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Establishment of reference values for endocrine tests – part V: acromegaly

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ABSTRACT

Background: Plasma insulin-like growth factor 1 (IGF-1) and the response of growth hormone (GH) to oral glucose are frequently used in the evaluation of patients suspected of acromegaly. Because of the implementation of new assay methodology for GH and IGF-1, we have established the reference values for these tests, as well as for urinary GH excretion.

Methods: From the general population, 50 subjects were recruited, equally distributed according to sex and age between 20 and 70 years. Two consecutive 24-hour urine samples were collected to determine urinary GH. Plasma IGF-1 was measured as well as the GH response during an oral glucose tolerance test (OGTT) with 100 g glucose. Basal plasma IGF-1 was also measured in 250 subjects recruited likewise from the general population who had participated in previous studies on reference values.

Results: The following reference ranges were established: urinary GH <5-46 μU/24 h; nadir GH after OGTT ≤1.5 mU/l for males and ≤2.0 mU/l for females. IGF-1 was divided into age groups: 20-30 years 8-61 nmol/l; 31-40 years 8-41 nmol/l; 41-50 years 7-36 nmol/l; 51-60 years 5-37 nmol/l; and 61-70 years 7-27 nmol/l.

Conclusion: We have established reference values with state-of-the-art assay methodology for the diagnostic tests frequently used in the evaluation of patients suspected of acromegaly.

KEYWORDS

Acromegaly, endocrine tests, reference values

INTRODUCTION

Acromegaly, a rare disease with a prevalence of 40 to 70 cases per million,[1] is usually caused by overproduction of growth hormone (GH) by a pituitary adenoma. The delay between onset of GH overproduction and clear clinical manifestation can be more than ten years.[2]

A random blood sample for measuring GH is not a reliable test for diagnosing acromegaly because GH is secreted in a pulsatile manner; peaks of more than 40 mU/l are followed by episodes of immeasurable GH concentrations. A 24-hour plasma sampling every 15 to 30 minutes is used to determine 24-hour GH production, but this test is time-consuming and costly. GH is secreted in the urine, so determination of GH in a 24-hour urine sample can give a measure of 24-hour GH production as well.[3]

The degree of GH suppression during an oral glucose tolerance test (OGTT) is the traditional test[4] in the biochemical diagnosis of acromegaly, although the OGTT is not 100% sensitive.[5] The serum concentration of insulin-like growth factor I (IGF-1) may reflect overall GH secretion and therefore be a good marker for GH overproduction.[2] Thus, serum IGF-1 is frequently used as a screening test for acromegaly.

In recent years much has changed in immunoassay methodology, also for the determination of GH and IGF-1. Older polyclonal radioimmunoassays or immunoradiometric assays have been replaced by (automated, monoclonal) immunochemiluminometric assays. In addition, for GH, International Reference Preparations (IRP 66/217, IRP 80/505) have been replaced by International Standards (IS 88/624 and IS 98/574). The IRPs were of pituitary origin; the ISs are recombinant DNA-derived human GH. With these changes, the cut-off values reported for GH suppression after a OGTT have changed from 2.5 to 5 μg/l to 0.4 to 1 μg/l.[6]

The consensus[7] for baseline biochemical parameters states that a random GH level of <0.4 μg/l and an IGF-

1 within the age- and gender-matched reference range excludes acromegaly. If either of these levels is not achieved, an OGTT should be performed with subsequent measurements of glucose and GH every 30 minutes over two hours. GH concentration should fall to ≤1 μg/l to exclude acromegaly. In recent years there has been some debate about reducing the nadir GH after OGTT to 0.4 μg/l[8] or even to 0.21 μg/l.[9] Under ideal conditions any assay should be validated with a normal range for suppressed GH levels after an oral glucose load.[10]
In the Netherlands there were discussions on which unit should be used to express GH concentration, i.e. μg/l or mU/l. Although the IS is expressed as μg/l, clinical endocrinologists and the board of the Endocrinology Section of the Dutch Foundation for Quality Assessment in Clinical Laboratories (SKML) have decided to use mU/l, because GH has a heterogeneous molecular composition. To reduce the inter-laboratory variation (especially for the diagnosis of GH deficiency) caused by, among other things, different specificities of the anti-GH antibodies for the different GH isoforms, GH assays have been harmonised in the Netherlands. A harmonisation sample from native serum with an assigned consensus value is used for this purpose.

