Effects of Single Nightly Injections of Growth Hormone–Releasing Hormone (GHRH 1-29) in Healthy Elderly Men

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Age-related reductions in growth hormone (GH) and insulin-like growth factor-I (IGF-I) may contribute to decreased muscle mass and strength in older persons. The relationship of this phenomenon to skeletal muscle bioenergetics has not been reported. We sought to determine whether administration of GH-releasing hormone (GHRH) would sustain increases in GH and IGF-I and improve skeletal muscle function and selected measures of body composition and metabolism. We measured GH secretion, muscle strength, muscle histology, and muscle energy metabolism by phosphorus nuclear magnetic resonance spectroscopy (^{31}P-NMRS), body composition, and endocrine-metabolic functions before and after 6 weeks of treatment. Eleven healthy, ambulatory, non-obese men aged 64 to 76 years with low baseline IGF-I levels were treated at home as outpatients by nightly subcutaneous self-injections of 2 mg GHRH for 6 weeks. We measured GH levels in blood samples obtained every 20 minutes from 8:00 PM to 8:00 AM; AM serum levels of IGF-I, IGF binding protein-3 (IGFBP-3), and GH binding protein (GHBP); muscle strength; muscle histology; the normalized phosphocreatine abundance, PCr/[PCr + Pi], and intracellular pH in forearm muscle by NMRS during both sustained and ramped exercise; body composition by dual-energy x-ray absorptiometry (DEXA); lipid levels; and glucose, insulin, and GH levels during an oral glucose tolerance test (OGTT). GHRH treatment increased mean nocturnal GH release ($P < .02$), the area under the GH peak ([AUPGH] $P < .006$), and GH peak amplitude ($P < .05$), with no change in GH pulse frequency or in levels of IGF-I, IGFBP-3, or GHBP. Two of six measures of muscle strength, upright row ($P < .02$) and shoulder press ($P < .04$), and a test of muscle endurance, abdominal crunch ($P < .03$), improved. GHRH treatment did not alter exercise-mediated changes in PCr/[PCr + Pi] or intracellular pH, but decreased or abolished significant relationships between changes in PCr/[PCr + Pi] or pH and indices of muscle strength. GHRH treatment did not change weight, body mass index, waist to hip ratio, DEXA measures of muscle and fat, muscle histology, glucose, insulin, or GH responses to OGTT, or lipids. No significant adverse effects were observed. These data suggest that single nightly doses of GHRH are less effective than multiple daily doses of GHRH in eliciting GH- and/or IGF-I–mediated effects. GHRH treatment may increase muscle strength, and it alters baseline relationships between muscle strength and muscle bioenergetics in a manner consistent with a reduced need for anaerobic metabolism during exercise. Thus, an optimized regimen of GHRH administration might attenuate some of the effects of aging on skeletal muscle function in older persons.

AGE-RELATED DECLINES in skeletal muscle mass and strength[1-4] are well documented, but the etiology of these changes is unknown. Aging is also associated with decreased levels of growth hormone (GH) and insulin-like growth factor-I (IGF-I)[5] and increased body fat.[6] GH treatment of GH-deficient nonelderly adults increases lean body mass,[7] skeletal muscle mass and strength,[8] and aerobic capacity.[9] Studies of GH administration to healthy older men have reported increases in lean body mass[10] and hand-grip strength.[11]

We reported that 2 weeks of twice-daily subcutaneous injections[12] or continuous infusions[13] of GH-releasing hormone (GHRH) in healthy elderly men reversed subnormal GH secretory profiles and serum IGF-I to levels similar to those in younger men, without discernible adverse effects. Thus, GHRH (or other GH secretagogues) may provide a more physiological and safer method for reversing age-related decrements in the GH–IGF-I axis.

We now report the effects of 6 weeks of single nightly subcutaneous injections of GHRH in healthy elderly men on muscle strength, muscle histology, bioenergetic responses of forearm muscle to acute exercise as measured by ^{31}P nuclear magnetic resonance spectroscopy (^{31}P-NMRS), and selected measures of body composition and endocrine-metabolic function.

SUBJECTS AND METHODS

Subjects

Eleven men aged 64 to 76 years (mean, 69.1) were recruited from the community. Of these, nine had participated in one or both of our prior studies of GHRH administration.[12,13] All subjects were healthy as determined by medical history, a screening physical examination, routine serum analyses, electrocardiogram, and urinalysis. None were cigarette smokers at the time of study or took any medications known to influence the GH–IGF-I axis. Body mass index ranged from 20 to 31 kg/m^2 (mean, 26.9), and all subjects had baseline serum IGF-I values less than 250 μg/L. The study was approved by the Institutional Review Board of the Johns Hopkins Bayview Medical Center/Gerontology Research Center, and written informed consent was obtained from all subjects.

