A high-sensitivity test in the assessment of adrenocortical insufficiency: 10 µg vs 250 µg cosyntropin dose assessment of adrenocortical insufficiency

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Abstract

The short cosyntropin (synthetic ACTH) test is recognized as the best screening manoeuvre in the assessment of adrenocortical insufficiency. Recent data, however, suggest that i.v. administration of 250 µg cosyntropin could be a pharmacological rather than a physiological stimulus, losing sensitivity for detecting adrenocortical failure. Our objective was to compare 10 vs 250 µg cosyntropin in order to find differences in serum cortisol peaks in healthy individuals, the adrenocortical response in a variety of hypothalamic–pituitary–adrenal axis disorders and the highest sensitivity and specificity serum cortisol cut-off point values.

The subjects were 83 healthy people and 37 patients, the latter having Addison's disease (11), pituitary adenomas (7), Sheehan's syndrome (9) and recent use of glucocorticoid therapy (10). Forty-six healthy subjects and

all patients underwent low- and standard-dose cosyntropin testing. In addition, 37 controls underwent the low-dose test.

On comparing low- and standard-dose cosyntropin testing in healthy subjects there were no statistical differences in baseline and peaks of serum cortisol. In the group of patients, 2 out of 11 cases of Addison's disease showed normal cortisol criterion values during the standard test but abnormal during the low-dose test. In our group of patients and controls, the statistical analysis displayed a better sensitivity of the low-dose vs standard-dose ACTH test at 30 and 60 min.

In conclusion, these results suggest that the use of 10 µg rather than 250 µg cosyntropin i.v. in the assessment of suspicious adrenocortical dysfunction gives better results.

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Introduction

Assessment of the adrenal gland functional reserve with the short cosyntropin (synthetic adrenocorticotrophin (ACTH)) test has long been accepted as the most reliable diagnostic screening procedure in patients with a clinical picture suggestive of chronic adrenocortical hypofunction or in high-risk populations (Grinspoon & Biller 1994). Classically, the recommended test is carried out with 250 µg synthetic ACTH 1–24, taking blood samples for serum cortisol at baseline, 30 and 60 min after i.v. or i.m. stimulation. In spite of some controversy in interpretation of results, a peak serum cortisol above 18 µg/dl (496 nmol/l) is accepted as a normal adrenal response (Orth *et al.* 1992, Grinspoon & Biller 1994, Miller & Blake-Tyrrel 1995).

Recent evidence, however, suggests that i.v. administration of 250 µg ACTH 1–24 could be a provocative

pharmacological rather than a physiological stimulus (Dickstein *et al.* 1990, 1997, Crowley *et al.* 1991, Daidoh *et al.* 1995). Trials with a reduced number of healthy individuals have shown that 1, 5, 10 and 250 µg ACTH 1–24 i.v. are able to produce similar serum cortisol peaks (Graybeal & Fang 1985, Dickstein *et al.* 1990, 1997, Crowley *et al.* 1991, Broide *et al.* 1995, Daidoh *et al.* 1995, Tordjman *et al.* 1995, Weintrob *et al.* 1998). In addition, Dickstein *et al.* (1990) evaluating patients on long-term glucocorticoid therapy, found low sensitivity of the standard ACTH testing at identifying secondary adrenal failure when contrasting it to 1 µg ACTH. Furthermore, Graybeal & Fang (1985) generating physiological stress with insulin-induced hypoglycaemia found peaks of plasma ACTH and cortisol equivalents applying 4–6% of the standard 250 µg ACTH dose. The recommended ACTH dose was 0.2 µg/kg body weight, which makes a dose of 10–15 µg in a 50–75 kg adult. Despite all these

data, state-of-the-art endocrinology textbooks still suggest employing the i.v. administration of 250 µg ACTH as the standard diagnostic procedure (Orth *et al.* 1992, Loriaux & McDonald 1995, Miller & Blake-Tyrrel 1995).

With the above in mind, we contrasted the i.v. administration of 10 vs 250 µg ACTH in order to establish: (1) differences in serum cortisol peaks to these doses in healthy individuals, (2) interchangeability of these tests in healthy subjects, (3) the adrenocortical response in a variety of hypothalamic–pituitary–adrenal axis disorders, and (4) the highest sensitivity and specificity serum cortisol cut-off point values with healthy subjects and patients.

