

Original Paper

Effects of Exogenous Recombinant Human Growth Hormone on an Animal Model of Suboptimal Nutrition

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Key words: suboptimal nutrition, growth hormone, dosage, malnutrition, nutritional dwarfing

Background: Nutritional dwarfing, a form of suboptimal nutrition, has been identified as a frequent cause of short stature and delayed sexual development in children. Retarded growth is an adaptive response to suboptimal nutrition.

Objective: To assess whether recombinant human growth hormone (rhGH) may promote growth during various levels of suboptimal nutrition.

Methods: Using a previously developed rat model of suboptimal nutrition, six groups of rats (six rats/group) were fed a balanced 1:1 carbohydrate:fat ratio diet for 4 weeks. Three of the groups were administered daily injections of rhGH (0.1 mg/100 g BW) subcutaneously in the back while the other three groups were kept as controls and were given similar dosages of normal saline solution (NSS). Restricted rats within each treatment group were pair fed 80 and 60% of the ad-libitum rats intake. Daily intake of the 80 and 60% fed groups were determined based on the intake of the ad-libitum fed groups. Serum IGF-I and insulin were determined after 4 weeks of dietary treatment by radioimmunoassay while IGFBP-3 was determined by an immunoradiometric assay. Body composition was assessed in all rats by carcass analysis.

Results: After 4 weeks, total weight gain and tail growth were higher ($p < 0.05$) in the rhGH treated group at 80 and 60% of-libitum energy intake. Serum levels of IGF-I and IGFBP-3 were higher ($p < 0.05$) in rhGH treated rats fed at 60% of ad-libitum. In comparison to the NSS groups, administration of rhGH in rats fed ad-libitum increased total body water. Energy restriction caused decreased fat percentage ($p < 0.05$) in both rhGH and NSS groups without differences among treated groups.

Conclusion: These results suggest that the anabolic effects of rhGH may overcome mild to moderate energy restriction.

INTRODUCTION

Suboptimal nutrition in children is a common cause of short stature [1]. Patients with nutritional dwarfing (ND) show deteriorating linear growth that is preceded or accompanied by inadequate weight gain [2]. In developed countries energy and fat intake may be restricted for several reasons, including fear of obesity [3], eating disorders [4] and fear of hypercholesterolemia [5]. Other pathologic conditions such as Crohn's disease [6], celiac disease [7], acquired immunodeficiency syndrome, immunodeficiency virus infection [8] as well as any condition that causes malabsorption may also lead to ND [9]. In a previous study in children with inappropriate growth due to

suboptimal nutrition, the standard biochemical indices of malnutrition remained within normal limits, however, reduced erythrocyte Na^+ , K^+ -ATPase activity was found to be the only marker of adaptation to decreased nutrient intake [10,11].

Nutritional status and intake of dietary energy and protein are critical regulators of growth hormone (GH), insulin-like growth factor-I (IGF-I) and insulin-like growth factor binding protein (IGFBP) [12–14]. Children with ND have decreased spontaneous GH secretion during puberty and the response to GH-releasing hormone is increased before adolescence [15].

Using a rat model of suboptimal nutrition, erythrocyte Na^+ , K^+ ATPase activity as well as serum IGF-I concentrations decreased along with energy restriction [16]. Growth hormone

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secretory episodes and suppression of high amplitude GH bursts were associated with food deprivation [17]. Protein malnutrition caused failure to promote growth despite infusion of IGF-I [18]. Possible explanations for the association of growth retardation and IGF-I are down regulation of tissue GH receptors [19], decreased IGF-I gene expression [20], alterations in IGFBP's and resistance to the anabolic actions of IGF-I [21].

The present study was designed to evaluate if exogenous GH enhances growth in an animal model of suboptimal nutrition.

