Recombinant human growth hormone improves muscle amino acid uptake and whole-body protein metabolism in chronic hemodialysis patients[1–3]

Lara B Pupim, Paul J Flakoll, Chang Yu, and T Alp Ikizler

ABSTRACT

Background: Intradialytic parenteral nutrition (IDPN), with or without exercise, has been shown to reverse the net negative whole-body and forearm muscle protein balances observed during hemodialysis. Pharmacologic doses of recombinant human growth hormone (rhGH) constitute another potential anabolic therapy in chronic hemodialysis patients.

Objective: Our goal was to examine the potential additive anabolic effects of rhGH compared with IDPN and exercise on protein and energy homeostasis.

Design: We studied 7 chronic hemodialysis patients in a crossover design study in which each subject participated in 2 protocols: GH (rhGH + IDPN + exercise) and no GH (IDPN + exercise). During the GH protocol, the subjects were studied after 3 daily doses of rhGH. Each subject was studied 2 h before, 4 h during, and 2 h after a hemodialysis session with the use of a primed, constant infusion of L-[1-^{13}C]leucine.

Results: Whole-body net protein balance was -0.50 ± 0.07 mg · kg fat-free mass^{-1} · min^{-1} when the patients did not receive rhGH and -0.39 ± 0.04 mg · kg fat-free mass^{-1} · min^{-1} when the patients received rhGH, a 22% improvement in prehemodialysis whole-body protein homeostasis ($P < 0.05$). Essential amino acid muscle loss was also significantly less during the prehemodialysis period when rhGH was administered (-18 ± 23 compared with -71 ± 20 mmol/L; $P < 0.05$). The whole-body anabolic effects of rhGH observed during the prehemodialysis period persisted throughout the entire study, as evidenced by a lack of significant interaction or main effect of treatment during hemodialysis and in the posthemodialysis period.

Conclusion: rhGH improves whole-body protein homeostasis in chronic hemodialysis patients. *Am J Clin Nutr* 2005;82: 1235–43.

KEY WORDS Hemodialysis, metabolism, intradialytic parenteral nutrition, exercise, growth hormone

INTRODUCTION

Mortality and morbidity rates in patients with end-stage renal disease are unacceptably high (1). Of the several factors that have been identified as predictors of this poor outcome, protein catabolism leading to uremic malnutrition is an important one because it is potentially reversible.

Several anabolic interventions have been proposed in chronic dialysis patients. Our laboratory previously showed that intradialytic parenteral nutrition (IDPN) robustly improves whole-body and muscle net protein homeostasis in chronic hemodialysis (CHD) patients (2, 3). The anabolic effects observed in these studies were very pronounced during IDPN infusion (ie, during hemodialysis) but were abruptly shut off once the infusion was terminated, which suggests that CHD patients may have a defect in maintaining the incorporation of nutrients provided by the IDPN. In a subsequent study in a similar population, we showed the additive beneficial effects of exercise along with IDPN administration on protein metabolism and found enhancements in muscle protein uptake in addition to what is observed with IDPN administration alone (3).

Another potential intervention to improve protein and energy homeostasis in end-stage renal disease patients is the administration of recombinant human growth hormone (rhGH), because there is resistance to the anabolic actions of GH in advanced uremia. Studies have shown that rhGH administered at pharmacologic doses induces a net anabolic action and also improves food utilization in uremic animal models (4, 5). Similar findings are reported in studies using different methods to assess protein homeostasis in patients with end-stage renal disease. Such findings include increases in serum creatinine, decreases in serum

[1] From the Department of Medicine, Division of Nephrology (LBP and TAI), and the Department of Biostatistics (CY), Vanderbilt University Medical Center, Nashville, TN, and the Center for Designing Foods to Improve Nutrition, Food Science and Human Nutrition, Iowa State University, Ames, IA (PJF).

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[3] Reprints not available. Address correspondence to TA Ikizler, Vanderbilt University Medical Center, 1161 21st Avenue South & Garland, Division of Nephrology, S-3223 MCN, Nashville, TN 37232-2372. E-mail: alp.ikizler@vanderbilt.edu.

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TABLE 1
Baseline characteristics of the study population[1]

Sex [*n* (%)]	
Male	5 (71)
Female	2 (29)
Race [*n* (%)]	
African American	5 (71)
White	2 (29)
Age (y)	42.4 ± 15.6[2]
Cause of ESRD (%)	
Hypertension	3
ADPKD	2
FSGS	2
Body weight (kg)	77.6 ± 19.8
BMI (kg/m^2)	26.1 ± 5.7
Fat mass by DXA (% of body wt)	25.6 ± 11.1

[1] ESRD, end-stage renal disease; ADPKD, autosomal dominant polycystic kidney disease; FSGS, focal segmental glomerulosclerosis; DXA, dual-energy X-ray absorptiometry.
[2] $\bar{x}$ ± SD (all such values).

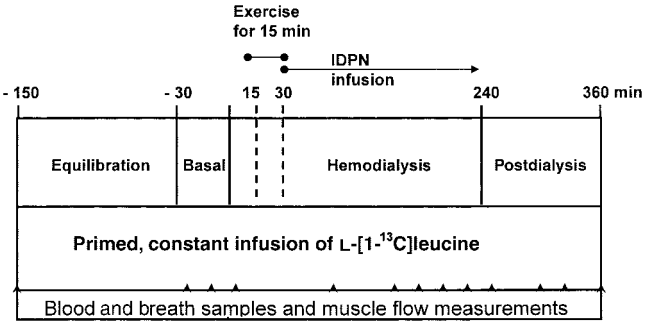

FIGURE 1. Schematic diagram of the metabolic study day protocol. The arrow heads denote the time points for blood draws, breath sample collections, and muscle plasma flow measurements. Subjects received a 75-mg · kg^{-1} · d^{-1} dose of recombinant human growth hormone subcutaneously for 3 nights before the study. A primed, constant infusion of L-[1-^{13}C]leucine and L-[ring-$^2H_5$]phenylalanine was maintained throughout the study (360 min). IDPN, intradialytic parenteral nutrition.

urea nitrogen, decreased dialysate and urinary urea nitrogen excretion rates, and decreased protein catabolic rate (6, 7). Similarly, Ziegler et al (8) studied the effects of a 2-wk therapy with rhGH after each hemodialysis section and found significant decreases in serum urea nitrogen, urea generation, and the protein catabolic rate. Anabolic effects of rhGH in CHD patients have also been noted on the muscle compartment, with increases of up to 3–4 kg in lean body mass with short and midterm administration (9, 10).

