Administration of recombinant human GHRH-1,44-amide for 3 months reduces abdominal visceral fat mass and increases physical performance measures in postmenopausal women

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Abstract

Objective: A recent study indicated that twice-daily s.c. administration of a high dose of recombinant human GHRH-1,44-amide (GHRH) for 90 days can alter body composition in healthy older men. No data establish whether this is also true in postmenopausal women. The present study tests the hypothesis that the same GHRH regimen applied in women will: (i) elevate both IGF-I and GH concentrations; and (ii) reduce abdominal visceral fat mass, augment total body water and enhance functional performance.

Design: Ten postmenopausal volunteers underwent baseline study and then received 1 mg GHRH twice daily s.c. for 3 months.

Methods: Statistical comparisons were made with preintervention baseline data.

Results: GHRH administration stimulated: (i) a mean 98\(^\pm\)14% elevation of overnight GH concentrations after administration of the peptide for 1 and 3 months (P < 0.005); (ii) a sustained 71\(^\pm\)3.5% rise in IGF-I concentrations over the interval from 2 weeks to 3 months (P < 0.0012); (iii) a 16\(^\pm\)7% reduction in abdominal visceral fat mass (P = 0.029) and a 14\(^\pm\)5% increase in triitated water space (P < 0.025); (iv) an abbreviation of the times required to walk 30 m (P = 0.015) and ascend two flights of stairs (P = 0.003). Most (70%) subjects experienced local skin reactivity. There were no systemic adverse events.

Conclusions: A 3-month regimen of GHRH supplementation in postmenopausal women can stimulate GH and IGF-I production, reduce abdominal visceral fat and improve selected measures of physical performance, while inducing significant local skin reactivity.

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Introduction

Healthy aging results in a progressive reduction in growth hormone (GH) secretion rates and insulin-like growth factor-I (IGF-I) concentrations (1). Associated physical features include sarcopenia, osteopenia, increased visceral fat mass, heightened atherosclerotic risk, impaired insulin action and reduced strength, well being and endurance (2). Many of these signs and symptoms are also characteristic of GH deficiency (3–5). Although IGF-I concentrations decline in aging, the hepatic actions of GH are preserved in older adults, since low doses of recombinant human (rh) GH stimulate comparable increments in total IGF-I concentrations in elderly and young volunteers (6, 7).

The age-related fall in GH secretion may reflect heightened hypothalamic somatostatinergic inhibition and/or diminished hypothalamo-pituitary drive by the potent secretagogues, GH-releasing hormone (GHRH) and/or GH-releasing peptide (GHRP, ghrelin) (1, 8, 9). Secretagogue deficiency is suggested but not proven by some interventional studies. For example, single or repeated daily injection or continuous s.c. infusion of GHRH or GHRP-2 elevates GH and IGF-I concentrations for intervals of 24 h to 4 months in elderly men (10–15). However, only one of these interventions altered body composition significantly (15). In contrast, GH supplementation consistently decreases intra-abdominal fat and increases lean body mass in GH-deficient adults (16, 17). Whether an inadequate dose and/or frequency of secretagogue administration accounts for the lack of whole-body effects in earlier regimens is not known. The issue is important, because a high dose (1 mg) of rhGHRH administered twice daily for 3 months doubled mean concentrations of GH and
IGF-I, reduced total abdominal fat content, increased lean body mass and improved certain measures of physical performance in older men (15). There are no comparable data in women.

Two studies of secretagogue supplementation have included postmenopausal women. One used GHRP but did not assess body composition (14), and the other found that nightly injection of GHRH 1.29 for 4 months did not alter body composition (13). In view of the recent experience in older men given a high dose of GHRH twice daily for 3 months (15), the present investigation tests the hypotheses that the same regimen will achieve comparable biochemical, body compositional and performance-related improvements in postmenopausal women.

