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Double Stimulation with Arginine Infusion for Assessment of Growth Hormone in Short Children

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Abstract

Aim: To verify whether the release of GH obtained by double stimulation with arginine (arginine plus arginine) is equal to that obtained using two different stimuli (arginine plus glucagon).

Patients: Fifty (50) children (27 M, 23 F), aged 9.54 ± 3.81 yr, 37 prepubertal and 13 at onset of puberty, who were being investigated for growth failure, were divided in two groups. Twenty-two subjects (group A) received arginine infusion followed by glucagon injection as the other provocative stimulus to confirm blunted GH response. Sixteen patients (group B) received arginine infusion as the first provocative stimulus followed by a second infusion of arginine, as they had presented risk factors for the use of glucagon as a second stimulus.

Results: No differences in serum GH peak levels were found between group A and group B patients receiving arginine monochloride infusion as first stimulus (4.14 ± 2.43 ng/ml *vs.* 4.54 ± 1.94 ng/ml, respectively). Moreover, no differences in serum GH peak concentrations were observed between group A, who received a glucagon injection as the second stimulus, and group B, who received another arginine infusion as the second stimulus (6.17 ± 1.94 ng/ml *vs.* 5.00 ± 2.00 ng/ml, respectively).

Conclusion: Repetition of arginine as a provocative test is as effective as other classical stimuli in evaluating GH secretion. Therefore, it can be used in particular cases where other stimuli may involve risks for the patient.

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Introduction

In children, the diagnosis of Growth Hormone Deficiency (GHD) is usually based on auxological evaluation and careful exclusion of other causes of growth failure [1,2]. Confirmation of the diagnosis requires a blunted GH response to at least two pharmacological stimuli [3]. Despite potential hazards [4], the use of insulin-induced hypoglycemia to provoke GH secretion remains the gold standard [5]. However, insulin hypoglycemia and glucagon injections can be dangerous in young children, in subjects with a history of seizures and in those with suspected complete GH deficiency. Therefore, the assessment of GH in response to other provocative tests is mandatory in these patients.

The aim of the present study was to verify whether double stimulation with arginine (arginine plus arginine) for the release of GH could match that obtained using two different stimuli (arginine plus glucagon).

Patients and Methods

Fifty (50) children (27 M, 23 F), aged 9.54 ± 3.81, 37 prepubertal and 13 at onset of puberty, were being investigated for growth failure, in accordance with international guidelines [1], which include assessment of GH response to at least two pharmacological stimulation tests [3], such as arginine infusion (500 mg/kg of arginine monochloride administered intravenously over a period of 30 minutes) and intramuscular injection of glucagon (0.03 mg/kg up to a maximum of 1 mg).

None of the patients presented diabetes insipidus, chromosomal abnormalities, dysmorphic syndromes, chronic diseases or acquired GHD.

Patients were divided into two groups:

1. Group A: It is consisting of 22 children: 11 males (5 prepubertal and 6 at onset of puberty) and 11 females (6 prepubertal and 5 at onset of puberty), who received arginine infusion followed by glucagon injection as the other provocative test to confirm the blunted GH response after the first stimulus. Auxological data of the patients are shown in Table 1.

2. Group B: It is consisting of 28 children: 16 males (15 prepubertal and 1 at onset of puberty) and 12 females (11 prepubertal and 1 at onset of puberty), who received arginine infusion as the first provocative stimulus and, then, arginine infusion again to confirm the blunted GH response after the first stimulus. As second stimulus, these patients received the same dose of arginine per kg as for the first, on a separate day, due to risk factors associated with the use of glucagon such as young age, history of neurological diseases, in particular seizures, suspected complete GH deficiency or because the parents had expressly requested it. Moreover, auxological data of the patients are shown in Table 1.

In both groups, the second test was carried out within 30 days from the first.

GH concentrations were measured in samples drawn at 0, 30, 60, 90 and 120 minutes after infusion with arginine and 0, 30, 60, 90, 120, 150 and 180 minutes after glucagon injection. All serum GH samples from the same individual were run in the same assay and measured using a fully automated immunochemistry analyzer, Immulite 2000 (Siemens Diagnostics). GH methods are based on solid phase, two-site immunometric sandwich assays with a chemiluminescent signal.

