Lack of effects of circulating thyroid hormone levels on serum leptin concentrations

Sabrina Corbetta, Piera Englaro, Salvatore Giambona, Luca Persani, Werner F Blum and Paolo Beck-Peccoz

Institute of Endocrine Sciences, University of Milan, Ospedale Maggiore di Milano-IRCCS, Centro Auxologico Italiano IRCCS and Istituto Clinico Humanitas, Milano, Italy and 1University Children’s Hospital, Giessen, Germany and Lilly Germany, Bad Homburg, Germany

(Correspondence should be addressed to P Beck-Peccoz, Istituto Clinico Humanitas, Via Manzoni 56, 20089 Rozzano-Milano, Italy)

Abstract

Leptin is the protein product of the \(ob\) gene, secreted by adipocytes. It has been suggested that it may play an important role in regulating appetite and energy expenditure. The aim of this study was to evaluate a possible interaction of thyroid hormones with the leptin system. We studied 114 adult patients (65 females and 49 males): 36 were affected with primary hypothyroidism (PH), 38 with central hypothyroidism (CH) and 40 with thyrotoxicosis (TT). Patients with CH were studied both before and after 6 months of \(l\)-thyroxine replacement therapy. Body mass index (BMI; kg/m\(^2\)), thyroid function and fasting serum leptin were assessed in all patients. Since BMI has been proved to be the major influencing variable of circulating leptin levels, data were expressed as standard deviation score (SDS) calculated from 393 male and 561 female controls matched for age and BMI.

No difference in SDS was recorded between males and females whatever the levels of circulating thyroid hormones. In males, no significant difference was recorded among the SDSs of PH (\(\bar{x} = 0.36 \pm 1.2\)), TT (\(\bar{x} = 0.35 \pm 1.2\)) and CH (0.01 \(\pm 0.7\)) patients. Females with PH had an SDS significantly lower than TT females (\(\bar{x} = 0.77 \pm 1.0\) vs \(\bar{x} = 0.06 \pm 1.2\); \(P < 0.02\)), while no significant differences between CH (\(\bar{x} = 0.34 \pm 0.7\)) and TT females or between CH and PH females were observed. SDS in CH patients after 6 months of \(l\)-thyroxine therapy significantly varied only in females (0.25 \(\pm 1.4\)). In conclusion, circulating thyroid hormones do not appear to play any relevant role in leptin synthesis and secretion. However, as females with either overt hypo- or hyper-thyroidism or central hypothyroidism after \(l\)-thyroxine therapy show differences in their SDSs, a subtle interaction between sex steroids and thyroid status in modulating leptin secretion, at least in women, may occur.

Introduction

Leptin is a 146-amino acid protein hormone encoded by the \(ob\) gene (1) and secreted by adipocytes in response to an increase in fat mass (2–4). It seems to be a key molecule in the feedback loop that regulates energy balance (5). Leptin has a dual action: it decreases the appetite and increases energy consumption, causing more fat to be burned. In fact, both \(ob/ob\) and \(db/db\) mice are characterized by numerous abnormalities, such as severe insulin resistance, infertility, decrease in lean body mass, cold intolerance and hypothermia (6, 7), while administration of leptin to \(ob/ob\) mice results in a reduction in food intake, an increase in locomotor activity (with an increase in oxygen consumption) and a reduction in body weight (8, 9). As substantial variability in serum leptin levels occurs among individuals with comparable degrees of obesity (10), other factors in addition to the amount of body fat appear to regulate leptin secretion. Recent reports suggest a role for insulin (11), glucocorticoids (12, 13), catecholamines (14, 15) and sex hormones (16).

In thyroid disorders there are changes in basal metabolic rate, oxygen consumption, appetite and body weight. Thyroid hormones are important regulators of both basal and total energy consumption, and modulate the activity of several enzymes involved in lipid metabolism (17, 18). They are therefore a major component in the regulation of energy balance and body composition in humans. In the present study, we evaluated the potential interaction of circulating thyroid hormones with the leptin system in patients with various thyroid disorders, ranging from severe primary hypothyroidism to overt thyrotoxicosis. In addition, we investigated serum leptin levels both before and after \(l\)-thyroxine (\(T_4\)) replacement therapy in patients with central hypothyroidism.

