Macimorelin as a Diagnostic Test for Adult GH Deficiency

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Purpose: The diagnosis of adult GH deficiency (AGHD) is challenging and often requires confirmation with a GH stimulation test (GHST). The insulin tolerance test (ITT) is considered the reference standard GHST but is labor intensive, can cause severe hypoglycemia, and is contraindicated for certain patients. Macimorelin, an orally active GH secretagogue, could be used to diagnose AGHD by measuring stimulated GH levels after an oral dose.

Materials and Methods: The present multicenter, open-label, randomized, two-way crossover trial was designed to validate the efficacy and safety of single-dose oral macimorelin for AGHD diagnosis compared with the ITT. Subjects with high (n = 38), intermediate (n = 37), and low (n = 39) likelihood for AGHD and healthy, matched controls (n = 25) were included in the efficacy analysis.

Results: After the first test, 99% of macimorelin tests and 82% of ITTs were evaluable. Using GH cutoff levels of 2.8 ng/mL for macimorelin and 5.1 ng/mL for ITTs, the negative agreement was 95.38% (95% CI, 87% to 99%), the positive agreement was 74.32% (95% CI, 63% to 84%), sensitivity was 87%, and specificity was 96%. On retesting, the reproducibility was 97% for macimorelin (n = 33). In post hoc analyses, a GH cutoff of 5.1 ng/mL for both tests resulted in 94% (95% CI, 85% to 98%) negative agreement, 82% (95% CI, 72% to 90%) positive agreement, 92% sensitivity, and 96% specificity. No serious adverse events were reported for macimorelin.

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Abbreviations: AE, adverse event; AGHD, adult GH deficiency; BMI, body mass index; DRC, data review committee; GHD, GH deficiency; GHS, GH secretagogue; GHST, GH stimulation test; ITT, insulin tolerance test; SAE, serious adverse event.

Conclusions: Oral macimorelin is a simple, well-tolerated, reproducible, and safe diagnostic test for AGHD with accuracy comparable to that of the ITT. A GH cutoff of 5.1 ng/mL for the macimorelin test provides an excellent balance between sensitivity and specificity. (***J Clin Endocrinol Metab*** **103: 3083–3093, 2018)**

GH therapy offers clinical benefits for individuals with adult GH deficiency (AGHD) (1–6). However, diagnosing this condition is often challenging and remains a barrier to initiating GH treatment. The measurement of random GH levels will not distinguish GH-deficient from GH-sufficient subjects reliably. Accordingly, the diagnosis of AGHD often depends on GH stimulation tests (GHSTs) using agents known to provoke GH release greater than a certain level in healthy individuals.

The insulin tolerance test (ITT) is considered the reference standard GHST; however, the test is labor intensive, can be unpleasant for patients, has potential risks (including severe hypoglycemia), and is contraindicated for elderly patients and those with seizure disorders or heart disease (7, 8). Other alternative provocative tests such as the arginine plus GHRH, arginine alone, and glucagon stimulation tests are either not available in the United States or have substantial limitations, including requiring intramuscular administration, a long duration, and/or low accuracy. Thus, an unmet medical need remains for alternative GHSTs that are safe and reliable and have been approved by a regulatory authority.

Ghrelin is known to potently stimulate GH release (9) mediated by specific ghrelin receptors in the pituitary and hypothalamus (10, 11). This effect is shared by synthetic agonists of this receptor known as ghrelin mimetics or GH secretagogues (GHSs). Macimorelin acetate is an oral ghrelin receptor agonist with GHS activity that is readily absorbed and effectively stimulates endogenous GH secretion in healthy volunteers with good tolerability (12).

The present trial was designed to validate the use of a single-dose oral macimorelin test for the diagnosis of AGHD using the ITT as the comparator. The secondary objective was to characterize the safety of macimorelin in this setting.

Materials and Methods

The present phase III study was an open-label, randomized, multicenter, two-way crossover study of the macimorelin test vs the ITT (core study). Additionally, a subset of patients (n = 33) underwent the macimorelin test twice to evaluate the reproducibility of the test (reproducibility substudy). The present study was conducted in five sites in the United States and 25 sites across Europe. The institutional review board at each institution approved the protocol, and the study was conducted in compliance with the Declarations of Helsinki and its amendments and the International Conference on Harmonization Guideline for Good Clinical Practices. Recruitment for the study occurred from September 2015 to November 2016.

