Circulating ghrelin in thyroid dysfunction is related to insulin resistance and not to hunger, food intake or anthropometric changes

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Abstract

Objective: Ghrelin is a gastric peptide that plays a role in appetite stimulation, energy balance and possibly in insulin resistance. Hyperthyroidism is a situation where negative energy balance and insulin resistance coexist, while in hypothyroidism a positive energy balance and normal insulin sensitivity predominate. We investigated ghrelin levels and their relationship with hunger, food intake and both anthropometric and insulin resistance parameters in patients with thyroid dysfunction.

Design and methods: We studied 24 hyperthyroid and 17 hypothyroid patients before and after normalisation of thyroid hormone levels and their respective body mass index (BMI)-matched control group. We measured plasma ghrelin levels, homeostasis model assessment of insulin resistance (HOMA-IR) index, a hunger score, mean three-day calorie intake and anthropometric parameters.

Results: In hyperthyroidism, HOMA-IR index was higher (3.21 ± 0.60 vs 1.67 ± 0.15 mM mU/l; \( P = 0.014, t \) test for independent data) and ghrelin levels were lower (463.6 ± 36.4 vs 561.1 ± 32.1 pg/ml; \( P = 0.041, \) Mann–Whitney U-test) than in its control group and both normalised after treatment (HOMA-IR: 2.28 ± 0.38 mM mU/l; \( P = 0.106, t \) test for independent data, and ghrelin: 539.7 ± 45.4 pg/ml; \( P = 0.549, \) Mann–Whitney U-test). Glucose, as a component of HOMA-IR index was the only predictor for ghrelin levels (\( \beta = -0.415, P = 0.044, \) stepwise multiple regression analysis). In hypothyroidism, HOMA-IR index and ghrelin levels were similar to those in its control group both before and after treatment. In both thyroid dysfunction states, no correlations were observed between changes in ghrelin levels and in free T4, free T3, anthropometric parameters, total calorie intake and hunger score.

Conclusions: In thyroid dysfunction states, ghrelin levels seemed to be in relation to insulin resistance and not to energy balance and food intake regulation, as seen in other physiological and pathological states.

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Introduction

Ghrelin is a novel enteric hormone that increases food intake, body weight and, growth hormone (GH) secretion as potently as any known peptide (1–6). A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans (7). It has been suggested that ghrelin is one of the peripheral hormones, together with leptin, insulin and glucocorticoids, which permit the central regulation of energy balance. It is thought that these peripheral hormones exert their effects on energy homeostasis either by activating or inhibiting the activity of the orexigenic or anorexigenic peptides within the hypothalamus (8).

Ghrelin stimulates food intake through the activation of neuropeptide Y (NPY)/agouti-related protein (AGRP) producing neurons (9).

In states of positive energy balance such as obesity or the postprandial state, ghrelin concentrations are suppressed (10–12) and, conversely, they are increased (13) in negative energy balance states such as anorexia nervosa. It appears that insulin may suppress circulating ghrelin levels (14–16) and that there is an inverse association between ghrelin and insulin resistance (17, 18).

Thyroid dysfunction is associated with changes in body weight, food intake and energy expenditure. Hyperthyroid patients usually experience a decrease
in body weight in spite of hyperphagia and thyroid hormone excess induces appreciable insulin resistance, while hypothyroidism is associated with weight increase and normal insulin sensitivity (19–22).

Data about ghrelin levels in human thyroid dysfunction are limited. To date, only one study has been performed in a small number of patients with hyperthyroidism, which reported suppressed circulating ghrelin levels despite being in a negative energy balance state (23). In this study food intake was not evaluated. On the other hand there is no information available on hypothyroid patients. Therefore, to elucidate the potential role of ghrelin in the regulation of food intake and energy balance during thyroid dysfunction, we have evaluated fasting plasma ghrelin levels in both hyper and hypothyroidism before and after normalisation of thyroid function and its relationship with hunger, caloric intake, and both anthropometric and insulin resistance parameters.

**Subjects and methods**

The study was performed in 41 patients with thyroid dysfunction recruited from the outpatient clinic of the Diabetes, Endocrinology and Nutrition Unit (Institut Universitari Parc Taulí, Sabadell, Spain). Forty-five euthyroid subjects served as the control group. The diagnosis of hyperthyroidism or hypothyroidism was based on clinical assessment and biochemical findings (free T4 (FT4), free T3 (FT3), and thyrotropin (TSH)).

