EXPERIMENTAL STUDY

Metabolic effects of 20 kDa and 22 kDa human growth hormones on adult male spontaneous dwarf rats

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Abstract

Background: Two molecular forms of human GH (hGH) have been shown to be biologically active. The 20 kDa form has been reported to have weaker diabetogenic and lipolytic actions than the 22 kDa form.

Objective: To analyze the carbohydrate metabolism of 20 kDa and 22 kDa hGH, using the adult male spontaneous dwarf rat (SDR), which is GH deficient.

Design: SDRs were given 20 kDa or 22 kDa hGH in doses of 125 μg/rat or 500 μg/rat, or saline, for 10 days, and their weight, serum IGF-I, glucose, insulin, leptin and body composition were measured.

Results: Weight and serum IGF-I increased both in the 20 kDa and 22 kDa groups, but IGF-I concentrations were significantly lower in the 20 kDa group than in the 22 kDa group. Serum glucose was not increased by either 20 kDa or 22 kDa hGH, whereas insulin was significantly increased after the higher dose of the 22 kDa hGH. Although blood concentrations of leptin were decreased by both 20 kDa and 22 kDa hGH, values were lower in the high-dose 20 kDa group than in the group given the same dose of 22 kDa hGH. Both forms of GH increased the percentage body water and body protein content, and decreased the percentage of body fat by the same degree. The observation that the higher dose of the 22 kDa hGH increased insulin concentrations without changing blood glucose demonstrates that this concentration of the hormone induces insulin resistance, whereas the same dose of 20 kDa hGH does not.

Conclusions: The results can be interpreted to indicate that the higher dose of the 22 kDa hGH has diabetogenic activity, as reported previously, whereas the 20 kDa hGH has lower diabetogenic activity. The 20 kDa form of hGH may therefore be more useful in treating adult GH deficiency, especially those with severe obesity.

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Introduction

The 22 kDa form of human growth hormone (hGH) is the most abundant form of GH. The primary form of pituitary hGH consists of 191 amino acids and has a molecular mass of 22 124 Daltons (1). The second most abundant hGH in the pituitary is the 20 000 Dalton variant (2), which is a single chain, 176 amino acid protein, similar to the 22 kDa form but with residues 32–46 deleted (3). It has been reported that the 20 kDa form accounts for 10–15% of the total pituitary GH. Recent specific measurement of this form revealed that it represents about 6% of serum hGH (4, 5).

It has been determined and recognized that certain of the actions of 20 kDa hGH, such as its diabetogenic and lipolytic actions, are different from those of 22 kDa hGH. It has been reported (6) that, in dogs, the 20 kDa form has a lower diabetogenic action because, in glucose tolerance tests, blood glucose did not change significantly after treatment with 20 kDa hGH compared with controls, whereas it did increase significantly after 22 kDa hGH treatment. It has also been reported that the lipolytic action is weaker after administration of the 20 kDa form than after the 22 kDa, as serum free fatty acids in hypophysectomised rats do not increase significantly 5 h after treatment with 20 kDa hGH, but do increase after 22 kDa hGH treatment (7). Some effects of 22 kDa hGH on body composition (8, 9), namely decreasing body fat and increasing the ratio of protein to fat, have been identified, but in general the effects
of the 20 kDa hGH variant on body composition have not been studied adequately.

Here, in the first report on the action of recombinant 20 kDa hGH in adult GH-deficient spontaneous dwarf rats (SDR) (10, 11) we studied the effect of 20 kDa hGH on carbohydrate metabolism and body composition.

Materials and methods

Animals and diet

The 7–8-month-old male adult SDRs, genetically segregated from Sprague–Dawley rats, were provided by Morishita Pharmaceutical Co. Ltd (Shiga, Japan) and the Department of Anatomy II, Jikei University School of Medicine, where the SDR gene mutation was investigated (10, 11). Eight-month-old Sprague–Dawley rats were also used. They were housed in controlled conditions of light (lights on 11 h each day) and temperature (23°C), and were given tap water and a standard diet.

Materials

Human 22 kDa GH (Norditropin 4 IU/V) was provided by Novo Nordisk A/S (Bagsvaerd, Denmark) and human 20 kDa hGH was prepared by Mitsui Pharmaceuticals Inc. (Tokyo, Japan) (12). The 22 kDa hGH was stored at 4°C and was dissolved on the day of the experiments to concentrations of 500 mg/ml and 2 mg/ml according to the procedure recommended by Novo Nordisk; the 20 kDa hGH was dissolved in saline to give concentrations of 500 μg/ml and 2 mg/ml.

