

# Metabolic Effects of 20-Kilodalton Human Growth Hormone (20K-hGH) for Adults with Growth Hormone Deficiency: Results of an Exploratory Uncontrolled Multicenter Clinical Trial of 20K-hGH

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The biological effects of 20-kDa human GH (20K-hGH), which is produced in the pituitary by alternative splicing of GH mRNA and comprises approximately 6% of all GH in serum, have not been reported.

We have investigated the metabolic effects of recombinant 20K-hGH in adult patients with GH deficiency in an exploratory study. Three doses of 20K-hGH (0.006, 0.012, and 0.024 mg/kg·d), were administered for 16 wk to three groups (consisting of 18 or 19 subjects), respectively. The 20K-hGH dose-dependently increased serum IGF-I and IGFBP-3 levels, and the lowest dose (0.006 mg/kg) was enough to normalize both hormones by wk 4. Serum osteocalcin levels and urinary deoxyypyridinoline excretion were also dose-dependently increased. There was a significant decrease in body fat mass with an increase of lean body mass at the lowest dose of 0.006

mg/kg·d. Blood glucose and serum insulin were increased significantly at 4 wk only in the high-dose group (0.024 mg/kg). Glucose tolerance was slightly impaired in 26–39% of patients in all treatment groups as judged by oral glucose tolerance tests, but there was no development of overt diabetes. The major adverse event in the 20K-hGH treatment was peripheral edema, similar to the incidence reported for 22K-hGH.

The data demonstrated that 20K-hGH had metabolic effects comparable to those of 22K-hGH in humans. The results suggest that 20K-hGH could be used to treat GH-deficient patients, although further studies may be required to investigate the optimum dose and superiority of 20K-hGH over 22K-hGH in a comparative study. (*J Clin Endocrinol Metab* 89: 1562–1571, 2004)

**H**UMAN GH (hGH) with a molecular mass of 20 kDa (20K-hGH) is produced in the human pituitary by an alternative splicing of the hGH-N gene (1) and comprises 6–7% of all the circulating hGH (2, 3). Recently, Uchida *et al.* (4) have produced recombinant 20K-hGH in amounts sufficient to test the biological activities in both experimental animals and humans. The 20K-hGH possesses a unique property in binding to hGH receptors (hGHR) due to the conformational change restricted to its site 1 binding region to hGHR. That is, 20K-hGH forms a 1:2 complex (hGH:hGHR) as an active form to the same extent as 22K-hGH but has difficulty in forming an inactive 1:1 complex (hGH:

hGHR) (5). This property might be related to the poor interaction of 20K-hGH with circulating hGH-binding protein (BP) (6). In addition, the lactogenic activity is also lower than that for 22K-hGH (7). Recombinant 20K-hGH has been shown to stimulate linear growth in spontaneous dwarf rats (8), to induce lipolysis in 3T3L-1 cells expressing hGHR (9), and to have an osteoanabolic effect in human osteoblast cells (10), all of which were comparable to the effects of 22K-hGH.

It is well known that hGH replacement treatment in patients with adult GH deficiency (GHD) is frequently associated with development of edema (11–13), which is ascribed to the antinatriuretic action of hGH. However, the antinatriuretic action of recombinant 20K-hGH was clearly less potent than that of 22K-hGH in rats (14). Furthermore, diabetogenic activity was also lower in 20K-hGH in the experiments using the euglycemic clamp in rats (15). Given the lower levels of diabetogenic and antinatriuretic activity, 20K-hGH might be preferable to 22K-hGH, but there is little information on the effect(s) of recombinant 20K-hGH in humans. Hashimoto *et al.* (16) have recently reported that single sc administration of 20K-hGH dose-dependently (0.003–0.1 mg/kg) increases serum IGF-I levels and stimulates lipolysis in normal subjects. Their results also showed that 20K-hGH administration significantly suppressed secretion of endogenous 22K-hGH.

Abbreviations: ALT, Alanine aminotransferase; ANP, atrial natriuretic peptide; AST, aspartate aminotransferase; BFM, body fat mass; BP, binding protein; Cho, cholesterol; CT, computed tomography; CV, coefficient(s) of variation; DL, detection limit(s); EF, ejection fraction; %FS, fractional shortening; GHD, GH deficiency or GH deficient; HbA1c, glycosylated hemoglobin; HDL, high-density lipoprotein; hGH, human GH; hGHR, human GH receptor(s); IGT, impaired glucose tolerance; IRMA, immunoradiometric assay(s); 20K-hGH, 20-kDa hGH; LBM, lean body mass; LDL, low-density lipoprotein; NEFA, nonesterified fatty acid; OGTT, oral glucose tolerance test; QOL, quality of life; V/S, visceral fat to sc fat ratio.

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In the phase I clinical trial, we also confirmed that 20K-hGH (0.1 mg/kg) administration for 7 d increased serum IGF-I levels in normal subjects without significant adverse events. The aim of the present study was to investigate the metabolic effects of three doses of recombinant 20K-hGH in adult patients with GHD as an exploratory study. This study was carried out in 27 institutions in Japan in compliance with the ethical principles set out in the Declaration of Helsinki and the Good Clinical Practice (GCP, a set of guidelines issued by the Japanese Ministry of Health, Labor and Welfare, Notification no. 28, 1997).

## Subjects and Methods

### Subjects

Sixty-five patients with a confirmed medical history of hypothalamo-pituitary disease were recruited from 27 medical institutions to participate in this study. The study protocol stipulated that patients undergo a GH provocative test within the 2 months before starting treatment. Only patients showing a peak response of less than 3 ng/ml to either the insulin tolerance test or the arginine tolerance test were allowed to enroll in the study. Exclusion criteria were GH treatment in the 12 months preceding recruitment, malignant tumors (or history of), hypertension (systolic blood pressure > 165 mm Hg and/or diastolic blood pressure > 95 mm Hg), and diabetes mellitus diagnosed by 75-g oral glucose tolerance test (OGTT) in accordance with the Japan Diabetes Society's classification and diagnostic criteria of diabetes mellitus (17). Of the 65 patients, one withdrew consent, and eight did not meet the inclusion criteria. Twenty-four patients showed an acceptable response to the insulin tolerance test, and 32 showed an acceptable response to the arginine tolerance test. Consequently, 56 patients started the 16-wk treatment of 20K-hGH.

