Low-Dose Growth Hormone and Hypocaloric Nutrition Attenuate the Protein-Catabolic Response After Major Operation

ZHU-MING JIANG, M.D., GUI-ZHEN HE, SI-YUAN ZHANG, M.D., XIU-RONG WANG, NAI-FAI YANG,
YU ZHU, M.D., and DOUGLAS W. WILMORE, M.D.*

To determine the effects of low-dose recombinant human growth hormone (GH) and hypocaloric nutrition on postoperative convalescence, we performed a placebo-controlled randomized double-blind trial in 18 patients after elective gastrectomy or colectomy. The subjects received parenteral nutrition containing 20 calories/kg per day and 1 g protein/kg per day. Daily injections of drug or placebo were given during the first postoperative week. The nine control subjects lost 3.3 kg (5.9% of preoperative weight) and had a cumulative nitrogen loss of 32.6 ± 4.2 g nitrogen at eight days. The patients receiving GH lost significantly less weight (1.3 kg) and nitrogen loss was 7.1 ± 3.1 g at eight days ($p < 0.001$). Kinetic studies demonstrated that the anabolic effects of GH were associated with increased protein synthesis, and amino acid flux studies across the forearm revealed increased uptake of amino acid nitrogen in the GH-treated patients. Body compositional analysis revealed that the patients receiving GH maintained their lean body mass despite the major surgical procedure. Analysis of hand grip force showed a 10% loss of strength in the control subjects; with GH the patients maintained their grip force throughout the postoperative period. We conclude that the postoperative catabolic response can be modified with GH and hypocaloric nutrition. These metabolic and physiologic effects should now be studied in a larger number of patients to determine if this approach can reduce morbidity, mortality, and length of hospital stay for surgical patients.

From the Department of Surgery, Peking Union Medical College Hospital, Beijing, China, and the Department of Surgery, Brigham and Women's Hospital, and the Harvard Medical School, Boston Massachusetts*

THE METABOLIC RESPONSE to a major elective intra-abdominal operation is characterized by weight loss and negative nitrogen balance. Postoperative patients increase the rate of urea production and excrete urinary nitrogen in excess of intake. The cumulative nitrogen loss is generally related to the patient's weight loss,[1] which averages about 6% of preoperative body weight over 2 weeks in the patient who has an uncomplicated postoperative course.[2]

The components of the weight loss are fat, protein, and body water.[2] While adipose tissue is available for metabolic energy, the loss of body protein is of concern because there is no "storage" form of body nitrogen and body protein represents structural and functional components of the body. Loss of body protein in the short term may be of little consequence to the healthy patient undergoing an elective operation, but in the more severely ill or metabolically or physiologically compromised individual the protein–catabolic state may increase susceptibility to infection, result in poor wound healing, and prolong convalescence. Because of the relationship between protein catabolism and outcome, a variety of techniques have been proposed to alter protein breakdown and these have included nutritional support,[3] thoracic epidural anesthesia,[4] and hypothermia.[5]

We have recently investigated the effects of hypocaloric feedings combined with the administration of recombinant human growth hormone (GH) on the catabolic response. Hypocaloric feedings are usually associated with negative nitrogen balance. However when only 800 to 1200 calories were given daily along with adequate quantities of protein and GH, positive nitrogen balance was achieved.[6,7,8] As part of our ongoing effort to evaluate this approach in catabolic patients, we studied the effects of GH and hypocaloric feedings in a group of individuals undergoing gastrectomy or colectomy. GH and hypocaloric feedings greatly attenuated the loss of body weight and negative nitrogen balance that has been believed to

---

Presented at the 109th Annual Meeting of the American Surgical Association, Colorado Springs, Colorado, April 10–12, 1989.

Correspondence and reprint requests to : Zhu-ming Jiang, M.D., c/o Douglas W. Wilmore, M.D., Department of Surgery, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115.

This work has been supported by a grant from the University Education Foundation, Contract 870060 with additional support from KabiVitrum Nutrition and Peptide Hormones, Stockholm, Sweden.

Accepted for publication: April 17, 1989.

be an indelible part of the postoperative catabolic response.

Materials and Methods

Patients

The patients were seen and evaluated in the general surgical clinic and the inpatient service of the Peking Union Medical College Hospital, Beijing, China. All individuals had a history and documented evidence of peptic ulcer disease, stable inflammatory bowel disease with stricture, or localized cancer requiring elective subtotal gastrectomy or hemicolectomy. In addition the patients satisfied the following eligibility criteria: (1) They were men between the ages of 25 and 65 years. (2) They had normal nutritional status as determined by body weight within 10% of ideal body weight, minimal recent weight loss, no evidence of nutritional deficiencies on physical examination, and normal nutritional status as determined by laboratory tests. (3) They had normal hepatic and renal function, determined by standard clinical and biochemical tests. (4) They exhibited no evidence of metastatic disease. (5) They showed no evidence of diabetes mellitus or other chronic disease and no long-term use of medications, including glucocorticoids.

The study protocol was approved by the Academic Committee of the Peking Union Medical College Hospital, which served as the institutional review board for the hospital. Written informed consent was obtained from all patients. Nineteen patients were entered into the study between December 1986 and December 1988.

Study Design

The subjects were admitted to the hospital at least four days before the date of their scheduled operative procedure. At this time they were taken to the operating theater and, using sterile technique, a triple lumen central venous catheter (Arrow, Arrow International, Reading, PA) was inserted on the right side *via* the percutaneous subclavian approach. On the morning (8:00 AM) of the third day before operation, blood was obtained for baseline studies and infusion of the parenteral nutrient solution was initiated (10:00 AM).

The nutrient solution contained 20 nonprotein calories/kg body weight per day with one half of the calories provided by carbohydrate and the remainder provided by fat emulsion (20% Intralipid, KabiVitrum, Sweden). Amino acids (Vamin-N, KabiVitrum, Sweden) were administered to provide 1 g protein/kg per day (150 mg nitrogen/kg per day). Vitamins (Vitalipid and Soluvit, KabiVitrum, Sweden) and trace elements (Addamel, KabiVitrum, Sweden) were added daily in fixed amounts to provide requirements; minerals were added daily in varying amounts to provide requirements and maintain normal blood levels. The carbohydrate, animo acid solution, vitamins, trace elements, and minerals were mixed in a 3-L bag in a laminar air flow hood. This mixture was infused at a constant continuous rate *via* the central lumen of the central venous catheter. The fat emulsion was infused separately over a 24-hour period *via* a second lumen. The total volume of the nutrient solution infused was approximately 2 L per day. Additional fluid volume (500 to 1000 mL) was administered by adding sterile water to the nutrient mixture to maintain adequate hydration. Constant nutrient infusion was maintained by using calibrated infusion pumps (Lifecare-3, Abbott, North Chicago, IL). No food or water was taken by mouth after this time until the eighth postoperative day.

The subjects were cared for in the Intensive Gastrointestinal Support Unit, which is a specialized two-bed area with dedicated nursing and pharmacy support to provide safe parenteral nutrition and collect and process all urine and other losses for analysis. Two days before operation body weight was measured and total body water was determined. Hand grip strength was also measured.

On the day of operation the patients received an epidural block to the T 6 to 9 level using Xylocaine (Astra, Sweden) and Dicaine (Beijing Pharmaceutical Co., Beijing, China) supplemented with an intravenous narcotic (meperidine) and an antihistamine (Phenergan; Wyeth Labs, Philadelphia, PA). The anesthesia was administered by a senior anesthesiologist and the operation was performed by the same team and same primary surgeon. One unit of blood was administered to each patient during the procedure. After the operation the epidural catheter was removed and the block was allowed to dissipate; narcotic was administered as required. The patient was moved back to the Unit and all urine and nasogastric losses were collected. The parenteral nutrition was administered at the same rate throughout the operative procedure. All patients received two days of perioperative prophylactic antibiotics.