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Location

Subjects were studied at the General Clinical Research Center (GCRC) of the Johns Hopkins Bayview Medical Center (JHBMC) and at the adjacent Gerontology Research Center of the National Institute on Aging.

Study Protocol

Subjects were admitted to the GCRC for 2 days of baseline evaluations. On day 1, after an overnight fast, blood was collected for determination of IGF-I, IGF binding protein-3 (IGFBP-3), GH-binding protein (GHBP), glucose, and lipids. Subjects then underwent NMRS of the flexor digitorum superficialis muscle of the dominant arm; dual-energy x-ray absorptiometry (DEXA) scanning (Lunar, Madison, WI) for assessment of total and regional muscle and fat mass; and one repetition–maximum strength testing using a Universal (Cedar Rapids, IA) weight machine with six stations: latissimus pull-down, leg extension, bench press, leg curl, shoulder press, and upright row. In addition, muscle endurance was assessed by the maximal number of abdominal crunches performed during 30 seconds. Twenty-four-hour urine specimens were collected for determination of creatinine, C-peptide, and nitrogen.

From 8:00 PM to 8:00 AM, subjects were fasted and blood samples (1 mL) were collected at 20-minute intervals. Serum was saved at −70°C for subsequent GH determinations.

On day 2, each subject underwent a 2-hour, 75-g oral glucose tolerance test with assays of glucose, insulin, and GH, followed by a percutaneous skeletal muscle biopsy (40 to 60 mg wet weight) obtained from the right vastus lateralis with a 5-mm Bergstrom needle (DePuy, Warsaw, IN). A portion of the muscle specimen was stored in O.C.T. embedding medium (Miles Diagnostics, Elkhart, IN) at −80°C.

After discharge, subjects self-administered 2 mg $GHRH^{1-29}$ subcutaneously nightly for 6 weeks. After 2 weeks, blood pressure was measured and blood was collected for assay of fasting glucose and serum IGF-I. At the end of 6 weeks, subjects were readmitted to the GCRC and repeated the 2-day inpatient protocol with continued administration of nocturnal GHRH injections. The second muscle biopsy was performed adjacent to the original incision. Each subject returned 2 weeks after completing the study for a blood pressure check and measurement of fasting blood glucose and IGF-I levels.

Measurements

Muscle histology. Transverse muscle sections (10 μm) were cut for histochemical analysis on a cryostat at −20°C. Muscle fiber-type distribution (% type I, slow-twitch, and type II, fast-twitch, fibers) was determined in sections stained for myosin adenosine triphosphatase activity, as previously described.[14] A Ken-A-Vision projection microscope was used to project the cross-sections at 150× magnification onto a four-quadrant grid system for fiber counting (mean no. of fibers counted, 398 ± 105 and 385 ± 73, before and after, respectively). A Numonics GridMaster digitizing tablet interfaced with an IBM computer with Easydij software (V 7.0; Geocomp Ltd, Golden, CO) was used to determine the area of type I and type II muscle fibers. Briefly, an artifact-free region of the section was examined, and one fiber was selected at random. The areas of the nearest 25 fibers of both type I and type II muscle fibers were measured. The reliability of repeated area determinations on 25 muscle fibers using this system yielded an r value of 0.98.

NMRS measurements. NMRS was performed using a 1.89-T Bruker Biospec Spectrometer (Billerica, MA) with a 30-cm horizontal bore. The operating frequency was 80.3 MHz for ^{1}H and 32.5 MHz for ^{31}P. A custom-built double-tuned elliptical surface coil measuring 2.9 × 3.8 cm was used for adiabatic excitation[15] and signal detection. The interpulse delay was 1.3 seconds. Magnetic field homogeneity was optimized by shimming on the proton signal to a line width of less than 30 Hz. Metabolites were quantified by integration of spectral resonances and corrected for saturation factors. Intracellular pH was determined by the chemical shift of P_i relative to PCr in parts per million.[16]

NMRS exercise protocols. The maximum hand-grip strength was determined for each subject before and after GHRH therapy. Each subject was then seated with the dominant forearm inserted into the NMRS magnet at shoulder height. The flexor digitorum superficialis muscle was located by palpation and visual inspection and secured over the surface coil.

After control data were obtained, spectra were collected over 30-second intervals during exercise and recovery. In the first protocol, the subject gripped the hand dynamometer at 25% of maximum grip strength for 3 minutes, and then rested for a 6-minute recovery period with continued spectral acquisition. The subject then rested outside the magnet for 20 minutes before further testing. For the second protocol, the subject performed incremental isometric exercise, gripping the hand dynamometer for 1 minute at 20%, 30%, and 40% of maximum grip strength, with a 1-minute rest between these exercise periods. This was again followed by 6 minutes of recovery.

