Enhancement of the Anabolic Effects of Growth Hormone and Insulin-like Growth Factor I by Use of Both Agents Simultaneously

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Abstract

The use of growth hormone (GH) as an anabolic agent is limited by its tendency to cause hyperglycemia and by its inability to reverse nitrogen wasting in some catabolic conditions. In a previous study comparing the anabolic actions of GH and IGF-I (insulin-like growth factor I), we observed that intravenous infusions of IGF-I (12 μg/kg ideal body wt [IBW]/h) attenuated nitrogen wasting to a degree comparable to GH given subcutaneously at a standard dose of 0.05 mg/kg IBW per d. IGF-I, however, had a tendency to cause hypoglycemia.

In the present study, we treated seven calorically restricted (20 kcal/kg IBW per d) normal volunteers with a combination of GH and IGF-I (using the same doses as in the previous study) and compared its effects on anabolism and carbohydrate metabolism to treatment with IGF-I alone. The GH/IGF-I combination caused significantly greater nitrogen retention (262±43 mmol/d, mean±SD) compared to IGF-I alone (108±29 mmol/d; $P < 0.001$). GH/IGF-I treatment resulted in substantial urinary potassium conservation (34±3 mmol/d, mean±SE; $P < 0.001$), suggesting that most protein accretion occurred in muscle and connective tissue. GH attenuated the hypoglycemia induced by IGF-I as indicated by fewer hypoglycemic episodes and higher capillary blood glucose concentrations on GH/IGF-I (4.3±1.0 mmol/liter, mean±SD) compared to IGF-I alone (3.8±0.8 mmol/liter; $P < 0.001$). IGF-I caused a marked decline in C-peptide (1,165±341 pmol/liter; mean±SD) compared to the GH/IGF-I combination (2,280±612 pmol/liter; $P < 0.001$), suggesting maintenance of normal carbohydrate metabolism with the latter regimen. GH/IGF-I produced higher serum IGF-I concentrations (1,854±708 μg/liter; mean±SD) compared to IGF-I only treatment (1,092±503 μg/liter; $P < 0.001$). This observation was associated with increased concentrations of IGF binding protein 3 and acid-labile subunit on GH/IGF-I treatment and decreased concentrations on IGF-I alone.

These results suggest that the combination of GH and IGF-I treatment is substantially more anabolic than either IGF-I or GH alone. GH/IGF-I treatment also attenuates the hypoglycemia caused by IGF-I alone. GH/IGF-I treatment could have important applications in diseases associated with catabolism. (J. Clin. Invest. 1993. 91:391–396.) Key words: growth hormone • insulin-like growth factor I • anabolism • insulin • glucose regulation

Introduction

Patients with acute or chronic illnesses are often in negative nitrogen balance despite adequate nutritional support (1–3). Growth hormone (GH)[1] can cause a partial reversal of nitrogen wasting in humans who are catabolic because of surgery (4), glucocorticoid treatment (5), chronic obstructive pulmonary disease (6), or dietary restriction (7, 8). However, GH has failed to improve nitrogen retention in some patients with sepsis (9) or burn injury (10). Furthermore, GH's tendency to cause insulin resistance (11) worsens the glucose intolerance associated with glucocorticoid therapy (5) or severe stress, such as that caused by extensive surface burns (10).

Insulin-like growth factor-I (IGF-I), a peptide that mediates the growth-promoting effects of GH (12, 13), has been shown to reverse the catabolic effects of caloric restriction in normal volunteers (14). In a study comparing the anabolic actions of GH with IGF-I (14), we observed that infusions of IGF-I (12 μg/kg per h) attenuate nitrogen wasting to a degree comparable to a standard treatment dose of GH (0.05 mg/kg per d). IGF-I infusion, however, had a tendency to cause hypoglycemia despite substantial suppression of insulin. In contrast, GH caused significant increases in both insulin and glucose concentrations, consistent with an increase in insulin resistance.

Based on these observations, we hypothesized that treatment with a combination of GH and IGF-I would ameliorate the hypoglycemia caused by IGF-I given alone, and that combined GH/IGF-I treatment would be more anabolic than either hormone given alone. Therefore, we treated calorically restricted normal volunteers with a combination of GH and IGF-I, and compared its effects to IGF-I alone.