Overall, these studies suggest that GH may be a useful adjunctive therapy to diminish body protein catabolism in this patient population. In the present study, we aimed to examine the anabolic effects of short-term administration of rhGH on protein and energy homeostasis in 2 separate settings in CHD patients: *1*) three consecutive daily administrations alone; and *2*) the potential additive effects of the combination of IDPN and exercise.

SUBJECTS AND METHODS

Patients

Patients were recruited from the Vanderbilt University Outpatient Dialysis Unit. The inclusion criteria for the study were a urine output of <100 mL/d and receipt of CHD therapy for >6 mo. The patients were receiving an adequate dose of dialysis (single pool Kt/$V \geq 1.4$) in a thrice-weekly hemodialysis program with a biocompatible hemodialysis membrane (Fresenius F80; Fresenius USA, Lexington, MA). Exclusion criteria consisted of active infectious or inflammatory disease (eg, vascular access infections and overt periodontal disease), hospitalizations within 3 mo before the study, recirculation in the vascular access, or vascular access blood flow <750 mL/min detected on the arteriovenous shunt. In addition, patients receiving steroids or immunosuppressive agents were excluded. The Institutional Review Board of Vanderbilt University Medical Center approved the study protocol, and written informed consent was obtained from all study patients. Patient characteristics are shown in **Table 1**.

Within 1 wk before each study, dual-energy X-ray absorptiometry was performed to estimate lean and fat body masses, and resting metabolic rate was measured by indirect calorimetry to acclimate the subjects to this technology. In addition, patients were brought to the General Clinical Research Center (GCRC) 1 wk before the study to estimate the workload required to achieve their maximal heart rate (11) and to test the subject's ability to sustain exercise at 40% of this level for 15 min. Heart rate and blood pressure were monitored while the patients pedaled on a recumbent stationary cycle (Ergonomics 800; Ergolin, Bandhagen, Sweden) with incremental (+10 W/min) changes in workload until the maximal heart rate was attained. Subsequently, heart rate, blood pressure, oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), energy expenditure, and respiratory quotient were monitored as the subjects cycled at 40% of their maximal heart rate for 15 min. Patients who were not able to tolerate this workload were excluded from the study (11).

rhGH (75 μg/kg subcutaneously) was administered 3 times before the metabolic study day. The first dose was given in the evening after a usual hemodialysis session, the second in the evening on the next day (a nondialysis day), and the third in the evening when the patients were admitted to the GCRC.

Design

This was a randomized crossover study. After they reviewed the inclusion and exclusion criteria, eligible patients completed 2 separate treatment protocols: *1*) IDPN + exercise + rhGH (GH protocol) or *2*) IDPN + exercise (no GH protocol). The order of the protocols was random. Randomization was done by using Pocock's table of random numbers and assigning random sequences of protocols for enrolling patients.

Metabolic study

The patients were admitted to the GCRC the night before the study at ≈1900. They received a meal from the GCRC bionutrition services on admission, after which they remained fasting. This meal was given ≥10 h before the start of the study for all patients and consisted of 18% protein and 30% lipid. Energy intake was kept at maintenance levels on the basis of the Harris-Benedict equation and each subject's sex, height, weight, and activity levels.

A schematic diagram of the metabolic study day protocol is depicted in **Figure 1**. Each study consisted of a prehemodialysis

phase (a 2-h equilibration phase followed by a 0.5-h basal sampling phase), a 4-h hemodialysis phase, and a 2-h posthemodialysis phase. Each metabolic study was initiated at ≈0600 by starting the infusion of isotopically labeled leucine, which was continued throughout the study. The hemodialysis phase was started with the initiation of hemodialysis. During the hemodialysis phase, the 15-min exercise bout was started 15 min after the initiation of hemodialysis. Furthermore, IDPN was started in both treatments 30 min into the phase and was continued throughout the hemodialysis phase. The posthemodialysis phase started immediately at the conclusion of hemodialysis.

A dialysis catheter was placed at the venous site of the arteriovenous shunt of the forearm at 0600 to collect a baseline blood sample (to assess baseline biochemical nutritional markers and isotopic backgrounds) and to then initiate the isotope infusion. An arteriovenous shunt is commonly used for vascular access with hemodialysis and is created by connecting an artery to a nearby vein either by direct surgical anastomosis of the native vessels (fistula) or with synthetic vascular material (graft). In the present study, 2 patients had a native fistula and 4 patients had an artificial graft. The arterial side of the arteriovenous shunt was the site of choice for sampling arterial blood. The only occasion that would affect the arterial purity of the samples would be if there were stenoses in the arteriovenous shunt causing the venous blood to mix with arterial blood (recirculation). Therefore, recirculation of the arteriovenous shunt as well as vascular access blood flow to assess stenoses in the arteriovenous shunts was checked in every patient before the study by means of the ultrasound dilution technique (Transonic Systems Inc, Ithaca, NY). Arterial vascular access obtained though the arterial side of the arteriovenous shunt was used to perform hemodialysis and to sample arterial blood. The venous site of the arteriovenous shunt was used to infuse the isotope (labeled leucine). Another catheter was placed in a superficial vein (on a retrograde insertion) of the contralateral forearm to sample blood draining the forearm muscle bed.

At the start of the experiment, the subjects received a bolus injection of $NaH^{13}CO_3$ (0.12 mg/kg), and L-[1-^{13}C]leucine (7.2 μmol/kg) to prime the carbon dioxide and leucine pools, respectively. Subsequently, a continuous infusion of L-[1-^{13}C]leucine (0.12 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) was started and continued throughout the remainder of the study.

Patients were dialyzed for 4 h with blood flow of 400 mL/min and dialysate flow of 500 mL/min. Ultrafiltration rates were determined by the patients' needs and "estimated dry weight" and were similar during both treatments. The composition of the dialysate used during the study was identical for all treatments and consisted of sodium (139 mmol/L), potassium (2 mmol/L), calcium (1.25 mmol/L), glucose (11.1 mmol/L), and bicarbonate (39 mmol/L).