Assays. GH level was measured using an immunoradiometric assay (Allegro HGH kit; Nichols Institute Diagnostics, San Juan Capistrano, CA).[12] Serum samples were assayed in duplicate, with an assay sensitivity of 0.05 μg/L. Serum IGF-I (after acid ethanol extraction),[17] IGFBP-3,[18] and urinary C-peptide levels were all measured by radioimmunoassay (RIA) at Endocrine Sciences Laboratories (Tarzana, CA). GHBP level was measured by a GH binding/immunoprecipitation assay[19] as modified by Ho et al.[20] All samples were analyzed in a single assay with an intraassay coefficient of variation (CV) of 9.7%. Serum glucose was determined by the glucose oxidase method, and serum insulin was assayed by RIA (Linco Research, St Louis, MO) with intraassay and interassay CVs of 11.5% and 6.9%, respectively. Cholesterol (total and high-density lipoprotein [HDL]) and triglycerides were assayed by standardized methods at the Johns Hopkins Lipid Research Clinics Laboratory. Serum testosterone and triiodothyronine (T_3) levels were measured by RIA (Diagnostic Products, Los Angeles, CA). Serum values for thyroxine (T_4), T_3 resin uptake, and thyrotropin, as well as routine serum and urine chemistries, were measured in the JHBMC Clinical Chemistry Laboratory.

Statistical Analysis

Two subjects inadvertently did not receive the last nocturnal GHRH injection during the second GCRC visit at 6 weeks. Consequently, analysis of GH data was performed in only nine of 11 men. For all other results, data from all 11 men were used.

Twelve-hour nocturnal GH secretory parameters were analyzed using the Pulsar computer program.[21] Peak detection cutoff values were G1 = 5, G2 = 4, G3 = 3.5, G4 = 3, and G5 = 2.5, with an expected false-positive rate of less than 1%. GH secretory parameters included mean GH levels, GH peak amplitude, duration of GH peaks, and GH peak number. Integrated areas under the nighttime GH peaks (IAUPs) were calculated by the method of trapezoidal integration.

For each subject in each of the two exercise protocols, a resting PCr/[PCr + Pi] ratio was obtained. The exercise-induced high-energy phosphate nadir was identified as the minimum ratio during exercise; the maximum change in the ratio (Δ) and the percent change (%Δ) from baseline were calculated. The postnadir recovery rate was estimated as the slope of the ratio per unit of time (ie, the ratio Δ per minute) calculated by linear regression of values from the nadir either to the first value that at least equalled one of the baseline values or, if postexercise values did not reach baseline, to the plateau (ie, the value after which PCr/[PCr + Pi] ceased to increase). Corresponding values for the baseline, nadir, Δ, %Δ, and recovery slope for pH measurements were identified using the same criteria.

All data are expressed as the mean ± SD. Values for GH secretory

indices, NMRS parameters, and muscle strength, body composition, hormone, and metabolic measurements were compared before and after GHRH treatment by paired t test. Linear regression analyses with Pearson correlation coefficients were used to test the hypothesis that baseline relationships between measures of muscle bioenergetics and of GH, IGF-I, and muscle performance were altered by GHRH treatment.

RESULTS

Effects of GHRH on Nocturnal GH Release

Pulsar analysis (Table 1) showed that GHRH treatment approximately doubled the 12-hour mean GH ($P < .02$) and the mean area under the GH peaks ([AUPGH] $P < .02$). The increase in mean peak amplitude was not significant, and there was no significant change in the mean number of peaks per 12 hours. The duration of GH peaks was slightly decreased ($P < .05$). Inspection of the nocturnal serum GH profiles shows that the marked increase in GH release after GHRH treatment was entirely confined to the 2 hours following GHRH injection (Fig 1). GH secretory profiles of the two men who did not receive the last GHRH injection were similar to their pretreatment profiles (data not shown).

Effects of GHRH on Circulating IGF-I, IGFBP-3, and GHBP

Baseline serum IGF-I levels (152 ± 51 μg/L) did not increase significantly after 2 (163 ± 48) and 6 (159 ± 49) weeks of GHRH administration. However, the mean IGF-I level at week 2 of treatment was higher than at 2 weeks after cessation of GHRH (137 ± 44 μg/L, $P < .01$). There were no significant changes (before v after 6 weeks of GHRH administration) in IGFBP-3 levels (2.4 ± 0.3 v 2.5 ± 0.4 μg/L) or GHBP activity (123% ± 25% v 121% ± 28% specific binding).

Effects of GHRH on Body Composition

There were no significant changes (before v after GHRH administration) in the total body weight (80.0 ± 8.4 v 80.1 ± 8.3 kg), body mass index (27.0 ± 2.7 v 27.2 ± 2.6 kg/m^2), or waist to hip ratio (0.96 ± 0.04 v 0.96 ± 0.04). Similarly, DEXA scans revealed no significant changes in percent total lean body mass, percent total fat mass, or percent lean or fat mass in the arms, legs, or trunk (data not shown).

