Human Growth Hormone Prevents the Protein Catabolic Side Effects of Prednisone in Humans

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Abstract

Prednisone treatment causes protein wasting and adds additional risks to a patient, whereas human growth hormone (hGH) treatment causes positive nitrogen balance. To determine whether concomitant administration of hGH prevents the protein catabolic effects of prednisone, four groups of eight healthy volunteers each were studied using isotope dilution and nitrogen balance techniques after 7 d of placebo, hGH alone (0.1 $mg \cdot kg^{-1} \cdot d^{-1}$), prednisone alone (0.8 $mg \cdot kg^{-1} \cdot d^{-1}$), or prednisone plus hGH ($n = 8$ in each group). Whether protein balance was calculated from the leucine kinetic data or nitrogen balance values, prednisone alone induced protein wasting ($P < 0.001$), whereas hGH alone resulted in positive ($P < 0.001$) protein balance, when compared to the placebo-treated subjects. When hGH was added to prednisone therapy, the glucocorticoid-induced protein catabolism was prevented. Using leucine kinetic data, negative protein balance during prednisone was due to increased ($P < 0.05$) proteolysis, whereas hGH had no effect on proteolysis and increased ($P < 0.01$) whole body protein synthesis. During combined treatment, estimates of proteolysis and protein synthesis were similar to those observed in the placebo treated control group. In conclusion, human growth hormone may have a distinct role in preventing the protein losses associated with the administration of pharmacologic doses of glucocorticosteroids in humans. (*J. Clin. Invest.* 1990. 86:265–272.) Key words: leucine • α-ketoisocaproate • radioactive tracers • stable isotopic tracers • insulin-like growth factor I

Introduction

Patients treated with glucocorticoids over prolonged periods of time have protein wasting, poor tissue healing, and an increased incidence of infections (1–5). The ongoing and, on occasion, severe losses of body protein (4) remain a major concern in the management of patients receiving glucocorticosteroid therapy. Identification of the factors leading to glucocorticosteroid-induced protein losses and of therapeutic modalities which might ameliorate (6–8) or prevent these losses may have major implications for the management of the large numbers of patients receiving long-term glucocorticoid therapy (e.g., allograft recipients, patients with autoimmune, dermatologic, or asthmatic diseases, etc.).

In the past, nitrogen balance measurements have been used to gain insights into protein metabolism; however, such measurements only allow estimates of net changes in whole body protein. In contrast, using isotope tracers of the essential amino acid leucine (which represents 8% of whole body proteins [9]) and isotope dilution methodology, rates of protein breakdown and protein synthesis can be measured in addition to estimates of protein balance (10).

In humans, short-term infusion of cortisol sufficient to increase plasma cortisol concentrations to the high physiologic range increases the plasma concentrations of amino acids, particularly those of the branched-chain amino acids (leucine, isoleucine, and valine) (11, 12). The rate of appearance of leucine in the postabsorptive state is increased, suggesting an increased rate of whole body proteolysis (12). In normal volunteers short-term high dose prednisone therapy (~ 60 mg/d) results in negative leucine balance in both the fasted and fed states when compared to the same subjects treated with placebo (13). These data provide clear evidence that even during short periods of exposure (5 d), high dose glucocorticoid therapy has deleterious effects on whole body protein metabolism.

Human growth hormone (hGH)[1] is a potent anabolic agent known to stimulate linear height in growth hormone deficient children (14) and protein synthesis as well as cell growth in vitro (15). In the past, the amount of hGH available from cadaver pituitary glands was insufficient to treat all children with known hGH deficiency and precluded exploration of its effects in a variety of disease states in adults. However, the commercial availability of recombinant DNA human growth hormone (rhGH) has made such studies possible. These studies have demonstrated improved nitrogen balance in hypercatabolic burn patients (16, 17), in normal volunteers during hypocaloric intravenous feeding (18), in calorically restricted obese volunteers (19, 20), in patients during parenteral nutrition (21), and in highly trained athletes (22).

Thus, the purpose of the present investigation was to determine whether concomitant daily injections of rhGH during short-term, high-dose administration of the glucocorticoid prednisone may reverse the protein catabolic state observed in subjects receiving prednisone alone.

Methods

Subjects

After review and approval of the protocol by the Mayo Institutional Review Board and the Clinical Research Center Advisory Committee,

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1. *Abbreviations used in this paper:* hGH, human growth hormone; IGF-I, insulin-like growth factor I; KIC, α-ketoisocaproate; LBM, lean body mass; NOLD, nonoxidative leucine disappearance; rhGH, recombinant DNA human growth hormone; SA, specific activity.

informed consent was obtained from 32 healthy adult volunteers who were between the ages of 18 to 36 yr and within 6% of their ideal body weight (see Table I). None had a family history of diabetes mellitus in first degree relatives, gastritis, peptic ulcer disease, or history of gastrointestinal bleeding. All had a normal 2-h postprandial plasma glucose concentration after a meal containing at least 100 g of glucose to exclude possible underlying carbohydrate intolerance. Only subjects with normal hematology, chemistry, urinalysis, and plasma thyroxine values were enrolled into the study. These laboratory values did not change as a result of drug or placebo therapy (see below).

Protocol

Subjects were randomized into one of four study groups (eight subjects per group) and received oral tablets and injections in a single blinded fashion under the supervision of the nursing staff of the Mayo CRC. Group I served as controls (placebo lactose tablets and saline injections). Group II (prednisone tablets and saline injections) received prednisone orally in three equal doses 15 min before breakfast, lunch, and dinner for 7 d at a dose of 0.8 $mg \cdot kg^{-1} \cdot d^{-1}$. Group III (placebo tablets and subcutaneous injections of rhGH, 0.1 $mg \cdot kg^{-1} \cdot d^{-1}$) received rhGH 15 min before dinner (daily alternation between both thighs) for 7 d before the study and for the 3 d of inpatient study. Group IV (prednisone tablets and rhGH injections) received prednisone and rhGH as described for groups II and III, respectively.