Experimental design

Seven- to eight-month-old male SDRs were divided into five groups of seven to nine animals each. The groups were treated with either the 20 kDa hGH or 22 kDa hGH in a concentration of either 125 μg/rat or 500 μg/rat, or with saline as the control. In the 20 kDa hGH and 22 kDa hGH-treated groups, the recombinant hormone was administered by subcutaneous injection in a volume of 0.250 ml per rat every morning for 10 days. The control groups received 0.250 ml saline in the same manner. In addition, 8-month-old male Sprague–Dawley rats were administered 0.250 ml saline as a normal control group (n = 8). All of the animals were weighed every morning before receiving their injection. On the morning of the 11th day, after overnight fasting (24 h after the 10th injection), all animals were decapitated, blood samples were collected from the heart for determination of serum glucose and insulin, and their carcasses weighted before being stored frozen at −70°C until required. The procedure was approved by the Committee on Animal Care and Use of the Jikei University School of Medicine.

Measurement of serum IGF-I, blood glucose, insulin and leptin

The serum concentration of IGF-I was measured by formic acid–acetone extraction and RIA (Eiken Chemical Co. Ltd, Tokyo, Japan) using the method of Bowsher et al. (13), with slight modifications. Briefly, a 100 μl aliquot of serum was acidified by adding 50 μl 8.0 M formic acid–0.5% Tween-20. Immediately after vortexing, 350 μl acetone was added and mixed by further vortexing. After centrifugation at 3000 r.p.m. for 20 min at 4°C, the supernatant was diluted with RIA buffer (1:20) and used directly for RIA, using rat IGF-I for the standards.

The serum concentration of blood sugar was measured by Reflotron (Yamanouchi Pharmaceuticals Inc., Tokyo, Japan) and serum insulin was measured using the Ab Bead Insulin ‘Eiken’ Radioimmunoassay kit (Eiken Chemical Co. Ltd). The serum concentration of leptin was analyzed using a rat leptin RIA kit (Linco Research Inc., St Louis, USA).

Measurement of body composition

The amounts of water, fat, protein in relation to body mass were measured from the minced carcasses of each rat, as previously described (14, 15). Water content was determined by freeze-drying the carcass to a constant weight, and fat content was measured using Folch’s method. Chloroform–methanol (2:1; 150 ml) was added to 10 g of the minced carcass, mixed well, and left undisturbed for a day. The mixture was then filtered using a G-4 glass filter. The residue was washed using chloroform–methanol, and the volume of the eluted solution was adjusted to 200 ml by the chloroform method. CaCl₂ (4 ml of 0.003 M) was added to 20 ml of the mixture, and the new mixture was shaken vigorously for 10 min and centrifuged at 3000 r.p.m. for 10 min. The organic layer was concentrated under N₂ at 40°C and reweighed. From these data, the total fat content was calculated. The protein content was calculated as (total body nitrogen content) × 6.25, and the nitrogen content of the carcass residue was measured by the method of keldahl using a kjeltec DS-20 autoanalyzer (Tecator Ltd, Bristol, Avon, UK).

Statistical analysis

Statistical analyses were performed by a parametric two-tailed comparisons using the Mann–Whitney U-tests in a Stat View (version 4.5) package from Abacus Concepts, Inc. (Berkeley, CA, USA).

Results

Body weight

Figure 1 shows the changes in body weight after 20 kDa or 22 kDa hGH. After 10 days of injections, body
Weights increased significantly, by approximately 8.6–14.1% \( (P < 0.05) \), in both the 20 kDa- and 22 kDa hGH-treated groups, whereas that in the SDR control group decreased to approximately 2.8%. Body weights of the normal Sprague–Dawley rats did not change significantly. However, after overnight fasting on the 11th day, the body weights of all SDR groups and the normal Sprague–Dawley rats decreased by 4.4±1.7% and 5.1±1.2% \( (\text{means±S.D.}) \) respectively. There was no significant difference in the percentage increase in body weight between the 20 kDa- and 22 kDa hGH-treated groups, or between those receiving concentrations of hGH 125 \( \mu \text{g/rat} \) or 500 \( \mu \text{g/rat} \), but the body weights of rats receiving 500 \( \mu \text{g} \) of the 20 kDa form of hGH were greater than those receiving 125 \( \mu \text{g} \) of the 20 kDa form.