Eighteen of 56 patients had childhood-onset GHD, and the remaining 38 had adult-onset GHD. The causes of GHD included hypothalamo-pituitary tumors (31 cases, craniopharyngioma, germinoma, pituitary adenoma, and other brain tumors), postpartum pituitary necrosis (four cases, Sheehan syndrome), and head trauma (four cases). In 11 patients, the cause of GHD was unknown (idiopathic). All patients with childhood-onset GHD had been treated with 22K-hGH. Fifty-four of the 56 patients had multiple pituitary hormone deficiency in various combi-

nations (Table 1) and were receiving adequate adrenal, thyroid, and/or gonadal hormone replacement therapy. The patient profiles are shown in Table 1. There was no difference among the three groups with respect to age, body weight, body mass index, height, or age of onset of GHD. Approximately 25% of the patients in the three groups were being treated with antihyperlipidemic agents, and they did not cease or change their medication dose throughout the study.

### Design

Using a randomization procedure with minimization algorithms for age (under/over 40 yr of age), gender (male/female), and timing of onset (childhood/adult), three groups (A, B, and C) of 18–19 patients each were randomly formed. Patients were asked to sc self-inject 0.006 (group A), 0.12 (group B), or 0.024 mg/kg body weight·d (group C) of recombinant 20K-hGH immediately before bedtime for 16 wk. The 20K-hGH was provided in a freeze-dried formulation containing 12 mg of 20K-hGH per vial with a 3-ml solvent. The injections were done using Ultrapen (13BY1096, Becton, Dickinson and Co., Franklin Lakes, NJ). The cartridge containing 20K-hGH solution was stored below 10 C. The patients were seen by their doctors immediately before the treatment; in wk 4, 8, 12, and 16 of the treatment period; and finally at 4 wk after administration had ended. The patients were monitored for physical status and various parameters as described below.

### Measurement

Body composition was measured in the patients in the supine position by bioelectrical impedance method using the FRM-96 fat rate meter (Metron, Yokohama, Japan). A 50-kHz, 800-mA current was applied as previously described (18). Distribution of abdominal and visceral fat was measured by computed tomography (CT) image scan at the level of the umbilicus and 3 cm above and below the umbilicus. The areas of the visceral and sc fat were determined by one expert with the following procedure to adjust CT films scanned under the various conditions at multiple institutions: 1) the computer-graphic image was made from an individual CT-scan film with a transmission scanner (model Scan JX 330, Sharp, Osaka, Japan). 2) The area of the sc fat in the image file was traced freehand. The mean and SD of the Hounsfield numbers were calculated by histogram analysis to determine the fat area threshold. The mean of the Hounsfield numbers for each file was varied. The area that fell within the mean  $\pm$  2 SD of Hounsfield numbers was regarded as fat tissue. 3) The areas of gas in the intestines, which showed similar Hounsfield

**TABLE 1.** Background of patients

	Group [dose (mg/kg·d)]			Total
	A (0.006)	B (0.012)	C (0.024)	
No.	19	18	19	56
Sex [male/female (male %)]	10/9 (52.6)	10/8 (55.6)	10/9 (52.6)	30/26 (53.6)
Age (yr) [mean (range)]	41.7 (23–62)	44.1 (21–64)	38.3 (20–59)	41.4 (20–64)
Onset of GHD [childhood/adult-hood (child %)]	7/12 (36.8)	6/12 (33.3)	5/14 (26.3)	18/38 (32.1)
BMI [mean (range)]	24.6 (16.0–30.1)	24.4 (19.4–32.7)	24.7 (18.0–36.4)	24.6 (16.0–36.4)
Organic disorder				
Yes (%)	12 (63.2)	16 (88.9)	17 (89.5)	45 (80.4)
Hypothalamo-pituitary tumors	8	12	11	31
Postpartum necrosis	0	2	2	4
Head trauma	0	1	3	4
Others	4	1	1	6
No (%)	7 (36.8)	2 (11.1)	2 (10.5)	11 (19.6)
Other deficient hormones				
Yes (%)	18 (94.7)	17 (94.4)	19 (100)	54 (96.4)
LH/FSH <sup>a</sup>	17	17	18	52
ACTH <sup>a</sup>	17	15	17	49
TSH <sup>a</sup>	17	15	17	49
PRL	8	7	8	23
ADH <sup>a</sup>	10	5	8	23
No (%)	1 (5.3)	1 (5.6)	0 (0.0)	2 (3.6)

BMI, Body mass index; PRL, prolactin; ADH, antidiuretic hormone.

<sup>a</sup> Gonadal steroids for LH/FSH deficiency, cortisol for ACTH, thyroid hormone for TSH, and desmopressin (DDAVP) for ADH were treated as replacement therapy, respectively.

numbers to visceral fat, were marked by freehand and totaled. 4) The area of visceral fat was then calculated by subtracting the total area of gas from the total abdominal cavity area shown by the range of Hounsfield numbers above. The above analysis was conducted for each CT film using the following: a 256-gray scale tone with special software (Adobe PhotoShop, version 2.5, Adobe Systems Inc., San Jose, CA; NIH Image Processing Toolbox, version 1.56, National Institutes of Health, Bethesda, MD), and personal computer (Macintosh, M7824J/A, Apple Computer, Inc., Cupertino, CA). Before the analysis in this study, it had been confirmed that a good correlation coefficient was shown between the above analysis and the automatic analysis program used by the CT device at the expert's institution for both the visceral fat ( $r = 0.85$ ) and the sc fat ( $r = 0.9$ ).

Cardiac functions, including the fractional shortening (%FS) and ejection fraction (EF), were evaluated by echocardiography (19, 20). Patients' grasping power was measured with a hand dynamometer. The effect of 20K-hGH treatment on quality of life (QOL) was evaluated using the Japanese version of the short-form 36-item health survey (SF-36), despite not being specifically designed for assessing the effects of GH. The SF-36 questionnaire has 36 self-administered items including (item 1) general health feeling, designed to measure perceived health problems and the extent/degree of any problems that affect patients' daily activities. The questionnaire yields scores in eight subsections. The subsections cover physical functions, role limitations due to physical problems, bodily pain, vitality, role limitations due to emotional problems, mental health, social functioning, and general health. The Japanese version was validated for its suitability and reliability before our study (21). The scores were calculated using special analysis software (MAP-R for Windows, QualityMetric Inc., Lincoln, RI).

### Metabolic parameters

Serum IGF-I and IGFBP-3 were measured by immunoradiometric assay(s) (IRMA) (IGF-I, Daiichi Radioisotope Laboratories, Ltd., Tokyo, Japan; IGFBP-3, Eiken Chemical Co. Ltd., Tokyo, Japan). Coefficient(s) of variation (CV) and detection limit(s) (DL) of these two measurements were 1.1–3.4% (CV) and 0.2 ng/ml (DL) for IGF-I and 3.4–3.9% (CV) and 2 ng/ml (DL) for IGFBP-3. TSH, free  $T_4$ , and free  $T_3$  were assayed by IRMA. Serum osteocalcin and urinary deoxypyridinoline were determined by IRMA and enzyme immunoassay, respectively. Plasma renin activity, aldosterone and human atrial natriuretic peptide (hANP) were determined by RIA or IRMA. The assays were performed at the SRL Medisearch, Inc., laboratories (Tokyo, Japan). Blood glucose, serum insulin, and glycosylated hemoglobin (HbA1c) levels were measured by glucose oxidase method, double antibody RIA, and latex coagulation method, respectively. In addition, OGTT was conducted after 16-wk treatment by the same method described in *Subjects and Methods*. Total cholesterol (Cho), low-density lipoprotein (LDL)-cholesterol (LDL-Cho), triglyceride, and nonesterified fatty acid (NEFA) levels were determined using standard methods, and the LDL-particle size was assayed by LDL-Rf as described (22, 23). 20K-hGH antibody was determined with an ELISA system developed at our laboratories.