IDPN was infused via the venous port of the bubble trap on the hemodialysis tubing and was continued throughout the entire hemodialysis procedure (total of 3.5 h of IDPN infusion). The IDPN treatment was based on existing recommendations (12). The solution was given at a rate of 150 mL/h and consisted of 300 mL of an amino acid solution (15% Clinisol; Baxter Healthcare Corporation, Deerfield, IL), 150 mL of a 50%-dextrose solution (Abbott Laboratories, Abbott Park, IL), and 150 mL of a lipid solution (20% IntraLipid; Kabi Pharmacia Inc, Clayton, NC). Each 100 mL of the amino acid solution contained 1.18 g lysine, 1.04 g leucine, 1.04 g phenylalanine, 960 mg valine, 894 mg histidine, 749 mg isoleucine, 749 mg methionine, 749 mg threonine, 250 mg tryptophan, 2.17 g alanine, 1.47 g arginine, 1.04 g glycine, 894 mg proline, 749 mg glutamate, 592 mg serine, 434 mg aspartate, and 39 mg tyrosine. The total solution provided 786.6 kJ/h (188 kcal/h) or 14.6 kJ · kg fat-free mass $(FFM)^{-1} \cdot h^{-1}$ (3.5 kcal · kg $FFM^{-1} \cdot h^{-1}$). The extra volume, as well as the electrolytes that IDPN provided to the patients, was accounted for and removed during hemodialysis.

Fifteen minutes after the start of hemodialysis, the patients began the exercise session on a recumbent stationary bicycle. The workload during exercise was set at 40% of maximal heart rate, as previously explained (11). Exercise was continued for 15 min, during which time heart rate, $\dot{V}O_2$, $\dot{V}CO_2$, respiratory quotient, and energy expenditure were monitored. At the conclusion of exercise, the patients were moved back to their dialysis chair.

Simultaneous blood and breath samples were collected once before the start of the study, 3 times during the basal sampling phase, 6 times during IDPN and dialysis, and 3 times during the posthemodialysis phase. Blood samples were obtained from arterial and forearm venous sampling sites. Breath samples were collected from the subjects via a Douglas bag with duplicate 20-mL samples placed into nonsiliconized glass evacuated tubes for measurement of breath $^{13}CO_2$ enrichment. Subjects were asked to breathe through a mask for 1 min each time blood was collected. In addition, forearm blood flow was estimated by using capacitance plethysmography (Hokanson Inc, Bellevue, WA). Simultaneous energy expenditure and respiratory quotient were determined by indirect calorimetry with the use of a metabolic cart (model 2900; Sensormedics Palo Alto, CA) to measure ventilation rates, carbon dioxide production, and oxygen consumption. Metabolic cart assessment was also done during exercise.

Once hemodialysis was finished, the dialysis lines were disconnected, and the 2-h posthemodialysis phase ensued. After the posthemodialysis phase, all catheters were removed. The patients were given a meal and observed at the GCRC until stable, at which time they were discharged. Patients continued their CHD therapy at the outpatient dialysis unit as scheduled.

Analytic procedures

Blood samples were collected into Venoject tubes containing 15 mg Na_2EDTA (Terumo Medical Corp, Elkton, MD). A 3-mL sample of blood was transferred to a tube containing EDTA and reduced glutathione, and the plasma was stored at −80 °C for later measurement of plasma epinephrine and norepinephrine concentrations by HPLC (13). The remaining blood was spun in a refrigerated (4 °C) centrifuge (Beckman Instruments, Fullerton, CA) at 3 × *g*, 10 min, 5 °C, and plasma was extracted and stored at −80 °C for later analysis. Plasma glucose concentrations were measured by the glucose oxidase method (Beckman Instruments, Fullerton, CA).

Nutritional biochemical variables were measured at specialized end-stage renal disease clinical and special chemistry laboratories (Spectra Laboratories, San Juan, CA, and RenaLab Inc, Richland, MS). Serum albumin was analyzed by the bromcresol green technique. Serum prealbumin was analyzed by an antigen-antibody complex assay, and serum transferrin was analyzed by turbidimetric reading (Boehringer Mannheim, Indianapolis, IN). Serum CRP was measured by nephelometric analysis at the Vanderbilt University Medical Center clinical chemistry laboratory.

Immunoreactive insulin was measured in plasma by use of a double-antibody system. Plasma aliquots for glucagon measurement were placed in tubes containing 25 kallikrein-inhibitor units of aprotinin (FBA Pharmaceutical, New York, NY) and were later measured by use of an established radioimmunoassay with a double-antibody system modified from the method of Morgan and Lazarow (14) for insulin. Insulin and glucagon antisera and standards, as well as ^{125}I-labeled hormones, were obtained from RL Gingerich (Linco Research, St Louis, MO). Clinical Assays Gammacoat Radioimmunoassay kit (Travenol-GenTech, Cambridge, MA) was used to measure plasma cortisol concentrations. Plasma insulin-like growth factor I (IGF-I) concentrations were measured by use of a radioimmunoassay acid-extraction procedure (Nichols Institute Diagnostics, San Juan Capistrano, CA). Plasma amino acid concentrations were measured by reversed-phase HPLC after derivatization with phenylisothiocyanate (15). Individual amino acids were also placed into groups for analysis purposes. These groups included branched-chain amino acids (the sum of leucine, isoleucine, and valine), essential amino acids (the sum of arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine), total amino acids (the sum of all individual amino acids), and nonessential amino acids (the difference between total and essential amino acids).

Plasma enrichments of [^{13}C]leucine and [^{13}C]ketoisocaproate (KIC) were measured by use of gas chromatography–mass spectrometry (Hewlett-Packard, San Fernando, CA). Plasma was deproteinized with 4% perchloric acid, and the supernatant fluid was passed over a cation-exchange resin to separate the keto and amino acids. The keto acids were further extracted with methylene chloride and 0.5 mol ammonium hydroxide/L (16). After drying under nitrogen gas, the keto and amino acid fractions were derivatized (17) with *N*-methyl-*N*-(*t*-butyldimethylsilyl)-trifluoroacetamide containing 1% *t*-butyldimethylchlorosilane (MtBDSTFA + 1% tBDMCS; Regis Technologies, Inc, Morton Grove, IL). The derivatized samples were then analyzed by gas chromatography–mass spectrometry for plasma leucine and KIC enrichments with selected ion monitoring. The major fragments analyzed for the tBDMCS derivative of KIC and [^{13}C]KIC were the (M-57) ion fragments 301 *m/z* and 302 *m/z*, respectively. The enrichment was quantified in plasma as the ratio of [^{13}C]KIC:KIC (ion abundance of 301/302 *m/z*). Enrichment measurements were made in duplicate, and duplicates had a CV $<$ 3%. Breath $^{13}CO_2$ was measured by isotope ratio mass spectrometry (Metabolic Solutions, Nashua, NH) (18).