Effects of GHRH on Muscle Strength

The physical activity of the men, defined by self-report of household, occupational, and sports tasks as moderate, hard, or very hard, remained constant over the GHRH treatment period. Muscle strength testing showed a highly significant increase in the mean value for upright row (21%, $P < .001$), and less significant or borderline increases in shoulder press (3%, $P < .04$), leg curl (12%, $P < .07$), and abdominal crunch (11%, $P < .03$). The other exercise parameters remained unchanged.

Table 1. GH Secretory Parameters in Healthy Old Men Before and After GHRH

Variable	12-Hour Mean GH (μg/L)	Peak No.	Peak Amplitude (μg/L)	Peak Duration (min)	AUPGH (μg · min/L)
Before GHRH	1.1 ± 0.9	6.5 ± 1.5	1.8 ± 1.6	71.0 ± 14.2	1,114 ± 931
After GHRH	2.2 ± 1.9	7.1 ± 1.9	2.4 ± 2.1	60.4 ± 15.5	2,032 ± 1,728
P	<.02	NS	NS	<.05	<.02

Abbreviation: NS, nonsignificant.

Effects of GHRH on Muscle Energy Metabolism

The time courses for high-energy phosphate utilization expressed as PCr/[PCr + Pi] (Fig 2A) and for intracellular pH (Fig 2B) during exercise protocol 1 were indistinguishable before and after GHRH administration. Values for PCr/[PCr + Pi] declined progressively during sustained handgrip to a nadir of approximately 0.45. During the 6-minute recovery period, the ratios returned gradually to preexercise levels, achieving resting values at approximately 4 minutes of recovery. Values for pH also decreased during exercise to a nadir at 7 minutes and returned to baseline at 12 minutes.

The effects of 6 weeks of GHRH administration on mean values for PCr/[PCr + Pi] and pH at rest, at the nadir, Δ, %Δ, and the recovery slopes showed no significant differences between pre- and post-GHRH values (Table 2).

The time courses for PCr/[PCr + Pi] and intracellular pH measured during exercise protocol 2 were also indistinguishable before and after GHRH treatment (data not shown). Resting values for PCr/[PCr + Pi] were stable, then declined in response to handgrip exercise with a return toward baseline during each intervening 1-minute recovery period. By the end of the 6-minute recovery period, PCr/[PCr + Pi] recovered to about 80% of the previous resting value. A stepwise decline in pH was also seen, with a mean nadir value of 6.6 reached at 40% maximum grip strength. Recovery to baseline had not occurred by the end of the 6-minute recovery period.

Before GHRH treatment, there were positive correlations of borderline significance between resting PCr/[PCr + Pi] and AUPGH ($r = .56, P = .07$) and serum IGF-I ($r = .57, P = .06$). After treatment, these relationships were no longer present. Moreover, there were no significant correlations of AUPGH or IGF-I with the ΔPCr/[PCr + Pi], or with the PCr/[PCr + Pi] nadir or PCr/[PCr + Pi] recovery slopes (data not shown) before or after GHRH treatment. Regression analysis of the corresponding pH variables showed a positive correlation of marginal significance between ΔpH and IGF-I ($r = .59$, $P = .06$). There were no significant correlations between the other derived pH indices and AUPGH or IGF-I before or after GHRH treatment.

We examined the relationships between the decreases in high-energy phosphate and in pH during exercise protocol 1 with values for measures of muscle strength and endurance. Before GHRH treatment, there was an inverse relationship between resting PCr/[PCr + Pi] and upright row ($r = -.66$, $P = .03$), whereas the relationships for resting PCr/[PCr + Pi] with other muscle strength variables were nonsignificant (Table 3). In contrast, ΔPCr/[PCr + Pi] was significantly and inversely related to three of six strength measurements: leg extension ($r = -.71, P = .01$), leg curl ($r = -.70, P < .02$), and latissimus pull-down ($r = -.70, P = .02$). No significant relationships were observed between the PCr/[PCr + Pi] recovery slope and any measurement of muscle strength (data not shown). The significance of all relationships between measures of phosphate utilization and measures of muscle strength disappeared after GHRH administration.

