CHAPTER 4

Pharmacokinetics and pharmacodynamics of orally administered clonidine – a model-based approach

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Abstract

BACKGROUND The oral clonidine test is a diagnostic procedure performed in children with suspected growth hormone (GH) deficiency. It is associated with untoward effects, including bradycardia, hypotension and sedation. Serum clonidine levels have not previously been assessed during this test.

METHOD In 40 children referred for an oral clonidine test, blood samples were drawn for clonidine and GH. Vital statistics and sedation scores were recorded until 210 minutes post-dose. We explored the relationship between clonidine concentrations and effects such as GH and blood pressure.

RESULTS Of 40 participants, 5 children were GH deficient. Peak clonidine concentrations of 0.846±0.288 ng/mL were reached after one hour. Serum levels declined slowly, with concentrations of 0.701±0.189 ng/mL 210 minutes post-dose. A large inter-individual variation of serum levels was observed. During the procedure systolic BP dropped by 12.8%, diastolic BP by 19.7% and heart rate by 8.4%. Moderate sedation levels were observed. Concentration-effect modeling showed that the amount of GH available for secretion as determined by previous bursts was an important factor influencing GH response.

CONCLUSIONS Clonidine concentrations during the test were higher than necessary according to model-based predictions. A lower clonidine dose may be sufficient, and may produce less side-effects.

Introduction

Children with short stature are frequently referred to a pediatrician or pediatric endocrinologist for assessment of an underlying cause. Short stature may be caused by a variety of conditions, one of which is a growth hormone (GH) deficiency. Several diagnostic procedures are currently employed to assess the adequacy of pituitary GH secretion. Many clinicians use serum IGF-I as a screening parameter, and if serum IGF-I is below the mean for age, one or two GH provocation tests are carried out. These tests are based on the effect on GH secretion of a number of stimuli, including insulin-induced hypoglycemia (insulin-tolerance test, ITT), exercise, sleep (12 or 24 hour GH profile) and a range of pharmacological challenges (centrally acting alfa-receptor agonists, beta receptor antagonists, dopaminergic agonists and serotonergic agonists) (1). Because of the risks of severe hypoglycemia during the ITT (2), in many clinics one of the other pharmacological tests is used, particularly the clonidine test.

Clonidine, a centrally acting alfa2 receptor agonist, was first reported as being a useful tool in assessing the GH axis in the 1970's (3, 4). It was administered in a dose of 0.15 mg per square meter of body surface area (BSA), the dose still used in clinical practice today. Clonidine was demonstrated to elicit a substantial release of GH in healthy controls as compared to patients with hypopituitarism (3).

The diagnostic procedure in suspected GH deficiency currently consists of oral administration of clonidine in a fasted patient, followed by repeated blood samples until 150 minutes post-dose (1). In a non-deficient patient, this generally leads to a significant rise in serum GH levels with a peak usually occurring within 90 minutes after administration of clonidine (5). The diagnosis of GH deficiency will be rejected if GH rises above a pre-defined cut-off during the procedure. Currently, a cut-off level of 6.7 µg/L is used in many centers. The test is associated with several untoward effects (6), including hypotension, bradycardia, moderate sedation and hypoglycemia. At higher dose levels (accidental intoxication),

more serious effects such as coma, respiratory depression and generalized hypotonia have been reported (7). Both the intended (GH release) as unintended effects may well be related to serum levels of clonidine during the test, but this has not been studied so far. In this study we investigated serum levels and pharmacodynamic properties of oral clonidine in children with short stature during the oral clonidine test. To predict the effects of lower dose levels of clonidine, an approach using non-linear mixed effects modeling was employed.

Patients and Methods

40 patients suspected of having GH deficiency and admitted for a clonidine test were included in this study. The study protocol was approved by the Medical Ethics Committee of the Leiden University Medical Center. All study procedures were in accordance with the Declaration of Helsinki. Study participants were recruited from the Leiden University Medical Center (Leiden, the Netherlands), The Juliana Children's Hospital (The Hague, the Netherlands), and the Reinier de Graaf hospital (Delft, the Netherlands). Patients and/or their parents were approached prior to admission, and written informed consent was obtained from parents prior to study participation. All patients with an indication for a clonidine test were eligible for study participation and there were no exclusion criteria.

CLONIDINE ADMINISTRATION

A dose of 150 µg clonidine per square meter body surface area was administered orally after completion of baseline measurements. We used the IV solution (Catapresan®, Boehringer Ingelheim, Germany). In children with a BSA exceeding 1 square meter, no more than 150 µg of clonidine was administered according to the local protocol.