Eligibility criteria

Inclusion criteria were age 18 to 65 years and suspected GH deficiency (GHD) determined by one of the following: structural hypothalamic/pituitary disease, surgery or irradiation in these areas, head trauma as an adult, evidence of other pituitary hormone deficiencies, or idiopathic childhood-onset GHD (1). The exclusion criteria were as follows: GH therapy within the previous month; performance of a GH stimulation test in the previous 7 days; the presence of an untreated thyroid disorder or hypogonadism or unstable disease with substitution treatment; treatment with drugs affecting GH secretion or somatostatin; the use of antimuscarinic agents or CYP3A4 inducers; ongoing symptomatic severe psychiatric disorders, Parkinson disease, active Cushing disease, or receipt of supraphysiologic glucocorticoid therapy; type 1 diabetes or poorly controlled type 2 diabetes mellitus (glycated Hb >8%); body mass index (BMI) ≥40 kg/m^2; participation in a trial with investigational drugs within 30 days; vigorous physical exercise within 24 hours before each GHST; clinically important cardiovascular or cerebrovascular disease; a prolonged QT interval (QTc >500 ms); concomitant treatment with drugs that prolong the QT/QTc; hepatic or renal dysfunction; a history of seizure disorders or immunosuppression; active malignancy other than nonmelanoma skin cancer; and breastfeeding, positive urine pregnancy test result, or women of childbearing age without contraception. Postmenopausal status was not considered an exclusion criterion.

Subjects with a high, intermediate, and low likelihood for AGHD were included in the study (≥25% of subjects with AGHD were in the high and low likelihood groups). The high likelihood group (group A) was defined as those with a structural hypothalamic or pituitary lesion and low IGF-1 levels, three or more pituitary hormone deficiencies and low IGF-1 levels, or childhood onset GHD with structural lesions and low IGF-1 levels. The low likelihood group (group C) was defined as those with one risk factor for AGHD, such as a history of distant traumatic brain injury, only one pituitary hormone deficiency, or childhood-onset isolated GHD. Subjects were included in the intermediate likelihood group (group B) if they did not meet the criteria for group A or C. A group of healthy subjects (group D) matched with the group A subjects by sex, age, BMI, and estrogen status was also included. A subset of subjects from groups A to C underwent a second macimorelin GHST and were included in the reproducibility substudy.

Study procedures

The subjects were randomized to a sequence of both tests (macimorelin GHST followed by the ITT or vice versa) performed 7 to 30 days apart after fasting for 8 hours before the start of the test and continued throughout the test. A test result

was classified as "positive" for GHD when the peak GH value was less than the cutpoint established *a priori*, suggesting the patient had the disease. A test result was classified as "negative" for GHD when the peak GH value was greater than the cutpoint, suggesting the patient did not have the disease.

Macimorelin test

The macimorelin oral solution was prepared by the trial personnel at a dose of 0.5 mg/kg body weight to be administered within 30 minutes. Blood samples for GH serum levels were collected before administration and at 30, 45, 60, and 90 minutes (±5-minute window) after administration of macimorelin.

Insulin tolerance test

The ITT was performed with regular human insulin administered intravenously at 0.1 U/kg (0.15 U/kg if the BMI was >30 kg/m^2). Glucose was monitored in capillary or venous blood every 15 minutes until 60 minutes after insulin administration and, thereafter, every 30 minutes and when evidence was present of symptomatic hypoglycemia with diaphoresis or cognitive symptoms. As soon as clinical signs of hypoglycemia were achieved, blood for plasma glucose testing was taken for confirmation, defined as a glucose value <2.2 mmol/L (<40 mg/dL). An additional insulin bolus of 0.05 U/kg was administered if a glucose value <2.2 mmol/L (<40 mg/dL) and symptomatic hypoglycemia had not been achieved within 45 minutes after the initial dose. Blood samples to determine the serum GH concentrations were collected before dosing and 15, 30, 45, 60, 90, and 120 minutes (±5-minute window) after insulin administration. Intravenous glucose/dextrose was administered if a subject developed severe symptoms of neuroglycopenia (*i.e.*, seizures). Oral glucose administration was allowed if the patient had a glucose level <2.2 mmol/L (<40 mg/dL) and moderate symptoms of neuroglycopenia (*e.g.*, confusion).

Determination of evaluable tests

The cutoff values determined *a priori* for the stimulated GH levels measured using the IDS-iSYS human GH assay (Immunodiagnostic Systems, Ltd.; Tyne & Wear, United Kingdom) were 2.8 ng/mL for the macimorelin test and 5.1 ng/mL for the ITT, in accordance with previously reported data (13, 14). A data review committee (DRC), which included four investigators and representatives from the sponsor reviewed and qualified each test as "evaluable" or "not evaluable" before the availability of the GH results. The reasons for the DRC to designate a test as not evaluable included major deviation in the blood sampling protocol, not reaching the target glucose level and symptomatic hypoglycemia (for the ITT), and incomplete intake of the dose or vomiting after drinking for the macimorelin test. Whenever possible, a test declared not evaluable by the DRC was repeated after ≥7 days. The DRC also reviewed the assignment of study participants to the AGHD likelihood groups (groups A to C).

GH measurements

Serum GH concentrations were measured centrally (Synevo Central Laboratory, Warsaw, Poland) using a validated immunochemiluminometric assay (IDS-iSYS human GH) (15, 16). This assay is standardized to the recombinant GH calibration standard World Health Organization 98/574 and complies with recommendations on assay standardization (17).