In the light of these changes, we decided to obtain reference values for GH concentrations after OGTT, urinary GH excretion and IGF-1 in 50 subjects recruited from the general population, not suspected of having acromegaly. Because of the small number of subjects for establishing age- and gender-matched IGF-1 concentrations, we included 250 subjects from previous projects, equally distributed according to sex and age between 20 and 70 years. Reference values for other endocrine tests have previously been published in this journal.[11-14]

SUBJECTS AND METHODS

Subjects

Volunteers were recruited through an advertisement in a local newspaper with a free house-to-house distribution in the Amsterdam region: 104 subjects responded. The respondents were asked by telephone whether they met the inclusion criteria. The inclusion criteria were age between 20 and 69 years and self-reported good health. Exclusion criteria were diabetes mellitus or intravenous drug abuse, recent surgical treatment, hospital admission in the past month, having given birth in the past six months and breastfeeding at the time of the study.
The first 50 volunteers who met the inclusion criteria were invited to our department, where they were interviewed about drinking and smoking habits, current use of medication and their racial background. Females were asked about their periods (first day of the last menstrual cycle). Informed consent was obtained from all subjects and the study was approved by the local hospital's ethics committee.
The 50 subjects were divided into five age groups, ranging from 20-70 years. Each age group consisted of ten subjects (five males and five females). One subject did not complete the tests because of a problem with inserting an indwelling venous catheter. The first volunteer on the backup list meeting the age and sex criteria, replaced this subject. Three subjects were excluded later on, because their test results showed that they had diabetes mellitus. They were not replaced.
The 250 added subjects for establishing age- and gender-specific IGF-1 reference values met the same inclusion and exclusion criteria.

Tests

The tests were performed in the following order:
- 24-hour urinary excretion of GH
- Basal plasma values of IGF-1
- GH response after oral glucose load

Twenty-four hour urinary excretion of GH

On two successive days prior to the OGTT, two 24-hour urine collections were taken in two separate containers. During the collection period the urine was kept cool. Heavy physical exercise was not allowed while the urine was being saved, because it is known that GH production is stimulated by exercise.
The total volume per 24 hours of the two separate urine samples was measured, as well as the concentration of GH, glucose and creatinine. Total creatinine excretion was measured to check whether collection was complete. Some 15 to 20% of the difference in intra-individual creatinine excretion may be due to variations in dietary intake. Since urine collection took place in an outpatient setting, an intra-individual difference of up to 30% was accepted. If total creatinine excretion in the sample with the highest creatinine excretion was more than 150% of the creatinine excretion in the other sample, both samples were excluded.[14]

Basal serum levels of IGF-I

The OGTT was performed the day after the second urine collection. We combined the IGF-I measurement with the OGTT. The subjects were in the postabsorptive state and were not allowed to smoke before or during the test. Body height and weight were obtained in advance. An indwelling venous catheter was inserted (t = -30 min) in an antecubital vein and blood samples were taken at t = -15 min and t = 0 min for measuring GH and glucose (see hereafter). IGF-1 was measured in the t = 0 min blood sample. In the extra 250 subjects, the IGF-1 was measured in a basal sample.

GH response after oral glucose load

After the blood sample at t = 0 min the volunteers drank a solution of 100 g glucose in 200 ml water. After 30, 60 and 90 minutes, respectively, blood samples were obtained for measuring GH and glucose. The subjects were in a sitting position during the test.

Analytical methods

Glucose and creatinine were measured according to standard clinical chemical techniques.