The subjects consumed only a prescribed diet of 35 $kcal \cdot kg^{-1} \cdot d^{-1}$ containing ~ 53%, 29%, and 18% carbohydrate, fat and protein, respectively, for 7 d in the Clinical Research Center. Each subject consumed identical daily meals at breakfast, lunch, and dinner, and was required to eat everything provided and not to eat off the Unit. Throughout the study, 24-h urine and stool collections were obtained to determine nitrogen balance and urinary creatinine excretion. In addition, on each of the days, plasma glucose and creatinine concentrations were determined.

Subsequently, all subjects were admitted to the Mayo Clinical Research Center in the afternoon of the seventh treatment day and were hospitalized for the next 3 d. At ~ 1700 hours on the day of admission, ^3H_2O (20 μCi) was administered orally to each subject and urine was collected before and after 2, 4, and 6 h to determine whole body water specific activity (SA) from which lean body mass (LBM) was calculated as previously described (23). At ~ 1730 hours on the afternoon of admission, an intravenous catheter was placed in an antecubital vein and a continuous infusion of prednisolone (0.8 $mg \cdot kg^{-1} \cdot d^{-1}$, groups II and IV) or 0.9% saline (Group I and III) was started and continued throughout the three inpatient study days.

Three different study protocols (A, B, and C) were carried out over the 3 d of hospitalization in each subject (see below). After completion of each study, subjects received their full daily diet in two meals.

Studies

Study A. Study A was designed to: (*a*) assess the effects of hGH and/or prednisone on whole body proteolysis (as estimated by the rate of leucine appearance using [1-^{14}C]leucine), leucine oxidation (corrected for individual CO_2 fixation using steady-state sodium [^{14}C]bicarbonate infusions), and whole body protein synthesis (which was calculated by subtracting leucine oxidation from the rate of appearance of leucine) in the postabsorptive state; (*b*) trace the systemic entry from the gastrointestinal tract and oxidation of leucine in the postabsorptive state by infusing [1-^{13}C]leucine via a constant enteral infusion; and (*c*) control for the constancy of absorption of a hydrogen labeled tracer in experiments A, B, and C by infusion [^{3}H]leucine via the enterally placed tube.

At 0330 hours the morning after admission, the exact body weight was obtained and another intravenous site was established in a contralateral hand vein for arterialized-venous blood sampling (24, 25). At 0400 hours, a primed, constant infusion of sodium [^{14}C]bicarbonate (5 μCi and 0.08 $\mu Ci \cdot kg^{-1} \cdot min^{-1}$) was initiated and continued for 3.5 h to estimate the fractional recovery of $^{14}CO_2$ in breath. After 0600 hours, breath samples (but no blood samples) were collected every 20 min. At ~ 0730 hours, a standard enteral feeding tube (8 French, 109 cm [Keofeed; IVAC, San Diego, CA]) was placed via the nose into the duodenum. A primed constant intravenous infusion of [1-^{14}C]leucine (group I, 9.3±0.4; group II, 10.2±1.1; group III, 14.9±1.1; and group IV, 10.6±1.4 × 10^3 $dpm \cdot kg\ LBM^{-1} \cdot min^{-1}$, respectively) was initiated at ~ 0800 hours and continued for 6 h. In addition, tracer amounts of [1-^{13}C]leucine (group I, 53±3; group II, 49±3; group III, 50±2; and group IV, 50±2 $nmol \cdot kg\ LBM^{-1} \cdot min^{-1}$, respectively) and [4,5-^{3}H]-leucine (group I, 17.9±2.5; group II, 18.2±2.9; group III, 24.9±1.8; and group IV, 16.3±2.5 × 10^3 $dpm \cdot kg\ LBM^{-1} \cdot min^{-1}$, respectively) were infused at a constant rate via the enteral tube over the 6 h of study. These latter isotopes were added to the enteral infusions to trace the systemic entry of the amino acids and to permit the partitioning of the circulating leucine into endogenous and exogenous components. The higher rates of [^{3}H] and [^{14}C]leucine were necessary because of the lower plasma concentrations of both leucine and KIC observed during rhGH treatment. In addition, 0.45% saline was infused at 160 ml/h using a Travenol infusion pump, a volume equal to that of the test meal infused in studies B and C (see below). After 4 h of isotope infusion, blood and breath samples were obtained at 20-min intervals for the remaining 2 h (see below).

Study B. Study B was designed to: (*a*) assess the effects of hGH and/or prednisone on whole body proteolysis (as estimated by the rate of leucine appearance using [1-^{14}C]leucine), leucine oxidation, and whole body protein synthesis in the fed state; (*b*) quantitate in the fed state (at isotope and substrate steady state) the systemic entry and oxidation of enterally infused leucine in a "meal" consisting of amino acids and glucose by infusing [1-^{13}C]leucine via a constant enteral infusion; and (*c*) quantitate the endogenous rate of appearance of leucine by subtracting the infusion rate of cold leucine from the total rate of appearance of leucine to the plasma space.

