Utility of Salivary Cortisol Measurements in Cushing's Syndrome and Adrenal Insufficiency

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Context: The measurement of cortisol in saliva is a simple, reproducible, and reliable test to evaluate the normal and disordered control of the hypothalamic-pituitary-adrenal (HPA) axis. There are a variety of simple methods to obtain saliva samples without stress, making this a robust test applicable to many different experimental and clinical situations.

Evidence Acquisition: Ovid Medline and PubMed from 1950 to present were searched using the following strategies: [<saliva or salivary>and<cortisol or hydrocortisone>and<Cushing or Cushing's>] and [<saliva or salivary>and<cortisol or hydrocortisone>and<adrenal insufficiency or hypoadrenalism or hypopituitarism or Addison's disease>]. The bibliographies of all relevant citations were evaluated for any additional appropriate citations.

Evidence Synthesis: Measurement of an elevated late-night (2300 to 2400 h) salivary cortisol has a greater than 90% sensitivity and specificity for the diagnosis of endogenous Cushing's syndrome. Late-night salivary cortisol measurements are also useful to monitor patients for remission and/or recurrence after pituitary surgery for Cushing's disease. Because it is a surrogate for plasma free cortisol, the measurement of salivary cortisol may be useful during an ACTH stimulation test in patients with increased plasma binding protein concentrations due to increased estrogen, or decreased plasma binding protein concentrations during critical illness. Most reference laboratories now offer salivary cortisol testing.

Conclusions: It is expected that the use of the measurement of salivary cortisol will become routine in the evaluation of patients with disorders of the HPA axis. (***J Clin Endocrinol Metab*** **94: 3647–3655, 2009)**

One of the hallmarks of the hypothalamic-pituitary-adrenal (HPA) axis is its central role in the stress response (1). As a result, clinical evaluation of the HPA axis is predicated on the notion that biochemical assessment should be performed in a low-stress environment, particularly when increases in cortisol secretion are suspected. In most situations, a typical blood draw does bring with it some degree of stress, particularly in children. Therefore, less invasive techniques have been developed, including the 24-h collection of urine for the measurement of free cortisol. A major advance in assessing the dynamics of the HPA axis in a low-stress environment is the measurement of salivary cortisol.

Salivary cortisol measurements have been in use since the 1960s. The majority of studies published using salivary cortisol involved its application to studying the stress response and psychoneuroendocrinology, rather than for the clinical evaluation of patients (2, 3). An Ovid Medline search of <saliva or salivary>and<cortisol or hydrocortisone> for 1950 to March 2009 yielded 2433 citations, including 1247 citations with a further focus on "stress." It is beyond the scope of this review to discuss the large

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Abbreviations: CBG, Corticosteroid-binding globulin; HPA, hypothalamic-pituitary-adrenal; LC/TMS, liquid chromatography/tandem mass spectrometry.

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number of studies that used salivary cortisol measurements to examine the stress response. Suffice it to say that acquiring samples of saliva for the analysis of cortisol is a robust and accessible technique that can be applied to almost any experimental scenario.

The Ovid Medline search described above was refined to focus on salivary cortisol and Cushing's syndrome, yielding 104 citations, and on salivary cortisol and adrenal insufficiency, yielding 25 citations. Recently, there has been a rapid increase in interest in the use of salivary cortisol measurements for the diagnosis of disorders of the HPA axis highlighted by several recent reviews (4–11). This Clinical Review will focus on newer studies using salivary cortisol measurements in the diagnosis of Cushing's syndrome and in the evaluation of adrenal insufficiency.

Methodological Considerations

The biologically active component of the HPA axis is plasma free cortisol that is in equilibrium with salivary cortisol (8). There are a variety of approaches to obtain saliva for the measurement of cortisol, including expectoration, passive drool, aspiration (particularly in infants), and chewing on a cotton or polyester swab using commercial devices (8). The method of obtaining saliva can affect the assessment of steroids including cortisol, so the reference ranges established should be internally consistent with the sampling method (12–15). Saliva obtained from a commercially available cotton sampling device like the Salivette, which is easy to use and transport, appears to give salivary cortisol results that are very reliable predictors of total and free plasma cortisol levels (16).

