

A Physiologic Approach to Diagnosis of the Cushing Syndrome

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Clinical Principles*		Physiologic Principles
General	Neuropsychiatric	The hypothalamic–pituitary–adrenal axis
Obesity	Depression	Control of adrenal function and growth
Hypertension	Cognitive impairment	Hypothalamic control of pituitary function
Metabolic	Emotional lability	Glucocorticoid-negative feedback
Diabetes mellitus or impaired glucose tolerance	Euphoria	Circadian rhythm
Hyperlipidemia	Psychosis	The stress response
Nephrolithiasis	Gonadal dysfunction	Posttranslational processing of proopiomelanocortin to adrenocorticotrophic hormone
Polyuria	Oligomenorrhea or amenorrhea	Cortisol plasma binding, metabolism, and excretion
Skin	Impotence, decreased libido	Glucocorticoid action
Plethora	Immune suppression (susceptible to opportunistic infections)	11 β -hydroxysteroid dehydrogenase type 1 and 11 β -hydroxysteroid dehydrogenase type 2
Hirsutism		
Striae		
Acne		
Bruising		
Musculoskeletal		
Osteopenia or osteoporosis		
Proximal myopathy		

*Notice that many of the features of the Cushing syndrome resemble those of the metabolic syndrome (e.g., obesity, hypertension, impaired glucose tolerance, hyperlipidemia, hirsutism, acne, and gonadal dysfunction).

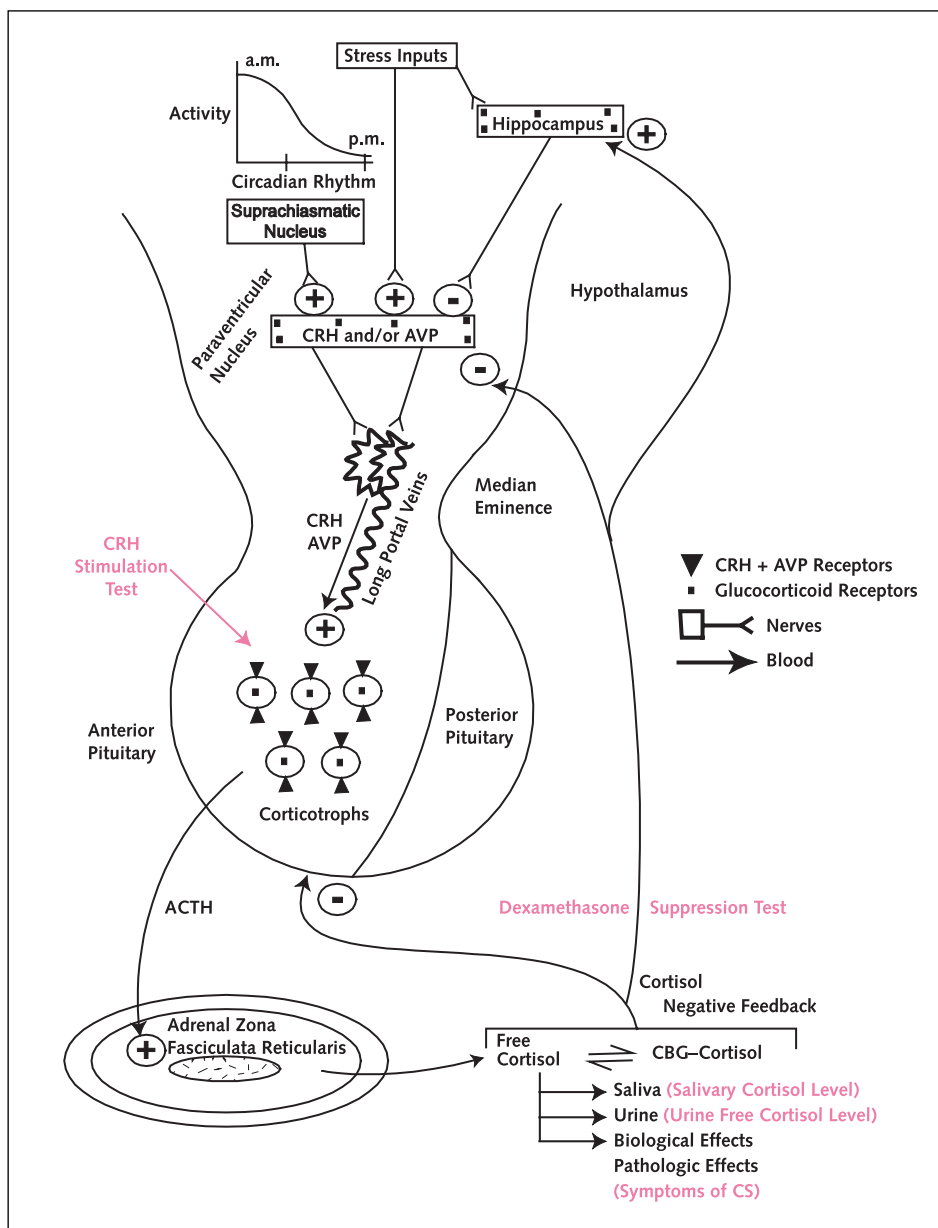
The relationship among obesity, impaired glucose tolerance or diabetes, hypertension, and gonadal dysfunction was initially recognized in two clinical syndromes described early in the 20th century. In 1932, Harvey Cushing reported these findings as well as other features of endogenous hypercortisolism in patients with small basophilic pituitary adenomas (1). A decade earlier, two French physicians, Drs. Archard and Thiers, described a similar phenotype in the syndrome that is now recognized as the syndrome of insulin resistance (the metabolic syndrome) and the polycystic ovary syndrome (2).

Recently, increasing evidence has shown that the Cushing syndrome is a reversible cause of the metabolic syndrome (3) and that it may be more common than previously thought. Some patients with incidentally discovered adrenocortical tumors have subclinical hypercortisolism and experience clinical improvement in diabetes, hypertension,

and obesity after adrenalectomy; this illustrates the importance of discovering even mild cases of the Cushing syndrome (4). In addition, two recent studies found that 2% to 3% of patients with poorly controlled type 2 diabetes mellitus may have unrecognized Cushing syndrome (5, 6).