PHARMACOKINETIC SAMPLING AND ANALYSIS

In addition to blood samples taken for GH analysis, a total of 9 samples per patient were drawn from an intravenous cannula for measurement of clonidine levels (before, and at 10, 20, 30, 60, 90, 120, 150 and 210 minutes after clonidine dosing). Serum samples were stored at -40°C until analysis. Clonidine was determined in serum using High Performance Liquid Chromatography (LC 20-A, Shimadzu corporation, Kyoto, Japan), linked to a tandem mass spectrometer (API-4000, Applied Biosystems, Carlsbad CA, USA) at the Clinical Pharmacology Research Center of the Peking Union Medical College Hospital, Beijing, China. The assay had a lower limit of quantitation of 0.1 ng/mL, and the assay was validated using quality control (QC) samples of 0.25 (low), 5 (medium) and 80 (high) ng/mL using codeine as internal standard. The inter-day coefficient of variation was 8.1% for the low QC sample, 2.6% for the medium QC sample and 5.9% for the high QC sample. The intra-day coefficient of variation was 8.3-10.4 % for the low QC sample, 2.3-5.4% for the medium QC sample and 5-11.5% for the high QC sample.

PHARMACODYNAMIC ASSESSMENTS

Vital signs (blood pressure, heart rate, respiratory rate and oxygen saturation) were recorded at 15 minute intervals during the test. Sedation levels were recorded using the modified Ramsay sedation score (MRSS) (8). The definitions of consciousness states of the MRSS are shown in table 1. All assessments in all 40 patients were performed by the same investigator (RK). Serum levels of GH and cortisol were measured in blood samples taken at baseline, and at 10, 20, 30, 60, 90, 120, 150 and 210 minutes after administration of clonidine.

GH was analysed on an Immulite 2500 immunoanalyser (Immuno-LuminoMetric-Assay, ILMA, Siemens Healthcare Diagnostics, Tarrytown (NY), USA). The analytical variation was 5.5%. GH results were expressed

in mass units, in accordance with the most recent consensus statement (IS 98/574; 1 mg equals 3 IU) (9). Cortisol was analysed on a Modular E170 Immunoanalyser (Roche Diagnostics, Mannheim, Germany). The analytical variation was 3.4%. Glucose was analysed using a routine hexokinase method on a Modular P800 analyser (Roche Diagnostics). The analytical variation was 2.5%.

STATISTICS

Paired t-tests were performed to compare the baseline measurements with the mean post-dose measurements and to compare the baseline measurements with the maximum effect values per patient. Analyses of GH, cortisol and serum glucose were performed for two separate groups (GH deficient vs. non-GH deficient). The measurements of the variables GH and cortisol were log-transformed prior to analysis to correct for the expected log-normal distribution of the data. As normal values for blood pressure and heart rate are age dependent, an additional analysis was performed on calculated percentage change from baseline measurements. A one-sample t-test was performed to test if the percentage change from baseline values differed from 0.

A mixed model analysis of variance (with time, sub-group, time*sub-group as fixed factors and subject as random factor) was employed to determine whether presence of GH deficiency influenced the obtained pharmacodynamic measurements. All calculations were performed using SAS for windows V9.1.3 (SAS Institute, Inc., Cary, NC, USA).

The data were analyzed with a non-linear mixed effects modeling approach to explore the relationship between clonidine concentrations and effects using the software package NONMEM v7.2.0 (10). This analysis included modeling of the clonidine pharmacokinetics and the effects of clonidine on GH release, blood pressure and heart rate. The approach entails simultaneous estimation of typical PK and PD model parameters as well as the variability of these parameters within the study population.

Additionally, parameters characterizing individual subjects were derived from the estimated typical values and their variability. With this approach it is possible to plot the effect profiles over time, while the clonidine concentrations are taken into account and determine the shape of the profile.

Results

Characteristics of the study population are shown in table 2. In 33 patients the clonidine test resulted in a GH response above the threshold of 6.7 µg/L, precluding a diagnosis of GH deficiency. Of the remaining 7 patients, a diagnosis of GH deficiency was rejected in 2 patients after normal GH responses during a subsequent arginine test, resulting in 5 patients diagnosed with GH deficiency.

The serum clonidine concentrations increased rapidly with average (± SD) peak concentrations of 0.846 ± 0.288 ng/mL at 1 hour (figure 1). The decline in clonidine serum concentrations was slow, with an average clonidine concentration of 0.701 ± 0.189 ng/mL at 210 minutes post-dose. The data could be well described with a one-compartment model.

Measurements of GH, cortisol and glucose are represented graphically in graphs 2a-c, and summarized in table 3. In non-deficient patients, GH levels increased to a peak around 60 minutes, with an average GH concentration of 15 µg/L at 60 minutes post-dose, and 13 had GH levels exceeding 6.7 µg/L before administration of clonidine. All patients with a GH response >6.7 µg/L had reached this cut-off at or before 90 minutes post-dose. Cortisol levels declined slightly in both deficient and non-deficient patients. Glucose levels did not change significantly during the clonidine test. Three procedures had to be terminated due to hypoglycemia (2.9 mmol/L at 30 minutes in a GH deficient patient aged 4.2 yrs; 2.4 mmol/L at 90 minutes in a GH deficient patient aged 5.1 yrs; and 2.5 mmol/L at 120 minutes in a non-GH deficient patient aged 3.8 yrs

respectively). Mixed model analysis of variance showed that a diagnosis of GH deficiency was associated with a greater maximal decrease in serum glucose (effect size -0.6 mmol/L, 95% CI -1.1 to 0.0, p=0.0571) and minimum serum cortisol (effect size 69.5%, 95% CI -0.4 to 188.6%, p=0.0519).

