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# Advances in Adrenal Function Testing: A Review Article

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**Abstract**:

This article aims to review recent advances in adrenal function testing, focusing on how new biochemical assays, imaging techniques, and multimarket approaches improve the diagnosis and monitoring of adrenal disorders. This highlights the importance of adrenal function evaluation in the diagnosis of conditions such as adrenal insufficiency and Cushing's syndrome. The article also discusses the evolution of testing methods, from clinical observation to advanced techniques such as ACTH stimulation tests and dexamethasone suppression tests.

Biochemical markers and imaging techniques diagnostic accuracy, whereas emerging technologies such as genomics and machine learning contribute to personalized testing. Despite progress, challenges remain in standardizing reference ranges and improving assay precision. The goal of this research is to increase the accuracy of adrenal function tests and make them more accessible and precise worldwide, with a focus on new technologies and ongoing challenges in the field.

**Keywords:** Cortisol; ACTH stimulation; Diagnostic imaging; Emerging technologies.

# التطورات في اختبار وظائف الغدة الكظرية: مقال مراجعة

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**مستخلص:**

يهدف المقال إلى استعراض التقدمات الحديثة في اختبارات وظائف الغدة الكظرية، مع التركيز على كيفية تحسين التشخيص ومراقبة الاضطرابات الكظرية من خلال الاختبارات البيوكيميائية الجديدة، وتقنيات التصوير، والنهج متعدد المؤشرات. كما يوضح أهمية تقييم وظائف الغدة الكظرية في تشخيص اضطرابات مثل قصور الغدة الكظرية ومتلازمة كوشينغ. و يناقش تطور الاختبارات من الملاحظة السريرية إلى استخدام تقنيات متقدمة مثل اختبارتحفيز ACTH ، واختبارات تثبيط الديكساميثازون. تساعد المؤشرات البيوكيميائية وتقنيات التصوير في تحسين دقة التشخيص، بينما تسهم التقنيات الناشئة مثل الجينوميات وتعلم الآلة في تقديم اختبارات شخصية. رغم التقدم، هناك تحديات مستمرة مثل توحيد النطاقات المرجعية ودقة الاختبارات. الهدف من البحث هو تحسين دقة اختبارات وظائف الغدة الكظرية وجعلها أكثر إتاحة ودقة على مستوى العالم، من خلال التركيز على التقنيات الجديدة والتحديات المستمرة في هذا المجال.

**الكلمات المفتاحية:**الكورتيزول ، تحفيز ACTH ، التقنيات الناشئة ، التصوير التشخيصي .

## Introduction

The endocrine system maintains homeostasis in living organisms. Adrenal hormones are significant in this regard. They affect nutritional, psychological, and physiological processes and play a significant role in the stress response of humans [1]. Adrenocorticotropic hormone (ACTH) regulates the release of the main hormone in response to various stress mechanisms and causes the release of adrenal hormones. Most glucocorticoids in the circulation are produced by the glucocorticoid-producing zona fasciculata layer [2]. Other cells in the adrenal cortex produce mineralocorticoids, mainly aldosterone, in the zona glomerulosa and androgens in the zona fasciculata and zona reticularis. Even peripheral hormone production from the glucocorticoid and adrenal glands is tightly regulated by negative feedback inhibition exerted on the pituitary and hypothalamic portions of the hypothalamic–pituitary–adrenal axis (HPA axis). Patients with endogenous hyper cortisol levels were assessed at the next visit [3].

Adrenal function testing is a central feature in clinical practice. Adrenal glands regulate a number of physiological processes in health and disease, trying to maintain the internal milieu of an organism in a constant state via adrenal hormones [4]. There is a major pore-excavated product that removes the body from homeostasis generated during the process of puberty with the release of adrenal hormones, a reaction to physical stress and a short burst of catecholamines from the adrenal cells. Thus, a better understanding of patients with adrenal glands is essential. Despite the need for progress in adrenal function testing, the understanding of adrenal hormones in the body has increased over time. This understanding of adrenal function has been achieved with the knowledge and progress of recent cutting-edge research, which is more population-based, conscious, and specialized to diagnose and manage patients with adrenal disorders [5]. It is of paramount importance that we advance our knowledge continuously. Adrenal function testing methods are continuously being updated and modified. For this reason, the well-being of healthcare workers and assessment

approaches should be taken into account when new testing approaches are developed. Healthcare professionals need to be aware of these developing situations that also concern patient care [6].