There are several potential preanalytical errors in acquiring samples for salivary cortisol including contamination of the sample by the patient with over-the-counter hydrocortisone creams and ointments. Remember that hydrocortisone is authentic cortisol and cannot be distinguished from endogenous cortisol, even using analysis with chromatography and mass spectrometry. Also, the timing of the sampling is usually critical, and it is documented that compliance with this requirement can be less than optimal (17). Because patients often obtain saliva samples at home, the clinician should always carefully explain to the patient the importance of the timing of the sample as well as confirm that sampling was done correctly when the results are interpreted. It is fortunate that cortisol is stable in saliva at room temperature for at least a week, and samples can be mailed to the laboratory by standard postal service (18–20). Although blood contamination of saliva may alter other analyte concentrations, there seems to be no or a minimal effect on the measurement of salivary cortisol (21).

There are several methods for the measurement of cortisol in saliva. These include RIA, ELISA, platform immunoassays, and liquid chromatography/tandem mass spectrometry (LC/TMS) (4, 8, 11, 15, 22–26). Each approach has its pluses and minuses. In actuality, under most circumstances, any of these assay methods generally performs well. The advantages of immunoassays are that they detect many cortisol metabolites, are simple and inexpensive to run, and require small volumes of saliva. The disadvantage is the potential to cross-react with synthetic steroids like prednisone. LC/TMS avoids this problem. However, TMS may miss the patient with factitious Cushing's syndrome unless synthetic steroids are analyzed in every sample. Occult factitious Cushing's syndrome was detected in samples referred to my laboratory because of very high salivary cortisol levels detected by ELISA that were subsequently confirmed by LC/TMS for synthetic steroids and by the clinicians reinterviewing the patients. It is also important to point out again that TMS cannot distinguish between endogenous cortisol and contamination from hydrocortisone in over-the-counter ointments and creams, although an increased ratio of cortisol to cortisone in the sample may suggest contamination. Because immunoassays are standard in most laboratories and LC/TMS requires expensive and sophisticated equipment, immunoassays are usually acceptable as long as the caveats described above are kept in mind. Each methodology generates a different reference range, so it is vital that clinicians carefully interpret salivary cortisol results, taking into consideration the laboratory and method used particularly because there is no external proficiency-testing scheme or certified reference material currently available to harmonize assays (27).

There is a misconception that it is difficult for clinicians to obtain a salivary cortisol measurement for their patients through their clinical laboratory or institution. In fact, most, if not all reference laboratories now offer this test, although the methods used and reference ranges generated vary. Furthermore, a Food and Drug Administration-cleared ELISA, which is simple and inexpensive to perform, is commercially available (28). Therefore, there is no impediment to evaluating salivary cortisol in patients in the United States.

Cushing's Syndrome

The diagnosis of endogenous Cushing's syndrome is among the most difficult in clinical endocrinology. The phenotype is very common in the population, and the frequency of the disorder is relatively uncommon. Therefore,

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accurate, convenient, and relatively inexpensive screening tests are paramount if the large number of patients with suspected Cushing's syndrome can be evaluated. The comparison of the performance of the commonly used diagnostic tests for Cushing's syndrome has been extensively reviewed, and a detailed discussion of the comparison of these tests is beyond the scope of this review (1, 6, 7, 10, 29–34). Briefly, the standard approaches are the overnight low-dose dexamethasone suppression test, 24-h urine free cortisol, and late-night salivary cortisol measurement. All of these tests have potential pluses and minuses, but the general consensus is that late-night salivary cortisol has the highest sensitivity, whereas the low-dose dexamethasone suppression test can have the best specificity, depending on the serum cortisol cutoff used. Because these different tests evaluate different dynamic components of the HPA axis, it is advocated that they be used in concert to complement each other (4, 34).

Overt Cushing's syndrome

The circadian rhythm is another hallmark of the HPA axis. Because the failure to achieve a normal nadir between 2300 and 0100 h is characteristic of Cushing's syndrome, assessment of an elevated late-night or midnight cortisol level was initially shown to have a very high sensitivity and specificity when blood was sampled in sleeping patients under very controlled circumstances to reduce ambient stress (35, 36). This approach is highly impractical for most clinicians, so sampling for salivary cortisol has been advocated as a useful surrogate for blood sampling for cortisol.

