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ORIGINAL ARTICLE

Biochemical assessment of disease control in acromegaly: reappraisal of the glucose suppression test in somatostatin analogue (SA) treated patients

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Abstract
Background It is recommended not to measure growth hormone during oral glucose suppression (oral glucose tolerance test) during somatostatin analog treatment in acromegaly. However, we have observed that failure to suppress growth hormone in response to oral glucose tolerance test during somatostatin analog unmasks insufficient disease control and hypothesize that somatostatin analog also induces insufficient growth hormone suppression to mixed meals.
Methods We therefore compared serum growth hormone levels during two mixed meals in patients with controlled insulin-like growth factor-I levels after either surgery alone ($n = 9$) or somatostatin analog treatment ($n = 9$). The patients were unbiasedly matched for gender and insulin-like growth factor-I and studied twice in the following order: (1) during a 6 h growth hormone day curve including two mixed meals and (2) during a 3 h growth hormone profile including 60 min fasting followed by a 2-h oral glucose tolerance test.
Results During the day curve growth hormone levels were elevated in the somatostatin analog group ($P = 0.008$) and growth hormone levels 1 h after each meal declined significantly only in the surgery group ($P = 0.02$). During the oral glucose tolerance test the two groups had similar growth hormone levels prior to the glucose load ($P = 0.6$), whereas a significant 66% suppression was observed after glucose only in the surgery group ($P = 0.001$).
Conclusions (1) Patients controlled by somatostatin analog fail to suppress growth hormone in response to both mixed meals and oral glucose tolerance test (2) This phenomenon is likely to result in elevated serum growth hormone levels during everyday life in somatostatin analog-treated patients, (3) We postulate that measuring growth hormone levels during oral glucose tolerance test is useful to unmask potential somatostatin analog under-treatment in the presence of 'safe' insulin-like growth factor-I levels.

Keywords Acromegaly · GH · IGF-I · Oral glucose suppression test · Somatostatin analog

Introduction

Acromegaly is a disease characterized by excess production of growth hormone (GH) and insulin-like growth factor-I (IGF-I). Acromegaly is in 95% of the cases caused by a benign GH secreting pituitary adenoma, and disease control is important to avoid excess morbidity and mortality [1–3]. Transsphenoidal surgery remains primary treatment, but this is only effective in ≈50% due to the size and location of the adenoma [3, 4]. Medical treatment with somatostatin analogs (SA) suppresses GH secretion and reduces tumor size and is used when surgery is insufficient or unfeasible [3].

Monitoring of serum IGF-I and GH levels is used in the diagnosis and follow-up of the patients, and optimal disease control is defined by IGF-I level in the age and sex-adjusted normal range and a random GH level <1 µg/l [3, 5]. It is also generally accepted that measuring GH levels in combination with an oral glucose tolerance test (OGTT) adds information on disease control after surgical treatment.

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However, the OGTT is not uniformly used to monitor disease control in SA treated patient since it is considered not to offer useful additional information [3, 6, 7]. A high discordance rate between IGF-I levels and glucose-suppressed GH levels after treatment is also recognized [6, 8], which is particularly prevalent after SA treatment and characterized by elevated GH levels [6, 9]. In this regard it is noteworthy that somatostatin also suppresses insulin secretion [10], which may reduce hepatic IGF-I production and thereby "disproportionally" lower serum IGF-I levels [11].

We have previously shown that patients controlled by SA treatment display unsuppressed GH levels during OGTT as compared to surgically controlled patients with similar basal GH levels and serum IGF-I levels [12]. More importantly, the SA treated patients also exhibited a reduced disease-specific health status [12]. These data therefore suggested that measurement of serum GH levels during an OGTT in SA treated patients unmasked insufficient disease control despite normalized serum IGF-I levels. It is plausible that this lack of suppression also applies to post-prandial conditions, which would further strengthen our hypothesis that SA treated patients with controlled IGF-I levels may exhibit unrecognized residual disease activity.