**Anatomy and Physiology of the Adrenal Glands**

The adrenal glands are located atop the kidneys, cranial to the retroperitoneal space. Both are composed of two layers: the outermost layer, the renal capsule, and the innermost layer, the parenchyma. Additionally, both layers possess two blood supplies and perform different functions. Although functionally one organ, the cortex and medulla are indeed two distinct endocrine glands due to their separate origins [7].

The cortex produces corticosteroids that are essential for life processes such as glucose production and conversion, immune modulation, fat metabolism, stress adaptation, and electrolyte control. Two main parts compose the adrenal cortex: the zona glomerulosa and the zona fasciculata. Each zone secretes varying amounts and/or types of hormones according to the level of functioning of the hypothalamic–pituitary–adrenal axis. The medulla, which is derived from neural crest cells, produces catecholamines such as norepinephrine and epinephrine that act as neurotransmitters [8]. Developmentally, the adrenal cortex gives rise to the steroid hormone precursors dehydroepiandrosterone, androstenedione, and other androgen-related products. Furthermore, the cortisol produced can be deployed for further conversion to aldosterone, a mineralocorticoid, within the zona glomerulosa, as well as to androstenedione and the zona reticularis for further production of sex hormones [9]. Any dysregulation of the HPA axis, whether it leads to hypercortisolemia, hypercortisolism, or normal cortisol, has major health consequences for the patient. Therefore, the anatomy and physiology of the adrenal glands need to be appreciated for the purpose of performing tests in an informed way rather than in a mechanistic, unthinking way [10].

However, there is clear clinical utility in understanding adrenal physiology; the regulation of cortisol and aldosterone is the main reason for per-

forming adrenal function testing. As cortisol is essential in life, the body is capable of maintaining a normal diurnal rhythm at any cost in an otherwise healthy person, adjusting its cortisol requirement to its energy intake. Finally, adrenal function tests are conducted to diagnose and determine any issues associated with these pathways. Each test has an indication, and a clinician may choose to perform none, one, or several tests in an attempt to determine the right cortisol profile [11]. Figure 1 shows an illustrative image of the adrenal gland and its location inside the human body.