On the first postoperative day the patients received an intramuscular injection of either recombinant human growth hormone (GH, Somotonorm, Kabi-peptide, Sweden) 0.15 IU/kg per day (0.06 mg/kg per day) or placebo. The patients were randomized to control or study groups by a senior laboratory staff member who drew a card from an envelope that indicated a code letter. The code letter was matched with a set of seven vials that were assigned to the patient. Each set of seven vials contained either GH or carrier substance, but the contents of the vials were unknown to the physicians, nurses, and laboratory staff. The injectate was prepared from a new vial of the set each morning and the injection was given at 7:00 AM throughout the seven postoperative days.

The nasogastric tube was removed on the second or

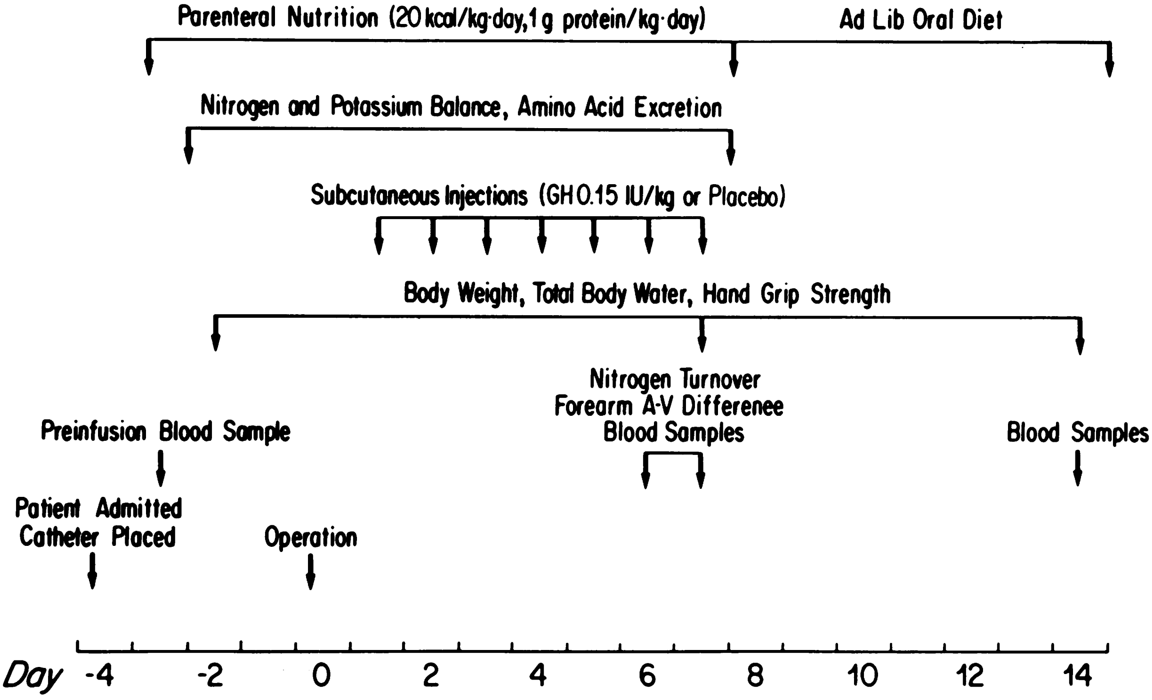

FIG 1. Outline of study plan.

third postoperative day but the patients were not allowed to eat until the eighth post operative day, at which time the parenteral nutrition was discontinued and *ad lib* food intake was initiated.

On the morning of the sixth postoperative day, an infusion containing N15 glycine was started (Institute of Chemical Industry of Shanghai, enrichment > 95% APE). A primed constant infusion was given through a separate lumen of the central venous catheter and continued for 48 hours. The priming dose was 0.04 mg N15/kg infused over 15 minutes followed by a constant infusion of 0.016 mg N15/kg per hour. The infused volume was approximately 10 mL/hour and the infusion was given by a volumetric pump (Imed 965 micro-volume pump, Imed Inc, San Diego, CA). On the seventh postoperative day, urine samples were collected every four hours for the next 24 hours and stored for subsequent analysis.

On the morning of the seventh postoperative day while the parenteral nutrients were infusing, amino acid exchange across the forearm was measured. First a catheter was inserted in a dorsal hand vein and the hand was warmed with a heating pad to arterialize the venous blood.[9] A catheter was also placed in the basilic vein of the contralateral arm in retrograde manner to obtain samples draining the forearm muscle bed. After at least 15 minutes of rest, two consecutive blood samples were simultaneously drawn from both catheters. The sampling catheters were removed after obtaining the second set of blood samples.

On the seventh and 14th postoperative days, blood was obtained for blood chemical determination, and body weight, total body water, and grip strength in both hands were measured using a hand dynamometer (Asimow Engineering Co., Los Angeles, CA). The protocol is summarized in Figure 1.

Sample Analysis

All urine and nasogastric tube losses were analyzed for nitrogen, potassium, sodium, creatinine, and 3-methylhistidine using previously described techniques.[10] The nitrogen content in each nutrient bag was also determined, and this value was multiplied by the infused volume to determine nitrogen intake. Blood chemical concentrations were measured using a standard hospital autoanalyzer (Encore Autoanalyzer, Baker Co., Allentown, PA). Plasma, whole blood, and urine were prepared as previously described[10] and amino acid concentrations were determined using a Beckman 119 CL amino acid analyzer using physiologic runs with internal standards. Blood hormone concentrations were analyzed using radioimmunoassay techniques.[7]

The N15 enrichment of urinary urea was determined using previously described methods.[7] The enrichment of urinary urea and ammonia was determined after distillation of the sample; analysis of the ammonia produced was measured using an isotope ratio mass spectrophotometer housed at the Atomic Energy Research Institute of Beijing (model MAT-251, Finnigan Corp., Cincinnati,

TABLE 1. *Characteristics of Patients and Some Admitting Laboratory Values (mean ± SEM)*

Characteristics/Values	Control	GH
n	9	9
Age (years)	54 ± 4	41 ± 4*
Weight (kg)	56.8 ± 2.1	58.6 ± 2.3
% predisease weight	96.3 ± 1.2	97.9 ± 2.3
Height (cm)	166 ± 2	169 ± 2
Diagnosis		
Duodenal orgastric ulcer	2	5
Gastric cancer	5	3
Colonic cancer	1	1
Crohn's disease	1	0
Blood pressure (mmHg)	119 ± 6	116 ± 4
	76 ± 2	77 ± 2
Hemoglobin (g/dL)	13.2 ± 0.8	13.9 ± 0.9
White blood count (cells/cmm)	8411 ± 801	7298 ± 548
Platelet count (#/cmm)	225,200 ± 34,500	203,300 ± 33,000
Lymphocyte count (cells/cmm)	2166 ± 252	2176 ± 206

* $p < 0.05$ when compared to control by nonpaired t test.

OH). Turnover, synthesis, and breakdown were determined by standard calculations.[11]

Total body water was determined by the intravenous administration of a known quantity (40mL) of deuterium oxide. After three hours of equilibration, plasma samples were obtained, the water purified, and the deuterium concentration measured by the falling drop method.[12,13]

Calculations and Statistical Analysis

Balance was calculated as the difference between intake and measured losses in the urine and from the nasogastric tube. Because the patients received intravenous nutrition, there was minimal stool loss and this was not included in the analysis.

The arterial-venous (A-V) difference of amino acids across the forearm was determined by subtraction of each value in the venous sample from its respective arterial sample set. The mean of the two sample sets was then taken as the A-V difference. Amino acids were summed to determine quantity of essential, nonessential, and branched-chain amino acids involved in the exchange. Total amino acid nitrogen was calculated by multiplying the value for each amino acid by the number of nitrogens in a respective amino acid and summing these values.