One of the first studies demonstrating the feasibility of using salivary cortisol measurements late at night for the clinical evaluation of endogenous hypercortisolism was in 1984 by Evans *et al.* (37) who demonstrated a dampening of the circadian rhythm and an elevation of midnight salivary cortisol in patients with Cushing's syndrome. They also demonstrated that assessment of salivary cortisol in the morning sample after an overnight dexamethasone suppression test is useful. The usefulness of a combination of the measurement of late-night salivary cortisol and a morning, dexamethasone-suppressed salivary cortisol to improve specificity has been confirmed by others (38–40).

Since the studies of Evans *et al.* in the 1980s, many studies have been published demonstrating a high sensitivity and specificity of late-night salivary cortisol measurements for Cushing's syndrome (Table 1). It is important to note that the number of cases and controls, the diagnostic criteria, the types of controls, the assay methodologies, the methods used to generate cut points, and the saliva sampling techniques varied between studies listed in this table. Even so, it is remarkable that these differences do not appear to be particularly important because most studies found similar sensitivities and specificities for Cushing's syndrome of more than 90%. It should also be noted that saliva sampling is particularly useful in children suspected of Cushing's syndrome in whom blood and urine sampling can be a challenge (41, 42).

TABLE 1. Studies of late-night salivary cortisol measurement in the diagnosis of Cushing's syndrome

First author, year (Ref.)	Sensitivity	Specificity
Adults		
Luthold, 1985 (89)	100%	100%
Laudat, 1988 (69)	100%	100%
Raff, 1998 (65)	92%	97%
Castro, 1999 (39)	93%	93%
Papanicolaou, 2002 (90)	95%	100%
Putignano, 2003 (91)	93%	93%
Yaneva, 2004 (92)	100%	94%
Trilck, 2005 (93)	98%	96%
Viardot, 2005 (94)	100%	100%
Baid, 2007 (23)	ND	85%,[a] 92%[b]
Vilar, 2008 (95)	100%	ND
Restituto, 2008 (43)	88%	82%
Doi, 2008 (96)	93%	100%
Nunes, 2009 (44)	100%	100%
Cardoso, 2009 (40)	100%	98%
Yaneva, 2009 (97)	93%	94%
Children		
Martinelli, 1999 (41)	100%	95%
Gafni, 2000 (42)	93%	100%

ND, Not done.

[a] Radioimmunoassay.

[b] LC/TMS.

Since our most recent comprehensive review of the literature (4), several papers have been published that should be considered. Restituto *et al.* (43) showed a lower sensitivity and specificity for Cushing's syndrome than most other studies (Table 1). Although a precise reason for this could not be pinpointed, it is noteworthy in this study that a midnight, sleeping serum cortisol yielded a sensitivity of only 50%, which is much lower than the seminal studies originally describing this approach (35, 36). One explanation may be the use of receiver operating characteristic curves to calculate sensitivity and specificity, whereas earlier studies used reference ranges and arbitrary cutoffs to do so. It is also noteworthy that the cutoffs for midnight levels in the study of Restituto *et al.* (43) were 291 nmol/liter (10.5 μg/dl) for serum cortisol, which is higher than previously found (36), and 2.2 nmol/liter (0.08 μg/dl) for salivary cortisol, which is lower than previously found using the same assay (25, 28). There may be something different about the general approach of Restituto *et al.* (43) in addition to the use of receiver operating characteristic curves that yielded lower diagnostic utility for late-night cortisol sampling whether in blood or saliva.

One of the possible problems using late-night salivary cortisol is the potential for a lower specificity for