Regression analysis of the corresponding protocol 1 pH

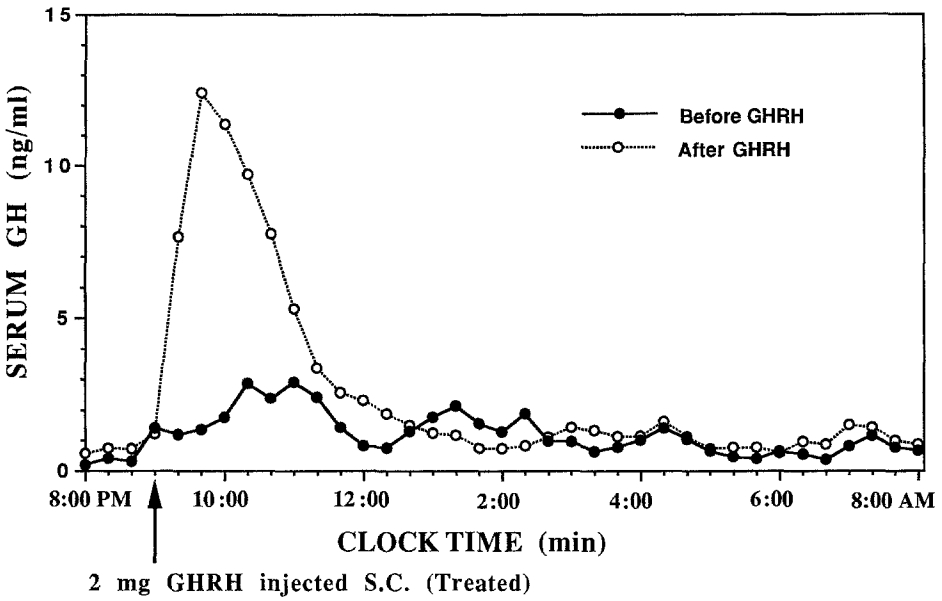

Fig 1. Mean nocturnal serum GH levels (μg/L) obtained at 20-minute intervals in 9 old men studied before and after 6 weeks of single nightly subcutaneous injection of GHRH (2 mg).

variables (Table 3) showed a significant positive correlation of pretreatment resting pH with leg curl ($r = .64$, $P = .03$), as well as inverse correlations of ΔpH with leg curl ($r = -.70$, $P = .02$), upright row ($r = -.64$, $P = .03$), shoulder press ($r = -.74$, $P = .01$), bench press ($r = -.62$, $P = .04$), and latissimus pull-down ($r = -.71$, $P = .01$). After GHRH treatment, leg extension was inversely correlated with resting pH ($r = -.71$, $P = .02$) and with Δ pH ($r = -.68$, $P = .02$), but all other pre-GHRH correlations for muscle measurements with pH were attenuated to the point of nonsignificance.

No significant relationships of PCr/[PCr + Pi] or pH indices with strength were seen in data derived from protocol 2 either at baseline or after GHRH.

Abdominal crunch, a measure of endurance rather than strength, was positively correlated with pretreatment resting pH ($r = .65$, $P = .03$), but not with any other measure of high-energy phosphate or pH during exercise.

To summarize, in protocol 1, administration of GHRH abolished three of three significant baseline inverse relationships between muscle strength and ΔPCr/[PCr + Pi]. Further, GHRH treatment attenuated all five inverse relationships between muscle strength and ΔpH.

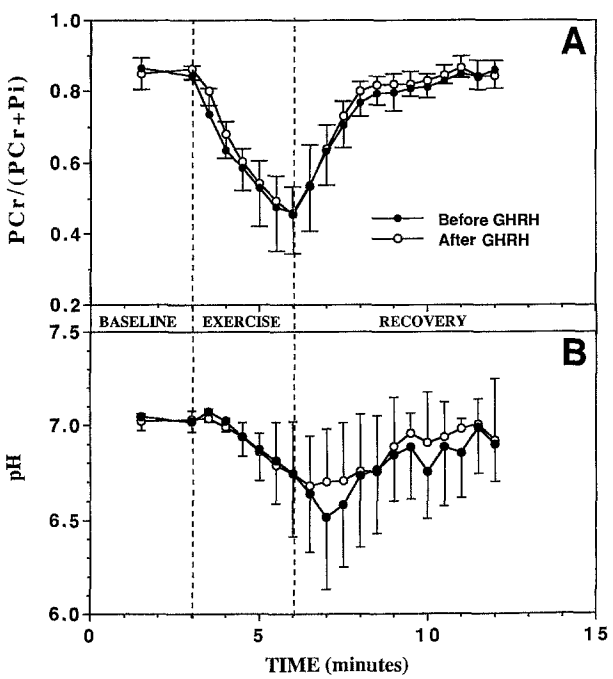

Fig 2. Effects of continuous isotonic exercise using a hand dynamometer at 25% of maximal grip strength for 3 minutes on (A) PCr/[PCr + Pi] and (B) intracellular pH. The mean ± SD for values measured by ^{31}P-NMRS in the flexor digitorum superficialis muscle of 11 men aged 64 to 76 years before and after 6 weeks of daily injections of GHRH are shown.

Effects of GHRH on Muscle Fiber Pattern

There were no significant changes (before *v* after GHRH administration) in the mean percentages of type I (58.9% ± 3.5% *v* 59.6% ± 4.7%) or type II (41.1% ± 3.5% *v* 40.7% ± 4.5%) muscle fibers, or in their respective fiber areas (5,182 ± 453 *v* 5,233 ± 355 μm^2 and 5,106 ± 907 *v* 5,318 ± 453 μm^2).