Total body water was determined using dilutional techniques.[14] Lean body mass and fat mass were determined from body weight and total body water using described methods.[14]

After 13 patients completed the study, the code that determined what the patients received was revealed by a physician not providing primary care. The data were analyzed and the decision made to continue the study to include approximately 20 patients or proceed until the end of December 1988. The code was revealed for all investigators in 1989 after all analysis had been performed.

Statistical calculations were performed on computer (IBM 1040 [Armonk, NY] and Macintosh S.E. [Apple Computers, Cupertino, CA]) using standard statistical software (Minitab, Copyright 1986, Pennsylvania State University and Statgraphics, State College, PA). Differences were tested by two-tailed unpaired t test or paired t test, when appropriate. Hand grip strength differences were compared using the Mann-Whitney test. The effect of treatment and time on grip strength was also assessed using multiple regression. Significance was accepted at the $p < 0.05$ level.

Results

Patient Groups

Individuals who met the study criteria were randomly selected for entry into the control and GH-treated groups. Nineteen patients satisfied this criteria and were entered into the study. One individual, subject 5 (control), developed bronchopneumonia on the first postoperative day and was dropped from the study on the morning of the second postoperative day. Thus 18 patients completed the study; nine were entered into the control arm and the remaining nine received GH. The patients were well matched in terms of weight, ideal weight and height (Table 1). There was an age difference, however, with the GH group being slightly but statistically younger than controls (41 ± 4 years *vs.* 54 ± 4 years, $p < 0.05$). Other clinical measurements and preoperative studies were comparable (also see Table 9 for other preoperative tests).

Operation and Postoperative Course

The operations were performed without complications in all patients using the same operative team and a senior anesthesiologist. Epidural anesthesia was used in all patients and the level of anesthesia did not rise higher than the sixth thoracic vertebrae. Supplemental intravenous anesthesia was also administered and the quantities used were comparable in the two groups (Table 2). There were 7 subtotal gastrectomies (> 70% of the stomach removed)

TABLE 2. *Description of the Operation in the Two Groups of Patients (mean ± SEM)*

	Control	GH
Duration (hours)	2.6 ± 0.1	2.6 ± 0.1
Blood loss (mL)	160 ± 40	170 ± 20
Duration of epidural (hours)	3.2 ± 0.1	3.1 ± 0.1
Intravenous anesthetics		
Meperidine (mg)	70 ± 10	70 ± 10
Phenergan (mg)	35 ± 6	50 ± 10
Blood transfusion (mL)	400 ± 0	400 ± 0
Postoperative narcotic		
Meperidine (mg)	150 ± 24	128 ± 25

TABLE 3. *Body Temperature, Nitrogen, and Potassium Balance and Creatine Excretion (mean ± SEM)*

Control	Preoperative Day	Day of Operation	1	2	3	4	5	6	7
Temperature (°C)	36.8 ± 0.1	36.9 ± 0.2	37.4 ± 0.2	37.5 ± 0.2	37.2 ± 0.1	37.0 ± 0.1	36.5 ± 0.1	36.4 ± 0.1	36.6 ± 0.1
Nitrogen intake (mg/kg·day)	155 ± 4	159 ± 2	156 ± 2	155 ± 3	160 ± 3	159 ± 2	159 ± 2	158 ± 2	159 ± 4
Nitrogen excretion (mg/kg·day)	161 ± 9	205 ± 11	258 ± 19	218 ± 11	236 ± 15	249 ± 25	245 ± 17	229 ± 20	216 ± 16
Nitrogen balance (mg/kg·day)	−6 ± 8	−46 ± 11	−102 ± 19	−63 ± 10	−75 ± 15	−90 ± 25	−86 ± 17	−70 ± 20	−57 ± 14
Potassium intake (mEq/day)	37 ± 8	22 ± 3	25 ± 4	31 ± 6	45 ± 7	47 ± 5	45 ± 7	44 ± 8	45 ± 6
Potassium excretion (mEq/day)	22 ± 4	46 ± 10	70 ± 23	33 ± 5	30 ± 6	30 ± 5	34 ± 4	39 ± 4	34 ± 4
Potassium balance (mEq/day)	15 ± 6	−25 ± 8	−44 ± 20	−2 ± 7	14 ± 11	17 ± 8	11 ± 6	4 ± 7	9 ± 5
Creatinine excretion (mg/kg·day)	21 ± 2	17 ± 1	19 ± 2	22 ± 2	25 ± 3	22 ± 2	18 ± 1	18 ± 1	20 ± 1
Growth Hormone									
Temperature (°C)	36.6 ± 0.1	37.1 ± 0.3	37.4 ± 0.1	37.5 ± 0.1	37.0 ± 0.2	36.7 ± 0.1	36.7 ± 0.1	36.8 ± 0.2	36.7 ± 0.2
Nitrogen intake (mg/kg·day)	154 ± 1	154 ± 1	150 ± 3	154 ± 2	155 ± 2	152 ± 2‡	154 ± 2	158 ± 2	158 ± 2
Nitrogen excretion (mg/kg·day)	156 ± 7	186 ± 26	226 ± 16	210 ± 18	159 ± 12†	156 ± 19†	150 ± 15‡	138 ± 9†	140 ± 5†
Nitrogen balance (mg/kg·day)	−2 ± 8	−32 ± 26	−76 ± 16	−56 ± 17	−4 ± 10†	−3 ± 19‡	4 ± 15†	19 ± 10†	17 ± 5‡
Potassium intake (mEq/day)	38 ± 7	31 ± 7	32 ± 5	44 ± 6	54 ± 8	49 ± 6	50 ± 7	51 ± 7	50 ± 6
Potassium excretion (mEq/day)	29 ± 6	59 ± 9	42 ± 5	27 ± 5	28 ± 6	33 ± 7	29 ± 6	34 ± 6	32 ± 6
Potassium balance (mEq/day)	9 ± 3	−28 ± 9	−10 ± 3	17 ± 4*	26 ± 6	16 ± 5	21 ± 5	17 ± 4	18 ± 5
Creatinine excretion (mg/kg·day)	20 ± 1	21 ± 2	24 ± 2	24 ± 2	21 ± 2	17 ± 2	19 ± 2	18 ± 2	21 ± 1

* $p < 0.05$, †$p < 0.01$, ‡$p < 0.001$ compared by nonpaired t test with controls.

and 2 hemicolectomies performed in the control subjects, and 8 subtotal gastrectomies and 1 hemicolectomy performed in the GH group. The operative time averaged 2.6 hours and the estimated blood loss was comparable in both groups. All subjects received 400 mL of whole blood during the operation.

The epidural anesthetic was allowed to dissipate shortly after the operation and the subjects received narcotics as indicated in the postoperative period. The dosage of meperidine administered was comparable in the two groups (Table 2).

There were no complications in the subjects who completed the study. Vital signs were comparable throughout the period of hospitalization and mean body temperature did not vary between groups (Table 3). All patients had nasogastric tubes inserted in the perioperative period and these were removed in all individuals between the second and third postoperative day.

Nutrient Intake, Excretion, and Balance

The subjects received their nutrient prescription without difficulty throughout the perioperative period, and there was comparable intravenous intake of fluid, calories, and nitrogen in both groups during the seven postoperative days (Table 3).

In the control group, nitrogen excretion exceeded intake on the day of operation and the succeeding seven postoperative days (Table 3), and the subjects were consistently in negative nitrogen balance (Fig. 2). In the patients receiving GH, negative nitrogen balance occurred on the day of operation and on the first two postoperative days. Thus this early metabolic response was similar to controls. On the third postoperative day, however, these subjects excreted significantly less urinary nitrogen then the control subjects. During the last four days of the balance study, nitrogen balance approached equilibrium and became positive toward the end of the first postoperative week. Potassium excretion tended to follow nitrogen balance, but balance became slightly positive in both groups earlier in the postoperative course (Fig. 2). Creatinine excretion was comparable in both groups of patients (Table 3).

