0021-972X/98/$03.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 83, No. 8
Printed in U.S.A.

Late-Night Salivary Cortisol as a Screening Test for Cushing's Syndrome*

HERSHEL RAFF, JONATHAN L. RAFF, AND JAMES W. FINDLING

Endocrine Research Laboratory and the Endocrine-Diabetes Center, St. Luke's Medical Center, Medical College of Wisconsin, Milwaukee, Wisconsin 53215

ABSTRACT

The clinical features of Cushing's syndrome (such as obesity, hypertension, and diabetes) are commonly encountered in clinical practice. Patients with Cushing's syndrome have been identified by an abnormal low-dose dexamethasone suppression test, elevated urine free cortisol (UFC), an absence of diurnal rhythm of plasma cortisol, or an elevated late-night plasma cortisol. Because the concentration of cortisol in the saliva is in equilibrium with the free (active) cortisol in the plasma, measurement of salivary cortisol in the evening (nadir) and morning (peak) may be a simple and convenient screening test for Cushing's syndrome. The purpose of this study was to evaluate the usefulness of the measurement of late-night and morning salivary cortisol in the diagnosis of Cushing's syndrome.

We studied 73 normal subjects and 78 patients referred for the diagnosis of Cushing's syndrome. Salivary cortisol was measured at 2300 h and 0700 h using a simple, commercially-available saliva collection device and a modification of a standard cortisol RIA. In addition, 24-h UFC was measured within 1 month of saliva sampling.

Patients with proven Cushing's syndrome (N = 39) had significantly elevated 2300-h salivary cortisol (24.0 ± 4.5 nmol/L), as compared with normal subjects (1.2 ± 0.1 nmol/L) or with patients referred with the clinical features of hypercortisolism in whom the diagnosis was excluded or not firmly established (1.6 ± 0.2 nmol/L; N = 39). Three of 39 patients with proven Cushing's had 2300-h salivary cortisol less than the calculated upper limit of the reference range (3.6 nmol/L), yielding a sensitivity of 92%; one of these 3 patients had intermittent hypercortisolism, and one had an abnormal diurnal rhythm (salivary cortisol 0700-h to 2300-h ratio <2). An elevated 2300-h salivary cortisol and/or an elevated UFC identified all 39 patients with proven Cushing's syndrome (100% sensitivity). Salivary cortisol measured at 0700 h demonstrated significant overlap between groups, even though it was significantly elevated in patients with proven Cushing's syndrome (23.0 ± 4.2 nmol/L), as compared with normal subjects (14.5 ± 0.8 nmol/L) or with patients in whom Cushing's was excluded or not firmly established (15.3 ± 1.5 nmol/L).

Late-night salivary cortisol measurement is a simple and reliable screening test for spontaneous Cushing's syndrome. In addition, late-night salivary cortisol measurements may simplify the evaluation of suspected intermittent hypercortisolism, and they may facilitate the screening of large high-risk populations (*e.g.* patients with diabetes mellitus). (*J Clin Endocrinol Metab* **83:** 2681–2686, 1998)

THE diagnosis of Cushing's syndrome requires biochemical verification of cortisol excess. The clinical features of endogenous hypercortisolism (in particular, weight gain with truncal obesity, hypertension, and glucose intolerance) are commonly encountered in clinical practice (1). The differentiation of patients with true spontaneous Cushing's syndrome from the large number patients with the Cushing's phenotype may often be clinically challenging, particularly if the degree of hypercortisolism is mild (2).

Biochemical screening studies for Cushing's syndrome have included low-dose dexamethasone suppression testing, urine free cortisol (UFC), assessment of diurnal rhythmicity, and (more recently) measurement of unstressed late-night serum cortisol level (3). These studies are often cumbersome and sometimes require hospitalization; and, because both false positive and false negative results are common, none of these studies are ideal screening tests (2, 3).

The disruption of the circadian rhythm has been considered a hallmark of Cushing's syndrome (2, 4). Normally, cortisol is secreted episodically with a diurnal rhythm paralleling the secretion of ACTH (4). Cortisol reaches a peak around the time of awakening and a nadir after the onset of sleep. The normal range for plasma cortisol in the morning is rather broad, and concentrations overlap with those in patients with Cushing's syndrome. Newell-Price *et al.* (3) have recently demonstrated that a single midnight serum cortisol concentration greater than 50 nmol/L (1.8 μg/dL) yielded a sensitivity of 100% for the diagnosis of Cushing's syndrome. It is neither practical nor cost effective to hospitalize patients with suspected Cushing's syndrome for 48 h or more to obtain an unstressed late-night serum cortisol level. Therefore, a method to assess adrenal function at bedtime, without disrupting a normal routine, might be a useful screening test for Cushing's syndrome.