A response of GH is considered to be pathological when GH peak is <8 ng/mL in both stimulation tests.

Thyroid and adrenal functions were evaluated measuring serum free T4 and TSH, and morning serum cortisol concentrations, respectively. Serum IGF-I concentrations were evaluated using immunometric sandwich assays with a chemiluminescent signal.

Bone age, assessed by means of the Greulich & Pyle method, was retarded by at least one year.

Brain Magnetic Resonance Imaging (MRI) was performed in order to rule out morphological abnormalities of the hypothalamus-pituitary region such as pituitary hypoplasia, ectopic posterior pituitary and a thin or absent pituitary stalk. Evidence of these conditions could be predictive for subsequent occurrences of other hormone deficiencies.

Once the diagnosis of GHD was made and before starting GH treatment, glucose tolerance was evaluated by performing an oral glucose tolerance test (OGTT).

The study was carried out according to the ethical standards involving all human participants. Informed consent regarding diagnostic approach was obtained from parents of all subjects prior to the start of investigations, using a format approved by the local bioethics committee.

Statistical Analysis

All quantitative variables were normally distributed (Shapiro-Wilk test) and hence expressed as the mean value and Standard Deviation (SD). Qualitative variables were summarized as counts and percentages. Student's t-test for independent data was used to compare quantitative variables between the two groups, while a chi square test was used for qualitative variables. $P<0.05$ was considered statistically significant. All tests were two-sided. The data analysis was performed using the STATA statistical package (release 15.0, 2017, Stata Corporation, College Station, Texas, USA).

Table 1: Pre-treatment auxological data in patients of group 1 and group 2.

	Group 1 (22)	Group 2 (28)	p value
Age	11.47 (2.57)	8.03 (3.97)	ns
Height sds	-1.53 (1.22)	-2.4 (0.62)	ns
Weight sds	-2.14 (0.92)	-2.44 (1.31)	ns
BMI sds	-1.28 (1.1)	-1.11 (1.25)	ns
Growth rate sds	-3.54 (-4.15)	-2.78 (-3.41)	ns

ns: not significant

Results

No differences in serum GH peak levels were found between the patients of group A and group B receiving arginine monochloride infusion as first stimulus (mean 4.14 [SD: 2.43] ng/ml *vs.* 4.54 [SD: 1.94] ng/ml, respectively). Moreover, no differences in serum GH peak concentrations were observed between group A, who received a glucagon injection as the second stimulus, and group B, who received another arginine monochloride infusion as the second stimulus (mean 6.17 [SD: 1.94] ng/ml *vs.* mean 5.00 [SD: 2.00] ng/ml, respectively).

Serum IGF-I concentration mean was low in both groups of patients, specifically 232.04 [SD: 195] ng/ml (-1 [SD: -1.55] in group A and 135 ng/ml [SD: 101] (-1.28 [SD: -1.79]) in group B. Both thyroid and adrenal functions were found to be normal. Moreover, OGTT showed normal oral glucose tolerance in all subjects. MRI did not detect any abnormalities of the patients' hypothalamus-pituitary region.

Before treatment, growth rate was low in both groups: group A 2.82 [SD: 2.38] cm/yr (-3.5 [SD: -4.15]) and group B 3.66 [SD: 3.12] cm/yr (-2.79 [SD: -3.4]). After one year of GH replacement therapy at the recommended weekly dosage of 0.23 mg/kg subdivided in 6 daily subcutaneous doses, both groups showed an increase in growth rate, i.e., 8.48 [SD: 2.98] cm/yr (3.38 [SD: 4.48]) in group A and 8.65 [SD: 1.74] cm/yr (2.24 [SD: 2.59]) in group B, indicating a significant growth-promoting effect of the drug (Table 2).

In both groups of patients, pre-treatment BMI values were within the normal range for age and sex (Table 1), and no changes were observed after one year of GH replacement treatment (Table 2).

