Serum Ghrelin Levels Are Increased in Hypothyroid Patients and Become Normalized by L-Thyroxine Treatment

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Context: An interaction between ghrelin, which is implicated in the regulation of short- and long-term energy balance, and thyroid function has been reported in hyperthyroidism in which ghrelin levels are reversibly suppressed. We measured serum ghrelin levels and metabolic indices in hypothyroid patients before and after L-thyroxine replacement.

Patients and Methods: Eleven patients were examined twice: 1) in the hypothyroid state and 2) after at least 2 months of euthyroidism. Ten healthy subjects served as a control group. Ghrelin was measured in conjunction with indirect calorimetry and a hyperinsulinemic euglycemic clamp.

Results: Serum ghrelin levels were increased by 32% under basal conditions in the hypothyroid state (PRE) as compared with posttreatment (POST) (picograms per milliliter): 976.4 $\pm$ 80.8 *vs.* 736.8 $\pm$ 67.1 ($P < 0.001$). This difference prevailed during the clamp, but a decline was observed in both states: 641.4 $\pm$ 82.2 *vs.* 444.3 $\pm$ 66.8 μg/ml ($P = 0.005$). The hypothyroid state was associated with decreased resting energy expenditure, increased respiratory quotient, and insulin resistance. Serum ghrelin levels as well as the metabolic aberrations became normalized after L-thyroxine replacement as compared with the control subjects.

Conclusion: Serum ghrelin levels are reversibly increased in hypothyroid patients. It remains to be investigated whether this represents a direct effect of iodothyronines on ghrelin secretion or clearance or a compensatory response to the abnormal energy metabolism in hypothyroid patients. (***J Clin Endocrinol Metab*** **93: 2277–2280, 2008)**

Ghrelin, an acylated 28-amino acid gut-derived peptide, is an endogenous ligand of the GH secretagogue type1a receptor (1). At the hypothalamic level, ghrelin stimulates GH release and regulates appetite and energy balance (2, 3). Increased levels are seen in catabolic conditions (4), and decreased levels are found in obese patients (5). Moreover, an inverse correlation between serum ghrelin levels and resting energy expenditure (REE) has been recorded in healthy women (6).

Thyroid disease is associated with changes in appetite, food intake, and REE. Previous studies have reported decreased levels of ghrelin in hyperthyroidism (7). Results from studies investigating hypothyroid patients are conflicting (8, 9).

In this study we measured ghrelin levels, REE, and substrate metabolism in patients with hypothyroidism before and after L-thyroxine replacement, compared with an age- and sex-matched group of healthy subjects.

Subjects and Methods

Eleven hypothyroid patients (seven women) were consecutively recruited from our outpatient clinic; the inclusion criterion was untreated hypo-

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Abbreviations: BMI, Body mass index; DEXA, dual-energy x-ray absorptiometry; FFA, free fatty acid; LBM, lean body mass; REE, resting energy expenditure.

thyroidism with serum TSH values above 20 mU/liter. The etiology in all cases was chronic autoimmune thyroiditis, with increased thyroperoxidase levels. Ten healthy volunteers served as control group, matched for sex, age, and body mass index (BMI) of the patients in the euthyroid state.

All participants provided written informed consent after receiving oral and written information. The study was performed in accordance with the Declaration of Helsinki and the Aarhus County Scientific Ethics Committee approved the protocol.

Study design

The patients were studied before (PRE) and after (POST) L-thyroxine replacement, when thyroid hormones had been normalized (normal ranges: T_3: 1.1–2.5 nmol/liter; T_4: 60–140 nmol/liter) for at least 2 months. Median (range) time duration between first and second examination was 7.0 (4–12) months. The healthy subjects (CTR) were studied once. Each study day started at 0800 h after a 12-h overnight fast. All subjects were studied during a 4-h basal period, and eight patients and seven volunteers were also studied during a 3-h hyperinsulinemic euglycemic clamp. To obtain comparable insulin levels during the glucose clamp in both hypothyroid and euthyroid subjects, insulin was infused at a rate of 0.6 mU/kg·min in the hypothyroid patients and 0.7 mU/kg·min in the patients in the euthyroid state and in the healthy controls. Euglycemia (~5 mmol/liter) was maintained by a variable iv infusion of 20% glucose (SAD, Copenhagen, Denmark). Every 10 min, plasma glucose was sampled and immediately measured in duplicate on a glucose analyzer (Beckman Instruments, Palo Alto, CA). Insulin sensitivity was calculated as glucose infusion rate divided by the measured mean insulin concentration during the second hour of the clamp [M/I_{clamp} (nanomoles glucose · kilogram lean body mass (LBM)$^{-1}$ · minute^{-1} per picomole · liter^{-1})].

