

Original Article

The effects of growth hormones on the growth velocities and serum index expressions in short stature children

Ping Zhou¹, Qing Lv²

¹Department of Child Health, Maternal and Child Hospital of Hubei Province, Wuhan 430070, Hubei, China; ²Department of Pediatrics, Shenzhen University General Hospital, Shenzhen 518055, China

Received February 22, 2021; Accepted April 12, 2021; Epub July 15, 2021; Published July 30, 2021

Abstract: Objective: To study the effect of recombinant human growth hormone (rhGH) treatment on the growth velocities and serum index expressions of short stature children. Methods: 56 short stature children admitted to our hospital from January 2018 to January 2020 were recruited as the study cohort. All the children were treated with rhGH. After six months of treatment, their serum indicators [ghrelin, Nesfatin-1, bone-specific alkaline phosphate (BAP), insulin-like growth factor 1 (IGF-1)], their growth velocity indicators [body mass index (BMI), height, growth velocity (GV)], their blood lipid levels [triglycerides (TG), total cholesterol (TC), low density lipoprotein (LDL), high and low density lipoprotein (HDL)], their insulin statuses [fasting insulin (FINS), their homeostasis model assessment of insulin resistance (HOMA-IR)], and their thyroid function index [thyroid stimulating hormone (TSH), 3'-triiodothyronine (T3), 4'-triiodothyronine (T4)] level changes before and after the treatment were compared. Results: (1) After the treatment, the children's serum ghrelin and IGF-1 levels increased in comparison with their pre-treatment levels ($P < 0.05$), and their nesfatin-1 levels decreased ($P < 0.05$). (2) After the treatment, the children's BMI, height, and GV increased in comparison with their pre-treatment levels ($P < 0.05$). (3) After the treatment, the children's TG levels were noticeably higher than they were before the treatment ($P < 0.05$), and the TC and LDL levels were remarkably lower than they were before the treatment ($P < 0.05$). (4) After the treatment, the children's T3 and T4 levels were significantly higher compared to their levels before the treatment ($P < 0.05$). Conclusion: GH can promote the development and growth of short stature children, improve their related serum indicator levels, and does not induce metabolic dysfunction.

Keywords: Short stature, growth hormone deficiency, idiopathic short stature, growth velocity, metabolic function

Introduction

Short stature children are children whose heights are considerably below average by at least two standard deviations compared to the heights of their peers, who have bone ages below the actual age by over two years, and whose annual growth velocity is less than 5 cm [1-3]. Short stature children present with symptoms such as short stature, slow growth velocity, abnormal metabolic function, and delayed bone maturation [4, 5]. The currently-known causes of short stature are a lack or a deficiency of growth hormone (GH), abnormal endocrine metabolism, genetics, etc. [6-8]. Short stature can cause serious adverse effects on the growth and development of children. As short stature children grow older, they can face multiple physical and psychological blows.

Ghrelin is an endogenous substance that is mainly synthesized by X/A-like cells in the fundus of the stomach and that regulates the secretions of GH from the pituitary gland by acting on the pituitary gland.

Ghrelin plays a key role in the release of GH it can stimulate the appetite, and it can regulate the energy balance, the gastric acid secretions, and the gastric motility. Nesfatin-1 is a type of secreted peptide, and it is widely distributed in the central nervous system of the human brainstem, hypothalamus, pancreatic islets, and peripheral gastrointestinal tissues, and it can regulate the feeding response function. Nesfatin-1 is related to insulin resistance and glucose metabolism dysfunction. Recombinant human growth hormone (rhGH) is commonly used for the treatment of short stature. In light

Growth hormones and short stature children

of the above findings, we conducted this research in order to objectively evaluate the curative effect of rhGH on the Nesfatin-1, ghrelin, and various endocrine and metabolism levels of short stature children.

Materials and methods

Subjects

This study was conducted with a cohort of 56 short stature children admitted to our hospital from January 2018 to January 2020. The diagnostic criteria we used were in accordance with the *Guidelines for the Diagnosis and Treatment of Children with Short Stature* and the recommendations on the clinical application of genetic recombinant human growth hormone in pediatrics [9, 10]. Inclusion criteria: ① Children who met the above-mentioned diagnostic criteria for short stature and the requirements for growth hormone treatment, ② Children 2 to 10 years old, ③ Informed consents were obtained from the children and their parents. Exclusion criteria: ① Children with congenital organic diseases, ② Children with thyroid disease, bone metabolism disease, or glucose and lipid metabolism diseases, ③ Children with severe malnutrition, ④ Children with heart, lung, liver, or kidney insufficiencies, ⑤ Children with a bone age over 16 years old. Approval for this study was granted by the ethics committee of our hospital. Among the enrolled children, 35 were males and 21 were females. Their ages ranged from 3 to 10 years old, with an average of (6.61 ± 2.01) years. Their symptoms included 37 cases of growth hormone deficiency (GHD) and 19 cases of idiopathic short stature (ISS).