Methods

Study design. The seven normal volunteers (four men and three women, ages 22–47 yr) who participated in this study were recruited by advertisement and were within 10% of ideal body weight (15). They were determined to be in good health by medical history, physical examination, and routine laboratory studies (complete blood count, electrolytes, urea nitrogen, creatinine, uric acid, total protein, albumin, total bilirubin, glucose, calcium, phosphorus, hepatic enzymes, lipid

1. *Abbreviations used in this paper:* ALS, acid-labile subunit of the 150-kD insulin-like growth factor binding protein complex; GH, growth hormone; IBW, ideal body weight; IGF-I, insulin-line growth factor I; IGFBP, IGF binding protein.

profile, and urinalysis). The study was approved by the University of North Carolina Institutional Committee for the Protection of the Rights of Human Subjects, and informed consent was obtained from each subject. The subjects were monitored on the General Clinical Research Center of the University of North Carolina (Chapel Hill, NC).

Subjects were permitted to continue most of their daily activities outside the hospital but were asked to refrain from vigorous exercise. During each of the two 2-wk diet periods, the subjects reported to the General Clinical Research Center daily for weighing, blood drawing, submission of 24-h urine collections, and consumption of meals. During weeks 1 and 2 of the study, each subject was fed a diet containing 20 kcal/kg IBW with 1 g protein/kg IBW. Nonprotein calories were provided as 50% carbohydrate and 50% lipid. The diet included 5 kcal/kg per d of carbohydrate in the form of juice divided into three aliquots and given at the hours of 0600, 2000, and 2400 each day. The nutrient content of the weighed food in each diet was determined from United States Department of Agriculture food tables (16). Dietary compliance was monitored by weighing the food left after each meal and by observing the constancy of weight loss and urinary nitrogen excretion.

During the last 4 d of week 1 and each day of week 2, 24-h urines were collected for urea nitrogen, creatinine, and potassium. During this same interval morning blood samples were collected before breakfast (0700 h) for glucose, urea nitrogen, electrolytes, creatinine, insulin, C-peptide, IGF-I, IGF binding proteins (IGFBP) 1, 2, and 3 and the acid-labile subunit (ALS) of the 150-kD IGF binding protein complex. On day 4 of week 2, blood samples were collected at 0700, 1600, 1800, 2000, and 2400 h to assess the pharmacokinetics of IGF-I. During week 2, subjects received one of two treatments: (a) daily intravenous infusions of recombinant human IGF-I (gift from Genentech, Inc., South San Francisco, CA) at a dose of 12 μg/kg IBW per h for 16 h between the hours of 1600 and 0800 for 5 d; or (b) combination of IGF-I (same conditions as above) and daily subcutaneous injections of recombinant human GH (Nutropin®, gift from Genentech, Inc.) at a dose of 0.05 mg/kg IBW that was given at 1600 h for 5 d. On the sixth day, the IGF-I infusion was given between 0800 and 2400 h, and the GH was administered at 0800. At times, the IGF-I infusion rate had to be reduced temporarily because of hypoglycemia (e.g., blood glucose < 55 mg/dl). After week 2, the subjects were fed a normal diet for 3 wk. During weeks 6 and 7, the diet restriction and treatment cycle that were carried out in weeks 1 and 2 were repeated using the alternate treatment. The sequence of the GH/IGF-I and IGF-I treatments was randomized among the subjects.

Measurements. Body weight was recorded daily. 24-h urinary excretion of creatinine, urea nitrogen, and potassium were measured by autoanalyzer (Hitachi 717; Boehringer Mannheim Diagnostic Lab Systems, Indianapolis, IN, and Synchron CX3; Beckman Instruments, Inc., Fullerton, CA). Urine collections for which the creatinine varied by > 15% from the mean for an individual subject were omitted from the analysis (2 of 154 collections were omitted). Daily nitrogen balance was calculated by subtracting the sum of the urinary urea nitrogen excretion and estimated stool and integument losses (245 mmol) from the daily nitrogen intake (16). The nitrogen content of food not eaten was subtracted from the total intake. Capillary blood glucose was measured during the IGF-I infusions at ~ 2-h intervals at the bedside (Chemstrip BG, Accucheck II; Boehringer-Mannheim). Serum potassium, glucose, urea nitrogen, and creatinine were measured by autoanalyzer (Synchron CX3; Beckman Instruments, Inc.).

