Impact of two or three daily subcutaneous injections of hexarelin, a synthetic growth hormone (GH) secretagogue, on 24-h GH, prolactin, adrenocorticotropin and cortisol secretion in humans

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Abstract

Objective: To extend the insights on the action of GH secretagogues (GHS) on pituitary function, we studied the impact of intermittent daily s.c. administration of a peptidyl GHS, hexarelin (HEX), on 24-h GH, PRL, ACTH and cortisol secretion in healthy volunteers.

Design: We investigated the impact of two or three times daily s.c. administration of a short-acting peptidyl GHS, the hexapeptide HEX (1.5 μg/kg) on 24-h GH, PRL, ACTH and cortisol secretion (sampling every 20 min) in six normal young men. To monitor possible down-regulation, the effect of 1 μg/kg i.v. HEX at the end of each 24-h sampling period was studied.

Methods: Multi-parameter deconvolution analysis was used to quantitate pulsatile GH, PRL, ACTH and cortisol secretion and estimate the corresponding hormone half-lives. Complementary to deconvolution analysis, approximate entropy was used as a scale- and model-independent statistic to quantify the serial orderliness or pattern regularity of hormone measurements.

Results: Mean and integrated (24-h) serum GH concentrations were increased from baseline values to the same extent by two and three HEX injections. Both HEX schedules equally increased GH secretory burst mass (but not burst frequency), mean daily GH production rate, GH half-life and irregularity of GH release patterns. No change occurred in the secretion of IGF-I, PRL, ACTH and cortisol. Intra-venuous HEX at the end of each spontaneous 24-h profile induced a significant rise in GH, PRL, ACTH and cortisol. Prior HEX administration blunted the GH response, abolished that of ACTH and cortisol and did not modify the PRL increase.

Conclusions: The study showed that two or three daily s.c. injections of HEX augmented 24-h GH secretion equally, amplifying selectively GH secretory pulse mass without altering lactotroph and corticotroph secretion. IGF-I levels were not modified by these 1-day HEX treatment schedules.

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Introduction

The neuroendocrine control of pulsatile somatotroph secretion is mediated by hypothalamic signals, intrapituitary mechanisms and multilevel feedback effects (1–3). The interplay between growth hormone-releasing hormone (GHRH) and somatostatin has a major role in the neural control of growth hormone (GH) secretion. In addition, GH secretion is subject to important influences by neurotransmitters, peripheral hormones and metabolic fuels, and possibly the natural ligand of the GH secretagogue (GHS) receptor (GHS-R) (2, 3). The last-mentioned family of agonists was discovered based on studies of synthetic peptidyl and non-peptidyl GHS (4–6). A natural ligand of the GHS-R, ghrelin, was recently purified from the stomach as a 28 amino acid peptide with a unique n-octanoyl esterification of the third serine residue (4). Ghrelin exerts potent stimulatory effects on somatotroph secretion, and is expressed in the brain and is measurable in the blood of rodents and the human (7, 8). The activity of ghrelin as well as that of non-natural GHS is not fully specific for GH; in fact, most GHSs also stimulate lactotroph and corticotroph secretion acutely (1, 5, 9).

Non-natural peptidyl and non-peptidyl GHSs can induce marked GH release after oral administration
and thus have received attention in recent years as potential alternatives to restore the activity of the GH/insulin-like growth factor-I (IGF-I) axis in various states of GH insufficiency (9–11), especially in conditions like the somatopause, which could reflect reduced activity of an endogenous GHS-R ligand (12, 13). GHSs act mainly at the hypothalamic level by eliciting GHRH release and functionally antagonizing somatostatin’s action (14, 15). The GH-releasing effect in the adult is generally independent of gender, but exhibits age-related variations (16, 17).

GHSs augment both basal non-pulsatile GH release and GH pulse mass, and increase the irregularity of 24-h GH secretion without changing GH pulse duration, interpulse interval, frequency or half-life (14, 15, 18). More prolonged administration elevates circulating IGF-I levels (19–21). The latter response could contribute to the progressive reduction of the stimulatory effects of GHS during chronic administration (22, 23). In addition, acute GHS infusion can induce homologous desensitization in humans as well as other animals (14, 23–28). Thus, the actions of GHS on somatotroph secretion may be influenced by intermittent versus continuous administration, as well as the pharmacokinetics of different molecules (19–21, 29, 30). Peptidyl GHSs have short-lasting effects, whereas a non-peptidyl GHS, MK-0677, possesses high bioavailability and exerts long-lasting effects (20, 31, 32). Single daily oral administration of MK-0677 increases activity of the GH/IGF-I axis, whereas recurrent administration of peptidyl GHSs or, alternatively, continuous parenteral infusion might be needed to achieve the same effect (9, 20).