Study B was carried out in an identical fashion to that of study A except that (*a*) a solution containing glucose and mixed amino acids (Travasol; Clintec Nutrition, Deerfield, IL) was infused at a rate to deliver 5.3±0.1 $mg \cdot kg\ LBM^{-1} \cdot min^{-1}$ of glucose and 1.1±0.1 $\mu mol \cdot kg\ LBM^{-1} \cdot min^{-1}$ of leucine. A total of 10 kcal per kilogram of body weight was infused over the 6 h of study to simulate the fed state; and (*b*) the infusion rates of the radiolabeled isotopes used in study A (see above) were increased in groups receiving prednisone ([1-^{14}C]-leucine: group I, 8.9±0.3; group II, 14.5±1.1; group III, 15.9±1.3; and group IV, 14.6±0.8 × 10^3 $dpm \cdot kg\ LBM^{-1} \cdot min^{-1}$, respectively; [^{3}H]-leucine: group I, 14.4±0.7; group II, 22.5±2.3; group III, 26.5±2.3; and group IV, 26.2±2.8 × 10^3 $dpm \cdot kg\ LBM^{-1} \cdot min^{-1}$, respectively; [1-^{13}C]leucine: group I, 53±3; group II, 48±3; group III, 49±4; and group IV, 49±3 $nmol \cdot kg\ LBM^{-1} \cdot min^{-1}$, respectively).

Study C. Study C was designed to: (*a*) control for the production of $^{13}CO_2$ derived from the oxidation of the enterally absorbed glucose and thus avoiding an overestimation in the rate of leucine oxidation from the enterally absorbed leucine in study B. No [^{13}C] tracer was infused during this study; (*b*) correct leucine oxidation for the individual CO_2 recovery in the fed state steady-state by infusing sodium [^{14}C]bicarbonate throughout the study; and (*c*) assure that the subjects were studied under comparable conditions in studies A and B by infusing [^{3}H]leucine intravenously and [$^2H_3$]leucine via the enterally placed tube.

This study was carried out in an identical fashion to that of study B except that (*a*) no [^{14}C]leucine or [^{13}C]leucine was administered; (*b*) [4,5^{3}H]leucine was infused intravenously (group I, 10.2±0.5; group II, 10.9±0.8; group III, 12.5±0.7; and group IV, 13.3±0.8 × 10^3 $dpm \cdot kg\ LBM^{-1} \cdot min^{-1}$, respectively) and [5,5,5-$^2H_3$]leucine (group I, 41±2; group II, 38±3; group III, 34±2; and group IV, 40±4 $nmol \cdot kg\ LBM^{-1} \cdot min^{-1}$, respectively) was infused with the meal; and (*c*) sodium [^{14}C] bicarbonate was infused throughout the entire study period (i.e., for 10 h).

Study B and study C were carried out in random order.

Blood and breath sampling in studies A, B and C

Blood (17 ml) and breath samples were collected at each sampling time (0, 260, 280, 300, 320, 340, and 360 min) in all studies (A, B, and C). At time point 0, 240, and 360, an additional 6 ml of blood was drawn to determine the plasma concentrations of glucose (YSI glucose ana-

lyzer [Yellow Springs Instrument Co., Yellow Springs, OH]), insulin, hGH, and insulin-like growth factor I (IGF-I, kindly determined by Dr. R. Hintz) (26–29). Blood samples were placed on ice, centrifuged at 4°C, and the plasma stored at −70°C until analyzed.

Procedures

ISOTOPES

L-[1-^{13}C]leucine (Merck, Sharp, and Dohme, St. Louis, MO), L-[4,5-^{3}H]leucine (> 55 mCi/mmol; Amersham Corp., Arlington Heights, IL), L-[1-^{14}C]leucine (> 55 mCi/mmol; Amersham Corp.), sodium[^{14}C]bicarbonate and $^{3}H_2O$ were determined to be pyrogen free (limulus amebocyte lysate) and > 99% pure and sterile before use. [1-^{13}C]Leucine and [$^{2}H_3$]leucine were determined to be > 95% enriched by gas chromatography/mass spectrometry (5985B Hewlett-Packard GC/MS; Hewlett-Packard Co., Palo Alto, CA), and to be exclusively in the L-isomer form (30). Recombinant DNA hGH was supplied by Genentech Inc. (San Francisco, CA) whereas prednisone (Upjohn Co., Kalamazoo, MI) and prednisolone (Hydeltrasol®; Merck, Sharp, and Dohme) were obtained from commercial sources.

SUBSTRATE, HORMONE, AND ISOTOPE MEASUREMENT

Plasma leucine and α-ketoisocaproate (KIC). Plasma concentrations and ^{3}H and ^{14}C specific activity of KIC and leucine were determined by high performance liquid chromatography (HPLC) as previously described (31). The enrichments of plasma [1-^{13}C]leucine, [$^{2}H_3$]leucine, [1-^{13}C]KIC, and [$^{2}H_3$]KIC were determined by GC/MS using the *t*-butyldimethylsilyl derivative (32). Plasma and meal amino acid concentrations and stable leucine isotope infusate concentrations were determined by ion exchange chromatography (26).

Determinations in urine, stools, and diet. The 24-h urine collections were assayed for creatinine and nitrogen and 24-h stool collections and the diet (breakfast, lunch, and dinner meals combined) were analyzed for nitrogen using methods commercially available through the Department of Laboratory Medicine at the Mayo Clinic.

Determination of $^{14}CO_2$ and $^{13}CO_2$ in expired air. Expired rates of $^{14}CO_2$ were determined by aspirating 2-min collections of expired air through an ethanolamine solution (33). In addition, the specific activity of breath $^{14}CO_2$ was determined at each breath sampling time by aspirating expired air through hydroxide of hyamine (33). Total CO_2 production (mmol · kg^{-1} · min^{-1}) was calculated by dividing expired $^{14}CO_2$ (dpm · kg^{-1} · min^{-1}) by the $^{14}CO_2$ SA (dpm · mmol^{-1}) (33).