IGF-I concentrations were measured by RIA after separating IGF-I from IGF binding proteins using octadecasilyl-silica cartridges (C-18 Sep-Paks; Waters Associates, Millipore Corp., Milford, MA) (17). The results were expressed as μg/liter after correcting for losses during extraction. The intra- and interassay coefficients of variation for this assay were 6.1%. Serum insulin was measured by RIA (18), and serum C-peptide was measured with a RIA kit (Incstar, Stillwater, MN). The lower limit of detection for the insulin assay is 18 pmol/liter, and for the C-peptide assay it is 92 pmol/liter. IGFBP-1 and 2 were assayed by disequilibrium RIA (19, 20). The lower limits of detection for the

IGFBP-1 and 2 assays are 2 and 0.2 ng/ml, respectively. The intra- and interassay coefficients of variation for IGFBP-1 were 9 and 15%, respectively. The intra- and interassay coefficients of variation for IGFBP-2 were 6 and 10%, respectively. IGFBP3 concentrations were measured by RIA using recombinant human IGFBP3 (gift from Genentech, Inc.) as a standard. The antiserum was prepared from rabbits immunized with human IGFBP3, as described previously for IGFBP1 (19). The antiserum bound 50% of the radiolabeled IGFBP3 at a 1:20,000 dilution. ^{125}I-IGFBP3 was prepared using a method described previously (19) and had a specific activity of 125 μCi/μg. The lower limit of detection is 1.0 ng/ml, and the intra- and interassay coefficients of variation were 8 and 13%, respectively. ALS concentrations were measured by RIA as previously described (21). The lower limit of detection is 0.5 μg/ml.

All values were expressed as the mean±SD, except for urinary potassium excretion, which was expressed as the mean±SE. The data were analyzed for statistical significance by the paired *t* test using the Bonferroni correction for repeated measurements. Unless otherwise stated, means and standard deviations in Results were calculated for the last 4 d of each diet and treatment week.

Results

The subjects lost 3.1±1.2 (mean±SD) and 2.7±0.6 kg during the week before IGF-I treatment and GH/IGF-I treatment, respectively (*P* = NS). The mean nitrogen balance was −139±48 mmol/d (mean±SD) during the IGF-I pretreatment week and −140±50 mmol/d during the GH/IGF-I pretreatment week (*P* = NS).

Anabolic effects. During the IGF-I treatment period, nitrogen balance improved from a pretreatment mean of −139±48 to −31±29 mmol/d during the last 4 d of treatment (*P* < 0.001; Fig. 1). GH/IGF-I treatment, however, induced entry into positive nitrogen balance, from a pretreatment mean of −140±50 to +122±43 mmol/d during the last 4 d of treatment
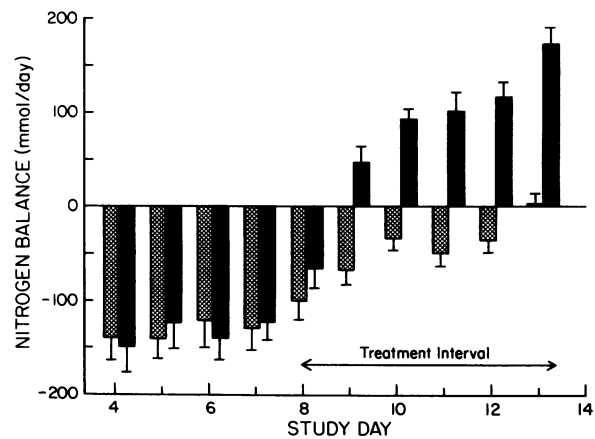

Figure 1. Changes in nitrogen balance in response to infusion of IGF-I (▨) or combined treatment with GH and IGF-I (■). IGF-I was given by continuous infusion (12 μg/kg IBW per h) for 16 h on days 8–12 between the hours of 1600 and 0800. On day 13, the infusion was given between 0800 and 2400 hours. GH was given by subcutaneous injection (0.05 mg/kg IBW) at 1600 h on days 8–12 and at 0800 on day 13. Daily nitrogen balance was calculated by subtracting the sum of the 24-h urinary urea nitrogen excretion and the estimate of stool and integument losses (245 mmol) from the daily nitrogen intake. The results are expressed as the mean±SD for seven subjects for the last 4 d of each diet and treatment week. The differences in nitrogen balance between treatment groups were significant (*P* < 0.01) on days 9–13.

