

Original Article

Three-Year Follow-up Study on Serum Leptin Levels in GH Deficient Children with GH Replacement Therapy

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Abstract. Interactions between GH and leptin have been extensively studied. However, results of long-term GH therapy on serum leptin levels in GH-deficient children were not consistent. Moreover, no such reports were available in Japanese children with this disease. We studied 35 Japanese patients with GH deficiency (26 boys and 9 girls, mean age: 9.8 ± 6.2 yr old), of whom 6 patients with complete and 29 with incomplete GH deficiency were identified by GH provocation test. Serum leptin levels, percent of ideal body weight (%IBW) and percent fat (%fat) were determined at 0, 1, 3, 6, 12, 18, 24, and 36 mo after beginning GH therapy. Baseline levels of %fat and leptin were significantly higher in girls than boys ($P < 0.05$), though serum leptin did not change throughout the study period in either group. Further, %IBW did not change significantly, whereas %fat exhibited significant changes after 6 mo in boys and remained virtually constant thereafter for up to 3 yr. In summary, serum leptin levels did not change in GH-deficient boys and girls during the 3-yr period after the start of GH replacement therapy, despite a decrease in %fat after 6 mo of therapy in the boys. Thus, it is conceivable that long-term GH replacement therapy can be employed without an effect on normal leptin secretion.

Key words: GH, leptin, percent fat

Introduction

GH has multiple properties other than growth in a variety of types of tissue metabolism in children and is known to play a critical role in lipolysis (1, 2). Interactions between GH and leptin have been studied since the discovery of leptin, a key molecule in the regulation of body fat (3), because of the importance of leptin function as a neuroendocrine hormone. An

alteration in leptin synthesis and/or degradation by GH therapy may have considerable effects on children, as they can have an effect on body weight regulation, as well as normal sexual maturation and development. Acute and chronic effects of GH treatment toward serum leptin levels have been reported in both adults and children with GH deficiency (4, 5). However, results of long-term GH replacement therapy on serum leptin in GH deficient children were not in agreement and such studies were not reported in Japanese children with this disease. The purpose of the present study was to determine whether long-term GH replacement (3 yr) has an influence on serum leptin levels in GH-deficient Japanese children.

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Table 1 Baseline characteristics of the subjects

	N	Age (y)	Bone age (y)	Height (cm)	Height-SDS	BW (kg)	%IBW	%fat	IGF-1 (ng/ml)	Leptin (ng/ml)
Total	35	9.8 ± 6.2	7.0 ± 5.1	119.3 ± 13.9	-2.69 ± 0.65	21.2 ± 7.0	104 ± 10	13.7 ± 4.4	141 ± 57	3.5 ± 1.9
Boys	26	10.2 ± 6.2	7.2 ± 5.3	121.1 ± 13.8	-2.72 ± 0.70	21.6 ± 7.4	104 ± 10	12.4 ± 4.1	146 ± 60	3.0 ± 1.3
Girls	9	8.7 ± 5.2	6.4 ± 4.7	114.1 ± 13.4	-2.60 ± 0.50	20.0 ± 5.3	106 ± 9	16.3 ± 3.9*	131 ± 51	5.0 ± 2.6*

Mean ± SD. N: number of patients, IBW: ideal body weight, *P<0.05 vs boys.

Subjects and Methods

We studied 35 prepubertal children with GH-deficiency (26 boys and 9 girls, mean age: 9.8 ± 6.2 yr old). The GH provocation test identified 6 patients with complete and 29 with incomplete GH deficiency. Subjects who showed features of puberty during the study were excluded from the analysis. The onset of puberty was defined according to the following criteria: 4 ml testis size in boys and Tanner stage 2 breast development in girls (6). This study was approved by the Human Study Committee of Nippon Medical School. Written informed consent was obtained from all subjects before enrolment.