Subjects and Methods

Healthy subjects

Forty-six healthy volunteers between 20 and 50 years of age were selected to contrast the cortisol response to 10 vs 250 µg synthetic ACTH. Before the investigation a complete clinical history and physical examination were carried out to exclude individuals with a past medical history of granulomatous, fungal, autoimmune, endocrine, psychiatric or neoplastic disorders and subjects taking any medication. Special attention was also taken to eliminate subjects with prior intake of glucocorticoids in the last 5 years.

Patients and controls

Thirty-seven patients with a variety of hypothalamic–pituitary–adrenal axis disorders underwent comparative ACTH testing (10 vs 250 µg). They included 11 patients with Addison's disease, 7 with clinically non-functioning pituitary adenomas, 9 cases of Sheehan's disease and 10 patients with recent glucocorticoid exposure. Patients with Addison's disease were studied at presentation (seven cases) or after therapy discontinuation for at least 4 days (four cases). Those cases with pituitary adenomas or Sheehan's disease were assessed after interruption of glucocorticoid replacement for 4 days. All these patients were carefully followed during this time without medication. Patients on recent glucocorticoid use were studied at least 4 months after glucocorticoid discontinuation. This population had been using glucocorticoids in a dose over 30 mg/day prednisone for at least 8 weeks. Thirty-seven healthy controls underwent only the 10 µg ACTH test to contrast their results with the patient group.

Study protocol

To perform the 10 µg ACTH test, one vial of 250 µg synthetic ACTH (Cortrosyn, Organon Inc., West Orange, NJ, USA) was diluted in 20 ml normal sterile

saline solution to achieve a concentration of 12.5 µg/ml. The vials were maintained refrigerated at 4 °C for a 3 month period. A previous study has shown stability of this preparation for up to 4 months (Dickstein *et al.* 1990).

All healthy subjects and controls underwent 10 µg ACTH dose testing (low-dose test). Healthy subjects also underwent the standard 250 µg ACTH test (standard-dose test) at least 1 week later. Patients with Addison's disease, pituitary adenomas, Sheehan's disease and recent glucocorticoid use underwent the 10 µg ACTH test and 2 days later the 250 µg ACTH test. After overnight fasting, between 0800 and 0900 h, an i.v. catheter (21 gauge scalp vein needle set, 30 cm length × ¼ inch, Asepto, Becton Dickinson Ind., Sao Paulo, Brazil) was inserted into the forearm vein; a baseline sample for serum cortisol was taken and then the set was heparinized. Subsequently, 10 or 250 µg ACTH were injected as a bolus and blood samples for serum cortisol were taken at 30 and 60 min. A serum cortisol value equal to or greater than 496 nmol/l at 30 or 60 min after stimulation was defined as a normal adrenocortical response.

The trial was approved by the Ethics Committee for Research in Humans of our university hospital. All participants signed an informed consent form after being given a detailed explanation of the biological effects of ACTH i.v. administration and test procedures.

Assays

Serum cortisol was measured using a commercial RIA kit (Diagnostic Products Corporation, Los Angeles, CA, USA). All samples were assayed twice. Intra-assay variation coefficients were 2.5, 3.2 and 4.3% for low, medium and high cortisol values respectively. Interassay variations were 3.5, 3.9 and 6.4% for low, medium and high cortisol values respectively.

Statistical analysis

All data are reported as means ± s.d. The paired Student's *t*-test was used to evaluate the significance of differences of means between the low- and standard-dose ACTH tests in the healthy subject group. A *P* value <0.05 was considered statistically significant. In addition, a graphical method, the Bland and Altman plot, was used for assessing agreement between the differences and the averages of serum cortisol values in both tests and to identify outliers (Bland & Altman 1986). ROC (receiver operating characteristic) curve analysis with cut-off point values was used to determine the sensitivity and specificity of the low- and standard-dose ACTH tests between the patients and the control group and to evaluate the test performance with healthy subjects and patient group (Zweig & Campbell 1993). Statistical calculations were performed using StatSoft (StatSoft Inc., Tulsa, OK, USA) and Med

Table 1 Serum cortisol values in healthy subjects after i.v. stimulation with 10 and 250 µg ACTH 1-24 (means ± s.d., range)