Methods

Subjects

Ten healthy ambulatory, community-dwelling, recreationally active women participated. Volunteers comprised postmenopausal women capable of administering GHRH twice daily by s.c. injection, performing intensive functional tests, and undergoing repetitive blood sampling at baseline and after 1 and 3 months of intervention. Subjects provided prior written voluntary informed consent approved by the Institutional Review Board. Physical characteristics were (mean with range in parentheses): age, 57 (51–73) years; height, 166 (161–172) cm; weight, 73 (69–78) kg. Each participant had an unremarkable medical history, physical examination and screening biochemical tests of hepatic, renal, metabolic, hematologic and endocrine function. Four women continued use of conventional postmenopausal hormone replacement therapy (HRT). Screening data revealed concentrations of follicle-stimulating hormone (FSH) > 30 IU/l (normal young-adult range, 2–8 IU/l); total testosterone (range) 10–58 ng/dl (multiply by 0.0347 for nmol/l); and estradiol 10–56 pg/ml (multiply by 3.67 for pmol/l). Exclusionary criteria were: recent weight loss or gain exceeding 3 kg in 6 weeks; an abnormal mammogram within the preceding year; acute or chronic systemic disease; 3 kg in 6 weeks; an abnormal mammogram within the preceding year; acute or chronic systemic disease; psychiatric illness; orthopedic handicaps that would limit exercise and balance testing; drug or alcohol abuse; concurrent use of psycho- or neuroactive medications; exposure to anabolic steroids or systemic glucocorticoids; anemia (hemoglobin < 14 g/l); cerebro-, cardio- or peripheral arteriovascular disease; reluctance to inject peptide twice daily for 3 months as an outpatient; lack of prompt access to the General Clinical Research Center (GCRC); and incompetence or unwillingness to provide written informed consent.

Clinical protocol

Volunteers were admitted to the GCRC on three occasions to undergo blood sampling for 12 hours overnight and metabolic studies the next day; viz. at baseline and after 1 and 3 months of twice-daily s.c. injection of 1 mg GHRH (rhGHRH-1,44-amide, BioNebaska, Inc., Lincoln, NE, USA). The peptide was administered at 2200 h and 0800 h in pre-filled syringes labeled with the volunteer’s name, protocol number and date. Peptide was reconstituted by dilution in 1 ml sterile water, and dispensed weekly by the Investigational Drug Pharmacy. Syringes were kept refrigerated. Volunteers continued their usual daily activities, dietary habits and recreational level of exercise. Four women continued HRT. The project was approved by the Food and Drug Administration (Washington, DC, USA) under an investigator-initiated new drug file.

In a pilot study, three out of the first four volunteers given 4 mg GHRH twice daily were removed from participation due to significant local edema at the site of injection (> 3 cm) and/or uticaria at sites remote from the injection. Hence, the 4 mg dose was discontinued due to adverse effects.

In-patient studies

To obviate confounding of GH and IGF-I measurements by variable nutrient intake, enrollees were admitted to the GCRC in the morning after ingesting a standardized breakfast at home (nutrient composition: 55% carbohydrate, 15% protein and 30% fat). Thereafter, participants received standardized meals (above) of 12.5 kcal/kg at 1200 h and 1700 h in the GCRC, and remained fasting overnight. At 1800 h, an indwelling i.v. catheter was placed in an antecubital vein to allow repetitive blood sampling (1.5 ml) every 10 min for 12 hours beginning at 2000 h. Vigorous exercise, hypnotics, caffeinated beverages, smoking and alcohol use were disallowed. Room lights were extinguished at 2300 h.

Functional assessments

Strength

Isokinetic eccentric and concentric strength of the quadriceps femoris and biceps femoris muscle groups was assessed using the Kin-Com II isokinetic dynamometer (Chattex, Corp., Hixson, TN, USA). After familiarization with the procedure, subjects performed three knee flexions and extensions at a calibrated velocity of 60 degrees per second. To estimate quadriceps strength: the lateral epicondyle of the knee was aligned with the axis of the dynamometer; the inferior edge of the force pad was placed directly superior to the medial malleolus; and Velcro straps were applied across the hips, thigh and ankle for stabilization. Gravity correction was performed with the knee at 0° flexion.

Balance

Quantitative measures were: (i) the duration of standing (in seconds) on one leg with eyes open and closed; (ii) dual-limb and single-limb stance protocols

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with eyes open and closed under static and dynamic conditions on the Chattex Balance System (Chattanooga Group Inc., Hixson, TN, USA); (ii) dynamic platform tilting to force plantar flexion and dorsiflexion; (iv) the sharpened Rhomberg (timed standing heel-to-toe with dominant foot in front and eyes closed) (18).