Calculation of cumulative balance demonstrated differences in nitrogen balance between control and GH-treated groups despite comparable nitrogen intake (Table

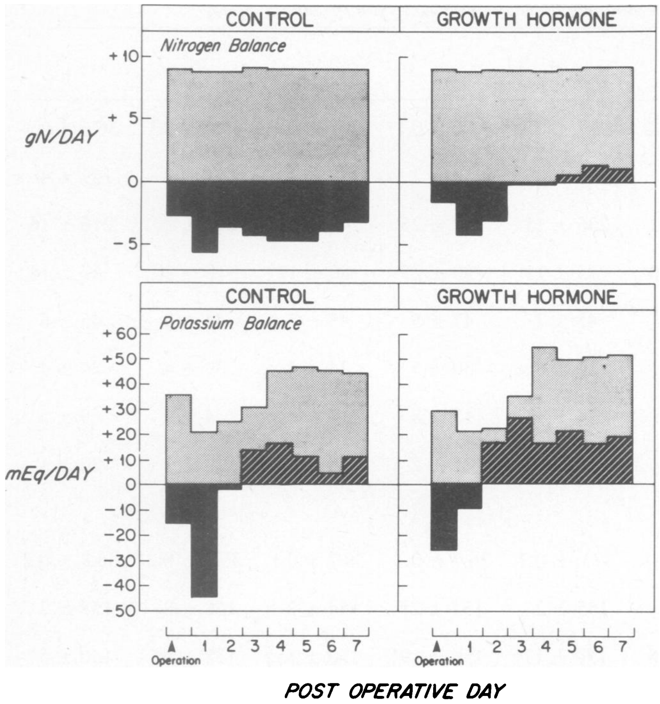

FIG. 2. Mean nitrogen and potassium balance for control and GH-treated patients. The upper line represents the quantity of the substances infused, while the black area shows negative balance and the shaded area shows positive balance.

4, Fig. 3). Potassium tended to follow this same pattern. Creatinine excretion, which reflects adequacy of urine collection, was comparable in both groups.

Nitrogen Kinetics and Amino Acid Studies

Changes in nitrogen balance can occur because of increased protein synthesis, decreased protein breakdown, or a combination of these two factors. Nitrogen turnover, calculated from the N15 enrichment of urinary urea nitrogen, tended to be greater in the GH-treated subjects (NS, Table 5).

However, the administration of GH was associated with a marked increase in nitrogen synthesis (2.19 ± 0.30 *vs.* 3.65 ± 0.47 g nitrogen/kg per day, $p < 0.05$). The rate of protein breakdown tended to increase in the GH subjects but this did not reach statistical significance.

Excretion of 3-methylhistidine, a marker of myofibrillar protein breakdown, rose from preoperative values of 4.4 ± 0.7 and 3.6 ± 0.3 μmol/kg per day in the control and GH subjects, respectively (NS), to 7.26 ± 1.01 and 4.4 ± 0.3 when assessed on postoperative day 7 ($p < 0.05$).

Before the initiation of parenteral nutrition, plasma vernous amino concentrations were in the normal range and there was no difference between groups. With amino acid infusion, these levels tended to rise slightly but not significantly (Table 6). The increase in plasma venous

TABLE 4. *Cumulative 8-Day Intake, Excretion, and Balance (mean ± SEM)*

	Control	GH
Nitrogen intake (g/8 days)	71.9 ± 2.8	72.3 ± 2.8
Nitrogen balance (g/8 days)	−32.6 ± 4.2	−7.1 ± 3.12†
Potassium intake (mEq/8 days)	303 ± 40	362 ± 42
Potassium balance (mEq/8 days)	−12 ± 37	+77 ± 22*
Creatinine excretion (g/8 days)	9.1 ± 0.5	9.7 ± 0.7

* p = 0.06, †p < 0.001 when compared to control by nonpaired t test.

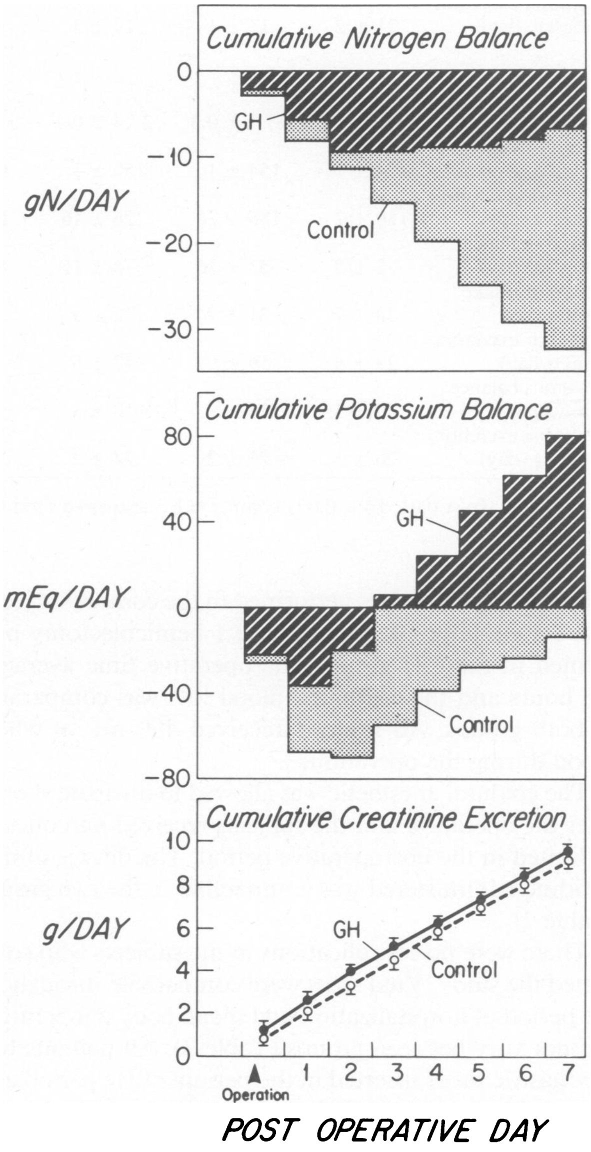

FIG. 3. The cumulative nitrogen balance was significantly different between groups from postoperative days 3 to 7. Potassium balance tended to follow nitrogen balance. Creatinine excretion was similar in both groups of patients, confirming the adequacy of urinary collection.

TABLE 5. *The Effect of GH on Nitrogen Balance and Nitrogen Kinetics Measured on Day 7 (mean ± SEM)*

	Control	GH
n	9	9
Nitrogen intake (g/kg • day)	0.16 ± 0.01	0.16 ± 0.01
Nitrogen excretion (g/kg • day)	0.22 ± 0.02	0.14 ± 0.01‡
Protein turnover (g/kg • day)	3.49 ± 0.32	4.55 ± 0.45*
Protein synthesis (g/kg • day)	2.19 ± 0.30	3.65 ± 0.47†
Protein breakdown (g/kg • day)	2.61 ± 0.30	3.55 ± 0.45*
3-methylhistidine excretion (μmoles/kg • day)	7.26 ± 1.01	4.44 ± 0.34†

* $p = 0.1$, †$p < 0.05$, ‡$p < 0.01$ when compared to control group.

amino acid concentration was similar in control and GH-treated subjects.

Forearm amino acid difference, measured on postoperative day 7 while the parenteral nutrients were being infused, was markedly different between groups (Fig. 4). There was continued release of amino acids from the forearm in the control subjects, while there was marked amino acid uptake in the GH-treated patients (A-V difference for total amino acid nitrogen was −398 ± 158 *vs.* +165 ± 61 μmoles/L, $p < 0.01$ with both values significantly different from zero). These alterations appeared to be due to selective changes in the release or uptake of specific amino acids, such as the branched-chain amino acids and glutamine (Table 6).

