Acute exogenous TSH administration stimulates leptin secretion in vivo

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Abbreviated title: TSH stimulates leptin secretion

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Abstract

Thyroid-stimulating hormone-receptor (TSH-r) has been found in a variety of cell types, including preadipocytes and adipocytes. In vitro, TSH-mediated preadipocyte and adipocyte responses include proliferation, differentiation, survival and lipolysis.

Objective: To measure the response of serum leptin to exogenous administration of recombinant human TSH (rhTSH) in vivo.

Patients: One hundred patients with differentiated thyroid cancer already treated with total thyroidectomy and $^{131}$I remnant ablation. Mean (± S.E.M.) body mass index (BMI) was 26.9 ± 0.6 Kg/m².

Methods: Patients received a standard dose of rhTSH for measurement of thyroglobulin in the follow-up of their disease. Blood samples were taken for the assay of TSH and leptin before the first administration of rhTSH (time 0) and after 24 h (time 1), 48 h (time 2), 72 h (time 3) and 96 h (time 4).

Results: Significant mean serum leptin increments, with respect to basal value, were 16 %, 13 %, 18 %, 11% at time 1, 2, 3 and 4 respectively. Significant positive correlations of leptin-area under the curve (AUC) with respect to basal leptin levels ($r = 0.43$; $P < 0.0001$) and BMI ($r = 0.32$; $P < 0.005$) were observed.

Conclusions: Acute rhTSH administration in hypothyroid subjects under L-thyroxine therapy produces a rise in serum leptin. This increase is proportional to the adipose mass suggesting that a functioning TSH-r is expressed on the surface of adipocytes. The role that TSH-r activation in adipocytes might play in physiological and pathological conditions remains a matter of investigation.
Introduction

The TSH-r is expressed on the surface of follicular thyroid cells, but mRNA and protein have been reported in a variety of cell types, including preadipocytes and adipocytes (1). High affinity TSH binding activity of Graves’ IgG has been shown in guinea pig adipocytes (2-4), and TSH-r cDNA fragments have been obtained by polymerase chain reaction from rat or human retro-orbital tissues, adipose tissue and fibroblasts (5-8). In vitro studies indicate that TSH-mediated responses on adipocytes include proliferation, differentiation, survival and stimulation of specific cellular pathways (9-17). In vitro studies with rat or human visceral adipocytes reveal opposite effects of TSH on leptin secretion. In rat epididymal adipocytes, TSH inhibited basal (18) and insulin-stimulated (19) leptin secretion. By converse, in a systematic study of organ cultures of human omental adipose tissue, TSH powerfully stimulated leptin secretion in vitro, while prolactin, ACTH, FSH and LH were devoid of action (14).

This study was designed to test whether acute administration of rhTSH to thyroidectomized patients is followed by changes in serum leptin concentrations, which would imply that the TSH-r is functionally active in human adipocytes in vivo.

Patients and Methods

One hundred consecutive patients (77 females, 23 males) turning to our Endocrine Unit were enrolled after total thyroidectomy and 131-iodine remnant ablation for differentiated thyroid cancer. Patients were scheduled to receive rhTSH (Thyrogen Genzyme, Cambridge, MA) for diagnostic purposes. Clinical, haematological, and instrumental examinations of each patient were performed following the appropriate protocol for management of their disease. The study was approved by the Institutional Reviewing Board. Age and BMI of patients are shown in Table I. Patients were on variable doses of L-T4, from substitutive to suppressive. Each patient received a standard dose of 0.9 mg rhTSH i.m. for two consecutive days according to the conventional protocol (20). Blood samples were taken between 8:00 and 9:00 a.m. after an overnight fasting for the assay of TSH and leptin, before the first administration of rhTSH (time 0) and 24 h (time 1), 48 h (time 2), 72 h (time 3) and 96 h (time 4) after the first administration of rhTSH. All patients attended for routine follow-up.
Serum TSH was measured using a solid-phase, two-site chemiluminescent immunometric assay (Immulite 2000, Third Generation TSH, Los Angeles, CA, USA. Analytical sensitivity 0.004 µU/ml; intra-assay coefficient of variation < 12%; linearity 85-104%). Serum leptin was measured by IRMA (Active Human Leptin IRMA, DSL-23100i, Webster, TX, USA. Analytical sensitivity 0.10 ng/ml; intra-assay coefficient of variation < 5%; linearity 80-110%).

