Effect of Gender and Age on Fasting Serum Growth Hormone Levels in Normal Subjects

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Summary

Fasting growth hormone (GH) level is an important reference level in dynamic tests of GH secretion. Other studies have demonstrated sex and age variation in the rate of GH secretion. We analysed fasting serum samples from 377 normal subjects (193 males and 184 females, age range 6 to 81 years old), using our in-house enzyme immunoassay. We found sex differences in fasting GH levels to be only significant in the prepubertal children (Tanner stage I), being higher in girls than in age-matched boys ($p<0.05$). Both sexes showed age-dependent changes in fasting GH levels ($p<0.001$); highest levels were achieved at puberty and subsequently declined with advancing age. Hence, the physiological sex difference and age-dependency in GH secretion can also be demonstrated in single fasting samples.

Key words: Growth hormone, ELISA.

Introduction

Growth hormone (GH) is secreted episodically, with low or undetectable daytime levels, while peak secretions occur during the early hours of the night following onset of deep sleep[1,2]. Results from physiological assessments of 12 or 24 hour integrated or spontaneous GH secretion in children or adults have shown that the GH secretion rate is age related, being highest during the periods of puberty and adolescence[3-6]. Rose *et al*[7] reported that GH spontaneous output in boys and girls differed significantly according to their pubertal development, whilst others[8,9] reported no sex difference in GH secretion. Similarly, in adults, spontaneous GH secretion has been shown to decline with age.

During provocative or suppression tests of GH secretion, the basal fasting level is also an important reference value besides the cut-off GH value used to distinguish GH-deficiency or GH-excess. The availability of various methods of determining serum GH concentration has, however, resulted in a number of problems, as these values can vary according to the type of assay used[12,13], causing discordant interpretations of results among centres[14].

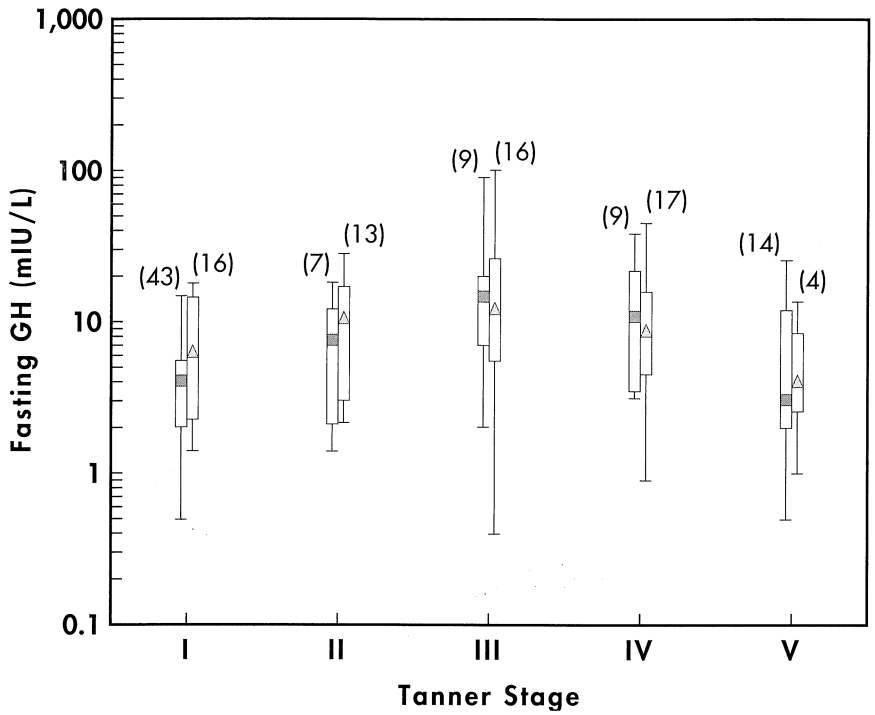

Fig 1: Sex dependent fasting GH levels of boys (□) and girls (△) in different Tanner stages (according to scrotum/breast development. Boxes and vertical bars represent inter-quartile ranges and 95% confidence limits respectively.

In this study, we used our own in-house enzyme-linked immunoabsorbent assay (ELISA) to measure fasting GH levels in a group of normal subjects, and examined the possible effect of gender, pubertal development and age of subjects on these levels.