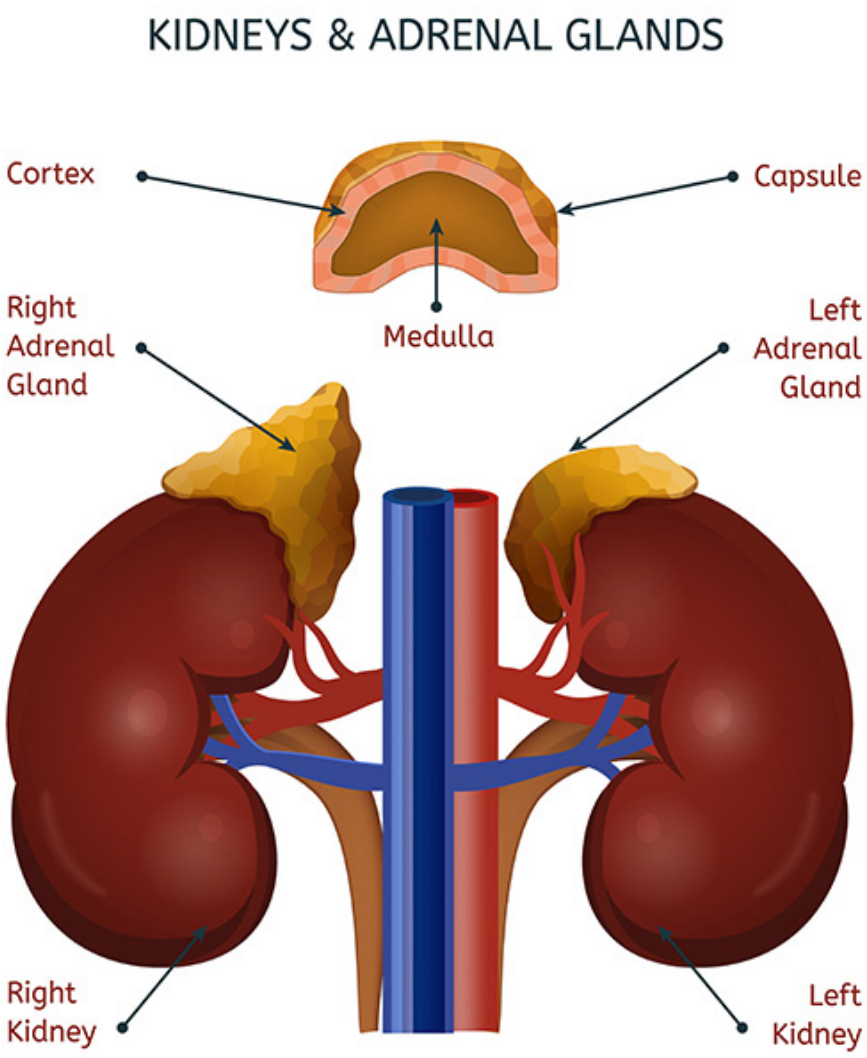


**Fig (1): Adrenal gland in the human body.**

**Historical Perspective on Adrenal Function Testing**

The rapid advances in medicine witnessed in the past 50 years have facilitated the identification of diseases involving these endocrine glands. Along with the wide spectrum of causative and concurrent pathophysiological mechanisms, practical endocrinology has responded with a vast array of sophisticated diagnostic techniques [12]. We attempt to present the evolution of the concepts of adrenal function, adrenal function tests, and their limitations from the earliest times to the present day. We discuss the impact of technical and conceptual innovations such as the introduction of radioimmunoassay, pituitary imaging, large-scale natural history studies, and quantitative epidemiological studies and evidence-based treatment approaches [13].

The evaluation of adrenal function has undergone substantial modification and refinement over the past century. The original concepts of adrenal function focused largely on the actions of the adrenal medulla and the classical mineralocorticoid effects of the adrenal cortex, which are typified by their high functional capacity and relative independence from adrenocorticotropic hormone [14]. The original clinical presentation of patients with 'adrenal insufficiency' consisted of a constellation of serious signs and symptoms often collectively referred to as 'Addison's disease,' the most extreme form of chronic hypercortisolism with concurrent mineralocorticoid deficiency. However, efforts to extrapolate the significance of the basic principles that emerged from the extensive descriptive and pathophysiological work on isolated or rare cases of chronic hypercortisolism are likely to be fallacious or oversimplified at best [15]. In essence, this was a large part of the impetus for the development of the need for more quantitative bases for the evaluation of adrenal function in health and disease. This principle certainly applies to modern practice, where the ability of a test to discriminate against the vast majority of individuals in health from those that are sick remains a standard yardstick [16].

**Biochemical Markers in Adrenal Function Testing**

The measurement of various bio-

chemical markers is crucial for the assessment of adrenal function. Cortisol and ACTH are the most important hormones in the evaluation of adrenal function, but additional markers for the assessment of mineralocorticoid function and hyperandrogenism are also important. A long list of tests, mostly dynamic tests, is available to evaluate adrenal functions involving these markers. A widely accepted alternative to these tests for use in daily clinical practice is still lacking in some situations but has become feasible in many other situations [17]. We therefore focus on the possible individual roles of biochemical markers in the clinical setting, even in studies published before or in the ten years since the guideline for the diagnosis of adrenal insufficiency in adults [18].

As shown in Table 1, various methods have been employed to test adrenal functions. These tests include the ACTH stimulation test, the insulin tolerance test, and the dexamethasone suppression test, each of which serve different diagnostic purposes.

**Table 1: Overview of Adrenal Function Testing Methods**

| Test Method | Description | Purpose |
|---|---|---|
| ACTH Stimulation Test | Measures the adrenal glands' response to ACTH | Assess adrenal hyperfunction and reserve capacity |
| Insulin Tolerance Test | Stimulates cortisol release by inducing hypoglycemia | Gold standard for diagnosing adrenal insufficiency |
| Dexamethasone Suppression Test | Involves giving dexamethasone and measuring cortisol levels | Differential diagnosis of hypercortisolism |
| Salivary Cortisol | Measures cortisol levels in saliva, which fluctuate diurnally | Diagnoses hypercortisolism helps track cortisol rhythm |
| Plasma Aldosterone and Renin | Plasma levels of aldosterone and renin | Evaluate mineralocorticoid function and the renin-angiotensin-aldosterone system |
| Imaging (CT, MRI) | Advanced imaging techniques to visualize adrenal lesions | Identify incidentalomas, differentiate functional from nonfunctional lesions |

