Normal spontaneous and stimulated GH levels despite decreased IGF-I concentrations in cystic fibrosis patients

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Abstract

Objective: The aim of the present study was to investigate whether patients with cystic fibrosis (CF) are GH resistant with increased GH release and decreased concentrations of IGF-I as a result of malabsorption, increased catabolism and impaired glucose tolerance.

Design: Twenty CF patients were included, ten with normal glucose tolerance (five male, five female, median age 25.5 years (range 20–31)) and ten with diabetes mellitus (five male, five female, median age 25.3 years (range 17–45)). Twenty healthy individuals served as controls (ten male, ten female, median age 28.4 years (range 18–36)).

Methods: GH status was evaluated by 12 h spontaneous GH release during the night time, arginine-stimulated GH release and the basal concentrations of IGF-I and insulin-like growth factor-binding protein-3 (IGFBP-3). Twelve hour spontaneous GH profiles were estimated using a constant blood withdrawal technique with sampling every 30 min and the Pulsar method was used for the analysis of profiles.

Results: No significant differences were found in spontaneous and stimulated GH release in CF patients compared with healthy controls, whereas IGF-I and IGFBP-3 were significantly decreased in CF patients compared with healthy controls. The combination of reduced IGF-I and IGFBP-3 with normal GH release points to a relative GH resistance or a disturbance in the pituitary axis in patients with CF. The spontaneous GH release, the stimulated GH release and the basal concentrations of IGF-I and IGFBP-3 were not significantly different in diabetic CF patients compared with CF patients with normal glucose tolerance and the presence of diabetes mellitus was not consistent with increased GH resistance in CF patients.

Conclusion: CF patients with normal glucose tolerance and diabetic CF patients had normal GH release and decreased concentrations of IGF-I indicating a relative GH resistance.

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Introduction

Patients with cystic fibrosis (CF) suffer from several different symptoms, among which pulmonary infections and malabsorption are the most prominent (1). Younger patients can usually be well controlled with intensive antibiotic treatment and pancreatic enzyme substitution (2). However, with increasing age CF patients often develop a catabolic condition with decline in body mass index (BMI), chronic pulmonary infection and impaired glucose metabolism (3). A delay in sexual maturation followed by a delay in the pubertal increment in several anabolic hormones such as sex hormones and growth hormone (GH) may also contribute to an increased catabolism (4–6).

We have previously shown diminished concentrations of insulin-like growth factor-I (IGF-I) in patients

with CF (7). This was later confirmed by Taylor *et al.* (8), who found the most pronounced abnormalities in IGF-I and insulin-like growth factor-binding protein-3 (IGFBP-3) in patients with CF during late puberty. The decreased concentrations of IGF-I in patients with CF might presumably cause an increase in GH secretion due to lack of negative feedback on somatotrophic cells in the anterior pituitary gland (9). Conversely, decreased IGF-I concentrations might be secondary to GH resistance (10, 11). GH resistance is characterized by a disturbance in the physiological relationship between GH secretion, IGF-I synthesis and the biological actions of GH. The classical form of primary GH resistance due to a defect in the GH receptor is by far the most common; however, GH resistance may also be secondary (10). Malnutrition is known to cause GH resistance, which is normalized in response to nutritional

supplementation (12). Catabolic states, as seen in severely ill patients may lead to GH resistance (13) and in poorly controlled patients with diabetes mellitus (DM) abnormally high GH secretion and relative IGF-I deficiency have been observed (14, 15).

The aim of this study was to investigate whether patients with CF are GH resistant. We measured the spontaneous as well as the stimulated GH release and compared the results with IGF-I and IGFBP-3, glucose tolerance and BMI in adult patients with CF and in healthy controls.

