

## Renability of the Low Dose Synacthen Test in Children Undergoing Pituitary Function Testing

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### ABSTRACT

**Background:** There are few data in the paediatric literature on the normal cortisol response to stimulation during the low dose synacthen test (LDST) (1 µg).

**Aim:** To examine the cortisol responses in children, subsequently presumed to be normal, who had an LDST during anterior pituitary function tests (APFTs).

**Methods:** A retrospective review of results in children with short stature and normal growth hormone levels.

**Results:** Of 33 children tested, seven had suboptimal cortisol responses based on accepted criteria (peak <500 nmol/l) - a false positive rate of 21%. Only three of these children had a repeat LDST, which was normal in all cases. The peak cortisol response (median 633, range, 417-1052 nmol/l) was inversely correlated with age ( $r = -0.44$ ,  $p < 0.05$ ).

**Conclusion:** One in five tests did not meet normal criteria. This false positive rate (21%) should be borne in mind when interpreting synacthen tests to prevent overdiagnosis of adrenal insufficiency.

### KEY WORDS

low dose synacthen, adrenal insufficiency

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### INTRODUCTION

Historically the insulin tolerance test has been the standard reference test for assessing the integrity of the hypothalamo-pituitary-adrenal (HPA) axis; however, it is intensive, unpleasant and potentially dangerous<sup>1-3</sup>. In the 1960s Wood *et al*<sup>4</sup> reported the cortisol response following an injection of 250 µg of adrenocorticotrophin (synacthen). This test has since gained in popularity, but this high dose may mask milder forms of adrenal insufficiency. In recent years, studies have confirmed that the low dose synacthen test (LDST) (using doses around 1 µg) is able to stimulate the adrenal gland maximally, thus increasing the sensitivity of the test, and it has been recommended as the screening test of choice in the investigation of the HPA axis<sup>5-8</sup>. This test is now widely used in paediatric practice.

Recent publications suggest that up to 40% of children on inhaled steroids have evidence of adrenal suppression<sup>9</sup>. This, combined with a number of suboptimal test responses in patients undergoing pituitary function tests in our unit, in whom we assessed the probability of adrenal insufficiency as very low, prompted us to examine the literature on which the cut-off values for normality or abnormality related to children undergoing the LDST are based. Major paediatric textbooks suggest a peak cortisol response greater than 550 nmol/l or an incremental increase from baseline of greater than 200 nmol/l constitutes a normal LDST response<sup>10</sup>. The references cited though refer to young adult males<sup>6</sup>. Other texts cite LDST references pertaining to the original standard dose (250 µg) synacthen test as opposed to the low dose synacthen test<sup>4,11,12</sup>. There appear to be few normative data for healthy children.

In our unit even when there is no clinical suspicion of adrenal insufficiency an LDST is routinely undertaken as part of the protocol for

anterior pituitary function testing, even when the primary indication for testing is to assess the growth hormone axis. We retrospectively examined the cortisol profiles of children who had undergone pituitary function testing for investigation of short stature in whom, based on clinical and biochemical criteria, we thought adrenal insufficiency was unlikely, and thus we presumed they had a normal HPA axis.

## METHODS

Between 2004 and 2005, 59 patients had full anterior pituitary function tests performed, which included a low dose synacthen test (1 µg) (Tetracosactide, Alliance Pharma, UK). Children were excluded if there was clinical suspicion of a problem with the HPA axis, an abnormal MRI, other co-existing pituitary hormone deficiencies, if they did not have a normal ACTH level, or if adrenal antibodies were positive. This resulted in a cohort of 33 patients, and the results of the LDST were analysed in an attempt to quantify the cortisol response in a population of children who we considered unlikely to have adrenal pathology.

Children were fasted from midnight and only clear fluid allowed up to the time of the test. Pituitary function was measured following an oral dose of clonidine (150 µg/m<sup>2</sup>) and intravenous thyrotrophin releasing hormone (TRH) (200 µg), luteinising releasing hormone (LHRH) (100 µg) and synacthen (1 µg). Synacthen was prepared by

dissolving 125 µg (0.5 ml) of synacthen in 500 ml of 0.9% saline and injecting 4 ml (1 µg) of the well-mixed solution. Samples were taken at baseline for ACTH and cortisol; thereafter cortisol levels were measured at 10, 20, 30 and 60 minutes. Serum cortisol was measured by immunoassay (Advia-Centaur, Bayer, New York, USA). Baseline cortisol, peak cortisol and the maximum increment of cortisol were recorded.

## Statistical analysis

The data are expressed as means, medians and ranges and analysed using the Pearson correlation coefficient. A p value <0.05 was considered to be significant. Data were analysed using SPSS software v 9.0 (SPSS Inc., Chicago, IL, USA) and Microsoft Excel 2003 (Microsoft Corp, Redmond, WA, USA).