$^{13}CO_2$ enrichments were determined using an automated isotope ratio mass spectrometer (13, 34, 35). The expired rate of $^{13}CO_2$ was determined by multiplying the $^{13}CO_2$ enrichment by the total CO_2 production (nmol · kg^{-1} · min^{-1}).

Determination of radioactivity. The ^{3}H and ^{14}C radioactivity in KIC and leucine and ^{14}C radioactivity in CO_2 were determined by using a liquid scintillation counter (LS9800 series; Beckman Instruments, Inc., Palo Alto, CA) using dual counting mode, which corrects the radioactivity for both quench and the spillover of ^{14}C radioactivity into the ^{3}H energy spectrum.

Calculations

The actual rates of stable isotope infusion were determined as the product of the infusate stable isotope concentration, isotope enrichment, and the pump infusion rate. Rates of radiolabeled isotope administration were determined by multiplying the dpm · ml^{-1} of infusate by the infusion rate of the pump used (ml · min^{-1}). Estimates of whole body leucine metabolism were made at near substrate and isotopic steady state between 260 and 360 min after starting the leucine tracer infusions using a reciprocal pool model as previously described (36, 37). During meal infusion, endogenous rate of appearance of leucine was calculated by subtracting the rate of the enterally infused leucine from the total leucine carbon flux as determined using the ^{14}C data. The systemic availability of leucine was calculated by dividing the [^{14}C]leucine rate of appearance (R_a, tracer infused systemically) by the [^{13}C] leucine R_a (tracer infused enterally). The rate of leucine oxidation (μmol · kg LBM^{-1} · min^{-1}) was calculated as previously described (36) using the [^{14}C]KIC SA (or [^{13}C]KIC enrichment) and was corrected for the CO_2 recovery obtained in each individual during NaH$^{14}CO_3$ infusions in the postabsorptive (mean value of studies A, B, and C) and the fed state during the course of study protocol C (see above). $^{14}CO_2$ recovery was calculated by dividing the rate of $^{14}CO_2$ expired in breath by the NaH$^{14}CO_2$ infusion rate.

Nitrogen balance was calculated by subtracting the meal nitrogen intake from the stool and urine nitrogen losses over the last three outpatient days (days 5, 6, and 7). No corrections were made for skin or other insensible losses. Protein balance was calculated from the nitrogen balance by multiplying the nitrogen balance (g/24 h) by 6.25 (38). Protein balance was also calculated from the leucine kinetic data assuming that (*a*) the subjects were in the absorptive phase of digestion for 16 h/d (0700–2300 hours) and in the postabsorptive phase for 8 h (2300–0700 hours) and (*b*) 8% of all protein is leucine (9). Thus protein balance (g · kg LBM^{-1} · 24 h^{-1}) equals:

$$\frac{\text{Leu bal}_{\text{fast}} + 2 \times \text{Leu bal}_{\text{fed}}}{3} \times \frac{1440 \times 131.2}{0.08 \times 10^6},$$

where LBM is the lean body mass, Leu bal$_{fast}$ and Leu bal$_{fed}$ are the leucine balance data in μmol · kg LBM^{-1} · min^{-1} in the fasted and fed states, respectively, 1,440 represent min/d, 131.2 g/mole of leucine, 0.08 is the fraction of body protein which is leucine and 10^6 converts μmol to mol.

Statistics

Data are expressed as mean±SEM. Groups were compared using ANOVA, and statistical differences among the groups were established using a post hoc Neuman-Keuls test. Comparisons within groups were done using the *t* test for paired observations. Values were considered to be significantly different with *P* values less than 0.05.

Results

Subjects' characteristics and diet composition (Table I). Body weight did not change as a result of any of the treatments. Body weight, height, and lean body mass were not significantly different among the four groups of subjects studied. Caloric intake and diet composition were not different among the groups investigated.

Plasma glucose, insulin, rhGH, and IGF-I concentrations. The postabsorptive overnight fasting (14 h) plasma glucose concentrations overall averaged 95±2 mg/dl and were similar in all four groups before initiation of drug and/or placebo treatment. During the 2 h of blood sampling in study A (postabsorptive state, 22 h fasting), the plasma glucose concentrations were 77±3 and 77±4 mg% in the control and rhGH alone treated groups, respectively. In the prednisone and prednisone plus rhGH group, the plasma glucose concentrations (106±4

Table I. Characteristics of Study Population

	Control	Prednisone*	rhGH‡	Prednisone + rhGH*‡
Body weight (*kg*)				
pre§	66±3	73±4	70±4	70±3
post‖	65±3	73±3	71±4	72±4
Lean body weight‖ (*kg*)	57±3	62±4	61±5	65±3
Height (*cm*)	171±2	174±3	173±3	177±2

Values are given as mean±SEM.
* 0.8 mg prednisone per kilogram body weight.
‡ 0.1 mg human growth hormone per kilogram body weight.
§ Fasting body weight on the morning of the first treatment day.
‖ Fasting value on the morning of the first inpatient study day.

and 120±8 mg%, respectively) were higher ($P < 0.01$, ANOVA) than those of the other two treatment groups. Plasma glucose concentrations were increased ($P < 0.01$) in all four groups during infusion of the "test meal" on both study days B and C. No differences in plasma glucose concentrations during meal infusion were observed among the control, prednisone alone, and rhGH alone groups (113±1, 140±6, 124±3 mg%, respectively, ANOVA); however, during combined treatment with rhGH and prednisone, the plasma glucose concentrations were elevated (204±33 mg%, $P < 0.001$, ANOVA). Since study day B and C were not significantly different, only glucose and hormone data from study day B are provided.

