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Laboratory Assessment of Adrenal Insufficiency*

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ADRENAL insufficiency results from dysfunction at one or more sites in the hypothalamic-pituitary-adrenal (HPA) axis. Primary hypoadrenalism is caused by bilateral adrenal gland destruction, whereas central hypoadrenalism is caused by CRH deficiency from hypothalamic dysfunction or ACTH deficiency from pituitary destruction. Central hypoadrenalism is sometimes termed secondary (pituitary) or tertiary (hypothalamic), depending on the level of dysfunction. The clinical spectrum of adrenal insufficiency is broad, ranging from clear hemodynamic compromise to subtle dysfunction manifest only with stress. Laboratory evaluation of the HPA axis is performed in two clinical settings. The first is the diagnostic evaluation of the patient presenting with symptoms suggestive of adrenal insufficiency. In this setting, the initial goal is to determine whether cortisol production is adequate. The Cortrosyn stimulation test (Organon Diagnostics, West Orange, NJ), the insulin tolerance test (ITT), the metyrapone test, and serum cortisol levels are routinely used for this purpose. Once the diagnosis of adrenal insufficiency has been made, the next step is to localize the defect, usually accomplished by drawing a plasma ACTH level. In some cases, a prolonged ACTH infusion or CRH test may be used to establish whether the defect is located centrally or at the adrenal glands.

The second clinical setting in which evaluation of the HPA axis is needed is the patient known to be at risk for the development of secondary hypoadrenalism. The most common risk factor is prior glucocorticoid therapy. Other predisposing factors include hypothalamic-pituitary disease and related surgery or radiation. Localization of the defect in such patients is usually obvious based on the specific risk factor, and the purpose of laboratory testing is to screen for the development of inadequate hypothalamic-pituitary reserve.

Biochemical evaluation is based on three important principles of HPA physiology: 1) cortisol exerts feedback inhibition at the pituitary and hypothalamic levels; 2) the adrenal glands depend on ACTH as a tropic hormone in such a way that ACTH deficiency results in a reversible inability to produce cortisol; and 3) the HPA axis can be activated by pharmacological and physiological stimuli that override the normal diurnal pattern of cortisol production. Numerous tests, both static and dynamic, are available to assess HPA function in patients who demonstrate symptoms of, or are at risk for, hypoadrenalism. The purpose of this article is to discuss the rationale, methods, criteria, limitations, and optimal use of these tests in the evaluation of adrenal insufficiency (see Table 1 for summary).

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Diagnostic Tests

Serum cortisol level

Cortisol production is a function of the coordinated activity of the entire HPA axis. Hormone release is pulsatile throughout the day, but exhibits a diurnal pattern with levels being the highest in the early morning. Production of cortisol increases in response to declining serum cortisol levels and to physiological stressors, such as hypoglycemia. Serum cortisol can be measured at a random point in time, as a morning value between 0600 and 0800 h, or as part of a dynamic test of HPA function. Interpretation of a serum cortisol level is complicated by a number of factors. Hydrocortisone, methylprednisolone, and prednisone, but not dexamethasone, cross-react in the cortisol assay and should be avoided within 24 h of testing. Because little of the hormone exists in the free or unbound state, the measured level of cortisol is a function of the predominant binding protein, cortisol-binding globulin (CBG). Estrogen stimulates hepatic production of CBG, thereby resulting in higher total serum cortisol levels. CBG is decreased in cirrhosis, in the nephrotic syndrome, and in hyperthyroidism, but not usually to an extent that affects serum cortisol levels. Urine-free cortisol (UFC) is unaffected by such variables but is a poor diagnostic test for adrenal insufficiency because it is normal in 20% of patients with adrenal insufficiency (1). The UFC is also a poor test to judge the adequacy of glucocorticoid replacement because it reflects only the fraction of circulating cortisol that exceeds the binding capacity of CBG.