METHODS

Animals and Experimental Design

Thirty-six prepubertal 4-week old male Sprague Dawley rats were housed individually under controlled environmental conditions (22°C; 12-hour lighting cycle). Rats were given water ad-libitum. Daily weight and estimate of food intake were obtained for each animal. Rats were weighed between 9 and 10 a.m., thereafter either recombinant human growth hormone (rhGH) or normal saline solution (NSS) were injected (0.1 mg/100 g BW) subcutaneously in the back. Within each group, rats were fed a balanced purified 1:1 carbohydrate to fat diet at three different levels of nutrient intake (ad-libitum, 80% and 60%) corresponding to three different groups of rats (n=6 rats/group) diet. Each rat in the ad-libitum group was paired with one rat in the 80% and 60% groups. The percentages of metabolizable energy from fat, carbohydrate and protein were adapted from McCargar et al [22], and were assigned to approximate those of typical American diets. Protein and micro-nutrient levels were maintained at constant levels by increasing the concentration of these nutrients based on the estimated intake in rats fed at 80 and 60% of ad-libitum. Food fed to the restricted rats was calculated according to the amount of food consumed by the respective rats in the ad-libitum fed groups. The amount of food given to the rats paired at 80% and 60% of energy restriction was calculated as follows: (food consumed by the ad-libitum fed rat in the previous day/weight of this rat in the previous day) × (0.8 or 0.6, depending on the restriction level) × (the current weight of the rat for which the food is calculated). Food and caloric intake were recorded daily. Weekly tail length was measured from the hairline to the tip of the tail. At the conclusion of the experimental period, all rats were anesthetized with sodium Nembutal (30–50g/kg BW) and exsanguinated by cardiac puncture. The blood obtained was allowed to coagulate at room temperature. Serum was removed and stored at –20°C for later analysis for IGF-I, IGFBP-3 and insulin. Carcasses were frozen for future measurement of body composition.

The animal protocol used in this study was approved by the Institutional Animal Care and Use Committee and the Maimonides Medical Center Research Committee.

Growth Hormone

Recombinant human growth hormone was generously provided by Eli Lilly & Company (Indianapolis, IN). The powdered form of the hormone was diluted 1:1 with normal bacteriostatic saline solution.

Radioimmunoassay (RIA) for IGF-I and Insulin

Serum IGF-I concentrations were measured in duplicate by double antibody RIA [23]. To decrease the interference of IGF binding proteins, the samples were prepared by acid-ethanol extraction followed by cryoprecipitation. The sensitivity of this RIA was equal to 0.06 ng/ml. The intra-assay coefficient of variation was equal to 2.4 and 3% at concentrations of 0.5 and 0.9 ng/ml, respectively. The inter-assay variance at 0.5 ng/ml and 0.8 ng/ml were equal to 5.2 and 8.4%, respectively. Insulin levels in plasma were measured by a solid phase RIA. The sensitivity was 1.5 mIU/ml. The intra-assay variation were determined for the following concentrations at 18.2, 36.5 and 91.5 mIU/ml and were 8.2, 4.2 and 5.4%, respectively.

Measurement of IGFBP-3

IGFBP-3 was measured by a two site immunoradiometric assay (IRMA) as described by Powell et al [24]. The minimum detection limit was 0.5 ng/ml. According to the manufacturer of the assay kits the intra-assay precision at 82.7, 27.5 and 7.3 ng/ml were 1.8, 3.2 and 4%, respectively. The inter-assay precision at 80, 21 and 8 ng/ml were 1.9, 0.5 and 0.6%, respectively.

Body Composition

Body composition was determined by carcass analysis as reported previously [25], however, a brief description of the procedure is presented. The carcasses were thawed and autoclaved in a beaker of distilled water for 1.5 hours, ground, and homogenized. Samples of the homogenate were stored at 4°C for analysis. All determinations were done gravimetrically in triplicate, using 1 g of homogenate per rat. Total body water was determined gravimetrically in triplicate using 1 g of homogenate for each rat. Weight of crucibles utilized for determinations were obtained after overnight drying in vacuum at 60°C. The next day, dry mass weights was recorded. The percentage of water was calculated as follows $1 - \frac{\text{dried homogenate weight}}{\text{water} + \text{carcass weight}} \times 100$. Fat mass was determined gravimetrically in triplicate using 1 g of homogenate for each rat. Then, 1 ml of methanol was added to dissolve polar substances. After 30 minutes, 14 ml of chloroform were added to solubilize all fats and allow to sit overnight. The solutions were then filtered using Whatman #1 filter paper into the corresponding pre-weighed centrifuge tubes. The tubes were centrifuged at 2300 g for 10 minutes and the methanol layer removed. After drying overnight in a vacuum oven at 60°C all tubes were weighed. The percentage of

fat was calculated based in following equation: [(fat weight/homogenate weight) × (water weight+carcass weight)/carcass weight] × 100.