To explore simple and convenient means for probing late-night cortisol secretion, we evaluated the concentration of cortisol in the saliva at 2300 h and 0700 h in a large group of patients with proven spontaneous Cushing's syndrome, a group of patients referred for possible Cushing's syndrome in whom other diagnostic studies excluded or did not confirm hypercortisolism, and in normal subjects. The concentration of cortisol in the saliva is in an equilibrium with free plasma cortisol and is independent of the rate of saliva production (5–7). Despite previous studies demonstrating the usefulness of the measurement of salivary cortisol to assess hypothalamic-pituitary-adrenal axis secretory activity and rhythm (5–8), it is surprising that this technique has not become more widely used. The current study provides evidence that measurement of a late-night salivary cortisol is a

Received April 7, 1998. Revised May 14, 1998. Accepted May 18, 1998.
Address all correspondence and requests for reprints to: Hershel Raff, Ph.D., St. Luke's Health Science Center, 2901 West KK River Parkway, Suite 503, Milwaukee, Wisconsin 53215. E-mail: hraff@post.its.mcw.edu.
* Supported by the Aurora Foundation.

simple and sensitive method for screening patients for spontaneous Cushing's syndrome.

Subjects and Methods

Experimental subjects

Normal subjects. Subjects (N = 73; age = 37 ± 11 SD; 35 male/38 female) were recruited from the student/staff/faculty population at the Medical College of Wisconsin, Veterans Administration Medical Center, and St. Luke's Medical Center. Confidentiality was maintained according to the Helsinki Declaration. Subjects in whom blood was sampled (cosyntropin stimulation test) gave written informed consent approved by the St. Luke's Medical Center Institutional Review Board. There were not sufficient numbers of women using oral contraceptives (N = 6) to accurately compare them with women not using oral contraceptives, so all data from normal female subjects were pooled. Subjects sampled their saliva at 2300 h and 0700 h (the following morning) after a routine evening in which alcohol intake was avoided. To determine whether excitement might confound this measurement, some subjects (15 Green Bay Packer fans) repeated saliva collection on the evening and morning after watching a professional football game. On another occasion, to correlate plasma and salivary cortisol, plasma and saliva were sampled (between 0800 and 1000 h) before and 30 min after administration of cosyntropin (1 μg iv) in some subjects (N = 9).

Patients. Patients evaluated for the diagnosis of Cushing's syndrome (N = 78) were referred because of their clinical history and features (*e.g.* obesity, hypertension, diabetes, hirsutism) and, in some cases, because of a single elevated UFC. Of these, the diagnosis of Cushing's syndrome was established in 39 (age = 42 ± 14 SD, 11 male/28 female) by clinical examination, repeatedly elevated UFC, and/or failure to suppress cortisol with a low-dose dexamethasone suppression test. The diagnosis of Cushing's disease (N = 30) was established by petrosal sinus sampling and cure by transsphenoidal microadenomectomy (9, 10). The diagnosis of ectopic ACTH (N = 4) was established by petrosal sinus sampling and successful removal of bronchial carcinoid tumors (9, 11). The diagnosis of adrenal Cushing's syndrome (N = 5) was established by the measurement of suppressed plasma ACTH and successful removal of an adrenal adenoma.

Cushing's syndrome was excluded or not firmly established in the remaining patients (N = 39; age = 44 ± 13 SD) independently of salivary cortisol measurement by subsequent normal UFC, normal dexamethasone suppression test, or identification of other causes of hypercortisolism (*e.g.* alcoholic pseudo-Cushing's). Follow-up was not routinely performed on these patients except where described in the results.

UFC was measured by high-performance liquid chromatography or RIA within one month of saliva sampling by the referring physician or by us. To consolidate these data, they were normalized by dividing by the upper limit of normal for each UFC assay used.

Methods

We used a simple device to collect and transport saliva samples (Plain Salivette, Sarstedt, Newton, NC). This device is composed of a cotton tube (similar to dental cotton), and two plastic tubes that fit one inside the other. Saliva was sampled at 2300 h and at 0700 h the following morning by chewing on the cotton tube for 2–3 min. The cotton tube was inserted inside the plastic tube, which was then capped. The Salivette can be stored at room temperature for at least 7 days and transported to the laboratory by mail or express carrier without any loss of cortisol activity (8). The saliva was separated from the cotton tube by centrifugation at 3000 RPM for 10 min and stored at −20 C or lower.

