CLINICAL STUDY

Ghrelin affects the hypothalamus–pituitary–thyroid axis in humans by increasing free thyroxine and decreasing TSH in plasma

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Abstract

Objective: Ghrelin promotes a positive energy balance, e.g. by increasing food intake. Stimulation of the activity of the hypothalamus–pituitary–thyroid (HPT) axis promotes a negative energy balance, e.g. by increasing energy expenditure. We therefore hypothesized that ghrelin suppresses the HPT axis in humans, counteracting its energy-saving effect.

Design and methods: In this single-blind, randomized, cross-over study, we determined secretion patterns of free triiodothyronine (fT₃), free thyroxine (fT₄), TSH, and thyroid-binding globulin (TBG) between 2000 and 0700 h in 20 healthy adults (10 males and 10 females, 25.3 ± 2.7 years) receiving 50 µg ghrelin or placebo at 2200, 2300, 0000, and 0100 h.

Results: fT₄ plasma levels were significantly higher after ghrelin administration than after placebo administration from 0000 h until 0620 h except for the time points at 0100, 0520, and 0600 h. TSH plasma levels were significantly lower from 0200 until the end of the study at 0700 h except for the time points at 0540, 0600, and 0620 h. The relative increase of fT₄ (area under the curve (AUC) 0130–0700 h (ng/dl × min): placebo: 1.31 ± 0.03; ghrelin: 1.39 ± 0.03; *P* = 0.001) was much weaker than the relative decrease of TSH (AUC 0130–0700 h (mIU/ml × min): placebo: 1.74 ± 0.12; ghrelin: 1.32 ± 0.12; *P* = 0.007). fT₃ and TBG were not affected.

Conclusions: This is the first study to report that ghrelin affects the HPT axis in humans. The early fT₄ increase was possibly induced by direct ghrelin action on the thyroid where ghrelin receptors have been identified. The TSH decrease might have been caused by ghrelin-mediated inhibition at hypothalamic level by feedback inhibition through fT₄, or both.

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Introduction

Ghrelin is the endogenous ligand of the GH secretagogue receptor (GHS-R). It is predominantly synthesized in the stomach, but it has also been detected in the bowels, kidney, pituitary, lung, lymphatic tissue, placenta, hypothalamus, and thyroid. Accordingly, a broad variety of endocrine, cardiovascular, immunological, reproductive, and behavioral effects have been described (1). Still, most studies have focussed on ghrelin's role in energy homeostasis and weight regulation: ghrelin is an orexigenic hormone that increases appetite and food intake (2, 3), and induces adiposity in rodents by decreasing lipid oxidation and increasing lipogenesis (2, 4, 5). Furthermore, it was shown to shift food preference towards high-fat diet (6). There is also evidence that ghrelin decreases thermogenesis and energy expenditure (7, 8). Thus, ghrelin causes a positive

energy balance. In humans, ghrelin plasma levels were found to be negatively correlated with weight and body fat (9, 10). Being decreased in obesity, plasma levels increased after weight loss (10). Conversely, plasma ghrelin levels were elevated in anorectic patients, and decreased after weight gain (11).

Hormones of the hypothalamus–pituitary–thyroid (HPT) axis are crucially involved in maintaining body temperature and energy homeostasis in mammals. They stimulate the metabolic rate of most tissues such as liver, heart, skin, bone, muscle, or adipose tissue, thereby increasing thermogenesis and energy expenditure (12–14). Consequently, hyperthyroid states are associated with weight loss and increased body temperature, whereas hypothyroid states are associated with weight gain and reduced body temperature (14). Taken together, ghrelin promotes energy-saving effects and weight gain, whereas the activation of the HPT axis

is associated with increased energy expenditure and weight loss.

These findings suggest that ghrelin may affect the HPT axis. In fact, a recent study in male rats has reported a decreased activity of the HPT axis after i.c.v. injection of ghrelin or placebo every 24 h for 5 days: pituitary TSH cells were smaller and TSH plasma levels were lower, and as a result, thyroid follicles were less active and thyroxine (T_4) plasma level was reduced compared with placebo-treated rats (15). Furthermore, another study found significantly lower TSH plasma levels after a single i.c.v. injection of ghrelin than after that of placebo after 20 min (16). But in studies on dogs (17, 18) and humans (19, 20), ghrelin did not change TSH levels. Also, the synthetic GHS-R agonist hexarelin was found to decrease TSH secretion after a single dose within 2 h (21). However, hexarelin had no effects on TSH levels in the long term, i.e. injected s.c. for 16 weeks twice daily (22). There are other findings indicating a role for ghrelin in the regulation of the HPT axis: ghrelin-binding sites, possibly different from the GHS-R, have been detected in the human thyroid (23, 24). In addition, ghrelin enhanced *in vitro* TSH-induced proliferation of rat thyrocytes (25), and diminished cell proliferation in human thyroid carcinoma cell lines (23).

