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Hunger–Satiety Signals in Patients with Graves' Thyrotoxicosis Before, During, and After Long-Term Pharmacological Treatment

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Patients with Graves' thyrotoxicosis lose weight despite increased appetite and food intake, thus suggesting a disturbed balance between energy intake and expenditure. Underlying mechanisms are not fully elucidated. The objective of this study was to investigate whether hormonal factors, known to affect hunger/satiety, change significantly over time as pharmacological treatment turns hyperthyroidism into euthyroidism. For that purpose 11 patients with Graves' thyrotoxicosis were given thiamazole and l-thyroxine for 18–20 mo. They were investigated on three occasions: *Test 1*: before pharmacological therapy; *Test 2*: during medication; *Test 3*: a few months after conclusion of the pharmacological treatment. Sixteen healthy subjects were also investigated for comparison. The participants were fasted overnight. Blood samples for determination of plasma glucose and serum concentrations of free T_3 and T_4, TSH, albumin, cortisol, insulin, GH, IGF-1, IGFBP-1, leptin, and ghrelin were drawn in the morning from an antecubital vein. Laboratory data obtained in test 1 were statistically compared with those in tests 2 and 3. The study showed that the free T_3 level declined from 42.8 ± 4.3 pmol/L in test 1 to 6.0 ± 0.8 pmol/L in test 2 (85 ± 2% decline), and 5.5 ± 0.3 pmol/L in test 3 (86 ± 2% decline). The free T_4 level fell concomitantly from 65.2 ± 4.8 to 16.6 ± 1.7 and 14.4 ± 1.2 pmol/L. The glucose level was significantly higher during hyperthyroidism (test 1) than during euthyroidism (tests 2 and 3), whereas cortisol, insulin, GH, IGF-1, and leptin levels were similar. The IGFBP-1 level was initially high (48.8 ± 8.5 µg/L in test 1), but with a relative decline in free T_3 of 85 ± 2% (test 2) the IGFBP-1 level declined by 34 ± 13%, and with a free T_3 decline of 86 ± 2% (test 3) the binding protein fell by 39 ± 29%. This brought about increased IGF-1 bioavailability as reflected by a rising IGF-1/IGFBP-1 ratio from 5.1 ± 1.1 to 13.8 ± 2.9 ($p < 0.01$). The ghrelin level was low (2454 ± 304 ng/L) in test 1. It increased to 3127 ± 397 ng/L in test 2 ($p < 0.05$), and to 3348 ± 279 ng/L in test 3 ($p < 0.01$). Conclusion: Both ghrelin secretion and IGF-1 bioavailability are low in patients with untreated thyrotoxicosis, but increase markedly as pharmacotherapy makes them euthyroid. These metabolic changes may be caused by the transition of hyperthyroidism into euthyroidism rather than by the pharmacotherapy per se, since the metabolic changes prevailed also in the posttreatment period.

Key Words: Hyperthyroidism; ghrelin; leptin; IGF-1; IGFBP-1.

Introduction

Weight loss—despite enhanced appetite and increased food intake—characterizes Graves' thyrotoxicosis *(1)*, and indicates impaired balance between energy intake and energy expenditure. The underlying mechanism is not fully understood, but it is well known that not only thyroid hormones, but other hormones as well *(2)* influence human energy homeostasis and could contribute to the energy imbalance in untreated thyrotoxic patients. Leptin is one such hormone that not only increases energy expenditure *(3)*, but also exerts long-term regulatory influence on food intake *(4,5)*. This regulatory effect is brought about by inhibition of neuropeptide Y (NPY) *(6,7)*, which under normal conditions stimulates appetite and augments intake of food *(8)*. Both cortisol and insulin stimulate leptin secretion *(9, 10)*. Hence, both of them should be of importance for balancing energy intake and expenditure. Ghrelin may also play a role in this context, as this GH secretagogue increases appetite and food intake *(11,12)* via stimulation of NPY *(13)*.