Statistical analysis

Statistical analyses were performed using SAS®, version 9.3 (SAS Institute, Inc., Cary, NC). All randomized subjects for whom both GHSTs were evaluable were included in the efficacy analyses. The criteria for an evaluable GHST were (1) the DRC adjudicated the GHST as evaluable, (2) a peak GH concentration equal to or greater than the cutoff, which rendered the test evaluable irrespective of the DRC adjudication, and (3) for the macimorelin GHST, 45- and 60-minute postdose GH concentrations available or imputable categorically. The safety population used for the primary safety analyses included all randomized subjects who had received at least one dose of the trial medication. The study was planned to include ≥110 subjects to achieve 55 subjects with GHD as assessed by ITT and 55 with negative ITT results for the GH test outcomes. The ITT was used as the comparator. The primary measures for diagnostic consistency were the percentage of positive agreement and the percentage of negative agreement. The estimated percentage agreements and the two-sided 95% CIs of the percentage agreements using the Clopper-Pearson method (18) were calculated.

The definition of the accuracy measures is presented in Table 1. The primary efficacy measures (negative and positive agreements) using the following four methods were analyzed by a hierarchical testing procedure with regard to the sampling time for the macimorelin test: (1) peak GH concentration among all post-baseline samples (30, 45, 60, and 90 minutes); (2) highest GH concentration for the 45- and 60-minute samples; (3) GH concentration at 60 minutes after the dose; and (4) GH concentration at 45 minutes after the dose. Adverse events (AEs), clinical laboratory results, and ECGs were evaluated using descriptive statistics. QTcF (Fridericia correction) was centrally recalculated for all ECGs using the formula: QT (ms)/RR (s)$^{(1/3)}$ (19).

For the exploratory analyses, the sensitivity and specificity for both GHSTs were estimated, assuming all subjects with a high probability (group A) of AGHD as "true" AGHD-positive subjects and all healthy matching subjects (group D) as "true" AGHD-negative subjects. The receiver operating characteristic analysis results are presented based on these assumptions. The reproducibility of the macimorelin test was analyzed using descriptive statistical analyses. Statistical tests were performed two-sided with a type I error (*P* value) of $\alpha = 0.05$.

Results

A total of 166 screened subjects were eligible and enrolled in the present study (137 with suspected AGHD and 29 healthy subjects). Of these subjects, 157 underwent at

Table 1. Definition of the Accuracy Measures

Macimorelin Test Outcome	ITT Outcome	
	Positive	Negative
Positive	*w*	*u*
Negative	*y*	*z*
Total	*w* + *y*	*x* + *z*

Positive percentage agreement (%) = 100% × *w*/(*w* + *y*).

Negative percentage agreement (%) = 100% × *u*/(*u* + *z*).

Overall percentage agreement (%) = 100% × (*w* + *z*)/(*w* + *u* + *y* + *z*).

least one GHST (safety population) and 154 underwent both GHSTs at least once; both GHSTs were considered evaluable by the DRC for 140 subjects. Of the 140 subjects, 1 showed no measurable macimorelin plasma level at the first macimorelin GHST and detectable macimorelin plasma levels and a GH increase during the macimorelin GHST in the reproducibility substudy. This was attributed to noncompliance or a dosing error, and this patient's data were removed from the efficacy analysis. The study design and patient disposition are shown in Fig. 1.

The baseline characteristics are shown in Table 2. In 27 of 157 subjects, the ITT provided a peak GH concentration <5.1 ng/mL, without confirmed hypoglycemia. For only 17 of these 27 subjects was the nonevaluable ITT repeated. Of these 17 subjects, the results for 4 were then classified as ITT negative; 14 of 27 subjects with a nonevaluable ITT could not be included in the efficacy analysis. Only 1 of 154 macimorelin GHSTs was considered not evaluable and had to be repeated. For that subject, the site had not collected blood samples for GH measurements at the initial macimorelin GHST. Of the 139 subjects in the efficacy population, 74 were classified as having GHD and 65 as having GH sufficient based on the ITT results. Of the 114 GHD subjects, 31 were in the United States and 83 were in Europe; all the subjects in group D were in Europe.

Negative and positive agreements between macimorelin GHST and ITT

The negative agreement was 95.38% (95% CI, 87.10% to 99.04%), and the positive agreement was 74.32% (95% CI, 62.84% to 83.78%) between the macimorelin GHST and the ITT with the prespecified