Discussion

In patients with clinical criteria for GHD, a single provocative test is deemed insufficient for the diagnosis of GHD given the considerable variability from one stimulation test to another. Moreover, poor reproducibility of response to any single test has been observed in patients, other than those with complete GHD. Despite potential hazards, insulin-induced hypoglycemia remains the "gold standard", although arginine appears to be equally effective. However, false negative responses have been found in children after both insulin and arginine, and discordant results from the two tests may occur in the same child. The explanation for the discordance of peak-provoked GH results for these tests is ascribed to biological variability or

Table 2: Auxological data in patients of group 1 and group 2 after the 1st year of GH replacement therapy.

	Group 1 (22)	Group 2(28)	p value
Age	12.58 (2.54)	8.98 (4.01)	ns
Height sds	-1.78 (0.74)	-2.03 (0.65)	ns
Weight sds	-2 (0.77)	-2.23 (1.14)	ns
BMI sds	-1.41 (0.79)	-1.24 (1.07)	ns
Growth rate sds	3.38 (4.48)	2.24 (2.59)	ns

ns: not significant

neuroregulatory abnormalities, although potential temporal effects in the sequential studies cannot be excluded. Moreover, serum GH cut-off values for pharmacological stimulation tests depend on the type of stimulus and the method used for determining serum GH [2,6].

Provocative tests also involve some elements of risk for the patient. Indeed, a child with severe GHD who receives insulin as a provocative stimulus may be particularly vulnerable. Deaths following insulin-induced hypoglycemia have been reported [4].

To obviate the use of GH provocative tests, measurements of GH-dependent peptides, such as serum insulin-like growth factor-I (IGF-I) and IGF-I binding protein-3 (IGFBP-3) have been used. However, levels of these growth-promoting factors can show overlap in short normal and normal children. Moreover, reference ranges for IGF-I and IGFBP-3, standardized for age and sex, depend on the assay used. There is general agreement that serum IGF-I and/or IGFBP-3 values associated with at least two provocative stimuli can increase the likelihood of diagnosis of GHD. It is therefore important that clinicians integrate all available data, i.e., clinical, auxological, radiological, and biochemical, when making a diagnosis.

Therefore, in clinical practice, at least two stimulation tests including different pharmacological stimuli are required to confirm the diagnosis of GHD. Provocative tests should be performed in pediatric endocrinology centers with experienced teams; particular attention is required when administering insulin and glucagon, due to the risk of symptomatic hypoglycemia. Furthermore, it is necessary to bear in mind that GH basal levels are not useful in confirming GH deficiency because of the variability of spontaneous GH secretion.

Our own results suggest that when patients present certain underlying risk factors (including young age, history of neurological diseases in particular seizures, suspicion of complete GH deficiency), a second dose of arginine as provocative stimulus can be used instead of glucagon to obtain the same results.

The mechanisms of action of glucagon and arginine on GH release have been widely studied. Glucagon has been used for many years as a stimulus for GH secretion in the diagnosis of GHD, but its mechanism of action has not yet been fully elucidated. Convincing experimental observations suggest that glucagon stimulates the secretion of noradrenaline, which in turn, may be responsible for GH release by acting on its alpha-adrenergic receptors [7,8]. Since GH response to alpha-adrenergic stimulation is mainly mediated by a decreased release of somatostatin [9-12], it can be hypothesized that glucagon stimulates GH secretion mainly through an inhibition of the hypothalamic somatostatin secretion mediated by noradrenaline release. The mechanism by which arginine stimulates the secretion of GH, on the other hand, is much clearer. In fact, almost all published studies agree that arginine stimulates GH secretion through inhibition of the somatostatin release [13-16]. Further confirmation of this mechanism derives from evidence that arginine can override the inhibition of GH secretion which occurs in many situations characterized by an increased somatostatinergic tone [17-25].

In conclusion, multiple stimuli induce GH release, but insulin-induced hypoglycemia has traditionally been considered the "gold standard" although arginine appears to be equally effective. Diagnosis of GHD in children can be supported by evidence of a blunted GH secretory response after two or more pharmacological stimuli and our study shows that repeating the same test with arginine is equally effective as other stimuli in evaluating GH secretion. It can be assumed, therefore, that glucagon and arginine share, at least in part, the same mechanism of action i.e., inhibition of the secretion of hypothalamic somatostatin. Therefore, arginine can be used in particular cases where other stimuli carry risks for the patient.

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