**Serum IGF-I**

IGF-I concentrations in control SDRs were markedly lower than those in normal Sprague–Dawley rats \( (87.9 \text{ng/ml and 178.3 ng/ml respectively}) \). The concentrations increased in a dose-dependent manner by both the 22 kDa hGH-treated groups, whereas in the SDR group given 20 kDa hGH there was no significant difference between those given saline and those given hGH 125 \( \mu \text{g/rat} \), but the concentration was significantly greater in those given hGH 500 \( \mu \text{g/rat} \) than in the saline group \( (\text{Fig. 2}) \). The concentration was also greater in 22 kDa hGH-treated groups than in 20 kDa hGH-treated groups, whether they received 125 \( \mu \text{g/rat} \) or 500 \( \mu \text{g/rat} \) \( (P < 0.01 \text{ and} P < 0.0005 \) respectively).

**Serum blood glucose and insulin**

The concentration of glucose was significantly lower in the SDR control group \( (129.1±14.2 \text{mg/dl}) \) than in normal Sprague–Dawley rats \( (161.0±12.4 \text{mg/dl}) \). The serum concentration of insulin was also significantly lower in SDR control group \( (10.1±2.5 \mu \text{U/ml}) \) than in Sprague–Dawley rats \( (49.1±19.3 \mu \text{U/ml}) \). Although there was no significant change in serum blood glucose after hGH administration in all the GH-treated animals \( (\text{Fig. 3A}) \), and no significant difference in blood glucose between the groups given 20 kDa or 22 kDa hGH, insulin concentrations were significantly greater in the group given 22 kDa hGH 500 \( \mu \text{g/rat} \) than in both the group given 500 \( \mu \text{g/rat} \) of the 20 kDa form and the SDR control group given saline \( (\text{Fig. 3B}) \).
Serum leptin

The serum leptin concentration was greater in the SDR control group than in Sprague–Dawley rats. The leptin concentration showed a decrease after hGH treatment and was significantly lower in the group given 500 mg of 20 kDa hGH than in those receiving 500 mg of the 22 kDa form (Fig. 4). The concentration of leptin was correlated to the percentage of fat mass \(P < 0.0001, r = 0.637\).

Body composition

The percentage of body protein and body water increased in response to GH treatment. The percentage of body fat decreased in these groups; however, there was no significant difference between the groups given the 20 kDa or the 22 kDa forms of hGH (Fig. 5).

Discussion

It has been determined and recognized that certain of the actions of 20 kDa hGH, such as its diabetogenic and lipolytic actions, are weaker than those of the 22 kDa form (3). In addition, some effects of the 22 kDa form on body composition (8, 9), namely decreasing body fat and increasing the protein-to-fat ratio, have been identified. However, the effects of 20 kDa hGH on body composition in general have not been studied. We therefore investigated the actions of 20 kDa hGH, in particular those on body composition in SDRs. The SDR is regarded as a suitable animal model in which to study isolated GH deficiency, because other pituitary hormones are preserved in this rat even though it is deficient in GH (10, 11).

The somatogenic activity of 20 kDa hGH has previously been studied in prepubertal SDRs and found to be almost same as that of 22 kDa hGH (16). In our present study, it became clear that administration of 20 kDa hGH increased the serum concentrations of IGF-I, but the activity was weaker than that of 22 kDa hGH. The differences between the two studies were the dose of GH administered and the age of the SDRs. The reason why the IGF-I production was greater in the group given 22 kDa hGH than in the group given 20 kDa hGH in adult rats remains unclear. However, a tendency towards a greater concentration of IGF-I in the 22 kDa hGH-treated group than in those treated with the 20 kDa hGH form was recognized in prepubertal male rats.