Physical performance Functional motor capability was assessed by a timed stair climb and 30 m walk. Subjects were asked to descend two flights of stairs, wait 1 min and ascend as quickly as possible using the railing for balance only. The timed 30 m walk was conducted twice on a level unobstructed passageway with 1 min of rest between to obtain a mean value.

Flexibility Hamstring extension/lower-back flexibility was assessed using a sit-and-reach apparatus. Volunteers sat with feet against the surface, and reached as far forward as possible with knees extended. Positive scores (mean of three trials) indicate the number of inches of extension beyond the toes (and, conversely, for negative scores).

Body composition analysis Total body water Participants received tritiated water (<0.12 mSv) orally at 0900 h on the second morning of study. Blood and urine samples were collected 1, 2, 3 and 4 hours later. Equilibrated radioactivity was quantitated by liquid scintillography. The density of water at body temperature was taken as 0.9937 kg/l (19).

Percentage body fat For hydrostatic densitometric estimates, subjects were weighed in air on an Accu-weigh beam scale accurate to 0.1 kg, and weighed again underwater on a Chatillon autopsy scale accurate to 10 g (20). Residual lung volume was measured by O₂ uptake at voluntary exhaustion. Forearm venous lactate concentrations were monitored at rest and during the last 15 s of each power stage (Yellow Springs Instruments, 2700 Select Biochemistry Analyzer, Yellow Springs, OH, USA). The LT was taken as the highest power output achieved before onset of the curvilinear increase in lactate concentrations (exceeding at least 0.2 mM). Oxygen consumption was quantitated by open-circuit spirometry (Sensormedics Metabolic Cart 229, Yorba Linda, CA, USA) and heart rate by electrocardiography (Marquette Max-1). \( V_{O_2,max} \) was defined as \( V_{O_2} \) uptake at voluntary exhaustion.

Hormone assays Mean GH concentrations were determined by automated immuno-chemiluminescence assay of sera collected every 10 min from 2000 h to 0800 h overnight (Nichols Institute Diagnostics, San Clemente, CA, USA) (24, 25). Assay sensitivity (at 3 S.D. above the zero-dose tube) was 0.005 µg/l. Median intra-assay and interassay coefficients of variation were 5.2 and 8.3% respectively. No values fell below 0.030 µg/l in the present study. Fasting (0800 h) concentrations of total IGF-I (Nichols Institute Diagnostics) and testosterone and estradiol (Diagnostic Products Inc., Webster, TX) were determined as reported previously (26).

Deconvolution analysis Biexponential deconvolution analysis was used to quantify basal and pulsatile GH secretion from each 12-h concentration profile (27). In view of parameter interdependence, estimation was conditioned statistically on mean populational GH kinetics and \( a \ priori \) pulse times (28); viz., GH half-lives were 3.5 and 20.8 min (fractional slow decay 0.63) (29), and putative pulse times were determined by Cluster (discrete peak-detection) analysis at a nominal \( P < 0.05 \) false-positive rate (2 \( \times 1 \) cluster size with \( t = 2.0/2.0 \)) (30). GH elimination kinetics and peak positions were held constant, allowing simultaneous estimation of the mass (or integral) of Gaussian secretory events and basal secretion (initially estimated as corresponding to the lowest 5% concentration). The outcomes were basal (time-invariant), pulsatile (sum of secretory-burst mass) and total (sum of basal and pulsatile) GH secretion expressed in µg/l per 12 hours. The foregoing modifications are required for

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reliable conditional parameter estimation, as established more recently by statistical verification and experimental validation (31, 32).

**Approximate entropy (ApEn)**

ApEn provides a scale- and model-independent regularity statistic to quantify the orderliness of serial measurements (33). Higher ApEn values denoted greater relative randomness or disorderliness of subpatterns. Mathematical models and clinical experiments establish that reduced pattern orderliness is a barometer of increased feedforward and/or decreased feedback within a neuroendocrine axis with high sensitivity and specificity (both > 90%) (34, 35).