($P < 0.001$). The improvement produced by the combination was significantly greater than that produced by IGF-I alone ($P < 0.001$).

The improvements in nitrogen balance were accompanied by decreases in serum urea nitrogen. From a pretreatment concentration of 5.54 ± 0.93 mmol/liter (mean±SD), IGF-I treatment decreased serum urea nitrogen to 2.35 ± 0.44 mmol/liter ($P < 0.001$). From a pretreatment mean of 5.63 ± 1.08 mmol/liter, GH/IGF-I treatment decreased serum urea nitrogen to 1.35 ± 0.33 mmol/liter ($P < 0.001$). The mean value during GH/IGF-I was significantly lower than that during IGF-I alone ($P < 0.001$).

In keeping with changes in nitrogen balance, the GH/IGF-I combination reduced urinary potassium excretion (pretreatment 67 ± 7 mmol/d vs. on treatment 33 ± 3 mmol/d, mean±SE; $P < 0.001$) (Fig. 2). With IGF-I alone, however, urinary potassium excretion did not change (pretreatment mean of 61 ± 5 mmol/day vs. on treatment mean of 61 ± 5 mmol/d). Serum potassium decreased on IGF-I alone, from a pretreatment concentration of 4.2 ± 0.2 (mean±SD) to 3.8 ± 0.3 mmol/liter ($P < 0.001$), and from 4.1 ± 0.2 to 3.6 ± 0.2 mmol/liter on GH/IGF-I ($P < 0.001$). The difference in serum potassium between the treatment groups was significant ($P = 0.002$).

Effects on IGF-I concentration. From a pretreatment concentration of 255 ± 94 μg/liter (mean±SD), serum IGF-I increased to $1,092\pm503$ μg/liter during IGF-I treatment ($P < 0.001$) (Fig. 3). GH/IGF-I treatment produced an even greater elevation in IGF-I concentration, from 273 ± 106 to $1,854\pm708$ μg/liter ($P < 0.001$). During the 8-h interval in which IGF-I was not infused (0800–1600) on day 11, serum IGF-I concentrations declined in both treatment groups ($1,114\pm483$ to 769 ± 633 μg/liter on IGF-I treatment and $1,841\pm637$ to $1,568\pm622$ μg/liter on GH/IGF-I treatment). When the IGF-I infusion was resumed at 1600 h, steady state IGF-I concentrations were achieved in both treatment groups within 4 h.
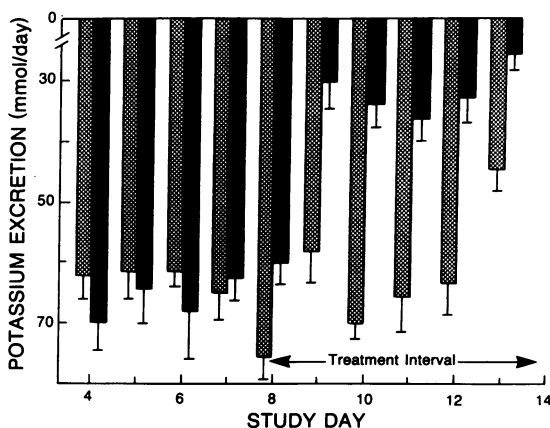

Figure 3. Changes in serum IGF-I concentration in response to GH/IGF-I (● — ●) or IGF-I (o - - - o) treatments. IGF-I and GH were administered as indicated in the legend in Fig. 1. Blood samples for measurement of IGF-I by RIA were collected at 0700, 1 h before the end of each IGF-I infusion on days 9–13 and 7 h after the end of the infusion on day 14. The results are expressed as the mean±SD for seven subjects. The differences in IGF-I concentrations between treatment groups were significant ($P < 0.01$) on days 11–14. Treatment interval (■).