Calculations

The steady-state rates of total whole-body leucine appearance (Ra) were calculated by dividing the [^{13}C]leucine infusion rate by the plasma [^{13}C]KIC enrichment (19). Plasma KIC provides a better estimate of intracellular leucine enrichment than does plasma leucine enrichment because KIC is derived from intracellular leucine metabolism (19). Steady-state conditions for KIC and carbon dioxide enrichments were achieved as evidenced by slopes within each phase that were not significantly different from zero (data not shown). The endogenous leucine Ra (an estimate of whole-body protein breakdown) was determined by subtracting the rate of leucine infusion via the IDPN from the total Ra (expressed as mg $\cdot$ kg FFM^{-1} $\cdot$ min^{-1}). Breath $^{13}CO_2$ production was determined by multiplying the total carbon dioxide production rate by breath $^{13}CO_2$ enrichment (19). The rate of whole-body leucine oxidation was calculated by dividing breath $^{13}CO_2$ production by 0.8 (correction factor for the retention of $^{13}CO_2$ in the bicarbonate pool) (20) and by the plasma KIC enrichment. The leucine rate of disappearance (Rd) during the dialysis phase was corrected for leucine loss into the ultrafiltration volume by measuring the ultrafiltration volume and the leucine concentration in the dialysate and by subtracting the leucine lost in the dialysate from the total Ra. The nonoxidative leucine Rd, an estimate of whole-body protein synthesis, was determined indirectly by subtracting leucine oxidation from the above-mentioned corrected total leucine Rd. Rates of whole-body protein breakdown, amino acid oxidation, and protein synthesis were calculated from the endogenous leucine Ra, the leucine oxidation rate, and the nonoxidative leucine Rd, respectively, assuming that leucine constitutes 7.8% of whole-body protein (21).

Rates of whole-body amino acid, carbohydrate, and lipid oxidation were determined from indirect calorimetry in combination with the leucine oxidation data. The energy expended due to amino acid oxidation was subtracted from total energy expenditure, and the net rates of carbohydrate and lipid oxidation were calculated on the basis of the nonprotein respiratory quotient (22). The assumptions and limitations of calculating substrate oxidation on the basis of indirect calorimetry measurements were reviewed previously (22).

Statistical analysis

The hypothesis of the present study was that rhGH administration would improve protein homeostasis in addition to the improvements already observed with IDPN + exercise. Specifically, we aimed at looking at the effects of rhGH on protein homeostasis at 3 separate phases: during the prehemodialysis period, during hemodialysis (along with 2 other known anabolic therapies: IDPN and exercise), and during the posthemodialysis period. The primary endpoint was net whole-body protein balance (synthesis minus breakdown) at each phase. For each protocol, mean variables for the prehemodialysis, hemodialysis, and posthemodialysis periods were calculated as the average of the time points for each of the 3 periods. To stabilize the variance in values for plasma GH concentration between the GH and no GH protocols, these values were log transformed for analysis. Nonetheless, the original data are presented in the manuscript for appropriate interpretation. We performed between-group (GH and no GH protocols) comparisons by using paired *t* tests for data with parametric distributions and Wilcoxon's signed-rank test for data with nonparametric distributions. We also performed repeated-measures analysis of variance with time and treatment as between-subjects factors to observe the influence of rhGH on the changes in the study variables over the 3 study phases (prehemodialysis, during hemodialysis, and posthemodialysis). SPSS version 12.0 (SPSS Inc, Chicago, IL) was used for all analyses. All tests were two-tailed, and P values $<$ 0.05 were considered to indicate statistical significance. Results are expressed in SI units as means $\pm$ SEMs, unless otherwise noted.

RESULTS

Blood chemistry

The patients were in an overall adequate nutritional state and there were no significant differences in nutritional measurements between the 2 protocols within patients (Table 1 and **Table 2**).

TABLE 2
Baseline biochemical nutritional indexes in the 2 study protocols[1]

	No GH	GH
Serum albumin (g/L)	41.3 ± 4.1	41.4 ± 2.6
Serum prealbumin (g/L)	0.373 ± 0.08	0.407 ± 0.08
Serum transferrin (g/L)	170.7 ± 34.9	179.3 ± 23.5
Serum cholesterol (mmol/L)	185.4 ± 24.3	184.4 ± 13.3
Total carbon dioxide (mmol/L)	22.7 ± 2.6	22.1 ± 1.7
C-reactive protein (mg/L)	8.70 ± 5.5	9.70 ± 6.1

[1] All values are $\bar{x}$ ± SD; $n = 7$. The GH protcol included recombinant human growth hormone + intradialytic parenteral nutrition + exercise; the no GH protocol included intradialytic parenteral nutrition + exercise. Conversion factors (from SI to conventional units): albumin, ÷10; prealbumin, ÷0.01; transferrin, ÷0.01; cholesterol, ÷0.0259; C-reactive protein, ÷10. There were no significant differences between protocols.

Measurement of pre- and posthemodialysis blood chemistry indexes, including serum urea nitrogen, serum creatinine, sodium, potassium, chloride, and total bicarbonate, showed the changes expected after hemodialysis treatment (data not shown).

Metabolic variables

The results for plasma metabolic hormones and glucose concentrations for the 2 study protocols are shown in **Table 3**. Plasma GH concentrations were significantly higher during the GH protocol before hemodialysis than during the no GH protocol, and this difference persisted during and after hemodialysis. These differences were not accompanied by significant differences in IGF-I concentrations. Concentrations of the stress hormone epinephrine were significantly lower before hemodialysis in the GH protocol and were significantly higher after hemodialysis than during hemodialysis in both protocols. Although there were no significant differences in norepinephrine concentrations between protocols before hemodialysis, norepinephrine concentrations were significantly higher during the posthemodialysis period than during hemodialysis in both protocols. Cortisol concentrations did not differ significantly between protocols during the prehemodialysis period and increased significantly during hemodialysis in both protocols. Insulin concentrations were not significantly different before hemodialysis between protocols but increased significantly during hemodialysis in both protocols (483% for the no GH protocol and 679% for the GH protocol). In the posthemodialysis period, insulin concentrations decreased significantly in both protocols compared with values during hemodialysis. Glucose concentrations did not differ significantly before hemodialysis for the 2 protocols and increased significantly during hemodialysis. In the posthemodialysis period, glucose concentrations decreased significantly in both protocols, returning to nearly prehemodialysis values. No significant differences in plasma glucagon concentrations were observed between protocols before hemodialysis, although glucagon concentrations decreased significantly during hemodialysis compared with before and increased toward prehemodialysis values after hemodialysis compared with during in both protocols.

Plasma concentrations and muscle uptake of amino acids

Plasma amino acid concentrations are shown in **Figure 2**. Plasma concentrations of the 3 functional amino acid groups (total, nonessential, and essential) were significantly lower during the prehemodialysis period in the GH protocol than in the no GH protocol. During hemodialysis, plasma concentrations of all 3 groups of amino acids increased significantly in both protocols. In the posthemodialysis period, plasma amino acid concentrations decreased significantly in both protocols. Muscle uptake of essential amino acids was significantly less negative in the prehemodialysis period in the GH protocol than in the no GH protocol (**Figure 3**). Muscle uptake of amino acids increased significantly during hemodialysis and decreased significantly after hemodialysis in both protocols (data not shown).

