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# Serum Concentrations of 20K Human Growth Hormone in Normal Adults and Patients with Various Endocrine Disorders

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**Abstract.** The 20K hGH isoform is produced by alternative splicing of GH mRNA, and comprises approximately 10% of all GH in the pituitary. The physiological role of 20K hGH remains to be determined partly because of the lack of a simple and specific assay. We have established sensitive enzyme-linked immunoadsorbent assays (ELISAs) specific to 20K and 22K hGH. The serum levels of 20K hGH after overnight fasting was 118±178 pg/mL (N=282) in normal women, significantly higher than in normal men (64±170 pg/mL, N=226). However, there was no difference in the proportion of 20K hGH to 20K plus 22K hGH between men (6.3±2.6%, N=176) and women (6.3±2.1%, N=263). No correlation was detected between the ratio of 20K hGH and age, body height, body weight or body fat mass in normal subjects. The proportion of 20K hGH was significantly ($P<0.001$) higher in patients with active acromegaly (9.2±2.2%, N=33) and in patients with anorexia nervosa (9.0±1.9, N=8), both of which are characterized by chronic elevation of circulating GH levels. The proportion of the 20K hGH in successfully treated acromegalic patients did not differ from that in normal subjects, suggesting that GH-producing pituitary tumors secrete a higher proportion of 20K hGH, or chronic excess of 22K hGH altering the metabolic clearance rate of 20K hGH. The values in patients with adult growth hormone deficiency (GHD), hyperthyroidism, primary hypothyroidism, or GH-independent short stature did not differ from those in normal subjects. The 20K ratio did not change after acute GH provocative tests such as insulin tolerance test and GRH test. These results suggest that secretion of 20K hGH from the pituitary is under the same control as that of 22K hGH.

*Key words*: 20K hGH, ELISA, Acromegaly, Anorexia nervosa

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**THERE** are several isoforms of human growth hormone (27K, 22K, 20K, and 19K hGH, and 5K hGH fragment) in both pituitary and serum [1]. The major species is 22K hGH, and the second most abundant isoform is 20K hGH, which lacks 32–46 residues of 22K hGH. The 20K hGH is produced by alternative splicing of 22K hGH mRNA [2],and this isoform has been shown to comprise approximately 10% of the pituitary GH. A number of studies have reported that 20K hGH differs from 22K hGH in receptor binding, metabolic clearance and biological activities [1, 3]. However, a recent study has shown that the binding affinity of recombinant 20K hGH to CHO cells stably transfected with hGH receptors is comparable to that of 22K hGH, and the 20K hGH exerts a full agonistic activity on cell proliferation [4]. Despite these studies, little information is available on the proportion of 20K hGH isoform in circulation

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under different physiological and pathophysiological conditions because of the lack of a specific assay. Serum concentrations of 20K hGH or non-22K hGH have been determined by several methods [5–8]. However, these methods require several steps and are not suitable for determining serum concentrations of the 20K hGH in a large number of samples. Recently, a monoclonal antibody (mAb) to 20K hGH was developed and used for sandwich enzyme immunoassay (EIA) specific to 20K hGH [9]. However, the sensitivity (0.2 nmol/L; 4 ng/mL) of the assay was not sufficient to determine the basal serum concentrations of the isoform in normal subjects. Recently, we developed a more sensitive enzyme-linked immunosorbent assay (ELISA) for the 20K variant [10]. Using the novel ELISA, we measured the concentrations of the 20K hGH in serum from normal subjects and patients with endocrine or metabolic disorders, and correlated the values with those of 22K hGH. The procedure of 20K- hGH ELISAs were described in detail eleswhere [10]. Briefly, monoclonal antibody (mAb) D05 specific to 20K hGH (Kd=440 pmol/L for 20K hGH) was coated onto a 96-well microtiter plate as a capture antibody. The assay was started by adding 0.1 mL of assay buffer or serum sample. The plates were incubated for 2 h at room temperature with constant shaking. The 20K hGH bound to the mAb D05 was allowed to react with horseradish peroxidase (POD)-conjugated mAb D14 which recognized both 20K hGH and 22K hGH with equal affinity. The wells were washed again and a substrate solution containing 3,3′,5,5′-tetramethylbenzidine and $H_2O_2$, pH 3.8 was added. The plates were incubated for 30 min in at room temperature, and the reaction was terminated by adding sulfuric acid. The absorbance was determined with a microtiter plate reader at 450 nm against reference of 620 nm. 22K hGH was measured in the same manner except that A36020047P, a monoclonal antibody specific to 22K hGH (BiosPacific Inc. Emeryville, CA) was used as the capture abtibody. The sensitivities of the assays were 5 pg/mL for 20K hGH and 50 pg/mL for 22K hGH. The 22K hGH, hGH fragment 44–191, hPRL and hPL did not cross-react in the ELISA for 20K hGH at concentrations up to 1000 ng/mL. Likewise, cross-reaction of hGH fragment 44–191, 20K hGH, hPL, and hPRL was negligible in the 22K hGH ELISA.