In the present study, we therefore compared two modes of assessing GH status in the same acromegalic patients. The first mode was a GH day curve obtained in combination with the intake of two mixed meals, whereas the second was performed in combination with an OGTT. We investigated patients controlled by either surgery-alone or by ongoing SA treatment.

Patients and methods

Patients

All patients were receiving standard treatment and care at the Department of Endocrinology and Internal Medicine at Aarhus University Hospital. We retrospectively identified records from patients diagnosed with acromegaly after the age of 18 years and subsequently treated with either trans-sphenoidal surgery alone or SA, who underwent regular assessment of disease control before and after September 2005. At this time point the department changed standard procedure from a 6 h 'GH day curve' including the response to two mixed meals, to an 'OGTT' including a 1 h fasting period followed by a 2 h OGTT. The patients of interest also had to be in biochemical remission for at least 6 months prior to the GH day curve, and prior or ongoing treatment with pegvisomant was not allowed. The definition of biochemical control was (1) serum IGF-I concentrations in the age and sex-adjusted normal range and (2) a random GH measurement less than 1 µg/l. Furthermore, to be included in the analysis no change in SA dose or any disease-specific treatment was allowed from 6 months prior to the GH day curve and until completion of the OGTT.

Complete biochemical records were retrievable from 52 patients of whom 12 were excluded from the analysis due to changes in SA dose ($n = 7$) or second surgery ($n = 5$). Seven additional patients were excluded due to inadequate disease control ($n = 5$), development of severe intercurrent illness ($n = 1$), or start of anti-estrogen therapy ($n = 1$), respectively. Thus, 33 patients were eligible to participate in the analysis of whom 23 were treated with surgery alone and 10 were on ongoing SA treatment. We furthermore strived for identical serum IGF-I levels in the two groups and therefore performed an unbiased pairwise matching based on serum IGF-I levels. We used the R command *find.matches* of the statistical package *Hmisc* (R version 3.1.3, R Foundation for Statistical Computing, Vienna, Austria, with Bioconductor 3.0) to provide the closest match based on IGF- I levels (tolerance set to 25) and gender. Five males and four females in each group fulfilled these criteria and were ultimately included in the analysis.

Methods

The GH day curve was performed after an overnight fast at the hospital, where the first blood sample was collected at 8 h, directly followed by continental breakfast. Blood samples were drawn every hour from 8–14 h, and a second meal was served at 12 h consisting of meat, potatoes and vegetables followed by a dessert. The OGTT was also performed after an overnight fast at the hospital and blood was sampled between 8 and 11 h with 10 min intervals during the first hour ($t = -60$ min to $t = 0$ min), followed by an oral glucose load (75 g) ($t = 0$) and sampling at $t = 30$, 45, 60, 90, and 120 min.

On the two study days, measurements of serum IGF-I and HbA1c were also obtained in addition to frequent glucose measurements during the OGTT. Serum GH was measured by a DELFIA assay (PerkinElmer, Turku, Finland), and serum IGF-I was measured by an in-house noncompetitive, time-resolved immunofluorometric assay [13].

Statistical analysis

Repeated measurements (GH concentrations in serum and blood glucose levels during the OGTT) were compared using a multivariate test (Stata) allowing for heterogeneity. During the OGTT we tested for interaction between group (SA vs. surgery-only) and time. Assumptions were checked by QQ-plot of the residuals, assessment of equal covariance's and by scrutinizing box-plots with respect to

outliers. For all other variables normality was assessed by QQ-plots of absolute or log-transformed values, and box-plots scrutinized for outliers. Student's *t*-test (given as mean ± SD or, if for transformed values, as geometric mean ± CI) or Mann–Whitney U-test (median with range) and their paired equivalent were used as appropriate. When dealing with paired data additional assumptions were checked by Bland-Altman plots and scatter plots. Comparisons of nominal variables were performed using Fisher's exact test. Bivariate associations of continuous variables were tested using Pearson's coefficient of correlation or Spearman's rank correlation coefficient. A P-value < 0.05 was considered significant. Statistical computations were performed using Stata Statistical Software: Release 12.1 (College Station, TX: StataCorp LP).

