Changes in ghrelin and nesfatin-1 in children with growth hormone deficiency treated by recombinant human growth hormone

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Abstract
This study aims to investigate the effects of recombinant human growth hormone (rhGH) on serum nesfatin-1 and ghrelin in children with growth hormone deficiency (GHD), in order to provide a reliable basis for the effectiveness and safety of applying rhGH in treating GHD children in the clinic. A total of 30 GHD pediatric patients were selected as the observation group. According to the peak of GH, these patients were divided into two subgroups: complete absence of growth hormone (CGHD) group and partial absence of growth hormone (PGHD) group. At the same time, 20 healthy children of normal height with matching age and gender were randomly selected as a normal control group. Serum ghrelin and nesfatin-1 levels were detected in children in the control group and observation group before rhGH treatment, and at 3 and 6 months after treatment. After 3 and 6 months of treatment, the height and growth rate of children in the PGHD and CGHD groups significantly increased (P < 0.05), but their body weights did not significantly change (P > 0.05), compared with those before treatment. Before treatment, ghrelin was higher in the PGHD group than in the control group, while ghrelin was lower in the CGHD group than in the control group. In addition, nesfatin-1 was higher in these two subgroups, compared with that in the control group. At pretreatment, and after 3 and 6 months of treatment, ghrelin and nesfatin-1 both decreased in the PGHD group, while ghrelin increased and nesfatin-1 decreased in the CGHD group. It was confirmed that ghrelin and nesfatin-1 were closely correlated with GHD. Furthermore, rhGH has a significant effect on children with GHD, and can significantly accelerate the annual growth rate.

Keywords
ghrelin, growth hormone deficiency, nesfatin-1, recombinant human growth hormone

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Introduction
Growth hormone deficiency (GHD) is one of the main causes of short stature in children. This disease is a growth and development disorder caused by the abnormal molecular structure of growth hormone (GH) and receptor, or the partial or complete deficiency of the secretion or synthesis of GH.¹ Ghrelin is an endogenous ligand of growth hormone secretin receptor (GHS-R), which can regulate the secretion of adenohypophysis by binding to the GH releasing hormone receptor, and promote the release of GH. Nesfatin-1 is a new satiety gene that was discovered in recent years. Its role is to extend the interval between meals and reduce food intake, inducing satiety to inhibit night feeding. Nesfatin-1 and
ghrelin are both involved in the regulation of the hypothalamus-pituitary-gonad axis, energy metabolism, feeding, and insulin secretion and the target organs are similar. It has been speculated that these two are closely correlated to GHD. However, at present, the effect of recombinant human growth hormone (rhGH) on nesfatin-1 and ghrelin after treatment for GHD has not been reported. In this study, the changes in serum nesfatin-1 and ghrelin levels in GHD children before and after rhGH treatment, and the correlation between these two were observed. Furthermore, the effect of rhGH on serum nesfatin-1 and ghrelin in GHD children was explored, in order to provide reliable evidence for the clinical efficacy and safety of rhGH in treating GHD children.

Materials and methods

Study subjects

A total of 30 children, who were diagnosed with GHD in the Department of Pediatrics of Hebei People’s Hospital from January 2017 to November 2017, were included in this study. These children were assigned as the observation group. Among these children, 19 children were boys and 11 children were girls, and the age of these children ranged within 3–7 years, with an average age at 4.5 ± 1.9 years. All the children were from Shijiazhuang and its surrounding cities and counties. The differences in eating habits, living environment and lifestyle were not statistically significant. In addition, 20 gender- and age-matched healthy children with normal height, who underwent physical examinations in our hospital, were included in this study. These children were assigned as the control group. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Hebei General Hospital. Written informed consent was obtained from the guardians of the participants. Diagnostic criteria of GHD: (1) the annual growth rate (AGR) of height was <4 cm, and bone age was less than the life age by 1.5 years; (2) height was less than the average height of gender-, age- and race-matched children by more than two standard deviations; (3) intelligence was normal and the figure was symmetrical dwarfism; and (4) the peak value of the GH secretion in the provocative test was <10 ng/mL. Pituitary magnetic resonance imaging (MRI) was performed to exclude pituitary organic lesions. In this study, the combination of clonidine hydrochloride and normal insulin was used to stimulate GH secretion. Based on the Guidelines for the diagnosis and treatment of short stature children, among these children, children with a peak value of GH of 5–10 ng/mL were assigned as the PGHD group, while children with a peak value of GH of <5 ng/mL were assigned as the CGHD group. In addition, children with a peak value of GH of ≥10 ng/mL were assigned as the idiopathic short stature (ISS) group. Exclusion criteria: (1) patients with chromosomal diseases, hereditary metabolic diseases, liver and kidney dysfunction, thyroid dysfunction, and chronic wasting diseases; (2) patients who received rhGH treatment in the past 3 months; and (3) patients with pituitary lesions revealed by pituitary MRI.