Patients and methods

Patients

Twenty adult patients with CF and chronic pulmonary infection with *Pseudomonas aeruginosa* were included, ten with normal glucose tolerance (NGT) (five male, five female, median age 25.5 years (range 20–31)), and ten with DM (five male, five female, median age 25.3 years (range 17–45)). Cut off values of 2 h post glucose load capillary plasma glucose concentration ≤ 8.8 mmol/l for NGT and ≥ 12.2 mmol/l for DM were used, according to WHO recommendations (16). The results were compared with an age- and sex-matched group of 20 healthy controls (10 male, 10 female, median age 28.4 years (range 18–36)). These controls had NGT as judged by fasting capillary plasma glucose (≤ 7.8 mmol/l) and glycated haemoglobin (≤ 0.063 mmol/l). The controls had significantly higher BMIs than did the CF patients (23.17 (19.5–26.2) vs 19.50 (15.4–25.1), $P < 0.0001$).

Ethics The study was carried out in accordance with the Helsinki II declaration and approved by the local ethics committee of Copenhagen, Denmark (approval no. 01–008/93 and 01–027/97). Written informed consent was obtained from each participant and from the parents of the participant below 18 years of age.

Methods

Spontaneous GH release was evaluated from a 12 h profile from approximately 19.00 h to 07.00 h with blood specimens being sampled every 30 min (24 samples). A constant withdrawal pump connected to a non-thrombogenic catheter was used according to the Cormed–Kowarski method (17). The stimulated GH release was evaluated by an arginine stimulation test (L-arginine monohydrochloride 0.5 g/kg body weight infused i.v. over 30 min) with blood samples 30 min before, immediately before and 10, 20, 30, 45, 60, 90, 120 and 150 min after the start of arginine infusion.

In diabetic CF patients, insulin therapy was withheld for the last 12 h before measuring spontaneous GH release and for the last 24 h before arginine stimulation.

GH was quantified using an immunofluorometric assay (trIFMA, Delfia, Wallac, Pharmacia Biosystems, Allerød, Denmark). In our laboratory the intra-assay coefficients of variation were 9.7, 2.2 and 1.2% at serum concentrations of 0.4, 14.1 and 59.8 mU/l respectively. Interassay coefficients of variation were 6.2, 6.0 and 2.3% at serum concentrations of 1.2, 16.4 and 58.2 mU/l respectively. The lower detection limit of the human GH kit was approximately 0.05 mU/l. All samples from each subject were measured simultaneously in the same assay.

The Pulsar method, which is based on computerized algorithms (18), was used to characterize the pattern of secretory episodes and identify GH pulses in the GH profiles. The following values were extracted from the Pulsar analysis for the evaluation of the spontaneous GH profiles: the smoothed 12 h mean, the maximum value, the number of peaks, the mean peak amplitude, the mean peak area, and the area under the curve (AUC) estimated above the calculated baseline. The Pulsar program was set up so that the probability of detecting a peak in a series without peaks was below 5% (19).

IGF-I was measured by RIA on acid-ethanol extracted serum including a cryoprecipitation step using monoiodinated [^{125} I-Tyr ^{31}]-des-(1–3)-IGF-I as radioligand. Interassay and intra-assay coefficients of variation were 8.7 and 3.9% respectively at a bound/free ratio of 0.4 (20). A specific RIA previously described by Blum *et al.* (21) was used to determine IGFBP-3.

Statistics

The statistical analyses were performed in a statistical package (SPSS/PC+). Non-parametric methods were used. The Mann–Whitney test was used to compare two different groups. The Kruskal–Wallis test was used for comparison of three groups.

Linear regression analysis was used to investigate the degree of correlation between variables.

Results

There were no significant differences in the spontaneous GH release (Table 1), the arginine-stimulated peak GH release (Table 2) or the basal concentrations of IGF-I and IGFBP-3 (Table 2) between patients with NGT and DM, and therefore data from the two groups were pooled for analysis.

None of the characteristics of the spontaneous GH release showed significant differences between CF patients and controls (Table 1). Figure 1 shows different examples of GH release profiles in individual CF patients and controls. Different types of secretory pattern were found equally in both groups (Fig. 1).