	Low-dose test (n=46)	Standard-dose test (n=46)	P value
Time			
Baseline (nmol/l)	374.4 ± 113.1, 221.0–771.1	363.5 ± 95.0, 211.3–575.5	0.5
30 min (nmol/l)	789.6 ± 165.7, 526.4–1220.0	809.3 ± 148.3, 569.7–1254.5	0.4
60 min (nmol/l)	950.7 ± 213.2, 531.1–1555.5	951.5 ± 188.1, 621.3–1560.0	0.9
0 min vs 30 min (nmol/l)	415.2 ± 170.8, 87.5–787.1	445.8 ± 131.9, 105.1–827.7	0.2
0 min vs 60 min (nmol/l)	576.3 ± 210.2, 117.0–1238.2	588.0 ± 168.7, 274.5–1037.9	0.6
0 min vs 30 min (%)	124.2 ± 66.9, 16.0–300.3	132.0 ± 53.7, 22.4–323.5	0.5
0 min vs 60 min (%)	169.5 ± 82.6, 21.4–390.3	172.7 ± 65.9, 55.7–374.4	0.8

To convert nmol/l to µg/dl divide by 2.759.

Calc (Broekstraat 52 B-9030 Mariakerke, Belgium) (Schoonjans *et al.* 1995).

within ± 1.96 s.d. (mean – 33.4 nmol/l, s.d. 78.1 to – 144.9 nmol/l).

Results

Healthy subjects

Our studied population were 46 subjects (23 females, 23 males) with a mean age of 33.22 ± 8.97 years (range 20–46). Mean baseline serum cortisol and 30 and 60 min responses during the low- and standard-dose tests are shown in Table 1. There were no statistical differences in baseline serum cortisol values between low- and standard-dose tests (374.4 ± 113.1 vs 363.5 ± 95 nmol/l respectively, $P=0.5$). The highest serum cortisol value was found 60 min after stimulation in all but seven tests; six subjects on the low-dose test and one subject on the standard-dose test. All individuals, however, had serum cortisol levels greater than 496 nmol/l at 30 and 60 min. On comparing serum cortisol values between low- and standard-dose tests there were no statistical differences either at 30 min (789.6 ± 165.7 vs 809.3 ± 148.3 nmol/l respectively, $P=0.4$) or at 60 min (950.7 ± 213.2 vs 951.5 ± 188.1 nmol/l respectively, $P=0.97$). There were no statistical differences in the percentage increment and absolute serum cortisol peak on contrasting low- and standard-dose tests at 30 and 60 min. On contrasting serum cortisol values at 30 vs 60 min on the low- and standard-dose tests, we found statistically higher cortisol levels at 60 min on each test (low-dose test, 789.6 ± 165.73 vs 950.7 ± 213.2, $P<0.001$, and standard-dose test, 809.3 ± 148.3 vs 951.5 ± 188.1, $P<0.001$). Furthermore, when contrasting the baseline serum cortisol level and its response after stimulation in the low- and standard-dose tests between males and females there were no statistical differences (data not shown). To assess interchangeability of the tests we used the Bland and Altman plot; the evaluation at 30 min identified 45 out of 46 subjects within ± 1.96 s.d. (mean – 20.5 nmol/l, s.d. 86.6 to – 127.6 nmol/l). At 60 min, 44 out of 46 cases were

Patients and controls

The patient group included 14 males and 23 females with a mean age of 47.05 ± 14.60 years (range 19–72). The control group contained 37 individuals (25 males, 12 females) with a mean age of 25.80 ± 3.60 years (range 20–40). The analysis of the patients by groups based on the variety of hypothalamic–pituitary–adrenal axis disorder did not show statistical differences when compared with baseline, 30 and 60 min serum cortisol responses to the low- and standard-dose tests except for 60 min in the recent glucocorticoid users group ($P=0.03$) (Table 2). Normal adrenocortical responses occurred in 14 out of 37 patients on the standard-dose test. On the other hand, there were 12 normal responses on the low-dose test.

In the Addison's disease group there were two cases having serum cortisol responses above 496 nmol/l at 30 and 60 min after stimulation with 250 µg ACTH. These patients were sent to us for assessment of adrenocortical insufficiency due to diffuse hyperpigmentation. The first patient's serum cortisol values were 446.4 and 475.1 nmol/l at 30 and 60 min on the low-dose test and 677.6 and 610.8 nmol/l at 30 and 60 min on the standard-dose test respectively. The second patient's serum cortisol levels at 30 and 60 min on the low-dose test were 436.8 and 361.4 nmol/l respectively and, on the standard-dose test 568.1 and 1012.6 nmol/l respectively. Their plasma ACTH levels, however, were quite elevated (1975.2 and 431.8 pg/ml, normal range 0–37 pg/ml); these findings support the diagnosis of primary adrenal failure (Betterle *et al.* 1988, 1997). They received glucocorticoid replacement therapy with gradual improvement of hyperpigmentation. Five months later the second patient interrupted medication developing unequivocal signs of acute adrenocortical insufficiency.