Morning cortisol level

The morning serum cortisol measurement has long been used as an index of adrenal function in the unstressed patient because it reflects peak endogenous activation of the HPA axis. However, the normal range, 9–25 μg/dL, is derived from morning cortisol levels in patients without HPA disease and does not necessarily differentiate normal subjects from those with adrenal dysfunction. For instance, in one series,

TABLE 1. Laboratory tests used in the evaluation of adrenal insufficiency

Name	Method	Criteria	Utility	Rationale	
Random cortisol[a,b]	Drawn emergently with simultaneous ACTH level	Normal test: F ≥ 18 μg/dL Presumptive AI: 5 μg/dL ≤ F ≤ 13 μg/dL Indeterminate test: 13 μg/dL < F < 18 μg/dL Definite AI: F < 5 μg/dL	Measure of HPA function in acutely ill patient	Stress activates HPA axis	O
Morning cortisol[a,b]	Drawn between 0600 and 0800 h	Normal test: F > 19 μg/dL Indeterminate test: 3 μg/dL < F < 19 μg/dL Definite AI: F ≤ 3 μg/dL	Measure of HPA function in stable patient	F production pulsatile; highest levels in morning	O
Cortrosyn stimulation test	250 μg ACTH iv; F sampled at 0, 30, and 60 min	Normal test: F ≥ 18 μg/dL at any point	Indirectly assesses entire HPA axis	Adrenal glands dependent on tropic effect of ACTH	F Fa
ITT	0.1–0.15 U/kg short acting insulin iv; glucose and F sampled at 0, 30, and 60 min	Normal test: F ≥ 18 μg/dL at any point AI: F ≤ 18 μg/dL and symptomatic hypoglycemia ≤40 mg/dL	Directly assesses entire HPA axis	Hypoglycemia a powerful stimulus of HPA axis	C Pl In
Overnight metyrapone test	30 mg/kg po at 2400 h in recumbent patient with 0800 h F and S	Normal test: S ≥ 7.0 μg/dL Indeterminate test: S < 7.0 μg/dL and F ≥ 5.0 μg/dl AI: S < 7.0 μg/dL and F < 5 μg/dL	Sensitive test for central AI	Hypocortisolemia from 11β-hydroxylase inhibition stimulates ACTH and steroidogenesis proximal to enzymatic blockade	M Pe Pl
24 h metyrapone test	500–750 mg po q 4 h × 6 with sequential 24-h urine for 17-OHS and cr collected at baseline, during, and day after metyrapone	Normal test: 17-OHS increase ≥2–3× over baseline AI: 17-OHS increase <2–3× over baseline	See above	See above	Se
ACTH level	Drawn after the diagnosis of AI is made except in the acutely ill patient, when drawn with simultaneous F	1° AI: ACTH (RIA) ≥ 100 pg/mL	Best test to separate 1° from central AI	In primary AI, ACTH levels rise because feedback inhibition is absent	N A M

Prolonged ACTH infusion test (3–5 days)	250 μg of ACTH IV over 8 h on each of 3–5 days with 24-h urine for 17-OHS and cr at baseline and during each subsequent day of the test	Central AI: 17-OHS increase ≥3× over baseline	Separates 1° from central AI	Adrenal atrophy in central AI reversed with prolonged exposure to ACTH	An t a h a l
Continuous ACTH infusion test (48 h)	3 μg/h ACTH iv over 2 days with 24-h urine for 17-OHS and cr on 1st and 2nd day of infusion	Central AI: 17-OHS ≥ 10 mg/24 h 1° AI: 17-OHS ≤ 4 mg/24 h	Less time consuming that 5-day test	As above	As
CRH test	1 μg/kg CRH iv; F and ACTH sampled at −15, 0, 15 30, 60, 90, and 120 min	See Fig. 1	Distinguishes 1°, 2°, and 3° AI, but not often necessary Possible future diagnostic test for AI	CRH produces an exaggerated ACTH response in hypothalamic disease and a flat response in pituitary disease	Flu
Plasma renin activity	Random sample	Primary AI: PRA > 3.0 ng/dL	Limited	PRA increases in response to mineralocorticoid deficiency	Te t t Af t s
Aldosterone stimulation test	Cortrosyn stimulation test with aldosterone levels at baseline and 60 min	Primary AI: Basal aldosterone < 5 ng/dL without rise after Cortrosyn	Limited	Aldosterone will not respond to Cortrosyn in 1° AI	As

[a] Prednisone, methylprednisolone, and hydrocortisone cross-react in the F assay.
[b] F is CBG dependent, and thus affected by estrogen, liver, thyroid, and renal status.
F, cortisol; S, compound S; cr, creatinine, 17-OHS, 17-hydroxysteroids; AI, adrenal insufficiency.