Methods

All the children were treated with rhGH. RhGH (S20030064) was given 0.1 IU/kg·d using subcutaneous injection at 0.5 h before going to bed in the evening around the umbilical cord. Six months was considered the course of treatment.

Observation indicators

After 6 months of treatment, the children's serum indicators [ghrelin, Nesfatin-1, bone-specific alkaline phosphate (BAP), insulin-like growth factor 1 (IGF-1)], their growth velocity indicators [body mass index (BMI), height, growth velocity (GV)], their blood lipid levels [tri-

lycerides (TG), total cholesterol (TC), low density lipoprotein (LDL), high and low density lipoprotein (HDL)], their insulin status [fasting insulin (FINS), homeostasis model assessment of insulin resistance (HOMA-IR)], and their thyroid function index [thyroid stimulating hormone (TSH), 3'-triiodothyronine (T3), 4'-triiodothyronine (T4)] level changes before and after the treatment were compared.

Before and after the treatment, 3 ml of fasting cubital venous blood samples were collected from all the children in the morning and equally divided into 2 parts. One part was placed in a centrifuge and processed at 3000 r/min for 10 min to obtain the serum for testing. Their ghrelin, nesfatin-1, BAP, and IGF-1 levels were measured using the immunoenzyme-linked adsorption method. Their FINS levels were measured using radioimmunoassays, and their thyroid function indicators were measured using the chemotherapy luminescence method. The other one used an automatic biochemical analyzer to measure their blood lipid index levels. $HOMA-IR = FINS \times \text{fasting blood glucose} \div 22.5$. The heights of all the children were measured every three months, and their height was calculated as the difference between the two heights/3×12.

Statistical analysis

SPSS 23.0 software was used as the statistical tool and GraphPad prism 8.0 was used as the drawing tool. The measurement data used paired t tests and were expressed in the $(\bar{x} \pm s)$ format. $P < 0.05$ was considered statistically significant.

Results

Serum indicators

After the treatment, the serum ghrelin and IGF-1 levels of the children were increased in comparison with their levels before the treatment ($P < 0.05$), and the nesfatin-1 were decreased ($P < 0.05$). However, no significant difference was noticed in their BAP levels before and after the treatment ($P > 0.05$). See **Table 1**.

Growth velocity index

Before the treatment, the BMI, height, and GV of the children were (18.58 ± 2.84) kg/m²,

Growth hormones and short stature children

Table 1. Comparison of serum index levels in the children before and after the treatment ($\bar{x} \pm s$)

Time	n	Ghrelin (pg/ml)	Nesfatin (pg/ml)	BAP (U/L)	IGF-1 (μ g/L)
Before treatment	56	3099.39 \pm 90.34	119.73 \pm 27.55	199.53 \pm 15.88	94.88 \pm 21.25
After treatment	56	3895.07 \pm 152.78	57.95 \pm 13.10	201.09 \pm 14.57	135.8 \pm 19.32
t		32.438	15.720	0.572	9.946
p		<0.001	<0.001	0.570	<0.001

Table 2. Comparison of the growth velocity indexes in the children before and after the treatment ($\bar{x} \pm s$)

Time	n	BMI (kg/m ²)	Height (cm)	GV (cm/year)
Before treatment	56	18.58 \pm 2.84	87.96 \pm 5.38	3.78 \pm 0.42
After treatment	56	20.09 \pm 2.22	96.15 \pm 7.44	12.05 \pm 2.12
t		3.059	7.073	29.194
p		0.003	<0.001	<0.001

Table 3. Comparison of blood lipid levels in the children before and after the treatment ($\bar{x} \pm s$)

Time	n	TG	TC	HDL	LDL
Before treatment	56	0.83 \pm 0.17	4.57 \pm 0.78	1.78 \pm 0.38	2.67 \pm 0.66
After treatment	56	1.09 \pm 0.32	4.05 \pm 0.71	1.71 \pm 0.55	2.17 \pm 0.66
t		5.951	3.686	0.795	4.500
p		<0.001	0.001	0.430	<0.001

(87.96 \pm 5.38) cm, and (3.78 \pm 0.42) cm/year. After the treatment, the above indicators were (20.09 \pm 2.22) kg/m², (96.15 \pm 7.44) cm, and (12.05 \pm 2.12) cm/year, so they were significantly increased in comparison with their pre-treatment levels (P<0.05). See **Table 2**.