We hypothesized that ghrelin suppresses the activity of the HPT axis in humans, which counteracts its energy-saving effects. We tested this hypothesis by determining secretion patterns of free triiodothyronine (fT_3), free T_4 (fT_4), TSH, and thyroid-binding globulin (TBG) after administration of ghrelin and placebo.

Research design and methods

Subjects

Twenty healthy adults, aged 20–30 years (10 males and 10 females, 25.3 ± 2.7 years; body mass index: 21.6 ± 1.6 ; range: 19.7–24.8), were included in this study. Exclusion criteria comprised a lifetime history of endocrine or psychiatric disorders, a pathological electroencephalogram (EEG) or electrocardiogram, or drug intake during the 3 months prior to study entry. All subjects had normal thyroid function as assessed by plasma levels of fT_3 , fT_4 , and TSH. The study was conducted in accordance with the guidelines in the Declaration of Helsinki. Written informed consent was obtained from all participants. Ethical review board approval was given.

Study design

This was a single-blind, placebo-controlled, randomized, cross-over study that comprised two blocks of two consecutive nights. In females, each block took place during the early follicular phase. Generally, both blocks occurred in two consecutive cycles, i.e. with an interval

of about 4 weeks. In males, the two blocks were separated by at least 1 week. The first night of each block served for adaptation to sleep laboratory settings. During the second night, 50 μ g of acylated ghrelin (Clinalfa, Lufelfingen, Switzerland) or placebo were injected at 2200, 2300, 0000, and 0100 h. In addition, 4 ml of blood was drawn every 30 min (2000–2200 h) and 20 min (2200–0700 h) respectively from the adjacent room using an i.v. cannula and a tubic extension. Furthermore, sleep EEGs were conducted between 2300 and 0700 h. These data have been presented elsewhere (26, 27). Substances (e.g. coffee and alcohol) or activities (e.g. naps during the day and excessive exercises) potentially influencing vigilance were restricted or prohibited. Food intake was not controlled prior to the study period, but all participants reported normal eating patterns including breakfast, lunch, and dinner. Apart from transient sweating in one female and one male subject in the ghrelin condition, no side effects occurred. None of the participants reported increased appetite.

Hormone analysis

Blood samples were centrifuged immediately, and plasma was frozen at -25°C . Concentrations of fT_3 , fT_4 , TSH, and TBG were determined using a solid-phase, two-site, sequential chemiluminescent immunometric assay in an automated analyzer (Immulite 2005, Siemens Medical Solutions, Erlangen, Germany). All samples of an individual were measured in the same assay run. Detection limits were 1.0 pg/ml for fT_3 , 0.12 ng/dl for fT_4 , 0.01 mIU/ml for TSH, and 1.6 μ g/ml for TBG. Intra- and interassay coefficients of variance were below 9 and 12% (fT_3 , fT_4 , and TBG), and below 6 and 8% (TSH) respectively.

Statistical methods

Treatment effects on hormone concentrations were identified by means of a MANOVA for the whole study period (2000–0700 h), the two halves of the study period (first half: 2000–0130 h; second half: 0130–0700 h), and the post-intervention period (2220–0700 h). Whenever significant treatment effects were found, three curve characteristics (area under the curve (AUC), mean location, and delta (highest value minus lowest value)) were calculated and tested for significant differences between treatment conditions with univariate *F*-tests. Differences of mean plasma levels of fT_3 , fT_4 , TSH, and TBG at single time points were tested for significance using a test with contrasts in a MANOVA (level of significance: $\alpha = 0.05$). The Holm–Sidak method was used for correction for multiple testing.

Pulses and pulse characteristics of TSH secretion were determined using Cluster8 from PulseXP software (Charlottesville, VA, USA) (28) for the whole study period and the post-intervention period. Number of peaks, mean peak interval, mean peak width, mean peak

height, and mean nadir were analyzed. Less than 2% of all the samples were missing, which were not replaced. Metric demographic variables are given as mean $\pm$ S.D., and hormone variables are given as mean $\pm$ S.E.M.