Cardiovascular measurements for the entire group of patients are represented in graphs 3a-c, and in table 3. After clonidine administration, systolic blood pressure dropped by 12.8% on average in participating patients. Diastolic blood pressure declined by 19.7%. A separate analysis on averaged minimum values per subject showed a decline in systolic blood pressure of 23%, and a decline in diastolic blood pressure of 35.7%. Heart rate declined by 8.4% on average, averaged minimum values were 20.3% below baseline. There were no differences in the cardiovascular measurements between patients with or without GH deficiency.

The observed modified Ramsay Sedation Score (mRSS) scores are represented in figure 4. As expected, all patients had a normal level of consciousness (mRSS 1) at baseline. After clonidine administration, its sedative effects rapidly become apparent, with effects persisting throughout the procedure. The highest levels of sedation were observed between 1 and 2 hours after administration of clonidine, with up to 83% of children having an mRSS of 3 or higher.

The pharmacokinetic model fitted well to the data and could be used to investigate the relationship between the clonidine concentrations and its effects on blood pressure, heart rate and GH. We first explored if there were differences in blood pressure and heart rate between normal and GH deficient children using a mixed model analysis of variance. This showed that there was no influence of presence or absence of a GH deficiency on cardiovascular measurements.

The model to describe the effect of clonidine on GH dynamics was based upon the assumption that it would be released according to a first-order process that was boosted by clonidine. It was further assumed that the GH reserve was finite, and depletion was a limiting factor for further release. Thus, GH release preceding clonidine administration was taken into account. The model described the individual GH-time profiles well for several conditions. Figure 5 shows some typical conditions that may be met during the test. This includes a patient (nr 6) with GH deficiency with low levels before and after clonidine administration. In patient 25 the pre-test GH levels were low, suggesting no previous burst, leading to high GH concentrations after clonidine administration. The model could also adequately capture situations as observed in patient 34 and 35 where GH levels before clonidine administration in subjects were elevated, most likely due to a previous burst. In these cases lower GH peak concentrations after clonidine administration were observed. Simulations based on the model (figure 6) with different doses of clonidine suggest that a substantial GH release (that will allow to confirm or refute the diagnosis of GH deficiency) can be reached with lower doses than the commonly used dose of 150 µg/m^2. The effects of different doses of clonidine on systolic and diastolic blood pressure measurements were also simulated and showed dose dependency (figure 6b).

Discussion

In this study, we collected both pharmacokinetic and pharmacodynamic data during the oral clonidine test. This test is frequently used to investigate the GH axis in children with short stature, to confirm or exclude GH deficiency as an underlying cause. Although frequently used, this procedure is associated with several adverse events, including hypotension, bradycardia and hypoglycemia. We demonstrated a rapid rise in serum clonidine levels after oral administration, with slow clearance. We also observed a significant decline of blood pressure and heart rate, as well as moderate levels of sedation during the test procedure.

Few formal pharmacokinetics studies of clonidine have been performed in children. Published data suggest a terminal half-life of clonidine of 9-12.5 hours (11, 12). This implies that the effects of clonidine can be

expected to be relatively long-lasting. In our study, peak clonidine serum levels were reached at about 60 minutes post-dose and concentrations declined only slowly thereafter. This observation matches previously published data, which showed peak plasma concentrations at 60 minutes post dose (13). The large inter-individual variation can in part be attributed to inter-individual differences in oral bio-availability, which is estimated at 46.9-65.4% (13). It may also be due to the fact that an intravenous formulation was administered orally.

As anticipated, a significant decline in blood pressure and heart rate was observed. These effects are mediated through clonidine's centrally acting alfa2 agonistic effect in a dose-dependent manner. This effect may create a safety issue for patients who undergo this procedure, as in some cases the fall in blood pressure can be clinically relevant. On the other hand, to our knowledge no permanent sequelae have been reported in the literature, arising from clonidine administration in this setting.

The sedative effect of clonidine was demonstrated using the MRSS during the 210 minute observation period in our study. We observed moderate levels of sedation. This is not surprising, as clonidine levels >0.3 ng/mL are viewed as adequate to achieve pre-operative sedation (14). Even at 210 minutes post dose, serum concentrations were 0.7 ng/mL on average. Hypoglycaemia has been associated with the oral clonidine test (15). In our series, 3 procedures had to be terminated due to hypoglycemia, 2 of which in children later confirmed to have a GH deficiency. If decreased serum glucose levels were a direct clonidine effect, one could expect this effect to become apparent in the data presented in figure 2c. As hypoglycemia seems to be a sporadic event during the oral clonidine test, one could hypothesize that individual susceptibility (e.g. GH deficiency) in combination with prolonged fasting for the clonidine test, rather than a generic clonidine effect, is an explanation for hypoglycemia. It has to be kept in mind that the procedure is performed under fasting conditions, so under these circumstances this individual susceptibility might become apparent independent from clonidine administration.

