REPRODUCIBILITY AND VARIABILITY OF THE ARGININE TEST IN NORMAL ADULTS

COMPARISON BETWEEN SEXES

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Abstract   The biochemical diagnosis of growth hormone deficiency in adults (AGHD) remains controversial, mainly as regards stimulation tests and suggested cut-off lines. The insulin tolerance test proved to be the most effective growth hormone (GH) secretagogue in normal males, but a poor intra-individual reproducibility has been reported. Given the safety of the arginine test (AST), we decided to evaluate the incidence of false negatives (non responder normal subjects), its reproducibility and variability. Twenty five healthy non-obese volunteers (16 males, 9 females) with a chronological age range between 19 and 40 years, (mean: 29.8) were evaluated. AST was performed (0.5 g/kg IV infusion for 30 min), measuring GH (IRMA) at baseline (B), 30, 60 and 90 minutes, and it was repeated in the same subject 7 to 30 days later; in females both tests were performed in the early follicular phase. Results (median and range) were: 1st test B: 0.61 (0.35-22.60) μg/L; maximal response (Mx Resp) 10.00 (0.48-48.80) μg/L. 2nd test B: 0.50 (0.38-27.0) μg/L; Mx Resp 11.00 (0.50-47.70) μg/L. The statistical evaluation (Wilcoxon signed rank test) showed no differences between B vs. B and Mx Resp vs Mx Resp. Separated by sex, males showed: 1st test: B 0.45 (0.35-4.30) μg/L; Mx Resp 6.30 (0.48-48.80) μg/L. 2nd test B 0.46 (0.38-8.80) μg/L; Mx Resp 10.90 (0.50-47.70) μg/L, while females showed 1st test: B 5.20 (0.50-22.60) μg/L; mx Resp 14.00 (3.50-36.70) μg/L. 2nd test B 3.60 (0.75-27.00) μg/L; Mx Resp 13.00 (3.70-28.10) μg/L. The statistical comparison (Mann Whitney test) showed significant differences between both sexes in basal values of the first and second test (p < 0.001), and in the maximal response of the first test (p < 0.03). The statistical analysis did not show significant differences in delta increases between males and females, neither in the first AST nor in the second one. Considering GH values ≥ 3 μg/L as a positive response, 4 males exhibited insufficient responses in both tests and other 2 males showed discordant results between tests 1 and 2. All females evaluated produced responses above 3 μg/L in both tests. The results of the present study demonstrate that, particularly in men, AST has no clear limit of normality while it shows good intra-individual reproducibility. In conclusion, at present the biochemical diagnosis of AGHD requires a clear and precise standardization which includes all variables that can modify the GH response to the stimulus used.

Resumen   *Reproductibilidad y variabilidad del test de arginina en adultos normales. Comparación entre ambos sexos.* El diagnóstico bioquímico de la deficiencia de hormona de crecimiento en el adulto (AGHD) es aún controvertido, principalmente en relación a las pruebas de estímulo y a las líneas de corte propuestas. La hipoglucemia insulínica demostró ser el secretagogo más efectivo de hormona de crecimiento en varones normales, pero con pobre reproductibilidad intraindividual. Dada la ausencia de efectos adversos del test de arginina (AST), nosotros decidimos evaluar la incidencia de falsos negativos (sujetos normales no respondientes), su reproductibilidad y variabilidad. Fueron evaluados veinticinco voluntarios sanos no obesos (16 varones, 9 mujeres), con edades cronológicas comprendidas entre 19 y 40 años (media 29.8). El AST fue realizado (0.5 g/kg en infusión endovenosa durante 30 minutos), midiendo GH (IRMA) en tiempos basal (B), 30, 60 y 90 minutos, y fue repetido en el mismo sujeto 7 a 30 días después; en las mujeres ambas pruebas fueron realizadas en fase folicular temprana. Los resultados (mediana y rango) fueron: 1° AST B: 0.61 (0.35-22.60) μg/L; Respuesta máxima (Mx Resp): 10.00 (0.48-48.80) μg/L. 2° AST B: 0.50 (0.38-27.0) μg/L; Mx Resp 11.00 (0.50-47.70) μg/L. La evaluación estadística (Wilcoxon signed rank test) no mostró diferencias entre B vs B y Mx Resp vs Mx Resp. Analizados por sexo, los varones mostraron: 1° AST: B 0.45 (0.35-4.30) μg/L; Mx Resp 6.30 (0.48-48.80) μg/L; 2° AST: B 0.46 (0.38-8.80) μg/L; Mx Resp 10.90 (0.50-47.70) μg/L, mientras que las mujeres mostraron: 1° AST: B 5.20 (0.50-22.60) μg/L; Mx Resp 14.00 (3.50-36.70) μg/L; 2° AST B 3.60 (0.75-27.00) μg/L; Mx Resp 13.00 (3.70-28.10) μg/L. La comparación estadística (test de Mann Whitney), mostró diferencias significativas entre ambos sexos en los valores basales de la primera y segunda prueba (p < 0.001), y en la respuesta máxima de la primera prueba (p < 0.03). En ninguno de los dos AST el análisis estadístico mostró diferencias significativas en los delta de incremento