Results

FT₃ and FT₄

FT₄ plasma levels were significantly ($P < 0.05$) higher after ghrelin administration than after placebo administration from 0000 h until 0620 h except for the time points at 0100, 0520, and 0600 h (Fig. 1). FT₃ plasma levels did not differ at any point in time. Consequently, a significant treatment effect on FT₄ secretion was observed during the whole study period ($P = 0.016$), the post-intervention period ($P = 0.019$), and the second half of the study period ($P = 0.005$), but not during the first half ($P = 0.063$; Table 1). The AUC (FT₄) was 1.1% higher after ghrelin injection than after placebo injection during the post-intervention period, and 5.8% higher during the second half of the study period. Significant treatment effects on secretion of FT₃ were not observed during any of these periods (Fig. 1).

Figure 1 Secretion profiles of free triiodothyronine (FT₃) and free thyroxine (FT₄) (mean $\pm$ S.E.M.) in 20 healthy subjects receiving ghrelin or placebo. *Significant difference between treatment groups.

TSH

Mean TSH plasma levels were almost identical in both treatment conditions until 0000 h, showing a strong increase peaking at 2300 h. TSH plasma levels were significantly ($P < 0.05$) lower in the ghrelin condition than in the placebo condition from 0200 h until the end of the study at 0700 h except for the time points at 0540, 0600, and 0620 h (Fig. 2). A significant treatment effect was observed during the whole study period ($P = 0.004$), the post-intervention period ($P = 0.003$), and the second half of the study period ($P = 0.003$), but not during the first half ($P = 0.214$). During the whole study period, delta was significantly larger in the ghrelin condition than in the placebo condition (Table 2). AUC and mean location were numerically but not significantly smaller. During the post-intervention period, all these findings reached statistical significance; AUC and mean location were about 15% smaller after ghrelin administration than after placebo administration (Table 2). During the second half of night, delta did not differ (placebo: 0.80 ± 0.10 ; ghrelin: 0.87 ± 0.09 mIU/ml), whereas AUC (placebo: 1.74 ± 0.18 ; ghrelin: 1.32 ± 0.12 mIU/ml $\times$ min; $P = 0.007$) and mean location (placebo: 1.85 ± 0.19 ; ghrelin: 1.41 ± 0.13 mIU/ml; $P = 0.008$) were 24% smaller after ghrelin administration than after placebo administration.

The mean nadir was smaller after ghrelin injection than after placebo injection (whole study period: $P = 0.088$; post-intervention period: $P = 0.040$). Other pulse characteristics, e.g. pulse frequency, did not differ (Table 2).

Thyroid-binding globulin

TBG plasma levels did not significantly differ at any time point between treatment conditions. Significant treatment effects were observed neither during the whole study period ($P = 0.832$) and the post-intervention period ($P = 0.738$), nor during one of the two halves of the study period (first half: $P = 0.785$; second half: $P = 0.240$).

Discussion

Ghrelin caused a subtle increase of FT₄ followed by a marked decrease of TSH ~ 2 h after that in our study. This decrease occurred after the physiological TSH surge (29) which was not affected. FT₃ plasma levels did not differ between placebo and ghrelin conditions at any time.

Potential reasons for elevated FT₄ plasma levels are an increased release from the thyroid or a decreased peripheral thyroid hormone metabolism, e.g. due to raised hepatobiliary clearance or reduced activity of deiodinases. In addition, reduced TBG plasma levels

Table 1 Curve characteristics of free thyroxine (fT₄) secretion in 20 adults receiving ghrelin or placebo; results (mean $\pm$ S.E.M.) during the following periods are depicted: whole study (2000–0700 h), post-intervention (2220–0700 h), and first half (2000–0130 h) and second half (0130–0700 h) of the study.