Little is known about how the above-mentioned hormones vary over time as long-term pharmacotherapy makes thyrotoxic patients euthyroid. Therefore, the aim of this investigation was not only to study how these hunger–satiety signals change during medication, but also to see whether there are additional changes in the posttreatment period.

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Table 1

		Test 1	Test 2	Test 3
BMI	P:	22.7 ± 0.8	24.6 ± 1.0**	25.6 ± 1.3***
(kg/m^2)	NR:	20–25		
Free T_3	P:	42.8 ± 4.3	6.0 ± 0.8***	5.5 ± 0.3***
(pmol/L)	NR:	3.0–6.5		
Free T_4	P:	65.2 ± 4.8	16.6 ± 1.7***	14.4 ± 1.2***
(pmol/L)	NR:	10–20		
T_4/T_3	P:	1.70 ± 0.28	2.90 ± 0.19***	2.65 ± 0.23**
TSH	P:	0.02 ± 0.00	2.07 ± 1.36	1.03 ± 0.28
(mU/L)	NR:	0.2–4.0		
Albumin	P:	36.2 ± 1.1	41.1 ± 1.1*	42.2 ± 0.8**
(g/L)	NR:	30–42		
Glucose	P:	5.2 ± 0.1	4.7 ± 0.2***	4.7 ± 0.1***
(mmol/L)	NR:	3.5–6.0		
Insulin	P:	8.7 ± 1.0	6.1 ± 0.7	7.0 ± 1.2
(mU/L)	NR:	<10		
Insulin/Glucose	P:	1.67 ± 0.18	1.27 ± 0.12	1.54 ± 0.31
Cortisol	P:	427 ± 51	364 ± 28	365 ± 49
(nmol/L)	NR:	200–700		
GH	P:	1.6 ± 0.4	2.5 ± 1.3	1.8 ± 0.8
(μg/L)	NR:	<5.0		
IGF-1	P:	191 ± 25	193 ± 27	157 ± 21
(μg/L)	NR:	130–350		
IGFBP-1	P:	48.8 ± 8.5	23.9 ± 3.3*	17.8 ± 4.8**
(μg/L)	NR:	17–30		
IGF-1/IGFBP-1	P:	5.1 ± 1.1	10.7 ± 2.7	13.8 ± 2.9**
Leptin	P:	11.5 ± 2.2	12.4 ± 3.4	15.3 ± 3.4
(μg/L)	M-NR:	<7.5		
	FM-NR:	<15.0		
Ghrelin	P:	2454 ± 304	3127 ± 397*	3348 ± 279**
(ng/L)	NR:	2497–5157		

[a]Results obtained in thyrotoxic patients before pharmacological treatment (test 1), after 7 mo of treatment (test 2), and 2.4 mo after cessation of the treatment (test 3). Values are means ± SEM. P = patient. NR = normal range. M-NR = normal range in men. M-FM = normal range in women. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ (comparison with pretreatment levels).

Results

BMI: The basal, pretreatment, value in test 1 was 22.7 ± 0.8 kg/m^2. It increased significantly in tests 2 and 3 as shown in Table 1 and Fig. 1.

Free T_3: Before therapy serum T_3 was markedly increased (42.8 ± 4.3 pmol/L). The level fell significantly in test 2 and continued to fall slightly in the period between tests 2 and 3.

Free T_4: Also the T_4 level was markedly increased in test 1 (65.2 ± 4.8 pmol/L). Pharmacological therapy normalized the hormone concentration. It stayed normal in the postherapy period as well.

T_4/T_3 ratio: In test 1 the T_4/T_3 ratio was 1.70 ± 0.28. It increased significantly during antithyroid treatment, and remained significantly increased in the posttreatment period.

TSH: A suppressed TSH level was found in test 1 (0.02 ± 0.00 mU/L), which increased to normal levels in tests 2 and 3.

Albumin: The basal serum albumin concentration was 36.2 ± 1.1 g/L. This level increased significantly in tests 2 and 3.

Glucose: The fasting plasma glucose concentration was 5.2 ± 0.1 mmol/L in test 1. It fell significantly in test 2, and remained at that level also in test 3 (Table 1 and Fig. 2).