In the postabsorptive state (study A), plasma insulin concentrations were similar (ANOVA) in the control, prednisone alone, and rhGH alone groups (34±8, 91±7, 52±6 pmol/liter, respectively), but elevated ($P < 0.01$) in the combined treatment group (237±57 pmol/liter). During meal infusion, plasma insulin concentrations were 226±44 pmol/liter in the control group but elevated ($P < 0.01$, ANOVA) in the other three groups of subjects when compared to the control group (667±72, 564±65, and 1,249±54 pmol/liter, prednisone, rhGH, and combined therapy, respectively). In addition, plasma concentrations of insulin were higher in the combined (rhGH and prednisone) treatment group during meal infusion, when compared to prednisone alone or rhGH alone treated subjects ($P < 0.01$).

In the postabsorptive state (study A), plasma hGH concentrations measured 20 h after the last injection were similar in the control, prednisone alone, and combined therapy groups (4.7±1.5, 2.3±1.1, and 3.1±1.2 ng/ml, respectively), whereas after rhGH treatment alone, the value was elevated (6.9±0.6 ng/ml, $P < 0.001$, ANOVA). During meal infusion (study B), similar values and statistical relationships were observed (2.5±0.8, 3.5±0.5, 5.7±0.7, and 4.9±1.0, respectively, placebo, prednisone alone, rhGH alone, and combined treatment groups; $P < 0.05$, ANOVA).

In the postabsorptive state (study A), plasma IGF-I concentrations were similar in the control and prednisone alone treated subjects (194±18 and 265±26 ng/ml, respectively). After rhGH treatment alone or in combination with prednisone, IGF-I concentrations were increased (677±82, 555±56 ng/ml, respectively, $P < 0.001$, ANOVA). In the fed state (study B) similar values and statistical relationships were observed (control: 204±19, prednisone alone: 309±25, rhGH alone: 622±76, rhGH and prednisone: 690±63 ng/ml, respectively).

Plasma leucine and KIC concentrations, SA, and enrichments and $^{14}CO_2$ recovery (Table II). In the postabsorptive state (study A) prednisone alone increased ($P < 0.001$) plasma KIC and leucine concentrations, whereas rhGH decreased ($P < 0.001$) plasma KIC concentrations when compared to the placebo-treated controls. In the fed state (study B), plasma KIC concentrations were increased ($P < 0.01$) in volunteers receiving combined prednisone and rhGH treatment when compared with those of the control subjects. With the exception of the group treated with combined rhGH and prednisone, KIC plasma concentrations decreased ($P < 0.001$) as a consequence of the test meal administration. After 4 h of isotope infusion, the plasma concentrations of leucine and KIC and their enrichments and SA were at steady-state, as were the expired breath rates of $^{13}CO_2$ and $^{14}CO_2$ excretion and total CO_2 production. The mean steady-state values for each study group (minutes 260 to 360) during study A, B, and C are depicted in Table II. In the postabsorptive state, $^{14}CO_2$ recovery in expired air during intravenous infusion of sodium[^{14}C]-bicarbonate was similar in the four groups of subjects (overall average: 70±2% of the infused radioactivity). As a consequence of meal infusion, the $^{14}CO_2$ recovery was increased ($P < 0.001$) in each group but, again, no differences were observed among the groups (overall average: 86±2%).

Leucine kinetics and plasma amino acid concentrations (Tables III and IV. Postabsorptive (study A). The rate of appearance of leucine into the plasma space was increased in subjects treated with prednisone alone in the postabsorptive

Table II. Infusion Rates of Leucine, Specific Radioactivities, Enrichments, and Plasma Concentrations of Leucine and KIC and $^{14}CO_2$ Values in Expired Air

	Control			Prednisone			rhGH			Prednisone + rhGH		
Study day	A	B	C	A	B	C	A	B	C	A	B	C
Concentrations												
Leucine (*μmol/liter*)	140±9	153±9	144±10	172±11‡	168±7	170±10	149±14	136±7	131±8	155±7	176±17	167±18
KIC (*μmol/liter*)	35±2	21±2§	20±2§	49±3*	27±1‡§	28±2‡§	25±3*	18±2§	17±2*§	40±3	34±4‡	33±3‡
Plasma specific activities (*dpm · nmol^{-1}*)												
[^{3}H]Leucine	8.7±1.0	6.6±0.4	5.0±0.4	9.2±1.3	9.7±1.3	5.3±0.5	12.3±1.3	10.5±0.9	7.6±1.7	8.3±1.4	10.8±1.3	6.0±0.4
[^{3}H]KIC	7.7±0.7	5.2±0.4	3.8±0.4	8.0±1.2	7.7±1.1	3.7±0.4	11.5±1.1	9.4±1.1	4.6±0.5	7.4±1.2	8.9±1.1	4.4±0.3
[^{14}C]Leucine	6.5±0.4	5.0±0.3	—	6.4±0.8	7.5±0.9	—	9.4±1.0	7.8±0.9	—	6.7±0.9	7.5±0.6	—
[^{14}C]KIC	4.8±0.3	3.5±0.3	—	4.7±0.7	5.4±0.7	—	7.2±0.7	5.7±0.7	—	5.0±0.6	5.4±0.4	—
Enrichments (*mole percent enrichment*)												
[^{13}C]Leucine	3.3±0.2	2.9±0.2	—	2.9±0.2	2.3±0.2	—	2.8±0.3	2.5±0.3	—	2.9±0.1	2.3±0.1	—
[^{13}C]KIC	3.0±0.2	2.4±0.2	—	2.6±0.2	1.9±0.2	—	2.5±0.2	2.1±0.2	—	2.6±0.1	1.9±0.1	—
[^{2}H$_3$]Leucine	—	—	1.9±0.1	—	—	1.6±0.2	—	—	1.7±0.2	—	—	1.7±0.1
[^{2}H$_3$]KIC	—	—	1.4±0.1	—	—	1.3±0.1	—	—	1.3±0.1	—	—	1.5±0.1
Breath												
$^{13}CO_2$‖	10.7±1.2	18.7±3.0	—	16.7±1.4	18.7±1.8	—	7.5±1.1	10.2±2.0	—	9.7±1.5	11.1±1.9	—
$^{14}CO_2$¶	14.3±1.3	21.1±1.4	—	19.8±2.9	37.2±4.3	—	12.2±1.9	26.4±3.4	—	14.4±1.7	33.5±2.7	—
CO_2 production**	152±6	175±6§	—	133±7	171±11§	—	152±10	167±6§	—	153±6	183±7§	—