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entre varones y mujeres. Considerando valores de GH ≥ 3 µg/L como respuesta positiva, 4 varones exhibieron respuestas insuficientes en ambas pruebas y otros dos mostraron resultados discordantes entre el AST 1 y 2. Todas las mujeres evaluadas respondieron por encima de 3 µg/L en ambas pruebas. La reproductibilidad del AST fue del 92%. Los resultados del presente estudio demuestran que, particularmente en hombres, el AST no tiene un límite claro de normalidad, aunque muestra buena reproductibilidad intraindividual. En conclusión, al presente el diagnóstico bioquímico del AGHD requiere una estandarización clara y precisa, la cual incluya todas las variables que puedan modificar la respuesta de GH al estímulo utilizado.

Key words: GH deficiency, adult growth hormone deficiency, arginine stimulation test

At present, the various clinical manifestations of Growth Hormone (GH) deficiency in adults (AGHD) are well known[1, 2]. GH substitution in these subjects appears to show promising results in the short and medium term, as regards improvement of some parameters of bone, lipids, hydrocarbonated (insulin-sensitivity) metabolism cardiovascular function and quality of life[3, 4]. Nevertheless, the diagnosis of AGHD is controversial, since there are still not sufficient data supporting defined criteria for the interpretation of GH response to different pharmacological stimuli[5, 6, 7, 8]. Recently, a GH response of less than 3 µg/L has been agreed upon to define severe GH deficiency[9]. On the other hand, some normal adults have exhibited responses to different stimuli below that value[10, 11]. Hence the need to establish a cut-off line and define, if possible, the best pharmacological stimuli having minimal adverse effects. In comparative studies between different provocative tests, the hypoglycemia induced by an insulin tolerance test (ITT) proved to be the most effective stimulus for GH secretion in normal males[11]. However, other authors have reported poor intra-individual reproducibility in repeated ITT and sex-dependent differences in the responses to such test[10]. Various stimuli have been used over the last years with the aim of establishing clear bases for diagnosis, and dissimilar results have been obtained[2, 6, 12]. The arginine stimulation test (AST), a relatively simple test having no significant adverse effects, has been one of the most widely used, but there is not enough experience in normal controls. Thus, we decided to evaluate this test in normal subjects of both sexes on two different occasions in order to study the incidence of false negatives (responses compatible with severe GH deficiency in normal subjects), their reproducibility and variability.