In the present study, we evaluated the impact of two or three daily injections of a peptidyl GHS, namely the hexapeptide, hexarelin (HEX), on 24-h somatotroph, lactotroph and corticotroph secretion in normal young volunteers. We applied two complementary technical strategies to appraise the dynamic regulation of somatotropic secretion: (a) deconvolution analysis to estimate underlying rates of pulsatile GH secretion and half-life from plasma GH concentration profiles (33) and (b) approximate entropy (ApEn) to quantify the regularity or orderliness of GH secretion (34, 35). Concurrently, we evaluated possible down-regulation of GH, prolactin (PRL) and adrenocorticotropic (ACTH) secretory responses to the twice- and three-times daily scheduled GHS administration.

Subjects and methods

Six healthy young male volunteers (age (means ± s.e.m.): 30 ± 1.2 years, range 27–35 years) were studied after providing written and voluntary informed consent. The study protocol had been approved by an independent local ethical committee.

Study design

All subjects were studied in three different sessions in a randomly assigned order with a wash-out period of at least 3 days. Volunteers were admitted to the Clinical Unit on the morning of the test session following an overnight fast. The cubital veins were cannulated at 0730 h and kept patent over 24 h by slow infusion of isotonic saline. During the day, subjects received a 200 kcal breakfast (60% carbohydrate) at 0900 h and a 800 kcal (50% carbohydrate, 30% lipid, 20% protein) lunch and dinner at 1300 h and 2000 h respectively.

At 0800, 1600, 2000 and 2400 h subjects received either 1 ml saline or HEX (1.5 μg/kg s.c.; 100 μg per vial; Europeptides, Argenteuil, France) according to the following schema. (A) HEX at 0800 and 2000 h; saline at 1600 and 2400 h. (B) HEX at 0800, 1600 and 2400 h; saline at 2000 h. (C) Saline alone. At the end of each session (i.e. at 0800 h on the following day), all subjects received HEX (1 μg/kg as an i.v. bolus).

Blood samples were withdrawn every 20 min for 26 h from 0800 on day 1 to 1000 h on the following day. GH, PRL, ACTH and cortisol were assayed in each sample (see below).

Hormone assays

Serum GH concentrations (μg/l) were measured by immunoradiometric assay (hGH-CTK; Sorin Biomedica, Saluggia, Italy). All samples from an individual subject were analyzed together in duplicate. The sensitivity of the assay was 0.15 μg/l. The ranges of inter- and intra-assay coefficients of variation were 4.9–6.5% and 1.5–2.9% respectively.

Serum PRL levels (μg/l) were measured by immunoradiometric assay (PRLCTK; Sorin Biomedica), as above. The sensitivity of the assay was 0.45 μg/l. The ranges of inter- and intra-assay coefficients of variation were 7.7–10.8% and 2.4–3.4% respectively.

Plasma ACTH levels (ng/l) were measured by immunoradiometric assay (ACTH; Nichols Institute Diagnostic, San Juan Capistrano, CA, USA). The sensitivity of the assay was 1 ng/l. The ranges of inter- and intra-assay coefficients of variation were 2.4–8.5% and 3.9–9.9% respectively.

Cortisol (nmol/l) was measured by radioimmunoassay (CORT-CTK125; Diasorin Diagnostic, Saluggia, Italy). The sensitivity of the assay was 0.66 nmol/l. The inter- and intra-assay coefficients of variation ranged from 4.3 to 14.6% and from 4.2 to 8.96% respectively.

Data analysis

Multiparameter deconvolution analysis was used to quantitate pulsatile GH, PRL, ACTH and cortisol secretion and estimate the corresponding hormone
half-lives (36). Basal hormone secretion represents the calculated time-invariant interpulse component of the release profile. The daily pulsatile production rate is the product of the number of secretory bursts and the mean mass of GH released per pulse. Secretory pulse identification required that secretory burst amplitudes and the basal secretion rate exceeded zero by 95% statistical confidence. The analyst was blinded to the randomization scheme.