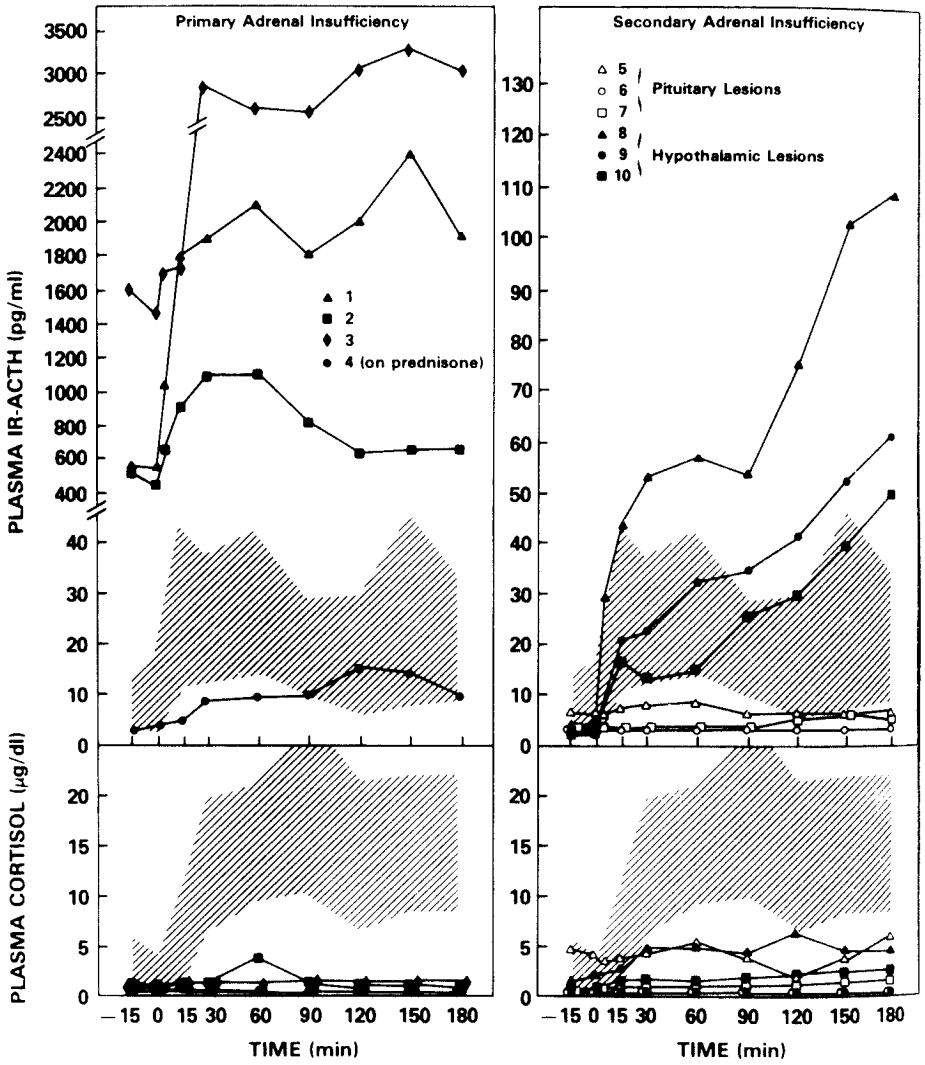

FIG. 1. Plasma ACTH (*top*) and cortisol (*bottom*) responses to CRH in subjects with primary adrenal insufficiency (*left*) or secondary adrenal insufficiency (*right*). Patients with hypothalamic lesions had clearly distinct ACTH responses to CRH, different from those in three patients with pituitary adrenal insufficiency. *Shaded area:* Absolute range from 15 normal subjects. Reproduced with permission from H. M. Schulte *et al, J Clin Endocrinol Metab (35)* and The Endocrine Society.