Subjects and Methods

Twenty five healthy non-obese adults (16 males, 9 females), with no history of endocrinological patologies nor clinical evidences of hormonal disturbances, were tested twice with the AST. Their chronological age range between 19 and 40 years (mean: 29.8). All of them had normal height and BMI between 20 and 25. Males were tested with an interval of 7 to 30 days between each test and females were tested in the early follicular phase (days 3 to 7) of two successive menstrual cycles. None of the subjects evaluated were receiving any medication. Tests were performed at 08.00 h after a 30-minute rest and an overnight fast. A cannula was inserted in an an-

tecubital vein. A 10% arginine hydrochloride solution was administered through this cannula for 30 minutes, at a dose of 0.5 g/kg body weight. Samples were drawn at 0 minutes (prior to infusion), and at 30, 60 and 90 min. GH was measured at 0, 30, 60 and 90 min. In all cases at 0 minutes, insulin growth factor-I (IGF-I), insulin growth factor-binding protein 3 (IGFBP3), were measured for a better caracterization of GH - IGF-I axis. Follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone (T) were measured in males in order to confirm the normality of hypotalamus-hypophiseal-testicular axis. Both gonadotrophins and estradiol (E) were measured in females in order to confirm early follicular phase values.

GH in serum was measured in duplicate by immunoradiometric assay (IRMA), using commercial kits (Serono Maia-Clone; Milan, Italy). The reference standard used was the 1st IRP 66/217. The intra-assay coefficient of variation was 3.7% for an average dose of 1.35 µg/L, 3.1% for an average dose of 6.7 µg/L and 2.1% for an average dose of 27.4 µg/L. The interassay coefficient of variation was 3.0%, 2.5% and 3.7% for average doses of 1.35, 6.7 and 27.4 µg/L, respectively. The analytical sensitivity corresponded to 0.23 µg/L. IGF-I and IGF-BP3 were measured by IRMA (Diagnostics Systems Labora-tories, Inc.; Texas, USA). IGF-I was measured after acid-alcohol extraction. Reference ranges were: IGF-I: 100-628 µg/L; IGF-BP3: 1.73-7.38 µg/L for males aged 20-40 years, and IGF-I: 96-521 µg/L; IGF-BP3: 2.05-7.60 µg/L for females of the same age. FSH and LH were measured by IRMA (Serono Maia-Clone; Milan, Italy). Values were normal in all cases and corresponded to the early follicular phase in females (values not shown). T and E were measured by RIA, using commercial kits (Diagnostic Systems Laboratories Inc.; Texas, USA). Reference values for T for adult males were 2.8-8.8 ng/mL. Reference values for E in the early follicular phase were 10-60 pg/mL (detection limit: 4 pg/mL).

All subjects studied were normal volunteers (many of them members of the medical staff). Informed consent was obtained from all subjects and the study protocol was submitted to the Education and Research Committee.

All values were expressed as medians and ranges, and the statistical evaluation was carried out using the Wilcoxon Signed Rank Test[13], to compare both the basal levels of the first test to those of the second one, and the maximal response of the first test to that of the second one. For comparison of basal levels, maximal responses and delta increases in relation to baseline (maximal response minus basal level) between both sexes, the Mann Whitney test[13] was used. In order to evaluate the variability of both tests considering GH values ≥ 3 µg/L as a positive response, the Sign test[13] was used.

Results

Whole Group:

Considering all the population evaluated, the GH basal level in test 1 was 0.61 µg/L (0.35-22.60 µg/L), while in

test 2 it was 0.50 µg/L (0.38-27.00 µg/L). The statistical comparison between basal levels of both tests did not show significant differences. The GH maximal response was 10.00 µg/L (0.48-48.8 µg/L) in test 1, and 11.00 µg/L (0.50-47.70 µg/L) in test 2. The comparison between maximal responses of both tests was not statistically significant. An 8% variability was observed when individual tests were evaluated, considering a value of GH ≥ 3 µg/L as a positive response (Sign test;   p = NS).

Males

When GH basal levels were separated by sex, in the first test, males showed: 0.45 µg/L (0.35-4.30 µg/L), and in the second test, 0.46 µg/L (0.38-8.80 µg/L); p = NS. GH maximal response in test 1 was 6.30 µg/L (0.48-48.80 µg/L) and in the second test, 10.90 µg/L (0.50-47.70 µg/L); p = NS. IGF-I, IGFBP3 and T levels were: 220 µg/L

(115-320 µg/L), 3.50 µg/L (1.80-4.20 µg/L) and 6.80 ng/mL (3.20-10 ng/mL), respectively.