**Approximate entropy**

ApEn was used as a scale- and model-independent statistic, which is complementary to deconvolution analysis (34). ApEn quantifies the serial orderliness or pattern regularity of hormone measurements. Normalized ApEn parameters of m = 1 (series length) and r = 20% (threshold) of the intraseries s.d. were used, as previously validated for 24-h time series (37). This statistic is thus designated ApEn (1, 20%). Increased ApEn (at equal series lengths and similar parameter values, as used here) indicates greater secretory process irregularity, consistent with altered within-axis feedforward feedback control (34).

The results are reported as the means±S.E.M. of absolute values as well as areas under curve (AUC) calculated by trapezoidal integration.

**Statistical analysis**

ANOVA with repeated measures was used to compare results among the different sessions. If a significant difference had occurred a post hoc analysis was carried out using Newman–Keuls test. P < 0.05 was considered statistically significant.

**Results**

Mean and integrated (24-h) serum GH concentrations were increased (P < 0.03) from baseline values of 0.6±0.2 µg/l and 890±220 µg/l per min to the same extent by two and three HEX injections daily:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Saline</th>
<th>HEX2</th>
<th>HEX3</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Basal secretion (concentration/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GH</td>
<td>86±72</td>
<td>29±14</td>
<td>14±14</td>
<td>ns</td>
</tr>
<tr>
<td>PRL</td>
<td>1.4±14</td>
<td>29±29</td>
<td>14±2±4</td>
<td>ns</td>
</tr>
<tr>
<td>ACTH</td>
<td>245±29</td>
<td>245±58</td>
<td>216±86</td>
<td>ns</td>
</tr>
<tr>
<td>Cortisol*</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>Mass (concentration units)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GH</td>
<td>4.7±1.2</td>
<td>23±3.3</td>
<td>18±1.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PRL</td>
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<tr>
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<td>44±16.7</td>
<td>57±22.8</td>
<td>ns</td>
</tr>
<tr>
<td>Cortisol</td>
<td>154±13</td>
<td>149±21</td>
<td>182±25</td>
<td>ns</td>
</tr>
<tr>
<td>Frequency (pulses/24h)</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>GH</td>
<td>7.5±1.0</td>
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<td>6.7±1.7</td>
<td>ns</td>
</tr>
<tr>
<td>PRL</td>
<td>13.1±0.7</td>
<td>15.8±0.8</td>
<td>12.5±1.0</td>
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<tr>
<td>ACTH</td>
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<td>19.8±0.7</td>
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<tr>
<td>Cortisol</td>
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<td>11.7±0.7</td>
<td>12.5±0.6</td>
<td>ns</td>
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<tr>
<td>Pulsatile production rate***</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GH</td>
<td>36±8.3</td>
<td>154±19.3</td>
<td>121±28</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PRL</td>
<td>68±8.5</td>
<td>69±7.5</td>
<td>73±9.3</td>
<td>ns</td>
</tr>
<tr>
<td>ACTH</td>
<td>11.1±0.3</td>
<td>9.9±0.3</td>
<td>1.1±0.5</td>
<td>ns</td>
</tr>
<tr>
<td>Cortisol</td>
<td>1.9±0.1</td>
<td>1.7±0.3</td>
<td>2.2±0.3</td>
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</tr>
<tr>
<td>Mean concentration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GH</td>
<td>0.6±0.2</td>
<td>3.7±0.7</td>
<td>3.3±1.1</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>PRL</td>
<td>5.6±0.7</td>
<td>6.0±0.5</td>
<td>6.1±0.9</td>
<td>ns</td>
</tr>
<tr>
<td>ACTH</td>
<td>19±2.3</td>
<td>18±1.7</td>
<td>18±1.4</td>
<td>ns</td>
</tr>
<tr>
<td>Cortisol</td>
<td>188±12</td>
<td>173±11</td>
<td>207±29</td>
<td>ns</td>
</tr>
<tr>
<td>Integrated concentration**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GH</td>
<td>0.9±0.2</td>
<td>5.3±1.0</td>
<td>4.3±1.2</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>PRLs</td>
<td>6.1±1.0</td>
<td>8.5±0.7</td>
<td>8.6±1.3</td>
<td>ns</td>
</tr>
<tr>
<td>ACTH</td>
<td>26±3.1</td>
<td>25±2.4</td>
<td>26±1.9</td>
<td>ns</td>
</tr>
<tr>
<td>Cortisol</td>
<td>265±18</td>
<td>243±16</td>
<td>248±58</td>
<td>ns</td>
</tr>
</tbody>
</table>

* Cortisol profiles were fit according to a model of zero basal secretion (see Methods).
** Integrated concentration of GH and PRL are reported in mg/l per min, ACTH in ng/l per min and cortisol in nmol/l per min.
*** GH production rate is reported in µg/l per day, ACTH in ng/l per day and cortisol in nmol/l per day.
ns, not significant.