Blood lipid levels

After the treatment, the TG levels in the children were noticeably higher than they were before the treatment (P<0.05), and the TC and LDL levels were remarkably lower than they were before the treatment (P<0.05). And no notable difference was identified in the children's HDL levels before and after the treatment (P>0.05), as shown in **Table 3**.

Insulin status

Before the treatment, the children's FINS and HOMA-IR levels were (11.08 \pm 2.60) μ U/mL and (2.43 \pm 0.74), and after the treatment, the children's FINS and HOMA-IR levels were (11.89 \pm 3.29) μ U/mL and (2.45 \pm 0.93) (P>0.05, **Figure 1**).

Thyroid function indicators

After the treatment, the children's T3 and T4 levels were significantly higher compared to their pre-treatment levels (P<0.05), and we observed no statistical difference in the children's TSH levels before and after the treatment (P>0.05, **Table 4**).

Discussion

Various indicators such as health levels, genetics, nutritional status, and other aspects can comprehensively reflect children's development. Human growth and final height are mainly affected by the synergistic effect of many factors such as height at birth, body weight, heredity, hormone levels, nutritional status, and the like. Short stature children suffer from growth retardation, and their heights and weights are below the averages of healthy children. The mechanism of the occurrence of short stature remains unclear, but it is known to be related to the state of the endocrine axis composed of the hypothalamus, the growth hormones, the IGF-1 levels, and the defects in the feedback transmission pathway in children [11, 12]. IGF-1 is a polypeptide protein, also known as growth-promoting factor, and its molecular structure is similar to insulin [13, 14]. IGF-1 plays an important role in the growth of infants and children and the continuous anabolic process in adults. Most short stature children have GH gene mutations, and exhibit a GH deficiency or reduced GH activity [15, 16]. Insufficient secretions of IGF-1 and GH and resistance to IGF-1 in the peripheral tissues can cause endocrine axis dysfunction and induce short stature. Ghrelin is a growth hormone releasing peptide

Growth hormones and short stature children

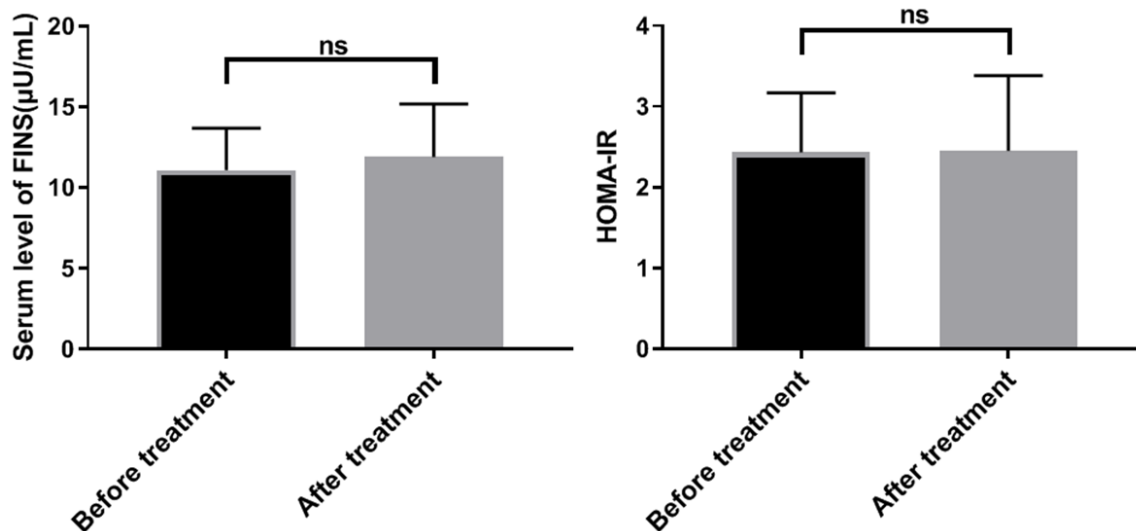


Figure 1. Comparison of the insulin indicators before and after the treatment. ns indicated $P > 0.05$.