TABLE 3
Plasma metabolic hormones and glucose concentrations before, during, and after hemodialysis (HD) in the 2 protocols[1]

	Before HD	During HD	After HD
Growth hormone (mmol/L)[2]			
No GH	1.3 ± 0.5	0.4 ± 0.1	2.3 ± 0.8
GH	69.7 ± 22.2[3]	61.6 ± 12.7	49.7 ± 9.6
IGF-I (nmol/L)			
No GH	31.7 ± 4.89	39.0 ± 4.03	30.4 ± 5.17
GH	43.2 ± 6.55	50.1 ± 7.94	41.3 ± 6.68
Epinephrine (pmol/L)[4]			
No GH	309 ± 77	276 ± 53	631 ± 98
GH	145 ± 36[3]	254 ± 47	530 ± 198
Norepinephrine (nmol/L)[4]			
No GH	4.14 ± 1.33	2.38 ± 0.62	3.04 ± 0.78
GH	2.59 ± 1.03	2.54 ± 0.46	3.17 ± 0.58
Cortisol (nmol/L)[5]			
No GH	281 ± 11	353 ± 55	579 ± 84
GH	254 ± 22	389 ± 63	337 ± 66
Insulin (pmol/L)[2,4–6]			
No GH	67 ± 13	391 ± 51	88 ± 21
GH	83 ± 10	647 ± 90	183 ± 33
Glucose (mmol/L)[4,5]			
No GH	4.53 ± 0.24	8.01 ± 0.32	4.34 ± 0.30
GH	5.07 ± 0.14	8.88 ± 0.44	4.80 ± 0.29
Glucagon (ng/mL)[4,5]			
No GH	165 ± 24	137 ± 19	151 ± 24
GH	174 ± 29	133 ± 17	147 ± 32

[1] All values are $\bar{x}$ ± SEM. The GH protocol included recombinant human growth hormone + intradialytic parenteral nutrition + exercise; the no GH protocol included intradialytic parenteral nutrition + exercise. IGF-I, insulin-like growth factor I. Conversion factors (SI to conventional units): glucose, ÷0.0555 mg/dL; insulin, ÷6.945 mIU/mL; glucagon, ÷1.0 pg/mL; IGF-I, ÷0.131 ng/mL; cortisol, ÷27.59 mg/dL; epinephrine, ÷5.46 pg/mL; norepinephrine, ÷0.00591 pg/mL. Growth hormone values for the GH protocol were log-transformed before analysis.

[2] Significant main effect of protocol.

[3] Significantly different from the no GH protocol, $P < 0.05$ (Wilcoxon's signed-rank test).

[4] Significant difference between during and after HD for both protocols, $P < 0.05$ (repeated-measures ANOVA).

[5] Significant difference between before and during HD for both protocols, $P < 0.05$ (repeated-measures ANOVA).

[6] Significant interaction term (protocol × time).

Energy expenditure and substrate oxidation

Energy expenditure and substrate oxidation data are shown in **Table 4**. There were no significant differences in energy expenditure before hemodialysis and there were significant increases

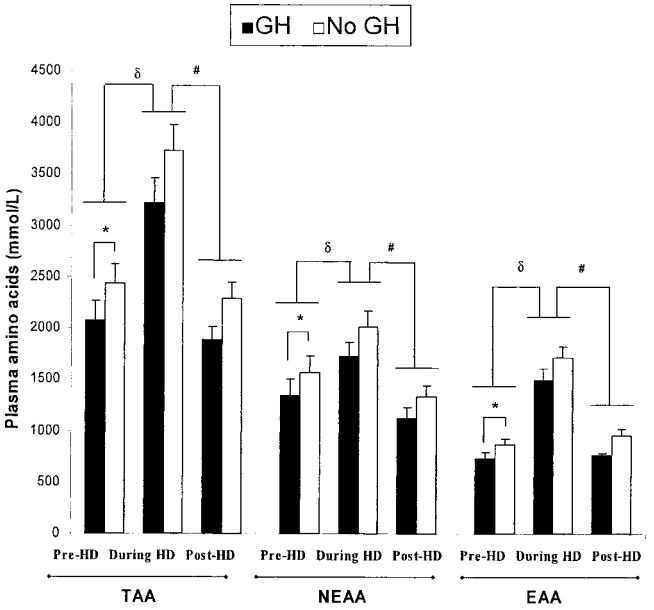

FIGURE 2. Mean (±SEM) plasma amino acid concentrations during the separate study periods in the GH [recombinant human growth hormone + intradialytic parenteral nutrition (IDPN) + exercise] and no GH (IDPN + exercise) protocols (n = 7). HD, hemodialysis; TAA, total amino acids; NEAA, nonessential amino acids; EAA, essential amino acids. The main effects of protocol and the time × protocol interaction were not significant. *Significant difference between the no GH and the GH protocols during the pre-HD period, $P < 0.05$ (Wilcoxon's signed-rank test). δSignificant difference between the pre-HD and the during HD periods for both protocols, $P < 0.05$ (repeated-measures ANOVA). #Significant difference between the during HD and the post-HD periods for both protocols, $P < 0.05$ (repeated-measures ANOVA).

during hemodialysis, with energy expenditure remaining elevated in the posthemodialysis period in both protocols. Fat oxidation did not differ significantly between protocols before hemodialysis and did not change significantly during hemodialysis in either protocol. In the posthemodialysis period, fat oxidation increased significantly in both protocols. Amino acid oxidation was significantly lower before hemodialysis in the GH protocol and increased significantly during hemodialysis in both protocols. Carbohydrate oxidation was significantly lower before hemodialysis in the GH protocol and, although there were no significant changes during hemodialysis for either protocol, decreased significantly after hemodialysis in both protocols.