Materials and Methods

Subjects

We studied 114 adult patients (65 females and 49 males) aged 48.0 \(\pm 16.9\) years (mean \(\pm\) S.D.) with thyroid
disorders: 36 were affected with autoimmune primary hypothyroidism (PH; 25 females, 11 males), 38 with central hypothyroidism mainly due to pituitary tumors (CH; 13 females, 25 males) and 40 with thyrotoxicosis due to either Graves’ disease or toxic nodular goiter (TT; 27 females, 13 males) (Table 1). CH patients were investigated during cortisol and/or sex steroid replacement therapy. All had growth hormone (GH) deficiency but none was being treated with recombinant human GH. Body weight, height and body mass index (BMI; kg/m²) were determined for each patient. Thyroid function was assessed by measuring serum fasting free triiodothyronine (FT₃), free thyroxine (FT₄), thyrotropin (TSH) and anti-thyroid autoantibody levels. None of the subjects was taking thyroid medications. CH patients were reinvestigated after 6 months of T₄ replacement therapy. Serum leptin levels were measured at 0800 h after an overnight fast. Reference ranges were derived from 393 healthy males and 561 females aged 20–81 years.

**Assays**

Serum leptin levels were determined by RIA with an inter- and intra-assay coefficients of variation of 6.5 and 0.8% respectively and a sensitivity of 0.03 µg/l (19). Circulating levels of TSH and FT₄ were measured by ultrasensitive immunofluorimetric assay and fluorometric immunoassay respectively, using the Delfia technique (Pharmacia, Milan, Italy). Serum FT₃ levels were measured by using FT₃ Amerlite MAB (Johnson & Johnson Clinical Diagnostics, Milan, Italy).

**Statistical methods**

Age, BMI, serum TSH, FT₄, FT₃ and leptin levels were compared among the various groups of patients using Student’s t-test and within each group by linear regression analysis. Data are expressed as means ± s.d. Leptin levels were adjusted for gender and BMI by calculating the standard deviation scores (SDS) according to the following formula (20): for males, $SDS = \ln leptin – \ln 0.0237 \times (0.1985 \times BMI)/0.63859$; for females, $SDS = \ln leptin – ln 0.3204 – (0.1448 \times BMI)/0.52483$.

**Results**

As seen in the control group, serum leptin levels in males with thyroid disorders were in general lower than those recorded in females. Leptin values correlated well with BMI in both male (Fig. 1) and female (Fig. 2) patients, with no evident relationship with the thyroid status, the various slopes of the correlation lines being similar in the different groups of patients. Serum leptin levels in PH and CH male patients were 6.4 ± 8.2 and 7.8 ± 6.9 µg/l, while in females they were 13.2 ± 16.0 and 33.1 ± 28.1 µg/l respectively (Table 1). The difference found between PH and CH

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**Table 1 Clinical and biochemical data of patients with various thyroid disorders. Data are expressed as means ± s.d. and ranges are in parentheses.**