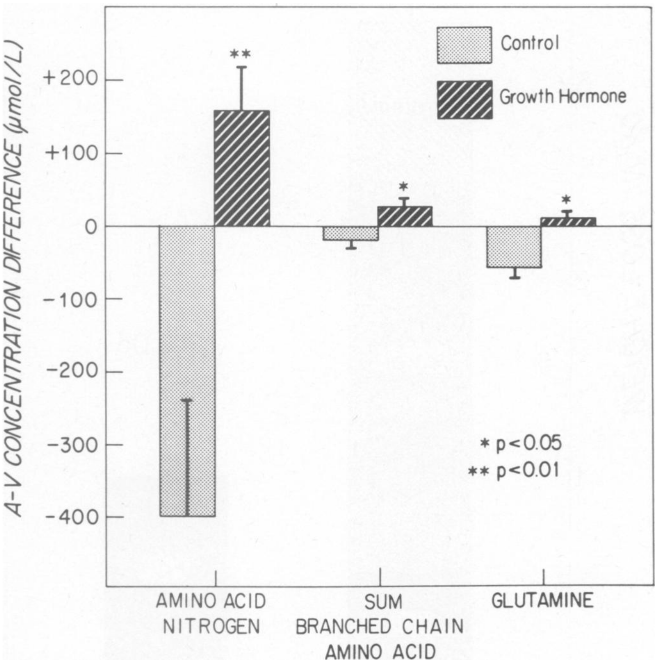

FIG. 4. The forearm of the control subjects was releasing amino acids, as shown by the negative A-V difference. In contrast, the forearm of the treated subjects' extracted amino acids. This in part could be explained by the difference between uptake and release of the branched-chain amino acids and glutamine.

Studies of Body Composition and Grip Strength

After operation, the control patients lost weight from 56.4 ± 1.9 kg to 53.2 ± 1.9 kg by the seventh postoperative day. In contrast the GH-treated patients lost weight from 58.6 ± 2.2 kg to 57.3 ± 2.3 kg (delta weight loss in controls 3.2 ± 0.3 kg *vs.* 1.3 ± 0.2 kg with GH, $p < 0.001$). Body weight tended to stabilize after the intravenous nutrition was discontinued on the morning of the eight postoperative day and *ad lib* dietary intake was started. Weight on the 14th postoperative day was 53.1 ± 2 kg for controls and 57.3 ± 2.1 kg for the study group ($p < 0.01$ for the weight change between groups when compared to preoperative values).

Total body water determinations were carried out in 15 of the 18 subjects. Before operation, total body water was approximately 35 L or roughly 60% of body weight (Table 7). While this is normal for the Chinese population,

TABLE 6. *Venous Plasma Amino Acid Concentrations Before and After Operation and Amino Acid Arterial-Venous Differences on Postoperative Day 7 (μmoles/L, mean ± SEM)*

	Control	GH
Before Infusion (3 days before operation)		
SUM essential amino acids	964 ± 82	915 ± 66
SUM BCAA	416 ± 44	415 ± 40
phenylalanine	70 ± 7	74 ± 9
SUM nonessential amino acids	2164 ± 221	1929 ± 55
alanine	351 ± 52	272 ± 26
glutamine	638 ± 56	572 ± 24
SUM amino acids nitrogen	4593 ± 432	4214 ± 161
Postoperative Day 7 (during IV infusion)		
Venous plasma concentration		
SUM essential amino acids	1046 ± 175	1125 ± 96
SUM BCAA	631 ± 55	514 ± 42
phenylalanine	105 ± 10	116 ± 13
SUM nonessential amino acids	2182 ± 140	1947 ± 107
alanine	242 ± 18	230 ± 20
glutamine	591 ± 57	528 ± 17
SUM amino acids nitrogen	4747 ± 320	4356 ± 268
A-V Difference		
SUM essential amino acids	−48 ± 40	+29 ± 27
SUM BCAA	−20 ± 16	+26 ± 12*
phenylalanine	−3 ± 6	+5 ± 5
SUM nonessential amino acids	−243 ± 77	+74 ± 50†
alanine	−24 ± 16	−3 ± 7
glutamine	−56 ± 22	+8 ± 11*
SUM amino acids nitrogen	−398 ± 158	+165 ± 61†

* $p < 0.05$, †$p < 0.01$ when compared to control by nonpaired t test. 0 = Essential amino acids contain: Val, Leu, Ile, Try, Phe Lys, Thr, and Met. 0 = Nonessential amino acids contain: Gln, Ala, Glu, Tyr, Glu, Pro, Ser, Arg, Orn, Asn, Asp, Tau, Cit, Cys, and His. − indicates release and + indicates uptake.

TABLE 7. *Changes in Body Weight and Body Water After Operation (mean ± SEM)*

	Control	GH
n	7	8
Body weight (kg)*		
preoperative	55.4 ± 1.9	57.6 ± 2.3
Day 7	52.3 ± 1.9	56.2 ± 2.3
Difference	−3.1 ± 0.4	−1.3 ± 0.2‡
Day 14	52.1 ± 2.1	56.6 ± 2.3
Difference	−3.3 ± 0.7	−1.0 ± 0.4‡
Total body water (L)		
preoperative	36.5 ± 1.4	33.7 ± 0.9
Day 7	34.1 ± 1.4	34.9 ± 0.9
Difference	−2.4 ± 0.7	+1.2 ± 0.3§
Day 14	34.5 ± 1.3	33.6 ± 0.8
Difference	−2.0 ± 1.1	−0.0 ± 0.5
Body water (mL/kg)		
preoperative	662 ± 29	589 ± 17
Day 7	657 ± 36	624 ± 15
Difference	−5 ± 17	+36 ± 6†
Day 14	666 ± 32	599 ± 16
Difference	+3 ± 17	+10 ± 6

* Listed only for those patients who had total body water measurements.

† $p < 0.05$, ‡$p < 0.01$, §$p < 0.001$ when compared to nonpaired t test with controls.

this degree of body hydration is greater than reported for individuals from Western societies. This difference reflects the increase in body fat found in Western individuals.

After operation, total body water decreased 2.4 L in the controls by the seventh postoperative day and tended to stabilize at this level throughout the next week. In contrast, the GH-treated patients maintained or increased their body water over the first postoperative week ($p < 0.001$ when compared to controls). With discontinuation of the hormone, total body water then tended to return to normal during the second postoperative week.

Calculation of body composition from this data uses standard equations that assume a normal degree of hydration of the lean body mass and do not account for expansion of the extracellular fluid compartment. Previous studies in normals and patients treated with GH show that some degree of fluid retention occurs with this treatment and that this cannot be totally accounted for by nitrogen retention.[8] Because this fluid is rapidly lost after termination of the therapy, the assumptions associated with the calculation of body composition are more appropriate for the 14th postoperative day when excess fluid has been excreted. These calculations demonstrate that the 6% loss in body weight that occurs in the control group is associated with a decrease in total body water of 2 L, a loss of lean body mass of 2.8 ± 1.46 kg (5.3% ± 2.9% of lean body mass), and a loss of 0.49 ± 1.30 kg (approximately 11%) of body fat (Table 7, Fig. 5). In contrast, the GH-treated patients lost 1.7% of body weight, essentially maintained body water and lean body mass (46 ± 1.3 kg before operation *vs.* 46 ± 1.1 kg on day 14), and lost 8.5% ± 0.4% (0.95 ± 0.46 kg) of body fat. These studies support the nitrogen balance measurements and amino acid flux studies that demonstrate that the lean body mass was preserved with this therapeutic approach. From the body compositional standpoint, the GH-treated patients had undergone a major elective operation and nearly maintained their preoperative body composition.

Hand grip strength was measured in 9 of the 18 subjects in an effort to access the functional response to this therapy. Hand grip strength fell with time in the control group (Table 8). In contrast the response in the GH-treated subjects was significantly different than controls ($p < 0.01$ for treatment effects using multiple regression analysis techniques); grip strength tended to increased with GH and was undistinguishable from the preoperative value with GH treatment at both postoperative days 7 and 14 (Fig. 6).