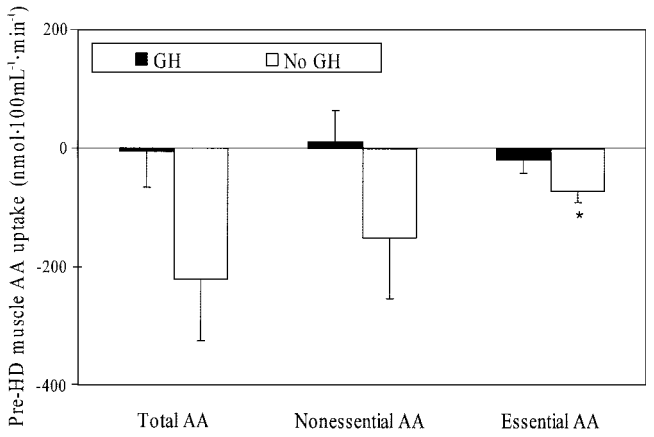

FIGURE 3. Mean (±SEM) forearm balance of amino acids (AAs) before hemodialysis (HD) for the GH [recombinant human growth hormone + intradialytic parenteral nutrition (IDPN) + exercise] and no GH (IDPN + exercise) protocols (n = 7). *Significantly different from the GH protocol, $P < 0.05$ (Wilcoxon's signed-rank test).

Whole-body protein metabolism

The components of whole-body protein homeostasis during all study periods are shown in **Table 5**. In the prehemodialysis period, there was significantly less whole-body protein loss (ie, negative balance between synthesis and breakdown) in the GH protocol than in the no GH protocol. During hemodialysis, there were significant increases in whole-body protein synthesis and decreases in whole-body protein breakdown, which resulted in significant increases in net whole-body protein balance (a change from a catabolic to a highly anabolic state) for the 2 study protocols. In the posthemodialysis period, whole-body protein synthesis decreased significantly and breakdown increased significantly, resulting in significantly negative net whole-body protein balance in the posthemodialysis period compared with during hemodialysis for both protocols.

DISCUSSION

The results of the present study indicate that short-term (3 consecutive daily administrations) rhGH therapy significantly improves net whole-body protein homeostasis in CHD patients, primarily through an 18% increase in whole-body protein synthesis. Previous studies of rhGH therapy in other situations support this conclusion. With the use of tracer techniques comparable with those used in the present study, rhGH administration was previously reported to increase whole-body protein synthesis in healthy, fed subjects (23); in surgical patients receiving parenteral nutrition (24); in GH-deficient young adults (25); in prepubertal children with cystic fibrosis (26); and in testosterone-treated prepubertal boys (27).

Acquired resistance to the anabolic actions of GH is a potential cause of the increased net protein catabolism in patients with advanced chronic kidney disease (28–31). Several studies have shown that the administration of rhGH at pharmacologic doses induces a net anabolic action and improves food utilization in uremic animal models (4, 5). Similar findings were reported in studies that used different methods to assess protein homeostasis in patients with end-stage renal disease (6–8). Using stable-isotope techniques to asses skeletal muscle protein homeostasis, Garibotto et al (32) showed significant improvement in net muscle protein balance (from -15 ± 2 to -8 ± 2 nmol · 100 mL^{-1} · min^{-1}) over a 6-wk administration of 50 μg rhGH in malnourished CHD patients. The current study corroborates these results: there was a greater net uptake of amino acids by the muscle tissues of the forearm. Furthermore, the current study extends these findings to the whole-body protein pool and suggests a significantly improved net whole-body protein balance. Notably, this beneficial effect is due to a combination of simultaneous improvements in protein synthesis and protein breakdown, which suggests the involvement of multiple mechanisms, such as direct actions of rhGH on protein synthesis and potential indirect actions through activation of an IGF-I–dependent decrease in protein breakdown.

One of the aims of the present study was to examine any additional beneficial effects of rhGH administration above and beyond what can be achieved with administration of IDPN and

TABLE 4
Components of energy expenditure before, during, and after hemodialysis (HD) in the 2 protocols

	Before HD	During HD	After HD
Energy expenditure (kJ · kg FFM^{-1} · h^{-1})[2]			
No GH	3.81 ± 0.42	4.23 ± 0.46	4.31 ± 0.63
GH	3.97 ± 0.58	4.18 ± 0.38	4.48 ± 0.38
Fat oxidation (mg · kg FFM^{-1} · min^{-1})[3,4]			
No GH	0.78 ± 0.13	0.82 ± 0.08	1.24 ± 0.22
GH	1.05 ± 0.06	1.03 ± 0.11	1.65 ± 0.20
Amino acid oxidation (mg · kg FFM^{-1} · min^{-1})[2]			
No GH	0.35 ± 0.04	0.68 ± 0.09	0.67 ± 0.15
GH	0.27 ± 0.03[5]	0.42 ± 0.06	0.38 ± 0.05
Carbohydrate oxidation (mg · kg FFM^{-1} · min^{-1})[3,4]			
No GH	1.88 ± 0.39	2.18 ± 0.34	1.29 ± 0.40
GH	1.39 ± 0.47[5]	1.64 ± 0.29	0.49 ± 0.24

[1] All values are $\bar{x}$ ± SEM; $n = 7$. The GH protocol included recombinant human growth hormone + intradialytic parenteral nutrition + exercise; the no GH protocol included intradialytic parenteral nutrition + exercise. FFM, fat-free mass. Conversion factor (SI to conventional units): energy, ÷4.184. The interaction terms (protocol × time) were not significant.
[2] Significant difference between before and during HD for both protocols, $P < 0.05$ (repeated-measures ANOVA).
[3] Significant difference between during and after HD for both protocols, $P < 0.05$ (repeated-measures ANOVA).
[4] Significant effect of protocol.
[5] Significantly different from the no GH protocol, $P < 0.05$ (Wilcoxon's signed-rank test).

exercise during hemodialysis. The adverse nutritional effects of the hemodialysis procedure have been well established and were confirmed by the present study. The available evidence indicates that the profound decrease in nutrient availability is likely the critical reason for increased protein catabolism during hemodialysis, because it can be completely reversed by replenishing the plasma amino acid pool through IDPN (2, 33, 34). Furthermore, the anabolic effects of IDPN can be augmented by additional maneuvers aimed at improving nutrient utilization, such as exercise or anabolic hormones. In a recent study, we examined the effects of exercise along with IDPN administration on protein metabolism in CHD patients and found enhancements in muscle protein uptake in addition to the ones observed with IDPN only (3). In the present study, the use of rhGH improved whole-body protein homeostasis during the prehemodialysis period; during hemodialysis and in the posthemodialysis periods, the observed benefits of rhGH seemed to be sustained, although at a small magnitude. This could be interpreted to suggest that rhGH provides enhancements in protein anabolism in addition to the proven benefits of IDPN and exercise.