	Area under the curve (ng/dl $\times$ min)			Mean location (ng/dl)			Delta (ng/dl)		
	Placebo	Ghrelin	P	Placebo	Ghrelin	P	Placebo	Ghrelin	P
fT ₄									
Whole study	1.31 (0.03)	1.33 (0.03)	0.005	1.22 (0.02)	1.27 (0.03)	0.006	0.26 (0.02)	0.30 (0.02)	NS
Post-intervention	1.27 (0.03)	1.28 (0.03)	0.004	1.21 (0.02)	1.27 (0.03)	0.005	0.25 (0.02)	0.28 (0.02)	NS
First half	1.31 (0.03)	1.27 (0.03)	NS	1.23 (0.03)	1.28 (0.03)	0.035	0.22 (0.02)	0.24 (0.02)	NS
Second half	1.31 (0.03)	1.39 (0.03)	0.001	1.20 (0.02)	1.27 (0.03)	0.001	0.20 (0.01)	0.24 (0.02)	NS

after ghrelin injection or T₄ displacement from TBG by ghrelin could be associated with higher fT₄ plasma levels. However, TBG plasma levels were similar for both treatments, and displacement of T₄ would have been probably accompanied by displacement of T₃ which is also bound to TBG (30, 31). More likely, increased fT₄ plasma levels could be caused by a decreased thyroid hormone metabolism: the hepatobiliary clearance of T₃ and T₄ occurs partly through different pathways, and substances which affect only the T₄ clearance have been described (32–34). Considering an altered activity of deiodinases, changed T₃ plasma levels might be actually expected since all deiodinases affect T₃. Deiodinase 1 and deiodinase 2 catalyze the conversion from T₄ to T₃. Deiodinase 3 catalyzes the degradation of both T₄ and T₃ (35). Yet surprisingly, deiodinase 1-deficient mice had elevated T₄ levels, while T₃ and TSH were normal (36). Accordingly, ghrelin could affect deiodinase 1 in humans. However, we assume that the increase of fT₄ was rather caused by release from the thyroid than by an altered metabolism since it occurred already 2 h after the first ghrelin injection. In contrast acute exposure to substances, which either induce or inhibit uridine diphosphoglucuronyl transferase (UGT), being crucially involved in thyroid hormone metabolism

(37, 38), led to significant changes of UGT activity not before 24 h after exposure (39, 40).

Assuming that the increase in fT₄ was due to release from the thyroid, it was apparently not induced by pituitary TSH since TSH plasma levels before the increase were similar for both treatments. We therefore suggest that ghrelin stimulated the fT₄ release directly from the thyroid where GHS-Rs were identified (24). A stimulatory action at thyroid level, namely a rapid activation of intracellular pathways involved in TSH-induced proliferation of thyrocytes, has been described in rats also (25). It can be argued that the TSH suppression observed in our study might be caused by a feedback inhibition by fT₄. Both the plasma half-life of TSH of roughly 1 h and our finding that the relative increase of fT₄ was about four times weaker than the relative decrease of TSH seem to be in line with this assumption since TSH and fT₄ have been shown to have an inverse log-linear relationship (41, 42). However, it is questionable whether the small increase of fT₄ can entirely explain the distinct and sustained suppression of TSH as several times stronger increases of fT₄ after administration of 125 or 250 μ g T₄ in healthy volunteers failed to be associated with a significant decrease of TSH (41).

In fact, there are several findings suggesting that ghrelin suppresses TSH secretion directly at hypothalamic level comparably to ghrelin's suppressive effect on the secretion of LH and FSH (43, 44): first, the same hypothalamic neurons that mediate ghrelin's orexiogenic action strongly affect the activity of TRH neurons, the hypothalamic releasing factor of TSH; ghrelin stimulates neurons containing the orexiogenic peptides neuropeptide Y (NPY) (45–47) and agouti-related protein (AgRP) (45, 47), which decrease the activity of TRH neurons (48, 49). Furthermore, ghrelin probably inhibits neurons containing the anorexigenic peptides α -melanocyte-stimulating hormone (α -MSH) (50) and cocaine- and amphetamine-regulated transcript (CART) (51), which increase the activity of TRH neurons (48, 49). Secondly, the hormone leptin that has antagonistic effects to ghrelin regarding appetite (decreasing) and TSH secretion (increasing) exerts these effects by an opposite action on NPY/AgRP neurons (inhibiting) (52, 53) and α -MSH/CART

Figure 2 Secretion profile of TSH (mean $\pm$ S.E.M.) in 20 healthy subjects receiving ghrelin or placebo. *Significant difference between treatment groups.

Table 2 Curve and pulse characteristics (mean $\pm$ s.e.m.) of TSH secretion during the whole study period (2000–0700 h) and the post-intervention period (2220–0700 h).

