Growth hormone and testosterone interact positively to enhance protein and energy metabolism in hypopituitary men

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Growth hormone and testosterone interact positively to enhance protein and energy metabolism in hypopituitary men. Am J Physiol Endocrinol Metab 289: E266–E271, 2005. First published February 22, 2005; doi:10.1152/ajpendo.00483.2004.—We investigated the impact of growth hormone (GH) alone, testosterone (T) alone, and combined GH and T on whole body protein metabolism. Twelve hypopituitary men participated in two studies. Study 1 compared the effects of GH alone with GH plus T, and study 2 compared the effects of T alone with GH plus T. IGF-I, resting energy expenditure (REE), and fat oxidation (Fox) and rates of whole body leucine appearance (Ra), oxidation (Lox), and nonoxidative leucine disposal (NOLD) were measured. In study 1, GH treatment increased mean plasma IGF-I (P < 0.001). GH did not change leucine Ra, but reduced Lox (P < 0.02) and increased NOLD (P < 0.02). Addition of T resulted in an additional increase in IGF-I (P < 0.05), reduction in Lox (P < 0.002), and increase in NOLD (P < 0.002). In study 2, T alone did not alter IGF-I levels. T alone did not change leucine Ra, but reduced Lox (P < 0.01) and increased NOLD (P < 0.01). Addition of GH further reduced Lox (P < 0.05) and increased NOLD (P < 0.05). In both studies, combined treatments on REE and Fox were greater than either alone. In summary, GH-induced increase of circulating IGF-I is augmented by T, which does not increase IGF-I in the absence of GH. T and GH exerted independent and additive effects on protein metabolism, Fox and REE. The anabolic effects of T are independent of circulating IGF-I. insulin-like growth factor I; protein turnover

Growth hormone (GH) and testosterone are potent anabolic hormones. There is strong evidence in children that both hormones interact positively to enhance growth and body composition (2, 22, 35). The mechanistic basis of this interaction is poorly understood.

Testosterone enhances the growth of boys with hypogonadism and those with hypopituitarism during GH treatment (2, 35). However, the effect of testosterone on somatic growth is poor in boys with hypopituitarism without concomitant GH treatment (2, 35). In hypopituitary adults who are not receiving GH replacement, testosterone exerts no effect on circulating IGF-I (18). These collective observations suggest that the growth promoting and anabolic effects of testosterone may be dependent on GH and possibly mediated in part by IGF-I.

How GH and testosterone interact to regulate protein metabolism in adult life is also poorly understood. There is evidence that both hormones are necessary to exert an optimal effect. Even after adequate androgen replacement, lean body mass is reduced in GH-deficient men (15). The observation that the effects of GH replacement are more marked in men than in women (9) provides further evidence that testosterone might enhance the anabolic effects of GH. The anabolic effects of GH are mediated by IGF-I, but whether IGF-I also plays a role in mediating the anabolic effects of testosterone is unknown. The aim of the study was to investigate how GH and testosterone interact to regulate anabolism by studying the independent and combined effects of these two hormones on IGF-I and protein metabolism.

SUBJECTS AND METHODS

Subjects. Twelve hypopituitary men with GH deficiency and hypogonadotropic hypogonadism were recruited from the Endocrine Outpatient Clinic at St. Vincent’s Hospital, Sydney, Australia. The clinical characteristics of these patients are shown in Table 1. Two studies were undertaken: the first compared the effects of GH alone with GH plus testosterone, and the second compared the effects of testosterone alone with GH plus testosterone. A three-period crossover study design was originally planned to investigate the effects of GH, testosterone, and combined (GH plus testosterone) treatment. However, the demands and logistics precluded the adoption of such a design, as many of the subjects were frail and lived outside Sydney. Instead, a design comprising two interrelated studies was adopted. GH deficiency was confirmed by a peak GH response to insulin-induced hypoglycemia of <3 ng/ml (14), and hypogonadotropic hypogonadism was defined as serum testosterone measured in a morning sample <4 nmol/l, accompanied by low serum luteinizing hormone level. The duration of hypopituitarism was ≥1 yr. All subjects were receiving stable hormone replacement for other deficiencies throughout and during the study periods. The Research Ethics Committee of St. Vincent’s Hospital approved the studies. Written informed consent was obtained from all subjects.