To evaluate diagnostic test performance by ROC curves we selected all cases with abnormal serum cortisol

Table 2 Serum cortisol values (nmol/l) in patients after i.v. stimulation with 10 and 250 µg ACTH (means ± s.d., range)

Group	Time (min)	Low-dose test	Standard-dose test	P value
Addison's disease (n=11)	Baseline	131.8 ± 157.3, 1.2–434.3	167.6 ± 177.1, 5.8–423.2	0.16
	30	158.6 ± 194.7, 0.3–475.9	213.3 ± 247.6, 4.6–677.6	0.08
	60	156.6 ± 190.7, 0.0–486.5	273.8 ± 328.4, 8.6–1012.5	0.09
Pituitary adenomas (n=7)	Baseline	237.3 ± 165.8, 1.5–375.8	235.1 ± 158.7, 3.3–365.6	0.89
	30	455.8 ± 307.5, 38.3–778.6	632.5 ± 482.5, 51.4–1403.8	0.08
	60	541.0 ± 385.3, 45.9–1005.4	724.5 ± 458.8, 90.0–1125.1	0.06
Steehan's syndrome (n=9)	Baseline	42.6 ± 63.4, 0.0–188.5	61.7 ± 89.0, 0.0–275.9	0.56
	30	70.8 ± 106.6, 1.0–336.3	66.4 ± 88.1, 3.3–288.2	0.51
	60	73.9 ± 107.1, 1.2–335.5	84.1 ± 118.0, 3.7–376.1	0.06
Recent glucocorticoid use (n=10)	Baseline	382.1 ± 191.7, 154.2–719.3	356.0 ± 142.3, 169.3–597.6	0.33
	30	619.1 ± 279.8, 233.5–1009.5	648.5 ± 287.1, 252.8–1078.5	0.12
	60	659.4 ± 280.5, 252.9–1042.9	733.8 ± 323.5, 279.5–1238.8	0.03

To convert nmol/l to µg/dl divide by 2.759.

responses on both ACTH tests (less than 496 nmol/l), as well as the two cases with Addison's disease with normal cortisol responses on the standard-dose test but abnormal on the low-dose test associated with elevated plasma ACTH levels and clinical response to glucocorticoid replacement. On the low-dose test, the analysis with patients (n=25) and controls (n=37) identified 525.6 and 543.5 nmol/l as the cut-off point values with the highest sensitivity and specificity (100 and 95% at 30 min and 96 and 97% at 60 min respectively). Sensitivity and specificity at 496 nmol/l standard value were 92 and 97% respectively. On the other hand, the sensitivity and specificity to both tests with patients (n=25) and healthy subjects (n=46) taking cut-off point values and 496 nmol/l are shown in Table 3. There was a higher sensitivity on the low-dose test taking serum cortisol cut-off point values and 496 nmol/l at 30 and 60 min.

Discussion

During the assessment of the functional reserve of the adrenal gland, a normal response to a short cosyntropin test

can be defined based on absolute serum cortisol peak values (>496 nmol/l) or an increment with reference to the baseline serum cortisol level (>200 nmol/l) (Speckart *et al.* 1971, Orth *et al.* 1992, Aron & Blake-Tyrrel 1994, Grinspoon & Biller 1994). There is evidence that the absolute serum cortisol peak value might be a better parameter to discriminate normal adrenal function from adrenocortical insufficiency (Aron & Blake-Tyrrel 1994, Grinspoon & Biller 1994). In some cases, however, a poor serum cortisol peak during an insulin tolerance test or a subnormal 11-deoxycortisol level on a metyrapone test has been detected in subjects with normal results to the standard cosyntropin test (Lindholm & Kehlet 1987, Cohen *et al.* 1996, Streeten *et al.* 1996). This situation may be due to the high plasma ACTH levels attained during this test, which are almost 20 times greater than those encountered during a physiological stress such as insulin-induced hypoglycaemia (Graybeal & Fang 1985).