IGF-I was measured by a fully automated, two-site chemiluminescent immunoassay (Nichols Advantage®; Nichols Institute Diagnostics, San Clemente, CA, USA). In this system the antibody to the C-terminal 62-70 amino acid sequence is biotinylated for capture and the antibody to the amino sequences of 1-23 and 42-61 is labelled with acridinium ester for detection. Samples are acidified to separate IGF-1 from IGF binding proteins (BP). Then, excess IGF-II is added in the assay to block the IGFBP binding sites from recombining with the released IGF-1. The acidified samples are incubated simultaneously with the biotinylated capture antibody, excess IGF-II, and the acridinium ester labelled tag antibody. During the first incubation, IGF-1 form a sandwich complex with the capture antibody and the acridinium ester labelled antibody in the samples. After the initial incubation period, streptavidin-coated magnetic particles are added to the reaction mixture and a second incubation follows. The streptavidin-coated particles allow for a highly specific and efficient means of binding the sandwich complex to the solid phase via the high-affinity interaction between biotin and streptavidin. Free-labelled antibody is separated from the labelled antibody bound to the magnetic particles by aspiration of the reaction mixture and subsequent washing. The wells containing the washed magnetic particles are transported into the system's luminometer, which automatically injects an acid hydrogen peroxide solution and a sodium hydroxide solution to initiate the chemiluminescence reaction. The light generated by the reaction is directly proportional to the amount of bound-labelled antibody and to the concentration of IGF-1. The assay is calibrated against the World Health Organisation's (WHO) International Reference Reagent 87/518. The inter-assay coefficient of variation (CV) is 6% (at 7 nmol/l) to 4% (at 55 nmol/l); the detection limit is set in our laboratory at 5 nmol/l.

Serum GH was measured using a two-site chemiluminescent immunoassay (Nichols Advantage; Nichols Institute Diagnostics, San Clemente, CA, USA). It utilises one mouse monoclonal antibody and one goat polyclonal antibody to GH. The monoclonal antibody is coupled to biotin, while the goat polyclonal antibody is labelled with an acridinium ester for detection; GH is 'sandwiched' between these antibodies. Separation and quantification is the same as in the IGF-1 assay. The inter-assay CV is 7% (at 2 mU/l) and 6% (at 18 mU/l); detection limit is 0.3 mU/l. The assay is calibrated against the WHO's 2nd International Standard 98/574. By definition 1 µg of this standard corresponds with 3 mU.[15] To achieve the assigned consensus value of the harmonisation sample, an additional multiplication factor of 1.85 is used to convert µg/l to mU/l. This was done for all serum GH values.

Urinary GH was measured by desalinating 2.5 ml of urine using a Sephadex G25 column, after which GH in the eluate was bound to a capture antibody coated to a tube (DiaSorin, MP Products, Amersfoort, the Netherlands) by rotating end over end for 18 hours and after washing followed by an incubation of a radioactive labelled detecting antibody. Standards were prepared from IS 98/574, range 0 to 2000 µU/l. Detection limit is 5 µU/l and inter-assay CV is 8% at 45µU/l and 12% at 15 µU/l.

Statistical methods

Alcohol intake was defined as the intake of at least two units of alcohol a day and smoking as smoking on a daily basis. The postabsorptive serum glucose and serum GH values were calculated as the average of the t = -15 min and t = 0 min values. The lowest GH value from t = 30, t = 60 and t = 90 was used for expressing GH suppression during the OGTT. Results under the detection limit were considered to have the value of 50% of this detection limit. Sex differences and the effect of smoking and alcohol intake were tested using the Mann-Whitney test. The Kruskal-Wallis test was used to evaluate the effects of age. Correlation between variables was tested by linear regression. The computer programme Graphpad Prism 3.0 was used for statistical analysis and composition of the figures. In all tests p values <0.05 were considered as statistically significant. Reference ranges were calculated as mean ± 2 SD if the values were normally distributed. In the case of GH after OGTT the lower and upper limits are given.