## Results and Discussion

The mean fasting serum 20K hGH level was 94±176 pg/mL in 508 normal subjects with a significantly higher level of 117±178 pg/mL (mean±SD, N=282) in women than in men 64±170 pg/mL for men (N=226). The higher 20K hGH values in women may be due to the higher levels of estradiol, as reported for 22K hGH [11]. Both 20K and 22K hGH concentrations were less than the assay limit in 50 out of 226 men, and 19 out of 282 women. There was a positive correlation between serum 20K and 22K hGH concentrations in normal subjects ($r=0.956$, $P<0.0001$), suggesting that both species of hGH are secreted in a parallel fashion.

The mean ratio of 20K hGH to 20K plus 22K hGH was 6.3±2.3% (range 0.9–23.1%), and there was sex difference. The majority of normal adults (76% of females and 61% of males) showed a ratio of 5–10%, and 19.8% of women and 30% of men had a value below 5%. The values were not very different from those determined by polyacrylamide gel electrophoresis [12]. Thus, circulating monomeric hGHs largely consist of 22K and 20K hGH, although Sinha and Jacobsen have recently reported that a 17 KDa fragment of hGH (hGH 44–191) circulates in blood at levels 1–2 times higher than that of 22K hGH [13].

There was no correlation between the ratio and age, sex, body weight, body height, or body mass index. Thus, the proportion of 20K hGH was not the determinant for these variables. Although an *in vitro* study [14] has shown that 20K hGH has a statistically higher lipolytic activity at low concentrations (1 and 10 ng/mL) than 22K, we could not find any correlation between the percentage of 20K hGH and percent body fat mass determined by the bioimpedance method. Thus, it remains to be determined whether 20K is involved in the regulation of lipid and carbohydrate metabolism.

The proportion of 20K hGH in patients with several hormonal or metabolic disorders is illustrated in Fig. 1. We found that the ratio of 20K hGH to 20K+22K hGH (9.2±2.2%, N=34; Mean±SD) is significantly higher than normal subjects ($P<0.001$, ANOVA) in patients with active acromegaly. The values decreased to normal range after succeful treatment. The result is similar to the result recently reported by Boguszewski CL *et al.* [15]. They

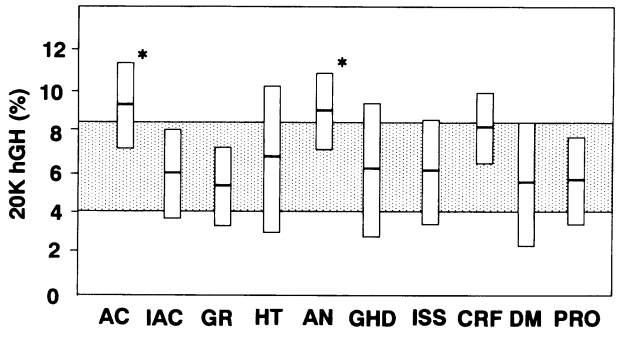


**Fig. 1.** The proportion of 20K hGH in various disorders. The ratio of 20K hGH to 20K plus 22K hGH was determined in active acromegaly (ACR; N=34), inactive acromegaly (IAC; N=10), untreated Graves' disease (GR; N=9), primary hypothyroidism (HT; N=10), anorexia nervosa (AN; N=8), idiopathic short stature (ISS; N=9), GH deficiency (GHD; N=31), chronic renal failure (CRF; N=10), diabetes mellitus (DM; N=11), and prolactinoma (PRO; N=5). The values are expressed as the meanSD. The ratios in active acromegaly and in anorexia nervosa are significantly (*$P<0.001$) higher than that for normal adults (dotted area).