Females

GH basal levels were 5.20 µg/L (0.50-22.60 µg/L) in test 1 and 3.60 µg/L (0.75-27.00 µg/L) in test 2; p = NS. GH maximal responses were in the first test 14.00 µg/L (3.50-36.70 µg/L) and in the second test 13.00 µg/L (3.70 - 28.10 µg/L); p = NS. IGF-I, IGFBP3 and E levels were 170 µg/L (115-400 µg/L), 3.80 µg/L (3.05-4.80 µg/L) and 31.0 pg/mL (12.5-54 pg/mL), respectively.

Comparison between sexes

The statistical comparison between both sexes showed significant differences among GH basal levels in the first test (p < 0.001), and in the second test (p < 0.001), and in
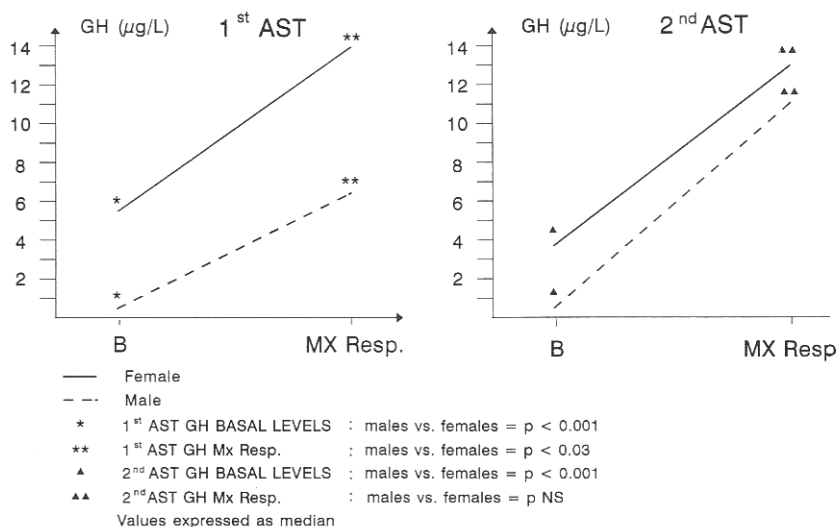

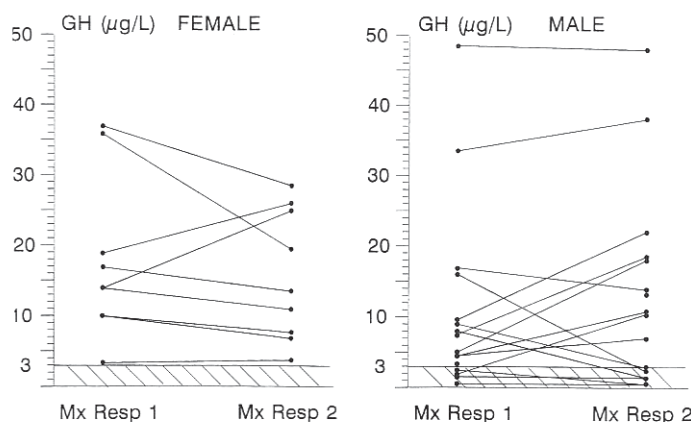Fig. 1.– Comparison of GH basal levels and maximal responses betwen sexes in 1st and 2nd AST.

Fig. 2.– GH maximal response in the1st and 2nd AST in both sexes

the GH maximal response following the first AST (p < 0.03). No significant differences were found between males and females in the GH maximal response in the second AST (Fig. 1).

The delta increase in relation to baseline in males was 3.6 µg/L (0.05-47.5 µg/L) in the first test, and 8.84 µg/L (0.05-43.6 µg/L) in the second test. In the first test, females had a delta of 6.0 µg/L (0.00-34.1 µg/L) and in the second one, a delta of 2.9 µg/L (0.00-22.7 µg/L). The statistical analysis did not show significant differences in delta increases between males and females, neither in the first AST nor in the second one. Considering GH values ≥ 3 µg/L as a positive response, 4 subjects (16%) exhibited insufficient responses in both tests, all subjects being males. Other 2 males showed discordant results between test 1 and 2 (Fig. 2). All females evaluated produced responses above 3 µg/L in both tests (Fig. 2).