Results

The SA group and the surgery-only groups were comparable at the time of diagnosis as regards age and sex and pertinent markers of disease (Table 1). However, the distribution of macroadenomas (maximal diameter >10 mm), microadenomas (<10 mm) and unknown tumor size was marginally different ($P = 0.047$) and characterized by the absence of microadenomas in the SA group, which on the other hand contained all three cases with an unknown tumor size. In the surgery-only group, the mean period of time since surgery was 6.5 years.

As predefined the two groups had comparable and normalized IGF- I levels at the time of the GH day curve and the mean age was not significantly different (Table 1). The corresponding IGF-I expressed as standard deviation scores ($IGF\text{-}I_{SDS}$) for age were 1.2 (CI: 0.8–1.7) vs. 1.1 (CI: 0.6–1.5) in the SA group and surgery group, respectively ($P = 0.5$). One patient in the SA-treated group received radiation therapy. None of the patients received dopamine agonist treatment. Overall, there was no difference as regards anterior pituitary function between the two groups. The prevalence of diagnosed diabetes mellitus was similar (Table 1) and no patients received insulin treatment.

Table 1 Patient characteristics

	SA treatment (9)	Surgery (9)	*P*-value
Time of diagnosis			
Age (years)	52 (31–65)	49 (29–68)	0.8
Sex (M/F)	5/4	5/4	1.0
Adenoma size			0.047
Macro	6	6	
Micro	0	3	
Unknown	3	0	
IGF-I (μg/l)	775 (534–1125)	844 (709–1005)	0.6
GH, nadir (μg/l)	8.50 (2.58–28.0)	9.81 (6.18–15.8)	0.8
Study start			
Age (years)	60 (39–80)	54 (34–75)	0.4
IGF-I (μg/l)	185 (157–213)	179 (147–212)	0.14
$IGF\text{-}I_{SDS}$	1.23 (0.78–1.68)	1.05 (0.61–1.49)	0.5
Surgery (y/n)	6/3	9/0	0.2
Radiation therapy (y/n)	0/9	0/9	1.0
Dopamine agonist (y/n)	0/9	0/9	NA
Diabetes mellitus (y/n)	1/8	1/8	1.0
Deficiency (y/n)	1/8	3/6	0.6

Serum GH levels during the GH day curve

The average GH levels were elevated in the SA group ($P = 0.008$), but the interaction with time from $t = 0$ to $t = 360$ min as assessed by ANOVA did not significantly differ between the two group during the GH day curve ($P = 0.2$) (Fig. 1a). The GHnadir:$IGF\text{-}I_{SDS}$ ratio during the GH day curve was also significantly elevated in the SA group ($P = 0.02$). The effect of each meal was expected *à priori* to occur 1 h postprandially, wherefore we compared the change in serum GH levels (μg/l) within the first hour following each meal, and recorded a significant decline in the surgery group after both the first meal [$P = 0.008$] and the second meal ($P = 0.02$) but not in the SA group ($P > 0.7$). All patients in the surgery group suppressed GH < 1 μg/l as opposed to 6 out of 9 patients in the SA group.

Serum GH levels during the OGTT

Serum GH levels were comparable during the first 60 min ($P = 0.6$), but a significant interaction between time and group was recorded after the oral glucose ($P = 0.01$), which also translated into a significant decline in GH levels after OGTT in the surgery group compared to the SA treated group ($P = 0.001$) (Fig. 1b). The mean (CI) GHnadir level (μg/l) in the surgery group was 0.25 (0.13–0.49) as compared to 0.99 (0.66–1.49) in the SA group ($P = 0.001$). This difference between the 2 groups became even more pronounced when expressed as % change from baseline values ($P = 0.0008$). Eight out of nine patients in the surgery group suppressed GH < 1 μg/l as opposed to five out of nine patients in the SA group. The GHnadir:$IGF\text{-}I_{SDS}$ ratio was also significantly elevated in the SA group ($P = 0.02$). The median (range) time to reach GHnadir (min after glucose load) was not different between the two groups (60 [30–120] (SA) vs. 90 [45–120)] surgery), $P = 0.6$).

