

CLINICAL STUDY

Discrepant results in the diagnosis of GH deficiency with the insulin-tolerance test and the GHRH plus arginine test in patients with traumatic brain injury

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Abstract

Objective: Patients with traumatic brain injury (TBI) are at moderate risk of GH deficiency (GHD), requiring a diagnostic test with high specificity. The GHRH + arginine (GHRH + ARG) test has been recommended as a reliable alternative to the insulin-tolerance test (ITT) as a standard test with a cut-off level of 9 ng/ml. However, it has recently been questioned for its low specificity in obese subjects, and now BMI-dependent cut-off levels are available. In this study, we compared the ITT and GHRH + ARG test in patients with TBI.

Design: A cross-sectional study

Methods: We performed an ITT and a GHRH + ARG test in 21 patients with TBI (6 women, 15 men; mean age 40.2 ± 12.1 years; BMI 30.7 ± 6.2). The number of patients classified discordantly as GH deficient by the ITT and the GHRH + ARG test with both classical and BMI-dependent cut-off levels was assessed.

Results: Using the GHRH + ARG test with the classical cut-off (≤ 9 ng/ml), we identified 12 patients as GH deficient who had a normal GH response to ITT (> 3 ng/ml), and one patient as GH sufficient who had a blunted GH response to ITT (discordance rate 61.9%). All patients discordantly classified as GH deficient by the GHRH + ARG test had a BMI of ≥ 28 . With the BMI-dependent cut-offs (4.2, 8.0, and 11.5 ng/ml in obese, overweight, and lean subjects respectively), only 3 of the 21 patients were discordantly classified (discordance rate 14.3%).

Conclusions: Our results discourage the use of a cut-off level of 9 ng/ml for the GHRH + ARG test in obese subjects. The diagnostic reliability of this test is improved with the BMI-dependent cut-offs.

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Introduction

Recent studies have stressed the role of traumatic brain injury (TBI) as a potential cause of growth hormone (GH) deficiency (GHD) reviewed in (1). However, as opposed to patients with pituitary–hypothalamic diseases and multiple pituitary deficits, who are at high risk of GHD, the risk of GHD ranges from 10 to 20% in TBI patients (1). In these patients, a test with a high specificity is necessary for the diagnosis of GHD to avoid a very high number of false-positive results (2). The insulin-tolerance test (ITT) has been shown to distinguish well between healthy subjects and patients with GHD (3) and has been recommended as the test of choice for the diagnosis of GHD by the growth hormone research society (4). Here, severe GHD was suggested to be defined at a cut-off level of 3 ng/ml or lower. However, this test is contraindicated in patients with several conditions, such as heart diseases and seizure disorders and causes more discomfort than other tests.

Ghigo *et al.* (5) suggested to use the GH-releasing hormone plus arginine (GHRH + ARG) test as a safe and sensitive alternative. They established a cut-off level of 9 ng/ml for the diagnosis of severe GHD in a lean population (5). However, recently, the GHRH + ARG test has been criticized to be strongly BMI dependent and to cause false-positive results in healthy obese and overweight subjects (6, 7). In TBI patients, GH levels decrease after GHRH + ARG stimulation, even after exclusion of obese subjects (8). Recently, Corneli *et al.* (9) have published BMI-dependent cut-off levels for the GHRH + ARG test. Another study, conducted mainly in an obese population, has suggested cut-off levels of 5.1 and 4.1 ng/ml for the ITT and GHRH + ARG test respectively (10). Yet, the cut-off of 9 ng/ml is still used in most endocrinological centers worldwide, irrespective of BMI, and this cut-off has been recommended for the diagnosis of GHD in recent reviews and consensus guidelines for both patients with known pituitary diseases and TBI (11, 12).

In this study, we wanted to compare the ITT and the GHRH+ARG test in patients with TBI with regard to both the classical and the new BMI-dependent cut-off levels.

Subjects and methods

Subjects

We studied 21 consecutive patients with TBI (6 women, 15 men; age (mean \pm s.d.) 40.2 ± 12.1 years; BMI 30.7 ± 6.2 ; time after trauma 9.5 ± 8.7 years). Fourteen patients were evaluated in the neuroendocrine outpatient clinic of the Max Planck Institute of Psychiatry. In these patients, ITT and GHRH+ARG test were done during routine endocrine evaluations and ITT was done after GHRH+ARG test either as a confirmation test in case of low or borderline low-GH response to GHRH+ARG, or, to exclude undetected hypothalamic deficiencies. All patients gave informed consent. Another seven patients were studied during a study of hypopituitarism after TBI in the University Hospital of Essen (13). These patients gave written informed consent and the study was approved by the local ethics committee.