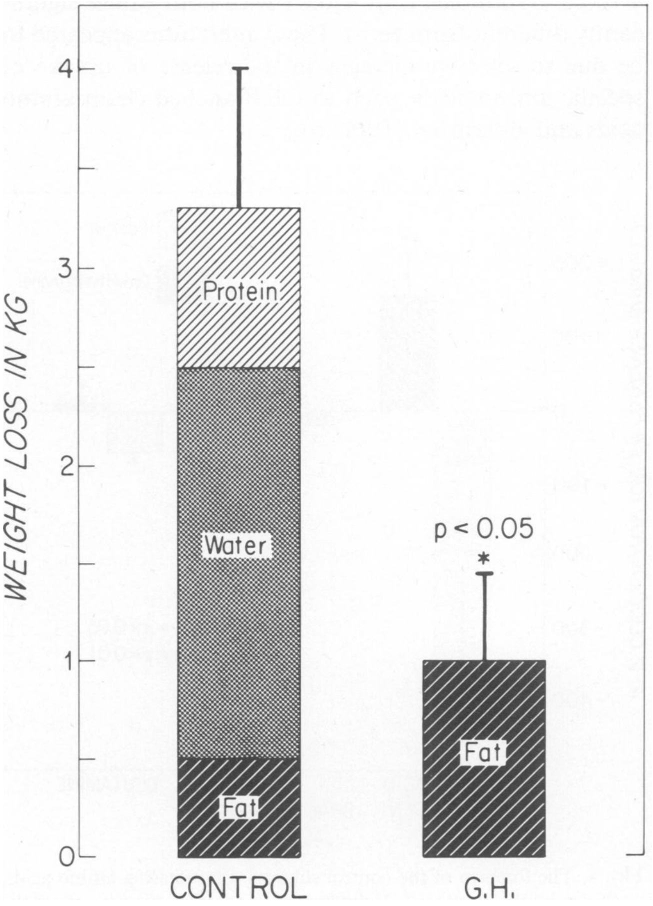

FIG. 5. The components of weight loss in controls and GH-treated patients following operation. The controls lost 490 g fat, 2 L water, and 800 g hydrated protein. In the contrast the GH-treated group only lost 950 g fat.

TABLE 8. *Hand Grip Strength Before and After Operation (kg force, mean ± SEM)*

Hand/Day	Control (n = 5)	GH (n = 4)
Right hand		
preoperative	38.2 ± 4.1	45.2 ± 5.2
Day 7	34.6 ± 4.6	46.3 ± 4.8
(change from preoperative)	−3.6 ± 2.2	+1.0 ± 0.8*
Day 14	33.8 ± 4.4	46.0 ± 5.2
(change from preoperative)	−4.4 ± 2.0	+0.8 ± 1.0
Left hand		
preoperative	38.0 ± 4.5	45.5 ± 4.7
Day 7	33.6 ± 4.8	46.8 ± 4.4
(change from preoperative)	−4.4 ± 1.9	+1.3 ± 1.26†
Day 14	33.6 ± 4.3	45.1 ± 4.4
(change from preoperative)	−4.4 ± 1.7	−0.4 ± 1.1

* Change from preoperative value (▲ response = postoperative value − preoperative value) significant difference than controls $p < 0.05$ by the Mann-Whitney Test.

† $p = 0.06$.

Blood and Hormone Values

Blood chemical values did not differ between groups when measured in the preoperative period. With operation there were moderate alterations in the liver transaminase level but there were no changes between groups. Cholesterol fell with intravenous therapy. Blood glucose levels were comparable in both groups of subjects. In contrast blood urea nitrogen level fell in the GH-treated patients (Table 9).

Serum insulin and glucagon were comparable in both groups of subjects at the time points measured. Growth hormone was elevated in the treated group and there was also a significant rise in insulin-like growth factor 1 (Table 10).

Discussion

Loss of body weight occurs after a major elective intra-abdominal surgical procedure. While the extent of weight loss varies depending on the age, sex, nutritional status, and postoperative nutritional support of the patient,[15] subjects closely monitored during their convalescence have lost approximately 6% of body weight by the 12th to 14th postoperative day.[2] This response is similar to that observed in our control subjects who lost 5.9% of preoperative weight by the end of the second week. The components of the weight loss were also similar to previous reported studies. Kinney[2] studied patients after similar operative procedures. The male subjects lost an average of 34 g nitrogen (range, 17 to 67 g) during the entire period of study, 267 g water per day, and 45 g fat per day. Our control subjects lost 32 g nitrogen in the first week, and averaged a loss of 143 g water per day and 35 g fat per day during the first 2 postoperative weeks. These compositional changes are within the error of the estimates

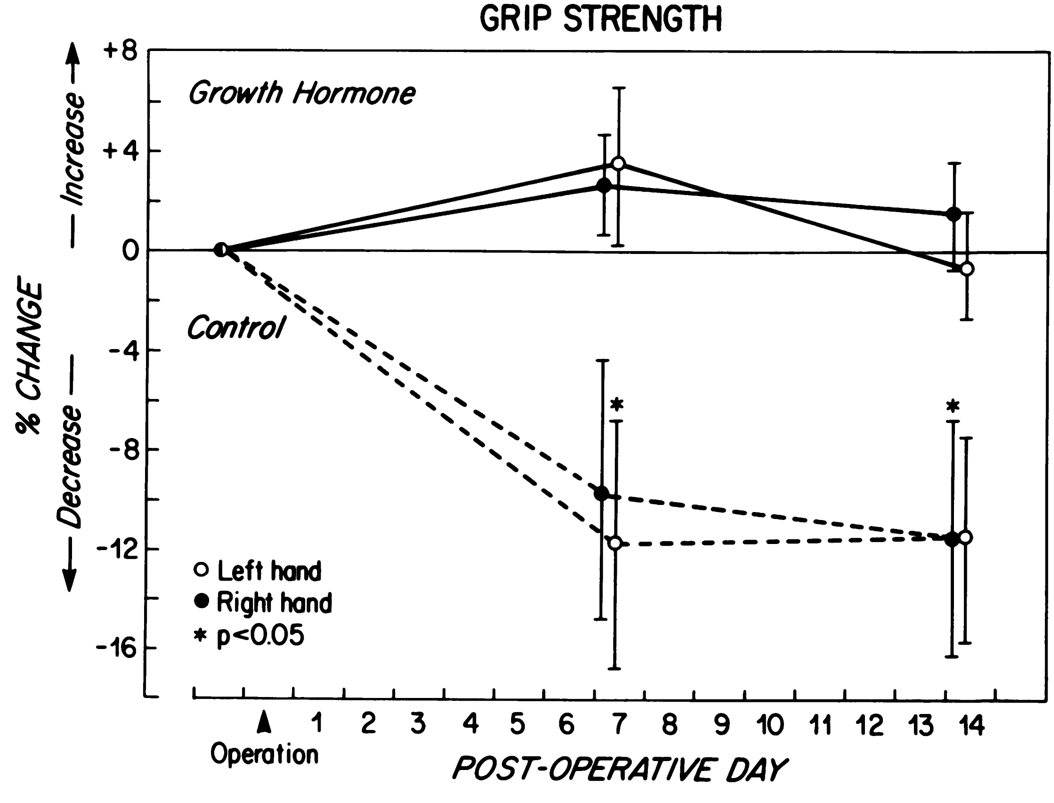

FIG. 6. After operation, the controls lost strength in both hands. However the GH-treated patients maintained grip strength.