Values are means±SEM. * $P < 0.001$ compared to controls by ANOVA; ‡ $P < 0.01$ compared to controls by ANOVA; § $P < 0.01$ compared to fasting values within each group. ‖ nmol · kg^{-1} · min^{-1}; ¶ 10^2 dpm · kg^{-1} · min^{-1}; **μmol · kg^{-1} · min^{-1}.

Table III. Effects of Treatment with Human Growth Hormone and/or Prednisone on Leucine Metabolism in Normal Healthy Volunteers (n = 8 in each group) in the Postabsorptive State

	Control	Prednisone*	rhGH‡	Prednisone + rhGH*‡
Leucine flux§	1.96±0.07	2.25±0.06‖	2.08±0.08	2.13±0.04
Leucine oxidation§	0.47±0.03	0.76±0.06¶	0.27±0.02¶	0.45±0.03
Nonoxidative leucine disappearance (NOLD)§	1.49±0.08	1.49±0.07	1.81±0.07**	1.68±0.06
Oxidation/NOLD	0.33±0.04	0.53±0.07¶	0.15±0.01¶	0.28±0.03
Systemic availability (%)	87±5	97±3	87±5	94±4

Values are given as mean±SEM and statistical differences among the groups were estimated using ANOVA. * 0.8 mg prednisone per kilogram body weight. ‡ 0.1 mg human growth hormone per kilogram body weight. § $\mu mol \cdot kg^{-1} \cdot min^{-1}$, using iv data. ‖ $P < 0.05$ of control subjects. ¶ $P < 0.01$ of all other groups. ** $P < 0.001$ of all other groups.

state ($P < 0.05$, ANOVA, Table III). Leucine oxidation, measured using the systemically infused tracer, was decreased ($P < 0.01$) in subjects receiving rhGH alone and was increased ($P < 0.01$) in subjects taking prednisone alone; in contrast, no difference was observed between placebo-treated subjects and volunteers treated with both prednisone and rhGH (Table III). In the postabsorptive state, the rate of leucine oxidation represents net leucine balance, i.e., the difference between the rates of leucine derived from proteolysis and leucine entering body protein (Fig. 1). Similar relationships and statistical differences were observed for the oxidation of the enterally infused [^{13}C]-leucine tracer (0.36±0.04, 0.67±0.07, 0.29±0.03, and 0.37±0.05 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in the placebo, prednisone, rhGH, and prednisone plus rhGH-treated groups, respectively). Nonoxidative leucine disappearance (NOLD), an indicator of whole body protein synthesis, was only increased in subjects treated with rhGH alone. The ratio of leucine oxidation to NOLD, an indicator of whole body leucine catabolism (39), was increased during prednisone treatment alone, decreased with rhGH therapy alone, but unchanged in subjects receiving both rhGH and prednisone when compared to placebo-treated subjects. The systemic availability of enterally infused [^{3}H]leucine and [^{13}C]leucine was 87% or greater in all groups, although slightly (but not significantly) higher in both groups treated with prednisone (Table III). For clarity of data presentation, the systemic availability are presented using only the [^{13}C]data (similar results were obtained using [^{3}H]leucine).

As expected from leucine kinetic data, plasma essential and nonessential amino acid concentrations were increased only in subjects treated with prednisone alone (Table IV).

During meal infusion (study B; Tables IV and V). Total and endogenous rates of appearance of leucine were not different among the groups during the meal infusion (Table V). However, endogenous leucine rate of appearance was decreased in all four groups investigated in the fed state, when compared to values obtained in the postabsorptive state ($P < 0.01$, Tables III and V). In all groups studied, leucine oxidation was greater ($P < 0.001$) during meal infusion than observed in the postabsorptive state ($P < 0.001$, Table V). Similar results were obtained using the enterally infused [^{13}C]leucine: 0.79±0.11, 1.04±0.12, 0.49±0.06, 0.62±0.10 $\mu mol \cdot kg\ LBM^{-1} \cdot min^{-1}$, placebo, prednisone alone, rhGH alone, and combined treatment groups, respectively). As was observed in the postabsorptive state, leucine oxidation was higher ($P < 0.05$) in subjects given prednisone alone and lower ($P < 0.05$) in those receiving rhGH alone when compared to control subjects. Subjects receiving rhGH and prednisone had values similar to those of the control group. When compared to the postabsorptive state, NOLD was greater ($P < 0.001$) during meal infusion in all groups of subjects investigated. Subjects given rhGH alone had greater ($P < 0.01$) whole body rates of leucine entering protein (NOLD) when compared with placebo-treated subjects. Again, in subjects receiving combined therapy, NOLD was not different from that of the controls. The mean systemic availability of enterally delivered leucine was > 92% (using either [^{3}H]leucine or [^{13}C]leucine as oral trace) during meal infusion and did not differ among the groups of subjects investigated (only values from study day B are depicted, Table V).