### Statistical analysis

The values are expressed as the mean  $\pm$  SD unless otherwise described. The significance of changes between data before and after treatment within groups was analyzed with a paired *t* test. The dose dependency of changes in each point was investigated using one-way ANOVA with a contrast defining dose dependency. *P* values less than 0.05 were considered statistically significant. Because this trial was conducted as an exploratory study for dose-finding, no analysis using techniques of adjustment for multiplicity was planned. The Bonferroni correction was, however, applied as a *post hoc* analysis if multiple comparisons had been made.

## Results

### Serum IGF-I and IGFBP-3

Serum IGF-I levels in the patients before treatment were  $54.5 \pm 39.1$ ,  $63.0 \pm 38.1$ , and  $70.4 \pm 49.1$  ng/ml in groups A, B, and C, respectively. The values were lower than those in

healthy Japanese subjects matched for sex and age ( $190 \pm 59$  ng/ml;  $n = 200$ ). Treatment with 20K-hGH resulted in a significant increase of IGF-I as shown in Fig. 1A. The effect was dose-dependent, and the values increased to normal levels at 4 wk, even with the lowest dose (0.006 mg/kg; group A). The values returned to pretreatment levels 4 wk after the end of therapy. It is noteworthy that the higher dose of 20K-hGH (groups B and C) increased serum IGF-I to the supraphysiological levels. Serum IGFBP-3 levels (initially  $1.5 \pm 0.3$ ,  $1.4 \pm 0.7$ , and  $1.6 \pm 0.3$   $\mu$ g/ml in groups A, B, and C, respectively) were also lower than in healthy Japanese subjects (17–35 yr old,  $3.1 \pm 0.5$   $\mu$ g/ml,  $n = 124$ ; 35–70 yr old,  $3.0 \pm 0.4$   $\mu$ g/ml,  $n = 53$ ). The treatment with 20K-hGH also increased the serum IGFBP-3 levels dose-dependently (Fig. 1B). Like IGF-I levels, the lowest dose of 20K-hGH was enough to restore the levels for 16 wk.

### Bone metabolism

Serum levels of osteocalcin, a marker of bone formation, and urinary excretion of deoxypyridinoline were also increased by 20K-hGH (Fig. 2, A and B). The effects were dependent on the dose of 20K-hGH. Again, the lowest dose of the hormone increased both bone markers. The values decreased after discontinuation of treatment but remained at higher than basal levels at 4 wk after the end of treatment.

### Serum lipids

The effect of 20K-hGH on the serum lipid profile is shown in Table 2. Total Cho and LDL-Cho levels tended to decrease, but the effect was minor and transient. High-density lipoprotein (HDL)-Cho did not change significantly during treatment but was higher than the basal levels in the three groups at 4 wk post treatment. LDL-Rf values in group C decreased significantly at 8 wk, but the effect was transient in other groups. There was no effect on serum triglyceride levels throughout the period. Plasma NEFA levels were increased at 4 and 8 wk in all of the groups, but there was no dose dependency.

### Body composition

Initial percentages of body fat were 32.0, 29.0, and 28.5% in groups A, B, and C, respectively; the values were significantly reduced by 20K-hGH treatment at 4 wk (by 4.5% in group A, 5.8% in group B, and 7.2% in group C); and the effect was still seen during the treatment period (Fig. 3A). A similar effect was observed for body fat mass (BFM) (Fig. 3B). At 16 wk, BFM decreased by 3.7 kg (from 21.5 to 17.8 kg) in group A, by 3.9 kg (from 18.6 to 14.7 kg) in group B, and by 6.0 kg (from 18.2 to 12.2 kg) in group C. On the contrary, lean body mass (LBM) and total body water increased significantly ( $P < 0.05$ ) in all the groups at 4–16 wk and returned to the basal levels 4 wk post treatment (Fig. 3, C and D).

### Abdominal fat areas

Abdominal sc and visceral fat areas determined by CT were also reduced dramatically by 20K-hGH treatment for 16 wk (Table 3). Subcutaneous fat areas decreased by 12.5% (from 154.9 to 135.5  $\text{cm}^2$ ) in group A, by 9.2% (from 128.3 to