RESULTS

Subject characteristics

The 50 subjects had a mean age of 44 ± 14 (SD, range 22 to 67) years, 19 were smokers (38%) and 20 used alcohol (40%). Body mass index (BMI) varied from 20.0 to 32.6 (median 25.1) in males and from 18.6 to 41.2 (median 23.9) in females. Eleven females were postmenopausal. After performing all tests three males (51, 55 and 66 years) with high serum glucose levels were taken out the study.

Urinary excretion of GH

One additional subject (female, age 29 years) was excluded on account of incomplete urine collection (the intra-individual difference in 24-hour creatinine excretion was more than 150%).

In subjects in whom urine collection was adequate, there was no significant difference in urinary excretion of GH between day 1 and 2, and for this reason the mean of the two days was used to determine the reference values. There were no significant effects of sex, smoking, alcohol, age or BMI on urinary excretion of GH in the included subjects. The mean was 23 μU/24 hour (range <5 to 46). There was no correlation between mean plasma GH concentration or nadir GH concentration and urinary GH excretion. The reference values are given in *table 1* and the distribution in *figure 1*.

Basal plasma levels of IGF-1

We found no sex differences in IGF-1 concentrations between males and females in our population (p=0.10); therefore, the males and females were analysed together in the age-groups. IGF-1 showed a significant correlation with age (p<0.001), with a decline with increasing age (*figure 2*). There was no correlation between mean basal plasma GH concentration, nadir GH concentration after oral glucose or urinary GH excretion and plasma IGF-1. The reference values are given in *table 1*.

Basal plasma levels of GH and GH response in OGTT

There was a significant difference between basal GH levels of males and females (p=0.002); the median for males was 2.1 (range <0.3 to 17 mU/l) and for females 6.8 (range 0.3 to 41 mU/l). The distribution of the GH values was non-Gaussian and could not be normalised after logarithmic transformation (*figure 3*).

During the OGTT 42 out of 47 subjects showed a decrease in GH. In four volunteers the GH concentration was undetectable at all time points and one subject showed a rise from 0.3 mU/l to 0.4 mU/l. Nadir GH was reached at 30 min in 24% of the subjects, at 60 min in 18% and after 90 min in 47%. In ten subjects there was a rise in GH concentration after the nadir. Males had a lower GH concentration after glucose (median <0.3; observed range <0.3 to 1.5 mU/l) than females (median 0.8; observed range <0.3 to 2 mU/l); p=0.01. No effects were seen in relation to age, BMI, alcohol intake or smoking. Also here no normalisation of the distribution could be achieved after logarithmic transformation. The results are depicted in *figure 4*.

DISCUSSION

In the biochemical diagnostic work-up for the diagnosis of acromegaly several tests are in use. The lack of widely available reference ranges for urinary GH, in addition to the difficulty in obtaining reliable 24-hour collection of urine samples, precludes this measurement in the routine diagnosis and follow-up of acromegaly.[2] Lack of information

Table 1. *Reference values for diagnostic tests in the evaluation of acromegaly*

	Mean	Reference interval*
Urinary GH	23 μU/24 hour	< 5 – 46 μU/24 hour
IGF-1 (age groups):		
• 20-30 years	34 nmol/l	8-61 nmol/l
• 31-40 years	25 nmol/l	8-41 nmol/l
• 41-50 years	22 nmol/l	7-36 nmol/l
• 51-60 years	21 nmol/l	5-37 nmol/l
• 61-70 years	17 nmol/l	7-27 nmol/l
GH after OGTT		
Male		≤1.5 mU/l (≤0.27 μg/l)
Female		≤2.0 mU/l (≤0.34 μg/l)

Reference interval: mean ± 2 SD, except for growth hormone (GH) after oral glucose tolerance test (OGTT).