BMI correlated significantly to mean GH secretion ($r = -0.41$, $P < 0.01$) but not to any other parameter used to characterize the spontaneous GH release.

Table 1 Characteristics of 12 h GH release in diabetic CF patients (CF-DM), patients with normal glucose tolerance (CF-NGT) and healthy controls. Values are median (range).

	Controls (n = 20)	CF-NGT (n = 10)	CF-DM (n = 10)	CF-NGT and CF-DM (n = 20)
12 h mean GH (mU/l)	2.66 (0.5–9.3)	2.99 (1.1–10.2) * <i>P</i> = 0.36	4.53 (1.45–14.3)	3.7 (1.1–14.3) ** <i>P</i> = 0.39
AUC to baseline (mU/l × 12 h)	51.85 (7.8–191.0)	42.74 (14.1–185.2) * <i>P</i> = 0.26	68.67 (28.0–106.7)	62.90 (14.1–185.2) ** <i>P</i> = 0.77
No. of GH peaks	4 (2–5)	4.5 (3–6) * <i>P</i> = 0.56	4.5 (3–6)	4.5 (3–6) ** <i>P</i> = 0.21
Maximum value of GH (mU/l)	13.70 (2.0–70.6)	14.30 (6.1–51.9) * <i>P</i> = 0.45	22.25 (0.3–38.3)	16.95 (0.3–51.9) ** <i>P</i> = 0.43
Peak amplitude of GH (mU/l)	5.73 (1.0–21.8)	5.35 (2.4–31.3) * <i>P</i> = 0.45	8.54 (2.9–11.0)	7.61 (2.4–31.3) ** <i>P</i> = 0.65
Peak area of GH (mU/l)	13.02 (1.9–47.5)	11.20 (2.7–61.2) * <i>P</i> = 0.41	15.6 (6.9–31.6)	12.1 (2.7–61.2) ** <i>P</i> = 0.53

*P values indicate CF-NGT compared with CF-DM, **P values indicate CF patients compared with controls.

Table 2 BMI, basal concentrations of IGF-I and IGFBP-3 and GH peak concentration in response to arginine stimulation in diabetic CF patients (CF-DM), patients with normal glucose tolerance (CF-NGT) and healthy controls. Values are median (range).

	Controls (n = 20)	CF-NGT (n = 10)	CF-DM (n = 10)	CF-NGT and CF-DM (n = 20)
BMI	23.17 (19.5–26.2)	19.64 (15.4–25.1) * <i>P</i> = 0.71	19.26 (16.7–23.2)	19.50 (15.4–25.1) ** <i>P</i> = 0.0001
IGF-I (ng/ml)	228 (134–393)	154 (82–264) * <i>P</i> = 1.0	163 (59–298)	157 (59–298) ** <i>P</i> = 0.003
IGFBP-3 (ng/ml)	3754 (2731–4779)	2896 (1626–4349) * <i>P</i> = 0.88	2860 (1332–3725)	2860 (1332–4349) ** <i>P</i> = 0.0008
GH peak values (mU/l)	16.1 (0.4–56.1)	29.4 (2.9–78.9) * <i>P</i> = 0.55	26.4 (0.9–59.7)	28.5 (0.9–78.9) ** <i>P</i> = 0.09

*P values indicate CF-NGT compared with CF-DM. **P values (Mann–Whitney) indicate CF patients compared with controls.

GH release in response to arginine stimulation was evaluated by peak GH concentration (Table 2). There was no significant difference between patients and controls. The patients seemed to obtain higher peak concentrations than controls; however, the range was wide and the comparison showed no statistically significant difference (Table 2).

There was a significant negative correlation between peak GH concentration during arginine stimulation and BMI ($r = -0.61$, $P < 0.0001$).

Basal levels of IGF-I and IGFBP-3 were significantly reduced in CF patients compared with controls (Table 2). IGF-I but not IGFBP-3 showed a significant positive correlation to BMI ($r = 0.42$, $P < 0.01$).