Methods

General data collection and physical examination

The age, gender, birth history (feeding history, history of childbirth, and history of chronic consumptive disease), exercise, diet, intelligence, sleep situation, AGR, height, and the development of family members were recorded for all children. The height and weight of all subjects were measured during the same time period by a specially assigned person, and the body mass index (BMI) was calculated. The development and stage of secondary sex characteristics were examined, and the presence of abnormalities in bones, muscles, and organs was determined.

Laboratory and imaging examinations

(1) At 6 a.m., fasting venous blood was collected from each subject, and was used for detecting fasting blood glucose, routine blood test indexes, liver and kidney functions, thyroid function, and basic GH level. (2) Bone age was measured using the bone age evaluation software for “Bone Age Assessment and Height Prediction, Chinese version.” (3) Head magnetic resonance imaging (MRI) examination was performed, and the morphology of the pituitary gland, the height of the anterior pituitary, the shape, position and coronal diameter of the pituitary stalk, the abnormal manifestation of the
sella and the parasellar tissues, and the position and size of the posterior pituitary were recorded. An enhanced scan was performed, when necessary.

Sample acquisition
Before the GH provocative test and at 3 and 6 months after rhGH treatment, 4 mL of fasting venous blood was collected in the morning from children in the observation group. Then, the blood samples were centrifuged using a TDZ4-WS low-speed tabletop centrifuge (Xiangyi Centrifuge Instrument Co., Ltd., China; 2500 r/min, 5 min) to obtain the serum, and the serum was stored in a low-temperature refrigerator at −80°C for the subsequent determination of serum ghrelin and nesfatin-1 levels. In addition, 2 mL of fasting venous blood was collected in the morning from children in the control group. Then, serum was stored and the levels of serum ghrelin and nesfatin-1 were determined in the same way.

Drug treatment and follow-up
Children in the experimental group were treated with domestic rhGH (Changchun Kinsey Pharmaceutical Co., Ltd.; National drug approval no.: S20050024), which was subcutaneously injected at 30 min before bedtime every night. The injection site was at the perichilum of the abdomen, and the dose was 0.1 IU/kg, once a day. Then, the injection site was 2 cm apart from the site of the last injection, and 6 months was set as the course of treatment. During the recheck, height and body weight were measured, and BMI and AGR were calculated. AGR = height after treatment − height before treatment/3 × 12 cm/year.

Sample detection
Serum ghrelin and nesfatin-1 levels were determined by enzyme-linked immunosorbent assay (ELISA), and the human Ghrelin/GHRL ELISA kit (NeoBioscience, China) and human nesfatin-1 ELISA Research Reagent kit (Wuhan Boster Biotechnology Ltd., China) were used.

Statistics analysis
A database was established using an Excel sheet, and the data were analyzed using statistical software SPSS 18.0. All data were tested for homogeneity and normal distribution. Normally distributed measurement data were expressed as mean ± standard deviation (X ± SD). Pairwise comparison was conducted using the Student–Newman–Keuls test, and multigroup comparison was conducted using univariate analysis of variance. P < 0.05 was considered statistically significant.