Ghrelin levels were determined in duplicate at the end of the baseline and clamp periods.

Methods

Serum ghrelin was determined by an in-house RIA. The assay recognizes the COOH-terminal of ghrelin and as such determines acylated as well as des-acylated ghrelin (10). The intraassay coefficient of variation is less than 2.6% and samples from each individual were analyzed in one assay. Thyroid hormones (total T_3 and T_4) and TSH were measured by immunoassay (Bayer ADVIA Centaur; Bayer Healthcare, Tarrytown, NY). A double-monoclonal immunofluorometric assay (DELFIA; Perkin-Elmer, Wallac, Turku, Finland) was used to measure serum GH. Serum insulin was determined by a commercial immunological kit (Dako, Glostrup, Denmark). Serum free fatty acid (FFA) levels were determined using a commercial kit (Wako Chemicals, Neuss, Germany). Serum leptin was measured by a commercialized ELISA kit (Linco Research, Inc., St. Charles, MO). Plasma glucagon was measured by an in-house RIA.

Indirect calorimetry (Deltatrac; Datex Instrumentarium, Inc., Helsinki, Finland) was performed to assess the respiratory quotient and REE. Anthropometrical measurements and whole-body dual-energy x-ray absorptiometry (DEXA) scanning (QDR 1000/2000/W scanner; Hologic, Inc., Waltham, MA) were performed in the patients before and after L-thyroxine therapy.

Statistics

Data are shown as mean ± SE. A Kolmogorov-Smirnov test was used to test data for normal distribution. When variables were not normally distributed, a Mann-Whitney *U* test was used for comparison of data between groups, and Wilcoxon signed-rank test was used to compare paired data before and after treatment. For normally distributed data, *t* tests for unpaired or paired data were used where appropriate. $P < 0.05$ was considered statistically significant. All calculations were carried out using SPSS 14 for Windows (SPSS, Inc., Chicago, IL).

Results

Body composition, energy metabolism, and thyroid function

The patients and healthy controls were comparable regarding age, sex, and BMI (Table 1). The patients were profoundly hypothyroid at study entry: pretreatment T_4 levels were 86% lower than after treatment (nanomoles per liter) [14.9 ± 3.7 (PRE) *vs.* 108.1 ± 4.4 (POST), $P < 0.001$] and 83% lower, compared with the healthy subjects ($P < 0.001$). Replacement with L-thyroxine resulted in a normalization of thyroid hormones (Table 1). Restoration of euthyroidism resulted in a significant reduction in BMI (kilograms per square meter) [26.8 ± 1.6 (PRE) *vs.* 25.8 ± 1.5 (POST), $P = 0.02$] corresponding to a 2.8 ± 1.1 kg weight loss ($P = 0.03$). Body composition as assessed by DEXA in the hypothyroid state was characterized by decreased fat mass ($P = 0.003$) and increased LBM ($P < 0.001$), compared with the euthyroid state (Table 1). Both fat mass and LBM normalized during treatment and became comparable with controls. REE was significantly reduced in the hypothyroid state and increased after L-thyroxine replacement to a level comparable with that of the control group (Table 1). The respiratory quotient was elevated in the hypothyroid state and became normalized after L-thyroxine substitution (Table 1).