Table 4. Comparison of the thyroid function indexes in the children before and after the treatment ($\bar{x} \pm s$)

Time	n	T3 (pmol/L)	T4 (pmol/L)	TSH (mU/L)
Before treatment	56	5.34±1.50	13.13±4.13	4.54±1.15
After treatment	56	6.13±0.32	17.07±3.02	4.74±1.69
t		4.064	5.664	0.684
p		<0.001	<0.001	0.497

containing 28 amino acids. The concentration of ghrelin in the blood gradually rises starting from birth, peaking at around 2 years old, and then it begins to decrease. In the late stage of puberty, the concentration of blood ghrelin can be reduced by approximately 30% to 50%, and the level of ghrelin during this period is negatively correlated with human age. Ghrelin is also an endogenous ligand of the growth hormone secretagogue receptor (GHS-R), which can promote the release of GH after binding to GHS-R, and exert the regulatory mechanism of the GH-IGF growth promoting axis [17]. The binding of ghrelin and GHS-R can stimulate the pituitary gland to secrete growth hormone release inhibitory hormone (GHRH) and inhibit the secretions of GHRH from the hypothalamus through a negative feedback mechanism, thereby increasing the activity of GH. Ghrelin also plays a regulatory role in bone development, promoting food intake, and maintaining energy balance in the body. Nesfatin-1 is a polypeptide produced by the hydrolysis of the amino terminus of nuclear histone 2 and con-

tains 82 amino acids. Nesfatin-1 is widely distributed in human cells, and it can inhibit food intake, reduce body fat content, and increase body energy consumption [18]. The nesfatin-1 levels in the serum of short stature children are mostly high, so the energy balance in the children's body is disturbed wherein energy is excessively consumed, and the body fat content is too low [19]. For

this reason, it hardly meets the needs of the children's normal growth, metabolism and nutritional needs, so they eventually develop a short stature.

BAP is a special marker that reflects the number and activity of osteoblasts. It is used in clinical practice, and it is also the most commonly-used indicator for evaluating bone formation and bone turnover. It has been stated that rhGH is an important way to treat short stature, but its efficacy and safety need to be further studied and analyzed [20].

We found that the children's serum ghrelin and IGF-1 levels increased after the treatment, and their nesfatin-1 levels decreased, suggesting that the rhGH treatment can effectively improve the ghrelin and IGF-1 levels, promote the secretions of GH and improve its activity. Meanwhile, the decreased nesfatin-1 levels can reduce the excessive energy consumption to promote the growth and development of the children. Furthermore, showing no difference in the BAP levels before and after the treatment indicates

that the application of rhGH will not cause any abnormal changes in the osteoblasts and their activity, and it also shows that the six-month treatment cycle is not enough to yield positive bone quality changes in children, and it needs to be further observed by prolonging the course of treatment. After the treatment, the children's BMI, height, and GV increased, suggesting that rhGH treatment can promote the growth and development of short stature children. In terms of blood lipids, the TG levels of the children after the treatment were increased compared with their pre-treatment levels, suggesting that the application of rhGH increases the TG level to meet or partially meet the growth and development needs of the child. In the meantime, the TC and LDL levels decreased compared to before the treatment, suggesting the application of rhGH can boost the availability of blood lipids and improve the blood lipid levels of children. However, the HDL level did not increase but was within the normal range, indicating that the rhGH treatment will not cause a reduction in HDL, and that it is safe and feasible in terms of the blood lipid levels. After the treatment, there was no statistical difference in the insulin status or the TSH levels of the children, but their T3 and T4 levels increased. This suggests that the application of rhGH treatment will not trigger serious abnormal changes in insulin secretions or thyroid function. There were some drawbacks to this study that should be mentioned. It was an observational study with a small cohort. There were confounding factors such as time and development. A randomized controlled study should be conducted to confirm this conclusion further.

To conclude, GH therapy is expected to be a preferable option for short stature children to boost their growth velocity and to improve their serum related indicator expression levels, thereby regulating their own GH secretion levels. The therapy causes no endocrine or metabolic disorders.

Disclosure of conflict of interest

None.

Address correspondence to: Ping Zhou, Department of Child Health, Maternal and Child Hospital of Hubei Province, Wuhan 430070, Hubei, China. Tel: +86-13628686200; E-mail: zhouping2021@163.com

