• BASIC RESEARCH •

The anabolic effects of recombinant human growth hormone and glutamine on parenterally fed, short bowel rats

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Received 2002-01-14   **Accepted** 2002-02-09

Abstract

AIM: **To evaluate the metabolic effects associated with administration of rhGH and/or Gln in parenterally fed, short-bowel rats.**

METHODS: **Forty SD rats subjected to 75 % intestinal resection and maintained with parenteral nutrition were randomly divided into 4 groups as follows: -rhGH, -Gln; -rhGH, +Gln; +rhGH, -Gln; +rhGH, +Gln. Body weight and nitrogen balance were evaluated daily. After 6 days of PN, rats were killed, various organs were dissected and weighted, the carcasses were used for analysis of body composition. Serum GH and IGF-1 were determined by RIA method.**

RESULTS: **Weight loss in rats with rhGH (17.4±12.8 g) and rhGH+Gln (23.8±3.5 g) was significantly less than rats with PN alone (29.6±6. 9 g) and rats with Gln-supplemented PN (31.85±12.8 g), $P<0.05$. The accumulated NB in rats with rhGH (1252.9±294.3 mg N/d) and rhGH+Gln (1261.7±85.5 mg N/d) was significantly greater than those with PN alone (704.8±379.0 mg N/d) and with Gln-supplemented PN (856.7±284.4 mg N/d), $P<0.05$. The absolute weight of gastrocnemius muscle in rats with rhGH (2683.9±341.6 mg) and rhGH+Gln (2579.1±359.5 mg) was greater than those with PN alone (2176.3±167.1 mg) and with Gln-supplemented PN (2141.9±353.6 mg). Although the absolute weight of remnant small intestine itself was not significantly different in 4 experimental groups, the weight/length of the segments was greater in rats with rhGH and/or Gln (48.7±5.5, 52.7±4.1 and 67.4±5.3 respectively) than those with PN alone(47.8±5.0), there were synergistic effects between rhGH and Gln in improvement of the weight/length of remnant small intestine, $P<0.05$. Analyses of body carcass composition showed that a higher percentage of carcass weight as protein and a lower percentage of carcass weight as fat were occurred in rats with rhGH (20.8±4.0,6.0±2.6) and rhGH+Gln(21.3±2.4,4.4±1.5) than those with PN alone (16.4±2.4,9.2±3.7) and with Gln-supplemented PN (17.8±3.0,6.3±2.0), rhGH had significant effects on alteration of body composition, $P<0.05$. Serum GH and IGF-1 concentration in rats with rhGH(5.221±0.8 and 425.1±19.2 ng/ml respectively)and rhGH+Gln (5.507±1.0 and 461.1±49.9 ng/ml respectively) were greater than those with PN alone(3.327±1.7 and 325.8±29.6 ng/ml respectively) and with Gln-supplemented PN(3.433±0.1 and 347.7±55.7 ng/ml respectively), $P<0.01$.**

CONCLUSION: **rhGH significantly improves the anabolism in parenterally fed. short bowel rats, anabolic effect with Gln is less dramatic, there is no synergistic effect between rhGH and Gln in improvement of whole body anabolism. IGF-1 plays an important part in growth-promoting effects of rhGH.**

INTRODUCTION

Short bowel syndrome follows therapeutic massive small bowel resection, following such surgery, affected patients often require long-term parenteral nutrition (PN) to provide adequate nutrition to meet the needs of the body[1-2]. However, long-term experience with conventional PN is unable to increase or even maintain body protein, the anabolic response to PN is often suboptical because of the concomitant presence of catabolism and /or alterations in the hormonal regulation of metabolism[3-4]. Thus the efficacy of treatment with anabolic opsonins, such as growth factors, glutamine (Gln) is currently under the investigation[5-7]. But few studies have made direct comparisons of the metabolic response to rhGH *vs.* Gln in the body. The objective of the present study was to evaluate the relative anabolic and metabolic effects associated with administration of rhGH, Gln and the combination of rhGH and Gln during PN in short-bowel rats and to gain insight regarding potential mechanism mediating the anabolic effects of these factors.