TABLE 9. *Blood Chemical Values and Hormone Concentrations Before and After Operation (mean ± SEM)*

	Control			G.H.		
	Preoperative Day	7 Days Postoperative	14 Days Postoperative	Preoperative Day	7 Days Postoperative	14 Days Postoperative
Glucose (mg/dL)	93 ± 6	109 ± 9	95 ± 4	99 ± 6	111 ± 10	100 ± 9
Blood urea nitrogen (mg/dL)	14 ± 1	23 ± 2	18 ± 1	15 ± 1	15 ± 2*	14 ± 2
Creatinine (mg/dL)	0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.9 ± 0.1
Albumin (g/dL)	4.0 ± 0.1	3.7 ± 0.1	3.8 ± 0.1	3.9 ± 0.2	3.7 ± 0.2	3.9 ± 0.2
SGPT (IU/L)	22 ± 1	27 ± 5	23 ± 3	22 ± 1	27 ± 4	24 ± 3
Total bilirubin (mg/dL)	0.7 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.8 ± 0.2
Cholesterol (mg/dL)	191 ± 18	164 ± 20	169 ± 17	208 ± 15	173 ± 12	192 ± 13
Triglycerides (mg/dL)	105 ± 23	107 ± 17	94 ± 12	111 ± 10	109 ± 19	115 ± 17
Total amino acid nitrogen (μmoles/L)	4981 ± 441	5011 ± 490	5225 ± 547	4214 ± 161	4356 ± 268	4045 ± 116
Insulin (mIU/L)	22.2 ± 4.6	27.1 ± 7.7	14.9 ± 2.6	14.2 ± 2.7	26.0 ± 4.4	31.8 ± 8.0
Glucagon (μg/L)	60 ± 10	56 ± 13	54 ± 12	46 ± 6	58 ± 9	59 ± 9

* $p < 0.05$ when compared to control by unpaired t test.

determined by Kinney et al.[2] and demonstrates that the fairly stereotypic responses that occur after an elective operation have not greatly changed in the past 20 years.

In contrast the patients receiving GH and hypocaloric feedings preserved their lean body mass. This was demonstrated by the nitrogen balance studies in the first week; cumulative nitrogen balance in the GH patients was − 7.1 ± 3.1 by postoperative day 7, a value that was indistinguishable from zero. The body compositional measurements demonstrated an increase in the state of hydration on day 7, and this corresponded to a decrease in body weight of 1.3 kg. Previous studies have also demonstrated that a slight increase in fluid retention occurs with GH therapy.[6,8] However these same studies demonstrate that the patients undergo a brisk diuresis with discontinuation of the hormone: after 1 week the relationship between lean tissue and body water had returned to normal. Thus the studies performed of postoperative day 14 are more appropriate for the calculation of body composition using standard equations. These calculations confirm that body water and lean body mass are unchanged in these patients; the 1 kg weight loss that did occur was attributable to loss of body fat, not protein. This is the first demonstration that a hormonal manipulation can maintain the lean body mass after a major elective intra-abdominal procedure.

TABLE 10. *The Effects of GH Administration on GH and IGF-1 Concentration (mean ± SEM)*

	Control	GH
Growth Hormone (μg/L)		
preoperative	5.5 ± 2.4	4.2 ± 1.7
Day 7	3.2 ± 1.7	65.6 ± 15.1†
IGF-1 (μg/L)		
preoperative	211 ± 16	249 ± 19
Day 7	237 ± 33	463 ± 44*

* $p < 0.05$, †$p < 0.01$ when compared to controls by nonpaired t test.

The catabolic effects of an operation are probably most evident on skeletal muscle, which represents the largest single tissue compartment of body protein. That the net catabolism of body protein is increased in the control subjects after operation is evident by the negative nitrogen balance, loss of lean body mass, increased amino acid efflux from the extremity, accelerated excretion of 3-methylhistidine (a marker of myofibular protein breakdown), an increase in the rate of protein breakdown over synthesis, and a decrease in muscle strength. With GH these alterations were all significantly reversed.

One of the most dramatic changes observed occurred with the uptake of amino acid across the forearm. This alteration in A-V difference was so apparent that it was not necessary to calculate a flux rate (the multiplication of forearm blood flow times A-V difference). This occurred because the control group continually lost amino acid from the forearm muscle bed while GH reversed this gradient, resulting in a significant decrease in venous amino acid concentration when compared to arterial concentration. Uptake of amino acid across resting skeletal muscle has not been observed after a long-term hormonal manipulation and previous reports of amino acid exchange across the extremity during the infusion of parenteral nutrition have consistently demonstrated that the skeletal muscle continues to release amino acids in spite of "adequate" nutrition.[16,17]

The measurement of nitrogen kinetics support the concept that the effects observed are related to increased protein synthesis. This is similar to previous studies in normal individuals receiving GH.[7] Although there was a trend toward increased nitrogen turnover and breakdown with GH, these alterations did not achieve statistical significance (Table 5). However other investigators have reported changes in all components of nitrogen kinetics in postoperative patients receiving GH,[18] and it is probable that the rate of movement of amino acids through many metabolic pathways is increased, a state associated with the support of tissue growth and maintenance of tissue turnover.

The trend to increase the rate of protein breakdown with GH seems inconsistent with the 3-methyl histidine excretion data, which suggests that a diminished rate of breakdown was occurring in the myofibular protein pool, represented in large part by skeletal muscle. It is probable that the rate of protein synthesis is somehow linked to the rate of protein breakdown and it may be that augmentation of skeletal muscle synthesis in postoperative patients receiving GH may dampen the accelerated rate of muscle breakdown. However one would expect this response to be reflected in the isotopic kinetic data, which demonstrated that breakdown tended to increase, not decrease. There may be several possible explanations for these results. First these techniques may not be sensitive enough to simultaneously detect the same changes. Conversely these measurements may be quantitating turnover in different pools of protein; breakdown rate may be decreasing in skeletal muscle while turnover is accelerated in other tissues, particularly abdominal organs.

Of most importance, however, is the association between improvement in protein metabolism and the increase in muscle strength. The postoperative loss of muscle strength has been carefully studied and commonly occurs after all major operations.[19] The components of this response are complex and are related in part to nutritional factors and both cardiovascular and exercise deconditioning. Our control subjects lost grip strength in both arms after operation; in contrast GH-treated individuals increased or maintained grip strength. The response to therapy as shown by the lack of change in hand grip force has not been previously observed. The initial preoperative measurements demonstrated that the GH-treated subjects tended to have a greater grip strength then control subjects (NS). It could be argued that this might account for the absolute differences observed, although there is no physiologic basis to explain why a baseline shift might explain why one group would have diminished grip strength in the postoperative period and the other group maintained grip force. Subjective influences affect this measurement, but it is difficult to understand how this could influence the results in a placebo-controlled double-blind trial. Thus we conclude that these effects are the result of GH treatment and this response is a functional manifestation of the metabolic results that have been documented in this study.

The changes in hand grip force confirm our previous observations that patients receiving GH have increased strength and activity. This may be demonstrated by increased self care, ambulation, and independence. In more seriously ill patients this may be seen by increased respiratory muscle strength and the ability to cough, breathe deeply, and to be weaned from the ventilator. If this response can be confirmed in additional studies, this type of approach may have specific advantages in accelerating postoperative recovery and shortening surgical convalescence as we appreciate it today.

Finally the metabolic response to operative stress was markedly altered with minimal patient risk. The hypocaloric nutrients represent a nutrient formulation that contains 150 to 200 g glucose and this can be infused by peripheral vein. Hyperglycemia did not occur and unexpected alterations in electrolyte or amino acid concentrations were not observed. The dose of growth hormone used was approximately 9 IU/day (about 3.3 mg/day) or approximately one third of the dose we have administered to more catabolic patients. This lower dose may account for the reduced side effects, and use of such doses also reduces cost.

This study demonstrates the direct influence that the revolution in biotechnology has had on surgical care. GH was not previously available in large quantities because the only source was the human pituitary gland. Bioengineering developed a method of hormone manufacture and this agent is now readily available. In this study in postoperative patients, GH and hypocaloric feedings markedly attenuated the catabolic effects of a major surgical procedure. This was associated with maintenance of the lean body mass at preoperative levels, attenuation of urinary nitrogen loss, uptake of amino acids across the forearm, and increase or maintenance of skeletal muscle strength. Studies are now underway to determine if these metabolic and physiologic effects can be correlated with reduced morbidity and mortality and diminished hospital stay in surgical patients.