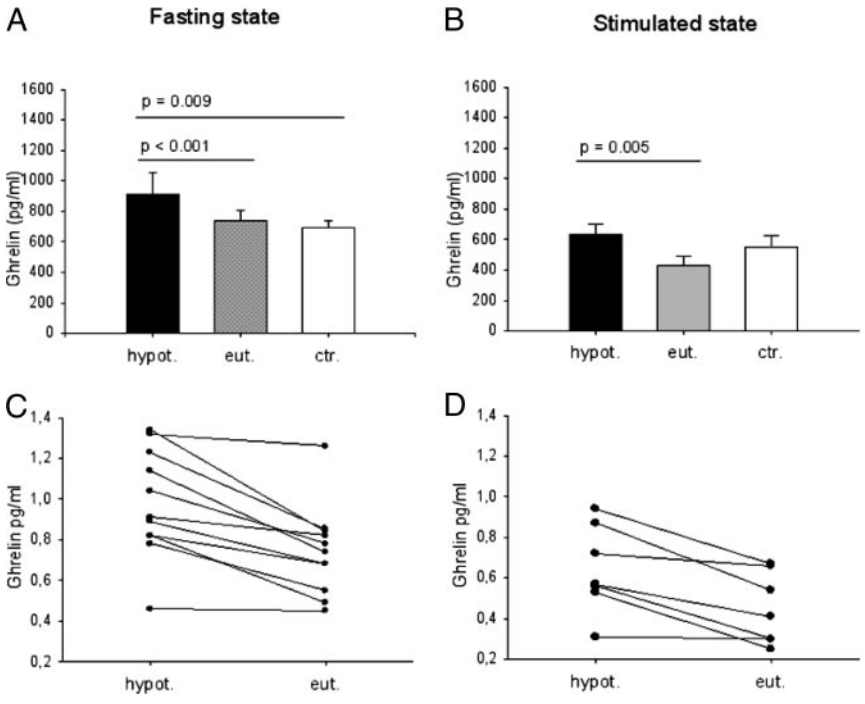

FIG. 1. Ghrelin levels in hypothyroid patients before (hypot) and after (eut) treatment and in healthy controls in the fasting state (A) and in the stimulated state (B) are shown Ghrelin levels for each patient before and after treatment in the fasting state (C) and the stimulated state (D) are also shown.

Hormones and metabolites

Fasting serum ghrelin levels (picograms per milliliter) in the hypothyroid state were elevated by 32%, compared with posttreat-

TABLE 1. Comparison of patients and healthy controls

	Patients					
	Hypothyroid	Euthyroid	Hypothyroid *vs.* euthyroid (*P* value)	Control subjects	Hypothyroid *vs.* control (*P* value)	Euthyroid *vs.* control (*P* value)
BMI (kg/m^2)	26.8 ± 1.6	25.8 ± 1.5	0.02	26.4 ± 1.2	ns	ns
DEXA total fat (kg)	21.3 ± 2.9	23.7 ± 2.9	0.003	23.6 ± 3.0	ns	ns
DEXA LBM (kg)	50.4 ± 2.7	45.9 ± 2.3	<0.001	52.3 ± 2.8	ns	ns
Total T_3 (1.1–2.6 nmol/liter)	0.68 ± 0.09	1.90 ± 0.07	<0.001	2.08 ± 0.09	<0.001	ns
Total T_4 (58–161 nmol/liter)	14.91 ± 3.68	108.18 ± 9.52	<0.001	86.6 ± 4.4	<0.001	ns
TSH (0, 1–4, 0 mU/liter)	149.9 ± 60.4	4.2 ± 2.2	0.03	1.3 ± 0.2	0.03	ns
TPO (kU/liter)	1636.5 ± 402.0					
RQ	0.86 ± 0.01	0.81 ± 0.01	<0.001	0.79 ± 0.01	<0.001	ns
EE (kcal per 24 h)	1160.9 ± 57.6	1487.3 ± 57.7	<0.001	1689.0 ± 81.5	<0.001	ns
Ghrelin (pg/ml)	976.4 ± 80.8	736.8 ± 67.1	0.001	686 ± 53.7	0.009	ns
Ghrelin stimulation (pg/ml)	641.4 ± 82.2	444.3 ± 66.8	0.005	550.0 ± 74.1	ns	ns

EE, Energy expenditure; TPO, thyroperoxidase; RQ, respiratory quotient; ns, not significant.

ment levels [976 ± 81 (PRE) *vs.* 737 ± 67 (POST), $P < 0.001$] (Fig. 1). L-Thyroxine replacement resulted in ghrelin levels comparable with healthy controls (Fig. 1). Circulating ghrelin levels decreased significantly during the glucose clamp in the patients, regardless of thyroid status, as well as in the healthy subjects, but these levels remained increased in the hypothyroid state, compared with the euthyroid state ($P = 0.005$) (Table 1). A negative correlation was found between the change in ghrelin and baseline T_4 ($P = 0.038$; $r = -0.629$); no correlation was found between the change in ghrelin and T_3 or TSH. No correlation was found between ghrelin and either REE or insulin sensitivity. Insulin sensitivity was 39% lower in hypothyroid patients, compared with after treatment. Fasting levels of insulin, glucagon, leptin, and GH levels were not significantly influenced by thyroid status, but IGF-I levels were significantly decreased [95.2 ± 7.4 (PRE) *vs.* 123.0 ± 8.7 (POST), $P = 0.004$].