MATERIALS AND METHODS

Animals and experimental design

Forty male Sprague-Dawley rats weighing 180-220 g were housed and underwent an acclimatization period of 7 days. After overnight fasting, they were anesthetized with 0.5 % pentobarbital (40 mg/kg) shortly before surgery. Two operations were performed: 1, placement of PN catheter in the superior vena cava via the external jugular vein; 2, 75 % small intestinal resection. After operation, the rats were placed in individual metabolic cages[8].

The experimental design was a randomized complete block with four treatment groups arranged in a 2×2 factorial design as follows: -rhGH, -Gln; -rhGH, +Gln; +rhGH, -Gln; +rhGH, +Gln. The final sample size was 10 rats per group. In addition, an orally fed, nonoperated, age-matched group (*n*=8) was included to serve as reference for determination of net changes in total body content of protein, water and fat. After surgery, on day 0 only saline was provided through the catheter. From day 1 to day 6, PN solution was given and water was provided ad libitum. Rats received rhGH and/or Gln for 6 days, during which PN provided the sole source of nutrition.

At the end of the experiment, the rats were reanesthesized and laparotomy was performed under sterile conditions. Blood was collected by cardiac puncture, and thymus, heart, lung, spleen, liver ,kidney, adrenal, testis and gastrocnemius muscles were dissected, weighted, frozen and stored at -70 ℃.The entire gastrointestinal tract was removed, flushed with cold saline, the wet weight of stomach, remnant small intestine (from pylorus to cecum), and cecum was recorded. The length of remnant small intestine segment was also determined. After removal of the above tissues, carcass weights were recorded and carcasses were stored at -70 ℃ for body composition analysis.

Recombinant human growth hormone (rhGH)
rhGH (Saizen) was provided by Serono Singapore Pte. Ltd, Singapore. The dose of rhGH was 1 U/kg· d (0.33 mg/kg· d) administered once a day as a subcutaneous injection over a hind limb beginning on day 1 and continued for 6 days. Sham saline injection was given to rats not treated with rhGH[9].

Compositions of PN solution
The PN solution was prepared aseptically using 7 % Vamine (SSPC), 50 % dextrose, 20 % Intralipid (SSPC), vitamins, trace elements and electrolytes. Gly-Gln (Pharmacia &Upjohn Co. Germany) was added to the Gln-PN solution and Gly-Ala-Ser-Pro was added to the standard PN solution to equalize the total nitrogen to 8.62 g/L in both solutions. All rats received 166.4 Kcal/kg· d and 1.4 g N/kg· d parenterally with an infusion pump. Compositions of the Gln-PN and standard PN are shown in Table 1.

Table 1 Compositions of Gln-PN and standard-PN solution (100ml)

	Gln-PN	Standard-PN
50 % Dextrose (ml)	33	33
20 % Intralipid (ml)	17	17
7 % Vamine (ml)	30	30
17.54 % Gly-Gln (ml)	16	0
Gly-Ala-Ser-Pro (ml)	0	16
10 % KCL (ml)	1	1
10 %NaCL (ml)	1	1
Addamel(SSPC) (ml)	1	1
Soluvit(SSPC) (ml)	1	1
Non-protein Calories (Kcal)	104	104
Total nitrogen (g)	0.862	0.862

Body composition
Body composition analysis was performed on rat carcasses to determine the percent composition of water, protein and fat[10,11]. Total carcass water was determined by freeze-drying the carcasses to obtain a constant dry carcass weight and then subtracting it from total carcass weight. The freeze-dried carcasses were then homogenized, and aliquots of dried rat homogenate were assayed for nitrogen and lipid concentrations[10]. Carcass nitrogen concentration was determined by micro-Kjeldahl analysis of 0.50 g dried rat homogenate[12], and carcass protein concentration was calculated by multiplying carcass nitrogen concentration by 6.25. carcass lipid concentration was determined by extracting 1.0 g dried rat homogenate with 2:1 (v/v) Chloroform-methanol, and then determine lipid concentration gravimetrically[10].