The fact that insulin concentrations increased after administration of the high dose of 22 kDa hGH, without associated changes in blood glucose, demonstrates that high doses of this variant induce insulin resistance whereas the 20 kDa form does not. This is comparable to the observation that fasting insulin in adult patients with GH deficiency increased significantly after 6 or 24 months of GH therapy (17). The action of 20 kDa hGH in increasing the fasting insulin concentration was weaker than that of the 22 kDa form in SDRs, although body fat mass, which has been considered to be related to insulin resistance, was almost the same in both the 20 kDa and 22 kDa hGH-treated groups. It has been reported that treatment with recombinant 22 kDa hGH caused insulin resistance, although body fat mass decreased (17, 18). A retrospective analysis of data from an international pharmacepidemiological survey of children treated with GH was undertaken to determine the incidence of impaired glucose tolerance, and both insulin-dependent and non-insulin-dependent
diabetes mellitus (NIDDM). Results showed that the incidence of NIDDM was sixfold greater than that reported in children not treated with GH (19). Therefore, it is speculated that the decreases in fat in response to GH treatment are not sufficient to compensate for the hyperinsulinemia caused by the anti-insulin effect of 22 kDa hGH, especially in higher doses. This supports the premise that the 20 kDa form of hGH has a weaker anti-insulin effect than the 22 kDa form. Kostyo et al. (20) determined the diabetogenic activity of 20 kDa hGH using female ob/ob mice. They demonstrated that fasting blood sugar was increased after the administration of 20 kDa hGH-hGH for 3 days in doses of 25 or 50 μg/day, but not after doses of 5 or 10 μg/day. In another study, Kostyo and colleagues (21) determined the diabetogenic activity of 20 kDa hGH using female ob/ob mice. They demonstrated that fasting blood sugar increased after the administration of 20 kDa hGH-hGH for 3 days in doses of 25 or 50 μg/day, but not after doses of 5 or 10 μg/day. In our study, the concentration of insulin was increased only in the group treated with 22 kDa hGH, which is the most abundant form of GH in the pituitary. In our study, the concentration of insulin was increased only in the group treated with 22 kDa hGH 500 μg/rat per day, whereas the concentration of blood glucose was not increased in any group. These differences in findings might be caused by factors such as sex and GH deficiency. These details should be determined in the future.

The percent body fat in the adult SDRs was high compared with that in normal rats. Obesity is one of the characteristics of human adult GH deficiency (22), and is known as a risk factor for NIDDM, hypertension and atherosclerosis. The body fat mass decreased after the administration of both the 20 kDa hGH and the 22 kDa forms of hGH, indicating that the 20 kDa variant has same potency to reduce the increased fat mass caused by GH deficiency as has the 22 kDa form.

Leptin has been reported to be secreted from adipose tissue (23, 24). It is also reported that the serum leptin concentration was greater in patients with GH

Figure 4 Effects of the administration of 20 kDa hGH and 22 kDa hGH on serum leptin in SDRs and normal Sprague–Dawley rats (SD) (means±s.d.).
deficiency than in normal individuals (25), and that long-term GH replacement therapy results in decreased serum leptin concentrations (25, 26). Miyakawa et al. (27) determined the effect of GH on serum leptin in patients with acromegaly and GH deficiency, and suggested that GH or IGF-I reduces serum leptin concentrations by reducing body fat mass. In our study, serum leptin decreased in response to GH treatment; leptin concentrations also correlated significantly with the percentage to body fat (P = 0.0001). There is no difference between the 22 kDa hGH- and 20 kDa hGH-treated groups with respect to percentage of body fat mass, although the leptin concentration was significantly lower in the latter group given high doses of hormone. These data suggest that GH reduces leptin by unknown mechanisms, different from those producing body fat mass reduction.

Finally, because we used human GH in rats, we should take into account the differences between the GH receptors (GHR) of humans and rats. We previously determined the somatogenic activity of hGH in prepubertal SDRs (16). The somatogenic activity of 20 kDa hGH in SDRs was almost same as that of the 22 kDa form in the high-dose GH (100 µg/rat) administration groups, the percentage increase in body weight on SDRs being almost the same as in normal Sprague–Dawley rats. Comparisons of the affinity of 20 kDa hGH and 22 kDa hGH for the GHR have been reported in several species. Barnard et al. (30) determined the potency of 20 kDa hGH at the rat, rabbit and human GHRs, and showed that its relative potency in rat liver, rat adipose tissue, rabbit liver and rabbit adipose tissue were 18, 6, 10 and 6% those of 22 kDa form respectively. Barnard et al. (30) also reported that the relative potency of 20 kDa hGH was slightly lower than that of 22 kDa hGH in IM9 cells, which is a human lymphoma cell line. As the results were very different among species, the result of our present study in rats might not be applicable to humans. The somatogenic and metabolic effects of 20 kDa hGH should be determined in human cells such as hepatocytes in vitro in a future study.

In conclusion, we suggest that the 20 kDa GH variant has a lower diabetogenic action than the 22 kDa form, and that it has more potency in reducing body fat in SDRs.

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