A time-average analysis of baseline-corrected serum leptin and TSH concentrations (area under the curve, \( \text{AUC}_{4\text{days}} \)) was performed. One-way repeated measures ANOVA was conducted to compare serum leptin concentrations at various time points. Post-hoc comparisons were conducted using Bonferroni’s correction when necessary. A mixed between-within subjects ANOVA was also conducted in order to evaluate the effect of gender on leptin serum concentrations during time. Simple linear regression analysis was applied when indicated. Statistical significance was defined as \( p < 0.05 \). Data are presented as mean ± SE.

**Results**

Mean values of basal TSH did not show a significant difference between females (0.5 µU/ml ± 0.2, range 0.003 – 6.5 µU/ml) and males (0.5 µU/ml ± 1.21, range 0.003 – 3.6 µU/ml).

After rhTSH administration, a wide spectrum of values of serum TSH was observed. As expected (20), the TSH-\( \text{AUC}_{4\text{days}} \) was significantly lower in males (215.8 µU/ml ± 14.6, range 96.3 - 418.5) than in females (275.5 µU/ml ± 12, range 68.8 - 718.7). TSH-\( \text{AUC}_{4\text{days}} \) was negatively related to BMI (\( r = 0.26, p < 0.05 \)) and positively associated with age (\( r = 0.27, p < 0.05 \)).

The values of serum leptin at several time points after rhTSH administration are reported in table 2. A significant difference in serum leptin concentrations (Wilks’ Lambda = 0.82, \( F = 5.824, p<0.005 \), multivariate partial \( \eta^2 = 0.18 \)) by one-way repeated measures ANOVA, was observed. Post-hoc comparisons using the Bonferroni’s correction indicated that the mean serum leptin concentration at time 0 was significantly different from that at time 1 (\( p<0.001 \)), time 2 (\( p<0.05 \)), time 3 (\( p<0.005 \)) and time 4 (\( p<0.05 \)).

A mixed between-within subjects ANOVA was conducted to explore the impact of gender and time on serum leptin concentrations. There was a statistically significant main effect for time (Wilks’ Lambda = 0.886, \( F= 3.064, p<0.05 \), multivariate partial \( \eta^2 = 0.11 \)) and for gender (\( F = 8.138, p<0.01 \), partial \( \eta^2 = 0.07 \)) with females showing higher mean values of serum leptin concentrations than males. The interaction effect
gender-time did not reach statistical significance. Since distribution of basal serum leptin concentrations spanned a broad range, we have assessed the TSH-induced increase in serum leptin within groups of subjects identified by quartiles of serum leptin at time 0. Results are shown in figure 1. Although not always reaching statistical significance (due to the low number of subjects in each group), a clear increase is observed in each quartile.

A linear positive correlation between serum leptin at time 0 and BMI was observed in the total population ($r = 0.75; p < 0.0001$), in females ($r = 0.81; p < 0.0001$) and in males ($r = 0.43; p < 0.05$).

The mean value of leptin-$\text{AUC}_{4\text{days}}$ in the total population was $6.2 \pm 1.5 \text{ ng/ml d}$, being higher in females ($7.1 \pm 1.9 \text{ ng/ml d}$) than in males ($3.3 \pm 1.3 \text{ ng/ml d}$).

Serum leptin-$\text{AUC}_{4\text{days}}$ was positively associated with serum leptin at time 0 (Figure 2A) in the total population as well as in females and in males, separately. A significant positive correlation between serum leptin-$\text{AUC}$ and BMI (Figure 2B) in the total population and in females was also observed. No significant association between serum TSH-$\text{AUC}_{4\text{days}}$ and serum leptin-$\text{AUC}_{4\text{days}}$ was observed.

**Discussion**

Leptin is a hormone produced mainly by adipocytes that acts on specific neurons within well defined centers, by relaying information regarding the level of energy stored in the adipose tissue. Leptin participates to the regulation of energy homeostasis, and serum leptin concentrations are proportional to the total fat mass. Hormones and nutrients may influence leptin gene expression or regulate translational efficiency and mRNA stability (21-24).

