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The arginine and GHRP-2 tests as alternatives to the insulin tolerance test for the diagnosis of adult GH deficiency in Japanese patients: A comparison

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Abstract. The arginine + GHRH test has been established as an alternative to the insulin tolerance test (ITT) for the diagnosis of adult GH deficiency (AGHD). However, the glucagon, arginine, and GH releasing peptide-2 (GHRP-2) test are recommended as alternatives in Japan. The objective of this study was to evaluate the arginine and GHRP-2 tests as alternatives to the ITT for the diagnosis of AGHD in a Japanese population. Three stimulation tests (ITT, arginine test, and GHRP-2 test) were conducted in 71 pre-operative adult patients with pituitary tumors (age, 18-65 years). The peak GH responses to each test were examined. The peak GH responses were significantly lower with the ARG test (median 4.43 μg/L) \( (p < 0.0001) \) than with the ITT (median 9.38 μg/L), and the peak GH responses with the GHRP-2 test (median 28.88 μg/L) were higher \( (p < 0.0001) \). However, among the AGHD patients, there was no significant difference between the peak GH responses to the ARG test and the ITT. The sensitivities and specificities of the ARG / GHRP-2 tests compared to the ITT for the diagnosis of severe AGHD (peak GH responses to ITT \( \leq 1.8 \mu g/L \)) were 93.8% / 81.3% and 85.5% / 94.5%, respectively. The arginine and GHRP-2 stimulation tests are acceptable alternatives to the ITT for the diagnosis of AGHD in Japanese patients. The method and criterion for the diagnosis of AGHD should be reconsidered and adjusted to each population.

Key words: Arginine test, GH releasing peptide-2 test, Insulin tolerance test (ITT), GH stimulation test, Adult GH deficiency

IT HAS BEEN well recognized that adult GH deficiency (AGHD) is associated with a number of metabolic abnormalities and a decline in exercise capacity, and that GH replacement improves these impairments [1], but the treatment of AGHD is very expensive, with a high cost of long-term care and drug therapy. In Japan, hypopituitarism (including AGHD) was designated as a Specified Rare and Intractable Disease from October 2009, and the treatment of AGHD is publicly subsidized. Now, accurate diagnosis is required to manage AGHD for individual patients, as well as for Japanese society.

The insulin tolerance test (ITT) has been considered the international “gold standard” test, but it may carry increased risk in patients with seizure disorders or cardiovascular disease [1]. The Guideline for the Diagnosis and Treatment of AGHD issued by the Hypothalamic-Pituitary Dysfunction Study Group of the Ministry of Health, Labour, and Welfare, Japan includes the arginine (ARG) test, the glucagon test, and the GH releasing peptide-2 (GHRP-2) test, in addition to the ITT, as stimulation tests [2]. On the other hand, the ARG alone as a stimulation test should be considered an unreliable alternative for the diagnosis of AGHD because of its inability to stimulate GH secretion [3-5]. The ARG + GHRH test has recently been reported as an excellent alternative to the ITT for the diagnosis of AGHD [1, 6-10].

The GHRP-2 test has been reported as a useful and safe stimulation test for the diagnosis of AGHD [11].
and it has recently come to be frequently performed in Japan [12]. However, the GHRP-2 is not commercially available in other countries, and its diagnostic value compared to the ITT or the ARG + GHRH test is less well established. The aim of this present study was to confirm the diagnostic reliability of the ARG and the GHRP-2 tests for the diagnosis of AGHD in a large Japanese population.