In our study, all of the patients with a GH response >6.7 µg/L during the oral clonidine test had reached this cut-off level at or before 90 minutes post-dose. This finding supports the suggestion by Galluzzi et al (5) to limit the procedure to 90 minutes. On the other hand, one could wonder whether it would be safe to discharge patients at this timepoint, given the persisting effects on blood pressure and sedation.

The exploratory analyses that we performed on the relationship between clonidine concentrations and pharmacodynamics responses (cardiovascular and GH) seem to suggest that the clonidine levels may be higher than necessary. Indeed simulations performed with lower doses suggest that it is possible to select a lower clonidine test dose that is still suitable as GH provocation test while having less effects on blood pressure. In our opinion, this observation should renew interest in a short publication by Laron et al (16), in which a much lower oral dose of 0.025 mg clonidine was shown to be effective in eliciting a substantial release of GH. On the other hand, later studies with clonidine doses of 0.025, 0.050 and 0.1 mg have shown lower peak levels of GH as well as a lower fraction of children reaching the GH cut-off level (17, 18). In this respect our model could be helpful as it will take into account also the GH concentrations before the test and thus putting the GH concentrations after the test in a better perspective. The data suggest that following this approach and taking 3-4 samples for GH before the start of the test would improve the current test's diagnostic sensitivity. This would allow detecting if the patient had a GH peak before the clonidine administration which could influence the response to the test. We also advocate using standardized oral clonidine formulations as it seems likely that the kinetics and the effects depend on the formulation. It may even be considered to use IV administration to reduce the inter-patient variability, but this advantage may be off-set by the expected stronger effects on blood pressure and heart rate.

The safety of the clonidine test might improve by performing a pharmacological intervention during the procedure, thereby stabilizing blood

pressure. For instance, atropine is frequently combined with clonidine as pre-medication in anaesthesiology (19). However, any additional intervention during the clonidine test would have to be scrutinized prior to implementation in practice, as this intervention in its own may have an effect on the GH axis, thereby rendering this combination unsuitable for a clonidine test. For instance, the combination with atropine would completely suppress GH secretion (20). Another intervention of interest could be (oral) fluid loading during the procedure. In a cohort of children who drank 10 ml/kg of diet sprite twice during a combined arginine-clonidine test procedure, less requirement for intravenous fluids and higher blood pressure were observed, with no apparent effect on GH secretion (21).

In conclusion, our study has demonstrated that during the routinely applied clonidine test for the evaluation of GH deficiency high clonidine concentrations were reached. The concentrations were generally higher when compared to target levels used in anesthetic practice. As expected, significant untoward effects were observed, in particular hypotension and sedation. As our results suggest that clonidine concentrations are well above levels required for the intended effect (GH release), we propose a modification of the clonidine challenge with a lower dose. The sampling period of GH could be limited to 90 minutes, as previous literature and our study have demonstrated that maximum GH concentrations have been reached at or before this time point. However, it could be argued that a clinical observation well beyond 90 minutes post-dose is warranted to monitor for clonidine's adverse effects.

TABLE 1 The modified Ramsay sedation score

Score	Definition
1	Awake and alert, minimal or no cognitive impairment
2	Awake but tranquil, purposeful responses to verbal commands at conversation level
3	Appears asleep, purposeful responses to verbal commands at conversation level
4	Appears asleep, purposeful responses to verbal commands but at louder than usual conversation level or requiring light glabellar tap
5	Asleep, sluggish purposeful responses only to loud verbal commands or strong glabellar tap
6	Asleep, sluggish purposeful responses only to painful stimuli
7	Asleep, reflex withdrawal to painful stimuli only (no purposeful response)
8	Unresponsive to external stimuli, including pain

TABLE 2 Study population characteristics*

	GH deficient	Not GH deficient
Age (yrs)	6.00 (± 3.60)	9.30 (± 3.85)
Sex	3 male; 2 female	19 male; 16 female
Height (cm)	107.2 (± 20.7)	123.6 cm (± 20.5 cm)
Height SDS	-2.20 (± 0.31)	-2.46 (± 0.63)
Weight (kg)	19.70 (± 9.39)	25.08 kg (± 9.78 kg)
BMI SDS	0.32 (± 0.78)	-0.65 (± 1.00)

* Mean (± SD)

TABLE 3 Summary of cortisol, serum glucose, growth hormone and vital statistics. Baseline measurements, the difference of mean post-dose measurements compared to baseline and the difference of averaged minimum** measurements for every patient compared to baseline are presented with 95% confidence intervals.