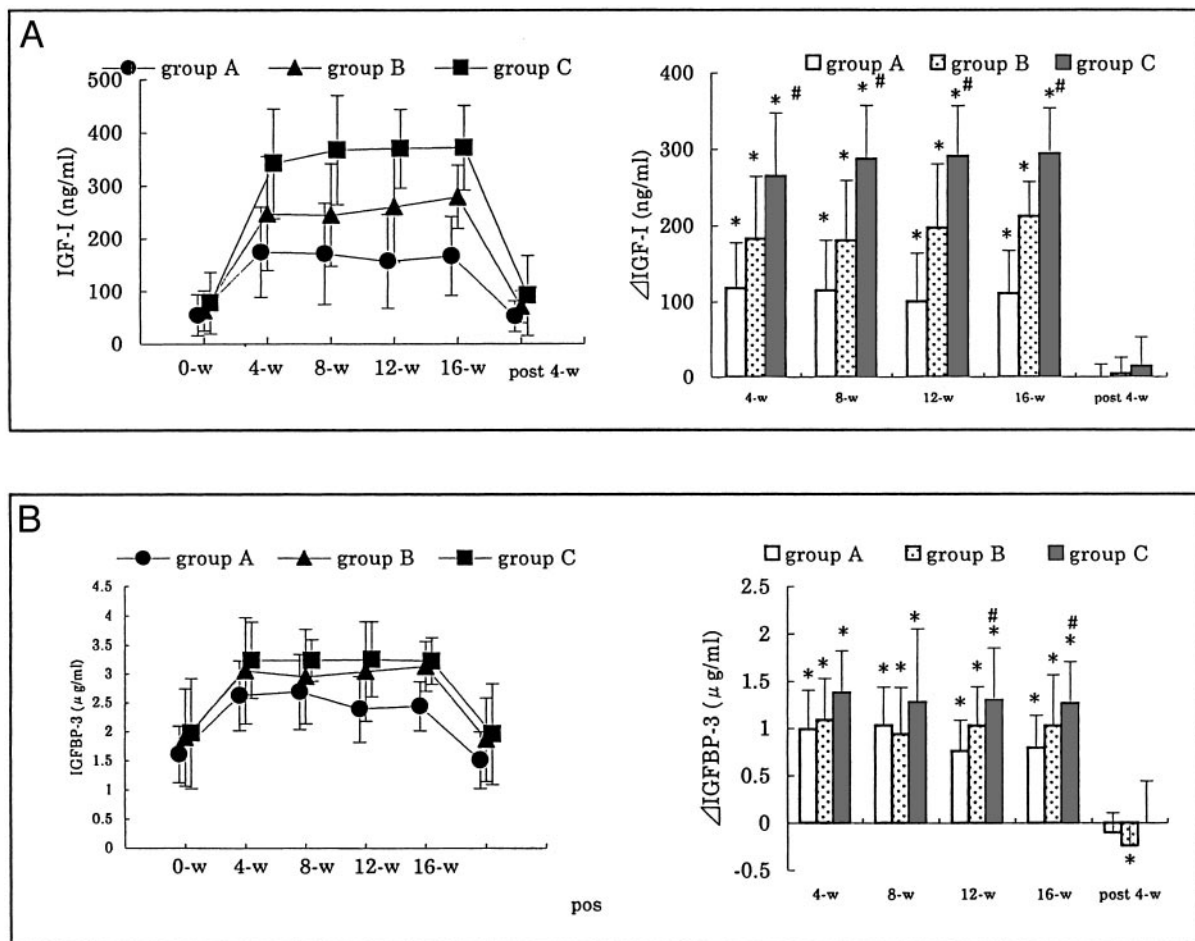


FIG. 1. Changes in serum IGF-I (A) and IGFBP-3 (B) (left, actual values; right, changed values from baseline). Each value represents mean  $\pm$  SD. Significant differences compared with initial values were investigated with paired *t* test (\*,  $P < 0.05$ ), and dose dependency of changed values was investigated with one-way ANOVA with a contrast defining dose dependency (#,  $P < 0.05$ ).

116.5 cm<sup>2</sup>) in group B, and by 28.5% (from 143.1 to 102.3 cm<sup>2</sup>) in group C at 16 wk. As for visceral fat areas, a more marked improvement was observed. These values in each group at 16 wk were reduced by 36.2% (from 73.0 to 46.5 cm<sup>2</sup>) in group A, by 33.1% (from 65.3 to 43.7 cm<sup>2</sup>) in group B, and by 49.1% (from 49.8 to 25.3 cm<sup>2</sup>) in group C. The visceral fat to sc fat ratio (V/S) of each group at 16 wk decreased, which clearly showed that 20K-hGH reduced more visceral than sc fat.

#### Glucose metabolism

Slight and significant increases of both blood glucose and serum insulin levels were observed at 4 wk in group C (Table 4), although there was no significant change in these parameters in groups A and B. Serum HbA1c values increased in groups B and C at 12 wk and in all groups at 16 wk, although they were within normal range. Results of the 75-g OGTT performed before and at 16 wk of treatment were summarized in Table 5. Although glucose tolerance was normal in 17 patients in group A before GH treatment, it was slightly impaired in five patients [four had impaired glucose tolerance (IGT)] at 16 wk. Similarly, glucose tolerance was minimally impaired in approximately 30% of the patients in

groups B and C. In total, two patients (one in group A and one in group C) showed diabetic pattern in glucose tolerance. In three of nine patients (one in each group) who showed a borderline pattern before treatment, glucose tolerance improved at 16 wk contrarily.

#### Cardiac functions

Cardiac functions determined by echocardiography (%FS and EF) were in the normal range in all of the patients before treatment, and there was no change in these functions after 16 wk of treatment with 20K-hGH.

#### QOL

There was no significant change in scores of physical functioning, vitality, mental health, and general health during GH treatment (0, 16 wk, and 4 wk post treatment) in group A. There were a few elements that showed increased scores at 16 wk when compared with the baseline, *e.g.* physical functioning, mental health, and general health in group B and/or group C, but the change was minimal and not related to the dose of 20K-hGH (data not shown).