The clinical and biochemical discrimination of true Cushing syndrome from the Cushing phenotype and the metabolic syndrome may be very difficult, particularly when hypercortisolism is mild. Since obesity, hypertension, diabetes, and lipid disorders are common in the general population, it is important for internists and other primary care physicians to be able to identify patients with the Cushing syndrome. This review demonstrates that an understanding of the physiologic characteristics of the hypothalamic–pituitary–adrenal axis is essential in formulating strategies to confirm the diagnosis of the Cushing syndrome as well as establish its cause.

Figure 1. The hypothalamic–pituitary–adrenal control system.



Neural pathways into the paraventricular nucleus of the hypothalamus can be classified as “stress” inputs (for example, hypoglycemia) directly and through the hippocampus and daily rhythm input (circadian rhythm). These inputs result in an activation of parvocellular neurons that release corticotrophin-releasing factor (*CRH*) and arginine vasopressin (*AVP*) into the capillary plexus of the median eminence, which forms long portal veins. These drain into the anterior pituitary, where *CRH* and *AVP* influence the corticotrophs to increase release of adrenocorticotrophic hormone (*ACTH*). The hormone enters the systemic circulation and stimulates the adrenal cortex to increase cortisol production. Cortisol exerts its biological effects through the glucocorticoid receptor and appears in the saliva and the urine. The hypothalamic–pituitary–adrenal axis loop is completed through glucocorticoid-negative feedback exerted at the anterior pituitary, hypothalamus, and hippocampus. Diagnostic tests associated with each physiologic process are shown in pink. CBG = corticosteroid-binding globulin; CS = Cushing syndrome.

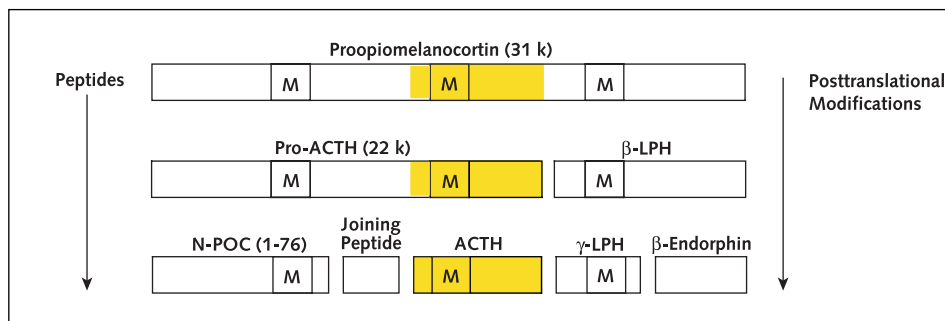
THE BASIC ORGANIZATION OF THE HYPOTHALAMIC–PITUITARY–ADRENAL AXIS

The general organization of the hypothalamic–pituitary–adrenal axis (Figure 1) has been appreciated for almost half a century. However, many recently described new concepts have significant implications for the understanding of the pathophysiologic characteristics, diagnosis, and treatment of the Cushing syndrome.

Control of Adrenal Function

The primary controller of the synthesis and release of cortisol from the adrenal zona fasciculata is adrenocorticotrophic hormone (*ACTH*) (7). This hormone exerts acute control, causing the plasma cortisol level to increase within minutes of an elevation in *ACTH* level. The main mechanism of action of *ACTH* is to activate adenylate cyclase activity, increase cytosolic cyclic adenosine 3',5'-mono-

Figure 2. Production of adrenocorticotrophic hormone (ACTH) in the anterior pituitary corticotroph cell.



The large protein proopiomelanocortin (POMC) is produced by transcription and translation of the POMC gene. Adrenocorticotrophic hormone is then produced by posttranslational processing. Note that other products of POMC can be produced (for example, γ -lipotropic hormone [*LPH*], *N*-terminal POMC fragment [*N-POC*], and melanocyte-stimulating hormone [*M*]). Ectopic ACTH-secreting tumors can perform the same processing but often produce large amounts of precursors (particularly pro-*ACTH*). Modified from Findling JW, Raff H. Ectopic ACTH. In: Mazzaferri EL, Samaan NA, eds. *Endocrine Tumors*. Cambridge, MA: Blackwell Scientific; 1993:554-66, with permission from Blackwell Science Ltd. (8).

phosphate (cAMP), and activate protein kinase A. This leads to an increase in the rate-limiting step: cholesterol transport from the cytosol across the mitochondrial membrane to provide substrate for the first enzyme in the steroidogenic pathway (P450 side-chain cleavage). At least two proteins expressed within the steroidogenic cell—steroidogenic acute regulatory protein and the peripheral-type benzodiazepine receptor—mediate this mitochondrial transport step. The mechanisms of action and control of expression of these two proteins are currently an area of intense investigation that has implications for a variety of adrenocortical disorders.

Adrenocorticotrophic hormone also exerts long-term trophic effects on the adrenal cortex. Prolonged stimulation leads to adrenal hypertrophy (as in bilateral adrenal hyperplasia), and prolonged decreases in ACTH level lead to adrenal atrophy (as in so-called secondary adrenal insufficiency and as observed with long-term glucocorticoid

therapy). It is also possible that the steroidogenic acute regulatory protein, the peripheral-type benzodiazepine receptor, or both are involved in the control of adrenocortical growth and atrophy.