Acknowledgments

The authors wish to express their appreciation to Lars Ekman, M.D., Ph.D., Director of Research and Development, KabiVitrum Nutrition, AB, for his support throughout this study. We also thank Drs. Rolf Gunnarsson, Director of Research, and Gunnar Johansson, Vice Director of Research, KabiVitrum Peptide Hormones, AB for their kind support.

References

1. Mullholland J, Tui C, Wright A, et al. Nitrogen metabolism, caloric intake and weight loss in postoperative convalescence. Ann Surg 1943; 117:512-517.
2. Kinney JM, Long CL, Gump FE, et al. Tissue composition of weight loss in surgical patients 1. Elective operation. Ann Surg 1968; 68:459-474.
3. Holter AR, Fischer JE. The effect of perioperative hyperalimentation on complication in patient with carcinoma and weight loss. J Surg Res. 1977; 23:31-35.
4. Kehlet H, Brandt MR, Prange HA, et al. Effect of epidural analgesia on metabolic profiles during and after surgery. Br J Surg 1979; 66:543-548.
5. Johnson DJ, Brooks DC, Pressler V, et al. Hypothemic anesthesia attenuates postoperative proteolysis. Ann Surg 1986; 204:419-429.

6. Manson JM, Wilmore DW. Positive nitrogen balance with growth hormone and hypocaloric intravenous feeding. Surgery 1986; 100: 188-197.
7. Manson JM, Smith RJ, Wilmore DW. Growth hormone stimulates protein synthesis during hypocaloric parenteral nutrition. Ann Surg 1988; 208:136-142.
8. Ziegler TR, Young LS, Wilmore DW, et al. Metabolic effects of recombinant human growth hormone in patients receiving parenteral nutrition. Ann Surg 1988; 208:6-16.
9. Ambumrad, NN, Rabin D, Diamond MP, et al. Use of a heated superficial hand vein as an alternative site for the measurement of amino acid concentrations and the study of glucose and alanine kinetics in man. Metabolism 1981; 30:936-940.
10. Jiang ZM, Zhang FS, Wilmore DW, et al. Evaluation of parenteral nutrition in the postoperative patient. Surg Gynecol Obstet 1988; 166:115-120.
11. Picou D, Taylor-Roberts T. The Measurement of total protein synthesis and catabolism and nitrogen turnover in infants in different nutritional states and receiving different amount of dietary protein. Clin Sci 1969; 35:283-296.
12. Schloerb PR, Fris-Hasen BJ, Moore FD, et al. The measurement of deuterium oxide in body fluids by the falling drop method. J Lab & Clin Med 1951; 37:653-661.
13. Tseng HC, Jiang ZM, Fei LM. Total body water measurement by heavy water dilution. Chinese J Medicine 1965; 51:612-617 (in Chinese).
14. Moore FD, Olsen KH, Ball MR, et al. The body cell mass and its supporting environment body composition in health and disease. Philadelphia: WB Saunders, 1963. pp. 19-28.
15. Holden DW, Krieger H, Levey S, et al. The effect of nutrition on nitrogen metabolism in the surgical patient. Ann Surg 1957; 146: 563-570.
16. Clowes GHA, Heideman M, Linberg B, et al. Effects of parenteral alimentation on amino acid metabolism in septic patients. Surgery, 1980; 88:531-543.
17. Fong YM, Rosenbaum M, Lowry SF, et al. Influence of substrate background on peripheral tissue responses to growth hormone. J Surg Res 1988; 44:702-708.
18. Pointing GA, Halliday D, Sim AJW, et al. Postoperative positive nitrogen balance with intravenous hyponutrition and growth hormone. Lancet 1988 (Feb 27): 438-440.
19. Hunt DR, Rowland BJ, Johnston D. Hand grip strength—a simple prognostic indicator in surgical patients. JPEN 1985; 9:701-704.

DISCUSSION

DR. PAUL SCHLOERB (Kansas City, Kansas): This is another landmark article from the Wilmore group—this time from Beijing. The paper presents many questions. How little growth hormone can be given to achieve this effect? Do these amounts of calories, carbohydrate, and protein represent minimum necessary quantities? What is the role of exercise? Some of the kinetic data presented in the paper would suggest that exercise might indeed play a part and influence these kinetics.

How long was the blinded status maintained? As I understand it, there is a euphoric effect from giving growth hormone. I wonder if the patients themselves may have given the secret away.

What about the side effects? Is there a depressive effect when it is stopped? Is hypersensitivity to growth hormone used in these circumstances a real consideration?

These studies were done on elective operations. It is a tribute to the surgeon that there were no complications in either the control or the experimental groups. Of course the real bottom line is the treatment of patients with complications such as sepsis and fistula. Do you have any experience, albeit anecdotal, on the treatment of patients with complications?

Finally, I would emphasize that this work has very wide spread implications for the understanding and the further supportive care of our surgical patients.

DR. BASIL A. PRUITT, JR. (Fort Sam Houston, Texas): Dr. Jiang, Dr. Wilmore, and their colleagues have presented data suggesting that it is possible to modify and, in part, abrogate the postoperative catabolic response. Even more impressive is their finding that recombinant human growth hormone, even in a hypocaloric setting, can maintain muscle strength and perhaps accelerate convalescence.

There are some puzzling and perhaps paradoxical aspects of this randomized prospective study that I hope the authors will clarify to assist us in interpreting their findings.

Although the operations performed on the study patients appear to have been generally comparable, were the metabolic rates measured and, if so, were the metabolic rates of the two groups comparable?

It is puzzling that the preintervention growth hormone levels were higher in the older patients because it is well known that growth hormone levels decrease with age. Do the authors have an explanation for that finding?

The authors note that additional fluids were added to the standard daily nutritional volume to maintain adequate hydration and it appears in Table 7 that the difference in weight change between the control and treatment groups at seven days can be entirely explained by the 3.6-L difference in total body water favoring the growth hormone group. The observed difference in BUN levels is certainly consistent with that possibility. Even at day 14, the 2.3-kg weight difference seems to be consistent with the 2-L difference in total body water; I wonder if the intake and output records of these patients confirm or refute that possibility?

The authors note a temporal dissociation between potassium and nitrogen balance. Since those balances tend to move in parallel, how do the authors explain the observed dissociation? The greater positive potassium balance is offered as a beneficial effect of the growth hormone therapy, but it is of concern to note that the growth hormone group received 19% more potassium. That in itself may account for the observed difference in potassium balance.

The authors explain the finding of increased amino acid uptake in a setting of increased protein breakdown on the basis of a differential effect of growth hormone on viscera and carcass. In this regard the timing of the forearm amino acid exchange studies vis-a-vis the daily growth hormone injection is of concern because the rapid stimulating effects of growth hormone on amino acid transport and protein synthesis are expressions of the insulinlike action of growth hormone and not manifestations of its growth-promoting action. To eliminate such a transient time-related effect, were similar studies done at other times of the day to confirm that this was a manifestation of persistent growth activity?

The authors note that body composition changes indicate that the growth hormone patients lost predominantly body fat, and certainly lipolysis is a well-known effect of growth hormone. As corroboration for the importance of that effect, do you have data showing that those patients treated with growth hormone had increased circulating levels of glycerol and free fatty acids?

I again compliment these authors on an important study that suggests that it is possible to shorten hospital stay after major surgery.

DR. JAMES MACKENZIE (New Brunswick, New Jersey): Dr. Mannick, I, too was fascinated with all of the implications of the study. I have one question. Has this study been done by the authors or others with comparison of operative and nonoperative groups? In other words, with merely infusion of the growth hormone or a placebo?

DR. STANLEY R. FRIESEN (Kansas City, Kansas): I, too, congratulate Dr. Wilmore, a former Kansas student, on this beautiful clinical investigation.

I would like to ask one question. What would he expect if he had substituted insulin or androgens as other anabolic products for growth hormone in this study?