The hyperthyroid group consisted of 24 patients (19 women and five men, mean age 42.7±2.7 years). The aetiology of the hyperthyroidism was Graves’ disease (n = 23) and toxic multinodular goitre (n = 1). The hypothyroid group consisted of 17 women (mean age 46.9±3.7 years) and the aetiology of the hypothyroidism was chronic autoimmune thyroiditis (n = 6), radioactive iodine therapy (n = 6), withdrawal of thyroid hormone therapy before a total body scan for papillary thyroid carcinoma (n = 3), non-autoimmune hypothyroidism (n = 1) and postpartum thyroiditis (n = 1).

The control group consisted of 45 euthyroid subjects, of which 25 (21 women and four men, mean age 40.2±2.4 years) matched for body mass index (BMI) served as control group for hyperthyroidism, and 20 women (mean age 43.7±3.2 years) also matched for BMI served for hypothyroid patients.

Patients were evaluated at the time of diagnosis and after normalisation of thyroid function (at least normal FT4 for hyperthyroidism and both normal FT4 and TSH for hypothyroidism) with appropriate medical therapy. Hyperthyroid patients were treated with methimazole. L-thyroxine was prescribed to hypothyroid patients to establish biochemical euthyroidism. Healthy subjects were tested once. All participants were admitted to the Endocrine Unit (Institut Universitari Parc Taulí) in the morning between 0800 h and 0900 h after a 12 h overnight fast. We performed a complete physical examination, including the measure of the waist-to-hip ratio and body composition by bioelectrical impedanciometry after emptying the urine bladder. Blood samples were obtained to measure serum concentrations FT4, FT3, TSH and plasma ghrelin concentrations. Thyroid peroxidase autoantibodies (TPOAbs), thyroid-stimulating immunoglobulins and other laboratory and complementary investigations were carried out in patients with thyroid dysfunction as part of the diagnostic work-up. Participants with thyroid dysfunction were asked to record their food intake over a period of 3 days in the week following the study (one at the weekend and two on consecutive working days) and hunger was evaluated using 100-mm visual analog scale preceded by the question ‘Do you feel hungry?’. The scale was anchored with ‘not at all’ and ‘extremely’ at the left and right ends, respectively. The distance from the extreme left to the subject’s vertical dash represented the rating score, expressed in mm as previously described (24). All the subjects were interviewed by a dietician about their food intake. Macronutrient composition and daily calorie intake were calculated with the use of food composition tables (Diet Source 2.0, Novartis Consumer Health, S.A. Barcelona, 1977–2003, www.novartismedicalnutrition.com.es).

Body composition was measured by bioelectric impedanciometry (body composition analyzer TBF-300, TANITA, Tokyo, Japan).

The homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated using fasting insulin and glucose concentrations as previously described (25).

Blood samples were processed immediately and the plasma and serum was stored at −80°C until assayed. Human plasma ghrelin was measured with a commercially available RIA (Phoenix Pharmaceuticals, Inc, Belmont, CA, USA) which measures total circulating ghrelin concentrations. The intra- and interassay coefficients of variation were 6.2% and 9.0% respectively. TSH, FT4 and FT3 were measured by commercially available electrochemiluminescence immunoassays (Roche Diagnostics GmbH, Mannheim, Germany). Interassay coefficients of variation were 8.7% at 0.034 μU/ml and 3.6% at 3.96 μU/ml for TSH; 3.5% at 0.68 ng/dl and 3.3% at 3.95 ng/dl for FT4 and 2.8% at 1.86 pg/ml and 2.7% at 12.7 pg/ml for FT3.

Insulin was determined by an immunochemiluminescence method (IMMULITE DPC, Los Angeles, CA, USA). The antiserum is specific for insulin with a low cross-reactivity for proinsulin (13.3%). Interassay coefficients of variation at 14.9 μU/ml and 49.3 μU/ml levels were 4.8% and 6.5% respectively. Glucose was determined by the oxidase method.

The local Ethics Committee approved the study protocol and all subjects gave written informed consent.

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Statistical analysis

Data are shown as the means±S.E.M. Kolmogorov–Smirnov test was used to test data for normal distribution. When the variables did not normally distribute, Mann–Whitney U-test was used for comparison of data between groups and Wilcoxon signed-rank test was used to compare data before and after treatment. For normally distributed data, t tests for independent or paired data were used for the same purposes. Correlations between variables were assessed using Spearman’s correlation analysis. Stepwise multiple regression analysis was also performed to find which variables predicted ghrelin levels. A value of \( P < 0.05 \) was considered statistically significant. All calculations were carried out using SPSS 11.5 for Windows (SPSS, Inc. Chicago, IL, USA).