Materials and Methods

Subjects

A total of 377 clinically normal, healthy subjects were studied. The children were classified according to their pubertal Tanner stage; Tanner stage I (43 males and 31 females, mean age 9.1±1.7 years and 8.5±1.5 years respectively), Tanner stage II (7 males and 13 females, mean age 10.8±1.2 years and 10.9±1.0 respectively), Tanner stage III (9 males and 16 females, mean age 13.2±1.1 years and 12.1±1.6 years respectively), Tanner stage IV (9 males and 17 females, mean age 13.9±1.0 years and 14.6±2.4 years respectively) and Tanner stage V (14 males and 4 females, mean age 15.5±1.7 years and 16.2±1.7 years respectively). There were also adults aged 20 years (27 males and 27 females) and aged 21 to 81 years (84 males and 76 females). Informed consent was obtained from subjects, as well as from parents in the case of children.

Protocol

After an overnight 10 to 12 hour fast, about 5 ml of blood was obtained from the forearm vein between 8 am and 9 am. Subjects were rested for at least 30 minutes prior to blood collection. Sera were aliquoted accordingly and immediately stored at -20°C until assayed. The heights and weights of the children were also recorded.

Hormone assay

Samples were assayed in duplicate by an in-house GH ELISA[15] using 96-well microtitre plate coated with rabbit polyclonal anti-GH. Cross-reaction of our polyclonal anti-GH with prolactin or human placental

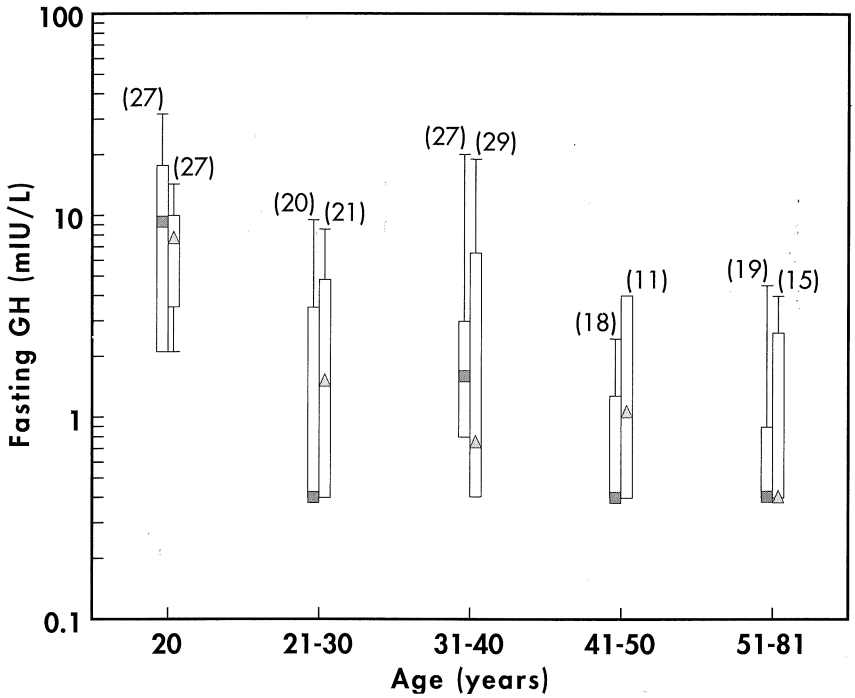

Fig 2: Sex dependent fasting GH levels in adult males (□) and females (△). Boxes and vertical bars represent inter-quartile ranges and 95% confidence limits respectively.

lactogen was only 0.2%. Monoclonal anti-GH was purchased from Chemicon, while HRP-labelled anti-mouse was obtained from Biorad. GH reference standard was I.S. 80/505. All undetectable levels were taken to be equal to 0.4 mIU/L, the detection limit of the assay. Intra-assay coefficient of variations (CVs) at concentrations 3.4 mIU/L, 11.8 mIU/L, 19.1 mIU/L and 55.7 mIU/L were 6.2%, 4.8%, 5.3% and 7.9% respectively. All samples from a particular group were analysed in a single assay.