Data herein presented show that acute rhTSH administration produces a significant increase in serum leptin concentrations in vivo. This model has been previously used to demonstrate an increase of bone remodeling markers in humans, suggesting that TSH can modulate bone remodeling independent of thyroid hormones (25).

The TSH-induced increase in serum leptin may have biological relevance in two areas: the in vivo demonstration of a functional TSH-r on the surface of human adipocytes and the potential role of TSH in the control of leptin secretion. The increase in serum leptin concentrations was proportional to BMI and basal serum leptin concentrations, both indexes of the total adipose mass. These relationships suggest that the
adipose tissue is responsible for the TSH-induced elevation of serum leptin and strongly support the occurrence of an active TSH-r on adipocytes. Yet, the possibility of an indirect effect of TSH via the receptors expressed in the pituitary or brain (1) cannot be ruled out. Whether rhTSH effect on serum leptin persists beyond the length of this study cannot be established at this time, since blood sampling was stopped at 96 h after the first rhTSH administration, according to the standard protocol for thyroglobulin measurement in thyroid cancer patients. In a recent study, no significant variations in serum leptin concentrations after in vivo stimulation with rhTSH were reported (26). The small number of subjects and different times of observation of TSH effects might explain the lack of significant changes in serum leptin observed in that study.

The action of the thyroid axis components on leptin secretion has been previously investigated. However, no data have demonstrated in a definitive way that thyroid dysfunction affect circulating leptin, and the minor leptin changes occasionally observed might be explained by parallel alterations in body weight of the subjects under observation. In particular, serum leptin in hypothyroid patients has been found lower, higher or unchanged, as compared with euthyroid subjects (27-35). It should be noted that in primary hypo- and hyperthyroidism conflicting effects on leptin secretion and/or clearance could be exerted by simultaneous opposite changes in serum thyroid hormones and TSH, which may result in incoherent variations of serum leptin concentrations. Furthermore, in protracted critical illness such as severe hypo- and hyperthyroidism, additional mechanisms beside thyroid hormone and TSH may intervene in the regulation of leptin secretion. The balance between free and bound leptin might have an impact since the free hormone is the one that selectively represents the fat mass (36), and this might explain the conflicting results obtained when measuring total leptin in the majority of the studies of thyroid patients. Another explanation for the lack of leptin changes in primary hypothyroidism is that the latter condition is characterized by chronically elevated serum TSH that may not necessarily generate a measurable effect on leptin secretion, at variance with what we observed after an acute stimulation. Finally, the discrepancies observed among various studies may be related to the presence of TSH-r antibody in serum of patients with autoimmune thyroid disease. This autoantibody, by directly activating TSH-r or by inhibiting TSH action, could produce a confounding effect on TSH-mediated regulation of leptin secretion. However, in this regard, no correlation between serum leptin concentrations and the title of TSH-r antibody has been reported in patients with Graves’ disease (37).
Based on our results we cannot infer about a role of TSH on the adipocyte function in physiological or pathological conditions, and no evidence currently allows us to define the relationship and possible mechanisms of mutual adjustment between leptin and TSH. It could be speculated that TSH-induced changes of leptin secretion may concur to modulate the feeding behavior observed in hypo- and hyperthyroidism. We believe that a leptin-mediated effect of TSH on appetite is unlikely since leptin changes induced by TSH are modest as compared to the magnitude of appetite changes observed in thyroid dysfunction. However, it is worthwhile observing that leptin action is not limited to appetite regulation: Indeed recent evidence, obtained through PET imaging, indicates that in both rodents and primates bone marrow and kidney represent major sites for leptin uptake (38).

Mostly important, in our view, is the support given by our results to the existence of a functioning TSH-r on the surface of adipocytes, with regards to the pathogenesis of some extrathyroidal manifestations of autoimmune thyroid disease. These receptors, when bound by specific autoantibody, could play a major role in the development of thyroid-associated ophtalmopathy (TAO), a feature of Graves’ disease characterized by an increased volume of adipose/connective tissue within the orbit (39). The initiation of TAO has been postulated to depend on expression of TSH-r in orbital pre-adipocytes (40). Orbital fibroblasts subjected to adipocytic differentiation increase TSH-r expression in vitro, and the receptor is able to respond specifically to TSH and TSH antibody (41). Although the pathogenesis of TAO remains largely unknown, binding of TSH-r antibody to TSH-r seems to play a major role.