Effects of GHRH on Glucose and Insulin Tolerance and Lipids

Blood glucose concentrations in the fasted state were normal at baseline, and remained unchanged throughout the study (data

Table 2. Indices Derived From PCr/[PCr + Pi] and pH Values in Forearm Muscle During 3 Minutes of Isotonic Handgrip (protocol 1) Before and After GHRH Treatment

	PCr/[PCr + Pi]			pH		
Parameter	Before GHRH	After GHRH	*P*	Before GHRH	After GHRH	*P*
Resting	0.86 ± 0.03	0.86 ± 0.02	.9	7.03 ± 0.03	7.02 ± 0.04	.7
Nadir	0.45 ± 0.10	0.44 ± 0.11	.8	6.42 ± 0.34	6.60 ± 0.27	.1
Δ to nadir	0.40 ± 0.12	0.41 ± 0.12	.9	0.61 ± .33	0.42 ± 0.27	.1
%Δ	47.0 ± 12.8	48.0 ± 13.4	.8	8.7 ± 4.8	6.0 ± 3.8	.1
Recovery slope	0.13 ± 0.04	0.16 ± 0.08	.4	0.38 ± 0.51	0.21 ± 0.29	.4

Table 3. *P* Values for Correlations of Derived PCr/[PCr + Pi] and pH Variables With Hormone and Muscle Performance Measures in Protocol 1 Before and After GHRH Treatment

	Before GHRH				After GHRH			
	PCr/[PCr + Pi]		pH		PCr/[PCr + Pi]		pH	
Derived Variable	Mean Resting	Δ to Nadir	Mean Resting	Δ to Nadir	Mean Resting	Δ to Nadir	Mean Resting	Δ to Nadir
Hormones								
AUPGH	.07	.13	.49	.67	.36	.28	.9	.46
IGF-I	.06	.13	.24	.06	.63	.13	.53	.55
Muscle strength								
Leg extension	.09	.01	.24	.23	.65	.98	.015	.02
Leg curl	.18	.015	.03	.02	.51	.77	.67	.09
Upright row	.03	.21	.28	.03	.69	.99	.99	.7
Shoulder press	.16	.33	.15	.01	.28	.67	.89	.08
Bench press	.09	.34	.11	.04	.3	.69	.96	.17
Latissimus pull-down	.07	.02	.26	.01	.83	.66	.17	>.05
Abdominal crunch	.32	.54	.03	.74	.37	.60	.32	.46

not shown). After 6 weeks of GHRH treatment, blood glucose values at 30 and 60 minutes of the oral glucose tolerance test were slightly but significantly lower than the corresponding pretreatment values ($P < .03$ and .06, respectively); however, GHRH treatment elicited no overall effect on the area under the glucose curve. There were no effects of GHRH administration on fasting or postglucose plasma insulin levels, or on GH responses to an oral glucose load. Moreover, there were no significant changes (before *v* after GHRH) in 24-hour urinary C-peptide excretion (69.5 ± 27.4 *v* 65.4 ± 17.6 μg/24 h) or levels (mmol/L) of total (4.9 ± 0.8 *v* 4.7 ± 0.8), low-density lipoprotein ([LDL] 3.3 ± 0.7 *v* 3.1 ± 0.8), or HDL (1.1 ± 0.3 *v* 1.1 ± 0.2) cholesterol, or triglycerides (1.3 ± 0.7 *v* 1.2 ± 0.4).

Effects of GHRH on Other Hormones

There were no significant changes in levels of testosterone (515 ± 160 *v* 501 ± 143 ng/dL), total T_4 (6.9 ± 1.2 *v* 6.4 ± 1.0 μg/dL), total T_3 (100 ± 17 *v* 96 ± 12 ng/dL), or thyrotropin (1.6 ± 1.1 *v* 2.0 ± 1.3 μU/mL) during GHRH treatment. In contrast, values for T_3 resin uptake decreased (0.93 ± 0.09 *v* 0.86 ± 0.06, $P < .02$), and values for the free T_4 index increased (6.2 ± 1.7 *v* 6.9 ± 0.4 μg/dL, $P < .001$).

Clinical Side Effects

For all subjects, mean systolic blood pressure decreased from 135 to 125 mm Hg ($P < .04$), with no change in diastolic pressure. No edema, carpal tunnel syndrome, or other adverse effects were reported.

DISCUSSION

The men in the current study had a mean baseline IGF-I level of 152 ± 51 μg/L (range, 64 to 220). These results are comparable to the serum IGF-I values of 150 ± 52 (mean ± SD) μg/L (range, 16 to 295) that we obtained from a sample of 116 healthy old men (aged 60 to 79 years; mean, 68.7) in the Baltimore Longitudinal Study on Aging.[5] Endocrine Sciences, which performed the IGF-I RIA determinations in this study, reports a mean of 157 μg/L (range, 52 to 223) on a sample from similarly aged men and a mean of 272 μg/L (range, 160 to 453) from healthy younger men (aged 20 to 39 years). Thus, the men in the current study, although selected to have baseline IGF-I levels less than 250 μg/L, exhibited IGF-I values typical for men their age.

