CLINICAL STUDY

Plasma ghrelin levels during exercise in healthy subjects and in growth hormone-deficient patients

Rolf Dall, Jill Kanaley¹, Troels Krarup Hansen, Niels Møller, Jens S Christiansen, Hiroshi Hosoda², Kenji Kangawa² and Jens O L Jørgensen

Medical Department M (Endocrinology and Diabetes) and Institute of Experimental Clinical Research, Aarhus University Hospital, Aarhus, Denmark, ¹Department of Exercise Science, Syracuse University, Syracuse, New York, USA and ²Department of Biochemistry, National Cardiovascular Research Institute, Osaka, Japan

(Correspondence should be addressed to J O L Jørgensen, Medical Department M, Aarhus Kommunehospital, DK 8000 C, Denmark; Email: jolj@dadlnet.dk)

Abstract

Objective: To characterise plasma levels of the recently identified endogenous ligand for the GH secretagogue receptor (ghrelin) during submaximal aerobic exercise in healthy adults and in GH-deficient adults.

Design: Eight healthy males (mean $\pm$ s.e. age, 40.8 ± 2.9 years) and eight hypopituitary males with verified GH deficiency (mean $\pm$ s.e. age, 40.8 ± 4.7 years) underwent a baseline test of their peak aerobic capacity (VO_2 peak) and lactate threshold (LT) on a cycle ergometer, as well as an evaluation of body composition. The patients were then studied on two occasions in random order when they exercised for 45 min at their LT. On one occasion, GH replacement had been discontinued from the evening before, whereas on the other occasion they received their evening GH in addition to an intravenous infusion of GH (0.4 IU) during exercise the following day. The healthy subjects exercised at their LT on one occasion without GH.

Results: The patients were significantly more obese and had lower VO_2 max (corrected for body weight) and LT as compared with the control subjects. Exercise induced a peak in serum GH concentrations after 45 min in the control group ($11.43 \pm 3.61 \mu\text{g/l}$). Infusion of GH in the patients resulted in a peak level after 45 min, whereas no increase was detected when exercising without GH (9.77 ± 2.40 (GH) vs $0.11 \pm 0.07 \mu\text{g/l}$ (no GH)). Plasma ghrelin levels did not change significantly with time in either study, and no correlations were detected between ghrelin levels and parameters such as GH and IGF-I levels, age or body composition. Plasma ghrelin levels were significantly lower during the study period with GH as compared with the study with no GH.

Conclusions: Submaximal aerobic exercise of an intensity sufficient to stimulate GH release was not associated with significant alterations in plasma ghrelin concentrations, which indicated that systemic ghrelin is not involved in the exercise-induced stimulation of GH secretion. The observation that ghrelin levels were lower during GH replacement suggests that GH may feedback-inhibit systemic ghrelin release.

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Introduction

Acute physical exercise is a well-recognised stimulator of growth hormone (GH) release in man (1). GH release is delayed until 10–20 min into exercise and usually remains elevated for 1–2 h after exercise. In addition, above a certain threshold a linear dose–response relationship appears between exercise intensity and GH release, while repeated bouts of exercise do not attenuate the magnitude of the response (2). A large inter-subject variability in exercise-induced peak GH levels is present, which can be only partly attributed to factors such as level of training, age, gender and

body composition (2). It is also well established that the response is aggravated in patients with poorly controlled diabetes mellitus (3). The neurotransmitters participating in the control of exercise-induced GH release remain uncertain despite numerous studies, but the final common pathway is assumed to involve either GH-releasing hormone secretion and/or inhibition of somatostatin release (4).

The recently identified endogenous ligand for the GH secretagogue receptor, ghrelin, has been shown to strongly stimulate GH release in healthy adults (5, 6). The principal site of ghrelin synthesis is the stomach, and the hormone has been shown to convey orexigenic

signals and to increase gastric motility and acid secretion (7, 8). It has also been reported that plasma ghrelin levels are decreased in obese subjects (9) and following food intake (10), whereas short-term fasting and intended weight loss induce moderately elevated ghrelin levels (10, 11). However, little is known about plasma ghrelin levels during other conditions associated with alterations in GH secretion.

In the present study, we have evaluated the effect of moderate submaximal exercise on plasma ghrelin levels, as well as pertinent components of the GH–insulin-like growth factor (IGF) axis. We performed serial plasma ghrelin measurements before, during and after 45 min of submaximal cycle ergometer exercise in eight GH-deficient adult males studied both with and without concomitant GH replacement, as well as eight age-matched healthy adults studied on one occasion.

