (%BW) were significantly (P<0.05) lower in patients with hypothyroidism. In female Graves’ disease, the ECM (extracellular mass)/BCM (body cell mass) ratio was significantly increased compared to those in normal controls (1.24±0.24 vs 1.03±0.14, P<0.001) which was the result of marked decrease of BCM with concomitant expansion of ECM. There was a similar result in male Graves’ patients compared to those in normal men. There was a strong positive relationship between se-

![Fig. 1](image-url)

Fig. 1. The mean differences in percentage of body components in patients with female Graves’ disease (the black column) and hypothyroidism (the dotted column) compared to normal female controls. Data were expressed as mean±SD. LB=lean body, BW=body water, BCM=body cell mass, ICM=intracellular mass, ECW=extracellular water, ECM=extracellular mass. These parameters were calculated from resistance and reactance by using the computer program Bodycomp2.55 supplied with the instrument. *P<0.05, **P<0.01, ***P<0.0001 compared with controls.
rum free T4 levels and the ECM/BCM ratio ($r = 0.67$, $P < 0.001$) and a negative correlation between $f$T4 levels and BCM ($r = -0.44$, $P < 0.001$) in all studied groups.

**Serum leptin concentrations**

To study whether thyroid hormone status was linked to the changes in serum leptin concentrations, serum leptin levels were measured in patients with Graves’ disease and hypothyroidism. In Graves’ disease with thyrotoxicosis, the mean serum leptin level was $2.3 \pm 0.7$ ng/ml in men ($n=12$) and $8.8 \pm 5.9$ ng/ml in women ($n=28$), respectively (Fig. 2). In male Graves’ patients serum leptin levels were significantly ($P < 0.05$) lower than those in normal men or female Graves’ patients. In patients with hypothyroidism ($n=11$), serum leptin levels were $9.5 \pm 7.6$ ng/ml, which was not different from those of normal controls. As leptin levels are influenced by body fat mass, we compared the leptin levels among the three groups after adjusting the percent fat mass, with a significant ($P < 0.05$) decrease of serum leptin levels being observed only in patients with male Graves’ disease (data not shown). In our previous study in normal subjects, a strong positive correlation was observed between log-transformed concentrations of leptin and %FM in both men ($r=0.606$, $P<0.0001$) and women ($r=0.707$, $P<0.0001$) [28]. As shown in Table 2, there was a similar positive correlation between serum leptin levels and %FM in female Graves’ patients ($r=0.635$, $P=0.001$) and hypothyroid patients ($r=0.801$, $P=0.014$), however, serum leptin levels in male Graves’ patients were not significantly related to %FM ($r=0.184$, $P=0.620$). Neither fT4, TSH, TRAb nor TSAb was directly correlated with the serum leptin levels in patients with thyroid disease. Only in normal subjects including men and women, log-transformed serum leptin levels significantly correlated with serum TSH levels ($N=66$, $r=0.431$, $P<0.001$, Fig. 3), and concomitantly, a negative correlation was observed between serum fT4/TSH.

![Fig. 2. Serum leptin levels in normal subjects, in patients with Graves' disease and hypothyroidism. Leptin levels were significantly lower in male groups (M) than those in female groups (F) in either normal subjects or Graves' patients (by ANOVA). There is no difference in serum leptin levels among female groups of normal subjects, Graves' patients or hypothyroid patients.](image)

<table>
<thead>
<tr>
<th>Table 2. Simple regression analysis with log-transformed concentration of leptin as a dependent variable</th>
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<tr>
<td>variable</td>
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<tr>
<td></td>
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<tr>
<td>BMI</td>
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<tr>
<td>fat mass (%)</td>
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<tr>
<td>fat mass (kg)</td>
</tr>
<tr>
<td>TRAb (%)</td>
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<tr>
<td>TSAb (%)</td>
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<td>fT4</td>
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<td>TSH</td>
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ratios and log-transformed leptin levels ($r = -0.32$, $P < 0.01$). Although body fat mass is the major determinant of serum leptin levels, there was no correlation between serum fT3 or fT4 levels and leptin levels expressed as the concentrations per absolute body fat (kg) in Graves' patients. We next performed multiple regression analysis with log-transformed leptin as the dependent variable, and gender, %FM, serum TSH or fT4 as independent variables. However, even in this model, only gender and %FM significantly account for the variability in serum leptin levels (data not shown). Among other body composition parameters, serum leptin levels were positively correlated with BCM ($r = 0.611$, $P < 0.0001$) and ECM ($r = 0.315$, $P < 0.01$), and inversely, were negatively correlated with LB% ($r = -0.563$, $P < 0.0001$) and BW% ($r = -0.644$, $P < 0.0001$) in all studied female subjects. In male groups, serum leptin levels were correlated only with LB% ($r = -0.583$, $P < 0.0001$).