Study design. Both studies were of open-label randomized crossover design and together allowed comparison of the individual and combined effects of testosterone and GH while taking time-dependent effects into consideration. Before commencement of each study, subjects underwent a 6-wk run-in period, when testosterone and GH were withdrawn. During this time and throughout the studies, they were instructed to follow their usual diet and habitual activities. In both studies, GH (Humatrope, Lilly Australia) was administered at a dose of 0.5 mg daily by self-injection at 8 PM, and testosterone enanthate (Primoteston) was administered as 250 mg intramuscularly 2 wk before measurement. The dose and timing of the testosterone injection were based on known pharmacokinetics of testosterone enanthate and aimed to expose subjects to physiological plasma testosterone levels during the 2 wk preceding the metabolic studies.

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EFFECTS OF TESTOSTERONE AND GH ON PROTEIN METABOLISM

Table 1. Characteristics of hypogonadal GH-deficient patients

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Study</th>
<th>Age, yr</th>
<th>Diagnosis</th>
<th>Height, cm</th>
<th>Weight, kg</th>
<th>BMI, kg/m²</th>
<th>Treatment</th>
<th>Hormone Replacement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1, 2</td>
<td>66</td>
<td>Pituitary macroadenoma</td>
<td>1.65</td>
<td>83.2</td>
<td>30.6</td>
<td>S/X</td>
<td>A, T, G</td>
</tr>
<tr>
<td>2</td>
<td>1, 2</td>
<td>61</td>
<td>Rathke’s cyst</td>
<td>1.67</td>
<td>61.9</td>
<td>22.2</td>
<td>S</td>
<td>A, T, G, D</td>
</tr>
<tr>
<td>3</td>
<td>1, 2</td>
<td>50</td>
<td>Pituitary macroadenoma</td>
<td>1.87</td>
<td>114.7</td>
<td>33.0</td>
<td>S</td>
<td>G</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>60</td>
<td>Pituitary macroadenoma</td>
<td>1.85</td>
<td>75.4</td>
<td>22.0</td>
<td>S</td>
<td>G</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>50</td>
<td>Craniohypophyseoma</td>
<td>1.73</td>
<td>111.0</td>
<td>37.1</td>
<td>S/X</td>
<td>A, T, G</td>
</tr>
<tr>
<td>6</td>
<td>1, 2</td>
<td>66</td>
<td>Pituitary macroadenoma</td>
<td>1.75</td>
<td>85.9</td>
<td>28.0</td>
<td>S</td>
<td>A, T, G</td>
</tr>
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<td>7</td>
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<td>46</td>
<td>Pituitary macroadenoma</td>
<td>1.78</td>
<td>84.8</td>
<td>26.8</td>
<td>S</td>
<td>A, T, G</td>
</tr>
<tr>
<td>8</td>
<td>1, 2</td>
<td>77</td>
<td>Pituitary macroadenoma</td>
<td>1.71</td>
<td>40.4</td>
<td>30.9</td>
<td>S</td>
<td>G</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>33</td>
<td>Craniohypophyseoma</td>
<td>1.60</td>
<td>60.6</td>
<td>23.7</td>
<td>S/X</td>
<td>A, T, G</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
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<td>Pituitary macroadenoma</td>
<td>1.76</td>
<td>99.7</td>
<td>32.2</td>
<td>S</td>
<td>T, D, G</td>
</tr>
<tr>
<td>12</td>
<td>1, 2</td>
<td>66</td>
<td>Pituitary macroadenoma</td>
<td>1.71</td>
<td>84.4</td>
<td>30.2</td>
<td>S</td>
<td>T, G</td>
</tr>
</tbody>
</table>

BMI, body mass index; S, surgery; X, irradiation; A, adrenal replacement; T, thyroid replacement; G, gonadal replacement; D, [deamino-Cys¹,D-Arg⁸] vasopressin.