## RESULTS

Of the 33 patients, there were 10 females and 23 males, mean age 10.9 years (range 2-17). The mean (standard deviation [SD]) and median peak cortisol responses are shown in Table 1. There was no significant difference in the mean peak cortisol response between males and females. Peak cortisol was inversely related to age ( $r = -0.443$ ,  $p < 0.05$ ) with younger children having a higher peak response. The mean incremental increase in cortisol from baseline was 322 nmol/l (SD 17 nmol/l) with

**TABLE 1**  
Peak cortisol response

		n	Peak cortisol response (nmol/l)		
			mean (SD)	median	Range
<b>Total</b>		33	646 (183)	623	363-1,052
<b>Sex</b>	<b>Female</b>	10	644 (201)	652	363-980
	<b>Male</b>	23	647 (179)	605	417-1,052
<b>Age</b>	<b>&lt;10 years</b>	13	745 (144)	777	489-980
	<b>&gt;10 years</b>	20	582 (180)	558	363-1,052

a median of 266 nmol/l. There was no relationship between the height SD scores and the peak cortisol response.

Seven (three females, four males) out of the 33 children had what may be considered suboptimal cortisol responses (peak <500 nmol/l and a rise in cortisol of <200 nmol/l), giving a false positive rate of 21%. The mean peak cortisol in this group was 432 nmol/l (SD 49). Three out of these seven children had a repeat LDST which was normal on retesting; mean cortisol peak of 567 (SD 70). In view of the otherwise normal pituitary function test, it was not felt appropriate to repeat the test in all children who 'failed' a synacthen test. No other investigations of the pituitary adrenal axis were undertaken.

### DISCUSSION

This retrospective study suggests that stimulated cortisol values following an LDST may result in a suboptimal response in up to 21% of tests in a paediatric population, which are often normal on repeat testing. It demonstrates the need for age and assay specific responses to the LDST in children. In a similar manner to our study, Crofton *et al.*<sup>13</sup> also noted that the peak cortisol response following insulin induced hypoglycaemia decreases with age, and Lashansky *et al.* demonstrated that following a 250 µg dose of ACTH in healthy children the peak cortisol response varied with age, gender and pubertal status<sup>14</sup>. The lower limit of the cortisol range may be below 500 nmol/l in both boys and girls. This difference was more pronounced during puberty, often the time when pituitary function tests are undertaken to assess short stature or disorders of puberty. In healthy adults, the peak cortisol response following an LDST has been shown to be less than 500 nmol/l in 7% of the population<sup>15</sup>. Considerable variation exists in the quoted sensitivity and specificity of the test. Some of these discrepancies may be due to the radioimmunoassay used, therefore kit-specific normal ranges have been advocated<sup>16,17</sup>.

We made the fundamental assumption that this group of children did not have adrenal insufficiency, which is a weakness of the study. This group is not representative of the normal population as

they were referred for further, albeit normal assessment, of short stature and did not require any treatment. There is also a preponderance of males in our group which reflects the nature of referrals for short stature to our endocrine unit. However, there are no studies which have looked at cortisol responses in a true control group of children, therefore the ranges have often been extrapolated from adult data. Other researchers have also used data obtained during pituitary function testing to establish the 'normal' ranges for children. In a study of the effects of inhaled corticosteroids on adrenal suppression, Raux Demay *et al.*<sup>18</sup> used ranges derived from 40 children investigated for short stature. The mean peak cortisol value of their control group was 580 nmol/l (range 439-726). Interestingly, to define a suboptimal response they used a cut-off of two standard deviations below the mean value for the controls (below 422 nmol/l). Using this criterion resulted in subnormal values in 28% of their steroid treated group. Meanwhile, Paton *et al.*<sup>9</sup> have suggested abnormal responses in nearly 40% of children treated with inhaled fluticasone propionate, utilising more traditional cut-off limits for peak cortisol of over 500 nmol/l.

An interaction between clonidine and the cortisol response to synacthen is a potential confounding factor that needs to be considered. Following the administration of clonidine alone in children undergoing growth hormone stimulation tests, cortisol levels tend to fall slightly, raising the possibility that it may have a suppressive effect on cortisol<sup>19,20</sup>. However, a study by Weintrob *et al.*<sup>21</sup> showed no difference between the peak cortisol response in children who had a combined clonidine and synacthen test compared to those who had a synacthen test alone. It seems unlikely therefore that clonidine has a significant effect on the cortisol response to stimulation by synacthen.

Given the high false positive rate we observed we believe that caution should be used when concluding that an individual has significant adrenal suppression based solely on the results of an LDST. There are no reliable paediatric standards as to what may constitute an abnormal response. Up to one in five children with a presumed normal HPA axis may not reach the traditional cut-off of a peak cortisol of 500 nmol/l or rise in cortisol of

>200 nmol/l. Age is also likely to affect the responses and thus data to establish the appropriate ranges in children are required.