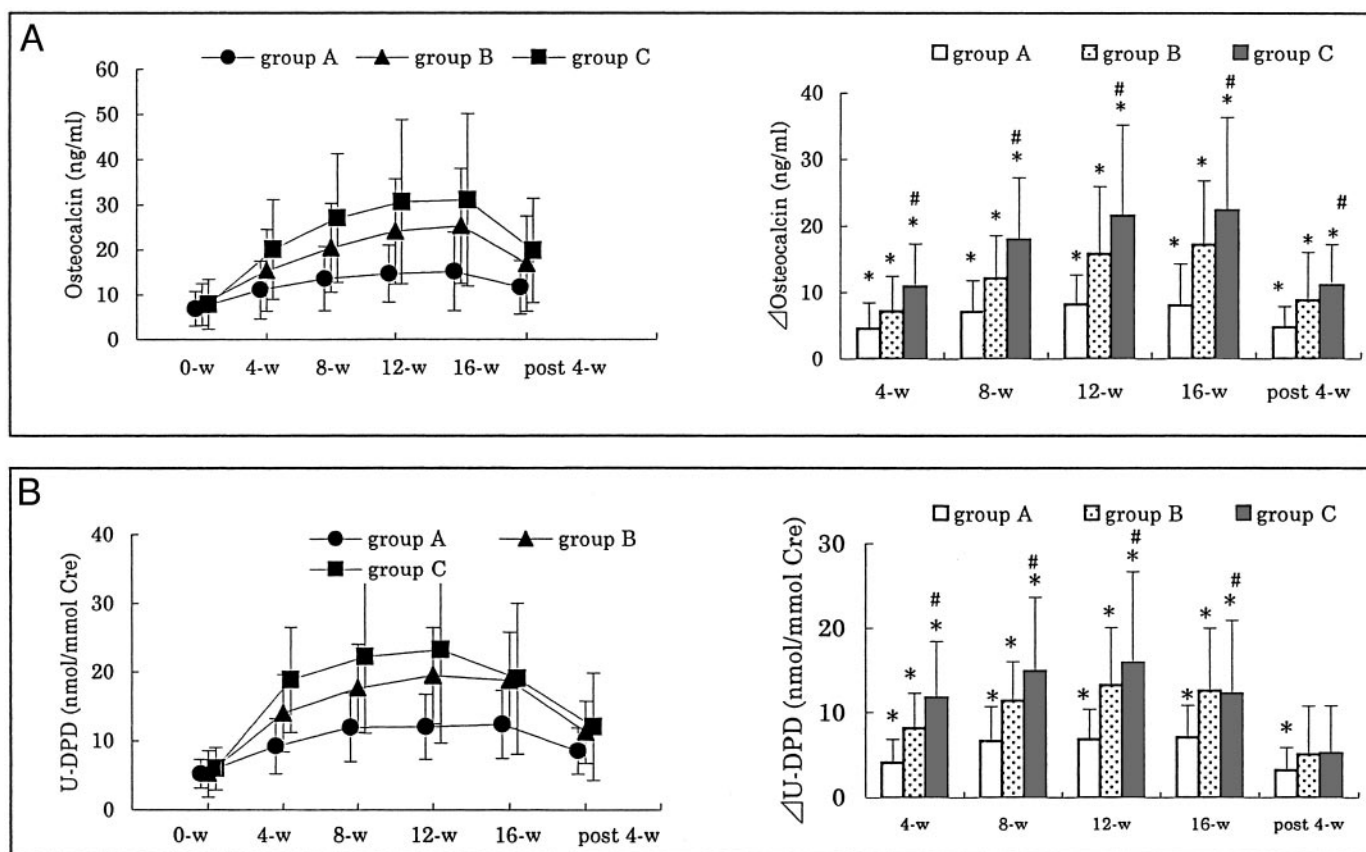


FIG. 2. Changes in serum osteocalcin (A) and urinary deoxypridinoline (U-DPD) (B) (left, actual values; right, changed values from baseline). Each value represents mean  $\pm$  SD. Significant differences compared with initial values were investigated with paired *t* test (\*;  $P < 0.05$ ), and dose dependency of changed values was investigated with one-way ANOVA with a contrast defining dose dependency (#,  $P < 0.05$ ). Cre, Creatinine.

### Others

Grasping power did not change in the majority of patients, but for some patients in groups B and C, grasping power unexpectedly decreased at 4 and 8 wk. This may be due to arthralgia, which was seen in these patients.

### Adverse events

Peripheral edema developed in six patients in group A (31.6%), 11 in group B (61.1%), and 15 (78.9%) in group C. Because of severe edema, GH treatment was discontinued in three patients in group B and seven patients in group C. Arthralgia at joints (wrist and/or knee) developed in two patients in group A, five in group B, and three in group C. One patient in group B and five patients in group C reported headache. Eczema hypoesthesia and palpitation were also noted in some patients. Slight increases (a less than 2-fold increase from basal) in aspartate aminotransferase (AST), alanine aminotransferase (ALT), or  $\gamma$ -GTP levels were seen in some patients. In groups A, B, and C, the numbers of subjects who showed transient liver enzyme abnormalities were one (5.3%), four (22.2%), and four (21.1%) for AST; three (15.8%), six (33.3%), and five (26.3%) for ALT; and two (10.5%), two (11.1%), and two (10.5%) for  $\gamma$ -GTP, respectively. These changes were observed mostly in the early stages of the treatment, but the values returned to a normal

range without any medical treatment. There was no significant effect of 20K-hGH on blood pressure, body temperature, or heart rate. No abnormalities were detected in electrocardiogram measurements throughout the study period.

### Discussion

Numerous studies have shown that GH treatment in adult patients with GHD produces beneficial effects, including improvement of body composition, bone metabolism, physical performance, cardiovascular function, and psychological well-being. Several adverse effects such as peripheral edema (11–13) have been reported for treatment with 22K-hGH. Recent studies have shown that 20K-hGH has somatogenic effects similar to 22K-hGH *in vitro* and in animal studies (8–10). It has been shown, however, that both the antinatriuretic and diabetogenic activity of 20K-hGH are much lower compared with those of 22K-hGH in rats (14, 15). If this is the case for humans, 20K-hGH might be superior to 22K-hGH. Here, we have studied the efficacy and safety of recombinant 20K-hGH in the three doses of treatment for Japanese adult patients with GHD in the exploratory study.

As expected from the results in animal experiments, 20K-hGH significantly increased serum IGF-I and IGFBP-3 levels within 4 wk. A dose as low as 0.006 mg/kg was enough to normalize serum IGF-I concentrations, and the higher doses

**TABLE 2.** Effect of 20K-hGH on serum lipids

Group (dose, mg/kg·d)	Initial values 0 wk	Changed values				
		4 wk	8 wk	12 wk	16 wk	4 wk post treatment
<b>Total-Cho (mg/dl)</b>						
A (0.006)	207 ± 33 (19)	-6.1 ± 19.6 (18)	-13.5 ± 22.9 (18)	-5.3 ± 22.2 (18)	-4.3 ± 30.1 (18)	4.8 ± 22.0 (19)
B (0.012)	216 ± 34 (17)	-20.5 ± 29.5 (14)	-17.6 ± 32.7 (12)	-16.8 ± 36.9 (12)	-11.4 ± 36.1 (11)	-1.8 ± 26.8 (11)
C (0.024)	227 ± 46 (19)	-24.9 ± 35.3 (13)	-27.0 ± 38.7 (10)	-12.0 ± 45.4 (10)	-18.0 ± 36.5 (11)	24.0 ± 40.3 (11)
<b>HDL-Cho (mg/dl)</b>						
A (0.006)	54 ± 15 (19)	1.2 ± 7.9 (18)	2.3 ± 9.9 (18)	4.5 ± 8.3 (18)	4.7 ± 8.9 (18)	12.6 ± 8.9 (19) <sup>a</sup>
B (0.012)	57 ± 24 (17)	-2.4 ± 9.6 (14)	0.7 ± 11.5 (12)	1.3 ± 11.7 (12)	2.4 ± 10.4 (11)	14.1 ± 13.1 (11) <sup>a</sup>
C (0.024)	52 ± 18 (19)	-3.2 ± 10.6 (13)	-0.2 ± 13.0 (10)	5.7 ± 11.7 (10)	4.3 ± 10.7 (11)	17.8 ± 9.0 (11) <sup>a</sup>
<b>LDL-Cho (mg/dl)</b>						
A (0.006)	131 ± 24 (19)	-8.3 ± 13.3 (18)	-17.7 ± 17.3 (18) <sup>a</sup>	-8.7 ± 19.1 (18)	-7.7 ± 26.4 (18)	-3.3 ± 19.7 (19)
B (0.012)	133 ± 32 (17)	-13.9 ± 24.3 (14)	-12.2 ± 23.4 (12)	-16.5 ± 24.0 (12)	-12.5 ± 26.3 (11)	-2.9 ± 23.7 (11)
C (0.024)	137 ± 32 (19)	-19.2 ± 27.6 (13)	-18.0 ± 29.4 (10)	-6.6 ± 34.5 (10)	-11.4 ± 31.1 (11)	23.0 ± 35.3 (11) <sup>b</sup>
<b>LDL-Rf value</b>						
A (0.006)	0.36 ± 0.03 (19)	-0.01 ± 0.03 (18)	-0.01 ± 0.02 (18)	-0.02 ± 0.04 (18)	0.00 ± 0.04 (18)	-0.01 ± 0.03 (19)
B (0.012)	0.37 ± 0.03 (17)	-0.01 ± 0.03 (14)	-0.02 ± 0.02 (12)	-0.02 ± 0.03 (12)	-0.02 ± 0.03 (11)	-0.02 ± 0.03 (11)
C (0.024)	0.39 ± 0.04 (19)	-0.04 ± 0.04 (13) <sup>a,b</sup>	-0.04 ± 0.04 (10)	-0.03 ± 0.05 (10)	-0.04 ± 0.03 (11) <sup>a,b</sup>	-0.04 ± 0.03 (11) <sup>a,b</sup>
<b>TG (mg/dl)</b>						
A (0.006)	136 ± 81 (19)	24.7 ± 82.1 (19)	23.7 ± 72.3 (19)	14.5 ± 61.6 (19)	10.7 ± 76.1 (18)	-9.2 ± 41.4 (19)
B (0.012)	148 ± 70 (18)	8.9 ± 47.6 (16)	-25.6 ± 68.0 (15)	23.7 ± 79.0 (15)	-7.6 ± 84.8 (14)	-40.2 ± 80.5 (14)
C (0.024)	190 ± 164 (19)	-1.9 ± 107.3 (16)	-42.2 ± 114.0 (12)	-51.8 ± 146.3 (12)	-41.8 ± 113.6 (12)	-75.9 ± 142.2 (12)
<b>NEFA (mEq/liter)</b>						
A (0.006)	0.52 ± 0.21 (19)	0.17 ± 0.25 (19) <sup>a</sup>	0.20 ± 0.33 (19)	0.21 ± 0.23 (19) <sup>a</sup>	0.14 ± 0.29 (18)	-0.11 ± 0.18 (19)
B (0.012)	0.38 ± 0.21 (18)	0.37 ± 0.34 (16) <sup>a</sup>	0.41 ± 0.25 (15) <sup>a</sup>	0.31 ± 0.38 (15) <sup>a</sup>	0.29 ± 0.28 (14) <sup>a</sup>	-0.04 ± 0.23 (14)
C (0.024)	0.40 ± 0.21 (19)	0.41 ± 0.39 (16) <sup>a</sup>	0.24 ± 0.35 (12)	0.32 ± 0.63 (12)	0.20 ± 0.42 (12)	-0.12 ± 0.26 (12)