Data analysis

All assay results were analysed using the LKB-WALLAC RIACALC programme. Comparisons between groups were made by non-parametric analyses, namely Kruskal Wallis and Wilcoxon rank sum tests using the Statistical Analysis System (SAS) software. Correlations between fasting GH levels and heights were assessed by Spearman's rank correlation. Statistical significance was accepted at $p<0.05$.

Results

The median, inter-quartile ranges and 95% confidence limits of fasting GH levels measured in children (at different Tanner stages) is graphically presented in Fig 1. Significant sex difference in fasting GH levels was found only in the prepubertal group. Prepubertal boys had significantly lower fasting GH (median=4.0 mIU/L, inter-quartile range=2.1-6.2 mIU/L, n=43, $p<0.05$) than females (median=7.1mIU/L, inter-quartile range=2.5-13.2 mIU/L, n=31). However, sex difference was no longer significant in the pubertal and adult groups (Figs 1 and 2 respectively).

To study the effects of age on fasting GH levels within the same sex, data were analysed according to the pubertal status of the subjects; prepubertals (Tanner I), pubertal (Tanner II, III and IV) and adults (21 to 44 and 44 to 81 years old) (Fig 3). Fasting GH levels were highly correlated to age ($p<0.001$). For both

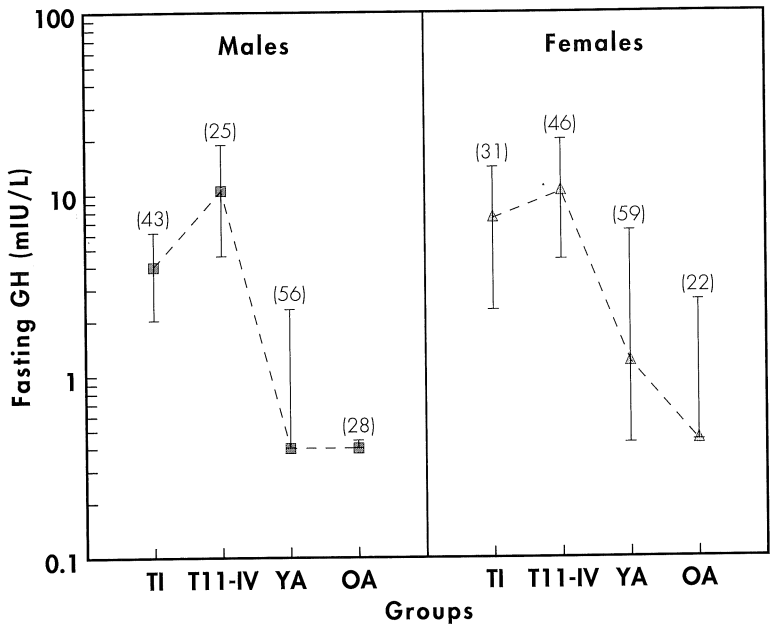

Fig 3: Age-dependent fasting GH levels in males (□) and females (△) at prepuberty (TI), puberty (TII-IV), young adults (YA) and old adults (OA). Vertical bars represent inter-quartile ranges.

