Circadian patterns of serum insulin-like growth factor (IGF) II and IGF binding protein 3 in growth hormone-deficient patients and age- and sex-matched normal subjects

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Abstract. Knowledge of the circadian patterns of serum IGF-II and the large molecular weight IGF binding protein, IGFBP-3 might, apart from its physiological relevance, be of clinical interest, inasmuch as measurements of these parameters are being introduced into the evaluation of GH deficiency. We therefore evaluated the 24-h (08.00-08.00 h) patterns of serum IGF-II and IGFBP-3 in 8 GH-deficient patients who were studied during three periods when receiving 1. GH (2 IU) at 20.00 h; 2. GH (2 IU) at 08.00 h and 3. no GH. For comparison, 10 age- and sex-matched untreated healthy subjects were studied once under similar conditions. The serum IGF-II levels of the patients were relatively stable over the 24-h periods, yielding mean levels which were significantly lower during no GH: 553±28 (evening GH), 554±54 (morning GH), and 429±65 μg/l (no GH). The mean IGF-II level in the normal subjects was 635±29 μg/l, which was significantly higher than in either patient study. Similarly, stable 24-h levels of IGFBP-3 were recorded in all studies. The mean IGFBP-3 level of the patients was significantly lower when they received no GH, and the mean level in the healthy subjects was higher than in any of the patient studies: 1853±301 (no GH), 2755±317 (evening GH), 2904±269 (morning GH), and 3856±186 μg/l (healthy subjects). However, minute but significant changes over time, characterised by slight decrements at night, were observed for both parameters in several of the studies. Nevertheless, since both IGF-II and IGFBP-3 display rather stable 24-h levels in the individual, it is concluded that measurements of these parameters in evaluation of growth retardation can be based on a single daytime sample.

Measurement of serum IGF-1 is becoming widely accepted as a supplement in the diagnosis of GH deficiency, and it has also in some (1-4), but not all (5,6) studies proven useful as a predictor of growth response during GH therapy. Circulating IGF-II, on the other hand, has been far less extensively studied in clinical growth disorders. In contrast to serum IGF-I, IGF-II is almost constant with age beyond the first year of life (7,8), which theoretically makes serum IGF-II more applicable as a diagnostic tool and therapeutic predictor.

However, any interpretation of circulating IGF levels is complicated by the existence of at least 4 specific binding proteins (IGFBP) (9-14). According to a recent suggestion (15), they are now designated IGFBP-1, IGFBP-2, IGFBP-3 and CSF-IGFBP, the latter a binding protein isolated from cerebrospinal fluid (14). IGFBP-1 exhibits distinct diurnal fluctuations, which seem to be GH-independent but rather influenced by nutritional variables (16-18). So far, little is known about the occurrence and regulation of IGFBP-2 and CSF-
IGFBP. The quantitatively most important IGFBP in postnatal life is IGFBP-3, which consists of a non-binding subunit, IGFBP-3α, and a binding-subunit, IGFBP-3β (19). It is GH-dependent with low levels in GH deficiency and high levels in acromegaly (20,21) and is presumed to function as a reservoir and buffer for IGFs (22). It has been proposed that concomitant measurements of serum IGF-I, IGF-II and IGFBP-3 may serve as both a screening for GH deficiency and as a growth predictor during treatment (23). But before introducing single measurements of the latter two parameters in the clinical evaluation of GH-deficient patients, it would seem pertinent to establish whether they display circadian variations. We have therefore investigated the 24-h patterns of circulating IGF-II and IGF BP-3 in GH-deficient patients, both in the untreated state and following GH therapy given in different modes, and in an age- and sex-matched group of untreated normal subjects.

Subjects and Methods

Eight GH-deficient patients participated: 2 females and 6 males, mean chronologic age 15.3 years (range 11-20). The pubertal development according to Tanner comprised stage 1 (N=2), stage 2 (N=3), stage 3 (N=1), and stage 4 (N=2). The diagnosis was based on auxiological criteria (height and height velocity >2 SD below the mean for sex and age, retardation of bone age) and on a peak serum GH <5 μg/l following 2 stimulation tests. The mean serum IGF-I level in the patients during periods off treatment was 75.3±21.1 μg/l. Prior to the study all patients were in current treatment with GH (Norditropin®, Nordisk Gentofte, Gentofte, Denmark) at a daily dose of 2 IU administered sc in the evening. Three patients received additional pituitary replacement therapy, which was continued unchanged throughout the study period. The group of healthy subjects consisted of 10 individuals, 3 girls and 7 boys with a mean age of 14.9±1.6 years (±SEM). The pubertal staging was stage 1 (N=2), stage 2 (N=1), stage 3 (N=4), and stage 5 (N=3). None had a previous medical history or received any medications.

Study design

All patients underwent 3 consecutive 4-week study periods in random order receiving either 1. daily sc injections of 2 IU GH (Norditropin®, 4 IU/ml) given in the evening (at 20.00 h); 2. daily sc injections of 2 IU GH given in the morning (at 08.00 h), or 3. no GH. At the end of each period the patients were hospitalized in the evening for 24 h frequent blood sampling; this started the following day at 08.00 h by means of a cannula inserted in an antecubital vein and kept patent by isotonic saline. The total amount of blood drawn at each study was 180 ml. Meals, standardized for each subject with respect to composition and total energy content, were served at 08.00 h, 12.00 h and 17.00 h. No energy intake was allowed in between. Normal daily activities other than sport were allowed until 20.00 h, whereafter the patients stayed in bed. The normal subjects were hospitalized once under similar conditions, but without receiving any treatment. The protocol was approved by the local ethical committee. Separate data derived from this study protocol have been published previously (24).