Values are expressed as mean ± SD (N). TG, Triglyceride.

<sup>a</sup> Paired *t* test, significant difference compared to baseline (*P* < 0.05).

<sup>b</sup> One-way ANOVA with a contrast defining dose dependency (*P* < 0.05).

of 20K-hGH (0.012 and 0.024 mg/kg) were apparently excessive, judged by the supraphysiological IGF-I levels. The potency of 20K-hGH to increase serum IGF-I appears to be equivalent to that previously reported for 22K-hGH.

There are several lines of evidence that long-term GH treatment stimulates bone turnover and increases bone mineral density (24, 25). The data presented here demonstrated that 20K-hGH dose-dependently increased both serum osteocalcin levels and urinary pyridinoline excretion, indicating that 20K-hGH also has the same effects in bone metabolism as 22K-hGH. As with IGF-I production, stimulation of bone turnover was evident at 4 wk at the lowest dose (0.006 mg/kg) of 20K-hGH. The effects of 20K-hGH on body composition (reduction of fat mass and increase of LBM) and abdominal fat distribution (decrease of visceral fat area) were also consistent with those reported for 22K-hGH (10–12, 26–30), for which the doses of 0.005–0.025 mg/kg·d were used. In the study using bioelectrical impedance, the body fat decreased significantly at 4 wk of treatment. This rapid change might be, at least in part, due to changes in body water. With respect to 22K-hGH, Bengtsson *et al.* (13) reported that BFM was decreased by approximately 25% in the bioelectrical impedance analysis after 6 months of treatment with 22K-hGH and sc fat tissues were changed by 22K-hGH, indicating that sc fat tissue decreased by 13% in the abdominal CT scan, whereas visceral fat tissue decreased by 30%. Thus, the effect of 20K-hGH on fat tissue was comparable to that of 22K-hGH.

The reported effects of 22K-hGH on serum lipid profiles in GHD patients are not consistent (11, 29, 30). Generally, GH treatment was reported to produce a mild reduction of total Cho without changes in triglycerides levels. The effects of

20K-hGH on total, HDL-, and LDL-Cho levels were minor, and there was no change in serum triglyceride concentrations. The slight change of LDL-Rf value was only transient. In contrast, treatment with 20K-hGH clearly increased plasma FFA levels during 4–12 wk of treatment, which is compatible with the potent lipolytic activity of 20K-hGH.

There are many reports showing that GHD is associated with abnormalities of cardiac function (31, 32), and GH treatment enhances cardiac function, increases cardiac mass, and reverses diastolic abnormalities in adults with hypopituitarism and GHD (33). In the present study, we found that both %FS and EF were within the normal range before treatment and remained unchanged throughout the study period. The failure to detect any favorable cardiac effects may be due to the relatively short period of treatment (16 wk), as well as the condition of the patients before treatment in this study, in which no patient showed a decrease in cardiac function. GH has also been reported to increase muscle volume, but the effect of muscle strength has been inconsistent (34, 35). Although 20K-hGH significantly increased LBM, it had no effect on grasping power. Long-term treatment may be required to increase muscle strength.

There are a number of reports regarding QOL of GHD patients. Adults with GHD frequently complain of lack of energy, fatigue, and social isolation resulting in a perception of low QOL. However, the effects of GH therapy on QOL have been conflicting, possibly because of sample heterogeneity (age of GHD onset, duration of GHD, different ethnic or cultural background), length of GH treatment, and different measures of QOL. McGauley (36) reported that 22K-hGH replacement for 6 months [0.07 IU/kg·d (approximately 0.02 mg/kg·d)] was associated with an improvement in