**Statistical methods**

Outcomes are reported as the arithmetic mean ± S.E.M. For discussion purposes, relative responses (fold-effects) are given as the geometric mean ratio (and 95% statistical confidence intervals [CIs]) of the value observed at 1 month or 3 months to that estimated at baseline in the same individual. To adjust for within-subject correlations, the statistical model comprised hierarchical mixed-effect one-way ANCOVA, wherein the baseline outcome served as the covariate (36). The model specification parameter was the duration of intervention (1 and 3 months) (37). Logarithmic transformation was utilized to limit heterogeneity of variance. The equal-slopes assumption of the ANCOVA structure was verified by a generalized $F$-ratio test, followed by restricted maximum-likelihood estimation of parameters. Rejection of pre-specified hypotheses was based on a multiple-comparison experiment-wise Type I error rate of < 0.05 using Fisher’s least-significant difference (LSD) test (36). Computations were performed using PROC MIXED in SAS version 8.0 (SAS Institute Inc., Cary, NC, USA).

**Results**

GHRH administration twice daily increased mean overnight GH concentrations ($\mu g/l$) from a baseline value of $0.71 \pm 0.18$ to $1.4 \pm 0.26$ (1 month) and $1.4 \pm 0.20$ (3 months) (both $P < 0.005$ vs baseline; $P = NS$ for 1 vs 3 months comparison; Fig. 1a). The incremental increase over baseline at both time points averaged $98 \pm 14\%$. Fasting serum IGF-I concentrations, measured at baseline and after 2, 4, 8 and 12 weeks of intervention, rose from a baseline mean of $109 \pm 14 \mu g/l$ to $204 \pm 35 \mu g/l$. ($P = 0.006$), and remained comparably elevated at all time points thereafter ($0.012 > P > 0.001$ vs baseline; Fig. 1b). The average percentage increase above baseline was $71 \pm 3.5\%$. Exploratory subgroup analysis showed that IGF-I increments in women receiving HRT ($n = 4$) did not differ from those in individuals not receiving HRT ($n = 6$).

The principal effects of GHRH were to decrease intra-abdominal visceral fat area by 16% (2–28%, 95% CI; $P = 0.029$) and increase total body water by 14% (4–23%; $P < 0.025$; Fig. 2). Lean body mass did not increase significantly.

Body compositional data that did not change are reported in Table 1 (hydrodensitometry), Table 2 (CT estimates of total abdominal fat and thigh muscle area) and Table 3 (DEXA measurements of total body fat, water and bone mineral content).

Figure 3 depicts 30 m walking times, which decreased by 14% (range, 9–19%; $P = 0.015$; Fig. 3b) and stair-climb times, which fell by 9% (range, 4–13%; $P = 0.003$; Fig. 3b) at 3 months compared with baseline.

Stable performance outcomes ($P > 0.05$ for 3 months vs baseline) are summarized in Table 4 (exercise function), Table 5 (strength at knees) and Table 6 (balance measures and sit-and-reach distance).
Pulsatile and basal GH secretion rates were estimated from the 12-h overnight (10-min) GH concentration profiles using deconvolution analysis. As in the case of mean GH concentrations, pulsatile GH release rose by more than 2-fold at 1 month and remained similarly elevated at 3 months of intervention ($P = 0.014$ overall effect; Fig. 4. For comparison, we also give previously unanalyzed overnight pulsatile GH secretion rates in men administered 1 mg GHRH twice daily (15). By two-way ANCOVA, stimulated GH secretion did not differ in women and men, but IGF-I rose less in women ($P = 0.05$). GH pulse number, secretory-burst

Table 1 Hydrodensitometry estimates.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Baseline</th>
<th>3 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body density (g/cm³)</td>
<td>1.006±0.007</td>
<td>1.011±0.008</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>40±2.2</td>
<td>38±3.9</td>
</tr>
<tr>
<td>FM (kg)</td>
<td>32±4.5</td>
<td>30±5.0</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>46±2.9</td>
<td>50±3.6</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>78±7.2</td>
<td>78±7.1</td>
</tr>
</tbody>
</table>

Data are the means±S.E.M. ($n = 10$). Values at baseline and at 3 months do not differ significantly. FM, fat mass; FFM, free-fat mass.