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Cushing's syndrome. This could be due to difficulties in reproducibility of the sample in patients with mild Cushing's syndrome as well as proximate stress in patients without Cushing's syndrome. This was recently studied by Cardoso *et al.* (40) who found an intraclass coefficient of variation of 0.89 in patients with Cushing's syndrome and 0.83 in control patients between two late-night salivary cortisol samples taken 48 h apart. This suggests that variation of two salivary cortisol measurements in the same patient is 17% or less. Considering that the assay used had an intraassay coefficient of variation of about 6%, and that normal subjects have low salivary cortisol levels at which assays generally do not perform as well as at higher concentrations, a biological variability of 17% is excellent. However, one can imagine that one of two samples could be abnormal in someone without Cushing's but with higher physiological HPA axis activity, and one of two samples could be normal in a patient with mild Cushing's syndrome. Nunes *et al.* (44) found a calculated concordance correlation coefficient by regression of approximately 0.65 for outpatients, suggesting a lower reproducibility than that of Cardoso *et al.* (40). We recently demonstrated that late-night salivary cortisol levels can be elevated in as few as 14% of the samples in patients with proven but very mild Cushing's syndrome (45). Interestingly, 24-h urine free cortisol was consistently normal in several of these patients. Only persistence and a high index of clinical suspicion can allow the accurate diagnosis of these challenging patients. It has been my experience that waiting 1 or 2 months and then repeating the salivary cortisol measurement often resolves a false-positive, discordant result.

A lower specificity of late-night salivary cortisol could also be due to other factors increasing late-night salivary cortisol in patients without Cushing's syndrome. Just about any stressor proximal to obtaining a late-night saliva sample could theoretically lead to a false-positive result, as could differences in lifestyle (46). For example, a recent study demonstrated that elderly male veterans with type 2 diabetes mellitus had an increased frequency of false-positive salivary cortisol results (47). Much of this may have been due to the population studied that included patients with difficult living situations, abnormal sleep patterns, and prior addiction problems. Another potential source of error is the venue at which the samples are obtained. Despite this concern, Nunes *et al.* (44) recently demonstrated a high concordance between late-night saliva samples obtained on the same patients in the inpatient *vs.* outpatient setting.

We recently published a meta-analysis of the performance of salivary cortisol for the diagnosis of Cushing's syndrome using the publications through 2007 shown in Table 1 (48). A total of 947 patients (339 with Cushing's syndrome) were identified in the seven studies that met the stringent criteria for meta-analysis. The sensitivity of late-night salivary cortisol for Cushing's syndrome was 92%, the specificity was 96%, and the diagnostic odds ratio was 312. This analysis establishes late-night salivary cortisol measurement as one of the primary methods of choice in general clinical practice to screen any patient suspected of Cushing's syndrome.

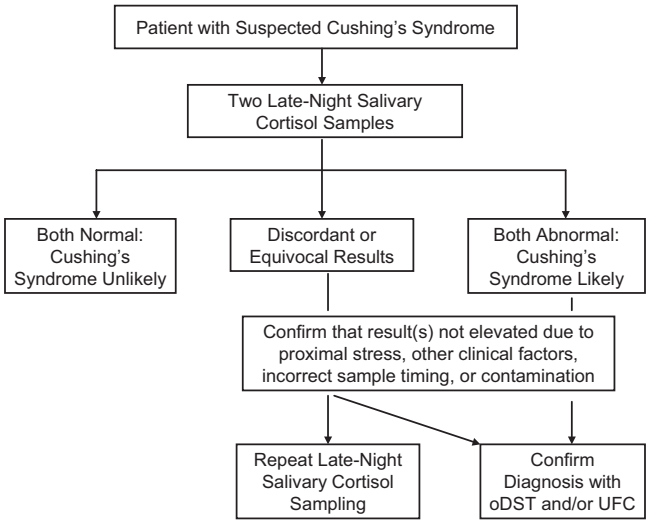

FIG. 1. A paradigm using salivary cortisol as the initial test to evaluate patients with suspected Cushing's syndrome (adapted from Ref. 4). oDST, Overnight 1 mg dexamethasone suppression test; UFC, 24-h urine free cortisol.

It is recommended that two late-night salivary cortisol tests be performed 24–48 h apart as the initial testing for Cushing's syndrome (Fig. 1). If they are both normal, Cushing's syndrome is excluded with about 90–95% certainty. If they are both above the reference range, Cushing's syndrome is proven with about 90–95% certainty. However, before a patient is subjected to a differential diagnostic workup, it is prudent to confirm the diagnosis with complementary testing such as the dexamethasone suppression test and 24-h urine free cortisol measurement. It is also prudent to carefully interview the patient for any factors that could have caused a physiological increase in cortisol release as well as to confirm that the samples were obtained at the correct time and without contamination. If the two salivary cortisol results are discordant, they can be repeated, or complementary testing can be performed.