Figure 1. *24-hour urinary GH concentrations in 46 healthy volunteers; mean of two 24-hour collections*

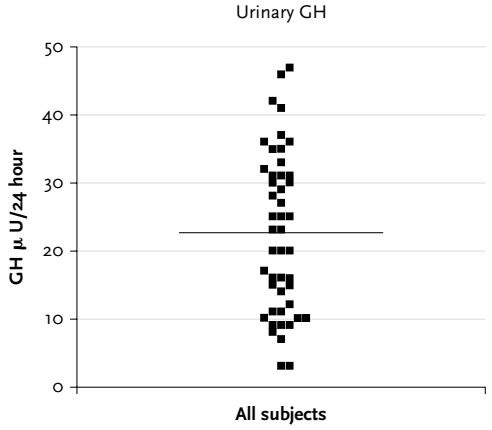


Figure 2. *Plasma IGF-1 concentrations in 297 healthy volunteers according to age groups*

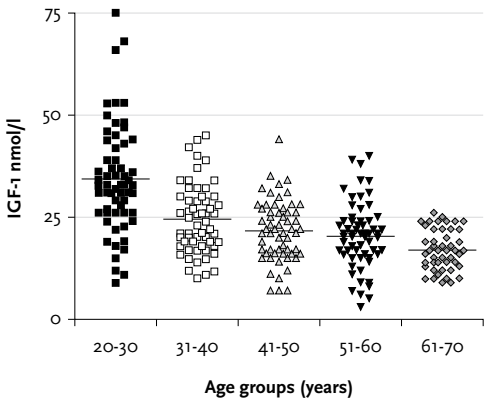
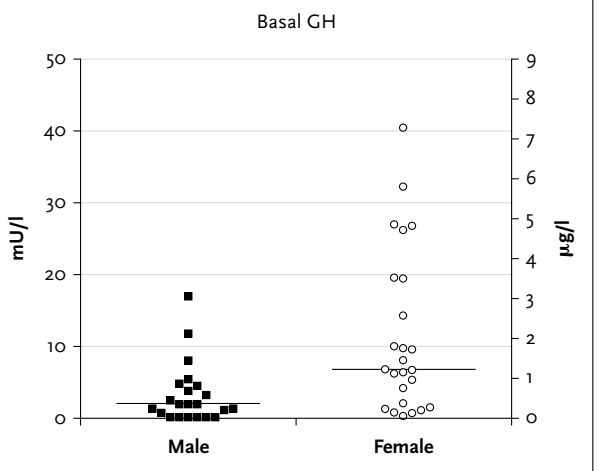
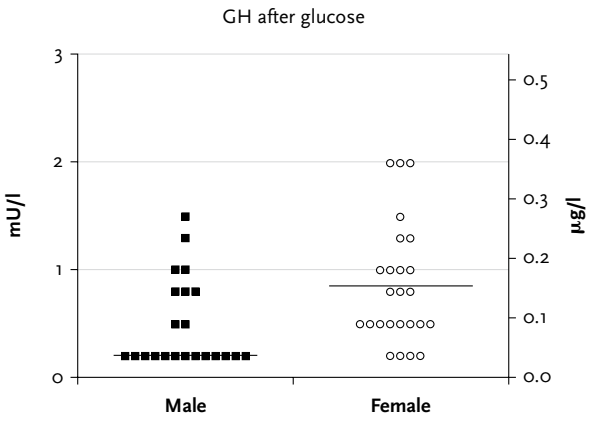


Figure 3. *Basal plasma GH concentrations in 22 male and 25 female healthy volunteers*



Conversion factor from µg/l to mU/l: 5.55.