15% of patients with documented primary adrenal insufficiency had morning cortisol levels between 9 and 19 μg/dL (1). Furthermore, it is not clear from the literature what level of morning serum cortisol accurately predicts an adequate response to stress. In one study, a level of 11 μg/dL predicted an adequate response to the ITT in 51 of 52 patients evaluated for adrenal insufficiency (2). In another study, no patient with a morning cortisol level above 14 μg/dL failed an ITT (3). Thus, it is difficult to know what cutoff to choose, but it is well accepted that patients with morning cortisol levels above 19 do not need further testing.

The morning cortisol level has also been used to predict the adequacy of adrenal function in the setting of recent pituitary surgery and chronic glucocorticoid therapy. It was shown in one study that a morning cortisol level over 9 μg/dL several days after pituitary surgery predicts a normal response to the ITT 2–3 days later (4). These data have not, however, been reproduced. Debate also exists about the use of the morning cortisol level in patients on chronic glucocorticoid therapy. Only half of the patients with a morning cortisol level greater than 5 μg/dL demonstrated a normal response to CRH in a recent study (5). It was concluded that the morning cortisol level does not reliably predict the response to dynamic testing in this patient population. However, the morning cortisol level that was chosen to define normal adrenal function, 5 μg/dL, was arbitrary and quite low. A higher level might better have predicted a normal response to dynamic testing.

Although a low morning cortisol level may simply reflect a nadir between glucocorticoid pulses, there are substantial data which confirm that a level of less than 3 μg/dL is always diagnostic of adrenal insufficiency (2, 4, 5). The major drawback of the morning cortisol level is that many patients have indeterminate levels and require additional testing. Nevertheless, the test is easy to perform and is a good first assessment of adrenal function in the stable patient because a very low or high level obviates the need for further tests.

Random cortisol level

A random cortisol level is sometimes useful to evaluate adrenal function in the clinical setting of severe stress (when

when there is no time to wait for a morning level or dynamic testing. In the acutely ill patient, glucocorticoid therapy should be given immediately after blood is drawn, with further testing performed at a later time. If dexamethasone is chosen for glucocorticoid replacement, a Cortrosyn test can subsequently be performed.

It is debated what level of cortisol production is adequate in the acutely ill patient. A cortisol level below 18 μg/dL during severe stress is often taken as evidence of inadequate adrenal function, but it is extrapolated from the literature regarding the rapid ACTH test. The range of serum cortisol levels in the acutely ill patient is broad. Mean random cortisol levels of 22, 40, and 45 μg/dL have been reported in patients with gastrointestinal bleeding, respiratory failure, and sepsis respectively. Intensive care patients with cortisol levels below 13 μg/dL exhibit inadequate Cortrosyn stimulation, increased mortality, and improvement in clinical condition after glucocorticoid administration (6). However, critically ill patients with random cortisol levels as low as 5 μg/dL have been known to survive and exhibit adequate stimulation to ACTH (7). A random cortisol level below 5 μg/dL during severe stress is definitive evidence of adrenal insufficiency. A level below 13 μg/dL is presumptive evidence of this disorder mandating glucocorticoid therapy to reduce potential morbidity. Levels above 13 μg/dL but below 18 μg/dL are indeterminate and necessitate further testing and interim glucocorticoid therapy.