**Figure 1** Mean GH concentration, pulsatile production rate and ApEn during saline or two (HEX2) or three (HEX3) daily s.c. injections of 1.5 µg/kg HEX in normal volunteers. Values are means±S.E.M.
i.e. to 3.7±0.7 vs 3.3±1.1 μg/l and 5300±1030 vs 4300±1200 μg/l per min (Fig. 1).

Based on deconvolution analysis (Table 1), HEX augmented, to the same extent in response to two and three HEX treatments (P < 0.01), the daily pulsatile GH production rate by three- to fourfold and mean GH secretory burst mass from 4.7±1.2 μg/l at baseline to 23±3.3 and 18±1.7 μg/l per min respectively. Pulse frequency did not change. GH half-life increased slightly from 15±0.8 at baseline to 19±1.5 and 22±1.9 min after two or three HEX administrations respectively (P < 0.05) (Figs 1 and 2).

Both two and three HEX injections elevated ApEn values of the 24-h GH profiles (P < 0.01) (Table 2 and Fig. 1).

GH peaks following the first two s.c. HEX stimuli were comparable, whereas that following the third injection was decreased.

Deconvolution analysis showed no changes in the parameters of PRL, ACTH and cortisol secretion after two or three HEX infusions. ApEn values of these hormones were also independent of intervention (Tables 1 and 2).

Cross (X)-ApEn analysis showed that there was a disjunction (i.e. synchronicity loss) of ACTH and cortisol secretion during two vs three daily HEX injections.

Acute challenge with i.v. HEX at the end of the spontaneous 24-h profile induced a marked GH rise (peak: 60±4.4 μg/l) and a significant increase in serum PRL (13±1.9 μg/l), ACTH (77±7.7 ng/l) and cortisol (552±26 nmol/l) concentrations (Table 3 and Fig. 3).

Two and three s.c. HEX administrations the day before blunted (P < 0.05) the GH response to acute i.v. HEX challenge to the same extent (peak: 39 and 35 μg/l; AUC: 2.5±0.6 and 2.1±0.5 mg/l per min) when compared with the response recorded after 24-h saline administration (peak: 66 μg/l; AUC: 4.4±0.4 mg/l per min). In addition, two and three s.c. HEX administrations abolished the ACTH and cortisol but did not modify the PRL response to the acute i.v. HEX challenge (Table 3 and Fig. 3).

Serum IGF-I concentrations at the end of the various testing sessions were similar.

**Side-effects**

No side-effects were evident after two or three s.c. injections of HEX daily. On the other hand, i.v. administration of HEX induced transient facial flushing in three subjects.

**Discussion**

The present study establishes the ability of two or three s.c. injections of a peptidyl GHS, HEX, to augment 24-h pulsatile GH secretion equivalently and significantly in young men. The increase in daily GH secretion driven by HEX reflected a preferential (three- to fourfold) increase in burst mass without any significant change in frequency. HEX also elevated the ApEn (irregularity) of GH pulsatility and blunted the GH response to a subsequent acute i.v. challenge with the hexapeptide at the end of 24 h. Circulating total IGF-I levels did not change over this interval. Likewise, lactotroph and corticotroph secretion was not modified by either two or three s.c. injections of HEX. However, HEX pretreatment abolished the acute ACTH and cortisol (but not PRL) response to a delayed challenge with this hexapeptide.

In an earlier study in healthy older humans, continuous i.v. infusion of GH-releasing peptide-2 (GHRP-2) also augmented 24-h serum GH concentrations due to an enhancement of the physiological pattern.