<table>
<thead>
<tr>
<th>No.</th>
<th>TSH (mU/l)</th>
<th>FT₄ (pmol/l)</th>
<th>FT₃ (pmol/l)</th>
<th>BMI (kg/m²)</th>
<th>Leptin (µg/l)</th>
<th>SDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PH 11</td>
<td>22.8 ± 17.8 e</td>
<td>7.6 ± 3.8 e</td>
<td>3.7 ± 1.4 e</td>
<td>26.0 ± 6.0</td>
<td>6.4 ± 8.2</td>
<td>-0.363 ± 1.21</td>
</tr>
<tr>
<td></td>
<td>(6.0–64.2)</td>
<td>(2.0–10.6)</td>
<td>(1.7–5.7)</td>
<td>(17.2–35.7)</td>
<td>(0.3–29.1)</td>
<td>(−1.97–1.72)</td>
</tr>
<tr>
<td></td>
<td>1.8 ± 2.3</td>
<td>3.8 ± 2.2 e</td>
<td>3.1 ± 1.0 e</td>
<td>27.7 ± 3.9 e</td>
<td>7.8 ± 6.9</td>
<td>0.012 ± 1.38</td>
</tr>
<tr>
<td>CH basal 25</td>
<td>(0.04–9.1)</td>
<td>(0.5–8.4)</td>
<td>(1.5–5.4)</td>
<td>(19.3–35.5)</td>
<td>(1.1–30.4)</td>
<td>(−3.99–2.19)</td>
</tr>
<tr>
<td>CH treated 25</td>
<td>0.1 ± 0.2 e</td>
<td>14.0 ± 3.3</td>
<td>5.5 ± 1.0</td>
<td>27.1 ± 3.8 e</td>
<td>7.7 ± 6.0</td>
<td>0.211 ± 1.22</td>
</tr>
<tr>
<td>TT 13</td>
<td>(&lt; 0.01–0.7)</td>
<td>(9.8–25.5)</td>
<td>(4.2–8.2)</td>
<td>(19.1–33.6)</td>
<td>(1.3–23.7)</td>
<td>(−2.69–2.25)</td>
</tr>
<tr>
<td>Controls 393</td>
<td>0.3–4.0</td>
<td>10–18</td>
<td>4–8</td>
<td>24.6 ± 3.3</td>
<td>4.1 ± 3.6</td>
<td>-2.00–2.00</td>
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<tr>
<td></td>
<td>(18.3–67.5)</td>
<td>(8.0–19.1)</td>
<td>(16.5–34)</td>
<td>(0.1–21.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PH 25</td>
<td>54.6 ± 12.6 e</td>
<td>4.4 ± 2.8 e</td>
<td>3.0 ± 1.1 e</td>
<td>25.0 ± 6.3</td>
<td>13.2 ± 16.0</td>
<td>-0.771 ± 1.00</td>
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<td></td>
<td>(6.1–315.0)</td>
<td>(1.0–9.1)</td>
<td>(1.0–4.9)</td>
<td>(19.0–41.9)</td>
<td>(1.2–68.9)</td>
<td>(−2.74–1.79)</td>
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<tr>
<td>CH basal 13</td>
<td>2.1 ± 1.8</td>
<td>3.7 ± 2.1 e</td>
<td>2.7 ± 0.9 e</td>
<td>29.0 ± 4.8 e</td>
<td>33.1 ± 28.1 e</td>
<td>-0.335 ± 0.66</td>
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<td>(0.2–6.9)</td>
<td>(0.5–7.4)</td>
<td>(1.3–4.4)</td>
<td>(21.8–39.1)</td>
<td>(7.6–114.4)</td>
<td>(−1.46–0.56)</td>
</tr>
<tr>
<td>CH treated 13</td>
<td>0.15 ± 0.3 e</td>
<td>11.5 ± 3.5</td>
<td>5.0 ± 0.9</td>
<td>28.6 ± 4.7 e</td>
<td>27.5 ± 16.9 e</td>
<td>0.254 ± 1.35</td>
</tr>
<tr>
<td>TT 27</td>
<td>(&lt; 0.01–1.2)</td>
<td>(4.6–19.4)</td>
<td>(3.9–6.7)</td>
<td>(22.1–37.2)</td>
<td>(9.5–62.5)</td>
<td>(−2.17–2.28)</td>
</tr>
<tr>
<td>Controls 561</td>
<td>0.3–4.0</td>
<td>10–18</td>
<td>4–8</td>
<td>24.4 ± 4.0</td>
<td>14.4 ± 11.5</td>
<td>-2.00–2.00</td>
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<td>(19.5–92.2)</td>
<td>(5.6–72.2)</td>
<td>(15.8–43.1)</td>
<td>(0.6–94.4)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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No: Number of patients; SDS: Standard deviation scores; e: Expressed in parentheses.