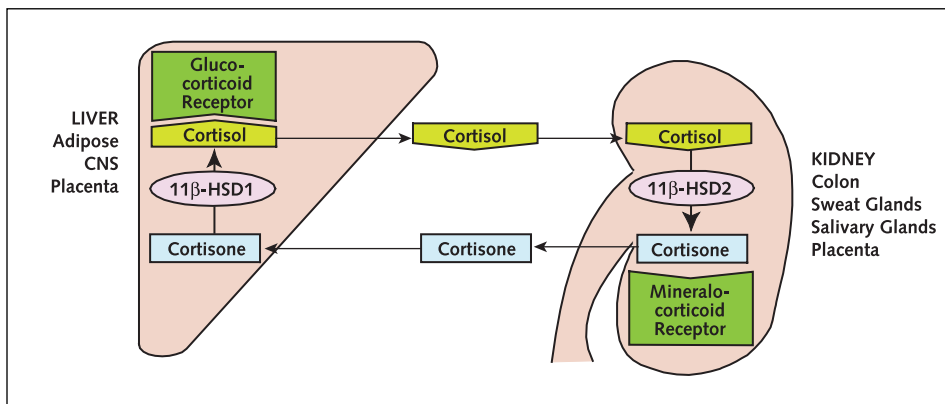
Synthesis of ACTH

Adrenocorticotrophic hormone is synthesized as part of a large precursor molecule called proopiomelanocortin (8). Along with other peptides such as lipotropin, ACTH is released from proopiomelanocortin by posttranslational processing within the pituitary corticotroph cells (Figure 2). Furthermore, nonpituitary tumors, which have undergone abnormal differentiation, can synthesize proopiomelanocortin and some or all of its posttranslational products; this explains the ectopic ACTH syndrome.

Hypothalamic–Pituitary Function

The release of ACTH from the normal anterior pituitary is controlled by stimulatory and inhibitory inputs (9–

Figure 3. The cortisol–cortisone shuttle.



The effect of cortisol on the mineralocorticoid receptor (for example, in the kidney) is prevented by metabolism to inactive cortisone by the enzyme 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2). Cortisone can be reactivated to cortisol by the enzyme 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1). When cortisol level is very high (as in the Cushing syndrome), not all of the excess cortisol can be inactivated to cortisone, and the effects of mineralocorticoid excess (for example, hypertension and hypokalemia) can occur. Modified from Seckl JR, Walker BR. Minireview: 11 β -hydroxysteroid dehydrogenase type 1—a tissue-specific amplifier of glucocorticoid action. *Endocrinology*. 2001;142(4):1371-6, with permission from The Endocrine Society (19). CNS = central nervous system.

11). Most of our knowledge about this system comes from experimental studies in animals. The primary stimulatory input is corticotropin-releasing hormone (CRH), which is synthesized in the parvocellular nerves of the paraventricular nucleus of the hypothalamus and released from their nerve terminals into capillaries in the median eminence. Arginine vasopressin (AVP) synthesized in parvocellular nerves of the paraventricular nucleus is also involved in the control of ACTH (12). This is distinct from AVP released from magnocellular nerve terminals in the posterior pituitary, which controls free water clearance in the kidney (13). Corticotropin-releasing hormone and AVP, once released into capillaries in the median eminence, drain into the anterior pituitary through the long portal veins and increase ACTH release. The other main controller of ACTH release is inhibition through glucocorticoid-negative feedback, which causes cortisol released from the adrenal to ultimately restrain its own release (10, 11). Glucocorticoid-negative feedback is the physiologic basis of the dexamethasone suppression test described later in this review.

The release of CRH (and AVP) from parvocellular neurons of the paraventricular nucleus of the hypothalamus into the portal veins is controlled by a variety of neural and hormonal inputs. The response to stress is mediated by neural inputs from several areas within the brain (9–11). For example, hypoglycemia is sensed by the hypothalamic glucose sensors, which have input into the paraventricular nucleus. In addition, the circadian rhythm of cortisol described later in this review is mediated by neural input from the suprachiasmatic nuclei of the hypothalamus. Glucocorticoid-negative feedback also suppresses CRH and AVP by direct action on parvocellular neurons. A newly appreciated mechanism of negative feedback is that glucocorticoids inhibit CRH release from the hypothalamus through input from the hippocampus, which expresses high levels of glucocorticoid receptor (10). In fact, many areas of the central nervous system with input into the hypothalamus can express high levels of the glucocorticoid receptor (14).

Circadian Rhythm

Cortisol is released episodically. The main rhythm is circadian, in which cortisol level is at its peak around the time of awakening (approximately 7:00 or 8:00 a.m.) and at its nadir at or soon after midnight (14, 15). Changes in this circadian rhythm, ranging from subtle alterations to complete disruption, are common in patients with the Cushing syndrome (15). Detection of these changes can be exploited as a diagnostic tool.

CORTISOL METABOLISM AND MECHANISMS OF ACTION

Metabolism

Cortisol circulates in the plasma both in the free (biologically active) form (approximately 5%) and while bound to corticosteroid-binding globulin and albumin (approximately 95%) (16). Cortisol is cleared from the circu-

lation primarily after conjugation to glucuronide in the liver and excretion in the urine. In addition, plasma free cortisol is filtered in the renal glomerulus and appears in the urine as free cortisol. This is the basis for using urine free cortisol level to diagnose hypercortisolism.

Mechanisms of Action

Appreciation of the distribution of the glucocorticoid receptor and its mechanism of action helps to explain the very wide range of symptoms found in patients with the Cushing syndrome. Glucocorticoid receptors are located within the cell, primarily in the cytoplasm. Binding of cortisol to the glucocorticoid receptor ultimately increases the expression of specific genes (transcription) and synthesis of new proteins (translation). A current area of investigation is the nongenomic actions of cortisol, which may be transduced through the cell membrane. The notion that steroids can activate cellular phenomena through transduction at the cell membrane is a new and exciting area, but its clinical significance is not yet clear (17). Glucocorticoid receptors in the anterior pituitary, hypothalamus, and hippocampus account for glucocorticoid-negative feedback (10, 14, 18). Glucocorticoid receptor expression throughout the brain accounts for many of the neuropsychiatric findings in the Cushing syndrome (18), and glucocorticoid receptors in muscle account for wasting. Glucose intolerance or diabetes, as well as hyperlipidemia, is explained by glucocorticoid receptor expression in muscle and adipose tissue and in the liver.