Table 2 CT estimates of body composition (cm²).

<table>
<thead>
<tr>
<th>Measure</th>
<th>Baseline</th>
<th>3 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total abdominal fat</td>
<td>543±72</td>
<td>503±87</td>
</tr>
<tr>
<td>Thigh fat area</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>105±18</td>
<td>110±11</td>
</tr>
<tr>
<td>Left</td>
<td>105±20</td>
<td>110±25</td>
</tr>
<tr>
<td>Thigh muscle area</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>95±13</td>
<td>109±12</td>
</tr>
<tr>
<td>Left</td>
<td>94±12</td>
<td>107±87</td>
</tr>
</tbody>
</table>

Comparisons at 3-months do not differ. Values are means±S.E.M. ($n = 10$). Visceral fat area is shown in Fig. 2.

Table 3 DEXA estimates of body composition.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Baseline</th>
<th>3 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMC ($g \times 10^3$)</td>
<td>2.4±0.14</td>
<td>2.4±0.13</td>
</tr>
<tr>
<td>BMD (g/cm²)</td>
<td>1.151±0.041</td>
<td>1.144±0.040</td>
</tr>
<tr>
<td>Total body fat (%)</td>
<td>43±3.1</td>
<td>39±3.5</td>
</tr>
<tr>
<td>Total body fat (kg)</td>
<td>34±4.8</td>
<td>32±5.1</td>
</tr>
</tbody>
</table>

Data are the means±S.E.M. ($n = 10$). Total body water and lean body mass are shown in Fig. 2. BMC, bone mineral content; BMD, bone mineral density.

Figure 2 Intra-abdominal visceral fat mass (AVF), total body water (TBW) and lean body mass (LBM) in ten postmenopausal women assessed at baseline and after 3 months of rhGHRH administration. Data are presented as described in the legend of Fig. 1, except that statistical values apply to the indicated paired outcomes.

Figure 3 Time required to ascend two flights of stairs (a) or walk 30 m (b) assessed at baseline (time zero) and after 1 and 3 months of GHRH administration in ten postmenopausal individuals. The data format is that given in Fig. 1.
duration and basal secretion rates were not affected by GHRH administration (not shown).

The ApEn statistic identifies feedback adaptations (regularity of patterned hormone release) with high sensitivity and specificity (see Methods section). Thus, ApEn was used to test the hypothesis that GHRH-stimulated pulsatile GH secretion remains orderly. Figure 5 shows that prolonged GHRH drive in the face of jointly elevated GH and IGF-I concentrations markedly enhances orderliness of GH release patterns (overall \( P < 0.001 \)), as indicated by a fall in GH ApEn at both 1 and 3 months (\( P < 0.001 \) in women and \( P < 0.01 \) in men vs baseline). There was no gender effect, and values at 1 month and 3 months did not differ from each other.

Seven of ten volunteers experienced significant skin reactivity at the injection site, defined by any two or more a priori features of localized edema (>2.0 cm), soreness, erythema or pruritus persisting for >24 hours after GHRH injection. None of the volunteers reported signs or symptoms of systemic histamine release, defined as unexplained hypotension, duration and basal secretion rates were not affected by GHRH administration (not shown).

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generalized urticaria, angioedema, vocal hoarseness or wheezing. Two subjects described weight gain > 2 kg and/or arthralgias, and one developed mild carpal-tunnel syndrome. The average weight gain for the cohort was 0.04 ± 1.3 kg. None of the volunteers discontinued participation. There were no significant changes in general chemistry, blood glucose or insulin, or clinical cardiac function.

Discussion

The present investigation demonstrates that administration of a high dose of rhGHRH-1,44-amide (1 mg s.c. twice daily) for 3 months doubles 12-h mean GH concentrations and increases IGF-I concentrations by 71% in postmenopausal women. The GHRH intervention decreased abdominal visceral fat by 16%, increased total body water by 14%, and reduced the 30 m walk time by 14% and the stair-climb time by 9%. Aerobic capacity, flexibility, balance, bone mineral density, lean body mass and muscle strength did not change significantly over the 3 months. Seventy per cent of the volunteers met two or more criteria for bothersome local skin reactions. None exhibited systemic histamine release. Taken together, these data extend earlier findings in women by establishing proof-of-principle that an adequate amount and schedule of GHRH administration can stimulate GH and IGF-I production for as long as 3 months, alter body composition and enhance certain performance measures in healthy postmenopausal individuals. Side effects include significant local skin reactivity to this dose and route of peptide administration.