Effects on carbohydrate metabolism. Capillary blood glucose concentrations measured every 2 h during IGF-I infusions were consistently higher on GH/IGF-I (4.3 ± 1.0 mmol/liter; mean±SD) compared to IGF-I alone (3.8 ± 0.8 mmol/liter) ($P < 0.001$; Fig. 4). Furthermore, 17.3% of values fell below 3.05 mmol/liter (55 mg/dl) during IGF-I treatment, whereas only 8.1% were low during GH/IGF-I treatment. Euglycemia was maintained better on the GH/IGF-I regimen compared to IGF-I treatment. This was apparent even though the IGF-I infusion had to be stopped or slowed several times during treatment with IGF-I alone, whereas the GH/IGF-I subjects continued to receive a constant rate of infusion. A total of five episodes of symptomatic hypoglycemia (tremulousness, light-headedness, diaphoresis, and headache) occurred in four subjects on IGF-I treatment compared to two episodes in two subjects on GH/IGF-I treatment. Both episodes on GH/IGF-I occurred during the first day of treatment. Morning (0700 h) serum glucose concentrations (1–2 h after 1.7 kcal/kg of carbohydrate) were also higher during GH/IGF-I treatment (5.7 ± 0.7 mmol/liter; mean±SD) than during IGF-I alone (4.3 ± 1.1 mmol/liter; $P < 0.001$).

Figure 2. Changes in urinary potassium excretion in response to infusion of IGF-I (▨) or combined treatment with GH and IGF-I (■). IGF-I and GH were administered as indicated in the legend in Fig. 1. Daily urinary potassium excretion was measured from 24-h urine collections. The results are expressed as the mean±SE for seven subjects for the last 4 d of each diet and treatment week. The differences in urinary potassium excretion between treatment groups were significant ($P < 0.01$) on days 9–13.
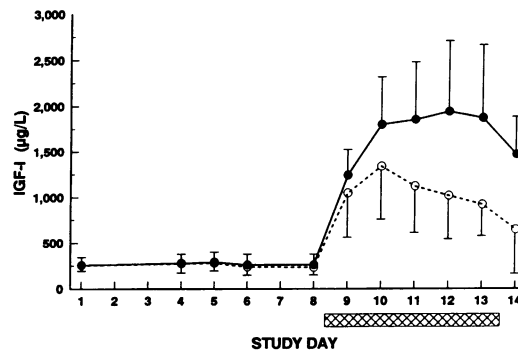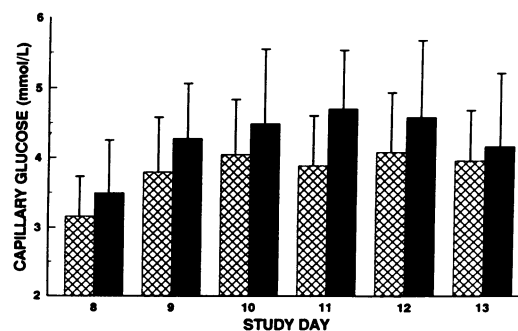

Figure 4. Effect of GH/IGF-I (■) or IGF-I (▨) treatments on capillary glucose. Capillary glucose was measured every 2 h during the IGF-I infusion by a reflectance meter (Accucheck II; Boehringer Mannheim). Each bar represents the mean±SD of 42 measurements. The differences in capillary glucoses between treatment groups were significant ($P < 0.01$) on days 9–12.
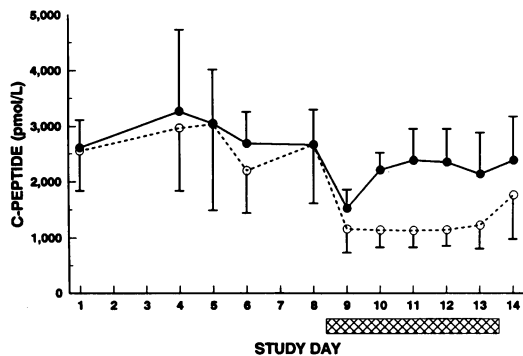
Figure 5. Effect of GH/IGF-I (● — ●) or IGF-I (o - - - o) treatments on C-peptide concentrations. Blood samples for C-peptide measurement by RIA were collected at 0700, 1 h before the end of each IGF-I infusion on days 9–13 and 7 h after the end of the infusion on day 14. The results are the mean±SD for seven subjects. The differences in C-peptide concentrations between treatment groups were significant on days 10–12. Treatment interval (▨).