Insulin: The basal insulin level (8.7 ± 1.1 mU/L) declined slightly, but not significantly, during, and after medication.

Insulin/glucose ratio: The insulin/glucose ratio was 1.67 ± 0.18 in test 1. It fell slightly but not significantly in tests 2 and 3.

Cortisol: In test 1 the serum cortisol concentration was 427 ± 51 nmol/L. It declined insignificantly after antithyroid medication.

GH: The basal serum GH level in test 1 (1.6 ± 0.4 μg/L) did not differ significantly from those in tests 2 and 3.

IGF-1: The IGF-1 level in test 1 was 191 ± 25 μg/L. The IGF-1-levels in tests 2 and 3 were of similar magnitude (Table 1 and Fig. 3).

IGFBP-1: The IGFBP-1 level was high in the thyrotoxic state (48.8 ± 8.5 μg/L). It fell significantly during treatment, and reached even lower levels after treatment.

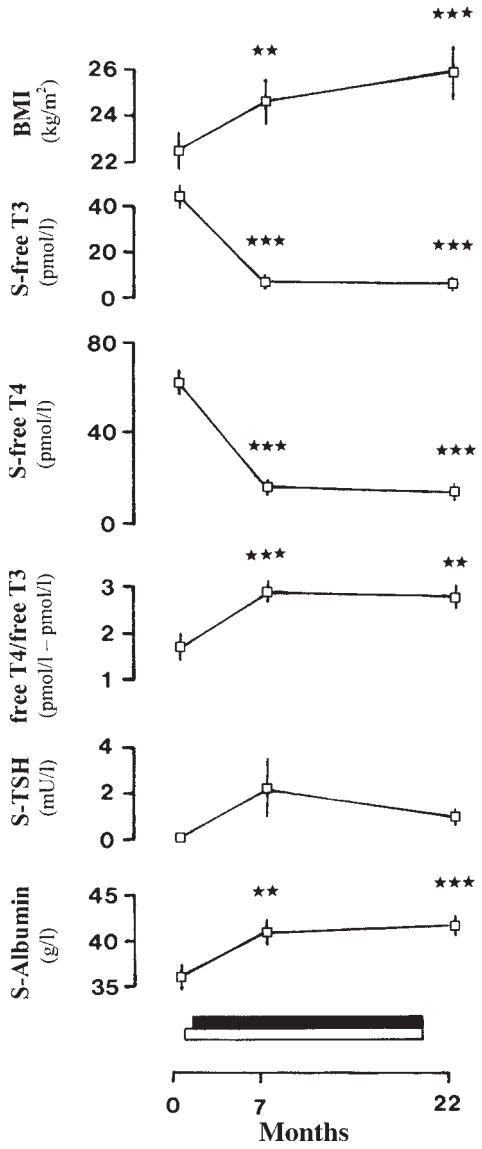

Fig. 1. BMI, serum T_3, T_4, T_4/T_3, TSH, and albumin measurements performed in 11 patients with Graves' thyrotoxicosis before, during, and after pharmacological treatment of the disease. Treatment was given with thiamazole (□) and thyroxine (■) for almost 20 mo. The final blood sample was collected approx 2 mo after conclusion of the pharmacological therapy. Values are means ± SEM. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ (comparison with pretreatment levels).

IGF-1/IGFBP-1 ratio: This ratio was 5.1 ± 1.1 in test 1. It increased slightly in test 2, and significantly in test 3.

Leptin: In test 1 the mean serum leptin concentration for both men and women was 11.5 ± 2.2 µg/L. No significant changes were noted in tests 2 and 3.

Ghrelin: In the thyrotoxic group the initial ghrelin concentration was 2454 ± 304 ng/L which was significantly lower than in the control group (4367 ± 219 ng/L; range: 2497–5157 ng/L; $p < 0.001$). During antithyroid therapy the ghrelin level increased to 3127 ± 397 ng/L ($p < 0.05$). It continued to increase in the posttherapy period, and reached a level of 3348 ± 279 ng/L ($p < 0.01$).