In the first study (study 1), GH was administered daily for 6 wk. Testosterone was administered either at baseline (group A, n = 5) or week 4 (group B, n = 5) of the study. Investigations were carried out at baseline, after 2 wk and 6 wk so that the effects of testosterone were assessed 2 wk after administration. Thus studies were carried out when subjects were not receiving GH or testosterone replacement, during replacement with GH alone, and during GH plus testosterone replacement (Fig. 1). In the second study (study 2), testosterone was administered at week 2 and week 6 of the study. GH was administered either during the first 4 wk (group A, n = 5) or the second 4 wk (group B, n = 4) of the study. Investigations were carried out at baseline, after 4 wk, and after 8 wk. Thus studies were carried out when subjects were not receiving GH or testosterone replacement, during replacement with testosterone alone, and during combined GH plus testosterone replacement (Fig. 1).

At each visit, all subjects underwent measurements of plasma IGF-I and whole body protein turnover using [1-13C]leucine tracer, as previously described (16, 34), and assessment of resting energy expenditure and fat and carbohydrate oxidation by use of indirect calorimetry (25). All subjects were studied at 8 AM after an overnight fast.

Study techniques. Whole body protein turnover was undertaken using a primed constant infusion of [1-13C]leucine. The technique has been extensively validated (1, 3, 10, 17, 27, 32) and has proved highly reproducible in our hands (12, 16, 28, 29, 34). NaH¹³CO₃ (99%) was obtained from Cambridge Isotope Laboratories (Woburn, MA), and 99% [1-13C]leucine was obtained from MassTrace (Woburn, MA). Solutions of each were prepared under sterile conditions using 0.9% saline.

Subjects were studied in the Clinical Research Facility, Garvan Institute of Medical Research, after an overnight fast. At 0800, canulae were inserted into both antecubital veins, one for isotope infusion and the other for blood sampling. A 0.1 mg/kg priming dose of NaH¹³CO₃ was followed immediately by [1-¹³C]leucine (prime, 0.5 mg/kg; infusion, 0.5 mg·kg⁻¹·h⁻¹). Blood and breath samples were collected before (−10, 0 min) and at the end of a 3-h infusion (140, 160, and 180 min), at which time we and others have previously demonstrated a physiological and isotopic steady state (7, 12, 16, 21, 30, 34). Blood was placed on ice, and plasma was separated and stored at −80°C until analysis. O₂ and CO₂ production rates were undertaken with an open circuit, ventilated hood system (Deltatrac monitor, Datex Instrumentation, Helsinki, Finland). The monitor was calibrated against standard gases before each study. Measurements were averaged over 20–40 and 160–180 min. The coefficient of variation for energy expenditure was 4.2%, and for substrate oxidation was 4% (24).

Calculation of whole body protein turnover. Rates of appearance (Rₑ) of leucine, nonoxidative leucine disposal (NOLD), and leucine oxidation were calculated as previously described (16, 34). Isotopic enrichment of plasma α-ketoisocaproic acid (KIC), which is believed to reflect intracellular leucine enrichment more closely than the isotopic enrichment of plasma leucine, was measured. Because leucine represents 8% of total body protein, or 590 µmol of leucine represents 1 g of protein, rates of protein turnover may be estimated using these constants (21, 26).