GH replacement therapy was started at a dose of 0.175 mg/kg/week. Serum leptin levels as well as body weight and body fat were measured at the start and after 1, 3, 6, 12, 18, 24 and 36 mo of therapy. Serum leptin was measured by RIA using a commercial kit (LINCO research, USA). Serum total IGF-1 was determined by immunoradiometric assay following acid-ethanol extraction. Percent of ideal body weight (%IBW) was calculated based on a standard body weight table (7) categorized by gender, age and height, and percent fat (%fat) was determined using bioelectrical impedance analysis with a body fat monitoring scale TBF-305 (Tanita, Japan).

Statistical analysis was performed using either one-way analysis of variance (ANOVA) followed by Dunnett's test for multiple

comparisons (Table 2), or with an unpaired *t*-test (Table 1), with P<0.05 considered to be statistically significant.

Results

Baseline values for serum leptin, %IBW, and %fat

The baseline characteristics of the subjects are presented in Table 1. Serum leptin levels in the study subjects were in a range between 0.9–11.3 ng/ml (mean 3.5 ng/ml). Girls (2.8–11.3 ng/ml, mean 5.0 ng/ml) had significantly higher leptin values than boys (0.9–6.8 ng/ml, mean 3.0 ng/ml). Further, the value for %fat was also significantly higher in girls (9.9–23.0, mean 16.3) than in boys (6.1–19.1, mean 12.4), though no other gender differences were found in the other values, including %IBW and IGF-1.

Changes in height-SDS, %IBW, %fat, and serum leptin values during the study period

The changes in height-SDS, %IBW, %fat and serum leptin levels over the period of GH therapy are shown in Table 2. Height-SDS steadily increased over time in both boys and girls, with the increase becoming significant at 12 mo and thereafter for all of the subjects. Percent IBW values did not change throughout the study. The values for %fat for all subjects were significantly decreased after 6 mo and 12 mo (both P<0.05) and then remained virtually constant thereafter in all of the subjects. The changes were noted in boys, while %fat in girls appeared to remain

Table 2 Changes of Height-SDS, %IBW, %Fat and serum leptin values during the study period

	0 mo	1 mo	3 mo	6 mo	12 mo	18 mo	24 mo	36 mo
Height-SDS								
Total	-2.69±0.11	-2.62±0.12	-2.56±0.11	-2.46±0.11	-2.28±0.11*	-2.18±0.11**	-1.99±0.12**	-1.74±0.13**
Boys	-2.72±0.14	-2.66±0.15	-2.60±0.13	-2.49±0.13	-2.29±0.14	-2.22±0.15	-2.08±0.15*	-1.84±0.17**
Girls	-2.60±0.17	-2.53±0.18	-2.48±0.17	-2.36±0.18	-2.23±0.16	-2.07±0.12	-1.79±0.10**	-1.47±0.12**
%IBW								
Total	105±2	104±2	104±1	102±1	102±2	102±2	101±2	103±2
Boys	104±2	103±2	103±2	102±2	102±2	101±2	100±2	103±3
Girls	106±3	106±3	105±2	103±3	103±2	105±3	105±3	104±4
%Fat								
Total	13.7±0.8	11.8±1.2	11.7±0.8	10.6±1.0*	10.8±1.0*	9.9±1.4	10.2±1.0	10.6±1.1
Boys	12.4±1.0	9.8±1.2	10.2±0.8	8.6±1.1*	9.8±1.2	8.7±2.1	8.2±1.6	8.6±1.4
Girls	16.3±1.3	15.2±1.4	14.3±1.0	14.6±1.1	14.7±1.1	12.8±0.7	15.3±0.8	14.6±1.0
Leptin								
Total	3.5±0.3	3.0±0.3	2.7±0.2	3.2±0.3	3.1±0.3	3.2±0.5	2.9±0.4	2.8±0.4
Boys	3.0±0.3	2.6±0.3	2.5±0.2	3.0±0.4	2.9±0.3	2.0±0.2	2.1±0.3	2.1±0.3
Girls	5.0±0.9	4.0±0.8	3.6±1.0	3.9±0.4	3.7±0.7	5.1±1.1	4.9±0.9	5.0±1.2

Mean ± SE. *P<005, **P<0.01 vs 0 mo.

virtually constant. No significant changes were detected in serum leptin concentrations in either boys or girls.