Our results indicate that the observed responses to rhGH during the prehemodialysis period and in combination with IDPN and exercise are mediated by different mechanisms. GH-associated improvements in protein metabolism may occur either directly through GH stimulation of initiation factors or indirectly through changes in circulating unbound IGF-I and IGF-binding proteins (35). It is difficult to separate these effects in the present study. Although rhGH treatment resulted in an ≈30–35% increase in IGF-I in each of the protocols, which suggests that the effects of GH could have been mediated via these peptides, the variation was such that the differences in IGF-I were not statistically significant. Plasma IGF-I concentrations do not always significantly increase after

TABLE 5
Components of whole-body protein homeostasis before, during, and after hemodialysis (HD) in the 2 protocols[1]

	Before HD	During HD	After HD
Protein synthesis (mg · kg FFM^{-1} · min^{-1})[2,3]			
No GH	3.23 ± 0.19	5.04 ± 0.44	3.44 ± 0.29
GH	3.94 ± 0.55	5.06 ± 0.43	3.41 ± 0.13
Protein breakdown (mg · kg FFM^{-1} · min^{-1})[2,3]			
No GH	3.79 ± 0.15	1.60 ± 0.18	4.60 ± 0.49
GH	4.33 ± 0.55	1.33 ± 0.34	4.06 ± 0.11
Net protein balance (mg · kg FFM^{-1} · min^{-1})[2,3]			
No GH	−0.50 ± 0.07	3.44 ± 0.37	−1.16 ± 0.45
GH	−0.39 ± 0.04[4]	3.73 ± 0.57	−0.65 ± 0.15

[1] All values are $\bar{x}$ ± SEM; $n = 7$. The GH protocol included recombinant human growth hormone + intradialytic parenteral nutrition + exercise; the no GH protocol included intradialytic parenteral nutrition + exercise. FFM, fat-free mass. The interaction terms and the main effects of treatment were not significant.
[2] Significant difference between before and during HD for both protocols, $P < 0.05$ (repeated-measures ANOVA).
[3] Significant difference between during and after HD for both protocols, $P < 0.05$ (repeated-measures ANOVA).
[4] Significantly different from the no GH protocol, $P < 0.05$ (Wilcoxon's signed-rank test).

rhGH administration (36). However, IGF-I increases similar to those found in the present study have been shown to positively act on protein homeostasis through the same mode and possibly the same receptors as insulin.

Direct effects of insulin are not likely to be involved in the changes in protein metabolism observed during the prehemodialysis period because the protein anabolic effects of rhGH were observed in lieu of no significant changes in basal glucose, insulin, and glucagon concentrations. However, during combined treatment, insulin concentrations were significantly higher with GH, which may explain the observed beneficial effect on proteolysis rather than an increase in synthesis.

We observed significant changes in substrate metabolism in response to rhGH administration. During the prehemodialysis period, fat oxidation was slightly higher after 3-d rhGH administration, a finding consistent with the actions of GH. During the posthemodialysis period, fat utilization was significantly higher with rhGH despite substantially increased insulin concentrations. These changes were accompanied by better, albeit not statistically significant, whole-body protein balance. Overall, these findings indicate that rhGH may overcome the antilipolytic effects of insulin, which could be one of the mechanisms by which we observed improvements in protein homeostasis in the GH protocol (37).

The significance of the differences noted with GH treatment in the present study can be evaluated only when extrapolated to a longer time period. Therefore, assuming that the body's FFM is 73% water, that the patients would be dialyzed 3 times/wk, and that the differences in whole-body protein balance noted for each period could be sustained for 1 y, our data would translate to an advantage of 8.2 kg FFM for the GH treatment over 1 y. Indeed, placebo-controlled studies report increases of 3.14 $\pm$ 0.41 kg FFM after 6 mo of daily rhGH administration (9) and of 3.9 $\pm$ 2.0 kg in response to thrice-weekly doses (after hemodialysis) (10) in CHD patients.

The results of the present study need to be interpreted with caution because of the study's limitations. First, no placebo injection of rhGH was used. However, it is not likely that a traditional placebo effect could have significant effects on the outcomes of protein kinetics. Second, we did not have nonexercise or non-IDPN control groups. Third, the decrease in circulating epinephrine and norepinephrine (nonsignificant) concentrations was not anticipated. Interestingly, concentrations of these catecholamines were not significantly different during and after hemodialysis. The reason for these changes is unclear. Increases in epinephrine, however, are not thought to cause increases in whole-body protein breakdown, but rather appear to exert a whole-body protein-sparing effect (38), even when insulin is controlled (39). Finally, the patients included in this study were in adequate nutritional status as measured by serum protein concentrations. It is possible that malnourished hemodialysis subjects would be more or less responsive to IDPN $\pm$ GH and thus respond differently to GH in the 3 hemodialysis phases.

In summary, our results show that rhGH administration has potential as a therapeutic approach to overcome uremic malnutrition in CHD patients. Administration of rhGH alone significantly improves whole-body protein metabolism and slightly augments the already proven beneficial effects of combined administration of IDPN and exercise during and after hemodialysis. These improvements in net protein anabolism are likely secondary to multiple mechanisms including but not limited to enhanced amino acid and fat utilization and concomitant improvements in muscle amino acid uptake. Because our patient population was in overall adequate nutritional status, further research is needed to extend these findings to CHD patients with more diverse nutritional status, especially in the long term.