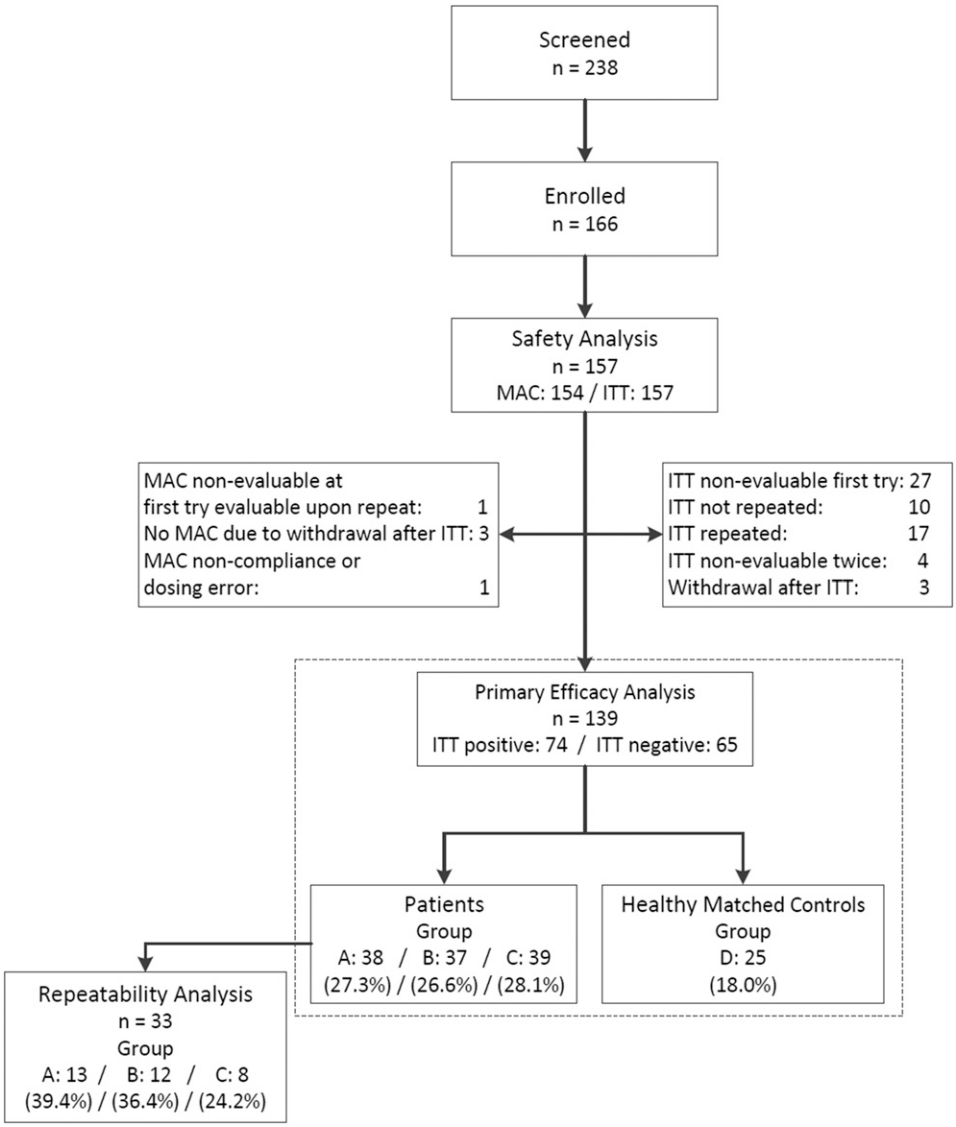

Figure 1. CONSORT (consolidating standards of reporting trials) diagram of study design and patient disposition. MAC, macimorelin stimulation test.

