Relationship between Cortisol Increment and Basal Cortisol: Implications for the Low-Dose Short Adrenocorticotropic Hormone Stimulation Test, Suhaib A.R. Dott, Ibrahim Lasheen, Khaldoon Al-Humood, and Kamal A.S. Al-Shoumer

Conclusions: We analyzed the low-dose (1 μg) rapid adrenocorticotropic hormone test (LDST) in 17 patients with a normal hypothalamic-pituitary-adrenal axis to determine reference intervals for the LDST on the basis of poststimulation cortisol increments. We studied 17 patients (14 females and 3 males; age range, 18–46 years) who had received a 2-mL aliquot of low-dose (1 μg) adrenocorticotropic hormone prepared from one 250-μg vial of Synacthen diluted in 500 mL of sterile normal saline solution. Sampling took place at 0, 20, 30, and 60 min post stimulation. The cortisol increment was plotted against basal cortisol.

Method: We analyzed test results for 17 patients (14 females and 3 males; age range, 18–46 years) who had received a 2-mL aliquot of low-dose (1 μg) adrenocorticotropic hormone prepared from one 250-μg vial of Synacthen diluted in 500 mL of sterile normal saline solution. Sampling took place at 0, 20, 30, and 60 min post stimulation. The cortisol increment was plotted against basal cortisol.

Results: We observed a marked interdependence of the basal cortisol concentration with the increase in cortisol concentration. The relationship was inverse and linear with the best fit observed at 30 min post stimulation. The lower 95% prediction limit for basal cortisol at the zero increment was 400 nmol/L with a mean concentration of 600 nmol/L.

Conclusions: We propose that a peak cortisol concentration <400 nmol/L is a sufficient single criterion for abnormal adrenal function as assessed by the LDST. Concentrations of 400–600 nmol/L are in the gray area, and those >600 nmol/L confirm normal adrenal function. Repeat analyses with larger sample sizes are warranted to confirm these observations.

The standard short high-dose (250 μg) adrenocorticotropic hormone (ACTH) stimulation test (HDST) with synthetic ACTH is the most widely used test for the detection of primary or prolonged secondary adrenocortical insufficiency (1). Whether this test accurately predicts early secondary adrenal insufficiency (2, 3) is an area of ongoing controversy because occasionally patients have a normal response to the HDST but a subnormal response to insulin-induced hypoglycemia (IIH), a test considered the gold standard test for evaluation of the hypothalamic-pituitary-adrenal (HPA) axis. The pharmacologic dose of 250 μg is useful for assessing the maximum secretory capacity of the adrenal cortex (1), but this dose may be excessive for the assessment of subclinical central hypocortisolism. The low-dose short ACTH stimulation test (LDST) has been shown to correlate well with IIH in such cases (4–7). Several centers are already using the HDST, and the higher sensitivity of the LDST in the diagnosis of mild forms of secondary adrenal insufficiency is already known (4, 8, 9). The LDST is also a sensitive test for mild forms of primary adrenal insufficiency (10) but is not more sensitive than the HDST, as demonstrated by sequential LDST-HDST studies (11).

Previous studies have evaluated the operating characteristics of the LDST in adult patients with normal and abnormal adrenal function. On the basis of the results of these studies, a serum cortisol concentration of 500 nmol/L (18 μg/dL) or higher (12, 13), before or after low-dose ACTH injection, is suggested to be indicative of normal adrenal function. Although retrospective analysis of the HDST in a large adult population showed a marked interdependence of the basal cortisol concentration, peak cortisol concentration, and increases in serum cortisol concentration (14), criteria that require a minimum increment in serum cortisol (13) are considered invalid because individuals who have a lower basal serum cortisol concentration because of recent ACTH deficiency may be maximally stimulated by the HDST and thus able to further increase cortisol secretion. The increment with a more physiologic (1 μg) dose may be expected to correlate better with the degree of adrenal stimulation. Persons with high-normal basal serum cortisol concentration may therefore have little or no increase after ACTH stimulation, whereas those with low-normal basal serum cortisol may have a maximal increase. With the HDST, however, this increase may still occur if the HPA axis is compromised. We therefore decided to investigate the relationship between the peak and basal cortisol values after the LDST.