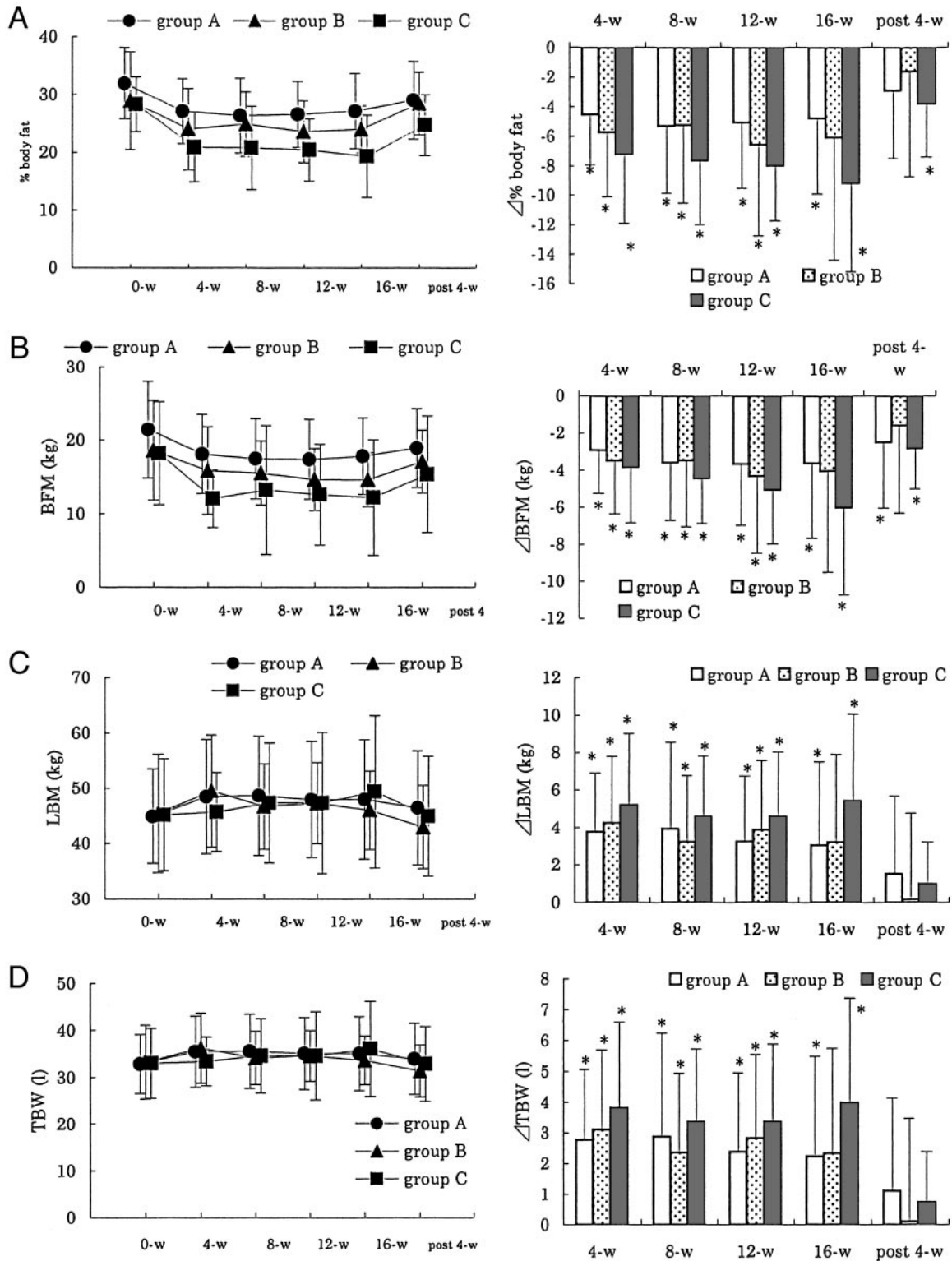


FIG. 3. Changes of body composition, percentage body fat (A), BFM (B), LBM (C), and total body water (TBW) (left, actual values; right, changed values from baseline). Each value represents mean  $\pm$  SD. Significant differences compared with initial values were investigated with paired *t* test (\*,  $P < 0.05$ ).

mood and energy in adult GHD. Furthermore, Bengtsson *et al.* (13) demonstrated a significant improvement on the Comprehensive Psychological Rating Scale using the same dose

of 22K-hGH replacement therapy, whereas Whitehead *et al.* (34) did not find any effect of 22K-hGH treatment on QOL. The present result with 20K-hGH was similar to that reported

**TABLE 3.** Changes in abdominal subcutaneous (S) and visceral (V) fat areas and V/S ratio

Group [dose (mg/kg·d)]	Initial	16 wk [Δ %]	4 wk post treatment [Δ %]
<b>S fat area (cm<sup>2</sup>)</b>			
A Low (0.006)	154.9 ± 57.7 (14)	135.5 ± 56.3 (14) [−12.5%] <sup>a</sup>	143.9 ± 68.5 (14) [−7.1%]
B Middle (0.012)	128.3 ± 49.0 (10)	116.5 ± 51.5 (10) [−9.2%]	133.5 ± 47.7 (10) [4.1%]
C High (0.024)	143.1 ± 78.4 (9)	102.3 ± 71.7 (9) [−28.5%] <sup>a</sup>	122.3 ± 78.6 (9) [−14.5%]
<b>V fat area (cm<sup>2</sup>)</b>			
A Low (0.006)	73.0 ± 31.1 (16)	46.5 ± 21.3 (16) [−36.3%] <sup>a</sup>	56.3 ± 30.1 (16) [−22.9%]
B Middle (0.012)	65.3 ± 47.6 (10)	43.7 ± 32.3 (10) [−33.1%] <sup>a</sup>	57.5 ± 36.1 (10) [−11.9%]
C High (0.024)	49.8 ± 27.2 (9)	25.3 ± 16.9 (9) [−49.2%] <sup>a</sup>	38.1 ± 29.2 (9) [−23.5%]
<b>V/S ratio</b>			
A Low (0.006)	0.48 ± 0.19 (14)	0.35 ± 0.15 (14) [−27.1%]	0.40 ± 0.18 (14) [−16.7%]
B Middle (0.012)	0.50 ± 0.32 (10)	0.37 ± 0.23 (10) [−26.0%]	0.45 ± 0.36 (10) [−10.0%]
C High (0.024)	0.42 ± 0.23 (9)	0.36 ± 0.24 (9) [−14.3%]	0.41 ± 0.27 (9) [−2.4%]

Values are expressed as mean ± SD (N).

<sup>a</sup> Paired *t* test, significant difference compared to baseline (*P* < 0.05).