Table 2. Baseline Characteristics

Parameter (Safety Population, n = 157)	AGHD Likelihood Group				Total
	A (High)	B (Intermediate)	C (Low)	D (Healthy)	
Sex					
Male	25 (59.5)	18 (42.9)	35 (79.6)	15 (51.7)	93 (59.2)
Female	17 (40.5)	24 (57.1)	9 (20.5)	14 (48.3)	64 (40.8)
Total	42 (100)	42 (100)	44(100)	29 (100)	157 (100)
Race					
Native Hawaiian or other Pacific Islander	0 (0)	0 (0)	1 (2.3)	0 (0)	1 (0.6)
Asian	2 (4.8)	1 (2.4)	2 (4.6)	0 (0)	5 (3.2)
White	36 (85.7)	36 (85.7)	34 (77.3)	29 (100)	135 (86.0)
Black or African American	0 (0)	1 (2.4)	2 (4.6)	0 (0)	3 (1.9)
Other	4 (9.5)	4 (9.5)	5 (11.4)	0 (0)	13 (8.3)
Total	42 (100)	42 (100)	44 (100)	29 (100)	157 (100)
Ethnicity					
Hispanic or Latino	4 (9.5)	9 (21.4)	2 (4.6)	0 (0)	15 (9.6)
Not Hispanic or Latino	34 (81)	29 (69.1)	36 (81.8)	29 (100)	128 (81.5)
Not reported	3 (7.1)	4 (9.5)	6 (13.6)	0 (0)	13 (8.3)
Unknown	1 (2.4)	0 (0)	0 (0)	0 (0)	1 (0.6)
Total	42 (100)	42 (100)	44 (100)	29 (100)	157 (100)
Pituitary adenoma				NA	
None	21 (13.4)	12 (7.6)	37 (23.6)		70 (44.6)
Macroprolactinoma	1 (0.6)	8 (5.1)	1 (0.6)		10 (6.4)
Microprolactinoma	1 (0.6)	0 (0)	2 (1.3)		3 (1.9)
Nonfunctioning	15 (9.6)	17 (10.8)	4 (2.6)		36 (22.9)
Acromegaly	2 (1.3)	2 (1.3)	0 (0)		4 (2.6)
History of Cushing disease	2 (1.3)	3 (1.9)	0 (0)		5 (3.2)
CNS tumors				NA	
None	32 (20.4)	34 (21.7)	39 (22.8)		105 (66.7)
Meningioma	0 (0)	5 (3.2)	2 (1.3)		7 (4.5)
Craniopharyngioma	9 (5.7)	1 (0.6)	0 (0)		10 (6.4)
Medulloblastoma	0 (0)	1 (0.6)	2 (1.3)		3 (1.9)
Other	1 (0.6)	1 (0.6)	1 (0.6)		3 (1.9)
Other abnormalities				NA	
None	26 (16.6)	35 (22.3)	10 (6.4)		71 (45.2)
Childhood-onset GHD (idiopathic)	4 (2.6)	2 (1.3)	6 (3.8)		12 (7.6)
Cyst (Rathke arachnoid, etc.)	4 (2.6)	0 (0)	2 (1.3)		6 (3.8)
Sheehan syndrome	1 (0.6)	0 (0)	0 (0)		1 (0.6)
Empty sella	1 (0.6)	0 (0)	0 (0)		1 (0.6)
Head trauma	1 (0.6)	3 (1.9)	21 (13.4)		25 (15.9)
Inflammatory disorder	1 (0.6)	0 (0)	0 (0)		1 (0.6)
Other	4 (2.6)	4 (2.6)	10 (6.4)		18 (11.5)
Subgroup (n = 139)					
BMI class, kg/m^2					
<30	27 (27.6)	21 (21.4)	27 (27.6)	23 (23.5)	98 (100)
30 but <35	7 (26.9)	10 (38.5)	7(29.6)	2 (7.7)	26 (100)
35 but <40	4 (26.7)	6 (40.0)	5 (33.3)	0 (0)	15 (100)
Total	38 (27.3)	37 (26.6)	39 (28.1)	25 (18.0)	139 (100)
Age, y					
18 but ≤25	7 (29.2)	2 (8.3)	10 (41.7)	5 (20.8)	24 (100)
>25	31 (27.0)	35 (30.4)	29 (25.2)	20 (17.4)	115 (100)
Total	38 (27.3)	37 (26.6)	39 (28.1)	25 (18.0)	139 (100)

Data presented as n (%).

Abbreviations: CNS, central nervous system; NA, not applicable.

cutoff points (2.8 ng/mL for the macimorelin GHST and 5.1 ng/mL for the ITT). In a post hoc analysis using a cutpoint of 5.1 ng/mL for both tests, the negative agreement was 93.85% (95% CI, 84.99% to 98.30%), and the positive agreement was 82.43% (95% CI, 71.83% to 90.30%). The performance of the macimorelin GHST using different cutoff points and the hierarchical stepwise approach is provided in Supplemental Tables 1 and 2, respectively.

Sensitivity and specificity of the macimorelin GHST

Because of the lack of a "standard of truth" to determine the true AGHD status of each participant, the sensitivity and specificity for both GHSTs could only be

estimated from the test outcomes in a subset of the efficacy population, with the assumption that all high likelihood AGHD subjects (group A) were true AGHD-positive subjects and all healthy matched subjects (group D) were true AGHD-negative subjects. Using the predefined cutoff points of 2.8 ng/mL for macimorelin, the sensitivity was 87% and the specificity was 96%. Figure 2 illustrates the effect of the varying GH cutoff points on the estimated sensitivity and specificity for the macimorelin GHST. The data showed that increasing the GH cutoff point for the macimorelin GHST from 2.8 ng/mL to ~8 ng/mL increased the sensitivity with a minimal effect on the specificity. When using a cutoff point of 5.1 ng/mL, the sensitivity and specificity of the macimorelin GHST were 92% and 96%, respectively.

Peak GH response in macimorelin GHST and ITT stratified by AGHD likelihood group

A greater peak GH level was seen in all groups with the macimorelin GHST compared with the ITT (Fig. 3A). Moreover, the peak GH levels were inversely related to the likelihood of having AGHD. A high correlation was found between the peak GH with the ITT and the macimorelin GHST (Fig. 3B).

Reproducibility of macimorelin GHST

The reproducibility of the macimorelin GHST was 94%. No substantial differences were found between the peak GH concentrations measured in the core study and those found in the reproducibility substudy (n = 33). The lack of a difference was shown, not only for the entire population of the repeatability study, but also for both subsets of positive and negative GHST outcomes in the core study (*i.e.,* stratified for subjects with a peak GH at <2.8 or >2.8 ng/mL; Supplemental Table 3) and for those subjects in groups A to C (Supplemental Fig. 1). The reproducibility of the macimorelin GHST was also maintained using different cutoff points and the hierarchical step-wise approach (Supplemental Tables 1 and 2).