Changes in serum leptin levels after treatment

We next investigated the changes in serum leptin levels in female Graves' patients and hypothyroid patients before and after thyroid function was normalized (Fig. 4). The mean duration of treatment were 5.6 ± 1.7 months in Graves' disease (n = 9) and 5.2 ± 3.9 months in hypothyroidism (n = 11), respectively. They were treated with antithyroid drugs (Methimazole or propylthiouracil) and thyroid hormone (thyroxine), respectively. As shown in Fig. 4, a slight but significant decrease of serum leptin levels was seen in female Graves' patients after treatment (10.4 ± 8.2 ng/ml vs 8.2 ± 6.4 ng/ml, $P = 0.016$), but, there was no significant difference in the values before and after treatment in hypothyroidism (8.4 ± 5.0 ng/ml vs 10.8 ± 7.2 ng/ml, $P = 0.127$). In male Graves' patients, there was no significant change in serum leptin levels between before and after treatment (n = 7, 2.4 ± 1.3 ng/ml and 2.3 ± 0.8 ng/ml, respectively).
Discussion

The effect of thyroid status on serum leptin concentrations has been a matter of controversy. Hyperthyroidism was associated with increased serum leptin concentrations in one study [22], but the levels were not different from those in normal subjects in other studies [19-21, 23, 24]. The values in hypothyroidism have been reported unchanged [20, 21, 23, 24], elevated [25], or even decreased [26]. It has also been shown that leptin mRNA expression increased in hypothyroid rats and decreased in response to T3 administration [18]. The reason for these discrepancies is not clear. Differences in the studied group not divided by gender, the severity of the disease, duration of thyroid dysfunction or timing of blood sampling (the influence of diurnal rhythm on leptin) may be responsible for these discrepancies.

To our knowledge, however, none of the previous studies has correlated serum leptin levels to body composition in patients with thyroid diseases. We measured body composition in normal subjects and patients with thyroid disorders, because the data in thyroid diseases have not been reported for Japanese. Among several parameters, the ECM/BCM ratio was the most sensitive in discriminating normal subjects and patients with thyroid disorders, because the data in thyroid diseases have not been reported for Japanese. We next correlated these body composition parameters with serum leptin concentrations. As reported by numerous studies including ours [28], there was a positive correlation between serum leptin levels and BMI or %FM in female patients with either hyper- or hypothyroidism. However, this correlation was not observed in male Graves' disease, which may be accounted for by the limited range of %FM and the influence of androgen. The serum leptin concentrations in male Graves' disease were also significantly lower than those in normal subjects matched for sex, age, and %FM. Thyroxine may directly inhibit leptin mRNA expression in adipocytes [32], however, it has been shown that thyroxine hormone stimulates the expression in adipocytes [17]. There was no correlation between serum fT4 and leptin levels in either male or female Graves' patients, as reported by Sreenan et al. [20]. Furthermore, normalization of thyroid function with antithyroid drugs only slightly affected serum leptin levels, indicating that the direct role of thyroxine on circulating leptin is minor, if any. Adrenergic stimulation of adipocytes have been shown to inhibit leptin production [33]. Since hyperthyroidism has been characterized as a functional hyperadrenergic state [34], this may be involved in suppression of leptin production. Whichever the case, it is not clear why serum leptin levels were significantly decreased only in male patients.

Reportedly adipocytes express TSH receptors [35]. TSH or its agonists, such as TSH receptor antibodies (TRAb) could inhibit leptin mRNA expression through cAMP pathways, because these pathways are inhibitory to leptin production [33, 36]. However, this is unlikely because there was no correlation between serum TSAb and leptin levels in either male or female Graves' disease.

The serum leptin levels in female patients with either hyper- or hypothyroidism were comparable to those in normal women whether or not the values were adjusted for sex, age, and % body fat mass. As with normal subjects [37, 38], the values in female patients with Graves' disease were significantly higher than those in male hyperthyroid patients, consistent with the inhibitory effect of androgen on leptin mRNA expression [15]. Failure of inhibition of leptin by hyperthyroidism in women may be due to relative resistance to leptin in this gender [37]. Again, there was no correlation between serum leptin levels and fT4 levels in female patients with thyroid disorders (Table 2), and neither were leptin levels affected by treatment with thyroxine, making the
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Although there was no correlation between serum TSH and leptin levels in hypothyroid patients, we have found a positive correlation between log-transformed serum leptin levels and serum TSH levels in normal subjects. There was also a negative correlation between leptin levels and fT4/TSH ratio in normal subjects. These findings were similar to the results reported by Pinkney et al. [25], who showed a positive correlation between plasma TSH levels and plasma leptin (r=0.61; P<0.001) or %FM (r=0.67; P<0.001) in obese subjects. Wesche et al. [39] also reported that the larger thyroid size in the obese subjects was associated with slightly but significantly higher TSH and lower fT4 serum concentrations compared to the non-obese subjects. The elevated TSH levels may be due to the stimulatory effects of leptin on the hypothalamic-TSH axis, since it has been shown that leptin per se prevents the fasting-induced suppression of hypothalamic pro-TRH gene expression and the amount of TRH, and thereby stimulates TSH [40]. Alternatively, leptin may make thyroid gland less sensitive to TSH stimulation. Furthermore, we have found that leptin receptor mRNA is expressed not only in FRTL-5 rat thyroid cells but also in cultured porcine thyroid cells, and that treatment with leptin attenuates TSH-induced iodide metabolism (Isozaki O, in preparation). These results suggest a communication between hypothalamic-TSH axis and adipocyte functions. It remains to be determined whether this putative relationship is disturbed in thyroid diseases.

In summary, the present study has shown that thyroid status has a minor effect on serum leptin levels despite the reported stimulatory effect of thyroid hormones on leptin mRNA expression in vitro. As was in normal subjects, regression analysis revealed that %FM or BMI accounted for the majority of leptin variance.

References