11 β -Hydroxysteroid Dehydrogenase

The mechanisms of the effects of cortisol on blood pressure, blood volume, adipose tissue, and renal function have recently been elucidated (19, 20). Another adrenal steroid, aldosterone, is the principle mineralocorticoid controlling sodium and potassium exchange in the distal nephron. The mineralocorticoid receptors in the distal nephron are responsible for this effect. Of interest, the sensitivities of glucocorticoid and mineralocorticoid receptors for cortisol *in vitro* are similar. However, small changes in aldosterone level affect sodium and potassium exchange in the kidney, despite the fact that the concentration of free (biologically active) plasma cortisol is higher. An intracellular enzyme, 11 β -hydroxysteroid dehydrogenase type 2, metabolizes cortisol to cortisone, which has much less biological activity. This protects the renal mineralocorticoid receptor from cortisol binding. However, when circulating cortisol levels are very high, as in severe Cushing syndrome, they can overwhelm this protective mechanism, allowing cortisol to bind to and activate the mineralocorticoid receptor. This newly described phenomenon explains the volume expansion, hypertension, and hypokalemia often observed in hypercortisolism of any cause.

Some tissues, like the liver, can convert inactive cortisone to cortisol through an enzyme called 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1). This com-

pletes the “cortisone–cortisol shuttle” because the kidney can inactivate cortisol to cortisone and the liver can reactivate cortisone to cortisol (Figure 3) (20).

A new area of interest is the role of 11 β -HSD1 in adipose tissue in the expression of abdominal obesity without biochemical hypercortisolism (21, 22). In a patient who had surgically proven pituitary-dependent Cushing syndrome but did not exhibit the classic phenotype of endogenous hypercortisolism, a partial defect of 11 β -HSD1 activity was recently demonstrated (21). It was hypothesized that the resultant increase in cortisol clearance protected the patient from the effects of cortisol excess. A groundbreaking study has recently been published describing a transgenic murine model resembling the metabolic syndrome in humans (22). In this model, targeted overexpression of 11 β -HSD1 led to an increase in visceral fat, presumably because of local production of active glucocorticoid within the adipocyte. This is a new area of research, and it is hoped that pharmaceutical agents targeted at adipose 11 β -HSD1 expression may ameliorate the abdominal obesity observed in the metabolic syndrome.

CLINICAL PRESENTATION OF THE CUSHING SYNDROME

The Cushing syndrome reflects the biological effects of excessive and sustained cortisol secretion or long-term glucocorticoid therapy (Figure 4). Although oral glucocorticoid therapy is the most common cause of exogenous Cushing syndrome, recent studies have shown that even intraarticular, epidural, and topical corticosteroids (inhaled, nasal, dermal) can cause features of the Cushing syndrome and suppression of the hypothalamic–pituitary–adrenal axis (23–26). Therefore, it is critical that all types

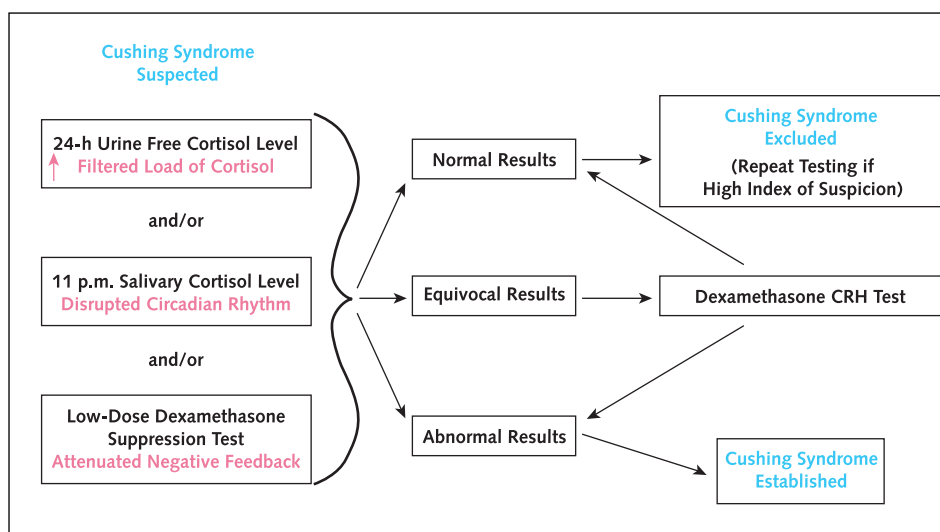
of glucocorticoid therapy be evaluated in patients with the metabolic syndrome.

The physiologic consequences of steroid excess produce a clinical syndrome and phenotype that are often indistinguishable from those of the metabolic syndrome (3, 26). Central obesity with insulin resistance, hypertension, dyslipidemia, and impaired glucose tolerance or diabetes mellitus and their sequelae of atherosclerosis and cardiovascular disease are associated with the Cushing syndrome. Facial rounding with plethora, supraclavicular fullness, osteoporosis, nephrolithiasis, neuropsychiatric problems, cutaneous wasting, or proximal myopathy are clinical findings that require evaluation for endogenous hypercortisolism in patients with the metabolic syndrome. In addition, the polycystic ovary syndrome is common in women with the Cushing syndrome. Therefore, women with the polycystic ovary syndrome should be tested to exclude endogenous hypercortisolism (27).

BIOCHEMICAL DIAGNOSIS OF THE CUSHING SYNDROME

Since correction of endogenous hypercortisolism may substantially improve the metabolic consequences of excess cortisol, even a relatively low index of suspicion should mandate at least a screening evaluation for the Cushing syndrome. Although the most appropriate diagnostic approach to patients with suspected endogenous hypercortisolism has not yet been determined, four diagnostic studies are currently used: late-night serum and salivary cortisol level, urine free cortisol level, low-dose dexamethasone suppression, and the dexamethasone–CRH test.

Figure 4. The diagnosis of endogenous cortisol excess (spontaneous Cushing syndrome).



Measurement of late-night (11:00 p.m.) salivary cortisol level and measurement of 24-hour urine free cortisol level are the initial diagnostic tests of choice. The low-dose dexamethasone suppression test must be used with caution with a stringent cutoff for serum cortisol level. If results are consistently abnormal, the Cushing syndrome is established. If the results of screening tests are equivocal, a dexamethasone–corticotropin-releasing hormone (CRH) test can be performed. The physiologic principles exploited by each test are shown in pink.