Adverse effects

No adverse events were observed in any of the subjects during both tests.

Discussion

The diagnosis of GH deficiency in the various age groups studied remains controversial. The variability of responses in a single subject, using the same provocative test[5] is well known in pediatric patients. On the other hand, we reported a variability of individual responses depending on the stimulus used and the cut-off line adopted, in children of normal height and growth velocity[14].

For many years, the diagnosis of AGHD has been of relative practical interest. The wide availability of recombinant human GH and the recognition of the clinical syndrome of AGHD make it necessary to have effective and reproducible studies with minimal side-effects. However, there are still not sufficient data in adults regarding reproducibility and variability of GH response to different tests.

Knowing the physiological decline of GH throughout adulthood[15, 16], in this study we decided to limit the age range of the evaluated population to a maximum of 40 years so as to leave out a variability factor. We have used the AST because it is considered as a reliable and well tolerated method. As no normality cut-off line has been established yet for the AST, we have tested a value of 3 µg/L as the cut-off line to define GH positive responses in adults. This criterion was adopted following the Gothenburg Consensus proposal[9], which established this cut-off value for ITT, even allowing for the influence of age and weight, further indicating that it was nonresponders who benefited the most from replacement therapy. Recently, Port Stephens Consensus[7] established that

most normal subjects respond to insulin-induced hypoglycemia, with a GH peak above 5 µg/L and severe GH deficiency is defined when the response is lower than 3 µg/L. These cut-off lines were set by using polyclonal RIA methods. Nevertheless, GH immunoassays vary depending on the various methods and, therefore, cut-off lines may need to be adjusted to each of them. It was also established that the other stimulation tests used for patients in whom the ITT is contraindicated should have their respective and appropriate cut-off lines[7]. For this reason, we were particularly interested in studying the variability and the potential existence of nonresponders in normal subjects with AST. The evaluation of the group as a whole showed good reproducibility (92%). On the other hand, we observed that females had greater basal values and reached higher post-stimulation levels than men. This does not mean that there was a difference in response between both sexes, since delta increases were similar. Considering a cut-off line of 3 ng/L, 4 males exhibited responses that did not reach such level, and in other 2, there was inconsistency between the responses from the first tests and those from the second one. This would raise some doubts as to whether the AST should be used in both sexes. It is worth mentioning that the age of males and females was similar, and that for females all tests were performed in the early follicular phase, with E levels corresponding to that moment to the cycle.

The joint analysis of our results and those obtained by other authors[17, 18, 19, 20], shows the complexity in GH regulation. The dissimilar finding would not only relate to the various neuroendocrine mechanisms involved depending on the stimulus used, but they would also be, at least partly, sex-dependent. Differences in responses to the same stimulus between both sexes had already been reported. In 1966 Merimeé et al.[21] showed that some males did not respond to the AST; a year later, Parker et al.[22] found higher responses to the same stimulus in females. Later, several authors found higher responses to the ITT in males[23, 24]. Recently, Hoeck et al reported a poor reproducibility of the test and lower responses in females[10]. The influence of sexual steroids on the GH secretion pattern is well known, with higher values being observed in the late phase of the menstrual cycle[25], and higher responses to various stimuli being produced after estrogen priming in prepuberty[26, 27, 28].

When analysing the various criteria for the diagnosis of AGHD, the laboratory assay used must also be considered[9, 7, 29]. The studies conducted by other authors and our own experience have shown the difficulties of extrapolating results obtained by different methodo-logies[30, 31, 32]. For this reason, a fixed cut-off line should not be used for all methods. Therefore, a better standar-dization is needed to unify criteria. All this is essential considering that the cut-off limit of 3 µg/L has not been established on the basis of appropiate population studies. It is evident that at

present the biochemical diagnosis of AGHD requires a clear and precise standardization which includes all the variables that can modify the GH response to each stimulus.

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