Peak GH concentrations during arginine stimulation correlated significantly to mean spontaneous GH concentration ($r = 0.43$, $P < 0.01$), maximal spontaneous GH concentration ($r = 0.43$, $P < 0.01$) and AUC to baseline ($r = 0.35$, $P < 0.05$).

Linear regression analysis for both patients and controls showed that the stimulated GH release did not correlate to IGF-I or IGFBP-3.

The spontaneous GH secretion correlated to IGF-I and IGFBP-3 since GH peak area and peak amplitude correlated to IGF-I ($r = 0.49$, $P < 0.05$ and $r = 0.59$, $P < 0.01$ respectively), and mean GH secretion, AUC to baseline and peak amplitude correlated to IGFBP-3 ($r = 0.55$, $P < 0.05$; $r = 0.45$, $P < 0.05$; $r = 0.49$, $P < 0.05$ respectively).

Discussion

We found normal spontaneous and stimulated GH levels in patients with CF. Previous studies on GH release in patients with CF have been conflicting as normal, increased and decreased GH concentrations have been demonstrated (Table 3). This inconsistency may be explained by several circumstances such as

Figure 1 GH profiles in three individual CF patients (left panels) and three healthy controls (right panels). Top panels, high GH release; middle panels, medium GH release; bottom panels, low GH release.

Table 3 Previous studies on GH release in patients with CF.

Author and year	CF patients	Age of patients	Result	Reference population	Method
Green <i>et al.</i> (1967) (22)	5 patients with height below third percentile	Children	Fasting GH↑ Hypoglycaemia stimulated GH→	13 normal children	RIA
Milunsky <i>et al.</i> (1971) (24)	15 patients	2.5–17.5 years	GH↑ after oral glucose load (not a standard stimulus)	16/15 normal controls with/without family history of DM	RIA
Biswas <i>et al.</i> (1975) (25)	69 patients	Children	GH→	12 children without CF	RIA
Lee <i>et al.</i> (1980) (23)	6 patients with height below third percentile	Children	Fasting GH→ L-dopa + exercise stimulated GH→	15 children followed for reason other than CF	RIA
Bedford <i>et al.</i> (1992) (26)	29 patients with 5 samples each	3.9–10.4 years	Overnight urinary GH→	174 school children	?
Culler & Meacham (1993) (27)	8 diabetic (4 males, 4 females)	19–29 years except one of 13 years	Arginine stimulated GH↑	8 age-matched healthy controls, all males	RIA
Huseman <i>et al.</i> (1996) (28)	9 prepubertal children	5.5–9.8 years	L-dopa: GH = 16.1 ± 2.8 ng/ml Hypoglycemia: 13.2 ± 3.2 ng/ml 18–24 h profiles: GH↑	16 short normal children were used as controls on profiles	RIA

differences in the study and reference populations and difficulties in the assessment of GH status.

Two reports dealt with severely growth-retarded children (22, 23). One study (24) did not use a standard stimulus for investigating GH release and one study (25) did not specify the circumstances under which GH was measured. Measurements of urinary GH (26) may be interfered with by the various medications taken by CF patients. Aminoglycosides may cause reversible or irreversible damage to the kidney and influence the urinary output of GH.

Three studies (23, 27, 28) used standard conditions and methods that are in accordance with the present methods used for GH assessment. The first (23) showed normal GH concentrations in growth-retarded children with CF, the second (27) showed increased GH concentrations in adult diabetic patients and the third (28) showed decreased concentrations of GH in prepubertal children with CF. Thus, a disturbance of the GH/IGF-I axis may be possible, but data are at present limited.