Calculation of energy expenditure and substrate oxidation. Energy expenditure (EE) and substrate oxidation were calculated from the following equations: EE = 3.91 Vₒ₂ + 1.10 Vₖₒ₂ − 0.53 protein oxidation; fat oxidation = 1.67 Vₒ₂ − 1.67 Vₖₒ₂ − 0.31 protein oxidation; and carbohydrate oxidation = 4.55 Vₖₒ₂ − 3.21 Vₒ₂ − 0.46 protein oxidation (11). Carbohydrate, lipid, and protein oxidation are expressed as grams per minute. Vₒ₂ represents O₂ consumption, and Vₖₒ₂ represents CO₂ production in liters per minute.

Analytic methods. KIC was extracted by the method of Nissen et al. (23). As previously described (16, 34), KIC enrichment was measured...
as the butyldimethylsilyl derivative by gas chromatography (model 5890, Hewlett-Packard, Palo Alto, CA)-mass spectrometry (MSD 5971A, Hewlett-Packard), with selective monitoring of ions 302 and 303 (31). CO₂ enrichment in breath was measured on a SIRA Series II isotope ratio mass spectrometer (VG Isotech, Cheshire, UK).

Statistics. Nonnormally distributed data were logarithmically transformed before analysis. Repeated measures ANOVA was used in each study to determine treatment effect, and paired t-tests were used to compare different time points. Results are expressed as means ± SE, and statistical significance was set at an α-level of 0.05.

RESULTS

There were no baseline differences between subjects who took part in the two studies. Both treatments alone and in combination were well tolerated, and no sequence effect was detected in either study.

IGF-I and testosterone. Mean IGF-I levels and testosterone concentrations in hypopituitary subjects from both study groups were subnormal (reference range for IGF-I: 15–35 nmol/l; testosterone: 12–30 nmol/l). In study 1, treatment with GH alone significantly increased IGF-I but did not alter plasma levels of testosterone, which remained in the hypogonadal range in all subjects (Table 2). Addition of testosterone to GH increased testosterone into the normal range in all subjects and resulted in a further uniform increase in plasma IGF-I levels (Table 2).

In study 2, treatment with testosterone alone increased testosterone into the normal range but did not alter mean plasma levels of IGF-I (Table 2). Compared with testosterone alone, treatment with GH plus testosterone did not result in any further change in plasma testosterone but increased IGF-I into the normal range (Table 2).

In summary, treatment with testosterone plus GH normalized plasma levels of testosterone and IGF-I, respectively, but testosterone increased IGF-I only during concomitant administration of GH.

Leucine turnover. In study 1, leucine Ra at baseline (147 ± 11 μmol/min) was not significantly different from that observed during GH treatment (148 ± 8 μmol/min) or combined treatment with testosterone (145 ± 11 μmol/min; Fig. 2). GH alone significantly reduced (P < 0.05) leucine oxidation from 41 ± 3 to 35 ± 2 μmol/min, and the addition of testosterone reduced leucine oxidation further (P < 0.05) to 27 ± 2 μmol/min (Fig. 2, left). When expressed as percent Ra, this corresponded to a fall in leucine oxidation from 25 ± 1% at baseline to 19 ± 1% with GH and to 17 ± 1% with combined treatment (Fig. 2, right). The absolute values for NOLD at baseline (129 ± 6 μmol/min), with testosterone (128 ± 6 μmol/min), and with combined treatment (135 ± 10 μmol/min) were not significantly different. However, when expressed as a proportion of Ra, NOLD increased significantly (P < 0.05) from 75 ± 1 to 80 ± 1% with testosterone, rising further to 83 ± 1% of Ra (P < 0.05) with combined treatment. These results did not differ when expressed in relation to body weight.

Resting EE and substrate metabolism. In study 1, GH alone induced an increase in resting EE, which narrowly failed to reach statistical significance (P = 0.07), but the addition of testosterone resulted in a cumulative increase that was significant (P < 0.05; Table 3) compared with baseline. GH alone increased fat oxidation, although the change did not reach statistical significance, whereas the addition of testosterone resulted in a further increase that was significant compared with GH alone and to baseline (P < 0.05).