Results

Body composition and anthropometric measures

At baseline, patients and their respective control groups were comparable by age, waist-to-hip ratio, BMI and % of body fat (Tables 1 and 2). After therapy, hyperthyroid patients gained an average of 1.9 kg of body weight with a significant increase in BMI, fat mass and waist-to-hip ratio (Table 1). Hypothyroid patients showed a significant decrease in weight with an average of 2 kg and a decrease in BMI without changes in % of body fat or waist-to-hip ratio (Table 2).

Thyroid function

The hyperthyroid group showed inhibited serum TSH concentrations and high serum levels of FT4 and/or FT3; while the hypothyroid group showed elevated serum TSH concentrations with low levels of FT4 which normalised after therapy (Tables 1 and 2). The mean time required to normalise thyroid function was 5.56±0.72 months for hyperthyroid patients and 7.70±0.66 months for hypothyroid patients.

Glucose and insulin concentrations and HOMA-IR:

Hyperthyroid patients showed higher fasting glucose and insulin levels and higher HOMA-IR than their controls. After normalisation of thyroid hormone levels, glucose and HOMA-IR decreased to the normal range (90.2±3.2 mg/dl vs 82.9±1.8 mg/dl, \( P = 0.06 \) and 2.28±0.38 mM mU/l vs 1.67±0.15 mM mU/l, \( P = 0.106 \) respectively); however, no changes in insulin concentrations were observed (Table 1).

Hypothyroid patients did not show differences in fasting glucose, insulin or HOMA-IR compared with their control group, but there was a mild increase of insulin levels after therapy without changes in glucose or HOMA-IR (Table 2).

Three day food-intake record and hunger score

Hyperthyroid patients showed a greater food intake before treatment than controls (2537.4±165.5 vs 1972.8±115.3 kcal; \( P = 0.021 \)) without differences in the percentage of carbohydrates, proteins or lipids (39.9%, 15.7% and 44.4% vs 40.3%, 14.8% and 44.9% respectively) and a significant decrease in food intake after treatment (2234.7±116.7 kcal; \( P = 0.026 \)) also without differences in the percentage of carbohydrates, proteins or lipids (40.8%, 15.1% and 44.1%). Before treatment, hypothyroid patients showed no differences in food intake (1781.9±106.8 vs 1988.4±102.4 kcal; \( P = 0.57 \)) or in the proportion of macronutrients (40.2%, 16.9% and 42.9% vs 39.8%, 15.6% and 44.6%) as compared to the control group. After treatment, no changes were observed in both parameters (1901.9±116.0 kcal, \( P = 0.48 \); 41.6%, 16.0% and 42.4%).

Table 1 Anthropometric and biochemical characteristics of hyperthyroid patients before and after treatment, and their control group.

<table>
<thead>
<tr>
<th>Patients with hyperthyroidism (n = 24)</th>
<th></th>
<th>Hyperthyroid control group (n = 25)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Before therapy</td>
<td>After therapy</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.1±1.2</td>
<td>26.7±1.2±a</td>
</tr>
<tr>
<td>WHR</td>
<td>0.80±0.01</td>
<td>0.83±0.01±a</td>
</tr>
<tr>
<td>% body fat mass</td>
<td>30.9±1.6</td>
<td>32.2±1.6±a</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>96.3±3.8±b</td>
<td>90.2±3.2±a</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>12.8±2.0±c</td>
<td>12.2±1.5±c</td>
</tr>
<tr>
<td>HOMA-IR index</td>
<td>3.21±0.60±d</td>
<td>2.28±0.38±a</td>
</tr>
<tr>
<td>TSH (µU/ml)</td>
<td>0.01±0.00±e</td>
<td>1.54±0.34±e</td>
</tr>
<tr>
<td>FT4 (ng/dl)</td>
<td>3.24±0.33±f</td>
<td>1.07±0.04±f</td>
</tr>
<tr>
<td>FT3 (pg/ml)</td>
<td>15.5±2.24±g</td>
<td>4.79±0.26±f</td>
</tr>
<tr>
<td>Ghrelin (pg/ml)</td>
<td>463.6±36.4±h</td>
<td>539.7±45.4±a</td>
</tr>
</tbody>
</table>

Values are means±S.E.M. \( aP < 0.05 \), \( bP < 0.001 \), before vs after therapy; \( cP < 0.05 \), \( dP < 0.001 \), patients vs control group. BMI, body mass index; HOMA-IR, homeostatic model assessment of insulin resistance index (mM mU/l); WHR, waist-to-hip ratio.
At baseline, hyperthyroid and hypothyroid patients presented the same hunger score as their respective control group (5.33 ± 0.46 vs 5.64 ± 0.44, P = 0.53 and 4.82 ± 0.49 vs 5.41 ± 0.63, P = 0.94), and remained unchanged after treatment, in both hyperthyroid (5.18 ± 0.35, P = 0.69) and hypothyroid (5.56 ± 0.36, P = 0.15) patients.