![Figure 2](https://via.placeholder.com/150)

**Figure 2** GH half-life and burst mass during saline or two (HEX2) or three (HEX3) daily s.c. injections of 1.5 μg/kg HEX in normal volunteers. Values are means ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>HEX2</th>
<th>HEX3</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH</td>
<td>0.16±0.04</td>
<td>0.32±0.03</td>
<td>0.34±0.03</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>PRL</td>
<td>1.01±0.07</td>
<td>1.07±0.05</td>
<td>1.09±0.04</td>
<td>ns</td>
</tr>
<tr>
<td>ACTH</td>
<td>0.91±0.06</td>
<td>1.05±0.07</td>
<td>1.05±0.09</td>
<td>ns</td>
</tr>
<tr>
<td>Cortisol</td>
<td>0.91±0.06</td>
<td>0.96±0.03</td>
<td>0.95±0.05</td>
<td>ns</td>
</tr>
</tbody>
</table>

ns, not significant.
of somatotroph secretion with an increase in pulse mass without any change in mean interpulse interval or pulse frequency (12, 15, 38). Subcutaneous GHRP-2 delivery and oral administration of non-peptidyl GHS likewise augmented 24-h GH secretion by increasing GH pulse amplitude without any change in pulse duration, interpulse interval, frequency and half-life (14, 15, 20). However, an increase in pulse frequency has been reported in one other analysis (23).

The present investigation highlights the stimulatory impact of two or three daily acute s.c. injections of a peptidyl GHS on 24-h GH pulsatility. First, our results show that intermittent HEX treatment increases 24-h GH secretion by three- to fourfold in normal young subjects, which is comparable to responses achieved by 24-h continuous i.v. infusion of GHRP-2 (15) or single daily oral administration of MK-0677 (20). The increase in daily GH secretion driven by HEX treatment primarily reflected augmented burst mass (see above) and a small (4–5 min) increase in GH half-life. The latter change probably reflects the concentration dependence of GH kinetics (17). Based on kinetics principles, the rise in 24-h integrated serum GH concentrations was due predominantly (>85%) to increased GH secretion (39).

The elevation of GH ApEn values is consistent with more disorderly patterns of 24-h GH release, as observed during fixed i.v. infusions of GHRH and GHRP-2 (15, 38, 40) and in healthy pubertal girls (41) and boys (41, 42). Aromatizable androgens and oestrogens also elevate GH ApEn (42, 43).

The magnitude of the effects of HEX on 24-h GH secretion was essentially equivalent whether two or three injections were administered, although the peak GH response to three consecutive s.c. injections was reduced. These results indicate that, at least under acute conditions, twice daily stimulation with a peptidyl GHS is sufficient to drive marked somatotroph secretion in young men, and that no further augmentation can be obtained by increasing the frequency of administration. Whether stimulation is maintained with more extended treatment, as observed for continuous s.c. GHRP-2 infusion (12), remains to be established.

At the end of the 24-h profile, the GH response to acute i.v. administration of the peptidyl GHS was blunted independently of the number of previous s.c. injections. Previous studies with continuous infusion (23, 28) or prolonged s.c. administration of GHSs (44) showed that the GH response to a following acute challenge with GHS is reduced, reflecting holo-

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Saline</th>
<th>HEX2</th>
<th>HEX3</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH (mg/l per min)</td>
<td>0.01±0.0</td>
<td>4.4±0.4#</td>
<td>2.5±0.6#</td>
<td>2.1±0.5#</td>
</tr>
<tr>
<td>PRL (mg/l per min)</td>
<td>0.4±0.1</td>
<td>1.0±0.2#</td>
<td>0.8±0.01#</td>
<td>1.0±0.2#</td>
</tr>
<tr>
<td>ACTH (ng/l per min)</td>
<td>2.5±0.4</td>
<td>4.8±0.4#</td>
<td>3.6±0.5*</td>
<td>3.1±0.3*</td>
</tr>
<tr>
<td>Cortisol (nmol/l per min)</td>
<td>36±4.4</td>
<td>55±2.2#</td>
<td>41±3.6*</td>
<td>36±3.1*</td>
</tr>
</tbody>
</table>

*P < 0.05 vs Saline; # P < 0.05 vs Basal.
Figure 3 Hormone responses to an acute i.v. challenge with 1 µg/kg HEX after each 24-h session in normal volunteers. GH, PRL and cortisol levels are reported in µg/l, ACTH in ng/l. Values are means ± S.E.M.
In conclusion, the present clinical investigation revealed that two or three daily s.c. injections of HEX increase 24-h GH secretion to an equivalent degree in healthy young men without altering concomitant lactotroph and corticotroph secretion. This dosing schedule blunts subsequent acute GH and abolishes delayed ACTH (but not PRL) responsiveness to an i.v. dose of the same hexapeptide. HEX selectively augments GH pulse mass and increases the irregularity of the GH secretory process but does not increase circulating IGF-I levels.

Acknowledgements

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