Concerning this issue, some investigators have found similar cortisol responses using lower ACTH doses when compared with the standard test (Graybeal & Fang 1985, Dickstein *et al.* 1990, 1997, Crowley *et al.* 1991, Broide *et al.* 1995, Daidoh *et al.* 1995, Tordjman *et al.* 1995,

Table 3 Comparison of the low vs standard ACTH dose tests in patients and healthy subjects at the higher sensitivity and specificity serum control cut-off point and the standard criterion value

	Low-dose test (10 µg) (n=71)		Standard-dose test (250 µg) (n=71)	
	30 min	60 min	30 min	60 min
Area under the curve	1.000	0.999	0.993	0.977
Standard error	0.000	0.003	0.009	0.017
95% CI	1.000 to 1.000	0.947 to 1.000	0.936 to 0.995	0.909 to 0.997
Cortisol value (nmol/l)	525.6*/496.0**	543.5*/496.0**	568.1*/496.0**	610.8*/496.0**
Sensitivity (%)	100/92	96/92	96/88	92/88
Specificity (%)	100/100	98/100	100/100	100/100

*Cut-off point values.
**Standard criterion value for normal adrenocortical response.

Weintrob *et al.* 1998). Most studies, however, have been performed on a reduced number of patients (fewer than ten subjects), making it difficult to arrive at a definitive conclusion.

In our study, a comparison of the adrenocortical response to 10 vs 250 µg ACTH in a large population of healthy volunteers revealed that all subjects reached the recommended criterion of normal adrenocortical response, equal serum cortisol peaks and compatibility of the tests. All publications evaluating the adrenal stimulation with very low ACTH doses (equal to or less than 1 µg) consistently show lower serum cortisol values at 60 min when contrasted to 30 min (Graybeal & Fang 1985, Dickstein *et al.* 1990, Broide *et al.* 1995, Daidoh *et al.* 1995, Tordjman *et al.* 1995, Weintrob *et al.* 1998). The clinical significance of a normal serum cortisol level at 30 min but subnormal at 60 min has not been determined in these very low ACTH dose protocols but it is known that serum cortisol levels increase progressively several hours after a stressful situation such as a surgical procedure (Streeten *et al.* 1996). Protocols with ACTH doses of 5 and 10 µg display cortisol levels increasing at 60 min (Graybeal & Fang 1985, Dickstein *et al.* 1990, Daidoh *et al.* 1995, Tordjman *et al.* 1995). Therefore, it is likely that these higher ACTH doses might indicate better the capability of the adrenal gland to respond to a more prolonged stress. In our study the serum cortisol levels at 60 min were always higher than 496 nmol/l, significantly higher when compared with the 30 min value and equal to the cortisol peak at 60 min during the standard ACTH test. These findings could indicate that the stimulation with ACTH doses lower than 0.2 µg/kg body weight may not be sufficient to stimulate further cortisol production, only revealing the adrenocortical ability to release stored cortisol in response to a minor stress but not enough to assess the response to a prolonged stressful condition. Furthermore, recent studies have pointed out false positive results in these very-low-dose protocols (1 µg ACTH) in the assessment of patients with pituitary hormone deficiencies, making it necessary to repeat the test in case of doubt (Cohen *et al.* 1996, Weintrob *et al.* 1998). On the other hand, Murphy *et al.* (1998) have recently shown that the type of device used to administer the ACTH in the 1 µg challenge affects the cortisol response, due to loss of material, probably as a consequence of ACTH binding to the plastic surface of the vein needle set.

False negative results in the evaluation of secondary adrenocortical failure by the standard ACTH test is a well known situation reported in up to 30–50% of reported cases (Cunningham *et al.* 1983, Lindholm & Kehlet 1987). In primary adrenocortical failure, however, this situation has been found in very few patients (Findling *et al.* 1987, Boscaro *et al.* 1994, El-Deiry *et al.* 1997). In our investigation, 2 out of 11 cases of Addison's disease showed normal cortisol criterion values during the standard test but abnormal values during the low-dose test. In our group of

patients and controls, the statistical analysis displayed a better sensitivity to the low-dose vs standard-dose ACTH test at 30 and 60 min. The application of cut-off point values showed higher cortisol values at 30 and 60 min, improving the sensitivity of the test. In addition, the excellent reliability of the 10 µg ACTH test could also be seen, since the cut-off point values were identical in both analysed groups.

Thus, the lack of difference of the adrenocortical responses to this low-dose trial in a large population of healthy subjects, its better sensitivity for identifying Addison's disease, its excellent reproducibility and the interchangeability with the results of the standard ACTH test, certainly justify the utilization of this low-dose ACTH test in the screening of suspected adrenocortical hypofunction. Finally, it is likely that further examination in primary adrenocortical failure high-risk populations, such as patients with granulomatous disorders or HIV infection by the 10 µg ACTH test will identify a higher prevalence of borderline adrenal involvement.

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