In study 2, testosterone alone significantly increased REE (P < 0.05), whereas combined treatment with testosterone and GH induced a further rise in REE that was significant compared with baseline (P < 0.05). Testosterone alone significantly increased fat oxidation, whereas the addition of GH resulted in a further increase that was significant compared with GH alone and with baseline (P < 0.05). In summary, combined treatment induced the greatest changes in REE and fat oxidation, whereas the effects of GH plus testosterone alone were intermediate.

DISCUSSION

These two open-label, randomized crossover studies demonstrate that testosterone increases circulating IGF-I in hypopituitary men during GH treatment but has no effect on circulating IGF-I in the absence of GH. GH and testosterone independently and additively increased resting energy expen-

Table 2. Plasma levels of IGF-I and testosterone in hypogonadal GH-deficient subjects at baseline and after treatment with GH alone and GH with testosterone (study 1) and at baseline and after treatment with testosterone alone and GH with testosterone (study 2)

<table>
<thead>
<tr>
<th></th>
<th>Study 1</th>
<th></th>
<th>Study 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>GH</td>
<td>GH + testosterone</td>
<td>Baseline</td>
</tr>
<tr>
<td>IGF-I (nmol/l)</td>
<td>8.4±0.9</td>
<td>28.4±3.2*</td>
<td>30.1±2.9†</td>
<td>11.9±1.1</td>
</tr>
<tr>
<td>Testosterone (mmol/l)</td>
<td>2.6±0.6</td>
<td>1.8±0.7</td>
<td>12.2±1.9*</td>
<td>2.0±0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 vs. baseline. †P < 0.05 vs. baseline and vs. growth hormone (GH) only. ‡P < 0.05 vs. baseline and vs. testosterone only.
diture and fat oxidation. Neither hormone affected protein breakdown, but each exerted independent and additive effects in suppressing protein oxidation and in stimulating protein synthesis. These are the first data to demonstrate that testosterone and GH interact positively to regulate energy expenditure, fat metabolism, and protein anabolism. Taken together, the data provide a mechanistic explanation for recent observations that the effects of combined GH and testosterone supplementation on body composition and muscle strength in elderly men are greater than those of GH or testosterone alone (4, 8).

Studies in children have provided strong evidence for a positive interaction between GH and androgens in growth and development.

Table 3. REE, PRox, Frox, and CHox in hypogonadal GH-deficient subjects at baseline, following treatment with GH alone and GH with testosterone (study 1), and at baseline and after treatment with testosterone alone and GH with testosterone (study 2)

<table>
<thead>
<tr>
<th>Study 1</th>
<th>Study 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td><strong>GH</strong></td>
</tr>
<tr>
<td>REE, kcal/24 h</td>
<td>1558±91</td>
</tr>
<tr>
<td>PRox, mg/min</td>
<td>73.3±6.3</td>
</tr>
<tr>
<td>Frox, mg/min</td>
<td>62.0±6.0</td>
</tr>
<tr>
<td>CHox, mg/min</td>
<td>86.3±8.9</td>
</tr>
</tbody>
</table>

Values are means ± SE. REE, resting energy expenditure; PRox, protein oxidation; Frox, fat oxidation; CHox, carbohydrate oxidation. ∗P < 0.05 vs. baseline. †P < 0.05 vs. baseline and vs. GH only. ‡P < 0.05 vs. baseline and vs. testosterone only.
Testosterone stimulates growth of prepubertal and hypogonadal children (35). As it is well established that testosterone increases GH release (33), greater GH secretion is one mechanism through which growth is augmented. However, the observation that androgens also accelerate growth in hypopituitary boys receiving a constant GH dose strongly suggests that these effects are independent of GH secretion (2). There is also evidence that in addition to its effects on growth and anabolism, GH augments other biological effects of testosterone. The development of secondary sexual characteristics in hypopituitary boys is modest unless GH is administered concurrently (35). In hypopituitary men, Blok et al. (5) observed that androgen-dependent hair growth is increased by GH in hypopituitary men receiving stable androgen replacement, despite unchanged or even reduced androgen levels.