We previously reported that twice-daily subcutaneous injections of GHRH in healthy old men, at cumulative dosages of 1 and 2 mg/d, increased diurnal GH release and serum IGF-I levels significantly after 2 weeks; GH secretory responses were greater after evening versus morning injections.[12] Consequently, in the current study, we simplified the GHRH regimen to single nightly subcutaneous injections of 2 mg GHRH. This regimen elicited significant increases in nighttime GH release by stimulating a single large GH peak. As was evident from the unchanged GH secretory profiles of the two men who missed the final GHRH injections, the enhancement of nocturnal GH release depended entirely on the immediately preceding GHRH injection, consistent with our prior observations.[12] In the present study, the magnitude of GH secretion resulting from a single 2-mg GHRH injection was as great as or greater than what we observed following two 1-mg injections.[12] Serum IGF-I began at a mean baseline value of approximately 150 μg/L in both studies, but increased to only 163 μg/L at 2 weeks (P = NS) in the current study as compared with 225 μg/L ($P < .005$) in the prior study. The fact that 2 mg of GHRH given once nightly exerted less effect on serum IGF-I levels than 1 mg given twice daily suggests that the frequency of GHRH administration may be an important factor in determining the subsequent circulating IGF-I response. This finding is consistent with the concept that physiological patterns of GH pulsatility are required to optimize target tissue effects.

Although circulating IGF-I is commonly used as an indicator of peripheral GH action, in GH-deficient children[22] and in nonelderly[23] and old[12] adults, serum IGF-I level is only weakly correlated with 24-hour GH release. Moreover, GH acts on a range of target tissues both directly and by local production of IGF-I. GH- or GH secretagogue–induced alterations in target tissue responses are thought to be more closely related to increments in circulating IGF-I than to putative changes in autocrine or paracrine effects of IGF-I. For example, Crist et al[24] reported GH-induced improvements in lipids and body composition in healthy adults in conjunction with elevations in circulating IGF-I, whereas in the current study the absence of such changes may be related to the failure of serum IGF-I to

increase significantly. Nonetheless, some GH-mediated tissue effects may result more from local IGF-I production than from effects of circulating IGF-I. Therefore, the enhanced GH release we observed could still have produced effects on muscle bioenergetics directly, or indirectly, via stimulation of local production of IGF-I in target tissues.

Circulating IGFBP-3 levels are GH-dependent, decrease with aging,[25] and are positively related to changes in IGF-I.[26] In this study, IGFBP-3, like IGF-I, did not increase significantly after GHRH administration. GHBP levels, which decrease with age,[27] did not change after GHRH treatment, perhaps because the increases in GH release were temporally confined to a single postinjection pulse.

We detected no changes in body composition after 6 weeks of GHRH administration. In contrast, prior longer-term GH intervention studies in healthy old men[10] and in GH-deficient adults[28] showed increases in lean body mass and decreases in percent body fat.

The magnitude of the strength increases we observed generally exceeded those expected from biological or technical variations in the measurements.[29] Our subjects reported no interval changes in their exercise habits, suggesting that strength gains were not due to a training effect. Because subjects were exposed to the weight apparatus only twice during the study, a practice effect was also unlikely. Several investigators have reported increased muscle mass by computed tomography, as well as improved muscle strength and/or significantly increased aerobic capacity during 4 or more months of GH treatment of GH-deficient adults.[7,9] Although our findings are consistent with those of prior studies, the current effects of GHRH on muscle strength and performance must still be considered inconclusive until a placebo-controlled trial with optimal doses of GHRH is performed.

The pre-GHRH percentages of type I and type II muscle fibers and fiber areas we observed were similar to those previously reported in this age group.[30,31] The lack of a detectable GHRH treatment effect on muscle fiber types and areas in samples taken from the vastus lateralis does not preclude a possible GHRH effect on the fiber composition or area of other muscle groups.

In the current study, we used NMRS to measure PCr/[PCr + Pi], which provides an estimate of the cellular energy charge in muscle.[32] Measurements of changes in this ratio and in pH during exercise and recovery provide estimates of muscle efficiency and oxidative phosphorylation capacity. Controversy exists as to whether normal human aging affects skeletal muscle bioenergetics. Both an age-related decrease in the ratio of PCr/Pi at rest and a decrease in the PCr recovery rate of gastrocnemius muscle following exercise,[33] and no age-related differences in forearm muscle metabolism[34-36] have been reported. These discrepancies may be due to greater stressing of the weaker (older) versus stronger (younger) individuals with relation to their respective maxima, or to differences between the specific muscles tested.