		Cortisol*	Glucose	Growth Hormone*	Systolic BP	Diastolic BP	Heart Rate	Respiratory Rate
Not GH def	Baseline (mean ± SD)	0.347 (±0.23) nmol/L	4.3 (±0.55) mmol/L	2.49 (±4.82) µg/L	104.3 (±9.18) mmHg	56.6 (±6.98) mmHg	85.0 (±16.35) bpm	20.3 (±3.7) /min
	Mean post-dose – Baseline	-45.71 (-64.6 to -29.0) %	0.0 (-0.1 to 0.2) mmol/L	43.03 (19.0 to 59.9) %	-12.3 (-13.9 to -10.7) %	-19.1 (-22.2 to -16.0) %	-9.5 (-12.7 to -6.3) %	-9.7 (-15.6 to -3.9) %
	Min. post-dose – Baseline**	-153.0 (-196.1 to -116.1) %	0.3 (0.1 to 0.5) mmol/L	82.45 (73.0 to 88.6) %	-22.7 (-24.9 to -20.4) %	-35.4 (-38.7 to -32.1) %	-20.4 (-23.6 to -17.1) %	-26.3 (-31.1 to -21.6) %
GH-def	Baseline (mean ± SD)	0.420 (±0.36) nmol/L	3.9 (±0.21) mmol/L	1.94 (±4.66) µg/L	108.0 (±28.36) mmHg	65.8 (±17.91) mmHg	87.4 (±20.38) bpm	24.0 (±1.73) /min
	Mean post-dose – Baseline	-18.58 (-42.1 to 1.0) %	-0.1 (-0.9 to 0.7) mmol/L	0.87 (-162.3 to 61.2) %	-16.3 (-34.2 to 1.5) %	-23.9(-36.5 to -11.2) %	-0.3 (-19.8 to 19.1) %	-14.4 (-60.0 to 31.2) %
	Min. post-dose – Baseline**	-80.6 (-173.8 to -19.2) %	0.2 (-0.6 to 0.9) mmol/L	33.51 (-50.9 to 70.7) %	-25.2 (-45.0 to -5.4) %	-38.2 (-5.7 to -24.7) %	-19.8 (-27.2 to -12.4) %	-37.8 (-76.3 to 0.6) %
All	Baseline (mean ± SD)	0.354 (±0.23) nmol/L	4.3 (±0.54) mmol/L	2.43 (±4.70) µg/L	104.8 (±12.55) mmHg	57.8 (±9.21) mmHg	85.3 (±16.62) bpm	20.6 (±3.71) /min
	Mean post-dose – Baseline	-42.66 (-59.6 to – 27.6) %	0.0 (-0.1 to 0.1) mmol/L	39.59 (16.5 to 56.3) %	-12.8 (-14.9 to -10.7) %	-19.7 (-22.7 to -16.8) %	-8.4 (-11.8 to -5.0) %	-10.1 (-15.7 to -4.6) %
	Min. post-dose – Baseline**	-144.4 (-182.9 to -111.1) %	0.3 (0.1 to 0.4) mmol/L	79.88 (69.7 to 86.6) %	-23.0 (-25.5 to -20.4) %	-35.7 (-38.8 to -32.6) %	-20.3 (-23.2 to -17.4) %	-27.3 (-31.9 to -22.7) %

* Analyses were performed on log-transformed data
** Max. post dose- baseline for Glucose and Growth hormone

FIGURE 1 Serum clonidine concentration-time profile after administration of oral solution of 150 µg clonidine/m² at t=0' in 40 pediatric patients. Individual measurements are represented with dots, the solid line indicates the mean profile based on the pharmacokinetic model that was constructed on the basis of the full dataset. The shaded area represents the 95% prediction interval around the mean.

FIGURE 2A-C GH, cortisol and glucose levels with SD error bars in GH deficient (•) and non-deficient (o) patients during the oral clonidine test.