Significant differences at the 0.01 level are indicated by an asterisk (*).
females was mainly due to the significantly different BMI in the two groups (25.0 ± 6.3 vs 29.0 ± 4.8 kg/m²; P < 0.015), and possibly to the concomitant untreated GH deficiency in the latter group. Leptin levels after T₄ treatment in CH patients were not significantly different from those recorded in pretreatment conditions (males: 7.7 ± 6.0 µg/l; females: 27.5 ± 16.9 µg/l), and a complete lack of correlation with FT₄ levels was seen. Serum leptin levels in male and female TT patients were 6.3 ± 7.6 and 10.9 ± 8.1 µg/l respectively. Interestingly, no significant differences in basal leptin concentrations were seen between TT and PH patients, as documented by the lack of correlation between leptin and FT₄ concentrations in PH and TT patients.

In order to avoid possible interference of BMI variations in the evaluation of serum leptin concentrations in the various groups of patients, we used the SDS calculated from normal controls matched for sex and BMI. Values of SDS higher than +2 or lower than −2 were seen in a minority of cases and were equally distributed among hyper- and hypothyroid patients. The correlations of SDS values and serum FT₄ concentrations in male and female patients with various thyroid disorders are reported in Figs 3 and 4 respectively.

The means of SDS values were similar in all groups of patients, with the exception of female CH patients before and after T₄ treatment (−0.34 ± 0.66 vs 0.25 ± 1.35, P < 0.04) and PH and TT females (−0.77 ± 1.00 vs −0.06 ± 1.20, P < 0.02) (Table 1). However, as depicted in Fig. 4, the SDS values overlapped greatly.
Discussion

The present results clearly suggest that circulating thyroid hormones do not play a major role in the regulation of leptin synthesis and secretion. Thus the ability of thyroid hormones to regulate energy expenditure does not operate through variations in serum leptin levels. Our results confirm and extend previous data showing that hypo- and hyperthyroidism in patients of both sexes (21) and short-term hyperthyroidism in males (22) do not alter circulating leptin concentrations. In contrast, they do not support previous studies in humans showing decreased leptin concentrations in hypothyroid patients (23) and in Zucker rats, demonstrating a decrease in leptin mRNA in response to T₄ administration (24). In the latter case, the significant loss of animal weight and the consequent decrease in adipose stores probably account for the reduced leptin gene expression.

In agreement with other reports (10, 11, 25, 26), we found that serum leptin concentrations recorded in both normal controls and patients with thyroid disorders were characterized by high variability, the major determinants of which are BMI and gender. This figure is documented by the significant correlation between leptin levels and BMI in any group of patients with thyroid disorders, and by the finding that leptin levels in males are consistently lower than those found in females. We therefore evaluated leptin data by using the SDS calculated from a huge number of controls matched for sex, age and BMI. The analysis of SDS data definitely indicates that no relationship between leptin and thyroid hormone levels exists. Indeed, only slight definite indicates that no relationship between leptin and thyroid hormone levels exists. Indeed, only slight differences were recorded between mean SDS in PH and thyroid hormone levels exists. Indeed, only slight differences were recorded between mean SDS in PH and TT females and in CH females before and after 6 months of T₄ replacement therapy. Interestingly, these differences were not recorded in males, although the BMI of patients of the two sexes were not significantly different. This may suggest that leptin regulatory mechanisms in females are more subtly regulated than in males, possibly through circulating sex steroid hormone variations. However, recent data failed to demonstrate a direct role for estrogens in leptin regulation (27), but other reports indicated that variations in serum androgens cause leptin synthesis to be partially inhibited. Since both hypo- and hyperthyroidism cause variations in circulating sex hormone-binding globulin (28, 29), as well as in peripheral sex hormone metabolism, the putative increase in or the relative prevalence of circulating androgenic compounds in female patients with thyroid disorders may account for the difference in SDS found in female patients.

In conclusion, the role of thyroid hormones in modulating leptin synthesis and secretion seems to be of little, if any, clinical or biological relevance.

Acknowledgements

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