While plasma concentrations of essential and nonessential amino acids were not different among the four study groups in the fed state, they were higher during meal absorption when compared to the postabsorptive study period ($P < 0.001$, Table IV).

Leucine balance (NOLD minus endogenous leucine rate of appearance) during meal infusion was similar in controls and in subjects treated with both rhGH and prednisone (0.26±0.04 vs. 0.33±0.04 $\mu mol \cdot kg\ LBM^{-1} \cdot min^{-1}$) (Fig. 1). In contrast, volunteers receiving prednisone alone exhibited a leucine bal-

Table IV. Effects of Treatment with Human Growth Hormone and/or Prednisone on Amino Acid Concentrations in Normal Healthy Volunteers (n = 8 in each group) in the Postabsorptive State

		Control	Prednisone*	rhGH‡	Prednisone + rhGH*‡
				μmol/liter	
Essential amino acids	fast	601±36	737±31§	598±29	647±25
	fed	990±43	1100±45	987±46	958±48
Nonessential amino acids	fast	771±53	893±54§	718±63	778±30
	fed	1852±122	1921±119	2018±94	1691±80

Values are expressed as mean±SEM. * 0.8 mg prednisone per kilogram body weight. ‡ 0.1 mg human growth hormone per kilogram body weight. § $P < 0.05$ of controls by ANOVA.

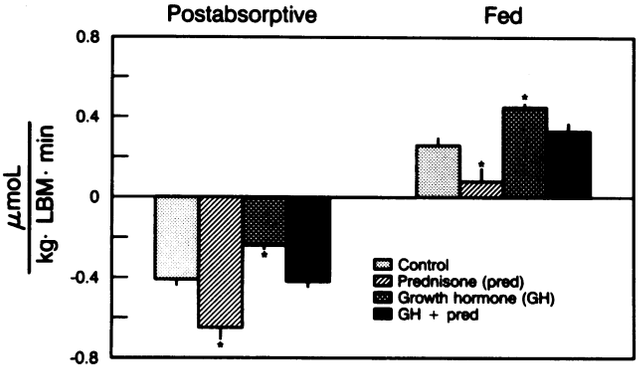

Figure 1. Leucine balance in the postabsorptive (*left side*) and fed state (*right side*) in the placebo treated subjects (control), subjects treated with prednisone (pred), recombinant DNA human growth hormone (GH), and combined treatment with rhGH and prednisone. *$P < 0.001$ when compared to placebo treated subjects by ANOVA.

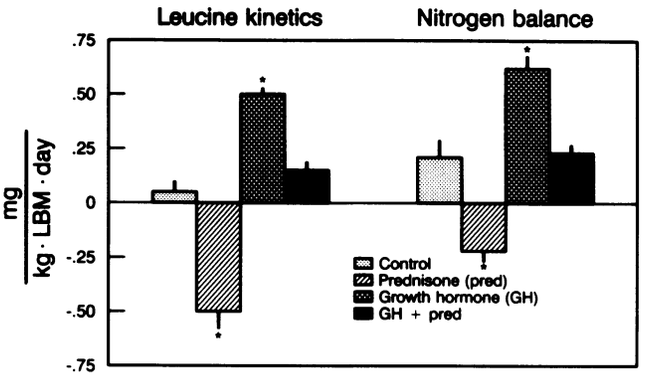

Figure 2. Protein balance calculated from leucine kinetic data (*left side*) and nitrogen balance (*right side*) in placebo treated subjects (control), patients treated with prednisone (pred), human growth hormone (GH), and combined treatment with human growth hormone and prednisone. Values are given as milligram of protein per kilogram LBM per day (see Methods for details). *$P < 0.001$ when compared to placebo treated subjects by ANOVA.

ance which was not significantly different from zero (0.09 ± 0.06 μmol · kg $LBM^{-1} \cdot min^{-1}$, $P < 0.001$ of controls and subjects treated with hGH and prednisone), whereas subjects receiving rhGH alone demonstrated a 70% higher (0.45 ± 0.02 μmol · kg $LBM^{-1} \cdot min^{-1}$) net leucine balance than placebo-treated control subjects ($P < 0.001$) and volunteers on combined treatment ($P < 0.001$) (Fig. 1).

Protein balance. Using nitrogen balance data, subjects receiving prednisone alone demonstrated negative protein balance ($P < 0.001$) when compared to both control and combined treatment subjects, whereas subjects on rhGH alone exhibited a positive protein balance, 2.5-fold greater ($P < 0.001$) than the controls. Subjects receiving both prednisone and rhGH treatment had a protein balance similar to that in the placebo-treated control subjects. The leucine kinetic data are not directly comparable with the nitrogen balance values, which reflect net protein balance over an entire 24-h period (i.e., both fasting and fed states). Extrapolating protein balance from the leucine kinetic data (see equation in Methods), similar patterns and significant differences were observed when compared with those derived from the nitrogen balance data (Fig. 2).

Discussion

The present studies using both traditional nitrogen balance and isotope dilution techniques demonstrate for the first time that the protein catabolic effects of pharmacologic doses of glucocorticosteroids can be prevented by the concomitant administration of rhGH in normal volunteers. Protein catabolism induced by the chronic administration of glucocorticosteroids is implicated in many, if not most, of the complications associated with use of these drugs. The conduct of these studies in normal healthy adult volunteers permitted us to assess the effects of prednisone and/or rhGH on whole body protein metabolism independent of any underlying disease and/or abnormal nutritional states. However, it would be inappropriate to assume that our conclusions can be extended to the wide variety of clinical conditions in which glucocorticoids are used for therapeutic purposes. Rather, the present studies provide reasonable justification for well-controlled prospective trials of rhGH in a number of selected clinical conditions in which the established therapeutic regimen is chronic glucocorticoid administration.