### REFERENCES

1. Plumpton FS, Besser GM. The adrenocortical response to surgery and insulin-induced hypoglycaemia in corticosteroid-treated and normal subjects. *Br J Surg* 1969; 56: 216-219.
2. Grinspoon SK, Biller BM. Laboratory assessment of adrenal insufficiency. *J Clin Endocrinol Metab* 1994; 79: 923-931.
3. Shah A, Stanhope R, Matthew D. Hazards of pharmacological tests of growth hormone secretion in childhood. *BMJ* 1992; 304: 173-174.
4. Wood JB, Frankland AW, James VH, Landon J. A rapid test of adrenocortical function. *Lancet* 1965; i: 243-245.
5. Dickstein G, Shechner C, Nicholson WE, Rosner I, Shen-Orr Z, Adawi F, Lahav M. Adrenocorticotropin stimulation test: effects of basal cortisol level, time of day, and suggested new sensitive low dose test. *J Clin Endocrinol Metab* 1991; 72: 773-778.
6. Crowley S, Hindmarsh PC, Holownia P, Honour JW, Brook CG. The use of low doses of ACTH in the investigation of adrenal function in man. *J Endocrinol* 1991; 130: 475-479.
7. Tordjman K, Jaffe A, Grazas N, Apter C, Stern N. The role of the low dose (1 microgram) adrenocorticotropin test in the evaluation of patients with pituitary diseases. *J Clin Endocrinol Metab* 1995; 80: 1301-1305.
8. Oelkers W. Dose-response aspects in the clinical assessment of the hypothalamo-pituitary-adrenal axis, and the low-dose adrenocorticotropin test. *Eur J Endocrinol* 1996; 135: 27-33.
9. Paton JY, Jardine E, McNeill E, Beaton S, Galloway P, Young D, Donaldson MD. Adrenal responses to low dose synthetic ACTH (synacthen) in children receiving high dose inhaled fluticasone. *Arch Dis Child* 2006; 91: 808-813.
10. Brooke CGD, Hindmarsh PC. Tests in paediatric endocrinology and normal values. In: *Clinical Paediatric Endocrinology*, 4<sup>th</sup> Ed. Oxford: Blackwell Science Ltd., 2001; pp 468.
11. Sperling MA. *Paediatric Endocrinology*. Amsterdam: Elsevier Science, 2<sup>nd</sup> Ed., 2002; pp 762.
12. Musa BU, Dowling JT. Rapid intravenous administration of corticotropin as a test of adrenocortical insufficiency. *JAMA* 1967; 201: 633-635.
13. Crofton PM, Don-Wauchope AC, Bath LE, Kelnar CJ. Cortisol responses to the insulin hypoglycaemia test in children. *Horm Res* 2004; 61: 92-97.
14. Lashansky G, Saenger P, Fishman K, Gautier T, Mayes D, Berg G, Di Martino-Nardi J, Reiter E. Normative data for adrenal steroidogenesis in a healthy pediatric population: age- and sex-related changes after adrenocorticotropin stimulation. *J Clin Endocrinol Metab* 1991; 73: 674-686.
15. Gonzalbez J, Villabona C, Ramon J, Navarro MA, Gimenez O, Ricart W, Soler J. Establishment of reference values for standard dose short synacthen test (250 microgram), low dose short synacthen test (1 microgram) and insulin tolerance test for assessment of the hypothalamo-pituitary-adrenal axis in normal subjects. *Clin Endocrinol* 2000; 53: 199-204.
16. Stewart PM, Corrie J, Seckl JR, Edwards CR, Padfield PL. A rational approach for assessing the hypothalamo-pituitary-adrenal axis. *Lancet* 1988; i: 1208-1210.
17. Clark PM, Neylon I, Raggatt PR, Sheppard MC, Stewart PM. Defining the normal cortisol response to the short synacthen test: implications for the investigation of hypothalamic-pituitary disorders. *Clin Endocrinol* 1998; 49: 287-292.
18. Raux Demay MC, Magny JP, Idres N, Grimfeld A, Le Bouc Y. Use of the low-dose corticotropin stimulation test for the monitoring of children with asthma treated with inhaled corticosteroids. *Horm Res* 2006; 66: 51-60.
19. Lanes R, Hurtado E. Oral clonidine—an effective growth hormone-releasing agent in prepubertal subjects. *J Pediatr* 1982; 100: 710-714.
20. Muñoz-Hoyos A, Fernández-García JM, Molina-Carballo A, Macías M, Escames G, Ruiz-Cosano C, Acuña-Castroviejo D. Effect of clonidine on plasma ACTH, cortisol and melatonin in children. *J Pineal Res* 2000; 29: 48-53.
21. Weintrob N, Sprecher E, Josefsberg Z, Vardi P, Weininger C, Aurbach-Klipper Y, Pertzalan A, Phillip M. Combined clonidine-short-ACTH test for the simultaneous assessment of growth hormone reserve and hypothalamic-pituitary-adrenal axis integrity in children. *Eur J Endocrinol* 2000; 143: 105-110.