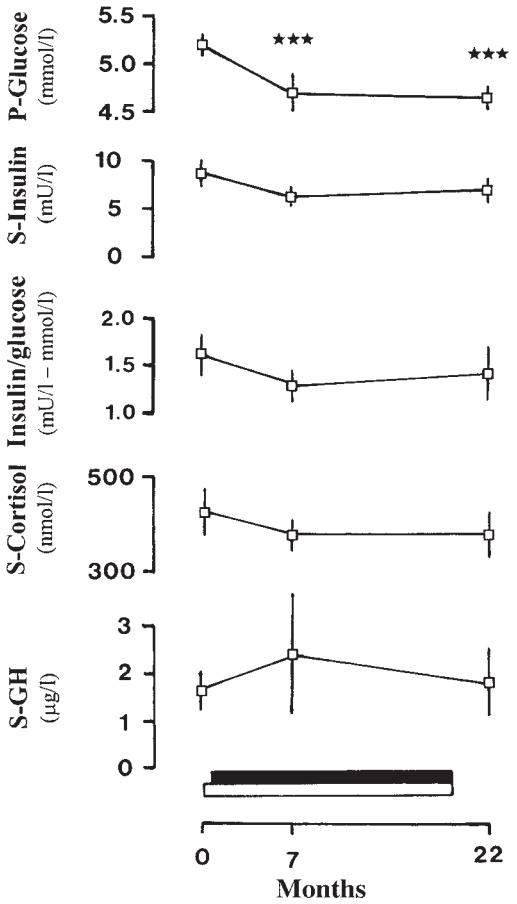

Fig. 2. Plasma glucose, and insulin, insulin/glucose, cortisol, and GH concentrations in serum of the same patients as described in Fig. 1.

Discussion

This study includes patients with newly diagnosed Graves' thyrotoxicosis. It is well known that such patients have a disturbed energy homeostasis *(1)*, but how this energy imbalance is brought about is not yet fully known. Hormones known to influence hunger–satiety signals may contribute. If so, it is reasonable to assume that the serum concentrations of these hormones change as long-term antithyroid medication transfers the thyrotoxic state into euthyroidism. In the current investigation this was studied by metabolic tests that were performed on three different occasions—before, during, and after pharmacological treatment of the disease. The surveillance program covered 22.0 ± 0.7 mo, and included a period of more than 2 mo during which the participants remained euthyroid without any form of medical treatment.

As predicted, increasing BMIs were recorded as long-term antithyroid treatment reduced the T_4 and T_3 secretion, inhibited the conversion of T_4 to T_3, and caused the T_4/T_3 ratio, and the thyroid hormone binding albumin concentration to increase.

The ghrelin picture was less predictable. A low serum ghrelin concentration was found in the thyrotoxic patients,