It has long been recognized that rhGH therapy leads to positive nitrogen balance and protein anabolism in both growth hormone deficient children (15, 40) and normal adult subjects (18, 22). Since nitrogen balance reflects only the net difference between protein synthesis and protein degradation, it has not been established if rhGH inhibits protein breakdown and/or increases protein synthesis in vivo. Using leucine isotope dilution methodology, the present studies demonstrate that 8 d of

Table V. Effects of Treatment with Human Growth Hormone and/or Prednisone on Leucine Metabolism in Normal Healthy Volunteers (n = 8 in each group) in the Fed State

	Control	Prednisone*	rhGH‡	Prednisone + rhGH*‡
Leucine rate of appearance§	2.61±0.14	2.86±0.07	2.82±0.10	2.77±0.08
Leucine infused orally§	1.11±0.04	1.10±0.03	1.07±0.03	1.07±0.02
Endogenous leucine rate of appearance§	1.50±0.10	1.75±0.07	1.75±0.10	1.70±0.09
Leucine oxidation§	0.85±0.07	1.02±0.06‖	0.62±0.05‖	0.73±0.03
Nonoxidative leucine disappearance (NOLD)§	1.81±0.10	1.86±0.07	2.20±0.06¶	2.03±0.06
Systemic availability (%)	93±5	94±3	96±3	92±5

Values are given as mean±SEM and statistical differences among the groups are estimated by ANOVA. * 0.8 mg prednisone per kilogram body weight. ‡ 0.1 mg human growth hormone per kilogram body weight. § $\mu mol \cdot kg^{-1} \cdot min^{-1}$, using iv data. ‖ $P < 0.05$ of control subjects. ¶ $P < 0.01$ of all other groups.

rhGH therapy result in increased protein synthesis (as indicated by the increment in the nonoxidative leucine disappearance rate), but does not decrease proteolysis (as indicated by the unchanged rate of leucine appearance) (Tables III and V).

The present data provide strong evidence that hGHs effects are mediated in vivo primarily by increasing whole body protein synthesis in both the postabsorptive and fed states. The mechanism(s) by which hGH increases whole body protein synthesis, whether in the presence or absence of glucocorticoid therapy, remains to be established. However, several possible mechanisms should be considered. (*a*) hGH and prednisone are known to cause insulin resistance with respect to carbohydrate metabolism. Therefore, the protein anabolic effects of rhGH observed in the present study could be mediated through the hyperinsulinemia observed in both the fed and postabsorptive states with combined therapy. However, previous studies (41, 42) have demonstrated that a primary effect of insulin on protein metabolism is to decrease proteolysis. In contrast, the administration of rhGH alone or in combination with prednisone in the present study did not affect the rate of appearance of leucine in either the fed or the postabsorptive state and would strongly suggest that the effect of rhGH is not mediated via insulin. (*b*) Increased plasma and tissue concentrations of both leucine and KIC have been demonstrated to decrease proteolysis and increase protein synthesis (43). However, the observation that increased protein synthesis induced by rhGH therapy occurred in the presence of normal or low concentrations of these two substrates would suggest that the effect of rhGH on protein synthesis is not leucine or KIC mediated. (*c*) The most likely mechanism is a direct effect of rhGH and/or IGF-I generated as a result of rhGH administration. Despite daily administration of rhGH, only the plasma concentrations of IGF-I were increased in a sustained fashion suggesting a more likely role for IGF-I in the observed effects of rhGH therapy. Were the effect of rhGH on protein metabolism primarily the result of IGF-I, it would raise questions about the physiologic relevance of recent studies demonstrating decreased proteolysis and protein synthesis as measured by leucine kinetics in rats infused with recombinant IGF-I (44). This question cannot be answered until sufficient amounts of IGF-I are available for clinical trials in humans.

At the present time, several potential drawbacks may exist when combined rhGH and prednisone administration is considered. (*a*) Using high doses of both rhGH and prednisone as used in the present studies, carbohydrate intolerance evolved within 8 d. This was an anticipated finding, since both hormones are known to cause insulin resistance (45). Whether reducing the daily dose of rhGH would preserve its anabolic effect but decrease its adverse effect on carbohydrate metabolism remains to be established. (*b*) rhGH has been shown in the present (data not shown), as well as in previous studies to increase glomerular filtration rates (46). Recent attention has been drawn to the progression of renal failure associated with glomerular hyperfiltration (47). Therefore, this potential long-term side effect must be considered in the design of any prospective trial using rhGH. (*c*) A known side effect of excessive hGH in adults is acromegaly and must be considered as a potential risk of therapy when long-term pharmacologic studies are entertained.

The present studies provide a rationale for the evaluation of rhGH in selective disease conditions in which short-term high dose glucocorticoids are used (lupus erythematosus, pemphigus, acute renal allograft rejection, etc.). However, the steroid dose used in the present study was nearly four times that usually prescribed to control a variety of underlying disease processes on a chronic basis. Therefore, it will be necessary to explore the effective dose of rhGH which may reverse the protein catabolic effects known to occur with chronic low dose glucocorticoid therapy (1–5) and to determine whether these effects are preserved over time (20). This presumed lower preventive dose of rhGH may decrease the risk of rhGH-associated side effects without compromising the beneficial effect on protein metabolism.

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