

# Anthropometric factors have significant influence on the outcome of the GHRH-arginine test – establishment of normative data

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## Introduction and objectives

- The diagnosis of growth hormone (GH) deficiency (GHD) in adults is based on provocative testing of GH secretion.
- The GHRH plus Arginine (GARG) test has been proposed as an alternative to the insulin tolerance test.
- To improve the diagnostic accuracy of the GARG, we aimed to establish normative data applying a newly developed automatic immunoassay specifically measuring 22kD hGH.

## Methods

- Two hundred healthy subjects (C) were prospectively stratified into three age groups (18-30, 31-50, >51 years), three BMI groups (<25, 25-29.9, ≥30kg/m<sup>2</sup>), and for both sexes, with at least 10 subjects in each subgroup.
- All subjects received GHRH 1 µg/kg i.v., followed by a 30-min infusion of L-ARG (30g), with blood samples drawn at 0', 30', 45', 60', 90', and 120'.
- In addition, eighty-seven patients with hypothalamic-pituitary disease were studied by GHRH-arginine test. Patients were classified according to the number of pituitary hormone deficiencies (PHD) and GHD was assumed when ≥2 PHD (in addition to GH) were present (n=51); the remaining 36 patients with < 2 PHD were considered GH sufficient (GHS).
- GH was measured with a newly developed GH immunoassay (IDS iSYS, Boldon, UK).
- ROC analysis was used to identify thresholds with at least 95% specificity.

## Results

### Influence of age, BMI, and sex on GH peak levels in healthy subjects.

- Multiple stepwise regression analysis in controls revealed that both BMI (21%, p<0.0001) and sex (20%, p<0.0001) accounted for most of the variability of GH peak levels during the GARG test, whereas the influence of age (5%, p<0.001) was much less dominant.
- Mean peak GH levels were significantly higher in females compared to males (27.4 ± 1.73 ng/ml vs. 13.7 ± 0.95 ng/ml, p<0.0001),