Ghrelin concentrations

In hyperthyroid patients, fasting ghrelin levels were significantly lower than in their control group (463.6 ± 36.4 vs 561.1 ± 32.1 pg/ml; P = 0.041). After normalisation of thyroid hormones, ghrelin concentrations increased up to the normal range (539.7 ± 45.4 pg/ml; P = 0.549) in spite of the increase in BMI, waist-to-hip ratio and % of body fat described above (Fig. 1A). In patients with hypothyroidism, ghrelin levels were similar to those of their control group both before (561.6 ± 49.9 vs 593.8 ± 55.1 pg/ml; P = 0.672) and after (590.2 ± 35.0 pg/ml; P = 0.958) treatment although BMI decreased (Fig. 1B).

Correlations

In hyperthyroidism, before treatment, ghrelin correlated negatively with BMI (r = −0.520, P = 0.009), insulin (r = −0.410, P = 0.046) (Fig. 2A) and glucose (r = −0.515, P = 0.01) (Fig. 2B) concentrations and the HOMA-IR index (r = −0.462, P = 0.023). No correlations were observed between ghrelin and the percentage of body fat, waist-to-hip ratio, TSH, FT4 and FT3. Although ghrelin correlated negatively with the hunger score (r = −0.476, P = 0.029) (Fig. 2C), no association between ghrelin and calorie intake or the percentage of macronutrients was observed. Using a stepwise multiple regression analysis with ghrelin as the dependent variable and BMI, insulin, glucose, FT3 and FT4 as the independent ones, only glucose entered into the model (β = −0.415, P = 0.044). The percentage of variation of ghrelin after treatment did not correlate with the percentage of variation of any independent variable.

In hypothyroidism, no correlations were observed between ghrelin and any of the variables mentioned either before or after treatment.

Table 2 Anthropometric and biochemical characteristics of hypothyroid patients before and after treatment, and their control group.

<table>
<thead>
<tr>
<th></th>
<th>Patients with hypothyroidism (n = 17)</th>
<th>Hypothyroid control group (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before therapy</td>
<td>After therapy</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.5±1.0a</td>
<td>27.7±1.1</td>
</tr>
<tr>
<td>WHR</td>
<td>0.83±0.01</td>
<td>0.84±0.01</td>
</tr>
<tr>
<td>% body fat mass</td>
<td>37.9±1.3</td>
<td>37.6±1.2</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>86.6±2.7</td>
<td>85.6±1.4</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>11.3±1.4a</td>
<td>13.5±1.1c</td>
</tr>
<tr>
<td>HOMA-IR index</td>
<td>2.46±0.31</td>
<td>2.73±0.31</td>
</tr>
<tr>
<td>TSH (µU/ml)</td>
<td>119.0±20.6d</td>
<td>2.10±0.19b</td>
</tr>
<tr>
<td>FT4 (ng/dl)</td>
<td>0.24±0.06d</td>
<td>1.29±0.07b</td>
</tr>
<tr>
<td>FT3 (pg/ml)</td>
<td>1.88±0.28d</td>
<td>4.32±0.22b</td>
</tr>
<tr>
<td>Ghrelin (pg/ml)</td>
<td>561.6±49.9</td>
<td>590.2±35.0</td>
</tr>
</tbody>
</table>

Values are means±S.E.M. aP < 0.05, bP < 0.001, before vs after therapy; cP < 0.05, dP < 0.001, patients vs control group. BMI, body mass index; HOMA-IR, homeostatic model assessment of insulin resistance index (mM mU/l); WHR, waist-to-hip ratio.

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In the control group, only insulin levels ($r = -0.347, P = 0.017$) and HOMA-IR index ($r = -0.354, P = 0.020$) correlated negatively with ghrelin concentrations.

**Discussion**

The present study evaluates the relationship between plasma ghrelin levels and hunger, food intake, anthropometric changes and insulin resistance in thyroid dysfunction states.