We confirmed the work of Lee *et al.* (23) by the finding of normal stimulated concentrations of GH, although the study of Lee *et al.* concerned growth-retarded children and the present study concerned adult patients. Furthermore, we found normal spontaneous GH release during the night. In contrast Culler & Meacham (27) found increased arginine-stimulated GH release in diabetic CF patients compared with healthy controls. However, the control group in Culler's study consisted of eight males whereas the group of CF patients consisted of four males and four females. As females have higher GH release than males (29, 30) this difference in gender may have biased the result.

In the study of Huseman *et al.* (28) of 18–24 h GH profiles, decreased GH secretion was found. The control group in this study was short normal children and it was not specified how these short children were classified as normal.

Meacham *et al.* (31) found that the basal levels of somatostatin were normal in diabetic CF patients, whereas peak concentrations of somatostatin after arginine stimulation were significantly increased compared with normal controls. This corresponds to the finding of a relative preservation of somatostatin secreting delta cells in pancreatic tissue from both diabetic CF patients and CF patients with NGT (32, 33). The study of stimulated GH release by Culler & Meacham (27) was done in continuation of this, and it was concluded that the increased somatostatin concentrations were not of sufficient magnitude to suppress pituitary GH release since GH release was increased rather than decreased in diabetic CF patients. As already discussed, the difference in gender in the controls may have biased the result and it may be possible that increased somatostatin concentrations prevented the expected GH increase in the present study.

We did not find any significant differences in the GH/IGF-I/IGFBP-3 axis in diabetic CF patients compared

with CF patients with NGT. This is in contrast to adolescents with insulin-dependent diabetes mellitus (IDDM) and in poorly controlled patients with IDDM where an abnormally high GH secretion and relative IGF-I deficiency have been observed (14, 15). It is, however, in accordance with our previous finding of equal concentrations of IGF-I and IGFBP-3 in diabetic CF patients compared with patients with NGT during the period of 5 years that preceded the diabetic diagnosis (34).

In accordance with our previous finding (7) IGF-I concentrations were reduced in CF patients compared with controls. Because of the reduced IGF-I concentrations we would have expected increased GH secretion due to the lack of negative feedback from IGF-I on the pituitary gland. The combination of normal GH secretion and reduced IGF-I and IGFBP-3 concentrations may point to a relative GH resistance or a disturbance in the pituitary axis. However, it may also be due to the difficulties associated with the assessment of GH status.

GH is difficult to assess and the stimulated GH release may vary considerably in healthy controls (35, 36). However, this should be partly compensated for by the design of this study with age- and sex-matched healthy controls. The evaluation of 12 or 24 h GH profiles has been suggested to be superior to stimulation tests (37, 38), but this is still controversial (39).

A relative GH resistance in CF may be due to increased catabolism, malnutrition or, in the older patients, chronic infection. There have been several former studies on GH and IGF-I secretion in various conditions of malnutrition and increased catabolism such as anorexia nervosa, kwashiorkor, marasmus and healthy starving subjects. An increased basal concentration of GH during malnutrition is consistently found in these studies (40–43). In both healthy starving subjects (42) and in patients with anorexia nervosa (41) the 24 h profiles of GH have revealed increased GH secretion. On the other hand, Soliman *et al.* (43) found a blunted response to arginine in severely malnourished children. However, fasting GH levels were increased and the blunted response may be caused by the circumstance that GH was already maximally stimulated by the condition of starvation (43). Hochberg *et al.* (44) found decreased concentrations of GH-binding protein, significantly lower binding capacity of GH and increased IGF-I binding on red blood cells in anorexia nervosa and proposed that anorexia nervosa presents a condition of GH resistance and IGF-I hypersensitivity.

Patients with CF cannot be compared with any of these conditions but there are some similarities. CF patients may present a catabolic condition by the combination of chronic pulmonary infection and poorly controlled malabsorption. Many older patients have reduced BMI although not to the same extent as patients with anorexia nervosa. The findings of normal or increased GH secretion in the various studies may

be related to changes in BMI and in our study GH secretion in response to arginine stimulation and mean GH secretion from the profiles showed a significant negative correlation to BMI.

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