Nitrogen balance
Nitrogen contents of urine samples were determined by micro-Kjeldahl procedure[12]. Daily nitrogen balance (NB) was determined by subtracting 24-hour urinary nitrogen from daily nitrogen intake, which was calculated from the volume of PN solution given. Accumulated NB was calculated by subtracting 6-day urinary nitrogen from 6-day nitrogen intake.

Serum GH and IGF-1 determination
Serum GH and IGF-1 concentrations were determined by radioimmunoassay in the isotope laboratory of Zhongshan Hospital[13]. Plasma IGF-1 was assayed with the DSL-2900 rat IGF-1 RIA kit (Diagnostic systems laboratories, Inc. USA), serum GH was assayed with the human GH RIA kit (Beifang biological technical institute, Beijing, China).

Statistical analysis
Data were analyzed by one-or two-way analysis of variance (ANOVA) using the Stata 5.0 program. The four PN groups in the factorial arrangement were compared in a two-way ANOVA, the reference group was excluded from the two-way ANOVA. Group means determined by the least-significant difference technique were considered significantly different at $P<0.05$. Values were expressed as means ±SE.

RESULTS

Body weight and body composition
Changes in body weight are summarized in Figure 1. There were no significant differences in body weight at the time of surgery and when PN was initiated, $P>0.05$. After 6 days of PN, rats given rhGH weighted significantly more than rats given without rhGH. Compared to the weight before the surgery, weight loss in rats with rhGH (17.4±12.18 g) and rhGH+Gln (23.8±3.46 g) was significantly less than those with PN alone (29.6±6.89 g) and with Gln-supplemented PN (31.8±12.75 g), $P<0.05$.

Results of body composition analysis are shown in Table 2. There were no significant differences in the absolute amount and percentage of water among the groups, $P>0.05$; the absolute amount of body fat in rats with rhGH+Gln was significantly decreased compared to those with PN alone and Gln-supplemented PN; however, rats with rhGH and rhGH + Gln showed a somewhat increase in the absolute amount and proportion of the body protein. rhGH had significant effects on alteration of body fat and body protein, $P<0.05$, Gln had no such effects, and there were no significant synergistic effects between rhGH and Gln in changes of body composition, $P>0.05$.
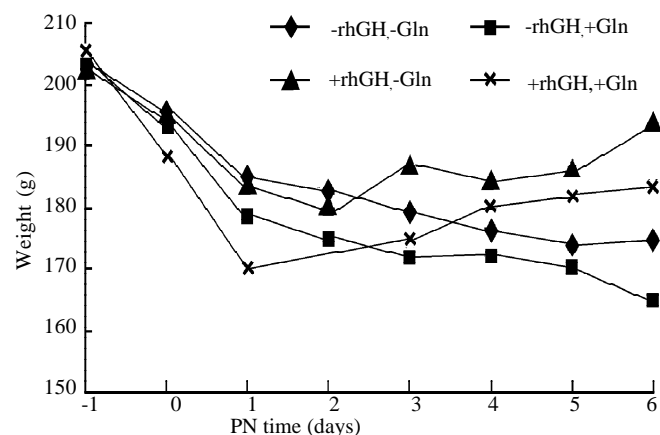

Figure 1 Daily changes in body weight in rats with PN

Table 2 Body composition of short-bowel rats maintained with PN and given rhGH and/or Gln

	Carcass Water		Carcass Fat		Carcass Protein	
	g	%	g	%	g	%
-rhGH						
-Gln	74.4±9.9	66.9±3.8	8.5±3.9	9.2±3.7	17.9±3.2	16.4±2.4
+Gln	71.0±12.9	68.8±3.4	6.0±1.7	6.3±2.0	18.2±2.5	17.8±3.0
+rhGH						
-Gln	76.5±14.5	71.0±6.2	5.4±2.3	6.0±2.6	22.2±3.0	20.8±4.0
+Gln	65.1±3.2	68.9±1.0	4.7±1.5[a]	4.4±1.5	20.3±3.1	21.3±2.4
Reference	77.7±2.3	73.1±1.0	7.2±1.7	7.6±1.9	18.0±1.8	16.9±1.5
two-way ANOVA						
Gln	NS	NS	0.0332	NS	NS	NS
rhGH	NS	NS	0.0153	0.0296	0.0134	0.0035
Gln+rhGH	NS	NS	NS	NS	NS	NS

[a]$P<0.05$ *vs* rats with PN alone, Gln-supplemented PN and reference rats. Reference group was not included in 2-way analysis of variance (ANOVA).