Plasma cortisol was measured by RIA (Coat-a-Count, Diagnostic Products, Los Angeles, CA). Salivary cortisol was measured using a modification of the same assay by increasing the analyte volume (from 25 to 200 μL), increasing the incubation time from 45 to 180 min, decreasing the incubation temperature from 37 C to room temperature, and diluting the provided calibrators 1:10 in distilled water (12). The minimal detectable salivary cortisol was 0.4 mmol/L. Samples diluted in parallel down to 1:5 dilution. The intraassay coefficient of variation was 3.0% (N = 15), and interassay coefficients of variation were 12.1% (low pool, N = 23) and 6.1% (high pool, N = 16).

Data were analyzed by ANOVA, followed by Student Newman-Keuls multiple-range test and linear regression using validated software (13). The ratio of 0700-h to 2300-h salivary cortisol was used as an index of diurnal rhythmicity. Reference ranges for data from normal subjects was calculated nonparametrically by the rank number method using 2.5–97.5 percentiles (14). Data are presented as mean ± SE, with $P < 0.05$ considered significant.

Results

Normal subjects

There was a significant correlation between plasma and salivary cortisol before and 30 min after administration of 1 μg cosyntropin (slope = 0.04, y-intercept = −4.3 nmol/L, n = 18, r = 0.86, $P < 0.001$). The slope of the relationship between plasma (x-axis) and salivary (y-axis) cortisol of 0.04 indicates that salivary cortisol represented about 4% of the total circulating plasma cortisol.

Average and individual data from normal subjects is shown in Table 1 and Fig. 1, respectively. Salivary cortisol was consistently and significantly greater at 0700 h than at 2300 h. There was no significant difference in the salivary cortisol levels between male and female normal subjects at either time point. Reference ranges for the salivary cortisol assay were calculated nonparametrically as <0.4–3.6 nmol/L at 2300 h and 4.7–32.0 nmol/L at 0700 h.

To determine whether nonspecific excitement or anxiety could influence these results, a subgroup of normal subjects (N = 15) were studied at 2300 h and 0700 h after watching a Sunday evening Green Bay Packers football game (1900–2200 h). There was no effect (despite a reported high level of excitement) on any of the subjects except one, whose 2300-h salivary cortisol (1 h after the game ended) was 22.8 nmol/L.

Patients

Table 1 shows the mean data of normal subjects, patients with proven Cushing's syndrome, and patients in whom Cushing's syndrome was excluded or not firmly established. The patients in whom Cushing's syndrome was excluded or not firmly established were statistically indistinguishable from normal subjects. Patients with proven Cushing's syndrome had 2300-h salivary cortisol that were, on average, almost 20 times higher than normal subjects or than patients in whom Cushing's syndrome was excluded or not firmly established. Salivary cortisol at 0700 h was also significantly higher in patients with Cushing's syndrome than in either of

TABLE 1. Mean ± SE for 2300-h and 0700-h salivary cortisol and the ratio of 0700-h to 2300-h salivary cortisol

	Salivary cortisol		
	nmol/L		0700-h:2300-h ratio
	2300 h	0700 h	
Normal subjects (N = 73)	1.2 ± 0.1	14.5 ± 0.8[a]	18.5 ± 1.9
Male (N = 35)	1.2 ± 0.1	15.6 ± 1.3[a]	
Female (N = 38)	1.2 ± 0.1	13.6 ± 1.0[a]	
Cushing's syndrome (N = 39)	24.0 ± 4.5[b]	23.0 ± 4.2[c]	1.8 ± 0.4[b]
R/O Cushing's (N = 39)	1.6 ± 0.2	15.3 ± 1.5[a]	14.7 ± 2.3

[a] 0700 h greater than 2300 h ($P < 0.001$); Cushing's syndrome different from other groups ([b] $P < 0.001$, [c] $P = 0.013$). R/O Cushing's was group in whom Cushing's syndrome was excluded or not firmly established.