Calculations

For each rat in each treatment group, weekly averages were determined for caloric intake, weight gain and food efficiency. Caloric intake was calculated by multiplying the amount of food consumed by the caloric density of the diet while food efficiency was calculated by dividing daily food intake by body weight gain. This ratio was then multiplied by 100. Total weight gain was calculated for each rat as the amount of weight gained above the previous day’s body weight.

Statistical Analysis

Statistical comparisons were analyzed by Student’s t-test. Data were determined to be significant at the 5% level of probability. All data were expressed as mean±standard deviation.

RESULTS

At the same level of energy intake, caloric intake corrected per 100 g of body weight was similar in both recombinant human growth hormone (rhGH) and normal saline solution

(NSS) treated rats (Table 1). Food efficiency showed different results during the study. Initially, during the first week of treatment, it increased (p<0.05) in rhGH-treated rats fed at ad-libitum but these effects were not seen thereafter. In contrast, in the restricted treatment groups, there were more profound differences. In those given 80% of energy intake the food efficiency was improved during the first and third week of treatment. Rats fed at 60% of ad-libitum intake increased (p<0.05) food efficiency when treated with rhGH as compared to NSS rats during the second and third weeks (Table 1).

The effect of exogenous rhGH or NSS on total weight gain and tail growth over the course of the experiment is shown in Fig. 1 and 2. Since the first week of the study, rhGH treated rats at 80% of ad-libitum energy intake showed a 20.5% increase (p<0.01) in total weight gain vs. the NSS group. The cumulative weight gain at 4 weeks remained 19.5% greater in the rhGH vs. the NSS groups. After the third week of the study, the cumulative weight gain was 59.6% greater (p<0.05) in the rhGH treated rats at 60% of ad-libitum energy intake in comparison to the NSS fed rats. Rats fed ad-libitum showed a difference (p<0.05) in weight gain only during the first week of the study (Fig. 1).

Since the first week of the study, rats fed at 80% ad-libitum showed increased cumulative tail growth of 47.5% in comparison to NSS fed rats. However, in rats fed at 60% of ad-libitum intake, the effects of rhGH on tail growth appeared only at the end of the study (Fig. 1).

Table 1. Dietary and Metabolic Results in Rats Administered rhGH and NSS (0.1 mg/100 g body weight) after 4 Weeks on a 1:1 CHO:FAT Diet

%EI	rhGH	NSS	%Δ	rhGH	NSS	%Δ
	Week 1			Week 2		
	Caloric Intake (kcal/day)					
100	62.0±4.1*	59.4±3.7	-4.2	46.0±5.9	47.2±2.9	2.6
80	39.0±4.8	38.3±2.4	-1.8	27.0±4.9	29.0±1.6	7.4
60	22.0±1.2	21.4±1.3	-2.7	15.0±2.7	16.4±0.6	9.3
	Food Efficiency (%)					
100	9.5±0.5 ^a	8.3±0.5	-12.6	8.1±1.0	8.6±0.8	6.1
80	19.0±9.8 ^b	10.0±1.6	-47.3	12.5±3.1	11.0±2.3	-8.3
60	12.0±1.5	11.0±2.9	-8.3	12.7±4.8 ^c	7.0±2.9	-44.8
	Week 3			Week 4		
	Caloric Intake (kcal/day)					
100	38.0±1.7	39.0±3.8	2.6	28.7±0.9	30.7±2.4	7.0
80	24.6±1.3	26.0±0.9	5.7	18.0±9.0	18.0±1.6	0.0
60	13.7±0.6	15.2±0.7 ^a	10.9	10.4±0.5	10.6±0.6	1.9
	Food Efficiency (%)					
100	8.5±1.6	11.0±7.5	29.4	8.7±1.3	8.8±1.4	1.1
80	11.0±2.5 ^b	8.6±1.6	-21.8	13.5±3.5	12.3±1.5	-8.9
60	13.5±7.0 ^c	3.5±3.3	-74.0	15.8±6.2	13.0±4.4	-17.7

^{a-c} p<0.05; Δ%=100-(value for NSS*100/value for rhGH).