Material and Methods

Patient population and study design

The institutional ethics committee approved the experimental protocol used (no. 053010, 2008). From November 2008 to January 2012, 79 patients were referred to Hiroshima University Hospital for the evaluation of pituitary function before surgery for pituitary tumors. All patients underwent three stimulation tests (ITT, ARG test, and GHRP-2 test) according to the standard procedures after overnight fasting. All three tests were conducted within 5 months before surgery (median 28.0 days). During that time, the patients’ physical status had been stable. Of these 79 patients, 8 patients without sufficiently induced hypoglycemia (blood glucose nadir ≤ 50 mg/dL or ≤ half of the pre-test blood glucose levels) [13] in the ITT were excluded. The remaining 71 patients (52 females, 19 males; median age, 38.0 years; age range, 18-65 years) were included. The patients’ clinical details are listed in Table 1. All patients underwent surgery for their pituitary lesions and were histopathologically diagnosed: pituitary adenoma not including GH secreting adenoma, 55 cases; Rathke’s cleft cyst, 8 cases; cranioopharyngioma, 3 cases; and others, 5 cases (germinoma, 1 case; Langerhans cell histiocytosis, 1 case; epidermoid cyst, 1 case; granulomatous hypophysitis, 1 case; and pituitary hyperplasia, 1 case). Five patients had tumors in the third ventricular floor and/or hypothalamus. In 3 of 5 patients, anterior and posterior pituitary functions were completely normal, and in the other 2 patients, pituitary functions were partly disordered (severe GH deficiency + hypogonadism + hyperprolactinemia and/or diabetes insipidus). No patient with hypothalamic dysfunction was enrolled in the present study (Supplemental Table).

Study procedures

1) For the ITT, regular human insulin at 0.10-0.15 U/

<table>
<thead>
<tr>
<th>Table 1</th>
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<td></td>
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<td>n</td>
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<td>Age (years)</td>
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<tr>
<td>Tumor size (mm)</td>
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</table>

Data are expressed as means ± standard deviations (medians). †No patient had sex steroid and GH replacement. ‡2 of 3 patients had thyroid hormone replacement due to primary hypothyroidism.
Tokyo, Japan), using a recombinant 22-kDa GH calibrator (International Reference Preparation (IRP) 98 / 574). The sensitivity of the assay was 0.1-60 μg/L.

Statistical analysis

All statistical analyses were performed using the software package SPSS 16.0 J for Windows (SPSS Inc., Chicago, IL, USA); the Mann-Whitney U-test and Spearman’s rank correlation coefficient were used for comparisons. Receiver-operating characteristic (ROC) curves were calculated to provide a cut-off point for the diagnosis of the normal GH response to the GHRP-2 test. The values are expressed as medians. Differences were considered significant at $p < 0.05$.

Results

Serum GH response to stimulation

The individual peak GH and the median GH responses to each stimulation test are shown in Fig. 1. The peak GH response to the ARG test (median 4.43 μg/L) was significantly lower than that to the ITT (median 9.38 μg/L) ($p < 0.0001$), and the peak GH response to the GHRP-2 test (median 28.88 μg/L) is significantly higher than that to the ITT ($**p < 0.0001$).

**Fig. 1** The individual peak GH response to the ITT (○), ARG (□) test, and GHRP-2 (Δ) test in all 71 patients. Fig. 1B shows the magnified area surrounded by the dashed line in Fig. 1A. The median peak GH level in each group is denoted with a bar. The peak GH response to the ARG test (median 4.43 μg/L) is significantly lower than that to the ITT (median 9.38 μg/L) ($^*p < 0.0001$), and the peak GH response to the GHRP-2 test (median 28.88 μg/L) is significantly higher than that to the ITT ($^{**}p < 0.0001$).

kg body weight was injected intravenously, and blood samples were taken before injection, and 30, 60, and 90 minutes after injection. To avoid unexpected cardiac events in elderly patients, the ITT was performed in our institution only for patients who were under 65 years old. 2) For the ARG test, 30 g of ARG hydrochloride (10% solution) were infused intravenously over 30 minutes, and blood samples were taken before infusion and 30, 60, 90, and 120 minutes after infusion. 3) For the GHRP-2 test, 100 µg of GHRP-2 (GHRP KAKEN 100, Kaken Pharmaceuticals, Tokyo, Japan) were administered intravenously, and blood samples were taken before injection and 15, 30, 45, and 60 minutes after injection.

The three tests were performed in all patients. According to the Guidelines for the Diagnosis and Treatment of AGHD in Japan [2], severe AGHD and mild AGHD were defined by peak GH responses of $\leq 1.8 \mu g/L$ and $\leq 3.0 \mu g/L$, respectively, to the ITT and the ARG test. In the GHRP-2 test, severe AGHD was defined by a peak GH response of $\leq 9.0 \mu g/L$ [2, 11, 12]. A criterion for mild AGHD on the GHRP-2 test had not yet been defined.