We studied 17 patients (14 females and 3 males; age range, 18–46 years) with suspected primary adrenal dysfunction that was subsequently ruled out via a normal cortisol response on the HDST. The patients were followed in the endocrinology clinic and underwent ACTH stimulation testing to exclude 21-hydroxylase deficiency and primary adrenal dysfunction suspected in the presence of other autoimmune disorders (such as hypothyroidism or diabetes mellitus). No patient was suspected of having ACTH deficiency, and none of the female patients was pregnant or taking oral contraceptives. Each patient was tested with the HDST, and if a normal result was obtained [peak >550 nmol/L (20 μg/dL)], then (within 1–2 weeks) with the LDST. We prepared low-dose (1 μg) ACTH in 2-mL aliquots with one 250-μg vial of Synacthen diluted in 500 mL of sterile normal saline solution. We obtained patient serum samples at 0, 20, 30, and 60 min after ACTH administration for the LDST. Each serum sample was separated and preserved in deep freezer at –20 °C in the laboratory until analysis. Plasma cortisol was determined by the DSL-2100 cortisol-coated tube RIA (DSL). The study was done after we obtained informed consent from all participants.

The basal cortisol concentrations varied widely (Fig. 1A), from 149 to 685 nmol/L (5.4–24.8 μg/dL); the mean (SD) concentration was 422.4 (138.2) nmol/L. The stimulated cortisol correlated inversely and linearly with basal
cortisol, and the best correlation between basal and stimulated cortisol was at 30 min, with a linear relationship between the proportional increase in cortisol (30-min cortisol/basal cortisol) and the basal cortisol (Fig. 1B). The 95% prediction limits obtained by linear regression analysis are shown in Fig. 1B, and the cutoff for the lower 95th percentile prediction limit at a stimulated/basal ratio of 1 was ≈400 nmol/L (14.5 µg/dL).

The false-negative rate for the LDST has been reported to be close to zero at cutoffs above 500–550 nmol/L (18–20 µg/dL) (4, 5, 7, 15–17). The LDST has also been advocated as a more sensitive test for ACTH deficiency than the HDST (18), but use of the same cutoff points for the LDST and HDST would serve to increase the apparent sensitivity of the LDST because peak cortisol responses are less after 1 µg than 250 µg of ACTH (19). At this cutoff point, however, false-positive rates are high, leading to low specificity (19).

We demonstrate here that the cortisol increment after LDST is inversely related to the basal cortisol, strongly suggesting that the peak cortisol depends on the state of adrenal stimulation at the time of the test. False positivity occurs because in many patients peak cortisol concentrations <500–550 nmol/L (18–20 µg/dL) represent normal variability, which has a wide range in humans, and not adrenal insufficiency. The incremental response to 1 µg of ACTH, however, is directly and inversely dependent on basal cortisol concentrations, decreasing to zero at a mean basal cortisol concentration of 600 nmol/L (21.7 µg/dL; Fig. 1B).

When devising a screening test, one aims for maximum sensitivity in the face of a reasonable specificity—in other words, excluding false negatives without including too many false positives. However, as is clear from Fig. 1B, with the LDST, the lowest basal cortisol concentration at which no increment may occur after stimulation is 400 nmol/L (14.5 µg/dL). This characteristic indicates that 100% sensitivity will be associated with markedly poor specificity, limiting the use of the LDST in this way. We therefore suggest that the lower 95% confidence interval for the basal cortisol at which the increment is zero be taken as the cutoff value associated with zero false positives. Any value <400 nmol/L (14.5 µg/dL) after LDST will therefore be abnormal. Any value above this and up to 600 nmol/L (21.7 µg/dL), our mean value at zero increment, needs a confirmatory test such as IIH or overnight metyrapone testing because the false-negative rate could be as high as 50% (19). Above 600 nmol/L (21.7 µg/dL), the test result is highly specific and need not be confirmed. Our gray zone is wide because of our small sample size; thus we recommend that this analysis be repeated with a larger sample size to obtain a more definitive gray zone with a lower upper limit.

References
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Fig. 1. Box-and-whisker plot of the spread of serum cortisol at the times sampled (A), and scatterplot of cortisol increment vs basal cortisol at 30 min post-LDST (B).

(A) □ mean; limits of the box, ±1 SD; whiskers, ±1.96 SD. (B), the gray area is the area between the regression line (solid line) and the lower 95% confidence line (lower dashed line). Regression equation for the line: ratio = −0.0031(basal cortisol) + 2.86 (r2 = 0.7; r < 0.001).


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