% EI=percent energy intake.

* Data are presented as mean±standard deviation.

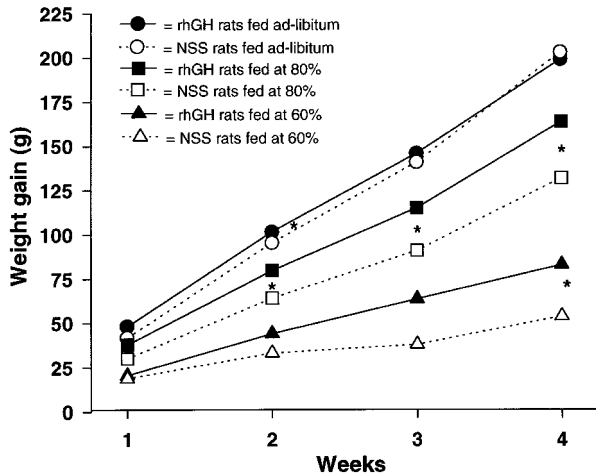


Fig. 1. Body weight gain over the course of the 4-week experiment. The asterics represents a difference ($p < 0.05$) between rhGH and NSS treatment groups at that particular week in the experiment.

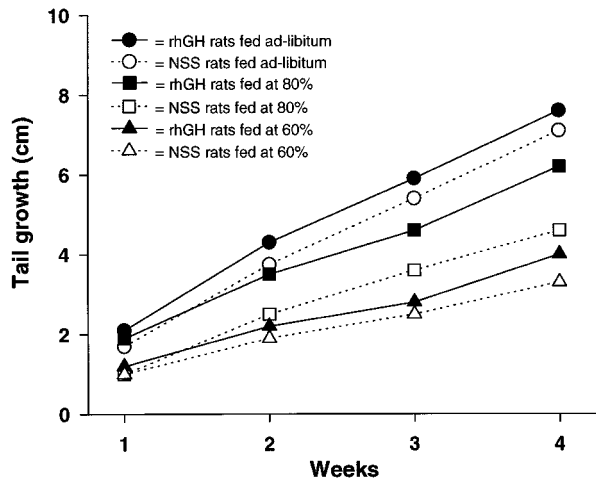


Fig. 2. Tail growth over the course of the 4-week experiment. The asterics represents a difference ($p < 0.05$) between rhGH and NSS treatment groups at that particular week in the experiment.

Biochemical changes in serum were assessed after 4 weeks of treatment (Table 2). In the rhGH treated rats at 60% energy intake serum IGF-I and IGFBP3 were higher than similarly fed NSS treated rats. This same effect was observed for IGFBP-3

Table 2. Serum IGF-I, IGFBP-3 and Insulin in Rats Administered rhGH and NSS (0.1 mg/100 g body weight) after 4 Weeks on a 1:1 CHO:FAT Diet

Energy intake	IGF-1 (ng/ml)		IGFBP-3 (ng/ml)		Insulin (mUI/ml)	
	rhGH	NSS	rhGH	NSS	rhGH	NSS
100%	882±310*	991±188	743±124	580±249	21.4±7.0	21.7±5.4
80%	1039±173	762±275	588±117 ^b	240±110	14.4±2.0	18.2±4.2
60%	485±188 ^a	183±34	337±166 ^b	101±22	5.1±1.3	3.4±2.3

^{a-b} $p < 0.05$.

* Data are presented as mean±standard deviation.

in rats fed at 80% of ad-libitum energy intake. Insulin levels were reduced in both treatment groups fed at 80 and 60% of ad-libitum energy intake.

Body Composition

In comparison to rats fed ad-libitum, fat percentage decreased ($p < 0.05$) in rats fed at 80 and 60% of ad-libitum energy intake within both the rhGH and NSS groups. However, no differences were found between rats treated with rhGH and NSS within the same level of energy restriction (Table 3). In comparison to ad-libitum fed NSS rats, total body water was increased in rats treated with rhGH ($p < 0.05$). However, total body water was reduced in rats treated with rhGH and fed at 60% of energy intake in comparison to the similarly fed NSS group.