Figure 6. Effect of GH/IGF-I or IGF-I treatments on (*A*) IGFBP3 and (*B*) ALS concentrations. Blood samples were obtained at 0700, 1 h before the end of each IGF-I infusion on days 9–13 and 7 h after the end of the infusion on day 14. IGFBP3 and ALS concentrations were determined by RIA. The results are expressed as the mean±SD for seven subjects for the last 3 d of each diet and treatment week. The differences in IGFBP3 and ALS between treatment groups were significant ($P < 0.01$) on days 11–14 and days 11–13, respectively. IGFBP3: GH/IGF-I (● — ●), IGF-I: (o - - - o); ALS: GH/IGF-I (■ — ■), IGF-I (□ - - - □); treatment interval (■).

While receiving IGF-I alone, the pretreatment morning insulin concentration decreased from 49.8±35.2 (mean±SD) to 16.6±12.7 pmol/liter ($P < 0.001$), and the pretreatment C-peptide concentration fell from 2,721±1,228 (mean±SD) to 1,165±341 pmol/liter ($P < 0.001$, Fig. 5). During GH/IGF-I, insulin decreased from a pretreatment mean of 44.9±26.8 to 33.4±16.7 pmol/liter ($P = 0.04$), and C-peptide declined from a pretreatment mean of 2,923±1,039 to 2,280±612 pmol/liter ($P = 0.02$; Fig. 5). The decreases in insulin and C-peptide concentrations were significantly greater during IGF-I treatment than during GH/IGF-I ($P < 0.001$ for C-peptide and $P = 0.001$ for insulin).

Effects on IGFBPs and the ALS of the 150-kD binding protein complex. GH/IGF-I increased IGFBP3 from 4.54±1.20 (mean±SD) to 6.40±1.70 μg/ml ($P < 0.01$), whereas IGF-I suppressed IGFBP3 from a pretreatment mean of 4.53±1.10 to 2.85±1.22 μg/ml ($P < 0.01$; Fig. 6 *A*). The difference in IGFBP3 concentration produced by each treatment was significant ($P < 0.001$). IGFBP2 concentration increased on IGF-I from 242±153 (mean±SD) to 497±268 ng/ml ($P = 0.002$), whereas GH/IGF-I treatment resulted in no change in the IGFBP2 concentration. IGF-I alone increased IGFBP1 from 68±20 (mean±SD) to 98±25 ng/ml ($P < 0.001$), whereas GH/IGF-I suppressed IGFBP1 (76±20 pretreatment to 65±8 ng/ml; $P = 0.02$). The difference between the two treatment groups was significant ($P < 0.001$).

IGF-I alone suppressed ALS from a pretreatment concentration of 33±12 (mean±SD) to 19±10 μg/ml ($P = 0.001$; Fig. 6 *B*), whereas GH/IGF-I maintained ALS concentration (32±10 μg/ml pretreatment to 40±13 μg/ml; $P = 0.07$). The difference in ALS concentration produced by each treatment was significant ($P < 0.001$).

Side effects. No significant adverse effects were observed during either the IGF-I or GH/IGF-I treatments. Six of the seven subjects reported discomfort over the area of the parotid glands during both treatments. Only one subject developed palpable enlargement of these glands, however, the serum amylase remained normal. Six subjects during GH/IGF-I treatment and four subjects during IGF-I treatment developed periorbital edema. One subject developed pitting edema in the extremities during GH/IGF-I treatment. During the treatment week, the
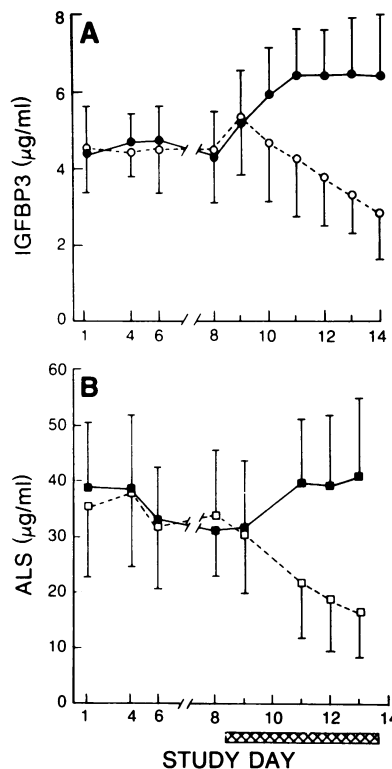
subjects gained 0.5±0.9 kg (mean±SD) on IGF-I alone and 2.1±1.7 kg on GH/IGF-I. The proportion of weight gain attributable to fluid retention or nitrogen accretion could not be determined because sodium balance was not analyzed. Most of the edema and all parotid tenderness resolved in every subject within 3 d after discontinuing treatment.