Assays

Serum GH levels were assayed by immunoenzymometric assay (E-test TOSHO II HGH, TOSHO co., Tokyo, Japan), using a recombinant 22-kDa GH calibrator (International Reference Preparation (IRP) 98 / 574). The sensitivity of the assay was 0.1-60 μg/L.
ITT was found ($p = 0.4887$) (Fig. 2A). In the Severe AGHD plus Mild AGHD group, there was also no difference between the peak GH response to the ARG test and to the ITT (data not shown).

Correlations of the peak GH responses to stimulation

The correlations between the peak GH responses to the ITT and the ARG / GHRP-2 test are shown in Fig. 3. The peak GH response to the ARG / GHRP-2 tests was

GH levels $\leq 1.8$ μg/L (Severe AGHD group, $n = 16$); 2) peak GH levels $1.8 - 3.0$ μg/L (Mild AGHD group, $n = 5$); and 3) peak GH levels $> 3.0$ μg/L (Normal Response group, $n = 50$). In the Normal Response group, the median of the peak GH response with the ARG test (6.73 μg/L) was also lower than with the ITT (12.30 μg/L) ($p < 0.0001$) (Fig. 2B), but interestingly, in the Severe AGHD group, no significant difference between the peak GH response to the ARG test and the ITT was found ($p = 0.4887$) (Fig. 2A). In the Severe AGHD plus Mild AGHD group, there was also no difference between the peak GH response to the ARG test and to the ITT (data not shown).

Fig. 2  The individual peak GH responses to the ITT (○) and ARG (□) test in 16 severe adult GH deficiency (sAGHD) patients (peak GH response to the ITT $\leq 1.8$ μg/L, Fig. 2A) and in 50 normal GH patients (peak GH response to the ITT $> 3.0$ μg/L, Fig. 2B). The median peak GH level in each group is denoted with a bar. Fig. 2A shows no difference between the peak GH response to the ITT (median 0.86 μg/L) and the ARG test (median 0.76 μg/L) in the sAGHD patients, but Fig. 2B shows that the peak GH response to the ARG test (median 6.73 μg/L) is significantly lower than that to the ITT (median 12.30 μg/L) in normal GH patients ($p < 0.0001$).

Fig. 3  Correlation between individual peak GH responses to the ARG (□) / GHRP-2 (△) tests and the ITT in all 71 patients. Fig. 3B shows the magnified area surrounded by the dashed line in Fig. 3A. The peak GH responses to the ARG and the GHRP-2 tests are significantly related to the ITT (ARG: $r = 0.7166$, $p < 0.0001$, GHRP-2: $r = 0.8745$, $p < 0.0001$).
Arginine and GHRP-2 tests for adult GHD

The effects of age, weight, and body mass index (BMI) on peak GH responses

Effect of age, weight, and BMI on peak GH responses

The effects of age, weight, and body mass index (BMI) on the peak GH responses to the ITT, the ARG test, and the GHRP-2 test were analyzed in 50 patients with normal GH secretion on the ITT (peak GH response to ITT > 3.0 μg/L), and the results are shown in Table 3. There were negative correlations between age and the peak GH responses to each stimulation test. Moreover, high weight and BMI had a tendency to suppress the peak GH responses to the stimulation tests.

Cut-off point for diagnosis of the normal GH response to the GHRP-2 test

In order to provide a cut-off point for the diagno-
This study also showed that the cut-off point corresponding to 1.8 μg/L on the ITT was 9.94 μg/L (sensitivity 90.9% and specificity 87.5%), which was similar to the established cut-off point of 9.0 μg/L on the GHRP-2 test for the diagnosis of severe AGHD in Japanese patients [2, 11, 12].

## Discussion

To compare the stimulation tests for GH deficiency, it is important to understand the different mechanisms of GH secretion in each test, because it is possible that a variety of mechanisms of GH secretion cause the different responses to these tests in patients with hypothalamic and pituitary disorders. Insulin-induced hypoglycemia and arginine stimulate GH secretion by suppressing endogenous somatostatin secretion in the hypothalamus [14, 15]. GHRP-2 is a potent synthetic GH secretagogue, which acts on a specific receptor at the hypothalamic arcuate nucleus-median eminence to potentiate GH-releasing activity [16, 17]. On the other hand, GHRH acts directly on the pituitary somatotroph to cause GH secretion. Since three stimulation tests (ITT, ARG, and GHRP-2) might be affected by hypothalamic lesions, it was confirmed that no patient with hypothalamic dysfunction was included in this study (Table 1 and Supplemental Table).