	Whole study period			Post-intervention period		
	Placebo	Ghrelin	P	Placebo	Ghrelin	P
Curve characteristics						
AUC (mIU/ml $\times$ min)	1.82 (0.18)	1.61 (0.15)	NS	1.85 (0.18)	1.57 (0.14)	0.044
Mean location (mIU/ml)	1.89 (0.18)	1.68 (0.16)	NS	1.86 (0.18)	1.58 (0.14)	0.046
Delta (mIU/ml)	1.28 (0.13)	1.68 (0.19)	0.012	1.23 (0.14)	1.65 (0.19)	0.009
Pulse characteristics						
Number of pulses	3.95 (0.18)	4.25 (0.28)	NS	3.40 (0.19)	3.60 (0.28)	NS
Mean peak interval (min)	126.4 (12.6)	114.2 (9.5)	NS	126.8 (12.7)	125.6 (11.1)	NS
Mean peak width (min)	95.3 (7.6)	85.5 (9.4)	NS	84.7 (6.6)	84.9 (9.9)	NS
Mean peak height (mIU/ml)	2.15 (0.21)	1.98 (0.17)	NS	2.10 (0.21)	1.90 (0.17)	NS
Mean nadir (mIU/ml)	1.54 (0.15)	1.30 (0.14)	NS	1.55 (0.15)	1.27 (0.13)	0.040

neurons (stimulating) (54, 55). Thirdly, TSH suppression in rats was induced by ghrelin injection in the CNS, suggesting a comparable mechanism in humans. In that study, ghrelin was given subchronically (5 days). TSH suppression was associated with a significant decrease in T_4 and a decrease in T_3 at a trend level ($P < 0.1$) (15).

Considering our and the other findings described, we propose that the observed decrease in TSH was caused to a greater extent by direct inhibition of hypothalamic neurons, and to a smaller extent by feedback inhibition by initially increased ft_4 . Yet, our study alone cannot definitely prove this assumption. The partly different findings in rats unequivocally showing a suppression of the HPT axis (TSH, T_4 , and T_3) (15) are probably caused by two factors: first, the central administration of ghrelin, rendering unlikely a direct stimulatory effect on the thyroid; secondly, the markedly longer treatment and observation period (5 days versus 9 h as judged from the first ghrelin injection in the present study). Taking the long half-life of T_4 of about 7 days into account, our study was too short to capture the effects on ft_4 consecutive to TSH suppression. Therefore, our results do not exclude that ghrelin overall suppresses the activity of the HPT axis.

Why did ft_3 plasma levels remain unchanged in our study? While regular administration of T_4 causes an increase of T_3 since more T_4 will be deiodinized to T_3 (56, 57), short-term administration of T_4 is not necessarily associated with changes in T_3 (41). Comparably, the small T_4 increase observed in our study was not followed by higher T_3 plasma levels. The study was conducted at night for capturing the TSH surge, because potential differences between treatments can be obviously more easily identified with higher plasma levels. In addition, potential inferences do occur more frequently during the day than during the night. Ghrelin doses given in our study (50 μ g corresponding to 0.6–0.8 μ g/kg body weight) were lower than those given in most other studies investigating ghrelin's effects in humans, which were usually 1 μ g/kg body weight

(58, 59). As a result, no side effects occurred apart from sweating in two subjects. Of interest, not even appetite induction of this actually appetite-inducing hormone (3) was reported. These clinical findings and the short half-life of acylated ghrelin of about 10 min (60) rebut the possible concern that effects on the HPT axis observed in the present study might have been caused by an accumulation of ghrelin.

To our knowledge, this is the first study showing that ghrelin affects the HPT axis in humans. Furthermore, it is the first study in mammals showing such an effect after peripheral administration of ghrelin. In rats, two groups reported a suppression of TSH plasma levels after i.c.v. injection (15, 16). However, most studies in animals and humans failed to detect an effect on the HPT axis possibly due to methodological limitations, e.g. lack of control condition (17–20), insufficient sample size and measurement period (17). In addition, several pulses of ghrelin might be required to elicit a suppressive effect since ghrelin physiologically exhibits a pulsatile secretion pattern (61).

In conclusion, we show for the first time that ghrelin affects the HPT axis in humans. The early ft_4 increase was possibly induced by direct ghrelin action on the thyroid where ghrelin receptors have been identified. The TSH decrease might have been caused by ghrelin-mediated inhibition at hypothalamic level by feedback inhibition through ft_4 , or both.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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