DISCUSSION

In this study, the effect of exogenous recombinant human growth hormone (rhGH) was evaluated using a previously developed animal model of suboptimal nutrition [16]. A pharmacological dose of rhGH (0.1 mg/100 g body weight) was selected to overcome the possible growth hormone resistance due to structural differences in growth hormone receptors in rats [26]. This model, unlike previous protein-energy malnutrition rat models [27–29] permits manipulation of energy restriction while maintaining adequate intake of protein and other nutrients. Using this new animal model, rats fed at 60 and 80% of ad-libitum intake had less weight gain, tail growth, insulin-like growth factor-I (IGF-I) and erythrocyte-Na+K+ ATPase activity [16].

It has been reported that inadequate food consumption could result in nutritional deficits that interfere with normal growth and sexual maturation [1,2]. However, growth is not totally inhibited nor does the patient require hospitalization as in protein-calorie mal-nutrition. Spontaneous overnight growth hormone secretion is attenuated in nutritional dwarfing patients during puberty and their growth hormone response to growth hormone releasing hormone is increased before adolescence. This may represent a compensatory mechanism to energy restriction [15].

Table 3. Body Composition in Rats Administered rhGH and NSS (0.1 mg/100 g body weight) after 4 Weeks on a 1:1 CHO:FAT Diet

Energy intake	Body fat (%)		Fat-free mass (%)		Total body water (%)	
	rhGH	NSS	rhGH	NSS	rhGH	NSS
100%	11.7±1.2*	11.5±1.5	88.3±7.5	88.5±1.5	67.4±2.1	60.4±2.7 ^a
80%	10.4±1.1	11.7±1.9	90.0±1.1	88.3±1.9	68.8±5.3	67.0±4.0
60%	6.9±0.8	7.4±1.3	93.0±0.8	92.6±10.9	64.9±2.6	69.6±2.9 ^a

^a p<0.05.

* Data are presented as mean±standard deviation.

Administration of rhGH showed increased weight gain and tail growth in rats fed at 80 and 60% of ad-libitum. The results of this study support evidence already available that growth hormone could exert its anabolic effects in the presence of hypocaloric intake and catabolic states [30]. Other studies had failed to demonstrate the growth-promoting effects based on the presence of growth hormone receptor resistance in animal models of protein-caloric malnutrition [26]. This may be due to reduced efficiency of rat growth hormone in comparison to higher doses of rhGH in rats during severe protein restriction. In this study we maintained an adequate protein intake overriding protein malnutrition. In agreement with others [31,32] growth hormone exerts some of its anabolic effects through IGF-I, which is believed to be one of the principal stimulators of balanced somatic growth [33–35]. In this study IGF-I levels were higher in recombinant human growth hormone treated rats fed 60% of ad-libitum energy intake compared with moderately restricted and non-restricted fed rats. Changes in serum IGF-I and insulin growth factor binding protein-3 (IGFBP-3) may be adaptive to caloric restriction in children [36]. It has been demonstrated that protein, not energy restriction, causes growth hormone resistance by a different mechanism and serum IGF-I concentrations are not restored to normal by daily injections (400 µg/100 g body weight) of growth hormone. Growth hormone given intermittently in the absence of growth-promoting effects suggests that nutritional sufficiency is essential for IGF-I to promote growth [26].

Growth hormone has different effects on body composition through its anabolic, lipolytic and antinatriuretic actions. However, differences in subject population, nutritional status, duration of growth hormone treatment, or the dose of growth hormone employed might explain the varied effects on body composition [30,37,38]. A reduction in fat percentage was found with a decrease in energy intake. However, there were no differences in fat percentage in rats treated with growth hormone compared with rats treated with normal saline. It is possible that the lipolytic effect of growth hormone is less evident under conditions of suboptimal nutrition [39,40]. Even though there were no differences in weight gain between both treatment groups in rats fed ad-libitum, the greater amount of total body water in rats treated with growth hormone may be

due to the antinatriuretic effect of growth hormone. Furthermore, the short duration of the study may also account for the minimal changes we observed in body composition.

In summary, growth hormone may have important clinical uses in children with suboptimal nutrition. Growth hormone administration may override mild to moderate reductions in growth associated with energy restriction. Based on these findings, future studies using this model will investigate the effects of lower amounts of growth hormone and the impact of different ratios of carbohydrate and fat.

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