Late-Night Serum and Salivary Cortisol Level

Alteration of the circadian rhythm—from subtle changes to complete disruption—is common in all patients with the Cushing syndrome, regardless of cause (15, 25, 26). Although the morning (peak) cortisol level is often within the reference range, the cortisol level does not adequately decrease at night when cortisol is usually at its nadir. Elevated late-night cortisol level appears to be the earliest and most sensitive marker for the Cushing syndrome (25, 26). Elevated nocturnal cortisol secretion may account for disrupted sleep cycles and some of the psychological problems seen in these patients (15, 28). The measurement of an elevated endogenous cortisol level at bedtime may be very helpful in identifying patients with the Cushing syndrome.

Several recent studies have demonstrated that an elevated midnight serum cortisol level (determined by testing a blood sample drawn while the patient is sleeping) is highly accurate in differentiating patients with the Cushing syndrome from normal persons and patients with some of the common features of the Cushing syndrome (29–31). However, in most clinical situations, it is not feasible to obtain a sleeping, unstressed midnight blood sample.

Recently, interest has focused on the measurement of nighttime salivary cortisol level as a simple and effective screening test if hypercortisolism is suspected. Many studies have demonstrated that increased bedtime salivary cortisol levels yield both a sensitivity and specificity of 90% to 95% for the diagnosis of the Cushing syndrome (32–38). A recent major study of more than 140 patients found a sensitivity of 93% and a specificity of 100% (39). The use of salivary cortisol level as the primary screening test shows great promise, although more clinical experience is necessary.

The U.S. Food and Drug Administration recently cleared a new enzyme-linked immunosorbent assay for salivary cortisol (40). On this assay, most normal persons will have late-night salivary cortisol levels less than 3.0 to 4.0 nmol/L (<0.11 to 0.15 $\mu\text{g/dL}$). In our experience, late-night salivary cortisol levels consistently greater than 7.0 nmol/L (>0.25 $\mu\text{g/dL}$) are diagnostic of the Cushing syndrome, and values between 3.0 and 7.0 nmol/L (0.11 to 0.25 $\mu\text{g/dL}$) require additional biochemical confirmation. These reference ranges vary depending on the assays used (41). Collection of saliva requires special sampling tubes, which then must be sent to an appropriate reference laboratory that uses a validated method.

Urine Free Cortisol

Cortisol appears in the urine both as conjugated metabolite produced in the liver and as the free form filtered in the glomerulus. An elevated serum free cortisol level leads to an increased filtered load of cortisol in the kidney, resulting in elevated urine free cortisol excretion. The reference range for urine free cortisol level depends on the type of assay used (42). High-performance liquid chroma-

tography or gas chromatography coupled with mass spectrometry provides the best specificity for measuring urine free cortisol. The upper range of normal with these methods is 110 to 138 nmol/d (40 to 50 $\mu\text{g/d}$). High-performance liquid chromatography or gas chromatography coupled with mass spectrometry is replacing older immunoassay methods because of their higher specificity.

Urine free cortisol determination is thought to have a high diagnostic sensitivity and specificity for the Cushing syndrome. However, a recent study comparing two different methods for measuring urine free cortisol (high-performance liquid chromatography vs. a competitive protein-binding method) demonstrated that 7 of 29 patients with proven Cushing syndrome had normal urinary free cortisol levels with either method (43). Needless to say, this new observation creates considerable concern about the negative predictive value of a single urine free cortisol measurement in the diagnosis of the Cushing syndrome. It has also been shown that in 10% to 15% of patients with the Cushing syndrome, at least one of four 24-hour determinations of urine free cortisol level are within the normal range (44). Moreover, elevated urine free cortisol level has also been reported in patients with so-called pseudo-Cushing conditions, such as endogenous depression, chronic alcoholism, and eating disorders. In addition, pseudohypercortisoluria—spurious elevations of urinary free cortisol level (measured by using high-performance liquid chromatography)—has been reported in patients taking certain medications, such as carbamazepine (45) and fenofibrate (46).

Other potential confounding problems in the assessment of urine free cortisol include kidney function and possibly urine volume. Since most filtered free cortisol is metabolized (by 11 β -hydroxysteroid hydrogenase type 2) or reabsorbed, increased fluid intake resulting in increased urine volume may reduce the fraction of filtered cortisol that is metabolized or reabsorbed, thereby increasing urine free cortisol excretion (47). Conversely, patients with even mild renal functional impairment may filter less cortisol or take longer to metabolize cortisol, spuriously lowering the urine cortisol determination (48). Urine free cortisol level continues to be the best means of assessing daily cortisol secretion. However, since many conditions other than the Cushing syndrome may cause elevated urine free cortisol levels, its specificity will always be less than optimal.

Low-Dose Dexamethasone Suppression Test

Low-dose dexamethasone suppression testing was introduced more than 40 years ago as a diagnostic aid in the evaluation of patients with suspected Cushing syndrome (49). The physiologic basis of this test involved the presence of glucocorticoid receptors in the corticotroph cells of the pituitary that synthesize ACTH. Corticotroph cells normally decrease ACTH release with low doses of dexamethasone, a potent glucocorticoid that is not detected in cortisol assays. A low dose of dexamethasone is intended to

mimic pathophysiologic elevations of cortisol. However, corticotroph cells that are transformed into adenomas often lose some sensitivity to glucocorticoid-negative feedback. Because of this, ACTH release can persist despite elevated levels of circulating cortisol. Theoretically, pathophysiologic elevations of glucocorticoid activity (as in the low-dose dexamethasone suppression test) do not fully suppress ACTH release from corticotroph adenomas, and cortisol release from the adrenal gland will therefore not be adequately decreased.

The classic 2-day low-dose dexamethasone suppression test (dexamethasone, 0.5 mg orally every 6 hours for 48 hours with measurement of urine steroids) has been shown to have a sensitivity of 79%, a specificity of 74%, and a diagnostic accuracy of only 71% in patients with mild Cushing syndrome (50). The overnight 1-mg dexamethasone suppression test, which is more commonly used, also has serious problems with sensitivity and specificity (26). The overnight test requires the administration of 1 mg of dexamethasone around 11:00 p.m.; a serum cortisol level is obtained the following morning. As immunoassays for cortisol have improved in sensitivity and specificity, reported cutoff values for the suppression of cortisol in normal patients have been reported as ranging from 50 to 200 nmol/L (1.8 to 7.3 $\mu\text{g/dL}$) (51).