Healthy older men given 1 or 4 mg GHRH s.c. twice daily for 3 months responded with elevated GH and IGF-I concentrations and dose-dependent changes in body composition (15). Pilot studies in women revealed that the 4 mg GHRH dose could not be tolerated due to marked skin reactivity triggered by histamine release. On the other hand, administration of smaller doses of GHRH-1,29) once daily for as long as 4 months stimulated GH release only briefly after each injection in elderly adults, and did not sustain IGF-I elevations or alter physical endpoints and performance outcomes (10, 13). The current intervention regimen of a high dose of GHRH administered twice daily significantly increased GH and IGF-I production and induced body-compositional and functional improvements in healthy older women. Whether the slightly longer half-life of GHRH-1,44 compared with that of GHRH-1,29 also contributed to these response differences is not known (1).

In the current investigation, GHRH administration in postmenopausal volunteers did not alter bone density or muscle mass over the 3-month intervention. The identical dose and schedule of GHRH supplementation in healthy older men augmented lean body mass, whereas a 4-fold higher GHRH dose was required to decrease abdominal visceral fat content (15). Post hoc comparisons of GHRH effects in men and women revealed comparable increases in pulsatile GH secretion and enhanced regularity of GH release (Figs 4 and 5), but lower IGF-I levels in women. Distinguishable tissue effects in women and men despite commensurately elevated GH concentrations would be consistent with sex differences in target-organ responsiveness, as inferred in hypopituitary adults treated with GH (38). Direct comparison between GH and GHRH supplementation would be of interest in this regard.

GHRH administration for 1 and 3 months enhanced the quantifiable orderliness of GH release patterns, as monitored by the approximate entropy statistic (33–35). Heightened GH regularity after GHRH exposure differs from the acute effects of this and other secretagogues, which reduce orderliness of GH release and thus mimic profiles observed in puberty (14, 39–41). Based upon biomathematical modeling, enhanced GH regularity following sustained GHRH stimulation would predict more effectual feedback restraint within the GH–IGF-I axis (35, 42, 43). Known feedback signals are elevated concentrations of GH and IGF-I, which putatively evoke central somatostatin outflow (1, 44). The latter would account for the present outcome, since somatostatin infusion also enforces more orderly GH secretion (45). A clinical distinction is that administration of GHRH (present data) but not GHRP-2 (14) for 1 month evokes more regular GH secretion. This contrast is consistent with the inferred capability of GHRP but not GHRH to attenuate somatostatinergic restraint (46).

Untoward local skin reactions were common after s.c. injection of 1 mg GHRH twice daily in elderly women. Systemic release of histamine was not observed. Two participants developed arthralgia and/or weight gain > 2 kg, and one reported carpal-tunnel syndrome. Such adverse events are consistent with an increase in total body water associated with elevated GH concentrations, as also observed during GH administration (16, 17). Clinical cardiac function and fasting glucose concentrations did not change.

Interpretative qualifications include selection of successfully aging volunteers capable of completing an intensive 3-month protocol. In the case of performance measures, a small training effect cannot be excluded in a longitudinal design. Four women were receiving postmenopausal HRT, but their responses were not distinguishable by subanalyses from those of the other six volunteers. The present results in healthy, community-living, unmedicated individuals would not necessarily apply equally to individuals with significant age-related frailty or systemic illness. In addition, the outcomes obtained here in ten women will require confirmation in a larger cohort of volunteers with longitudinal parallel placebo exposure.

In conclusion, twice-daily s.c. administration of a high dose of rhGHRH for 3 months elevates GH and
IGF-I concentrations in postmenopausal women, reduces abdominal visceral fat mass, increases total body water, improves certain measures of physical performance and elicits significant local skin reactions. Further investigations will be required to determine the impact of more prolonged delivery of GHRH on the GH–IGF-I axis, body composition and functional status in aging individuals.

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