Results
General information about children in the CGHD group, PGHD group, and control group
The 30 GHD children included 19 PGHD children (63.33%) and 11 CGHD children (36.67%). For all subjects, there were no significant difference in eating habits, living environment, and life styles. Differences in gender, age, and BMI among the CGHD group, PGHD group, and control group were not statistically significant (P > 0.05, for all). Furthermore, the height and AGR were significantly lower in the PGHD and CGHD groups than in the control group (88.45 ± 6.24, 86.32 ± 7.90 vs 97.33 ± 9.28; P < 0.05). Moreover, nesfatin-1 level was significantly higher in the PGHD and CGHD groups than in the control group (108.29 ± 20.19, 153.11 ± 25.22 vs 45.67 ± 12.01; P < 0.05). In addition, ghrelin level was higher in the PGHD group than in the control group (6502.11 ± 156.36 vs 4933.43 ± 232.91) and higher in the CGHD group than in the control group (3011.36 ± 102.33 vs 4933.43 ± 232.91). The differences in height, height growth rate, ghrelin, and nesfatin-1 levels between the PGHD and CGHD groups were all statistically significant (P < 0.05).

The change in growth rate before and after treatment
Compared with pretreatment, and at three and 6 months after treatment, the height and growth rate in the PGHD and CGHD groups significantly increased (P < 0.05), while BMI did not exhibit any significant change (P > 0.05).

Changes in serum ghrelin and nesfatin-1 levels before and after treatment
Compared with pretreatment, at three and 6 months after treatment, serum ghrelin level in the PGHD group significantly increased (P < 0.05), while serum nesfatin-1 level significantly decreased
(P < 0.05). Furthermore, serum ghrelin (P < 0.05) and nesfatin-1 (P < 0.05) levels both significantly decreased in the PGHD group. The details are present in Table 1.

**Discussion**

GHD is a type of endocrine disease caused by the lack of GH, and is mainly characterized by a slow growth rate of height and short stature. Short stature affects not only quality of life, but also the mental health of children, leading to psychological disorders such as inferiority and low self-consciousness. Since rhGH has been approved by the Food and Drug Administration of the United States for the treatment of GHD in 1985, its efficacy and safety have been proven. A study revealed that rhGH can significantly improve the growth rate of GHD children. In this study, after GHD children in the PGHD and CGHD groups were treated with rhGH, their height and growth rate significantly increased. However, BMI did not significantly change. Furthermore, with the time of treatment, height and growth rate of children in the PGHD and CGHD groups significantly increased. Previous studies have revealed that rhGH replacement therapy may affect the GHRH-GH-IGF-1 axis, liver function, the skeletal system, and glucose metabolism, and induce the development of insulin resistance, liver dysfunction, impaired glucose tolerance, femoral head spondylolisthesis, and scoliosis. Therefore, in order to ensure the safety of the treatment, it is very necessary to conduct long-term follow-ups for GHD patients treated with rhGH. Furthermore, in rhGH treatment, fasting blood glucose, liver and kidney functions, thyroid function, and other indicators should be regularly monitored, in order to avoid the occurrence of side effects.

Ghrelin is an endogenous ligand of growth hormone secretagogue receptor (GHS-R). Since it can promote GH secretion, it was named ghrelin. The results of this study revealed that the ghrelin level was significantly lower in the CGHD group than in the control group and significantly higher in the PGHD group than in the control group. These above results reveal that ghrelin plays an important role in the secretion of GH, and its deficiency may be an important cause for human GHD. In addition, there was a decrease in ghrelin level and a barrier to its growth-promoting function in GHD children. Therefore, the simultaneous detection of serum GH and ghrelin levels in GHD children is more valuable in diagnosing the disease, and in guiding the development of further treatment regimens, when compared to the detection of serum GH levels alone in the GH provocative test.

Nesfatin-1 has many physiological functions, such as delaying gastric emptying, inhibiting food intake and reducing weight, regulating blood glucose, and regulating body temperature. It was speculated that nesfatin-1 may play an important role in energy metabolism. In addition, nesfatin-1 is correlated to the nutritional metabolism state to a certain extent. In sum, serum nesfatin-1 levels are elevated in GHD children. Therefore, the feeding process is inhibited, and the state of the body’s nutrition and metabolism is simultaneously affected, which eventually affects the development level of children. The above results prove that ghrelin and nesfatin-1 are closely correlated to GHD. Ghrelin and nesfatin-1 participate in the regulation of growth and development through various mechanisms, promoting the height growth of GHD children.