The Cortrosyn stimulation test (1-h ACTH test)

The Cortrosyn stimulation test, in which cortisol is measured 0, 30, and 60 min after the iv administration of 250 μg synthetic $ACTH^{1-24}$, is an excellent diagnostic test for patients suspected of having chronic adrenal insufficiency. In most centers, it has supplanted the 8-h infusion test because it is easier to perform and achieves similar cortisol responses (8). Although this test directly measures only the functional integrity of the adrenal glands, it also provides an indirect assessment of hypothalamic and pituitary function because the adrenal glands depend on endogenous ACTH for its tropic effect. When ACTH production is impaired by pituitary or hypothalamic disease, the adrenal gland loses the capacity to respond to exogenous stimulation.

Two aspects of the Cortrosyn test have generated much debate: 1) selection of the best test criterion, *i.e.* peak cortisol response *vs.* increment; and 2) definition of an adequate level of cortisol response. Historically, both the increment in cortisol and the absolute level of cortisol were viewed as important. However, the increase in cortisol following Cortrosyn administration is an unreliable index of adrenal function because it fails to distinguish normal patients from those with adrenal insufficiency. This was demonstrated by a study in which one third of normal controls exhibited a rise in cortisol of less than or equal to 7 μg/dL (9). Because the cortisol increment is inversely proportional to the basal cortisol level, smaller increases are obtained in the morning, when endogenous ACTH and cortisol levels are already high (10, 11). The peak cortisol response to Cortrosyn, which is of adrenal function than the increment (12).

Peak cortisol levels ranging from 15–25 μg/dL have been proposed as criteria for establishing adequate adrenal function based on the Cortrosyn test. Values of 18–20 μg/dL are well supported in the literature. In one retrospective series, cortisol responses to Cortrosyn were examined in a heterogeneous population of 399 patients. In 95% of the tests, the peak cortisol level was greater than 19 μg/dL, although no data were provided regarding the actual incidence of adrenal insufficiency nor correlation made with other tests of adrenal function (10). In another series, a value of 18 μg/dL completely separated patients with known adrenal insufficiency from normal controls (9). The usefulness of the Cortrosyn test in detecting adrenal insufficiency is demonstrated by comparing it to other dynamic tests. Only 8 discrepancies were found when the Cortrosyn stimulation test and the ITT were compared in 200 consecutive patients evaluated prospectively for adrenal insufficiency. In 6 cases the discrepancies were either minor or were attributed to inadequate hypoglycemic response. In 2 cases of acute pituitary dysfunction, however, the Cortrosyn test was normal whereas the ITT was not (13).

Such cases illustrate the recognized phenomenon of a short window period of a few weeks after the onset of pituitary dysfunction, during which the adrenal glands are still capable of responding to exogenous ACTH (14). In the *de novo* evaluation of patients with hypothalamic-pituitary disease, the duration of adrenal insufficiency may not be known, and the Cortrosyn test may be misleading, as shown in a study demonstrating normal Cortrosyn test results but abnormal ITT responses in patients with pituitary disease (15). The most common clinical setting in which the test must not be used is immediately after pituitary surgery, when corticotrope damage may have occurred but the adrenal glands have not yet atrophied from the loss of ACTH effect. In this setting, it is essential to assess pituitary reserve with a morning cortisol, metyrapone test, or ITT. After a month, the test of choice is probably the Cortrosyn test, although normal Cortrosyn but abnormal ITT responses have been seen up to 3 months after pituitary surgery (16).

The most common cause of adrenal insufficiency is HPA axis suppression due to the use of exogenous glucocorticoids. A key question regarding patients on such therapy is whether they can safely tolerate the stress of a surgical procedure without steroid administration. Various tests of adrenal function, including the Cortrosyn stimulation test, metyrapone test, and ITT have been used to predict the adrenal response to surgery in this patient population. Cortisol responses to preoperative Cortrosyn stimulation correlate remarkably well with intraoperative cortisol levels (17). Furthermore, patients with a subnormal response to the Cortrosyn test demonstrate a smaller perioperative increase in cortisol than that seen in the patients with a normal Cortrosyn but abnormal insulin tolerance or metyrapone test (18). In contrast, abnormal metyrapone responses have been demonstrated in patients previously receiving high dose glucocorticoid therapy who have a normal Cortrosyn test. However, the data do not

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