Discussion

Our results show that the combination of GH and IGF-I treatment in calorically restricted humans is substantially more anabolic than either GH or IGF-I alone. The nitrogen retention induced by the GH/IGF-I combination was 2.4-fold greater than IGF-I alone (56±9 vs. 23±7%). Compared to results from our previous study (14), in which the effects of GH and IGF-I were analyzed individually, the GH/IGF-I combination caused 2.1-fold greater nitrogen retention than GH alone (56±9 vs. 26±8%). We consider the present experiments comparable to our previous study (14) because subject characteristics, study design, and GH and IGF-I doses were identical to the present study, and the responses in the each of the two studies to IGF-I alone were nearly identical.

The mean increase in nitrogen retention of 260 mmol/d caused by GH/IGF-I treatment is greater than that achieved in any previous trial using GH alone in catabolic subjects. Ward et al. treated postoperative gastrointestinal patients with GH (0.1 mg/kg per d) for 6 d and observed a mean increase in nitrogen retention of 127 mmol/d compared to placebo (4). In patients with chronic obstructive pulmonary disease, GH (0.05 mg/kg per d) caused a mean increase in nitrogen retention of 158 mmol/d compared to control (6). When obese subjects were calorically restricted (12 kcal/kg IBW per d) and treated with GH (0.1 mg/kg IBW every other day) for 5 wk, the mean

nitrogen retention improved by 128 mmol/d compared to placebo (22). Marcus et al. treated normal elderly subjects with GH (0.06 mg/kg per d) for 7 d and observed a mean increase in nitrogen retention of 193 mmol/d (23). The magnitude of nitrogen conservation in each of these trials of GH falls short of that which we observed with GH plus IGF-I.

Although a GH dose-response was observed in the study in elderly subjects (23), it is doubtful that higher doses of GH would have produced increases in nitrogen retention in catabolic patients that were equal to that caused by GH plus IGF-I. In obese, diet-restricted subjects, doubling the standard GH treatment dose to 0.1 mg/kg IBW per d caused only a 10% improvement in nitrogen retention (22, 24). Manson and Wilmore (8) observed a mean increase in nitrogen retention of 179 mmol/d in calorically restricted normal volunteers given high dose GH (0.14 mg/kg per d). More recently, Zeigler et al. reported an increase of 201 mmol/d in subjects receiving a high dose of GH (0.18 mg/kg per d) along with hyperalimentation therapy (25).

During catabolism a significant portion of the nitrogen excreted is derived from increased turnover of visceral protein. Since 83% of the total body potassium is contained in muscle and connective tissue compartments (26, 27), measurement of potassium balance is considered to be a good index of protein accretion in these compartments. We observed a marked decline in urinary potassium excretion on GH/IGF-I treatment suggesting that most nitrogen accretion occurred in muscle and nonvisceral protein. The failure of potassium excretion to decline during treatment with IGF-I alone might be the result of a less pronounced anabolic effect of IGF-I on muscle and nonvisceral protein accretion or a direct enhancement of renal potassium secretion that counterbalanced a positive effect of IGF-I on potassium accretion.

In a previous study (28), IGF-I infusions of 32 μg/kg per h in healthy volunteers on a normal diet were reported to cause hypoglycemia. However, when the IGF-I dose was lowered to 20 μg/kg per h, hypoglycemia did not occur (28). In contrast, we previously showed that an IGF-I infusion of only 12 μg/kg per h in calorically restricted humans caused hypoglycemia despite the fact that insulin levels were suppressed three- to fourfold (14). These observations suggest that catabolic subjects, who may have decreased caloric intake or increased substrate utilization, might be more sensitive than normal subjects to the hypoglycemic effects of IGF-I. Addition of GH to the treatment regimen attenuated the hypoglycemia despite the fact that the GH/IGF-I combination produced higher serum IGF-I concentrations than those on IGF-I treatment alone. This protective effect of GH may have resulted from the higher IGFBP3 concentrations, sequestering IGF-I and preventing its rapid efflux from the circulation (29). Another explanation might be that GH's tendency to cause insulin resistance counteracts the hypoglycemic effect of IGF-I (11). Conversely, the hyperglycemia and hyperinsulinemia associated with GH treatment alone were prevented by the combination treatment, suggesting that IGF-I counteracts GH-induced glucose intolerance (14).