**Declaration of conflicting interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

| Table 1. Serum ghrelin and nesfatin-1 of CGHD, PGHD, before, and after treatment. |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Index                           | Time               | PGHD group (n = 19) | CGHD group (n = 11) |
|---------------------------------|--------------------|---------------------|---------------------|
| Ghrelin (pg/mL)                 | Before treatment   | 6502.11 ± 156.36    | 3011.36 ± 102.33    |
|                                 | After 3 months     | 5920.39 ± 167.40*   | 3532.11 ± 135.22*   |
|                                 | After 6 months     | 4390.33 ± 172.11**  | 3938.35 ± 152.17**  |
| Nesfatin-1 (pg/mL)              | Before treatment   | 108.29 ± 20.19      | 153.11 ± 25.22      |
|                                 | After 3 months     | 78.39 ± 17.32*      | 89.39 ± 20.54*      |
|                                 | After 6 months     | 58.39 ± 15.22**     | 68.31 ± 19.36**     |

*P < 0.05 versus before treatment. ▲P < 0.05 versus after treatment for 3 months.
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**References**

1. Wu ZH, Li H and Sun SY (2011) Efficacy and prediction model of growth hormone deficiency treated by recombinant human growth hormone (in Chinese). Chinese Journal of Practical Pediatrics 26(4): 289–293.

2. Zhao Q and Li ZJ (2016) Efficacy of growth hormone deficiency and idiopathic short stature treated by recombinant human growth hormone in 45 children (in Chinese). Maternal and Child Health Care of China 31: 1300–1302.

3. Li X, Ban B, Qiao JM et al. (2017) Meta-analysis on the association of the relativity of neoplasms and the effect of recombinant human growth hormone therapy in growth hormone deficiency. Chinese Journal of Child Health Care 25: 55–58.

4. The Subspecialty Group of Endocrinologic, Hereditary and Metabolic Diseases; The Society of Pediatrics; Chinese Medical Association (2008) Guidelines for diagnosis and treatment of children with short stature (in Chinese). Chinese Journal of Pediatrics 6: 428–430.

5. Allo Miguel G, Serraclara Plá A, Partida Muñoz ML et al. (2016) Seven years of follow up of trabecular bone score, bone mineral density, body composition and quality of life in adults with growth hormone deficiency treated with rhGH replacement in a single center Therapeutic Advances in Endocrinology and Metabolism 7: 93–100.

6. Krebs A, Kratzin T, Doerfer J et al. (2016) Decrease of small dense LDL and lipoprotein-associated phospholipase A2 due to human growth hormone treatment in short children with growth hormone deficiency and small for gestational age status. Journal of Pediatric Endocrinology and Metabolism 29: 203–208.

7. Poyrazoğlu Ş, Akçay T, Arslanoğlu I et al. (2015) Current practice in diagnosis and treatment of growth hormone deficiency in childhood: A survey from Turkey. Journal of Clinical Research in Pediatric Endocrinology 7: 37–44.

8. Kawashima R, Uchida M, Yamaki T et al. (2016) Development of a transnasal delivery system for recombinant human growth hormone (rhGH): Effects of the concentration and molecular weight of poly-L-arginine on the nasal absorption of rhGH in rats. Biological and Pharmaceutical Bulletin 39: 329–335.

9. Clemmons DR, Miller S and Mamputu JC (2017) Safety and metabolic effects of tesamorelin, a growth hormone-releasing factor analogue, in patients with type 2 diabetes: A randomized, placebo-controlled trial. PLoS ONE 12: e0179538.

10. Weber MM, Biller BM, Pedersen BT et al. (2017) The effect of growth hormone (GH) replacement on blood glucose homeostasis in adult nondiabetic patients with GH deficiency: Real-life data from the NordiNet® International Outcome Study. Clinical Endocrinology (Oxford) 86: 192–198.

11. Kan JY, Yen MC, Wang JY et al. (2016) Nesfatin-1/Nucleobindin-2 enhances cell migration, invasion, and epithelial-mesenchymal transition via LKB1/AMPK/TORC1/ZEβ1 pathways in colon cancer. Oncotarget 7: 31336–31349.

12. Shimizu H, Oh IS, Okada S et al. (2009) Nesfatin-1: An overview and future clinical application. Endocrine Journal 56: 537–543.