** *p<0.001 TI vs respective TII-IV.*

* *p=0.02 TI vs respective TII-IV.*

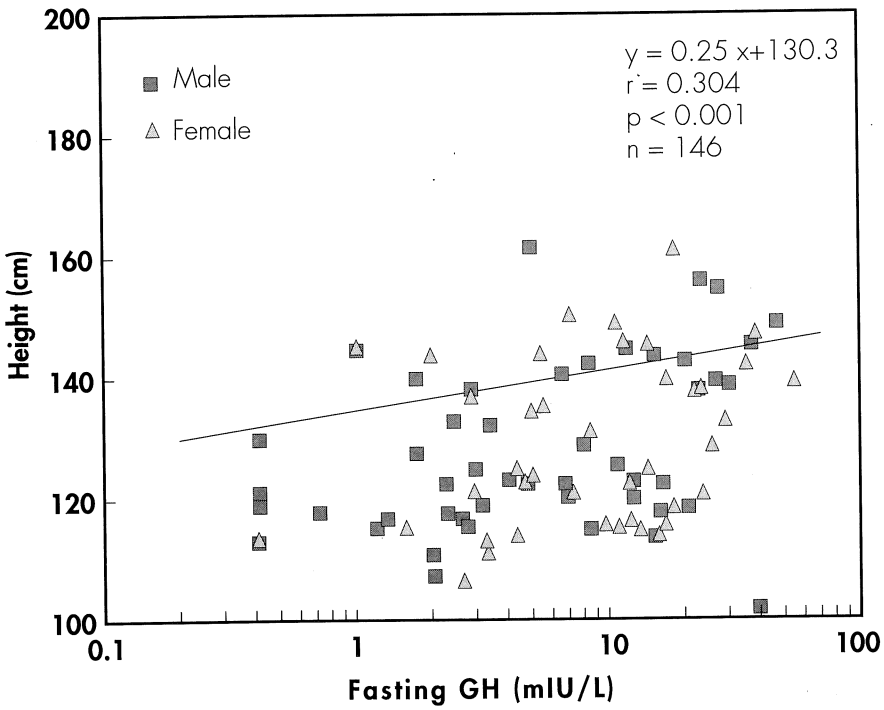

Fig 4: Correlation of fasting GH levels with heights in children of Tanner stages I to IV.

sexes, fasting GH peaked during the pubertal period (p=0.0001 and p=0.02 versus respective pubertal boys and girls) and subsequently declined with age, being lowest in adults aged 45 years and above (p=0.0001 versus prepubertals; p=0.002 and p=0.03 versus young adult males and females respectively). Table I summarises the median and 95% confidence limits of fasting GH levels established in this study for prepubertal and pubertal children, and 2 age groups of adults. Regression analysis showed that the fasting GH levels in children of Tanner I to IV correlated weakly but significantly with heights (r=0.304, p<0.001) (Fig 4).

Table I
Median and 95% confidence limits of fasting GH levels in normal subjects

	No.	Median	Fasting GH (mIU/L) 95% confidence limit
Prepubertal			
Male	43	4.0	0.5 - 13.2
Female	31	7.1	1.3 - 17.6[a]
Pubertal			
Tanner II - IV	71	9.8	1.4 - 44.0[b,c]
Tanner V	18	3.6	0.5 - 25.6
Adult			
21 - 44 years	115	1.2	0.4 - 18.2[d]
45 - 81 years	50	0.4	0.4 - 4.0

[a]p=0.02 vs prepubertal males.
[b]p<0.01 vs Tanner V group.
[c]p<0.001 vs prepubertal and young adults (21 - 44 years).
[d]p<0.01 vs old adults (45 - 81 years)

Discussion

Despite the limitations of measurement of GH levels in single fasting samples, we have been able to demonstrate that females in Tanner stage I had significantly higher fasting GH levels than Tanner-matched males. This finding is in agreement with several previous studies on spontaneous GH secretion in children[7] and adults[16-18]. Elevated fasting GH levels in prepubertal girls are probably due to estrogen, as proposed by Ho *et al*[19]. However, this sex difference was not observed in the pubertal children and adults. As shown by Miller *et al*[8] and Martha *et al*[20], GH secretion is enhanced by increased androgen production with advancing pubertal development.

Our study has shown that fasting GH levels are age dependent. Contrary to the findings by Thompson *et al*[21], and Butenandt *et al*[22], in which there was no difference in integrated growth hormone levels in boys at different pubertal stages, our study showed that fasting GH levels in children increased with pubertal development and were highest at Tanner stages I, II and IV. These levels then declined and remained low in adults. Similar observations have been reported by a number of investigators in which measurements of 12 or 24 hour integrated or spontaneous GH secretion were performed[3-6,10,11]. Despite our single sampling method, we could also show that the fasting GH levels correlated significantly to the heights of the children. This is comparable to the report by Albertsson-Wikland *et al*[9], who had carried out a more laborious study of measuring 24 hour GH secretion.

Clinical diagnostic usefulness of fasting GH level measurement still needs careful evaluation. Our observations have clearly demonstrated that the normalcy of a basal GH result should be interpreted with caution, taking into account the sex or pubertal development if the patient is a child, or age if the patient is an adult. As suggested by Whitehead *et al*[23], since there is physiological difference in GH secretion between age groups, it would be more appropriate that the criteria used for the diagnosis of GH deficiency in adults be different from that used for children.

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