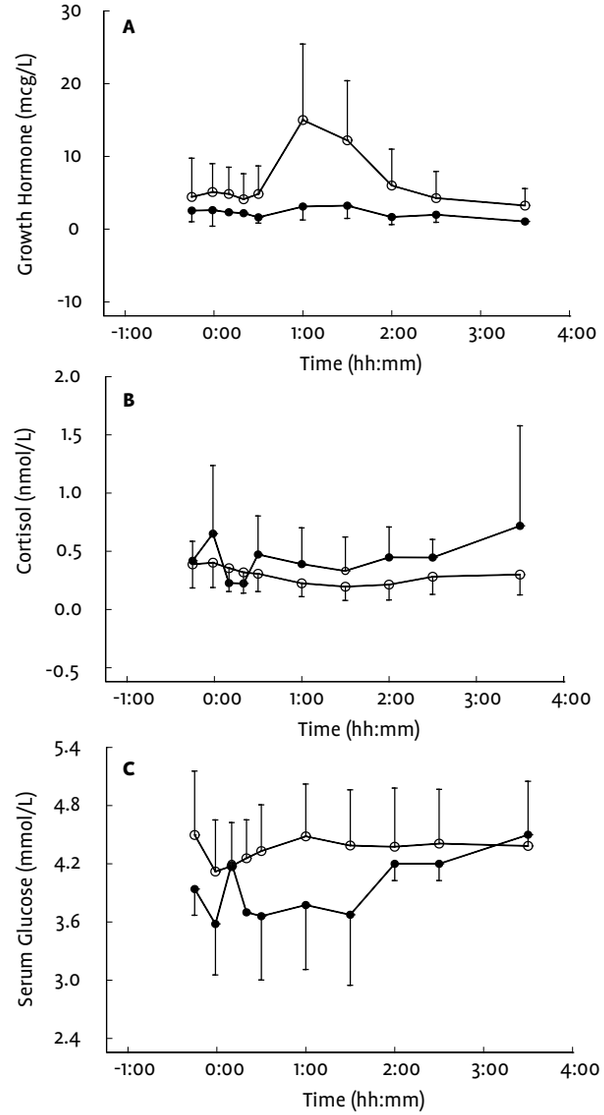


FIGURE 3A-C Blood pressure and heart rate (expressed as % change from baseline, with SD error bars) after 150 µg oral clonidine in children suspected of GH deficiency.

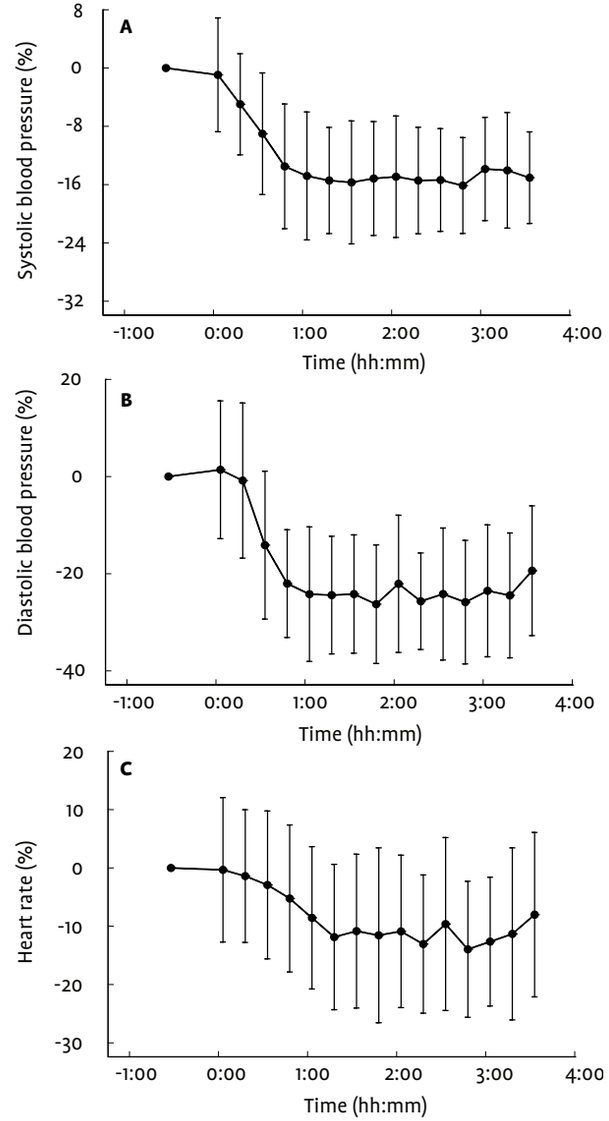


FIGURE 4 Modified Ramsay sedation scores during the oral clonidine test. Data of GH deficient and non-deficient patients were pooled.