In the present study, plasma levels of IGF-I were greater during combined treatment with GH and testosterone than during treatment with GH alone, indicating that testosterone enhances GH-induced IGF-I production. Because the liver is an androgen-responsive organ and is also the major source of circulating IGF-I (20), it is likely that testosterone increased hepatic production of IGF-I by GH. To our knowledge, this is the first study to address the effect of testosterone on IGF-I levels in hypogonadal GH-deficient subjects during GH replacement. Testosterone does not change IGF-I in hypogonadal GH-deficient subjects who are not receiving GH replacement (18) but increases plasma IGF-I levels in normal subjects (13, 33). This effect is at least partly due to increased pituitary GH secretion and is dependent on aromatization to estrogen (33).

Interestingly, studies in which testosterone and GH have been administered together and in combination to healthy older subjects have shown no additional effect of combined testosterone and GH to increase IGF-I compared with GH alone (4, 8). Differences between these observations and those in the present study might reflect the difference between augmenting low-normal testosterone in healthy subjects and normalizing testosterone in profoundly hypogonadal subjects. Although plasma testosterone levels were at the lower end of the normal range at the time of the metabolic studies, in view of the known pharmacokinetic profile of intramuscular testosterone enanthate, it is likely that subjects were exposed to higher testosterone levels over the preceding 14 days.

The finding that testosterone alone exerted significant protein anabolic effects was surprising in view of the observation that the growth response of hypopituitary children to androgens is very poor unless GH is replaced (6). This observation suggests that the anabolic effects of androgens are dependent on the presence of GH. Testosterone alone also significantly stimulated resting energy expenditure and fat oxidation in our hypopituitary subjects. These findings have important clinical and physiological implications. The observation that the metabolic effects of GH are enhanced during concomitant testosterone administration explains why sex steroid-replaced hypopituitary men are more responsive to GH replacement than hypopituitary women (9). Testosterone is probably not the sole determinant of this effect, however, as there is also evidence that orally administered estrogen reduces the metabolic effects of GH (34). The observation that GH and testosterone exert additive effects on protein metabolism and fat oxidation implies that administration of testosterone or GH alone may not maximize these processes; thus, in hypopituitary men, treatment with GH or testosterone alone is unlikely to normalize body protein or fat mass. In addition to the clinical implications of this finding, there are also economic implications. GH replacement therapy is expensive, and the cost is directly dependent on the dose used. The findings of the current study indicate that the effect of GH to stimulate protein anabolism is approximately doubled when testosterone is coadministered, suggesting that optimizing of concurrent androgen replacement will reduce GH dose requirements, providing a significant cost saving.

The findings also have wider relevance in the context of normal human aging. The decline in endogenous GH and testosterone production rates with increasing age (19) might contribute to some of the effects of aging, including reduced muscle mass and increased body fat. However, studies in which testosterone or GH have been administered in isolation to elderly subjects have demonstrated little or no clinically significant effect. Because our findings show that the optimization of the effects of GH and testosterone requires administration of both hormones simultaneously, there is a rationale for future studies to address the effects GH and testosterone administered in combination as well as or instead of in isolation. Results from clinical trials of combined treatments are encouraging (4, 8).

In summary, testosterone replacement in hypopituitary adults increased circulating IGF-I only during concomitant administration of GH. Testosterone and GH exerted independent and additive effects to reduce irreversible oxidative protein loss and increase protein synthesis. These findings suggest that testosterone enhances the anabolic effects of GH through IGF-I but exerts protein anabolic effects that are independent of GH action. Concurrent administration of testosterone and GH in GHD subjects is likely to be both physiologically and economically important. Further studies are needed to delineate the molecular mechanisms by which these effects occur.

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