showed that the proportion of non-22K hGH in serum was significantly higher than in normal subjects and decreased to the normal levels after the successful removal of pituitary tumors. Furthermore, the values correlated to tumor size, mean 24-h hGH concentration and serum PRL, suggesting that the GH-producing pituitary adenoma secretes a higher proportion of non-22K hGH, including 20K hGH. We were not able to correlate the proportion of 20K hGH to tumor size or mean 24 h hGH levels in this retrospective study, but there was a positive correlation between the 20K proportion and serum IGF-1 levels (data not shown), suggesting that the evaluation of 20K hGH isoform, like that of non-22K hGH, is useful in the follow-up of acromegalic patients. Likewise, the 20K proportion was significantly ($P<0.001$) higher in patients with anorexia nervosa ($9.0\pm1.9\%$). The reason for the higher 20K ratio in acromegaly and anorexia nervosa is not clear at present. Secretion of 20K-hGH from the pituitary may be higher relative to that of 22K hGH in these disorders. Alternatively, metabolism of the variant might be altered in the face of an excess of 22K hGH.

There was no significant difference in the 20K hGH ratio between normal subjects and patients with hypothyroidism, hyperthyroidism, GH-deficiency, non-insulin depenent diabetes mellitus or non-GH dependent short statue. Recently, it has been reported that the proportion of non-22 K hGH is elevated in a subset of children with non-GH-deficient short stature [16]. The authors showed that in children born small for date, the proportion of non-22K hGH isoforms was directly correlated with mean 24 h GH concentrations, and inversely correlated with height SD score. In the present study, however, there was no difference in the proportion of 20K hGH isoform between normal subjects and 10 subjects with idiopathic short stature. Furthermore, the results for non-GH dependent short statue in children [17] were consistent with our results. It is thought possible that other hGH isoforms distinct from 20K hGH may contribute to the higher proportion of the non-22K hGH.

We also determined whether acute stimulation of hGH secretion altered the ratio of 20K hGH. The concentrations of 20K- and 22K hGH were measured in serum obtained before, 30 and 60 min after administration of insulin (0.1 U/kg body weight) or GRH (100 mg) in patients with suspected hypopituitarism. The TRH test was also performed in 7 patients with active acromegaly. Confirming the results reported by others using SDS-PAGE [5, 6, 16] or the 22K GH exclusion assay [7], there was no significant difference in the ratio of 20K to 20K plus 22K hGH between before and after these provocative tests (Fig. 2), suggesting that secretion of both species of

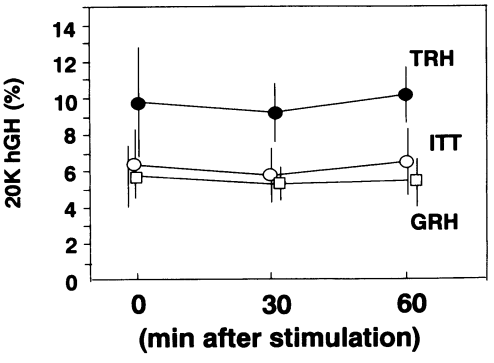


**Fig. 2.** Effect of GH provocative tests on 20K hGH ratio. Insulin tolerance test (0.1 U/kg body weight) and GRH test (100 $\mu$g) were carried out in 7 and 6 patients with suspected hypopituitarism, respectively. Eight patients with active acromegaly were challenged with TRH test (500 $\mu$g). In all these tests, there was no significant change in the 20K hGH proportion.

hGH is regulated in the same manner. Indeed, immunohistological studies have shown that 20K hGH is expressed in only 22K-hGH positive cells (San-noh et al, unpublished).

In conclusion, we showed that the ratio of 20K hGH to 20K plus 22K hGH is higher in active acromegaly and anorexia nervosa. The ratio for patients with GH deficiency, thyroid disorders, and in idiopathic short stature was not different from normal subjects. The 20K hGH ratio was fairly constant during GH provocative tests, confirming previous findings using other methods. The novel ELISA for 20K hGH was simple and sensitive, and will prove to be useful for understanding the physiological or pathophysiological role of the 20K hGH isoform.

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