Table 3 Organ and tissue weights of rats maintained with PN and given rhGH and/or Gln

	reference	-rhGH, -Gln	-rhGH, +Gln	+rhGH, -Gln	+rhGH, +Gln
Thymus (mg)	392.2±76.1	376.9±88.5	412.9±85.9	376.3±50.8	355.1±41.7
Heart (mg)	693.6±89.9	652.8±106.4	649.4±78.4	624.7±35.3	615.2±102.8
Lung (mg)	1086.1±143.7	1213.8±205.6	1032.3±137.2[b]	1318.7±168.2[a]	1067.9±119.9
Liver (mg)	8515.2±1201.7	7722.0±540.1	7819.5±1005.5	9608.9±884.7[c]	8180.5±556.6
Spleen (mg)	493.2±56.1	669.8±99.9[d]	467.8±153.8	589.7±112.1	580.3±93.3
Stomach (mg)	1067.5±156.8	1115.7±111.9	1087.1±181.4	1171.6±75.9	1216.9±171.9
Remnant intestine(mg)	1688.9±228.9	1658.0±264.9	1632.2±242.0	1679.0±270.9	1632.2±242.0
Weight/length of remnant intestine		47.8±5.0	48.7±5.5	52.7±4.1	67.4±5.3[e]
Cecum (mg)	724.9±61.3	628.2±90.4	620.8±130.2	548.0±147.5	608.6±80.3
Kidney (mg)	1747.2±265.7	1468.6±178.4	1485.9±208.2	1602.1±157.6	1408.1±62.6
Adrenal (mg)	54.5±8.2	42.7±11.4	48.0±8.9	47.1±3.8	44.1±7.7
Testis (mg)	2434.4±279.1	2385.1±164.8	2100.4±293.5	2383.2±397.8	2123.0±289.4
gastrocnemius muscles (mg)	2551.4±279.2[f]	2176.3±167.1	2141.9±353.6	2683.9±341.6[f]	2579.1±359.5[f]

[a]$P<0.05$ *vs* rats with rhGH+Gln and reference rats; [b]$P<0.05$ *vs* rats with rhGH and PN alone; [c]$P<0.05$ *vs* rats with PN alone,Gln-supplemented PN and rhGH+Gln; [d]$P<0.05$ *vs* rats with Gln-supplemented PN and reference rats; [e]$P<0.05$ *vs* rats with PN alone and Gln-supplemented PN; [f]$P<0.05$ *vs* rats with PN alone and Gln-supplemented PN.

Daily and accumulated nitrogen balance

Changes in daily nitrogen balance were shown in Figure 2. the improvements in daily NB was significantly higher in rats with rhGH and rhGH+Gln than those with PN alone and Gln-supplemented PN from day 4-6, $P<0.05$. the accumulated NB in rats with rhGH (1252.9±294.3 mg N/d) or rhGH+Gln (1261.7±85.5 mg N/d) was significantly greater than those with PN alone (704.8±379.0 mg N/d) and with Gln-supplemented PN (856.7±284.4 mg N/d), $P<0.05$. rhGH had significant effects on changes of NB, $P<0.01$, action of Gln was not significant statictically and there were no synergistic effects between rhGH and Gln.

Organ and tissue weights

The absolute weights of thymus, heart, lung, liver, spleen, stomach, remnant small intestine, cecum, kidney, adrenal, testis and gastrocnemius muscles are shown in table 3. Treatment with rhGH significantly increased the absolute weight of the gastrocnemius muscles compared to those not given rhGH, $P<0.01$, whereas Gln showed no significantly effect on the weight of this muscle tissue, $P>0.05$, there was no evidence of an additive effect of rhGH and Gln on the weight of gastrocnemius muscles. Although rhGH or Gln didn' t affected the absolute weight of the remnant small intestine, the ratio of weight/length was greater in rats treated with Gln, rhGH and rhGH+Gln than

those with PN alone (2-way ANOVA contrasting 4 experimental groups, $P=0.019$, 0.012 and 0.018 respectively), there were synergistic effects between rhGH and Gln in the increase of the weight/length of the remnant small intestine. Lung, liver and spleen weights were increased in rats treated with rhGH, others were not significantly affected by rhGH and/or Gln, $P>0.05$.