Hyperthyroidism is characterized by extensive weight loss despite normal or increased calorie intake. Weight loss reflects not only a depletion of body adipose tissue stores but also a loss of muscle mass caused by accelerated catabolism accompanied by increased oxygen consumption and heat elimination (26). As in other negative balance states (27), we expected that ghrelin concentrations would be high in hyperthyroidism as a response aimed to restore energy balance. However, consistently with a previous study in nine patients with hyperthyroidism (23) and another in rats (28), we found that plasma ghrelin levels were low and that they normalised after therapy. We hypothesise that the low ghrelin concentrations found in patients with hyperthyroidism could be explained, partly, by the high insulin levels and the elevated insulin resistance observed in this thyroid situation (19–21). Although some authors reported that the administration of insulin and glucose did not suppress ghrelin levels (12, 29), other studies stated that insulin may negatively regulate ghrelin concentrations. In agreement with our results, they also showed a negative correlation between ghrelin and insulin levels or insulin resistance (17, 18, 30, 31). Thus, the compensatory hyperinsulinemia associated with insulin resistance observed in the hyperthyroid group could contribute to the decreased ghrelin concentrations. However, after treatment, insulin sensitivity improved mainly due to a decrease in glucose levels while insulin concentrations remained unchanged. Furthermore, after a stepwise multiple regression analysis, glucose was the only variable that entered into the model to predict ghrelin levels. Therefore, a role of glucose in ghrelin suppression in hyperthyroidism needs to be considered. On the other hand, it is well-known that hyperthyroidism is associated with increased activity of the sympathetic nervous system and with abnormalities of GH/IGF-I axis (32, 33) which although they were not evaluated in this study may influence glucose homeostasis and insulin sensitivity and therefore, ghrelin levels. We cannot rule out the possibility of an increased metabolic clearance rate effect, similar to what has been shown with other hormones such as prolactin or growth hormone that show a decrease in their serum concentrations in hyperthyroid patients despite increased production rates (34–38). However, if this were the case, the opposite would be expected in hypothyroidism where ghrelin levels were similar to healthy controls before and after treatment. Furthermore, glucose and insulin concentrations and insulin sensitivity were normal in hypothyroidism, as previously described (23). This observation could contribute to explain why ghrelin levels were not altered in hypothyroidism. To our knowledge, this is the first report of ghrelin levels in human hypothyroidism. There is only one study in hypothyroid rats in which, in contrast to our findings, ghrelin m-RNA expression in the stomach and circulating plasma ghrelin were increased (28).

Ghrelin levels are modified along with changes in body weight. In this sense, ghrelin levels increase in obese patients after diet induced weight loss (39, 40) and decrease in anorexia nervosa after weight gain...
In the present study, ghrelin levels increased in hyperthyroidism after treatment, in spite of an increase in BMI, waist-to-hip ratio and percentage of body fat and, in hypothyroidism, BMI decreased without modification of ghrelin levels suggesting an absence of relationship between ghrelin and these anthropometric changes in thyroid dysfunction. Additionally, we did not find any correlation between ghrelin and thyroid hormone levels as previously described.

Hyperphagia is considered an important feature of hyperthyroidism and an alteration of the neurophysiology of food intake regulation has been suggested in Graves’ disease (41). In our study, food intake of hyperthyroid patients decreased after treatment with no relation to ghrelin levels. In addition, a negative correlation between plasma ghrelin concentrations and the hunger score was observed, in contrast to previous studies in healthy subjects (24). These findings would imply that the higher the ghrelin levels are the lower the appetite sensation is, which is not in accordance with the supposed role of ghrelin as an appetite stimulator as seen in other physiological conditions such as anorexia nervosa (42). All this information taken together it seems unlikely that ghrelin is the primary stimulator of appetite and food intake in hyperthyroidism. In hypothyroid patients food intake did not change after treatment and the hunger score did not correlate with ghrelin levels.

It has recently been shown that total ghrelin levels detected by using the commercially available Phoenix assay mostly reflect des-acyl ghrelin (43), which is devoid of orexigenic effects. Besides, the acylated ghrelin, which harbours the orexigenic effects, binds to some other particles in plasma such as HDL (44), which can be altered in situations of thyroid dysfunction. Thus, taking these two considerations together, it is possible that the detection of acylated ghrelin would have been more accurate to evaluate the relationship between this hormone and food intake in thyroid dysfunction.

In conclusion, ghrelin levels were decreased in hyperthyroidism and they seemed to be modulated by the state of insulin resistance. After treatment, ghrelin levels were restored along with insulin sensitivity. In hypothyroidism, ghrelin levels and insulin sensitivity were not altered neither before nor after treatment. In both thyroid dysfunctions, ghrelin levels were not related to hunger, food intake or changes in BMI, waist-to-hip ratio or the percentage of body fat. Therefore, in thyroid dysfunction states, a role for ghrelin in energy balance regulation as seen in other physiological and pathological situations was not stated.

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