Subclinical Cushing's syndrome

With the advent of CT scanning for general abdominal complaints, it has been found that adrenal neoplasms are common and some actually secrete cortisol autonomously. The Cushingoid signs and symptoms are usually few or absent, and only biochemical testing reveals subtle

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increases in cortisol secretion. This so-called subclinical Cushing's syndrome is now well documented, although there does not appear to be a consensus as to how this syndrome is defined (49–53). As one might expect, the performance of any test (including salivary cortisol) in the evaluation of subclinical Cushing's syndrome is less effective than in the diagnosis of overt Cushing's syndrome. The cutoff for an abnormal late-night salivary cortisol concentration had to be lowered, and, even then, the sensitivity and specificity was only about 90% in one study, which also showed that the overnight 1 mg dexamethasone suppression test may be an alternative of choice (44). A recent study, which also used other abnormal endocrine tests to establish subclinical Cushing's syndrome, found a much poorer performance with a sensitivity of only 23% and a specificity of 88% (54). Therefore, a normal late-night salivary cortisol concentration clearly does not rule out subclinical Cushing's syndrome (55).

Postsurgical follow-up

It is well known that recurrence of pituitary tumors in patients with Cushing's disease after pituitary surgery is fairly common (56). Periodic late-night salivary cortisol assessment in patients postoperatively would seem a simple and prudent approach to follow these patients. In two recent studies, an increased late-night salivary cortisol was considered a highly sensitive approach (90–100%) to detect a recurrence or surgical treatment failure in Cushing's disease (Fig. 2) (44, 57). Midnight salivary cortisol measurement performed as well as midnight plasma cortisol and is clearly easier to obtain. More importantly, 24-h urine free cortisol measurements had several false-negative results, confirming its lack of sensitivity for mild hypercortisolism (Fig. 2).

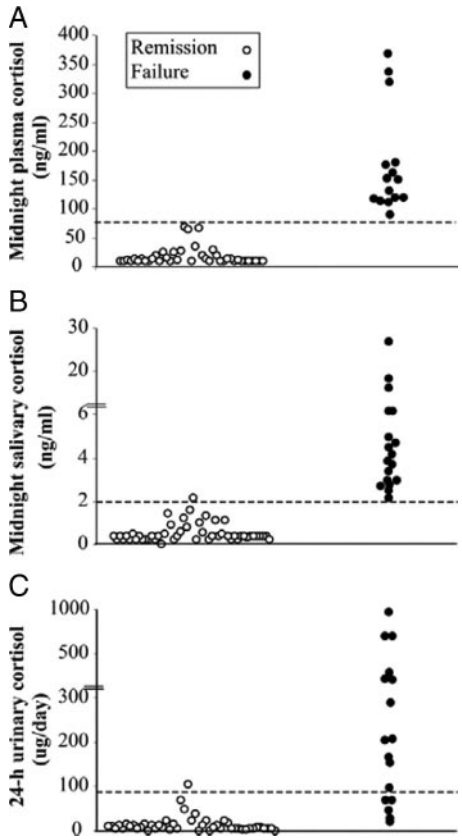

FIG. 2. A, Midnight plasma cortisol; B, midnight salivary cortisol; and C, 24-h urine free cortisol in postsurgical patients with Cushing's disease in remission compared with surgical failures. Each *circle* represents one patient. The *dashed line* corresponds to the cutoff of 75 ng/ml (207 nmol/liter) for midnight plasma cortisol (A), 2 ng/ml (5.52 nmol/liter) for midnight salivary cortisol (B), and 90 μg/24 h (248 nmol/24 h) for urinary cortisol excretion (C). From Ref. 57 with permission.

Cyclical or intermittent Cushing's syndrome

There are patients that, for pathophysiological reasons that are often unclear, have waxing and waning of their biochemical and clinical evidence of Cushing's syndrome (58–61). The assessment of late-night salivary cortisol over days, to weeks, to even years has been proposed as an efficient way to evaluate these patients (58–60). Furthermore, it is recommended that salivary cortisol be assessed the night before inferior petrosal sinus sampling for ACTH in the differential diagnosis of Cushing's disease and the occult ectopic ACTH syndrome to ensure that the testing is done when the ACTH-secreting tumor is active (62).