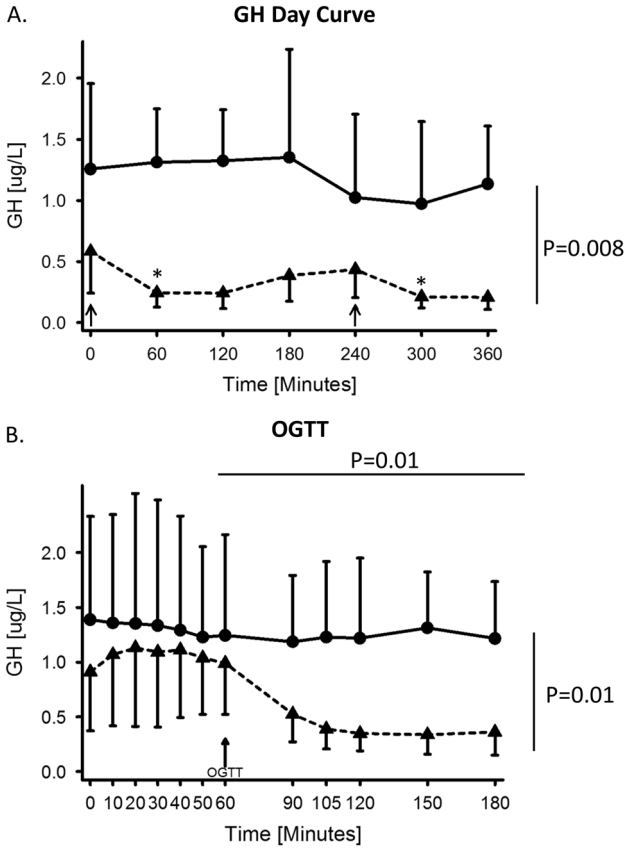

Fig. 1 Mean ± CI serum GH levels in acromegalic patients controlled by either SA patients (*circles* and *solid lines*) or surgery (*triangles* and *punctuated lines*) during a GH day curve (**a**) and during an OGTT (**b**). Two mixed meals were served as indicated by *arrows*. *Asterisks* indicate a significant decline in the surgery group at both the first meal and the second meal (**a**). The oral glucose load was administered as indicated by an *arrow* (**b**)

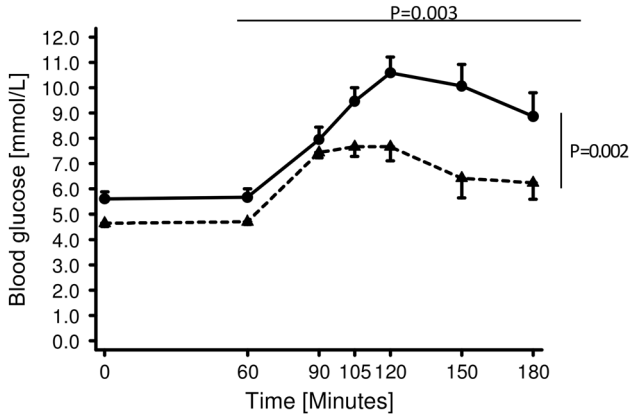

Fig. 2 Mean ± SE serum glucose levels in acromegalic patients controlled by either SA patients (*circles* and *solid lines*) or Surgery (*triangles* and *punctuated lines*) during the OGTT; the glucose load was administered at $t = 60$ min

Serum glucose levels during the OGTT

After the oral glucose load, we observed a significant interaction between time and group ($P = 0.003$) compatible with overall higher levels in the SA group when expressed as area under the curve ($P = 0.002$) (Fig. 2).

Discussion

The present study allowed a comparison between serum GH levels obtained in response to mixed meals with those obtained after an oral glucose load in two groups of patients with acromegaly controlled by either surgery-alone or SA treatment. Our hypothesis was that SA treated patients fail to suppress serum GH levels both during mixed meals and during OGTT. We did record that serum GH levels during SA treatment remained unsuppressed after mixed meals as well as an OGTT, which could imply residual disease activity in SA treated patients despite normalized IGF-I levels.