Recently, a consensus opinion by pathologists in the United Kingdom, which was based on available clinical data, stated that dexamethasone-induced suppression of plasma cortisol level to less than 50 nmol/L (<1.8 $\mu\text{g/dL}$) effectively excludes the Cushing syndrome (51). Since some patients with mild Cushing disease demonstrate unusual sensitivity to dexamethasone suppression, this much lower criterion should be used to provide adequate sensitivity for the test. Of course, the false-positive rate will increase, thereby decreasing the test's overall diagnostic utility. As a consequence, the low-dose dexamethasone suppression test should be used with some caution in the diagnosis of the Cushing syndrome. The traditional 2-day low-dose test can no longer be recommended. The overnight 1-mg test is useful because of its simplicity. A plasma cortisol level greater than 50 nmol/L (>1.8 $\mu\text{g/dL}$) after the overnight administration of 1 mg of dexamethasone merits further diagnostic evaluation. Recently, at an international workshop on diagnosis, complications, and treatment of the Cushing syndrome in Portonova, Italy (which one of the current authors attended), the application of this stringent cutoff to safely exclude the Cushing syndrome was one of the few criteria on which experts reached unanimous consensus.

Dexamethasone-CRH Test

The dexamethasone-CRH test was designed to take advantage of the physiologic impact of glucocorticoid-negative feedback on the hypothalamic-pituitary-adrenal axis, as well as the sensitivity of the pituitary-adrenal system to hypothalamic stimulation with CRH. It is intended to help

distinguish patients with true spontaneous Cushing syndrome from those with pseudo-Cushing conditions such as depression or alcoholism (50). Dexamethasone (0.5 mg every 6 hours) is given eight times, the first dose at noon and the last dose at 6:00 a.m., before the dynamic studies are performed. Corticotropin-releasing hormone (1 $\mu\text{g/kg}$ of body weight) is then administered intravenously at 8:00 a.m., and plasma cortisol and ACTH levels are obtained at 15-minute intervals for 1 hour. A plasma cortisol level greater than 39 nmol/L (>1.4 $\mu\text{g/dL}$) measured 15 minutes after the administration of CRH correctly identifies patients with the Cushing syndrome, and levels of 39 nmol/L or less ($\leq 1.4 \mu\text{g/dL}$) are considered normal. Criteria for a normal ACTH response have not been firmly established. In our experience, patients with the Cushing syndrome usually have a peak ACTH response exceeding 3.3 pmol/L (>15 pg/mL) during the test.

The dexamethasone-CRH test has substantial limitations. It is cumbersome and difficult to execute on an ambulatory basis. In addition, most commercially available assays for cortisol do not provide the necessary sensitivity to interpret the test properly. The dexamethasone-CRH test is usually reserved for patients with equivocal results on other diagnostic tests and a high index of suspicion for the Cushing syndrome. More experience with the dexamethasone-CRH test will be necessary before its widespread use can be advocated.

DIFFERENTIAL DIAGNOSIS OF THE SUBTYPES OF THE CUSHING SYNDROME

ACTH-Dependent versus ACTH-Independent Cushing Syndrome

Once the diagnosis of the Cushing syndrome is firmly established, the next step is differential diagnosis of the subtype (Table, Figure 5). Approximately 80% of patients with spontaneous Cushing syndrome have an ACTH-secreting neoplasm (ACTH-dependent Cushing syndrome) from a pituitary tumor (Cushing disease) or nonpituitary neoplasm (ectopic ACTH syndrome). Many patients with ectopic ACTH-secreting neoplasms (particularly those with neuroendocrine tumors, such as bronchial carcinoids) may present with hypercortisolism long before there is radiographic evidence of a neoplasm (occult ectopic ACTH syndrome [8]). These subtypes of ACTH-dependent Cushing syndrome are often clinically and biochemically indistinguishable from each other, and great care and expertise are required in their differential diagnosis.

Adrenocorticotrophic hormone-independent Cushing syndrome is due to autonomous adrenal production of cortisol (adrenal-dependent Cushing syndrome) or is caused by prolonged glucocorticoid therapy. Most patients with spontaneous ACTH-independent Cushing syndrome have a solitary, benign adrenocortical neoplasm, while a minority have bilateral nodular adrenal hyperplasia. Several molecular mechanisms for bilateral nodular adrenal hyperplasia

Table. Subtypes of the Cushing Syndrome*

ACTH dependent (excess ACTH despite increased glucocorticoid-negative feedback)
ACTH-secreting pituitary tumor (Cushing disease)
Ectopic ACTH syndrome
ACTH independent (primary increase in glucocorticoid activity that suppresses endogenous ACTH)
Exogenous glucocorticoid therapy (oral, intraarticular, dermal, inhaled, nasal)
Adrenal adenoma or carcinoma
Nodular adrenal hyperplasia
Carney complex (e.g., protein kinase A mutation)
Aberrant adrenal receptor expression (e.g., GIP)
McCune–Albright syndrome (mutations of $G_s\text{-}\alpha$)

* ACTH = adrenocorticotropic hormone; GIP = glucose-dependent insulinotropic polypeptide.