Discussion

Our study documents that serum ghrelin levels in the basal state as well as during a hyperinsulinemic glucose clamp are increased in hypothyroid patients and become normalized after substitution with L-thyroxine. We previously reported that hyperthyroidism was associated with suppressed ghrelin concentrations, which normalized when the patients became euthyroid (7). Taken together, we hypothesize that this reciprocal association between the circulating levels of ghrelin and T_4 in patients with thyroid disease may constitute either a direct effect of iodothyronines on gut-derived ghrelin secretion or clearance or a compensatory mechanism to balance the consequences of primary thyroid dysfunction on energy balance and substrate metabolism.

Our data contrast with those of Gimenez-Palop *et al.* (8), who recorded normal ghrelin levels and insulin sensitivity, as assessed by homeostasis model assessment, in 17 patients with hypothyroidism of different etiologies. Our study comprised solely patients with newly diagnosed and profound hypothyroidism due to autoimmune thyroiditis. Moreover, our patients also presented with overt signs of hypothyroidism in terms of reduced energy expenditure and insulin resistance. A similar study was carried out by Altinova *et al.* (9). They examined 47 hypothyroid patients and found no significant difference in pretreatment serum ghrelin levels, compared with posttreatment levels. They did, however, record pretreatment ghrelin levels in the patients to be significantly lower, compared with healthy subjects. In that study the degree of hypothyroidism assessed by TSH was less, compared with our patients (73.3 ± 6.8 *vs.* 149.9 ± 60.4 mU/liter).

Regarding alterations of glucose, lipid metabolism, and insulin sensitivity, our results are in line with previous reports (11, 12).

The increase in circulating ghrelin levels could be caused by reduced metabolic clearance rate because thyroid status is known to impact the clearance of, for example, insulin (13). The pharmacokinetics of ghrelin as a function of thyroid disease have not yet been investigated, but high-density lipoprotein cholesterol and higher BMI, both features of hypothyroidism, have been shown to increase the mean residence time of ghrelin in the body (14). The degradation of ghrelin is catalyzed by several esterases including butyrylcholinesterase (15), and thyroid hormone may influence the activity of these enzymes. Indeed, rodent studies have demonstrated accelerated butyrylcholinesterase activity in thyroxine-treated animals as well as delayed activity in hypothyroid rats (16). If assuming that the elevated ghrelin levels are caused by increased secretion, the available literature on the metabolic effects of exogenous ghrelin and determinants of endogenous ghrelin release offer no simple explanation for the observed reversible elevation in serum ghrelin levels in hypothyroidism.

In this study we used an in-house ghrelin assay, which measures total ghrelin. Total and acylated ghrelin levels, however, usually change in parallel (17). Whether thyroid function impacts the ratio between acylated and des-acylated ghrelin remains to be elucidated.

Systemic administration of ghrelin in human subjects increases appetite and food intake (3), stimulates GH secretion (2), and is also accompanied by moderate elevations in plasma glucose concentrations (18). In addition, we recently observed that exogenous ghrelin causes insulin resistance presumably via GH-independent mechanisms (19). Increased endogenous ghrelin levels are present in catabolic conditions such as weight loss (5) and anorexia nervosa (4), whereas ghrelin levels are suppressed in simple obesity. Hypothyroidism is associated with a moderate increase in BMI, which traditionally is attributed to reduced energy expenditure rather than

increased food intake and insulin resistance. In the present study, hypothyroidism was also associated with a small increase in BMI, but it was unexpected that DEXA scanning showed increased LBM and decreased fat mass. We are not aware of previous data using DEXA measurements in hypothyroidism, but we favor the explanation that the findings could be attributed to fluid retention, which will overestimate LBM and underestimate fat mass. We find it unlikely that the moderate differences in body composition explain the pronounced changes in ghrelin.

Stimulated GH release and IGF-I levels are reduced in hypothyroidism (20). The increase in ghrelin levels could represent a compensatory feedback to reduced GH secretion. The present study was not designed to assess endogenous GH secretion, but we did observe reduced IGF-I levels in the hypothyroid state. On the other hand, there is so far no clear evidence of a close association between endogenous systemic levels of GH and ghrelin in other conditions such as GH deficiency, acromegaly, fasting, or exercise (21).

The circulating levels of FFAs and ghrelin have previously been demonstrated to be inversely associated (22), whereas administration of ghrelin increases FFA levels in some (14) but not all studies (23). However, no correlation between FFA concentrations and ghrelin levels was observed in this study (data not shown), possibly due to the rather marginal reduction in FFA concentration in hypothyroid patients.

In conclusion, this study extends and supports a close reciprocal relationship between circulating levels of T_4 and ghrelin in patients with thyroid disease. The cause-effect relationship remains to be delineated.

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