References

- [1] Grimberg A and Allen DB. Growth hormone treatment for growth hormone deficiency and idiopathic short stature: new guidelines shaped by the presence and absence of evidence. *Curr Opin Pediatr* 2017; 29: 466-471.
- [2] Aguiar-Oliveira MH and Bartke A. Growth hormone deficiency: health and longevity. *Endocr Rev* 2019; 40: 575-601.
- [3] Chinoy A and Murray PG. Diagnosis of growth hormone deficiency in the paediatric and transitional age. *Best Pract Res Clin Endocrinol Metab* 2016; 30: 737-747.
- [4] Richmond E and Rogol AD. Treatment of growth hormone deficiency in children, adolescents and at the transitional age. *Best Pract Res Clin Endocrinol Metab* 2016; 30: 749-755.
- [5] Child CJ, Quigley CA, Cutler GB Jr, Moore WV, Wintergerst KA, Ross JL, Rosenfeld RG and Blum WF. Height gain and safety outcomes in growth hormone-treated children with idiopathic short stature: experience from a prospective observational study. *Horm Res Paediatr* 2019; 91: 241-251.
- [6] Al Shaikh A, Daftardar H, Alghamdi AA, Jamjoom M, Awidah S, Ahmed ME and Soliman AT. Effect of growth hormone treatment on children with idiopathic short stature (ISS), idiopathic growth hormone deficiency (IGHD), small for gestational age (SGA) and Turner syndrome (TS) in a tertiary care center. *Acta Biomed* 2020; 91: 29-40.
- [7] Yuen KCJ, Llahana S and Miller BS. Adult growth hormone deficiency: clinical advances and approaches to improve adherence. *Expert Rev Endocrinol Metab* 2019; 14: 419-436.
- [8] Molitch ME, Clemmons DR, Malozowski S, Merriam GR and Vance ML; Endocrine Society. Evaluation and treatment of adult growth hormone deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2011; 96: 1587-609.
- [9] Pozzobon G, Partenope C, Mora S, Garbetta G, Weber G and Barera G. Growth hormone therapy in children: predictive factors and short-term and long-term response criteria. *Endocrine* 2019; 66: 614-621.
- [10] He X and Barkan AL. Growth hormone therapy in adults with growth hormone deficiency: a critical assessment of the literature. *Pituitary* 2020; 23: 294-306.
- [11] Morrison M. Growth hormone, enhancement and the pharmaceuticalisation of short stature. *Soc Sci Med* 2015; 131: 305-12.
- [12] Derrick KM, Gomes WA and Gensure RC. Incidence and outcomes of pituitary microadenomas in children with short stature/growth hor-

Growth hormones and short stature children

- mone deficiency. *Horm Res Paediatr* 2018; 90: 151-160.
- [13] Kang MJ, Kim EY, Shim YS, Jeong HR, Lee HJ, Yang S and Hwang IT. Factors affecting bone age maturation during 3 years of growth hormone treatment in patients with idiopathic growth hormone deficiency and idiopathic short stature: analysis of data from the LG growth study. *Medicine (Baltimore)* 2019; 98: e14962.
- [14] Loche S, Di Iorgi N, Patti G, Noli S, Giaccardi M, Olivieri I, Ibba A and Maghnie M. Growth hormone deficiency in the transition age. *Endocr Dev* 2018; 33: 46-56.
- [15] Castagno M, Monzani A, Zanetta S, Genoni G, Giglione E, Ricotti R, Bona G, Prodham F and Bellone S. Evaluation of growth hormone response to GHRH plus arginine test in children with idiopathic short stature: role of peak time. *J Endocrinol Invest* 2018; 41: 977-983.
- [16] Hu Y, Liu X, Chen X, Chen T, Ye P, Jiang L, Fu Y, Xie X, Shan X and Yan Z. Differences in the functional connectivity density of the brain between individuals with growth hormone deficiency and idiopathic short stature. *Psychoneuroendocrinology* 2019; 103: 67-75.
- [17] Jensen EA, Young JA, Jackson Z, Busken J, List EO, Carroll RK, Kopchick JJ, Murphy ER and Berryman DE. Growth hormone deficiency and excess alter the gutmicrobiome in adult male mice. *Endocrinology* 2020; 161: bqaa026.
- [18] Bullinger M, Kołtowska-Häggström M, Sandberg D, Chaplin J, Wollmann H, Noeker M and Brütt AL. Health-related quality of life of children and adolescents with growth hormone deficiency or idiopathic short stature - part 2: available results and future directions. *Horm Res* 2009; 72: 74-81.
- [19] Isgaard J and Cittadini A. Growth hormone and the heart in growth hormone deficiency-what have we learned so far? *Endocrine* 2017; 55: 331-332.
- [20] Brütt AL, Sandberg DE, Chaplin J, Wollmann H, Noeker M, Kołtowska-Häggström M and Bullinger M. Assessment of health-related quality of life and patient satisfaction in children and adolescents with growth hormone deficiency or idiopathic short stature - part 1: a critical evaluation of available tools. *Horm Res* 2009; 72: 65-73.