Serum GH and IGF-1 concentrations

Rats treated with rhGH or rhGH+Gln showed significantly higher serum concentrations of GH and IGF-1 than those treated without rhGH, $P<0.05$. Plasma GH and IGF-1 concentrations increased by 61 % and 42 % in rats with rhGH application respectively. There was no significant difference in concentrations of GH and IGF-1 between rats treated with PN alone and those treated with Gln-supplemented PN and between rats treated with rhGH and those treated with rhGH+Gln, $P>0.05$. Plasma GH and IGF-1 concentrations were unaffected by Gln administration. See Figure 3.
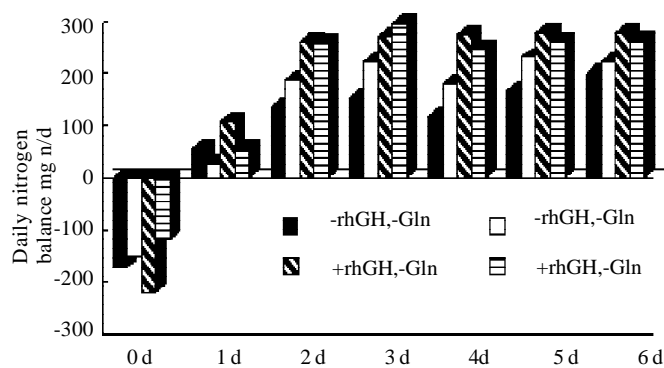

Figure 2 Daily changes of nitrogen balance in rats with PN
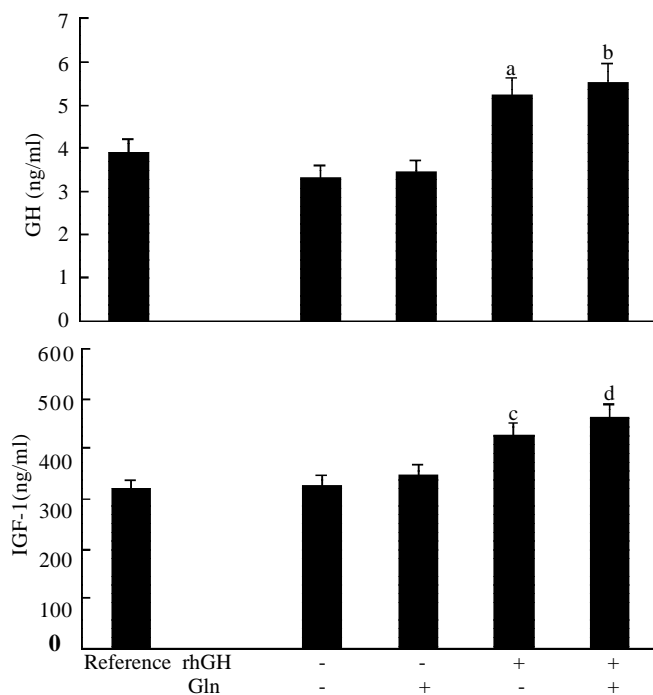

Figure 3 Concentrations in serum of GH and IGF-1 in rats maintained with PN and given rhGH and∕or Gln for 6 days after massive intestinal resection. [a]$P<0.05$ *vs* rats with Gln-

supplemented PN and reference rats; [b]$P<0.05$ *vs* rats with PN alone and rats with Gln-supplemented PN; [c]$P<0.05$ *vs* rats with PN alone and reference rats; [d]$P<0.05$ *vs* rats with PN alone, Gln-supplemented PN and reference rats.