Before GHRH administration, during sustained exercise we observed significant inverse correlations between pH reductions (change to nadir) and all muscle strength measurements except leg extension. In contrast, after GHRH treatment, all these correlations became nonsignificant. Leg extension was the single strength measure that significantly related to ΔpH after GHRH administration, and was the only measure to decrease compared with baseline. No significant correlations were seen during ramped exercise (protocol 2) before or after GHRH administration.

A larger decrease in pH implies greater reliance on anaerobic metabolism at high levels of stress.[37] Thus, the inverse relationships between muscle strength indices and the change to nadir in pH suggest that individuals with a higher level of muscle function were able to perform the exercises with significantly less reliance on anaerobic metabolism (ie, they had a greater aerobic capacity). Hence, decreased correlations between muscle performance indices and the change to nadir in pH after GHRH treatment may indicate that GHRH administration, by reducing the requirement for an anaerobic contribution, increased the intrinsic aerobic capacity at high workload. This interpretation is consistent with the observed trend toward a smaller decrease in mean pH during exercise following GHRH administration (Table 2). Possible tissue-level explanations for these observations include an increase in the proportion of type I (slow-twitch, aerobic) muscle fibers,[38] increased vascularization,[39] or increased functioning of creatine kinase[40] secondary to effects of increased GH and/or IGF-I on skeletal muscle.

Similar relationships were not observed in the ramped exercise protocol, perhaps because the rest periods in the intermittent exercise protocol permitted recovery of muscle bioenergetics. This interpretation is consistent with the observation that the manner in which work is performed can exert a profound influence on the bioenergetic response.[37]

Before GHRH treatment, there were significant inverse correlations between changes to nadir of PCr/[PCr + Pi] during sustained exercise and three of six indices of muscle strength, but these relationships were weaker than those for pH. These correlations were all nonsignificant after GHRH administration, despite the fact that the total change to nadir of PCr/[PCr + Pi] was unaltered. These findings are consistent with the observations made regarding pH, which is a more direct measure of anaerobic metabolism.

The lack of change in recovery slopes for PCr/[PCr + Pi] after GHRH administration is not inconsistent with the observed pH effects, because recovery slope reflects metabolism at low workload only. In contrast, the observations made above regarding aerobic reserve are applicable to the high workload used in this study.

There were no significant correlations between the abdominal crunch exercise and ΔPCr/[PCr + Pi] or ΔpH before or after GHRH treatment. The observation of a direct relationship between resting pH and abdominal crunch is of uncertain biological significance, as resting pH does not predict changes in muscle metabolism during exercise.

Treatment of GH-deficient younger adults with GH for 6 to 20 months reduces total-body and intraabdominal fat, decreases total and LDL cholesterol, and increases HDL cholesterol.[7,24,41] In contrast, in our prior[12,13] and present shorter-term studies, no changes in plasma levels of total cholesterol or other lipids, total or regional fat, or lean body mass were observed.

Hyperglycemia has been a common side effect in some[10,42]

but not in other[11] GH-replacement studies in elderly people, and appears to be dose-related.[42] Our observation that 6 weeks of GHRH administration led to no significant alterations in glucose or insulin levels in the fasted state or during glucose tolerance testing confirms and extends our prior studies, in which 2 weeks of GHRH administration did not change fasting blood glucose levels or 24-hour urinary C-peptide excretion.[12,13] Thus, GHRH administration may exert fewer adverse effects than GH on insulin and glucose homeostasis. The GH response to an oral glucose challenge was appropriately suppressed after GHRH administration, suggesting that the dynamics of the hypothalamic-pituitary somatotropic axis were relatively undisturbed by GHRH injections.

Hypothyroidism has been associated with reduced resting PCr/Pi, greater depletion of PCr, lower pH during exercise, and delayed recovery of PCr/Pi ratios after exercise,[43-45] which were reversed after thyroid hormone replacement.[43] Our findings that, save for a small increase in the free T_4 index, thyroid hormone and testosterone levels did not change significantly after GHRH administration suggest that neither hormone contributed to alterations in muscle strength or to the relationships between muscle strength and bioenergetics.

In summary, we observed changes consistent with increased aerobic reserve in forearm muscle in healthy elderly men treated with single nocturnal injections of GHRH. Enhanced pituitary GH release was temporally related to the GHRH injections. Despite minimal effects on circulating IGF-I, certain measures of muscle strength improved after GHRH administration, suggesting that GH-mediated effects occurred directly or via the actions of locally generated IGF-I. There were no adverse effects on glucose metabolism or blood pressure, or any complaints of arthralgia or symptoms of carpal tunnel syndrome. Taken together, the current data suggest that further clinical investigations of the effects of administration of GHRH or other GH secretagogues on muscle strength and bioenergetics are warranted.

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