Endocrine evaluations

We did a GHRH+ARG test and an ITT in all patients on 2 different days. All tests were performed in the morning after an overnight fast. For the ITT, 0.15 U/kg regular insulin (Actrapid Novo-Nordisk, Denmark), was given intravenously as a bolus at 0 min. Blood samples were taken every 15 min from -15 to +90 min. During ITT, glucose measurement was performed, and minimum plasma glucose level of 2.2 mmol/l or less was detected together with hypoglycemic symptoms. If necessary, a second bolus was added to achieve adequate hypoglycemia. For the GHRH+ARG test, different protocols were used in the two centers. In the Max Planck Institute, 50 μ g GHRH (Ferring) were given intravenously as a bolus and 30 g L-arginine in 250 ml physiological salt solution were administered as a 30-min infusion, and GH was measured at 0, 30, 45, 60, 90, and 120 min. This protocol is routinely used in this center as it was shown that synthetic GH-releasing factor causes a dose-dependent GH response up to a dose of 50 μ g in healthy subjects, but no further increase of GH response to doses of 50–200 μ g (14). In the University Hospital of Essen, 1 μ g GHRH/kg was administered by an i.v. bolus, followed by a 30-min infusion of 30 g arginine with the measurement of GH at -15, 0, 15, 30, 45, 60, 75, 90, 105, and 120 min. Further hormone deficiencies were recorded by standard clinical procedures of the respective centers (8, 13). To compare the two tests, we considered both the classical cut-offs of ≤ 3 and ≤ 9 ng/ml for the ITT (3, 4) and GHRH+ARG test (5) respectively, and the

BMI-dependent cut-off levels with ≤ 11.5 , ≤ 8.0 , and ≤ 4.2 ng/ml in lean (BMI < 25), overweight (BMI ≥ 25 to < 30), and obese (BMI ≥ 30) subjects respectively (9). Additionally, we also considered the cut-offs of ≤ 5.1 and ≤ 4.1 ng/ml for the ITT and GHRH+ARG respectively as suggested by Biller *et al.* (2002). For insulin-like growth factor (IGF)-I levels, age-dependent SDS were calculated (15).

GH and IGF-I were measured by chemiluminescence with the Nichols Advantage system (Nichols Institute Diagnostics, San Clemente, CA, USA). The maximal intra- and inter-assay coefficients of variation at different hormone concentrations were as follows: GH, 8% and 12%; IGF-I, 5% and 7% respectively.

Statistical analyses

Data are presented as mean \pm s.d. We analyzed the numbers and percentages classified concordantly and discordantly by the two tests using the different cut-off values. To test for significance between groups, we used the Mann-Whitney *U*-test. Correlations were calculated with the Spearman's correlations coefficient. A *P* value < 0.05 was considered significant.

Results

Mean peak GH levels after ITT and GHRH+ARG test were 11.6 ± 10.5 and 15.0 ± 19.9 ng/ml respectively. BMI correlated negatively with peak GH after GHRH+ARG test ($r=0.66$, $P=0.001$), but not after ITT ($r=-0.21$, $P=0.35$). These findings remained unchanged after adjustment for age.

Table 1 summarizes the results and clinical characteristics of the patients. The number of patients with peaks below the respective cut-off values is shown in Table 2. Only three patients (14.3%) had a GH response below or equal to the cut-off of 3 ng/ml to ITT, whereas 14 patients (66.7%) had GH responses ≤ 9 ng/ml to GHRH+ARG stimulation. Two patients had IGF-I levels < 2 s.d. below the age-related normal values. Both of these had a peak GH ≤ 3 ng/ml after ITT. Table 3 shows the number of patients classified concordantly or discordantly by the two tests using different cut-off values. The discordance rate was the highest when using the classical cut-offs of 3 and 9 ng/ml for the ITT and GHRH+ARG test respectively (61.9%) and the lowest when using 3 ng/ml for the ITT and the BMI-dependent cut-offs for the GHRH+ARG (14.3%). The one patient identified as GH deficient with the ITT and not the GHRH+ARG test, using classical cut-offs, had panhypopituitarism, hyperprolactinemia, and an IGF-I level of 21 ng/ml (< -4 s.d.). If we analyzed the patients of the clinical centers separately using the classical cut-offs, 8 of 14 patients from the Max Planck Institute and five out of seven patients from the University Hospital of Essen were classified discordantly (discordance rates 57.1% and 71.4% respectively). In detail, at the

Table 1 Patient characteristics.