Figure 4. *GH response on 100 g glucose in 22 male and 25 female healthy volunteers*



Conversion factor from µg/l to mU/l: 5.55.

on the standards used[16,17] and use of different standards have not been beneficial either for the establishment of uniform reference ranges. Our reference values are in agreement with a Danish study of 112 healthy adults, in which the upper reference limit was 49 µU/24 hours (using a conversion factor of 2.44 for ng to µU)[18] *vs* 46 µU/24 hour in our study. We did not find a sex difference in excretion of urinary growth hormone in contrast to other studies.[18,19] Whereas in some studies an age-related decline was observed in urinary GH excretion,[19,20] we did not found this relation. In acromegaly patients a (log) urinary GH and (log) serum GH correlation is found,[18,20] but not in healthy subjects; we did not find this correlation either. This discrepancy between patients and healthy subjects may reflect the large variation in urinary GH excretion in healthy controls as compared with the smaller variation in serum GH profiles.[18] This might be the explanation for us not finding a difference in urinary GH between males and females, while basal serum GH in females is higher than males. With our assays there was no relation between serum IGF-1 and urinary GH.

In recent years more robust assays have been developed to measure IGF-1. Since the world-wide calibration on WHO International Reference Reagent 87/518, published reference ranges are more in agreement with each other. The reference ranges established in this study are in a similar range to those of Brabant *et al.*,[21] who used the same methodology, although our upper limit of the reference range in all age groups is ± 5 nmol/l lower. In a recent study by Ranke *et al.*, comparing four immunoassays for IGF-1,[22] the measured reference range on the Advantage system is in agreement with our values. We did not find a gender difference in IGF-1 levels as other studies.[23] Before puberty, girls have significantly higher IGF-1 levels than boys, but after the age of 20 the values are more or less the same with a tendency of higher levels in males than in females.[21] Therefore, a reference range in age groups is more opportune than gender-related reference ranges.[23]

With the introduction of the WHO IS 98/574, based on recombinant DNA-derived GH, standardisation of GH assays has taken a tremendous step forward. Progress is, however, hindered by the discussion whether to express GH in mass units or arbitrary (activity) units. The supporters of mass units claim that the IS is derived by recombinant technology, so mass can be measured. The opponents say that GH in blood is not solely the 22 kd variant of GH and that different antibodies will not react in the same way with the other GH isoforms. This holds true in our case, where an additional multiplication factor of 1.85 (above the factor 3 for conversion from µg/l to mU/l) must be used. The endocrinologists in the Netherlands have chosen to use mU/l, so laboratories have agreed to harmonise their GH assays to an assigned consensus value of a native serum with a GH concentration of 17.5 mU/l. Also in a Belgian study such a harmonisation factor is proposed.[24]
In our study we saw an upper limit of nadir GH after OGTT of 1.5 mU/l in male and 2.0 mU/l in female volunteers. Of the male subjects, 56% reached undetectable GH concentrations *vs* 18% of the females. In the study by Freda *et al.* in 46 healthy volunteers (26 males, 20 females, age range 20-71 years) all had nadir GH after OGTT <0.14 µg/l;[25] in our situation this means <0.8 mU/l. They found no sex differences. In our study 7 male and 13 female

volunteers were above this level; we cannot explain this difference, other then perhaps the differences in antibodies used in the DSL kit *vs* the Advantage system. Chapman *et al*. studied only nine males and six females (age 21-34) and found a significantly higher nadir GH after OGTT in females than in males, with a mean of 0.25 µg/l *vs* 0.03 µg/l, and an upper limit of 0.72 µg/l *vs* 0.07 µg/l.[26] Other studies have set the cut-off level between disease and non-disease at 1 µg/l or 2 mU/l.[3,27,28] Looking at clinical studies, some report all acromegaly patients having GH after OGTT >2 mU/l,[28] or even >3 mU/l,[16] while Freda *et al*. found 5 out of 15 patients with a nadir GH between 0.4 and 1.0 µg/l.[5] Our levels of 1.5 mU/l (0.27 µg/l) for males and 2.0 mU/l (0.34 µg/l) for females are lower than that cut-off point, but still higher than in their healthy volunteers. On the other hand IGF-1 and perhaps urinary GH will add additional information, besides the clinical suspicion, for the diagnosis of acromegaly.

CONCLUSION

Urinary GH, IGF-1 and nadir GH after OGTT are used for the biochemical diagnosis of acromegaly. In our study we have established reference values for these tests.

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