Safety and tolerability

No serious AEs (SAEs) were reported after the ITT. One case of a broken arm was reported 1 day after a macimorelin GHST, which was considered unrelated to the test. Other non-SAEs were more common and of greater severity during the ITT than during the macimorelin GHST (Supplemental Material and Supplemental Table 4).

Discussion

Evaluation of AGHD should be based on the medical history, clinical findings, and use of the appropriate GHST for biochemical confirmation, except for patients with panhypopituitarism and low IGF-1 levels (1). The ITT remains the reference standard for the evaluation of AGHD; however, some endocrinologists are reluctant to perform it because of the potential risk of hypoglycemia

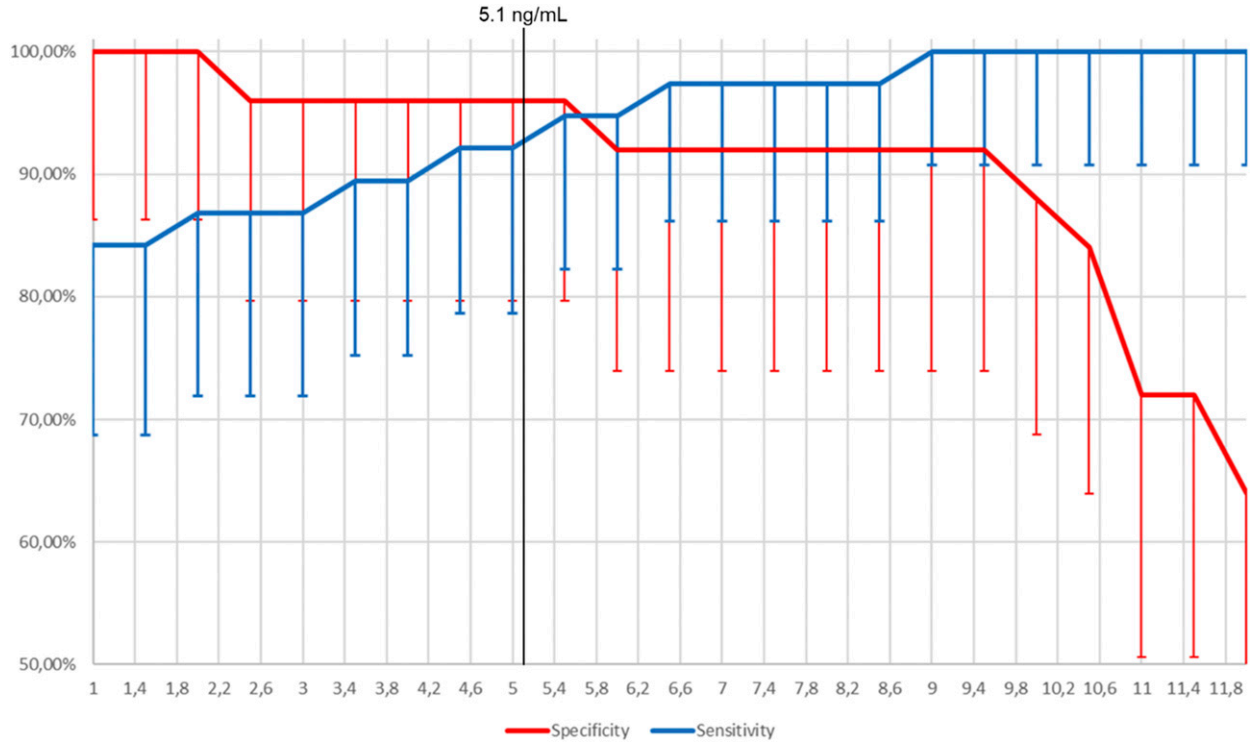

Figure 2. Specificity and sensitivity of the macimorelin GHST for varying GH cutoff points using data from group A and D subjects. Error bars represent 95% CIs.