Assays

IGF-II was measured by RIA after acid-ethanol extraction as described previously (8) using a highly specific polyclonal antibody produced against the C-peptide of human IGF-II. Residual IGFBP in the extract, which possibly interferes with the assay, was blocked by the addition of excess IGF-I (25 ng per tube) to the incubation mixture. Determination of IGFBP-3 was performed with a newly developed RIA using a polyclonal antibody against the acid-stable binding subunit (21). This assay recognizes the complete IGFBP-3 complex. Serum samples were diluted 1:336 with assay buffer before measurement.

Statistics

Analysis of variance was used to test for changes over time within and between (repeated measures) the study periods and for differences between the study periods in the 24 h means of each parameter, as calculated from the area under the individual curves. A p value <0.05 (two-tailed) was considered significant. Results are expressed as mean ± SEM.

Results

Serum IGF-II

Fig. 1 depicts the 24-h (08.00-08.00 h) mean levels in serum IGF-II both in the patients following evening and morning administration of GH and in the untreated state, and in the healthy subjects.

No significant circadian variation in IGF-II was revealed in any of the 3 patient studies, and when analysed by ANOVA for repeated measures, the time course of serum IGF-II did not differ between the studies where the patients received evening and morning GH, respectively. The 24-h mean levels following GH treatment were: 553.3±77.9 (evening GH) and 554.1±53.5 μg/l (morning GH) (NS). In the study period without GH administration a significantly lower IGF-II level was recorded: 428.9±64.5 μg/l (p<0.05). The average coefficient
of variation (CV) for the 24-h serum IGF-II was, respectively: 10.8 (evening GH), 10.1 (morning GH) and 7.8% (no GH). A significant albeit mild change in serum IGF-II with time was observed in the healthy subjects (p=0.02) with a decline during early night, although the average CV over the 24-h period was 4.9%. The mean IGF-II levels in this group was 635.4±28.8 μg/l, which was significantly higher than in either patient study (GH studies vs normals: p<0.05; no GH vs normals: p<0.001).

Serum IGFBP-3
Fig. 2 depicts the corresponding levels of IGFBP-3. Although the 24-h patterns appeared stable as also reflected by the average CVs between 6.9-7.8%, a significant variation with time of this parameter was observed in all patient studies, the most consistent finding being a decline in the night. The mean levels in the patients during GH therapy were 2755±317 (evening GH) and 2904±269 μg/l (morning GH) (NS), respectively. The mean level during no GH therapy was 1853±301 μg/l, which was significantly lower than in both GH treatment modes (p<0.01).

A mild but significant change over time of serum IGFBP-3 was also recorded in the normal subjects, again in spite of an average CV over the 24 h of only 6.3%. The mean level was 3856±186 μg/l, which was significantly higher than in either of the patient studies (p<0.001).

A positive correlation between the mean values at each time point of IGF-II and IGFBP-3 was observed in all studies and reached statistical significance except in the study where the patients did not receive GH.

Discussion
This study is the first to examine the circadian levels of serum IGF-II and IGFBP-3 in GH-deficient patients using highly specific RIA s (8,21). No gross circadian fluctuations were observed, although a small but significant change over time of both parameters was recorded in some of the studies, the consistent finding being a minor decline during night.

The 24-h mean level of IGF-II during GH treatment was significantly lower than in the normal subjects. This is in agreement with our previous experience with serum IGF-1 using the same daily GH dose (in average 1.5 IU/m² body surface) (24,25). The corresponding IGF-II level during the period of no GH therapy yielded values which were somewhat higher than expected for GH-deficient patients with this assay, although the mean level in
the control group also seemed higher than previously experienced (8). On the other hand, mean values above 400 µg/l in relatively large groups of GH-deficient patients have been reported in studies by other investigators (26,27). In these studies as well as in ours there was a trend towards somewhat higher levels in pubertal subjects (26) and in patients with craniopharyngeoma (27). At any rate, our data confirm previous observations of a relatively high frequency of near-normal IGF-II values in GH-deficient patients.

In the untreated state the IGFBP-3 level was substantially decreased compared with that in the healthy subjects. In fact, all patients had IGFBP-3 levels below -2SD of the healthy subjects except one patient, who just exceeded this value.

Our observation of relatively stable 24-h levels in the two parameters extends and confirms that of Yeoh & Baxter (16) and Baxter & Cowell (18). They studied a small number of healthy subjects for 24 h in whom no circadian variation in serum IGF-II or IGFBP-3 was observed. We did, on the other hand, record a slight but significant change of both parameters over time in some of the studies, which probably was related to decrements in the early night. Whether these minute changes reflect a true biological circadian variation of these parameters or a systematical pre-analytical variation caused by e.g. a change in the intravascular space is difficult to evaluate, but in all instances the changes are very small and hence of little practical relevance. The time course was independent of the mode of GH administration in the patients.

Our study was not designed to answer the question whether measurements of circulating IGF-II and IGFBP-3 should be used in the diagnosis of GH deficiency or as a monitor during GH therapy. This is so because our patients had been diagnosed and treated with GH for several years, and because we did not include a period of height velocity measurements. However, we find it an important clinical information that future measurements of these two parameters can be based on single samples, at least when collected at day-time, both for diagnostic and treatment evaluation purposes owing to the evidence of rather constant 24-h levels in both untreated and GH-treated patients, as well as in normal subjects.

References


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Received May 18th, 1990.
Accepted June 29th, 1990.

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