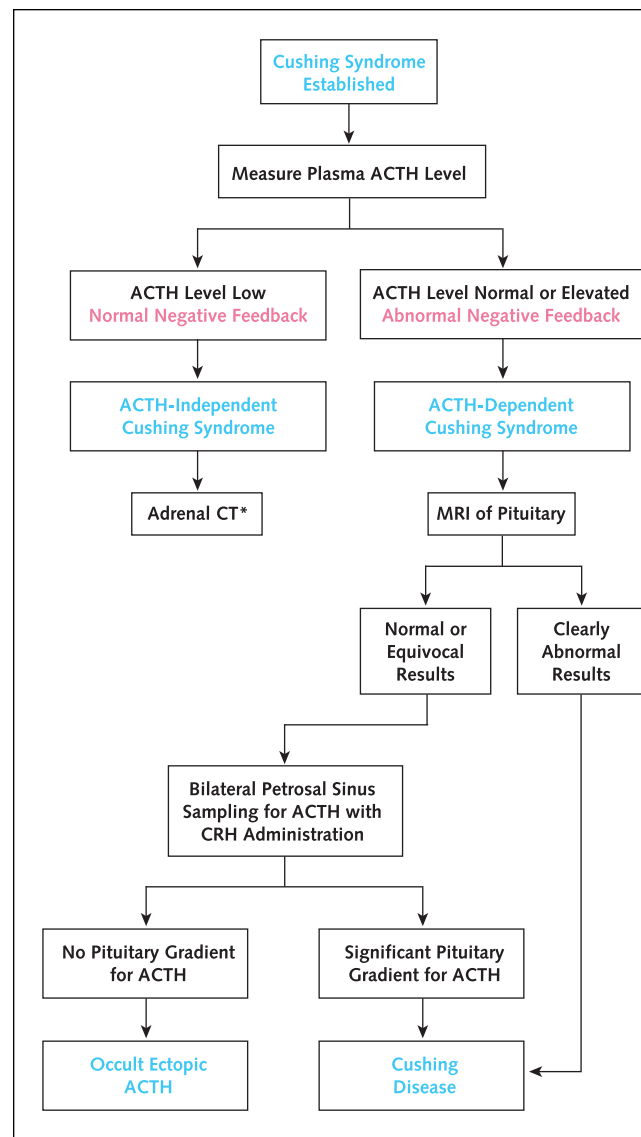
sia have recently been identified, including mutations in protein kinase A (Carney complex) (52); aberrant regulation of cortisol production by abnormal hormone receptors such as those for glucose-dependent insulinotropic polypeptide, vasopressin, and β -human chorionic gonadotropin/luteinizing hormone (β -HCG/LH) (53); or activating mutations of the receptor subunit $G_s\text{-}\alpha$ (McCune–Albright syndrome) (54). The molecular and biochemical mechanisms of nodular adrenal hyperplasia are currently being studied and are likely to provide insight into the biological characteristics of other endocrine neoplasms.

Measurement of ACTH

The initial step in the differential diagnosis of spontaneous Cushing syndrome requires the measurement of plasma ACTH. Two-site immunometric assays are sensitive, specific, and reliable and represent a major advance in clinical endocrinology over the past 15 years (55, 56). A suppressed ACTH concentration (<1.1 pmol/L [<5 pg/mL]) indicates adrenal-dependent Cushing syndrome caused by classic glucocorticoid-negative feedback both at the hypothalamus (to decrease CRH release) and directly at the pituitary (to decrease ACTH release). However, ACTH levels may not yet be fully suppressed in patients with adrenal Cushing syndrome who experience intermittent or modest increases in cortisol secretion. This has been particularly true in some patients with incidentally discovered adrenal masses in whom a mild degree of cortisol excess has been detected. Although plasma ACTH levels greater than 4.4 pmol/L (>20 pg/mL) certainly imply an ACTH-dependent cause, values between 1.1 and 4.4 pmol/L (5 to 20 pg/mL) usually require a CRH stimulation test for differential diagnosis. Patients with ACTH-independent Cushing syndrome usually have a subnormal peak ACTH response to CRH stimulation (usually <6.6 pmol/L [<30 pg/mL]). Such findings strongly suggest adrenal autonomy, and noncontrast computed tomography of the adrenal glands can usually distinguish a solitary adrenocortical neoplasm from bilateral nodular adrenal hyperplasia.

Normal or elevated plasma ACTH levels indicate an

ACTH-secreting neoplasm. Both pituitary and nonpituitary ACTH-secreting tumors may retain some sensitivity to glucocorticoid-negative feedback. As ACTH level increases early in the disease, its trophic effects increase the size of the zona fasciculata so that the adrenal gland can produce more cortisol with less ACTH. The increase in cortisol restrains the growth of the neoplasm and the se-

Figure 5. The differential diagnosis of the Cushing syndrome.

Once the diagnosis is established (see Figure 4), measurement of a suppressed plasma level of adrenocorticotropic hormone (ACTH) identifies ACTH-independent (adrenal) Cushing syndrome. Adrenal computed tomography (CT) is then performed, and a more detailed analysis is needed to differentiate among the subtypes of adrenal Cushing syndrome. The most challenging problem is the differential diagnosis of ACTH-dependent Cushing syndrome. The high-dose dexamethasone suppression test is no longer recommended. If the results of magnetic resonance imaging (MRI) of the pituitary are clearly abnormal, referral to a neurosurgeon is appropriate. If not, bilateral petrosal sinus sampling with administration of corticotropin-releasing hormone (CRH) is performed. This method reliably distinguishes pituitary Cushing disease from occult ectopic ACTH syndrome. * For a more thorough discussion, see text.

cretion of ACTH, but not completely. As the pituitary or nonpituitary tumor slowly grows (despite increased cortisol levels), ACTH level increases a bit more; however, the plasma level may certainly be within the reference range. Plasma ACTH levels are generally higher in patients with the ectopic ACTH syndrome than in patients with Cushing disease, but there is considerable overlap between these two disorders.

DIFFERENTIATION OF PITUITARY AND ECTOPIC ACTH-DEPENDENT CUSHING SYNDROME

The differentiation of pituitary and ectopic ACTH-dependent Cushing syndrome is a diagnostic challenge with several points of controversy. However, newer tests have made this process much more reliable.

High-Dose Dexamethasone Suppression Testing

High-dose dexamethasone suppression testing, much like its low-dose counterpart, was introduced over 40 years ago for the differential diagnosis of the Cushing syndrome (49). However, when this test was introduced, the ectopic ACTH syndrome had not even been described.