**Cortisol**

The measurement of cortisol in blood or saliva can be used for the diagnosis of adrenal insufficiency as well as an indicator of hyperfunction. It is usually measured in the morning or at diurnal levels. In healthy individuals, cortisol concentrations show a strong diurnal rhythm in blood and saliva [19]. These profiles differ, however, owing to different mechanisms of suppression in the parotid gland after the intake of food, which is typically used for salivary cortisol measurements. Additionally, the cortisol-binding globulin and albumin concentrations are different in blood and saliva; this may lead to slightly different concentrations in blood and saliva. The loss of salivary cortisol in dental plaque should also be considered, as should the technical and sampling aspects of saliva, to avoid derivation. Despite these differences, an increased concentration of salivary cortisol is a good marker for the diagnosis of overt hypercortisolism but should be confirmed with plasma cortisol measurements [20].

**Aldosterone**

Although aldosterone can be measured as random or 24-hour urine concentrations, measuring plasma aldosterone together with renin indicates the renin–angiotensin–aldosterone system, which is regulated by ACTH. Plasma aldosterone concentrations are relatively constant throughout the day and can be measured from a single plasma value [21]. There have been a number of assays available to measure plasma aldosterone concentrations directly, with increasing specificity and higher discrimination related to concentrations, owing to the widely available and preferred newer diagnostic methods [22][23].

**Endocrine Disorders of the Adrenal Glands**

Many endocrine disorders of the adrenal glands result in some degree of cortical insufficiency due to their disease pathology. The main focus of this review will be etiologies, diagnostic criteria, and testing for primary adrenal cortical failure, Cushing's syndrome, and adrenal hyperplasia. Adjunct aspects of adrenal testing involve the associations between disease and cortisol-producing adenomas and cancer

since they mimic Cushing's syndrome and the hypertensive encouragement that adrenal hyperplasia exerts. Isolated and subsequent mineralocorticoid hormone-deficient disturbances are recommended for other papers [24].

Disorders stemming from adrenal gland dysfunction: i) adrenal cortical insufficiency: 90% are primary Addison's disease, but atypical presentations are common; ii) multiple gland dysfunction: Cushing's syndrome of inadequacy in the production of excessive cortisol; iii) hyperplasia of adrenals: aldosterone excess and secondary hyperaldosteronism or renal sodium retention; iv) nonadrenal etiologies: certain suprarenal or neurological cancers. The atypical presentations of adrenal cortical insufficiency are critical for recognition in clinical practice because early, appropriate therapy can be lifesaving [25]. The etiologies vary from country to country, which might account in part for the vast differences in public health detection of the disease. The vulnerability of young females has likewise changed from tuberculosis to having an autoimmune origin in the West. The exact history must be excluded, particularly recent medications. Diagnosis is usually straightforward by atypical signs, basal serum cortisol, and provocative tests. Controversy in diagnosis can occur on the extreme borders of these tests when a single test is used. Patients with Addison syndrome need more comprehensive hormone evaluations because of their multiple gland vulnerability [26].

**Common Adrenal Function Tests**

The ACTH stimulation test is performed to assess adrenal hyperfunction and the reserve capacity of the adrenal cortex in the event of disease. It is the only test that can identify patients at risk of developing an adrenal crisis in situations of physiological stress. The test is based on the principle that ACTH increases cortisol levels [27]. The test was conducted under basal conditions first, after which 250 μg of ACTH was added. A pretest overnight fast is mandatory. Cortisol values below 550 nmol/L (20 μg/dL) as well as absolute increases in cortisol in response to ACTH below 200 nmol/L (7 μg/dL) are considered abnormal values in adult patients suffering from primary,

secondary, or tertiary adrenal insufficiency. The insulin tolerance test is the gold standard for identifying adrenal insufficiency. When performed according to the guidelines available, it is the only test able to diagnose both primary and secondary adrenal insufficiency [28]. In developing countries, where the ITT is not easy to perform and owing to the requirement of a physician specializing in internal medicine, short ACTH tests are increasingly utilized for diagnosing adrenal insufficiency. The dexamethasone suppression test is valuable in the differential diagnosis of hypercortisolism [29][30]. The detection of increased hormone levels helps to aid in the differential diagnosis of hypercortisolism and identifies pseudo-Cushing and evidence of normal responsiveness of the hypothalamic–pituitary–adrenal axis. The measurement of hormone levels is also useful for follow-up to verify the efficacy of Cushing's syndrome treatment. A pretest overnight fast without medication that could interfere with the results is required in the event of 1 mg and 8 mg DST [31]. No patient preparation is required in the case of 16 mg DST. Cortisol levels are measured in the serum because the binding capacity or binding protein properties are altered by treatments that interfere with hormone metabolism. Cortisol levels were measured between 8:00 and 9:00 AM. Normative cortisol levels are laboratory-dependent measurements taken later in the morning [32].