Patient	Sex	BMI	Age	Peak GH ITT	Peak GH GHRH + ARG	IGF-1 SDS	Other deficits/hormone disturbances
1	w	17.0	24	0.9	10.8	-4.22	LH/FSH, ACTH, TSH, hyperprolactinemia
2	w	18.4	44	8.2	81.6	-1.54	n
3	m	23.1	21	25.9	40.6	-0.14	ACTH
4	w	26.0	41	38.5	33.9	0.52	ACTH
5	m	27.1	56	14.1	14.8	0.20	n
6	m	27.7	46	33.5	47.7	-1.06	LH/FSH
7	m	28.0	31	25.3	8.8	-1.29	LH, hyperprolactinemia
8	m	28.7	57	8.1	7.1	-0.82	n
9	m	30.0	58	1.5	3.8	-0.93	ACTH
10	m	30.5	39	5.2	5.5	-0.91	n
11	m	30.6	24	13.2	5.0	-0.95	LH/FSH, ACTH
12	m	31.0	16	3.1	7.0	-1.02	LH
13	m	33.0	49	9.1	5.0	-0.15	n
14	m	34.0	47	2.2	2.8	-2.07	n
15	w	34.6	41	4.2	5.5	-0.62	n
16	m	35.0	37	4.8	7.4	-0.86	n
17	m	36.3	50	13.9	5.6	-0.25	LH/FSH
18	m	37.7	43	5.4	1.9	NA	n
19	m	38.0	34	9.5	6.7	0.13	LH/FSH
20	w	38.2	53	9.2	10.2	-0.43	n
21	w	39.5	34	7.9	3.5	-1.18	n

BMI, body mass index; GH, growth hormone; ITT, insulin tolerance test; GHRH + ARG, GH-releasing hormone + arginine test; IGF-1, insulin-like growth factor-1; w, women; m, men; LH, luteinizing hormone; FSH, follicle-stimulating hormone; ACTH, adrenocorticotropic hormone; TSH, thyroid-stimulating hormone; NA, not available; n, no deficit/hormone disturbance.

Table 2 Numbers and percentage of patients with peak GH levels below the respective cut-offs.

ITT Total	GHRH + ARG				
	≤3 ng/ml	≤5.1 ng/ml	≤9 ng/ml	≤BMI-dependent cut-off	≤4.1 ng/ml
n	21	3	6	14	6
%	100	14.3	28.6	66.7	28.6
					4
					19.0

ITT, insulin tolerance test; GHRH + ARG, GH-releasing hormone + arginine test; BMI, body mass index.

University Hospital of Essen, five patients were classified discordantly as GH deficient by the GHRH + ARG only, and two were classified concordantly GH deficient by both tests. At the Max Plack Institute, seven patients were identified discordantly by the GHRH + ARG test only and one patient by the ITT only.

Figure 1 depicts the association of peak responses to GHRH + ARG test and ITT in lean, overweight, and obese subjects and the different cut-offs of the respective

tests. Of the 12 patients discordantly classified as GH deficient by the GHRH + ARG test only using the classical cut-offs, ten were obese. The other two patients (patients 7 and 8) were overweight with BMIs of 28.0 and 28.7. Only one of all obese subjects had a peak GH above 9 ng/ml after GHRH + ARG test.

There were no significant differences among age, time after trauma, IGF-1, or IGF-1 SDS between obese and non-obese subjects.

Table 3 Numbers of patients classified as GH deficient by both tests (concordant classification) or only one test (discordant classification) using different cut-offs.

Cut-off used for definition of GHD	GHD both tests	GHD only ITT	GHD only GHRH + ARG	% Discordant classification
≤3 ng/ml (ITT) vs ≤9 ng/ml (GHRH + ARG)	2	1	12	61.9
≤3 ng/ml (ITT) versus ≤BMI-dependent cut-off (GHRH + ARG)	3	0	3	14.3
≤5.1 ng/ml (ITT) versus ≤BMI-dependent cut-off (GHRH + ARG)	3	3	3	28.6
≤5.1 ng/ml (ITT) vs ≤4.1 ng/ml (GHRH + ARG)	2	4	2	28.6

GHD, growth hormone deficiency; ITT, insulin tolerance test; GHRH + ARG, GH-releasing hormone + arginine test.