The physiologic theory behind the test is as follows. Adrenocorticotrophic hormone-secreting pituitary adenomas that cause Cushing disease arise from a normal corticotroph cell, leading to a monoclonal benign neoplasm. Because of these tumors' cellular lineage, glucocorticoid receptor is still expressed on them. Theoretically, if a high enough dose of dexamethasone were administered, the corticotroph tumors would decrease release of ACTH, leading to a suppressed cortisol level in the plasma or urine. However, this differentiation is not reliable. Some occult ectopic tumors have also been shown to be sensitive to glucocorticoid-negative feedback because of expression of glucocorticoid receptors (8). This phenomenon is particularly common in some neuroendocrine tumors, such as bronchial carcinoids. High-dose dexamethasone suppression testing yields a diagnostic sensitivity, specificity, and accuracy of only 80% when the pretest probability of pituitary Cushing syndrome is at least 90% (25, 26). We recently demonstrated that the results of high-dose dexamethasone suppression testing in patients with the ectopic ACTH syndrome are similar to those in patients with pituitary Cushing syndrome (57). Thus, this diagnostic approach can no longer be recommended.

CRH or Desmopressin Stimulation Test

Initial experience with exogenous CRH suggested that it could be used to differentiate between pituitary and ectopic ACTH-dependent Cushing syndrome (58). The physiologic theory is similar to the theory behind high-dose dexamethasone suppression testing, that is, that pituitary adenomas, because of their cellular lineage, still express the CRH receptor. However, ectopic tumors are not pituitary cells and would not be expected to respond to CRH. In contrast, some occult ectopic tumors express the

CRH receptor and do respond to CRH (59). It has also been suggested that desmopressin, the vasopressin V₂ agonist used to treat diabetes insipidus, could be used by following a similar receptor hypothesis (60). However, the use of desmopressin alone is not an improvement over CRH, and neither test provides adequate information to justify its use in the differential diagnosis of ACTH-dependent Cushing syndrome.

Pituitary Magnetic Resonance Imaging

New imaging techniques have been very promising but have also led to some overly optimistic expectations. Magnetic resonance imaging (MRI) of the pituitary gland with gadolinium enhancement should be performed in patients with ACTH-dependent Cushing syndrome. This procedure will demonstrate a discrete adenoma in approximately 35% to 60% of patients (61–63). When an unequivocal pituitary adenoma (>5 mm) is identified with MRI, further diagnostic evaluation may not be needed and referral to a pituitary neurosurgeon can be recommended. However, it should be remembered that at least 10% of the population 20 to 40 years of age have incidental tumors of the pituitary gland demonstrated on MRI (64). In our experience, 15% of patients with the ectopic ACTH syndrome have abnormal results on MRI of the pituitary. In patients without an unequivocal pituitary neoplasm on MRI or those whose clinical presentation (for example, male sex, hypokalemia, or rapid onset of symptoms) might suggest a nonpituitary neoplasm, a more definitive study is needed for differential diagnosis.

Inferior Petrosal Sinus Sampling

Bilateral simultaneous inferior petrosal sinus sampling for ACTH has emerged as the most reliable means of distinguishing between pituitary and nonpituitary ACTH-dependent Cushing syndrome (65, 66). This method takes advantage of the means by which anterior pituitary hormones such as ACTH reach the systemic circulation. Blood leaves the anterior lobe by numerous small hypophyseal veins that empty directly into the lateral adenohypophyseal veins. These converge into the confluent pituitary veins on the surface of the pituitary gland and then course laterally to join the cavernous sinus on the same side. The cavernous sinuses empty into the inferior petrosal sinuses, which course posteriorly and caudally from the cavernous sinuses to the end of the jugular bulbs at the base of the skull. A skilled invasive radiologist can safely catheterize each inferior petrosal sinus and, in some centers, the cavernous sinuses (67, 68). Blood samples are obtained from each inferior petrosal sinus and a peripheral vein in the basal state and at 2 or 3, 5, and 10 minutes after CRH (1 µg/kg) is administered intravenously. Ratios of right and left inferior petrosal sinus to peripheral ACTH are then calculated at each time point. A ratio greater than 3.0 after the administration of CRH is consistent with Cushing disease. Patients with the ectopic ACTH syndrome will have a ratio less than 2 before and after CRH

administration because endogenous hypercortisolism suppresses pituitary ACTH release through negative feedback.

Inferior petrosal sinus sampling has also been used to localize pituitary microadenomas associated with Cushing disease. An interpetrosal sinus ratio greater than 1.4 is consistent with ipsilateral localization of a corticotroph microadenoma. The rate of localization has been reported to range from 70% to 90% in most series. In fact, tumor localization by inferior petrosal sinus sampling has, in experienced hands, been shown to be more reliable than pituitary MRI (61, 69). More research is needed to confirm the utility of inferior petrosal sinus sampling in the lateralization of pituitary tumors.

Inferior petrosal sinus sampling has been associated with comorbid and even fatal complications, including deep venous thrombosis, pulmonary emboli, and brain stem vascular damage (70–73). In a series of more than 300 patients, we found that inferior petrosal sinus sampling was associated with one episode of deep venous thrombosis and no neurologic events. We strongly advocate the use of intravenous heparin during the procedure to help prevent thrombosis. Since the procedure is technical and invasive, experienced teams will have better results and fewer complications.

SUMMARY

As the metabolic syndrome has emerged as an important public health problem, the number of patients with a Cushing phenotype has increased. Determination of late-night salivary cortisol level is the simplest means of screening patients with suspected hypercortisolism. Repeated measurements of cortisol secretion (urine free cortisol or late-night salivary cortisol levels) over an extended period may be needed to establish a diagnosis. The low-dose dexamethasone suppression test (overnight 1-mg test) may be useful in some patients. Finally, the dexamethasone-CRH test is a reasonable approach in patients with equivocal data.

Reliable plasma ACTH measurements, sensitive pituitary and adrenal imaging studies, and inferior petrosal sinus ACTH sampling with CRH stimulation have provided the diagnostic tools necessary to establish the cause of the Cushing syndrome. Understanding the physiologic characteristics of the hypothalamic–pituitary–adrenal axis as well as the protean manifestations of excessive cortisol secretion is essential for formulating a diagnostic approach to patients with suspected Cushing syndrome. Appreciation of the subtleties of the different causes of the syndrome and the variety of modern, physiologically based tests available for diagnosis is likely to increase the detection and successful treatment of this challenging group of disorders.

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