Reproducibility of Growth Hormone Stimulation Tests (Arginine and Insulin), Insulin-like Growth Factor (IGF)-I and IGF-Binding Protein-3 Measurements

Yukihiro Hasegawa, Tomonobu Hasegawa, Shinobu Kotoh and Yutaka Tsuchiya

Division of Endocrinology and Metabolism, Tokyo Metropolitan Kiyose Children's Hospital, Tokyo, Japan

Abstract. Recently there have been some data suggesting that the results of GH stimulation tests are not reproducible. We studied here the reproducibility of GH stimulation tests (arginine and insulin) and compared it with the reproducibility of serum IGF-I and IGFBP-3 measurements. The interdaily variation of traditional GH tests can be higher than that of IGF-I and IGFBP-3 measurements. We should not over-rely upon traditional GH stimulation tests to diagnose GH insufficiency.

Key words: reproducibility, GH stimulation tests, arginine and insulin tests, IGF-I, IGFBP-3

Recently there have been some data suggesting that the results of GH stimulation tests, which have been used in the diagnosis of GH insufficiency, are not reproducible [1,2]. We studied here the reproducibility of GH stimulation tests (arginine and insulin) and compared it with the reproducibility of serum IGF-I and IGFBP-3 measurements.

Subjects

The subjects were 11 control children (CC: height > mean - 2 SD), 13 normal short children (NS: height < mean - 2 SD), and 11 patients with growth hormone insufficiency (GHI). In NS, at least one of the GH peaks of the GH stimulation tests was over 10 ng/ml.

In patients with GHI, all the GH peaks were less than 10 ng/ml.

The 15 subjects (2 CC and 13 NS) for checking the reproducibility of the GH stimulation tests were all prepubertal and their height SD scores were -3.13 ± 1.17 SD ($-1.35 \sim -5.52$ SD). The 28 subjects (9 CC, 8 NS and 11 GHI) for checking the reproducibility of IGF-I and IGFBP-3 were prepubertal or pubertal and their height SD scores ranged from mean + 1.0 SD to mean - 5.6 SD.

Method

- (1) GH was measured by IRMA (Eiken Kit).
- (2) IGF-I was measured with RIA after acid ethanol extraction [3].
- (3) IGFBP-3 was measured with Ranke and Blum's RIA kit [4-6], with a few modifications.
- (4) Arginine and insulin tests were done as

Correspondence: Dr. Yukihiro Hasegawa, Chief, Division of Endocrinology and Metabolism, Tokyo Metropolitan Kiyose Children's Hospital, 1-3-1, Umezono, Kiyose, Tokyo 204 Japan

Table 1. Arginine and insulin test
(Insulin is injected 2.5hr after starting arginine infusion)

follows (Table 1):

Arginine was infused (0.5 g/kg for 30 min), followed by insulin injection (0.1 U/kg one IV injection) two and half hours after starting arginine infusion. Blood was taken 0, 30, 60, 90, 120, 150 min after arginine infusion and 0, 15, 30, 45, 60, 90, 120 min after insulin injection.

(5) Reproducibility of GH stimulation tests was analysed within an interval of two years. Four out of 15 subjects were checked twice within an interval of two months (○ in Figure 1 and 2). Reproducibility of IGF-I and IGFBP-3 for prepubertal patients was checked at least three times within an interval of less than 6 months (usually three months), and for pubertal patients it was checked at least three times within an interval of one month.

(6) False positive ratio was arbitrarily determined on the presumption that GH secretion status was normal if any one of the GH peaks among GH stimulation tests was over 10 ng/ml.

Results

(1) The ratios of GH peaks in the first arginine and insulin tests to GH peaks in the second tests varied to a great extent (Figure 1 and 2). Twelve out of 56 showed false positive results,

Fig 1. GH peaks of two arginine tests (an interval of ○ and ● is < 2 months and < 2 years, respectively).

Fig 2. GH peaks of two insulin tests (an interval of ○ and ● is < 2 months and < 2 years, respectively).

Reproducibility of GH Stimulation Tests

Table 2. False positive ratios of GH stimulation tests in each height group or IGF-I group

height	< mean - 4SD	2/16(12.5%)
	< mean - 2SD	10/40(25%)
	> mean - 2SD	2/6 (33%)
IGF-I	< 5th percentile	3/12(25%)
	> 5th percentile	9/44(20%)

if based on the lower GH peaks. When we divided the subjects into subgroups with heights under mean - 2 SD, - 4 SD, and over mean - 2 SD, each false positive ratio is shown in Table 2. Similarly, false positive ratios for each low and normal IGF-I are shown in Table 2. There was no tendency that the higher the heights, the lower the false positive ratios were. Also there was no statistical difference in false positive ratio between subjects with low IGF-I (< 5th percentile of normal children [3]) and normal IGF-I (> 5th percentile of normal children [3]).

(2) Interdaily variation of IGF-I (percentage of each SD to mean) was $22.0 \pm 13.9\%$ ($n=28$, range, 2.5–45.9%). Mean IGF-I levels of each subject for this study ranged from 21 to 573 ng/ml.

(3) Interdaily variation of IGFBP-3 (percentage of each SD to mean) was $10.8 \pm 6.7\%$ ($n=27$, range, 0–25.4%). Mean IGFBP-3 levels of each subject for this study ranged from 0.21 to 4.44 mg/L.

Discussion

It has been reported that IGF-I and IGFBP-3 do not show much daily variation [7,8]. Our data about IGF-I and IGFBP-3 interdaily variation confirmed previous reports [7,8]. The interdaily variations of these two parameters were less than that of our GH stimulation tests at least in some patients, suggesting that these two values were more stable than results of GH stimulation tests.

There have been two reports that in some patients with short stature, GH stimulation

tests were not reproducible [1,2]. We confirmed the limitation of GH stimulation test in terms of reproducibility. Furthermore, the results of the false positive ratios of GH stimulation tests in each height group and IGF-I level group suggested that these false positive results might not reflect heights or IGF-I levels.

Taken together, we should not over-rely upon the results of GH stimulation tests, which have been used in the diagnosis of GH insufficiency for many years.

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