Subjects and methods

Subjects

Clinical characteristics of the GH-deficient patients and the healthy subjects are provided in Tables 1 and 2. The study included only male subjects. Hypopituitarism was secondary to verified juxtasellar pathology and/or its treatment. GH deficiency was diagnosed prior to the start of GH replacement by means of an insulin tolerance test, arginine infusion or both. Additional pituitary replacement where present was continued unchanged throughout the study. The healthy subjects were recruited from public advertisement and were matched with the patients for age. None of the participants had evidence of cardiovascular or pulmonary disease or hypertension. The protocol was approved by the Ethical Committee system, and included oral and written consent from the participants.

Exercise protocol

Each patient was examined on three occasions and the control subjects were studied on two occasions. On the first visit (baseline), all subjects underwent a test of

their peak aerobic capacity on the cycle ergometer to establish their level of cardiovascular fitness (VO_2 peak) and lactate threshold (LT), and underwent measures of body composition. The patients were then evaluated on two separate occasions for an 8-h period (study days). On both occasions, the patients were required to exercise for 45 min at their LT. On the study day of exercise without GH, the patients did not take their prescribed GH injection the evening before. During the exercise study with GH, the GH injection the evening before was taken and an intravenous GH infusion (0.4 IU Norditropin; Novo Nordisk, Bagsværd, Denmark) was administered during exercise. These study days were in randomised order. The control subjects were studied on one occasion (study day) with a protocol similar to the patients but did not receive any GH infusion during the exercise test.

VO_2 peak was determined using a continuous cycle ergometer protocol. Subjects started cycling at 100 W for the first 3 min and the work output was increased by 25 W every 3 min until volitional fatigue. Blood lactate samples were taken at the end of every 3-min stage to determine the LT. VO_2 peak was selected as the highest O_2 consumption and where a further work output increase resulted in less than a 200 ml increase in O_2 . In addition, all subjects had a rating of perceived exertion greater than 18 on the Borg scale, and all subjects reached their age-predicted maximal heart rate. Body composition was assessed using dual energy X-ray absorptiometry (DXA-Hologic QDR 2000, version 5.54, Waltham, MA, USA).

On a study day, subjects reported to the Research Centre at 0700 h at which time one catheter was placed retrogradely in a deep antecubital vein, one catheter was inserted retrogradely in a heated dorsal hand vein and one catheter was placed in the other antecubital vein for infusions. The subjects remained in the supine position throughout the study except for the 45 min of exercise. Resting blood samples were taken for 3 h prior to exercise ($t = -180, -60, -30, -15$ and 0 min), during exercise ($t = 15, 30$ and 45 min) and during 2.75 h of recovery ($t = 60, 90, 120, 180, 210, 225$ and 240 min). During exercise, subjects exercised at a workload that was equivalent

Table 1 Clinical characteristics of the patients.

Patient	Age (years)	BMI (kg/m^2)	Diagnosis	Stimulated peak GH ($\mu\text{g/l}$)	GH dose (mg)	Additional replacement
1	20	30.0	Glioma	0.64	0.70	T, T4
2	49	30.3	NFPA	0.48	0.50	
3	49	32.3	Cushing's disease	0.16	0.40	HC, T4
4	38	29.3	NFPA	1.12	0.50	HC, T4, T
5	45	25.4	Cushing's disease	0.05	0.70	HC, T, T4
6	24	27.9	Craniopharyngeoma	0.01	0.50	HC, T, T4
7	50	29.9	Cushing's disease	0.60	0.70	HC, T, T4
8	51	28.5	Prolactinoma	1.60	0.35	HC, T, T4

BMI, body mass index; NFPA, non-functioning pituitary adenoma; T, testosterone; T4, thyroxine; HC, hydrocortisone.

Table 2 Physical characteristics and exercise variables for the GH-deficient adults (GHDA) and control subjects.

	GHDA (n = 8)	Controls (n = 8)
Age (years)	40.8 ± 4.6	40.4 ± 4.2
BMI	29.2 ± 0.8**	23.6 ± 0.5
% fat	22.5 ± 1.9**	15.9 ± 0.9
LBM (kg)	69.8 ± 1.8	65.1 ± 1.8
Fat mass (kg)	20.7 ± 2.3**	12.4 ± 1.0
VO ₂ max (l/min)	2.8 ± 0.1	3.2 ± 0.2
VO ₂ max (ml/kg/min)	28.1 ± 2.1**	41.7 ± 2.6
Exercise workload (W)	120 ± 6.0**	155.1 ± 8.3

LBM, lean body mass; VO₂, aerobic capacity; W, watts.** $P < 0.01$ between groups.

to their LT for 45 min on the cycle ergometer. Subjects pedalled at a constant velocity to elicit an initial blood lactate concentration of approximately 2.5 mmol/l; this corresponded to approximately 62% of their VO₂ peak. Upon completion of the exercise, the subjects again remained in the supine position.