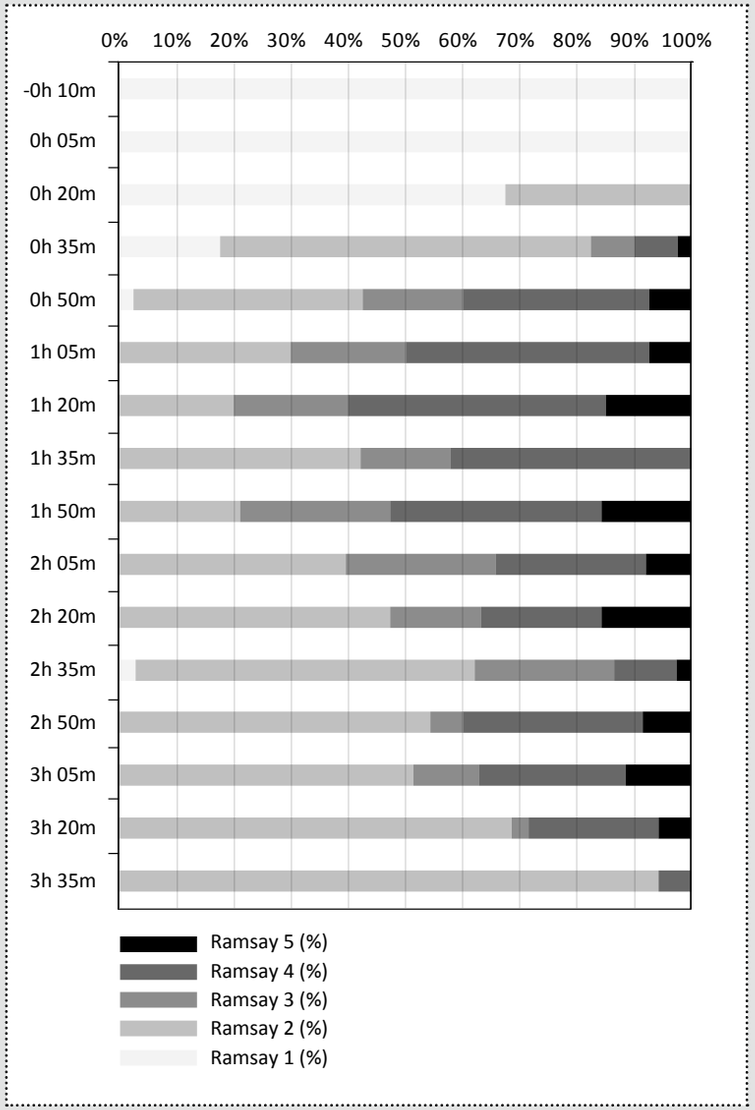


FIGURE 5 Observed GH profiles after clonidine administration for 4 typical patients. Symbols represent actual measurements, the lines show the model-based prediction of the GH response that could be expected taking into account the presence or absence of GH deficiency, as well as absence/presence and the timing of previous GH peaks. Patient 6 (triangle) shows an absent response in GH because of GH deficiency; patient 25 (dots) had low pre-test GH concentrations and the model predicted a robust GH response; patient 34 (rhombus) was supposed to have a peak occurring well before the clonidine administration and was expected to have a minimal increase in GH after clonidine and patient 35 (squares) may have had a robust GH peak just before the clonidine administration so that clonidine had only a minimal effect.