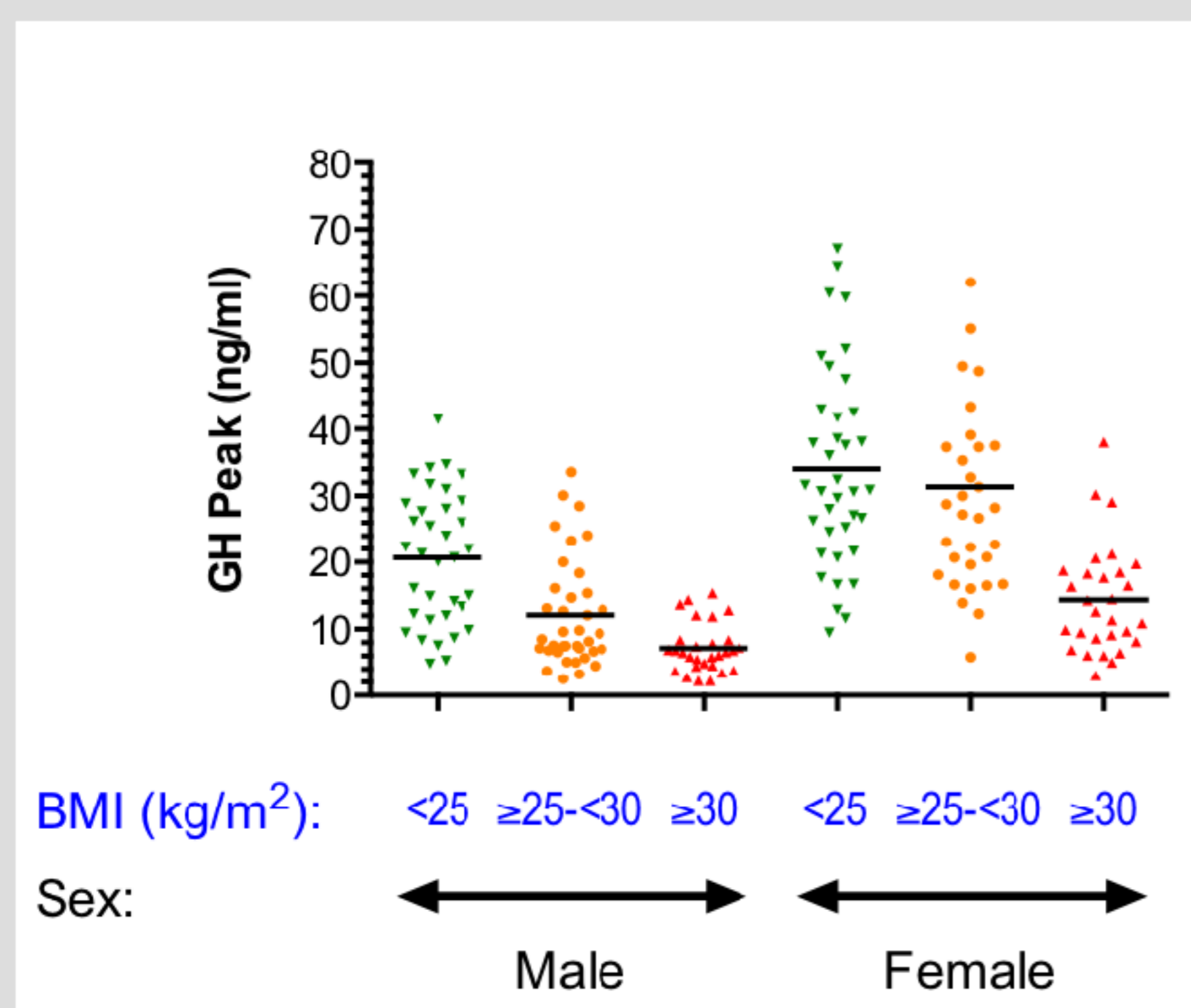


Fig. 1. Figure 1. Peak GH levels during the GARG test in 200 healthy subjects, stratified by sex and BMI. Dark lines represent the means for each group. Abbreviations are: GARG, growth hormone releasing hormone plus arginine; GH, growth hormone.

- Furthermore, mean peak GH levels were higher in lean (34.1 ± 2.46 ng/ml in females v. 20.7 ± 1.67 ng/ml in males, p<0.0001) compared to overweight (31.4 ± 3.38 ng/ml in females vs. 12.1 ± 1.34 ng/ml in males, p<0.0001) and obese subjects (14.4 ± 1.52 ng/ml in females vs. 7.19 ± 0.68 ng/ml in males, p<0.0001) (Figure 1).

### Establishment of new cutoffs for peak GH levels during the GHRH plus arginine test by comparison of growth hormone deficient patients and control subjects.

- Considering the significant influence of BMI and sex for the GARG in normal subjects, we aimed to establish diagnostic cutoffs that were adjusted accordingly.
- Comparison of peak GH levels during the GARG test of patients with GHD to those of a control group (consisting of patients with GHS and healthy subjects) allowed calculation of cutoffs for peak GH (either as an overall threshold or as BMI-dependent thresholds with at least 95% specificity for GHD, with the latter being separately provided for both sexes).
- As shown in the Table as well as in Figure 2, the overall cutoff for both sexes was 3.9 ng/ml (sensitivity 86% (95% confidence interval (CI) 74-94%), specificity 95% (95% CI 92-98%)); the BMI-adjusted thresholds were 6.5, 3.5, and 2.2 ng/ml for males, and 9.7, 8.5, and 4.4 ng/ml for females (always for lean, overweight, and obese subjects, respectively).