Summary of Cushing's syndrome

The measurement of late-night salivary cortisol, usually at 2300 to 2400 h, has proven to be a very useful approach to the diagnosis of Cushing's syndrome. Saliva can be sampled by patients at home and delivered or sent to the appropriate reference laboratory. It can also be sampled in hospitalized patients, but care should be taken to minimize stress in these circumstances. Late-night salivary cortisol sampling is also useful in assessing patients with suspected cyclical Cushing's syndrome as well as to follow patients after surgical treatment to monitor remission or recurrence.

Adrenal Insufficiency

Classic adrenal insufficiency has two general forms. Primary adrenal insufficiency is due to a failure of the adrenal gland to synthesize and release cortisol, usually due to an autoimmune or infectious process, and is also called Addison's disease (63). In secondary adrenal insufficiency, the adrenal cortex does not produce adequate cortisol because of a decrease in stimulation from pituitary ACTH. This can have many endogenous causes including infec-

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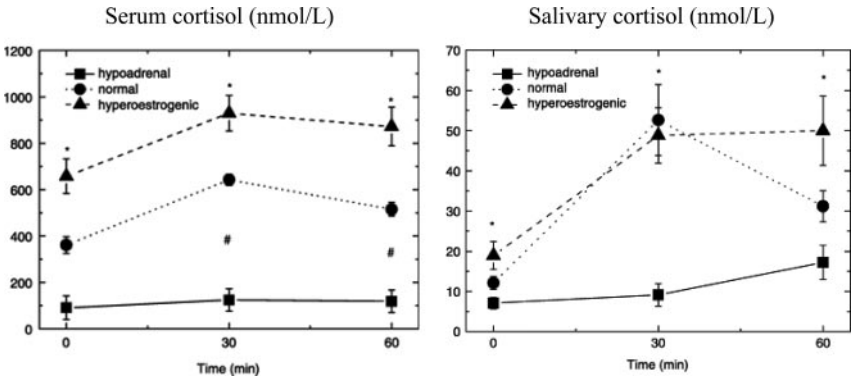

FIG. 3. Measurement of serum (*left*) and salivary (*right*) cortisol in response to the low-dose (1 μg) $ACTH_{1-24}$ in normal subjects, patients with secondary adrenal insufficiency (hypoadrenal), and patients with increased CBG due to oral contraceptives or hormone replacement therapy (hyperestrogenic). *, Hyperestrogenic women had increased cortisol levels; #, hypoadrenal patients had lower cortisol than normal subjects. From Ref. 67 with permission.

tion, autoimmune destruction, and infarction of the anterior pituitary gland (64). The most common exogenous cause is suppression of the HPA axis by exogenous steroid use (64). There are also a variety of clinical situations where HPA axis suppression or relative suppression is a concern, such as in critical illness. Although, in general, the diagnosis of frank adrenal insufficiency is not as challenging as Cushing's syndrome, salivary cortisol measurements have been used to evaluate the HPA axis in situations of suspected decreased adrenal function.

Morning salivary cortisol

Because cortisol release peaks in the morning during the HPA axis circadian rhythm, it is theoretically possible that assessment of a subnormal morning cortisol might be diagnostic for adrenal insufficiency. The problem with morning cortisol assessment is the wide range of morning serum and salivary cortisol in normal subjects (43, 65, 66). Not surprisingly, measurements of basal morning levels of salivary cortisol alone are not useful for the diagnosis of primary or secondary adrenal insufficiency (67). The sensitivity and specificity of the measurement of an 0800 h serum or salivary cortisol for primary adrenal insufficiency is less than 35% (43). Therefore, the measurement of morning salivary cortisol is not particularly useful by itself in the diagnosis of adrenal insufficiency.