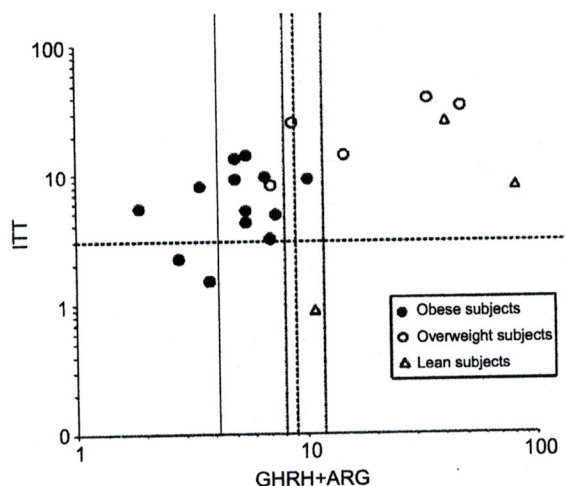


Figure 1 Correlation of peak growth hormone (GH) responses to insulin tolerance test (ITT) and GH-releasing hormone + arginine (GHRH+ARG) test. The perforated lines indicate the classical cut-off levels for ITT and GHRH+ARG test. The dotted lines indicate the cut-off levels for obese, overweight, and lean subjects (Corneli *et al.* 2005). Please note that scaling is logarithmic and axes do not start at 0.

Discussion

In this study, we have compared two stimulation tests for the diagnosis of GHD in identical patients with TBI, a condition that has only recently been highlighted to be at substantial risk of GHD. Our study shows that: (a) the majority of patients are classified as GH deficient with the GHRH + ARG test but not the ITT; (b) all of these patients have a BMI of 28 or higher; (c) there is a clear BMI dependence of the GHRH + ARG test but not the ITT; and (d) the concordance of the tests is increased if the BMI-dependent cut-offs are used.

TBI can cause pituitary dysfunction in 30–50% of patients (1, 8) and there is now a consensus that endocrine assessment should be implemented in the routine follow-up of TBI patients (16, 17). The GHRH + ARG test has been suggested as a safe and reliable alternative to the ITT (5) and the cut-off of 9 ng/ml is commonly used worldwide to diagnose severe GHD. Our data show a strong BMI dependence of this test in TBI patients and confirm similar findings from healthy subjects (6, 7). This leads to a large proportion of obese subjects classified as GH deficient using the classical cut-off for the GHRH + ARG test, even though a normal GH secretion is shown with the ITT. Thus, we must assume that these patients are misclassified as GH deficient. Therefore, if the decision to initiate GH replacement was done on the basis of the GHRH + ARG test with a cut-off of 9 ng/ml, the large majority of obese TBI patients would receive unjustified GH therapy.

The discordance rate is lower using the cut-offs suggested by Biller *et al.* (10) and lowest if the BMI-dependent cut-offs are used for the GHRH + ARG test, although some patients were still discordantly classified.

Even though we found no clear BMI dependence of the ITT, we cannot rule out that this was due to small patient number or too few normal weight subjects. Qu *et al.* (7) have shown BMI dependence of the ITT in healthy subjects, albeit less strong than for the GHRH + ARG test. Also BMI-dependent cut-offs may increase the accuracy of this test but they are not available. Moreover, theoretically, it is also possible that the ITT causes false-negative results. The high BMI in our population reflects the typical population of patients with TBI seen in our endocrine outpatient clinics. Most patients gain significant weight after TBI. Thus, it is of particular importance to take the possible effects of obesity into account when testing endocrine function in these patients.

The sensitivity and specificity of diagnostic testing increase if two or more different tests, instead of merely one test, are used. Therefore, a higher concordance rate of different tests indicates a higher accuracy of the single tests. Taking the above-mentioned limitations into account, the lower discordance rate indicates reasonable accuracy of the two tests.

It can be criticized that different protocols used for stimulation testing in the two centers might have influenced the results. However, this seems unlikely as the discordance rates in both centers were very similar. One patient with panhypopituitarism and hyperprolactinemia, indicative of hypothalamic dysfunction or stalk disruption was not identified as GH deficient by a cut-off of 9 ng/ml with the GHRH + ARG test. This shows that the GHRH + ARG test is less sensitive than the ITT for suprapituitary causes of dysfunction in these patients.

In summary, our data discourage the use of the classical cut-offs for the GHRH + ARG test in obese and maybe overweight patients for the diagnosis of GHD. The BMI-dependent cut-offs lead to better results, even though some patients with normal GH response to ITT may be classified as GH deficient with this test. Additionally, the GHRH + ARG test may be less sensitive to hypothalamic dysfunction. In clinical practice, the decision to initiate GH substitution should be ideally based on more than one adequate stimulation test with appropriate cut-offs in synopsis with clinical symptoms, additional deficiencies and IGF-I levels.

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