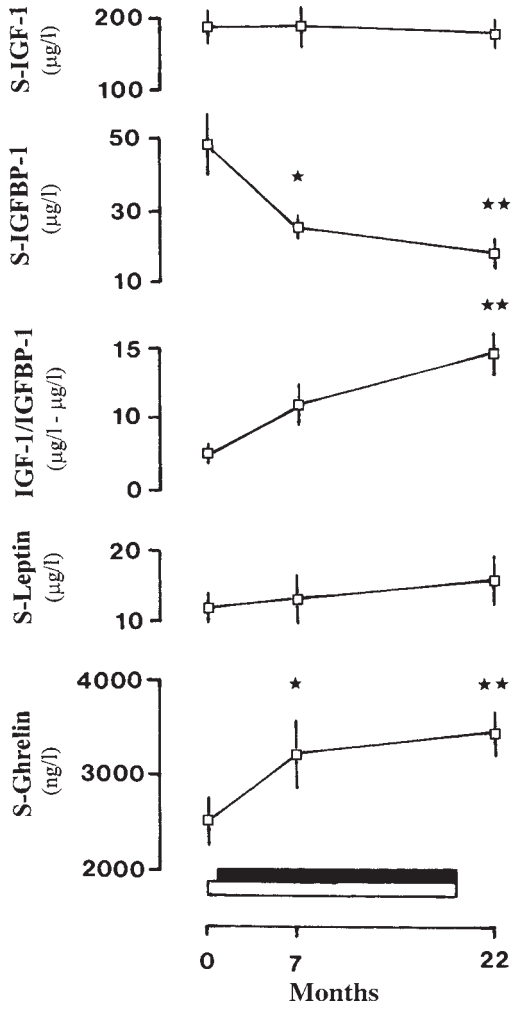

Fig. 3. IGF-1, IGFBP-1, IGF-1/IGFBP-1, leptin, and ghrelin concentrations in serum. Patients, treatment, and symbols are the same as described in Fig. 1 and 2.

which increased toward normal levels after pharmacological treatment. The low ghrelin level in the thyrotoxic state was a surprising finding taken into account that ghrelin stimulates appetite and food intake when given exogenously to normal subjects *(12)*. Moreover, increased circulating ghrelin levels have been found in anorectic patients *(14)*, and decreased levels in obese individuals *(15)*. With regard to these previous findings one would expect to find increased ghrelin concentrations in patients with untreated Graves' thyrotoxicosis. However, in contrast to that assumption, Riis et al. recently found low plasma ghrelin concentrations in patients with untreated hyperthyroidism, which increased significantly after a short period (2–3 mo) of antithyroid medication *(16)*. This could either reflect a stimulatory effect of the drugs on ghrelin secretion, or a change in the thyroid status when the thyrotoxic state was transformed into euthyroidism. Our present findings support the latter hypothesis in showing not only that the serum ghrelin level increased considerably after antithyroid medication, but also that the hormone concentration remained significantly increased for several weeks after conclusion of the long period of drug therapy.

It is not readily apparent why lower ghrelin levels were found in the hyperthyroid than in the euthyroid state, but one possible explanation is that total ghrelin concentrations were determined. Ghrelin has to be octanoylated at the third serine residue to be bioactive *(17)*. For that reason it cannot be taken for granted that observed changes in total ghrelin are reflected in similar changes in octnanoylated ghrelin. Another possibility is that hyperthyroid patients could be resistant, or respond differently, to changed secretion of ghrelin compared with euthyroid subjects.

If ghrelin does not seem to play an important role in the regulation of the energy homeostasis in thyreotoxic patients, other plausible candidates should be considered. One such candidate is leptin, which acts via NPY inhibition to reduce food intake in normal individuals. Plasma leptin has been found highly correlated with body mass index in humans as well as in rodents *(18)*. Accordingly, serum leptin levels are increased in obese subjects *(19)*, and decreased in patients with anorexia nervosa *(20)*. Most thyrotoxic patients lose weight despite increased intake of food *(1)*. After antithyroid medication both body weight and fat supplies tend to increase. This makes it reasonable to assume that fat-derived serum leptin concentrations should increase as pharmacological treatment makes thyrotoxic patients euthyroid. However, this was not the case in the present investigation. Instead, similar leptin levels were found in the hyperthyroid patients before and after normalization of their thyroid function. Valcavi et al. had made similar observation previously *(21)*, and Mantzoros et al. had shown that exogenous administration of T_3 to healthy volunteers did not affect their circulating serum leptin concentrations *(22)*. Taken together these observations imply that leptin can also be removed from the list of plausible regulators of the energy homeostasis in patients with hyperthyroidism.

Adipose tissue is a hormonally active system, which also produces adiponectin and resistin. These recently discovered adipocytokines are believed to play key roles in the regulation of energy metabolism, and it has been suggested that thyroid hormones are involved in the regulation of adiponectin and resistin *(23)*. How thyroid hormones influence the secretion of these hormones is currently a matter of debate. Noguerias et al. have shown that the secretion of resistin is severely decreased in hyperthyroid rats *(24)*. Iglesias et al. have made similar findings in hyperthyroid patients, but have also reported that the adiponectin secretion is normal in thyrotoxic patients *(25)*. Sanfini et al. have compared results obtained in euthyroid, hyperthyroid, and hypothyroid subjects and have found similar circulating levels of adiponectin and resistin in all three groups *(26)*. Yaturu et al. have obtained higher levels of both hormones during hyperthyroidism than during hypothyroidism *(23)*.