The observed failure to suppress serum GH levels during OGTT in SA treated patients as compared to patients controlled by surgery is in accordance with previous studies [6, 12], whereas failure of SA treated patients to suppress GH levels in response to mixed meals has not previously been reported. Since measurements of serum GH levels during mixed meals reflect everyday life circumstances, we believe that our data add important information and supports the notion that disease control in SA treated patients may not be adequately assessed by random GH levels and 'safe' or normalized IGF-I levels.

Several studies have recorded discordant IGF-I and GH levels during SA treatment [6–8, 12, 14]. This includes a study observing a high prevalence of elevated GH levels in the presence of normalized IGF-I levels, where it was concluded that the use of the OGTT provides no advantage for assessment of disease status compared with measuring basal GH measurements (6). This conclusion, however, was mainly based on the assumption that serum IGF-I levels adequately reflect disease activity during SA treatment, which may not be valid. Somatostatin suppresses the secretion of insulin [10] which, in turn, reduces hepatic IGF-I production also in humans [10], and data from rodents show that somatostatin directly suppresses the hepatic IGF-I production [15]. It is also well known from mice models that circulating IGF-I levels mainly derive from the liver, but at the same time it is recognized that the overall effects of GH only to a limited extent depend on circulating IGF-I levels [16]. Even though a relatively close positive correlation generally exists between serum levels of IGF-I and GH both in healthy subjects and patients with active acromegaly, it is well known that certain conditions such as fasting, oral estrogen treatment and poorly

controlled type 1 diabetes are associated with low IGF-I levels and elevated GH levels, the latter of which may promote extrahepatic GH effects that are independent of circulating IGF-I [17]. A similar discordance may also apply to SA treated patients with acromegaly as previously suggested [18]. Moreover, we have previously observed that SA treated patients with normalized IGF-I levels exhibit impaired disease-specific quality of health as compared to patients treated with surgery [12]. On the other hand, we have also reported that somatostatin may impact on GH signaling in peripheral tissues [19], which makes it plausible that SA treatment may protect against elevations in ambient GH levels.

It is important to emphasize that our data should not argue against the usefulness of SA treatment in acromegaly, but they open the possibility that a proportion of SA treated patients may require a higher dose targeted against a normalization of GH rather than IGF-I. Such an approach may also apply to treatment with pasireotide, a novel SA, which potently suppresses the secretion of both GH and insulin [20]. However, the usefulness of targeting GH levels during OGTT vs. IGF-I in SA treated patients should ideally be tested in a randomized therapeutic trial.

In accordance with previous data (12) we recorded higher glucose levels during the OGTT in SA treated patients, but it is uncertain whether this is of overt clinical significance [21].

Our data have limitations. First, it is a retrospective survey based on retrievable medical records. Second, even though the two groups were comparable at time of diagnoses, the SA-treated patients were selected on the basis of either failed surgery or ineligibility for surgery, which may indicate more severe disease and co-morbidity. However, the two groups were carefully and unbiasedly matched for gender and IGF-I levels.

In conclusion, normalized serum IGF-I levels in patients with acromegaly post treatment are associated suppression of GH levels after both OGTT and mixed meals in patients treated with surgery alone, but not in SA treated patients. We speculate that GH levels measured after an OGTT may aid to unmask a difference in disease control between patients treated with SA vs. surgery. Based also on previous data, we argue that this could be clinically relevant and suggest that biochemical assessment of SA treatment includes GH measurements during an OGTT.

Compliance with ethical standards

Conflict of interest Jens Otto L. Jorgensen has received consultant fees and unrestricted research grants from Novartis, IPSEN and Pfizer. The other authors have nothing to declare.

Ethical approval All data included in this retrospective study derive from routine clinical procedures at our department and was performed in accordance with the ethical standards of the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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