Assays

Plasma samples were collected in tubes with aprotinin/EDTA solution on ice and frozen immediately at -80°C . Total plasma ghrelin was measured with a radioimmunoassay (RIA) using polyclonal rabbit antibodies raised against the carboxy-terminal fragment (13-28) of ghrelin as previously described (12). In short, plasma was diluted with an equal volume of 0.9% saline after centrifugation at 4°C . The sample was loaded onto a Sep-Pak C18 cartridge (Waters, Milford, MA, USA) pre-equilibrated with 0.9% NaCl. The cartridge was washed and eluted, and the eluate (200 μl equivalent of plasma) lyophilised. Each RIA incubation mixture was composed of 100 ml standard ghrelin or unknown sample, and 200 ml anti-ghrelin [13-28] antiserum diluted 1/20 000 with RIA buffer containing 0.5% normal rabbit serum. After 12 h of incubation, 100 ml ^{125}I -labelled tracer (15 000 c.p.m.) were added. After an additional 36 h of incubation, 100 ml anti-rabbit IgG goat serum was added. Free and bound tracers were separated after incubation for 24 h by centrifugation at 3000 r.p.m. for 30 min. After aspiration of supernatant, radioactivity in the pellet was counted with a gamma counter (ARC-600; Aloka, Tokyo, Japan). All assay procedures were performed in duplicate at 4°C . The antiserum exhibited 100% cross-reactivity with human ghrelin, and no significant cross-reactivity with other peptides was observed. The intra- and interassay variability levels were less than 6% and 9% respectively. Day-to-day variation in normal subjects was less than 9%.

Serum GH was determined by a double monoclonal immunofluorometric assay (DELFI; Wallac Oy,

Turku, Finland). Intra- and interassay coefficients of variation ranged from 1.8 to 3.0% and 1.6 to 2.3% for 0.71–31.4 $\mu\text{g/l}$ GH respectively, and the detection limit was 0.01 $\mu\text{g/l}$. Serum IGF-I was measured with an in-house time-resolved immunofluorometric assay as previously described (13). Serum IGF-binding protein (IGFBP)-1 was measured by ELISA (Medix Biochemica, Kainainen, Finland) and IGFBP-3 by an immunoradiometric assay (Diagnostic System Laboratories, Webster, TX, USA).

Statistical methods

The Kolmogorov–Smirnov test was used to test for normality. The unpaired sample *t*-test or one-way analysis of variance (ANOVA) was used to evaluate the differences between groups. Two-way ANOVA for repeated measurements was used to estimate the changes over time in hormones, metabolites and binding proteins. Pearson's product moment correlation with two-tailed probability values was used to measure the strength of association between variables. Data are given as the mean $\pm$ S.E., and statistical significance was assumed at $P < 0.05$. All statistical calculations were performed with SPSS for Windows version 11.0 (SPSS, Chicago, IL, USA).

Results

The patients were significantly more obese as compared with the control group ($P < 0.01$, Table 2). Cardiovascular fitness (VO₂ peak) corrected for total body weight as well as LT were significantly lower in the patients as compared with the control subjects (Table 2). Serum GH concentrations increased significantly during exercise and reached a peak level after 45 min in the control

Figure 1 Serum GH concentrations (means $\pm$ S.E.) before, during and after exercise (shaded bar) in control subjects and in GH-deficient patients (GHDA) with or without GH substitution. For details on GH dosage, see text.

group (Fig. 1). Infusion of GH resulted in a peak GH level after 45 min, which did not differ significantly from the endogenous GH peak in the control group. As expected, GH levels did not change over time

when the patients exercised without GH (Fig. 1). Serum IGF-I levels were significantly higher in the patients on both study days as compared with the control group, whereas no significant changes with time

Figure 2 Serum concentrations (means $\pm$ S.E.) of IGF-I (upper panel), IGFBP-3 (middle panel) and IGFBP-1 (lower panel) before, during and after exercise (shaded bar) in control subjects (●) and in GH-deficient patients with (○) or without (▼) GH substitution.