**TABLE 4.** Effect of 20K-hGH on carbohydrate metabolism

Group (dose, mg/kg·d)	Initial values 0 wk	Changed values				
		4 wk	8 wk	12 wk	16 wk	4 wk post treatment
<b>Blood glucose (mg/dl)</b>						
A (0.006)	87 ± 12 (19)	4.5 ± 8.3 (19)	1.7 ± 10.2 (19)	2.4 ± 6.5 (19)	1.3 ± 8.8 (19)	−1.9 ± 10.9 (19)
B (0.012)	93 ± 15 (18)	5.2 ± 16.3 (16)	1.6 ± 16.6 (15)	2.8 ± 21.7 (15)	−1.4 ± 25.3 (15)	−2.7 ± 21.3 (15)
C (0.024)	88 ± 9 (19)	17.4 ± 14.2 (16) <sup>a,b</sup>	9.1 ± 13.7 (12)	8.9 ± 12.0 (12)	7.1 ± 12.8 (12)	−1.7 ± 7.9 (12)
<b>Serum insulin (μU/ml)</b>						
A (0.006)	11 ± 12 (19)	3.2 ± 14.5 (19)	0.2 ± 12.4 (19)	−1.1 ± 11.5 (19)	−0.9 ± 14.7 (19)	−3.9 ± 12.2 (19)
B (0.012)	9 ± 7 (18)	3.8 ± 10.5 (16)	0.5 ± 9.4 (15)	0.2 ± 9.6 (15)	1.3 ± 10.4 (15)	−2.2 ± 8.1 (15)
C (0.024)	6 ± 4 (18)	9.9 ± 9.8 (15) <sup>a</sup>	4.9 ± 5.1 (12)	3.6 ± 4.4 (11)	6.8 ± 10.2 (12)	−2.2 ± 3.0 (11)
<b>HbA1c (%)</b>						
A (0.006)	5 ± 0 (19)	0.06 ± 0.15 (19)	0.11 ± 0.3 (19)	0.10 ± 0.27 (19)	0.18 ± 0.23 (19) <sup>a</sup>	0.10 ± 0.28 (19)
B (0.012)	5 ± 1 (18)	0.06 ± 0.19 (16)	0.11 ± 0.3 (15)	0.23 ± 0.30 (15)	0.30 ± 0.24 (15) <sup>a</sup>	0.19 ± 0.17 (15) <sup>a</sup>
C (0.024)	5 ± 0 (19)	0.18 ± 0.45 (16)	0.30 ± 0.5 (12)	0.36 ± 0.38 (12) <sup>a</sup>	0.37 ± 0.27 (12) <sup>a</sup>	0.24 ± 0.19 (12) <sup>a</sup>

Values are expressed as mean ± SD (N).

<sup>a</sup> Paired *t* test, significant difference compared to baseline (*P* < 0.05).

<sup>b</sup> One-way ANOVA with a contrast defining dose dependency (*P* < 0.05).

**TABLE 5.** Results of 75 g OGTT performed before and after 16-wk administration of 20K-hGH

Group [Dose (mg/kg·d)]	Initial	16 wk			Incidence of IGT <sup>a</sup>
		Normal type	Borderline type	Diabetic type	
A (0.006)	Normal type	17	11	4	5/19 (26.3%)
	Borderline type	2	1	0	
B (0.012)	Normal type	12	7	5	5/15 (33.3%)
	Borderline type	3	1	0	
C (0.024)	Normal type	9	5	4	5/13 (38.5%)
	Borderline type	4	1	2	

The state of glycemia was classified into three categories on the basis of the plasma glucose 2 h after 75-g glucose load as follows: normal type (normoglycemia), below 140 mg/dl; borderline type (impaired glycemia), 140 mg/dl or higher to below 200 mg/dl; and diabetic type, 200 mg/dl or higher.

<sup>a</sup> IGT: patient who got worse classification at 16 wk compared to initial.

by Whitehead *et al.* (12), although there were a few elements that showed increased scores when compared with the baseline. The effects were marginal and not dependent on the doses of 20K-hGH. Thus, we were not able to determine the effect of 20K-hGH on QOL of adult GHD patients in the relatively short-term study.

Initiation of GH treatment in adults is frequently complicated by the development of symptomatic fluid retention (11–13). The mechanism by which GH causes fluid retention is not completely understood. Involvement of suppression of ANP was suggested in one study (36). However, Hoffman *et al.* (37) reported that GH affected neither aldosterone secretion nor ANP release and suggested a direct renal tubular

effect of GH. The renal effect may be mediated by increased Na-K ATPase (38). A recent study in our laboratory has shown that the water-retention effect of 20K-hGH is significantly lower than 22K-hGH in rats (14). We expected the same results in GHD patients. This was not the case, however. Incidence of edema in this study was comparable to that reported for 22K-hGH. The reason for the discrepancy between rats and humans is not clear at present.

Diabetogenic activity of 22K-hGH is well established; 22K-hGH inhibits the uptake and utilization of glucose in muscle and thereby produces insulin resistance (39). Previously, O'Neal *et al.* (29) reported that treatment with 22K-hGH (120 μg/kg·wk) induced a temporary and mild glucose intoler-

ance, hyperinsulinemia, and insulin resistance and raised NEFA levels at 1 wk; modest hyperinsulinemia persisted for 3 months in iv glucose tolerance test. The result of OGTT in the present study showed that five patients in each group had an IGT, although no one developed overt diabetes. This might indicate that 20K-hGH has a similar potency to 22K-hGH in inducing insulin resistance in adult GHD. We previously showed that the diabetic activity of recombinant 20K-hGH is much weaker than 22K-hGH in rats (15). The reason for the difference between rats and humans remains to be determined. It is known that there is a difference in the distribution of GH receptors in major target organs of GH, especially in the liver, between rats and humans. This may be responsible for the discrepancy. Although it has been reported that 20K-hGH has less affinity to GHBP when compared with 22K-hGH, the binding to circulating GHBPs was not investigated in this study. Furthermore, it has been shown that 20K-hGH is likely to form an active 1:2 complex (hGH:hGHR) in a way similar to 22K-hGH but has difficulty in forming an inactive 1:1 complex. The reason 20K-hGH does not show the characteristics that are revealed in cells and animal models has to be clarified in connection with the above molecular biological knowledge in further studies.

We found in this study that serum AST, ALT, or  $\gamma$ -GTP increased in some patients treated with 20K-hGH. None of them was alcoholic, and the liver enzyme values were in normal range in the test carried out immediately before the present study. In another series of studies using 52 patients, we have found that approximately 50% of the patients with GHD have fatty liver as judged by ultrasonographic findings, associated with slight and transient elevation in liver enzymes (our unpublished observation). We presume that the elevated liver enzymes may be due to the fatty liver. It appears that 20K-hGH is not involved in the liver dysfunction because these abnormalities normalized despite continuous treatment with GH. Therefore, these changes should be monitored carefully in the further studies, although all abnormal values returned to normal range without any medical treatment in this study.

Taken together, the results presented here have demonstrated for the first time that recombinant 20K-hGH has metabolic effects comparable to 22K-hGH in human GHD subjects. The biological potency appears to be equal to that of 22K-hGH. 20K-hGH was able to normalize serum IGF-I levels at a dose as low as 0.006 mg/kg·d. Incidence of unfavorable effects was also similar to that reported for 22K-hGH. We suggest that recombinant 20K-hGH could be used in the treatment of patients with GHD. However, further studies are required to investigate the optimum dose and superiority of 20K-hGH over 22K-hGH. This should be clarified in a comparative study.

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