ACTH stimulation test

The standardized ACTH stimulation test using synthetic $ACTH_{1-24}$ (cosyntropin, tetracosactrin) is the gold standard for evaluation of primary or secondary adrenal insufficiency (68). Briefly, the concept is that pharmacological stimulation of the adrenal gland will reveal either a primary decrease in adrenocortical function, or a decrease in adrenal sensitivity to ACTH due to prolonged loss of a corticotropic stimulus in secondary adrenal insufficiency. In the typical patient being evaluated as an outpatient, there is no theoretical advantage to saliva *vs.* serum sampling because proximal stress from blood drawing is not particularly relevant. The early studies of Laudat *et al.* (69) demonstrated that there was complete separation between ACTH-stimulated salivary and serum cortisol in patients with primary or secondary adrenal insufficiency compared with normal subjects. Because salivary cortisol is a much better estimate of serum free cortisol than serum total cortisol, measurement of salivary cortisol is particularly useful in patients with corticosteroid-binding globulin (CBG) deficiency (26) or with increased CBG levels found in pregnancy and in women on oral contraceptives (67, 70). However, Deutschbein *et al.* (71) recently demonstrated that assessment of salivary cortisol did not perform as well as serum cortisol in the diagnosis of adrenal insufficiency during a standard ACTH stimulation test.

A modified, low-dose ACTH stimulation test has been advocated as an improved test primarily because it assesses adrenal sensitivity rather than maximum secretory capacity (72). Although the merits of this test are controversial (73–75), several investigators have attempted to use the measurement of salivary cortisol to assess the adrenal response to low-dose synthetic ACTH. It has recently been demonstrated that measuring salivary cortisol is useful during a low-dose ACTH stimulation test in children and adult patients with secondary adrenal insufficiency (Fig. 3) (67, 76). Also notice in Fig. 3 that salivary cortisol measured 30 min after injection of ACTH was able to demonstrate normal adrenal function in hyperestrogenic women with increased serum cortisol, presumably due to increased serum binding proteins. Finally, Contreras *et al.* (77) developed a low-dose im ACTH stimulation test with assessment of salivary cortisol that does not require venous access. One can imagine, then, the ACTH stimulation test being performed in a variety of venues with the only requirements being an im injection of ACTH and the ability to sample saliva.

Monitoring glucocorticoid replacement

Another challenge in clinical endocrinology is how to monitor patients with adrenal insufficiency for adequate but not overreplacement of exogenous cortisol (hydrocortisone) therapy. In general, it has been found that monitor-

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ing salivary cortisol does not add significantly to the evaluation of adequate glucocorticoid replacement (78–82).

Critical illness

A major controversy spanning several specialties is the overuse of glucocorticoids in critically ill patients (83). Most of these patients do not have frank adrenal insufficiency, but they may have a lower total plasma cortisol level than expected for the degree of illness in part due to lower plasma binding proteins including CBG (84). Arafah *et al.* (85) have demonstrated that ACTH-stimulated salivary cortisol release correlated well with serum free cortisol levels in critically ill patients. We recently demonstrated that ACTH-stimulated salivary cortisol measurements can help avoid the unnecessary use of glucocorticoid therapy in hospitalized patients (86). Other examples of the usefulness of salivary cortisol measurements in chronic illness are in the evaluation of patients with end-stage renal disease and HIV infection (87, 88).

Summary of adrenal insufficiency

A random morning salivary or serum cortisol measurement is not a particularly useful method to make the diagnosis of adrenal insufficiency of any etiology. The measurement of salivary cortisol during an ACTH stimulation test may have some advantages over serum, particularly in patients with an increase in CBG levels due to pregnancy or estrogen therapy and decreased CBG levels due to critical illness. An exciting application of this approach is im ACTH injection with salivary cortisol measurement to evaluate adrenal function in the field. It may even be possible some day to measure salivary cortisol after ACTH injection in climbers on Mount Everest.

Summary

The advent of assay methods to measure steroids in small volumes of saliva has led to many new studies that have evaluated salivary cortisol for the diagnosis of pituitary-adrenal disorders. Clearly, the use of late-night salivary cortisol measurements in the diagnosis of Cushing's syndrome shows great promise and will probably supplant older more cumbersome testing over the next decade. The use of salivary cortisol measurements in the diagnosis of adrenal insufficiency is not as established and probably does not offer a major advance except in situations where venous sampling is difficult or plasma binding proteins are altered. It will be exciting to see many more studies evaluating this technology, and it is expected that the measurement of salivary cortisol will soon become routine in clinical practice for the evaluation of disorders of the HPA axis.

Acknowledgments

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