were disclosed in any situation (Fig. 2, upper panel). In all three studies, serum IGFBP-3 levels increased slightly at the end of exercise and returned to baseline levels at the end of the study period (Fig. 2, middle panel). Serum IGF-II levels remained stable over time without any between-group differences (data not shown). Serum concentrations of IGFBP-1 tended to increase after exercise but did not, however, reach statistical significance (Fig. 2, lower panel). Serum C-peptide levels did not change significantly in the three studies (data not shown). Plasma ghrelin levels did not significantly change before, during or after exercise in any of the three studies (Fig. 3, upper panel). Plasma ghrelin levels were significantly lower during the study day with GH as compared with the study day without GH (Fig. 3, lower panel). Mean plasma ghrelin levels did not significantly correlate with age, body composition or GH-related parameters (data not shown).

Figure 3 (Upper panel) Plasma ghrelin concentrations (means $\pm$ S.E.) before, during and after exercise (shaded bar) in control subjects (●) and in GH-deficient (GHDA) patients with (○) or without (▼) GH substitution. For details on GH dosage, see text. (Lower panel) Mean $\pm$ S.E. ghrelin levels during each study period.

Discussion

In the present study, we have evaluated plasma ghrelin concentrations during a standardised strenuous submaximal physical exercise protocol in healthy subjects, as well as in GH-deficient patients with and without concomitant GH administration. As expected, a significant increase in GH release was elicited by exercise in the control group. Circulating ghrelin levels, however, did not change significantly in any of the groups. Continued GH administration was associated with moderately reduced levels of ghrelin. These data indicate that exercise-induced GH release is not mediated via ghrelin, which is in accordance with one previous report (14).

The level of exercise intensity used in this study induced a significant increment in GH release in accordance with previous data (15). Moderate increments in circulating levels of IGF-I and IGFBP-3 were observed, although only changes in the latter reached statistical significance. An insignificant post-exercise increase in IGFBP-1 concentrations was recorded in all three studies. Similar observations have previously been reported, but the physiological significance, if any, remains uncertain (16).

In addition to exercise intensity, body composition and age are known to determine the exercise-induced GH response (2). As previously mentioned, the neural control of GH release during exercise remains unclear (4), but the present study strongly indicated that circulating ghrelin is not involved in exercise-induced GH release. Since ghrelin mRNA is also expressed in the arcuate nucleus it remains possible that locally released ghrelin may act at the hypothalamic or pituitary level to stimulate GH release (5).

The physiological role of ghrelin in humans has not yet been clarified, but it has been established that the stomach is the predominant site of production and that the circulating levels are associated with nutritional status and energy balance (10). Plasma ghrelin levels are decreased in obese subjects (9), and feeding is associated with a rapid decline (10). Patients with anorexia nervosa have elevated plasma ghrelin levels (10), and intended weight loss is accompanied by a moderate increase in ghrelin concentrations in obese subjects (11). It has recently been reported that ghrelin levels increase prior to food intake and, as such, may serve as an appetite signal (17). Ghrelin administration in human subjects is a very potent stimulator of GH release and ghrelin also increases the release of adrenocorticotrophin and prolactin (6). Moreover, intravenous administration of ghrelin has recently been shown to stimulate appetite and food intake in healthy human volunteers, both of which effects are undoubtedly independent or even opposite to those of GH (7). From these data, it can be inferred that alterations in ghrelin secretion may, to some extent, regulate GH release during conditions of acute or chronic perturbations in

energy balance, such as the postprandial phase, overt obesity and anorexia nervosa. Our observation that GH administration was associated with reduced ghrelin levels could also imply that GH may inhibit ghrelin secretion. In this regard, it is also noteworthy that the ghrelin levels in the patients when GH was discontinued appeared to be inappropriately elevated as compared with control subjects when considering that the patients were significantly more obese. The secretory dynamics of ghrelin remains to be elucidated during several other conditions with well-characterised differences or alterations in GH status. This includes puberty, gender and age-specific differences in GH secretion, catabolic states and poorly controlled diabetes mellitus. From the published data it is, however, tempting to speculate about whether the predominant physiological role of ghrelin may be regulation of food intake rather than stimulation of GH release.

In summary, submaximal aerobic exercise, which is a well-known stimulator of GH release, was not associated with alterations in circulating plasma ghrelin concentrations. The moderately suppressed ghrelin levels observed during GH administration in GH-deficient adults may suggest that GH suppresses ghrelin secretion. The degree to which ghrelin contributes to the complex regulation of GH secretion remains, however, to be investigated further.

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