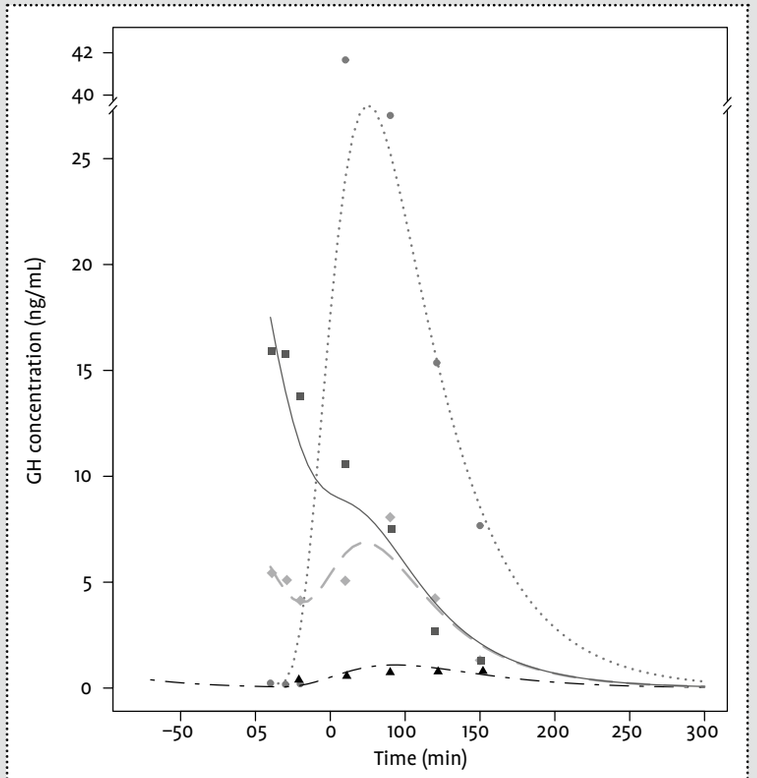
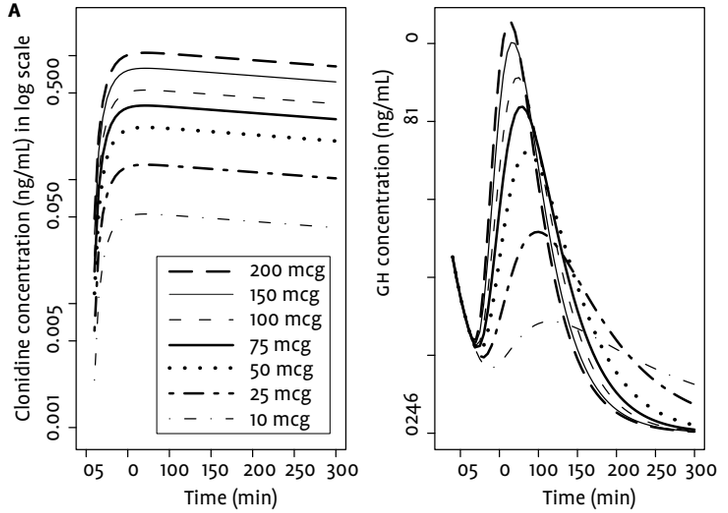
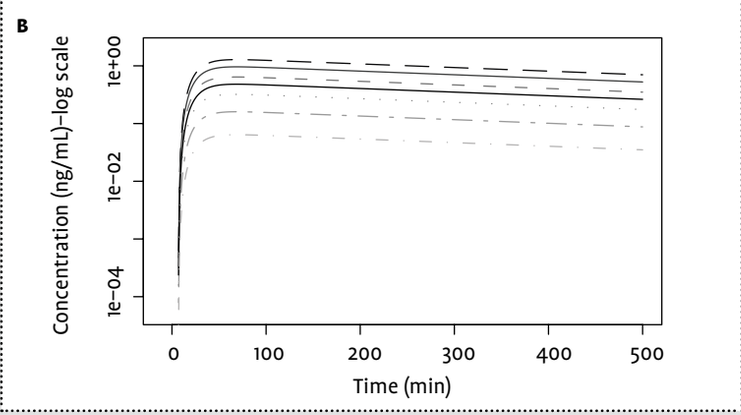
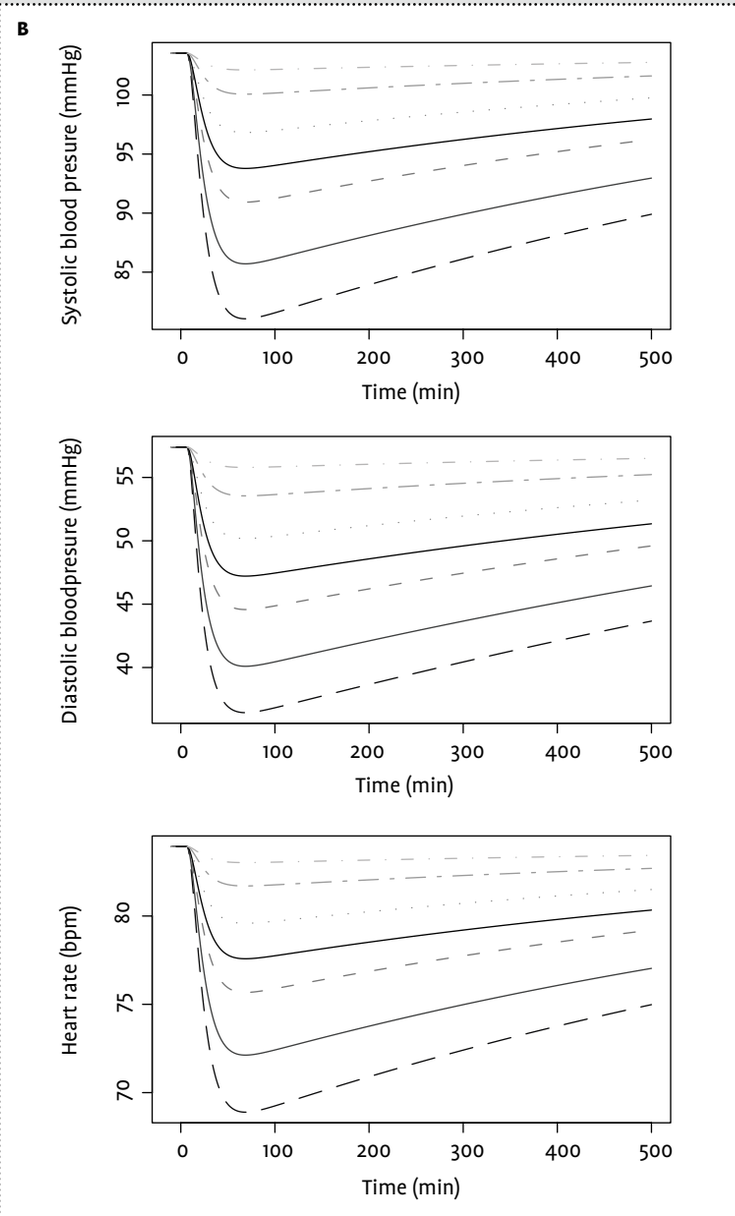


FIGURE 6A-B Simulations of GH profiles (a) and effects on blood pressure and heart rate (b) in a typical pediatric population using different doses of clonidine. Clonidine doses ranging from 200 to 10 µg (200, 150, 100, 75, 50, 25 and 10 µg) were used to simulate the response to the test. In these simulations, a body surface area of 0.903 m² was assumed (average of the study population).







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