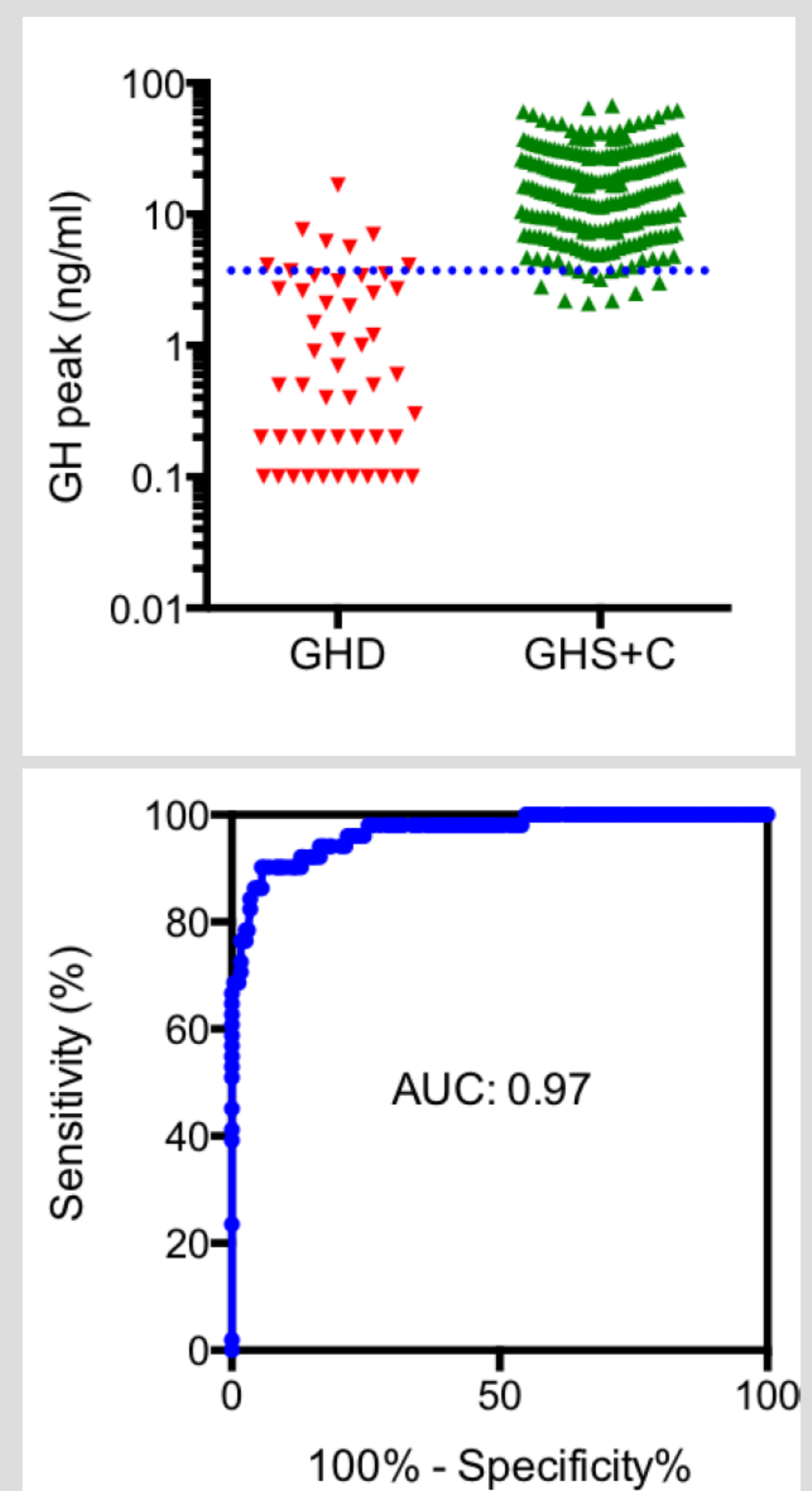


Fig. 2. Peak GH levels (top) during GHRH-arginine test and corresponding ROC curve (bottom). The broken line indicates the overall GH cutoff (3.9 ng/ml). Abbreviations are: C, healthy subjects; GHD, growth hormone deficiency; GHS, growth hormone sufficiency.

	Overall	Males			Females		
		Lean (BMI <25 kg/m <sup>2</sup> )	Over-weight (BMI ≥25-30 kg/m <sup>2</sup> )	Obese (BMI ≥30 kg/m <sup>2</sup> )	Lean (BMI <25 kg/m <sup>2</sup> )	Over-weight (BMI ≥25-30 kg/m <sup>2</sup> )	Obese (BMI ≥30 kg/m <sup>2</sup> )
Cutoff (ng/ml)	3.9	6.5	3.5	2.2	9.7	8.5	4.4
Sensitivity (%)	86	100	92	91	83	100	67
Specificity (%)	95	95	96	97	96	97	97
ROC AUC	0.97	1.00	0.99	0.99	0.97	1.00	0.95
p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0005	<0.0001	<0.001

## Conclusions

- We present thresholds for the GHRH-arginine test specific for the new IDS iSYS GH assay exclusively measuring 22kD hGH.
- The diagnostic accuracy of the GARG test is significantly improved by adjusting thresholds according to sex and BMI.
- Both may have important impact on the correct diagnosis in patients with suspicion of GHD.