Several mechanisms might explain the greater anabolic effect of the GH/IGF-I combination. First, GH/IGF-I treatment caused markedly higher serum IGF-I concentrations than IGF-I alone, possibly producing higher concentrations of IGF-I in tissues. Second, the addition of GH to IGF-I treatment reversed the insulin suppressive effect of IGF-I. These

higher insulin concentrations could enhance the anabolic effect of IGF-I, since insulin inhibits proteolysis (30, 31). Third, GH and IGF-I may each affect protein balance by different mechanisms. For example, GH administered to normal volunteers causes an increase in protein synthesis but does not significantly inhibit proteolysis (5). In contrast, the predominant mechanism that mediates the response to IGF-I infusion appears to be inhibition of proteolysis (30). Fourth, the induction of IGFBP3 and ALS by the GH/IGF-I combination might account for a more stable IGF-I pool, which in turn might provide more anabolic effect. IGFBP3 is the major carrier protein of IGF-I in serum (32), and its association with IGF-I and ALS in a 150-kD ternary complex (33) increases the half-life of IGF-I by more than 50-fold (34). In humans and hypophysectomized rats, GH induces IGFBP3 and ALS (35–37). Formation of the ternary complex in response to GH is likely to have facilitated stabilization of the IGF-I pool in the GH/IGF-I treatment group. In contrast, infusion of only IGF-I lowered IGFBP-3 and ALS, and is thereby likely to have caused a decrease in stability of IGF-I in the circulation. This effect might have resulted from suppression of GH secretion by IGF-I, although GH was not measured in this study. Stabilization of IGF-I during GH/IGF-I treatment may have allowed peripheral tissues to be exposed continuously to higher concentrations of IGF-I.

Insulin and IGF-I have qualitatively similar metabolic and anabolic effects in that both decrease serum glucose and inhibit proteolysis (30, 38). Since the mechanism of IGF-I action is unknown, we cannot rule out the possibility that the anabolic effects of IGF-I observed in this study might be mediated by an insulin-like mechanism.

Although treatment with GH/IGF-I and IGF-I alone caused fluid retention and parotid tenderness, these side effects were transient and well-tolerated. The fluid retention can probably be explained by the fact that both GH and IGF-I decrease renal sodium excretion and increase water reabsorption (13, 23, 39, 40). Parotid tenderness with intravenous IGF-I administration was observed in our previous study (14) and has been described by other investigators (Lowry, S. F., personal communication), however the cause of this effect is unknown. The hypoglycemia associated with the GH/IGF-I combination occurred mostly on the first day of treatment and may have been prevented entirely by administration of GH before initiating the IGF-I infusion. This strategy would be based on the observation that C-peptide concentrations initially declined and then recovered on the second day of GH/IGF-I treatment, suggesting that the insulin resistance induced by GH was delayed by 24–48 h.

Our results in this diet restriction, catabolic model suggest that the combination of GH/IGF-I could have important applications in diseases associated with catabolism. Since a variety of illnesses lead to either suppression of appetite or reduced nutrient absorption, therapy with GH and IGF-I could shorten the convalescent phase. Patients with extensive surface burns or sepsis have been reported to be refractory to GH (9, 10). The combination of GH and IGF-I could potentially be more effective in such patients. Maintenance of normal glucose tolerance by the GH/IGF-I combination suggests that treatment with both of these agents might be safer than IGF-I alone in patients who are predisposed to hypoglycemia because of reduced caloric intake. Finally, the hyperglycemia that sometimes develops in response to GH administration may be ame-

liorated by the combination therapy since the IGF-I dose could be used to adjust blood glucose. The challenge for the future is to determine whether this combination has efficacy in disease states that are known to result in significant catabolism.

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