In conclusion, our study indicates that acute rhTSH administration in hypothyroid subjects under L-thyroxine therapy produces a rise in serum leptin. This increase is proportional to the adipose mass suggesting that a functioning TSH-r is expressed on the surface of adipocytes. The role that TSH-r activation in adipocytes might play in physiological and pathological conditions remains a matter of investigation.
Disclosure

Declaration of interest: Furio Pacini is a member of the Advisory Board for Genzyme and recipient of speaker’s fees.

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References


LEGEND OF FIGURES

**Figure 1:** Serum leptin concentrations before (time 0) and 24 (time 1), 48 (time 2), 72 (time 3) and 96 (time 4) hours after the first administration of rhTSH. Subjects were divided according to quartiles of serum leptin at time 0: First quartile (A), second quartile (B), third quartile (C), fourth quartile (D). Data are shown as mean and S.E.M.. Results were analyzed by one-way repeated measures ANOVA. * p<0.05 after post-hoc Bonferroni’s correction versus time 0.

**Figure 2:** Correlations between baseline-corrected serum leptin-AUC$_{4\text{days}}$ after rhTSH administration and serum leptin at time 0 (A) or BMI (B).
Figure 1.
Figure 1.
Figure 2.

A

Leptin-AUC Log (ng/ ml·d)

Leptin Log (ng/ml)

p < 0.0001; r = 0.43
Figure 2.

- Leptin-AUC Log (ng/ml·d)
- BMI Log (Kg/m²)

p < 0.005; r = 0.32
Table 1: Age and BMI of the study group expressed as mean values ± S.E.M. and (range).

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<th>TOTAL</th>
<th>FEMALES</th>
<th>MALES</th>
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<td>Age (years)</td>
<td>47 ± 1.3 (16-76)</td>
<td>47.9 ± 1.4 (20-73)</td>
<td>45.8 ± 3.3 (16-76)</td>
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<tr>
<td>BMI (Kg/mq)</td>
<td>26.9 ± 0.6 (17.4-62.4)</td>
<td>26.7 ± 0.8 (17.4-62.4)</td>
<td>27.3 ± 0.8 (19.4-32.8)</td>
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Table 2: Values of serum leptin (ng/ml) at several time points after rhTSH administration expressed as mean ± SE, and percent variation (%) with respect to leptin at time 0. One-way repeated measures ANOVA in the total population, p<0.05. Significant differences after post-hoc Bonferroni’s correction: time 0 versus time 1 (p<0.001), time 2 (p<0.05), time 3 (p<0.005) and time 4 (p<0.05). A mixed between-within subjects ANOVA showed a statistically significant main effect for time (p<.05) and for gender (p<0.01).

<table>
<thead>
<tr>
<th></th>
<th>Total population</th>
<th>Females</th>
<th>Males</th>
</tr>
</thead>
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<tr>
<td><strong>Time 0</strong></td>
<td>11.6 (100) ± 1.1</td>
<td>13.3 (100) ± 1.3</td>
<td>5.9 (100) ± 0.9</td>
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<td><strong>Time 1</strong></td>
<td>13.5 (116) ± 1.3</td>
<td>15.4 (116) ± 1.6</td>
<td>7.1 (120) ± 1.1</td>
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<tr>
<td><strong>Time 2</strong></td>
<td>13.1 (113) ± 1.3</td>
<td>14.9 (112) ± 1.6</td>
<td>7 (119) ± 1.1</td>
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<tr>
<td><strong>Time 3</strong></td>
<td>13.7 (118) ± 1.5</td>
<td>15.8 (119) ± 1.8</td>
<td>6.4 (109) ± 0.9</td>
</tr>
<tr>
<td><strong>Time 4</strong></td>
<td>12.9 (111) ± 1.3</td>
<td>14.7 (111) ± 1.6</td>
<td>6.9 (117) ± 1.1</td>
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