DISCUSSION

Massive small bowel resection results in a marked decrease in the total surface area available for the absorption of luminal nutrients[14,15]. Affected patients often require long-term parenteral nutrition to provide adequate protein, energy and other essential nutrients to meet the needs of the body[16]. Although this approach attenuates protein losses, PN alone is often unable to increase or even maintain body protein[17], thus administration of rhGH and Gln have been of great interest in both of human and animal research in recent years[18-23]. Our experimental design provides new information about the relative anabolic and metabolic effects of rhGH *vs*. Gln and about interactive effects associated with simultaneous administration of these factors during PN.

Changes in body weight and nitrogen balance reflect the efficacy of PN[24]. We find in our study that PN alone doesn' t reserve the weight loss and nitrogen catabolism induced by short-bowel. Coinfusion of rhGH in short-bowel rats significantly reduced the weight loss and nitrogen catabolism as demonstrated by a significantly decrease in nitrogen excretion and significantly greater weight gain compared with rats without receiving rhGH, there is no significant difference between rats with rhGH and with rhGH+Gln. The anabolic effects of Gln in rats given PN without rhGH were less dramatic compared with rats with PN alone in terms of increased weight gain and decreased nitrogen excretion. Body composition analysis further confirmed that increases in carcass weights and nitrogen retention with rhGH were due to retention of carcass protein rather than fat or water retention. Gln had no significant effects on body composition. Our results demonstrated that the administration of rhGH could result in significant anabolic effects on body growth and improve the efficiency of PN.

GH is anabolic in protein metabolism[25-27]. Treatment with rhGH significantly increased the absolute weights of gastrocnemius muscles compared with those not given rhGH, whereas weights of thymus, heart, kidney, stomach, remnant small intestine, cecum, adrenal, testis were not affected by rhGH. Gln showed no significant effect on the weight of tissues mentioned above. This specific anabolic effects of rhGH may reflect differences in the distribution of GH receptors within various organs and tissues[28,29]. As skeletal muscle is the main effectors of GH action[11], stimulation of rhGH on skeletal muscle is more prominent compared to other organs. In our experiment, although we didn' t observe a rhGH and/or Gln effect on weights of small intestine, the ratio of weights/length of small intestine increased with rhGH and/or Gln administration, there is evidence of synergy, i.e., statistical interaction, between rhGH and Gln. Our studies of adaptation of the remnant small intestine in short-bowel rats also supported a synergistic response to rhGH and Gln[8]. Gln is the principal fuel utilized by the small intestine and is an essential precursor for purine and pyramiding synthesis required for DNA biosynthesis and cell division in the intestinal mucosa[30-32], Thus a constant supply of Gln to the intestinal mucosa in short-bowel is required and mandatory[33-35]. GH is another growth factor that may influence compensation of the small intestinal remnant[36-38]. Although opinions differ[39], GH is supposed to vigorously stimulate intestinal adaptation, it is suggested that GH takes part in the maintenance of the structure and function of the intestinal mucosa[40-42]. This study provides additional

evidence that simultaneous treatment with rhGH and Gln is more anabolic than administration of either rhGH or Gln alone in the remnant small intestine. In addition, in our study we find liver, spleen and lung weights increased with rhGH, but as the weight of these organs were easily affected by the deposited blood, further research is still needed to understand the effects of rhGH on these organs.

IGF-1 is an important peptide growth factor[43]. GH induces IGF-1 synthesis in the liver, which is thought to be the primary source of circulating IGF-1, and in many tissues with GH receptors including intestinal tissues[44-48]. IGF-1 has diverse regulatory functions, including stimulation of DNA and RNA synthesis, tissue amino acid uptake, and cellular protein accrual[49-51]. In our studies, circulating GH and IGF-1 concentrations were increased accordingly in rats treated with rhGH and with rhGH + Gln. However, supplemental Gln alone did not alter circulating GH or IGF-1 concentrations. Given the current experimental conditions, we may confirm in our study that the growth-promoting effects of rhGH are due to induction of IGF-1 production, enhanced IGF-1 synthesis contributes to the anabolic effects of rhGH.

In summary, our results indicate that treatment with rhGH during PN produced a whole body growth response that appeared to be mediated by greater concentrations of IGF-1, action of Gln is less dramatic, and there is no synergistic effect on the body anabolism between rhGH and Gln.

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Edited by Pagliarini R