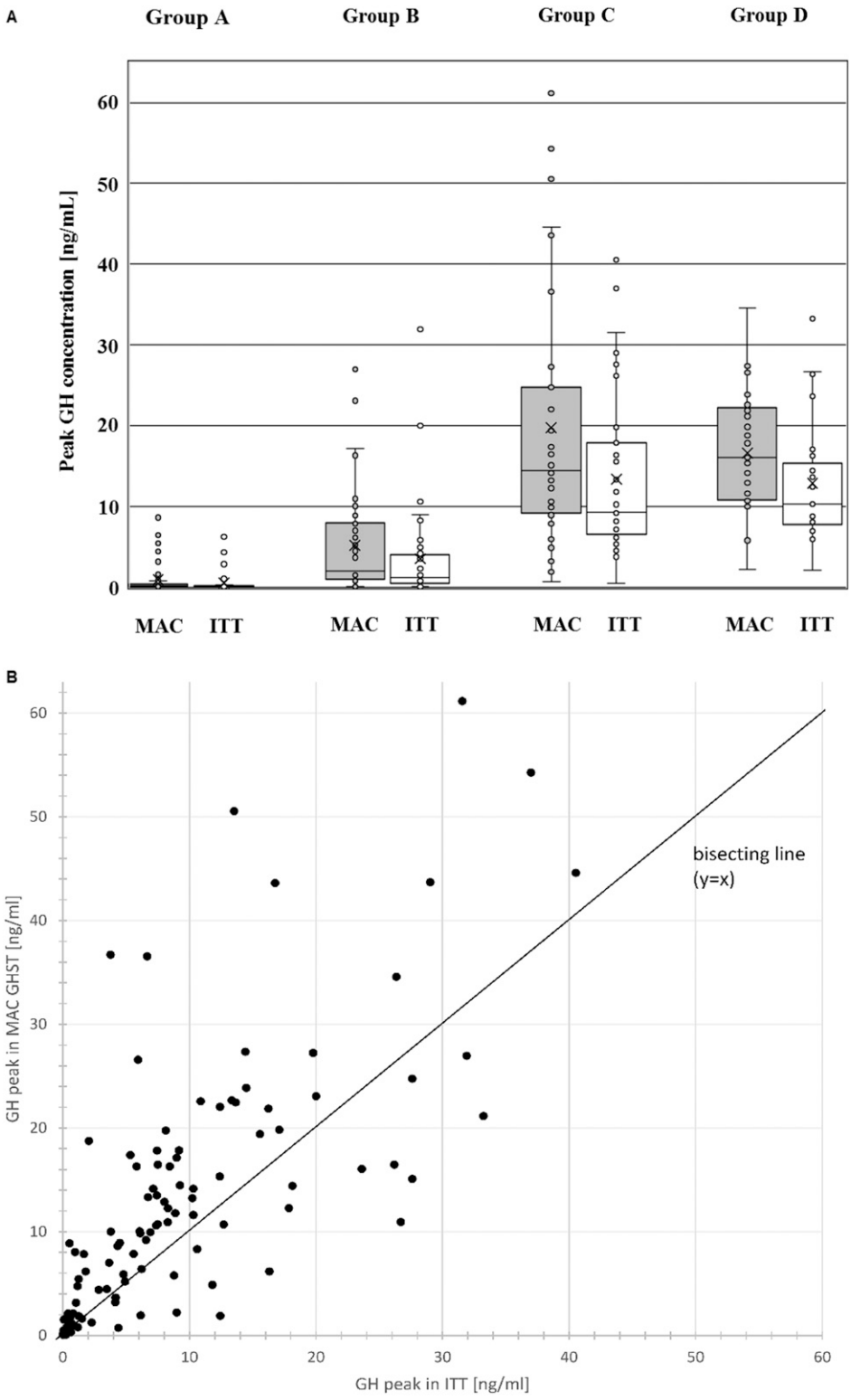

Figure 3. (A) Peak GH concentrations in macimorelin (MAC) stimulation test and ITT stratified by AGHD likelihood category (n = 139). The bottom and top of the boxes represent the first and third quartiles. The band inside the box is the median. The cross represents the mean, and the circles, the individual values. The whiskers are the lowest and highest data points within the 1.5 interquartile range of the lower and upper quartiles. (B) Scatterplot showing individual subjects' peak GH concentrations for both the MAC test and the ITT (n = 139). Most of the dots above the bisecting line (y = x) demonstrate the greater stimulation potential of the MAC test compared with the ITT. The solid line represents the bisecting line. Regression equation: y = 1.0694x + 2.5216; r^2 = 0.6.

and because it requires resources that might not be available in some settings (20). GHRH plus arginine was an alternative to ITT until 2008 when Geref® (EMD Serono; Rockland, MA), the only approved GHRH analog in the United States, was removed from the market, although it remains available in other countries (14). Recognizing the need for an alternative GHST to the ITT, we sought to validate the use of oral macimorelin as a diagnostic test for AGHD.

Acylated ghrelin (21) and agonists of its receptor (22, 23) have been evaluated as diagnostic tests for AGHD; however, none is commercially available in the United States. The ghrelin mimetic macimorelin is a pseudotripeptide with increased oral bioavailability compared with other GHSs (24). Previous studies have shown that a single oral dose will induce a strong dose-dependent increase in GH levels lasting 120 minutes, with peak plasma drug concentrations occurring between 50 and 75 minutes (12, 24).

A previous open-label, crossover, multicenter trial tested the diagnostic accuracy of a single oral dose of macimorelin (0.5 mg/kg) compared with arginine plus GHRH in patients with AGHD and healthy matched controls (13). The peak GH levels were 2.36 ± 5.69 and 17.71 ± 19.11 ng/mL in those with AGHD and healthy controls, respectively ($P < 0.0001$), with an optimal GH cutoff ranging from 2.7 to 5.2 ng/mL measured using a different immunochemiluminometric assay (Esoterix; LabCorp, Cranford, NJ) than the one used in the present study. However, after 43 patients with AGHD and 10 controls were tested, the GHRH analog Geref Diagnostic® (EMD Serono) was removed from the U.S. market, and 10 additional patients with AGHD and 38 controls only underwent testing with macimorelin, limiting the validity of that study (13).