As shown in Table 2, several key biochemical markers, including cortisol, ACTH, aldosterone, and renin, are used in the assessment of adrenal function.

**Table 2: Key Biochemical Markers in Adrenal Function Testing**

| Biochemical Marker | Description | Purpose |
|---|---|---|
| Cortisol | A glucocorticoid hormone produced by the adrenal cortex | Diagnosis of adrenal insufficiency, hypercortisolism |
| ACTH | Adrenocorticotropic hormone that stimulates cortisol production | Evaluate the integrity of the HPA axis |
| Aldosterone | A mineralocorticoid that regulates sodium and potassium balance | Assess adrenal function, especially in primary aldosteronism |
| Renin | A hormone that helps regulate blood pressure and fluid balance | Work in conjunction with aldosterone to evaluate the renin-angiotensin-aldosterone system |
| DHEA (Dehydroepiandrosterone) | A precursor to sex hormones, produced in the adrenal cortex | Evaluate androgen production and adrenal function |

**Future Directions and Research Opportunities**

There are several important issues where more basic science is needed [33]. The most pressing research requirement is the development and validation of simpler, easy-to-perform tests that can be standardized psychometrically. Although mass spectrometry has become more commercially accessible outside specialist referral centers, further research is needed to validate the utility of novel biomarkers to better define and subclassify adrenal function. More multicenter research trials are needed to set reference ranges across the life course [34][35]. Despite the increasing biases around sex and sex, studies on pregnant women and other more vulnerable research participants are lacking. Collaborative research that brings together endocrinologists, clinicians, nurses, psychologists, and basic and applied scientists to address these priorities is needed. Recruitment could be facilitated by the use of appropriate research networks [36]. The current unavailability of several synthetic ACTH

preparations and long-acting cortisol is a serious impediment to future clinical trials in patients with adrenal insufficiency [37].

Another area that requires further research is to define the absolute and relative levels and durations of cortisol values that predict clinical outcomes in patients with adrenal disease. This includes setting consensus guidelines for when to initiate ongoing treatment during illness that prevents ingestion or absorption of oral medication or during preterminal decline in patients with cancer. In addition to determining whether modified release medication is better than conventional medication in the long-term management of adrenal insufficiency, several medical and psychological interventions may be particularly beneficial to this cohort of first studies [38]. A research agenda made concrete steps to start resolving key clinical priorities across adrenal disorder research. The results of this research are known in their entirety to less than half of the total number of patients and clinicians involved in funded clinical care through conventional peer review publications. We now need to quantify short- and longer-term trial possibilities and begin to consider how research pertains to personalized medicine and public health [39]. Although publicly funded research informs public health medicine about improved health, well-being, and/or illness prevention, moving treatments and cognitive-induced health benefits through regulatory approval to routine national health provisions will have a direct effect on the provision and quality of care. A new global landscape of economies of health can be embraced to leverage research investment to maintain, develop, and increase the capacity of a world-class community [40].

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**Ethical Clearance**: This article is a review of previously published literature. It does not involve any studies with human participants or animals conducted by the authors. Therefore, ethical approval and informed consent were not needed.

## Conclusion

Research on adrenal function disorders has led to advancements in diag-

nostic tests and monitoring methods. New hormonal assays and imaging technologies now help track diseases such as primary aldosteronism and adrenocortical cancer. Collaboration among specialists supports multimarket testing and safer adrenal vein sampling with reduced radiation. However, key gaps remain, including the need for long-term data on daily variability diagnostic accuracy and reliable cutoff values. Patient involvement is crucial for selecting outcome measures and addressing conditions such as excess androgen that impair quality of life. Continued education and training are essential to keep pace with these rapid developments. Overall, adrenal function testing is moving toward patient-centered, widely applicable approaches even in resource-limited settings.

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