These inconsistent findings do not favor the notion that hyperorexia, associated with hyperthyroidism, is caused by changed secretion of adiponectin or resistin.

Hypoglycemia induces hunger and stimulates food intake in normal subjects *(27)*. But, hypoglycemic symptoms are, to the best of our knowledge, not more common among thyrotoxic patients than among euthyroid subjects, and none of the patients included in this series complained of such symptoms. Furthermore, our patients displayed a normal—but significantly higher—fasting plasma glucose level before pharmacological treatment than after. Although this does not necessarily exclude periods of hypoglycemia between tests, it is unlikely that hypoglycaemia is the mechanism that causes hyperorexia in thyrotoxic patients. Cortisol, insulin, GH, and IGF-1 levels were also normal in the hyperthyroid state, and all these levels changed very little after treatment. By contrast, the IGFBP-1 level was increased in the untreated patients, but it fell significantly after pharmacotherapy. IGFBP-1 binds circulating IGF-1. Only the free form of IGF-1 is considered biologically active *(28)*, and has strong blood glucose lowering potency *(29)*. The IGF-1/IGFBP-1 ratio reflects biologically active IGF-1 *(30)*. In the current study this ratio increased markedly after medication. An increased IGF-1 bioavailability after medication could well explain a concomitant plasma glucose decline, but a low IGF-1 bioavailability—as was found in the thyrotoxic state—is not compatible with hyperorexia, and can therefore hardly explain why patients with Graves' thyrotoxicosis have disturbed energy homeostasis.

It is possible that other IGF-1-binding proteins behave differently, and present decreased serum concentrations in untreated thyrotoxic patients. If so, this could, at least in part, explain why hyperorexia prevails in patients with thyrotoxicosis. However, until this possibility has been convincingly proved, it must be regarded merely hypothetical.

Changed serum concentrations of other hormone-binding proteins could also be of interest in this context. Previous studies have shown that thyroid hormone-binding globulin (TBG), and albumin concentrations are decreased in thyrotoxic patients *(31–34)*. Also in this investigation albumin levels were lower in the hyperthyroid than in the euthyroid state. However, at this point it is not known whether thyroid hormone transport proteins per se influence human energy homeostasis. This also applies to sex hormone–binding globulin, which, in contrast to TBG and albumin, is increased in patients with thyrotoxicosis *(35)*.

Increased thermogenesis, as a result of sympathetic overactivity *(36,37)*, could well contribute to energy expenditure associated with hyperthyroidism, but if, and how, such mechanisms influence IGF-1 bioavailability and ghrelin secretion, has to be determined in future studies.

Conclusion

In patients with untreated Graves' thyrotoxicosis the serum ghrelin concentration is low and the IGFBP-1 level high compared with normal individuals. During pharmacological treatment of the disease the ghrelin level increases and the IGFBP-1 level decreases. This tendency towards normalization continues also after cessation of the drug therapy, which suggests that the metabolic changes are caused by the changed thyroid status rather than by the medication per se.

Materials and Methods

Subjects

Eleven patients with Graves' thyrotoxicosis volunteered to participate. Nine were women and two men. Their mean age ± SEM was 36.6 ± 2.8 yr (range 25–51 yr). None of them suffered from other diseases, but all exhibited TSH receptor antibodies and markedly increased serum concentrations of free T_3 and free T_4 as shown in Table 1 and Fig. 1. Before therapy the BMIs ranged between 19.1 and 27.4 kg/m^2 (22.7 ± 0.8).

The patients were informed of the purpose of the study and gave their voluntary consent to participate in the project, which was approved by the ethics committee at Huddinge University Hospital in Stockholm.