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REFERENCES

1. USRDS. The United States Renal Data System. Am J Kidney Dis 2003; 42:1–230.
2. Pupim LB, Flakoll PJ, Brouillette JR, Levenhagen DK, Hakim RM, Ikizler TA. Intradialytic parenteral nutrition improves protein and energy homeostasis in chronic hemodialysis patients. J Clin Invest 2002; 110:483–92.
3. Pupim LB, Flakoll PJ, Levenhagen DK, Ikizler TA. Exercise augments the acute anabolic effects of intradialytic parenteral nutrition in chronic hemodialysis patients. Am J Physiol Endocrinol Metab 2004;286: E589–97.
4. Mehls O, Ritz E, Hunziker EB, Eggli P, Heinrich U, Zapf J. Improvement of growth and food utilization by human recombinant growth hormone in uremia. Kidney Int 1988;33:45–52.
5. Chan W, Valerie KC, Chan JCM. Expression of insulin-like growth factor-1 in uremic rats: growth hormone resistance and nutritional intake. Kidney Int 1993;43:790–5.
6. Ikizler TA, Wingard RL, Breyer JA, Schulman G, Parker RA, Hakim RM. Short-term effects of recombinant human growth hormone in CAPD patients. Kidney Int 1994;46:1178–83.
7. Ikizler TA, Wingard RL, Flakoll PJ, Schulman G, Parker RA, Hakim RM. Effects of recombinant human growth hormone on plasma and dialysate amino acid profiles in CAPD patients. Kidney Int 1996;50: 229–34.
8. Ziegler TR, Lazarus JM, Young LS, Hakim R, Wilmore DW. Effects of recombinant human growth hormone in adults receiving maintenance hemodialysis. J Am Soc Nephrol 1991;2:1130–5.
9. Hansen TB, Gram J, Jensen PB, et al. Influence of growth hormone on whole body and regional soft tissue composition in adult patients on hemodialysis. A double-blind, randomized, placebo-controlled study. Clin Nephrol 2000;53:99–107.
10. Johannsson G, Bengtsson BA, Ahlmen J. Double-blind, placebo-controlled study of growth hormone treatment in elderly patients undergoing chronic hemodialysis: anabolic effect and functional improvement. Am J Kidney Dis 1999;33:709–17.
11. Wasserman K. Principles of exercise testing & interpretation: including pathophysiology and clinical applications. 3rd ed. Philadelphia, PA: Lippincott Williams & Wilkins, 1999.
12. Kopple JD, Foulks CJ, Piraino B, Beto JA, Goldstein J. National Kidney Foundation position paper on proposed health care financing: administration guidelines for reimbursement of enteral and parenteral nutrition. J Renal Nutr 1996;6:45–7.
13. Goldstein DS, Feuerstein G, Izzo JL Jr, Kopin IJ, Keiser HR. Validity and reliability of liquid chromatography with electrochemical detection for measuring plasma levels of norepinephrine and epinephrine in man. Life Sci 1981;28:467–75.
14. Morgan CR, Lazarow A. Immunoassay of insulin: two antibody system. Plasma insulin levels of normal, subdiabetic, and diabetic rats. Diabetes 1963;12:115–26.
15. Heinrikson RI, Meredith SC. Amino acid analysis by reverse-phase high pressure liquid chromatography: precolumn derivatization with phenylisothiocyanate. Anal Biochem 1984;136:65–74.
16. Nissen SL, Van Huysen C, Haymond MW. Measurement of branched-chain α-ketoacids in plasma by high performance liquid chromatography. J Chromatogr 1982;232:170–5.

17. Schwenk WF, Berg PJ, Beaufrere B, Miles JM, Haymond MW. Use of *t*-butyldimethylsilylation in the gas chromatographic/mass spectrometric analysis of physiologic compounds found in plasma using electron-impact ionization. Anal Biochem 1984;141:101–9.
18. Scrimgeour CM, Rennie MJ. Automated measurement of the concentration and ^{13}C enrichment of carbon dioxide in breath and blood samples using the Finnigan MAT breath gas analysis system. Biomed Environ Mass Spectrom 1988;15:365–7.
19. Wolfe RR. Radioactive and stable isotope tracers in biomedicine: principles and practice of kinetic analysis. New York, NY: Wiley-Liss, 1992:283–316.
20. Allsop JR, Wolfe RR, Burke JF. Tracer priming the bicarbonate pool. J Appl Physiol 1978;45:137–9.
21. Garlick PJ, McNurlan MA, McHardy KC, et al. Rates of nutrient utilization in man measured by combined respiratory gas analysis and stable isotopic labeling: effect of food intake. Hum Nutr Clin Nutr 1987;41:177–91.
22. Jequier E, Acheson K, Schutz Y. Assessment of energy expenditure and fuel utilization in man. Annu Rev Nutr 1987;7:187–208.
23. Mauras N. Combined recombinant human growth hormone and recombinant human insulin-like growth factor I: lack of synergy on whole body protein anabolism in normally fed subjects. J Clin Endocrinol Metab 1995;80:2633–7.
24. Carli F, Webster JD, Halliday D. Growth hormone modulates amino acid oxidation in the surgical patient: leucine kinetics during the fasted and fed state using moderate nitrogenous and caloric diet and recombinant human growth hormone. Metabolism 1997;46:23–8.
25. Pfeifer M, Poredos P, Zizek B. Effect of growth hormone (GH) therapy on endothelial function in GH-deficient adults. J Clin Endocrinol Metab 2000;85:4923.
26. Darmaun D, Hayes V, Schaeffer D, Welch S, Mauras N. Effects of glutamine and recombinant human growth hormone on protein metabolism in prepubertal children with cystic fibrosis. J Clin Endocrinol Metab 2004;89:1146–52.
27. Mauras N, Rini A, Welch S, Sager B, Murphy SP. Synergistic effects of testosterone and growth hormone on protein metabolism and body composition in prepubertal boys. Metabolism 2003;52:964–9.
28. Blum WF, Ranke MB, Kietzmann K, Tonshoff B, Mehls O. Growth hormone resistance and inhibition of somatomedin activity by excess of insulin-like growth factor binding protein in uraemia. Pediatr Nephrol 1991;5:539–44.
29. Fouque D, Peng SC, Kopple JD. Impaired metabolic response to recombinant insulin-like growth factor-1 in dialysis patients. Kidney Int 1995; 47:876–83.
30. Fouque D, Peng SC, Kopple JD. Pharmacokinetics of recombinant human insulin-like growth factor-1 in dialysis patients. Kidney Int 1995; 47:869–75.
31. Ding H, Kopple JD, Cohen A, Hirschberg R. Recombinant human insulin-like growth factor-I accelerates recovery and reduces catabolism in rats with ischemic acute renal failure. J Clin Invest 1993;91:2281–7.
32. Garibotto G, Barreca A, Russo R, et al. Effects of recombinant human growth hormone on muscle protein turnover in malnourished hemodialysis patients. J Clin Invest 1997;99:97–105.
33. Wolfson M, Jones MR, Kopple JD. Amino acid losses during hemodialysis with infusion of amino acids and glucose. Kidney Int 1982;21: 500–6.
34. Ikizler TA, Flakoll PJ, Parker RA, Hakim RM. Amino acid and albumin losses during hemodialysis. Kidney Int 1994;46:830–7.
35. Davis TA, Bush JA, Vann RC, Suryawan A, Kimball SR, Burrin DG. Somatotropin regulation of protein metabolism in pigs. J Anim Sci 2004;82(E-suppl):E207–13.
36. Thissen JP, Ketelslegers JM, Underwood LE. Nutritional regulation of the insulin-like growth factors. Endocr Rev 1994;15:80–101.
37. Press M. Growth hormone and metabolism. Diabetes Metab Rev 1988; 4:391–414.
38. Keller U, Gerber PP, Stauffacher W. Stimulatory effect of norepinephrine on ketogenesis in normal and insulin-deficient humans. Am J Physiol 1984;247:E732–9.
39. Kraenzlin ME, Keller U, Keller A, Thelin A, Arnaud MJ, Stauffacher W. Elevation of plasma epinephrine concentrations inhibits proteolysis and leucine oxidation in man via beta-adrenergic mechanisms. J Clin Invest 1989;84:388–93.