In the present study, we validated the use of single-dose oral macimorelin for AGHD diagnostic testing, using the ITT as the comparator test. Macimorelin induced a robust increase in GH levels in healthy individuals and showed good agreement with the ITT in patients with AGHD with a range of pretest probabilities of having AGHD. The macimorelin test was easy to perform and well-tolerated, because it does not depend on the presence of hypoglycemia and only requires the collection of four venous blood samples after administration. The high repeatability (94%) and estimated sensitivity (92%) and specificity (96%) using a GH cutoff of 5.1 ng/mL were remarkable considering that the repeatability of the ITT has been shown to be 90% in one report (25) and to have a coefficient of variation of 58% in another study (26). The inverse relationship between the peak GH and the likelihood of having AGHD that we found is consistent with the reported data showing that peak GH levels are inversely related to the number of pituitary deficiencies (27, 28).

To minimize the potential for the overdiagnosis of AGHD, we selected *a priori* a cutoff point of 2.8 ng/mL, the low end of the range suggested by the previously available data, despite the use of different GH assays (13). The data from the present study indicate that the optimal cutoff point for macimorelin ranges from 4.6 to 8.1 ng/mL. Using 5.1 ng/mL as the cutoff point resulted in good negative and positive agreement (94% and 82%, respectively), with 92% sensitivity and 96% specificity. Because the measured GH concentrations will depend on the GH assay used, it is important to remember that our data are based on a recommended GH cutoff point of 5.1 ng/mL using the IDS-iSYS human GH assay (Immunodiagnostic Systems, Ltd.). This cutoff point is identical to the cutoff point recommended for the ITT, allowing endocrinologists using a different GH assay to apply a cutoff point related to the one used to evaluate ITT results in their local laboratory. Applying a higher GH cutoff point than used for the ITT will increase the sensitivity of macimorelin and lead to greater positive agreement with the ITT, owing to the higher stimulated GH concentrations in the macimorelin GHST compared with the ITT. However, this might also be associated with a greater risk of overdiagnosing AGHD.

The macimorelin GHST was safe and was not associated with frequent AEs or SAEs that would require specific precautions or close monitoring by medical personnel. The most frequently reported side effect was mild and transient dysgeusia. In a previous study, only one drug-related SAE, an asymptomatic QT interval prolongation on the ECG, had resolved spontaneously within 24 hours in an individual taking citalopram, a drug now known to be associated with QT prolongation (29). In the present study, no drug-related SAEs were observed, and no AE related to the QT interval was documented. In general, effects on the QT interval seem to be more pronounced during the ITT than during the macimorelin test. This is in line with a recent report showing QT prolongation in >20% of individuals undergoing an ITT (30).

The safety profile of macimorelin is particularly favorable compared with the ITT, which has potential for inducing severe side effects such as hypoglycemia-related seizure and exacerbation of cardiovascular and cerebrovascular disease. From a clinician's perspective, the macimorelin test is also more convenient, less time-consuming, and less resource-intensive than the ITT. This could increase the likelihood that at risk patients will be offered evaluation of AGHD. Another advantage of the macimorelin GHST is that in some individuals, the ITT will need to be repeated because of inadequate

hypoglycemia, likely due to insulin resistance. In contrast, 99% of the macimorelin tests were evaluable after the first attempt. The macimorelin GHST is also more convenient than other alternative tests such as the glucagon stimulation test, which requires 3 to 4 hours of testing and intramuscular administration, is associated with more side effects (*i.e.,* nausea, vomiting), and has questionable diagnostic accuracy in overweight and obese patients (31).

The present study had some limitations. The present study was relatively small. Also, the macimorelin test might not be an appropriate substitute test for the ITT or other provocative tests in all cases, at least until more data have been accumulated. For example, patients with uncontrolled diabetes, elderly patients, and pediatric patients were not evaluated in the present trial. Thus, further studies are needed in such groups. The results we have presented apply to the specific populations we tested: adults with a history compatible with the presence of AGHD. Also, only a small number of individuals with hypothalamic disease or with a BMI >35 kg/m^2 were included in the present study, limiting the generalizability of our findings to those groups. Owing to the lack of a "standard of truth" to determine the true AGHD status of each participant, it was not possible to measure the true sensitivity and specificity of the tests. The strengths of the study included the use of the ITT as a comparator, enrollment of matched healthy controls, evaluation of patients with a wide range of likelihood to have AGHD, and a state-of-the-art GH assay measured centrally. Future studies should assess patients suspected to have AGHD and amenable to GH replacement who are aged >65 years and have a BMI >40 kg/m^2. Also, the possible interactions between macimorelin and drugs that prolong QT should be further evaluated.

In conclusion, GH stimulation with oral macimorelin is a simple, well-tolerated, reproducible, and safe diagnostic test for AGHD, with accuracy comparable to that of the ITT. Evaluating the test at the same GH cutoff of 5.1 ng/mL used for the ITT limits the risk of a false-positive diagnosis and maintains a high detection rate for the affected patients because of the more potent GH stimulatory effect of macimorelin compared with the ITT.

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