### Table 3

<table>
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<th>Test</th>
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<td>GHRP-2</td>
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Normal GH secretion: peak GH response to the ITT > 3.0 μg/L
The classical stimulation test of GH secretion, the ARG test, has been widely used in clinical practice [13, 18]. However, the ARG test has been recognized as an unreliable test for the diagnosis of AGHD because of insufficient stimulation of GH secretion [3-5]. Then, the ARG + GHRH test was reported to be the most reliable candidate as a stimulation test for AGHD [7-9, 19], and the ARG + GHRH test was recommended in many guidelines for the diagnosis of AGHD [1, 6, 10, 20]. Though the ARG + GHRH test has recently become universal as an alternative to the ITT for the diagnosis of AGHD, the ARG test is still recommended in Japan [2]. This discrepancy raises the question whether the ARG test is appropriate for the diagnosis of AGHD in the Japanese population.

In the present study, the ARG test was an acceptable stimulation test for the diagnosis of AGHD in the Japanese population. This fact contradicts previous reports indicating that the ARG + GHRH was an appropriate alternative to the ITT, but not the ARG alone [1, 6-10, 19, 20]. This discrepancy may be due to differences in physique and body composition between patient populations in different countries. GH responses to stimulation tests are known to be influenced by obesity [21-23], BMI [24, 25], and waist circumference [24]. Obesity is associated with a state of chronic somatostatin hypersecretion [23], and free fatty acids block GHRH stimulation [26, 27], suppressing the GH peak in the stimulation tests. The ARG test is also affected by obesity [22, 23], and free fatty acids reduce stimulated GH secretion [28]. Moreover, the ARG was generally given by intravenous infusion of 0.5 g/kg with a maximum of 30 g, so larger patients tended to have weaker stimulation with the ARG. In the present study, the ARG stimulation test was also shown not to have much potency to stimulate GH secretion and to be weakened by high body weight and BMI. On the other hand, the Japanese physique [29] is smaller and less obese than the Caucasian physique. In the present study, only 7 of 71 (9.9%) patients had a higher weight, and only 12 of 71 (16.9%) patients had a higher BMI than average non-Hispanic white Americans [30]. Japanese anthropometry has also been reported to be smaller than that of Australian Caucasians [31]. These findings support the idea that the ARG test is sufficient and appropriate for the diagnosis of AGHD in the smaller and less obese Japanese population, despite the weak potency of the ARG test to stimulate GH secretion.

The GHRP-2 stimulation test has been reported to be a favorable alternative to the ITT for the diagnosis of AGHD, with only minimal side effects, and it can be completed in just one hour [11]. Thus, the GHRP-2 test is recommended as one of the GH stimulation tests in the Guideline for the Diagnosis and Treatment of AGHD in Japan [2]. In the present study, the GHRP-2 test was also shown to provide stronger stimulation to GH secretion than the ITT in the Japanese population. Then, the criterion for mild AGHD on the GHRP-2 test was supposed to be 12.9 μg/L. This test has the potential to be a more acceptable alternative to the ITT for the diagnosis of AGHD in the world, because of the strong stimulation, simplicity, and accuracy of the method. However, the compounds are not commercially available except in Japan, so that the evidence supporting the GHRP-2 test has not yet been sufficiently established. Further studies on the sensitivity and specificity of the GHRP-2 test should be performed in different ethnic and physique populations.

The ARG stimulation test and the GHRP-2 stimulation test are acceptable alternatives to the ITT for the diagnosis of adult GH deficiency in the Japanese population. Use of just one international standardized method or criterion for diagnosis of adult GH deficiency may result in a wrong diagnosis of adult GH deficiency in some populations. To accurately diagnose adult GH deficiency, more detailed methods and criteria of GH stimulation tests should be established according to different physiques, body compositions, and ethnic backgrounds.

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Disclosure Statement

We have no financial disclosure and conflicts of interest whatsoever.
### References