A group of 16 healthy, nonobese subjects (13 women and 3 men) had previously been investigated in our laboratory. They were of comparable age (range 24–63 yr) and had similar BMIs (range 18.4–28.0 kg/m^2) as the abovementioned thyrotoxic patients. Their serum ghrelin concentrations had been determined after an overnight fast (unpublished observations). This made it possible to compare the mean ghrelin level in our untreated thyrotoxic patients with the mean ghrelin concentration of this control group.

Protocol

All patients were given oral pharmacological therapy for 18–20 mo. During the initial 2 wk only oral antithyroid medication was given (Tiamazole 15 mg twice daily). Then, hormone substitution with l-thyroxine was added at a dose of 100–150 μg once daily (Figs. 1–3). This made the patients clinically euthyroid after approx 4–5 wk of treatment. They were tested on three occasions: before (test 1), during (test 2), and after (test 3) the pharmacological therapy. Tests 2 and 3 took place 7.0 ± 1.1 and 22.0 ± 0.7 mo, respectively, after test 1. On the third occasion the patients had been off therapy for 1–5 mo (2.4 ± 0.4 mo).

The tests were performed in the morning with the patients fasted for 8 h. Body weights were noted, and venous blood samples collected from an antecubital vein for determination of plasma glucose, and serum concentrations of free T_3, free T_4, TSH, albumin, insulin, cortisol, GH, IGF-1, IGFBP-1, leptin, and ghrelin. When the patients were on medication (test 2), they did not take their ordinary morning dosages of thiamazole and thyroxine until after the blood sampling procedure.

Assays

Plasma glucose was analyzed by a hexokinase method (Boehringer Ingelheim, Mannheim, Germany) on a Hitachi 917 analyzer (Tokyo, Japan). Serum insulin and GH levels were determined by use of commercial kits (Insulin RIA 100 from Pharmacia Upjohn, Stockholm, Sweden, and Delphia HGH fluoroimmunoassay from Wallac, Turku, Finland, respectively). A fluorescence immunoassay, provided by autoDELFIA, Wallac Oy, Turku, Finland, made it possible to measure serum cortisol concentrations. Concentrations of free thyroid hormones (T_3 and T_4), as well as TSH were also measured by commercial solid-phase fluoroimmunoassays by the same manufacturer. Determination of serum concentrations of albumin was a routine analysis performed by evaluation of dye dilution. IGF-1 was determined by RIA after separation of IGFs from IGFBPs by acid–ethanol extraction and cryoprecipitation. To minimize interference of remaining IGFBPs, des(1-3)-IGF-1 was used as radioligand *(38)*. The intra- and interassay coefficients of variance (CVs) were 4% and 11%, respectively. IGFBP-1 concentrations in serum were analyzed according to the method of Póvoa et al. *(39)*. The sensitivity of the RIA was 3 µg/L, and the intra- and interassay CVs were 3% and 10%, respectively. Serum leptin concentrations were measured by use of a commercial RIA kit provided by Linco (St. Charles, USA). The assay had a sensitivity of 0.5 µg/L, and intra- and interassay CVs of 3.8% and 6.5%, respectively, at serum leptin concentrations ranging between 2.5 and 8.2 µg/L. Ghrelin (total) concentrations in serum were also analyzed with a RIA kit from Linco (Ghrelin [total] RIA kit). The sensitivity of the assay was 100 ng/L, and the intra- and interassay CVs 4.4% and 16.7%, respectively, at serum ghrelin levels of approx 3000 ng/L.

Calculations and Statistical Analysis

Three ratios were formed: T_4/T_3, insulin/glucose, and IGF-1/IGFBP-1. Laboratory data obtained in test 1 were compared with those in test 2 and 3. Differences were statistically evaluated by use of one-way ANOVA (Dunnett's *post-hoc* test). When the mean ghrelin concentration in the untreated thyrotoxic patients (test 1) was compared with the corresponding mean ghrelin concentration in the nonobese, healthy subjects, Student's unpaired *t*-test was used. *p*-values <0.05 were considered significant. Values denoted are means ± SEM.

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