Discussion

We investigated the effects of long-term GH therapy on body fat metabolism, focusing on serum leptin concentration. Leptin was initially identified as a product of the mouse obese (*ob*) gene that regulates energy balance and body fat mass (8). In humans, leptin induces diverse actions in endocrine organs, in addition to fat metabolism (3, 9, 10). Leptin is a circulating cytokine mainly derived from adipose tissue, thus serum leptin levels are dependent on fat mass (11). However, gender, age, maturational stage and several other factors including steroids and insulin may have an effect on its level (12).

The present data regarding serum leptin values in GH deficient children (Table 1) are similar to findings in normal children including gender difference, as compared with the data by

Kodera *et al.* who reported that serum leptin levels in healthy non-obese Japanese boys and girls were 3.1 ± 0.2 and 4.0 ± 0.3 ng/ml (mean ± SE), respectively (13). This lack of impact of GH deficiency on serum leptin levels is consistent with the findings of an earlier study in which patients with adult GH deficiency were investigated (14). Significantly higher %fat values in girls (Table 1) probably made a major contribution to the gender difference.

Recent studies have suggested that significant interactions occur between leptin and GH (15). The effects of GH on serum leptin concentrations have been investigated over the past decade in a variety of types of patients. The results of studies performing short-term and long-term GH treatment are both conflicting. Short-term exposure to GH failed to influence leptin levels in some studies (16, 17), while Gill *et al.* reported an initial increase in serum leptin levels followed by a significant decrease from the baseline after a single bolus dose of GH in elderly subjects with GH deficiency (4). Further,

the direct effects of GH on leptin gene expression were inconsistent, depending on the tissues used (14). Chronic GH therapy, as performed in the present study, was also described by Gill *et al.* who reported no significant changes in serum leptin levels over 9 mo. However, down-regulation of serum leptin by chronic GH replacement therapy in GH-deficient children has been reported by a number of investigators (5, 18, 19), though the races and dosages used were different from those in our study. Leptin was reported to be significantly decreased after 10 d (5) or 1 mo (18, 19) of therapy, and remained constant up to 6 (18) and 12 mo (5, 19). In the present investigation, serum leptin levels did not change throughout the period of study. Since %fat was decreased after 6 mo in boys, serum leptin was expected to be down-regulated.

GH replacement therapy has multiple effects on various human organs including the endocrine system, thus other regulators of serum leptin concentration must be considered. We previously investigated the effects of GH treatment given with the same protocol on glucose tolerance in GH-deficient children (20). We found that plasma immunoreactive insulin (IRI) and C-peptide immunoreactivity (CPR) were significantly increased in an oral glucose test conducted after 1 yr. Since insulin up-regulates serum leptin, alteration of insulin synthesis may have an effect on the net effect of GH therapy toward serum leptin concentration. Leptin, in turn, may inhibit insulin secretion in the pancreas, and also enhance peripheral insulin sensitivity and glucose utilization (10). Other determinants of serum leptin levels such as alteration of leptin production by individual adipocyte, renal clearance and hepatic metabolism may operate, since GH receptors are present and functional in all three tissues (19). However, these possibilities remain to be tested.

Limitations of this study include methodology for measuring %fat and the characteristics of the patient population. For

measuring %fat, we used bioelectrical impedance method rather than dual energy x-ray absorptiometry. Although bioelectrical impedance analysis is commonly used for its convenience, noninvasiveness and reproducibility (21), this difference in methodology may exert some influence on the results. Regarding the patient population in the present study, majority of the patients were classified as incomplete form of GH deficiency. The dominance of milder form of the disease may be responsible for the inconsistency of the results between the current study and the previous reports. However, the previous studies did not classify the patients into complete and incomplete forms (5, 18, 19).

In summary, the present study suggests that conventional GH replacement therapy does not impair normal leptin secretion. Since leptin has diverse effects on endocrine organs, including the hypothalamus and gonads, preservation of serum leptin levels during long-term GH treatment may be important in children. It remains to be clarified how GH affects leptin function in target tissues at the receptor level.

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