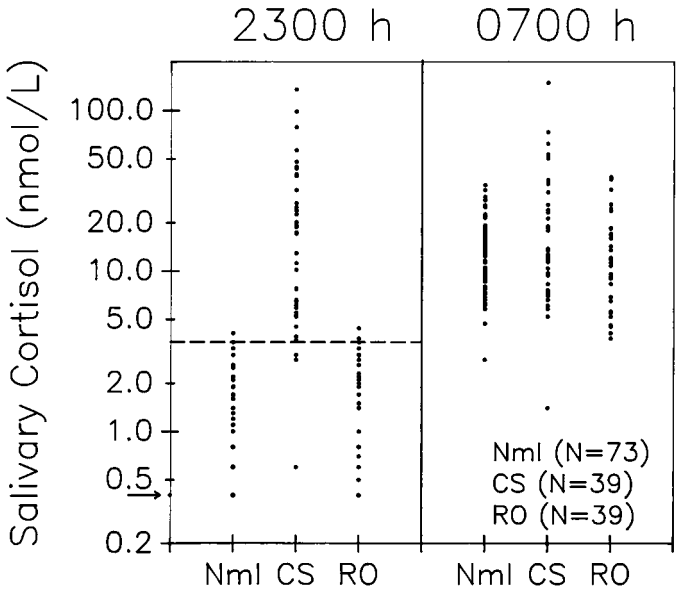

FIG. 1. Individual data points for salivary cortisol sampled at 2300 h and 0700 h in normal subjects (Nml), patients with proven Cushing's syndrome (CS), and patients in whom Cushing's syndrome was excluded or not firmly established (RO). The *broken line* indicates the upper limit of the reference range calculated from the 2300-h data of normal subjects. The *arrow* at 0.4 nmol/L of the ordinate indicates the limit of detection of the assay. For 0700-h cortisol, N = 38 for the CS group, and N = 38 for the RO group.

the two other groups. The loss of circadian rhythmicity was highlighted by a significantly lower ratio of 0700-h to 2300-h salivary cortisol in patients with Cushing's syndrome. In fact, this ratio was indistinguishable from no-rhythm (ratio = 1).

Figure 1 shows the individual data. The *broken line* in the *left panel* indicates the nonparametric estimate of the upper limit of the reference range for normal subjects (≥3.6 nmol/L). Most (36/39) patients with Cushing's syndrome had 2300-h salivary cortisol greater than the reference range. However, 3 patients with proven Cushing's disease had 2300-h salivary cortisol levels within the reference range. One of these 3 patients had very low 2300-h salivary cortisol (0.6 nmol/L) and a normal 0700-h to 2300-h ratio of salivary cortisol (17.3) and was probably not hypersecreting cortisol at the time of sampling. Therefore, the sensitivity of salivary cortisol measurement, using 2300-h salivary cortisol only (3.6 nmol/L as a cutoff), was 92% (36/39). If this cutoff was arbitrarily lowered to 2.7 nmol/L, the sensitivity of 2300-h salivary cortisol was increased to 97% (38/39). If a lack of diurnal rhythmicity was also included in the analysis (0700-h:2300-h <2), 1 patient was reclassified, yielding a sensitivity of 95% (37/39) if 3.6 nmol/L was used as a cutoff for 2300-h data. The sensitivity was not measurably improved if the arbitrary cutoff of 2.7 nmol/L for 2300-h salivary cortisol was combined with an 0700-h to 2300-h ratio less than 2, because of the 1 patient with intermittent Cushing's disease with completely normal 2300-h and 0700-h salivary cortisol.

Two of the 39 patients in the group in whom Cushing's syndrome was excluded or not firmly established had 2300-h salivary cortisol levels more than 3.6 nmol/L (4.4 and 3.8 nmol/L). These values were within the range of normal subjects. Furthermore, both patients had 0700-h to 2300-h ratios more than 2. Therefore, using both 2300-h less than 3.6 nmol/L, and a 0700-h to 2300-h ratio more than 2, the specificity of salivary cortisol was 100%. If the arbitrarily lower cutoff of 2.7 nmol/L was used, which improved sensitivity (see above), the specificity in the patients in whom Cushing's syndrome was not firmly established decreased to 77% (30/39). Interestingly, 69 of 73 normal subjects had 2300-h salivary cortisol less than 2.7 nmol/L, indicating that specificity (95%) of this lower cutoff was significantly better than in patients in whom Cushing's syndrome was ruled out or not firmly established ($\chi^2 = 7.67$, 1 degree of freedom, $P < 0.01$). There was significant overlap between 0700-h salivary cortisol for all groups.

Figure 2 shows the individual values for the ratio of 0700-h to 2300-h salivary cortisol (an index of diurnal rhythmicity). A ratio of 2 was calculated (nonparametrically) as the lower limit of the reference range for normal subjects. Of the 38 evaluable patients with Cushing's syndrome (one of 39 was missing the 0700-h measurement), 6 had 0700-h:2300-h ratios within the reference range for normal subjects.

A highly significant correlation of UFC (normalized by dividing by the upper limit of the assay reference range) and 2300-h salivary cortisol was found for patients with Cushing's syndrome (Fig. 3). The 1 patient with Cushing's syndrome in whom 2300-h salivary cortisol was less than 1.0 nmol/L had intermittent Cushing's disease and was prob-

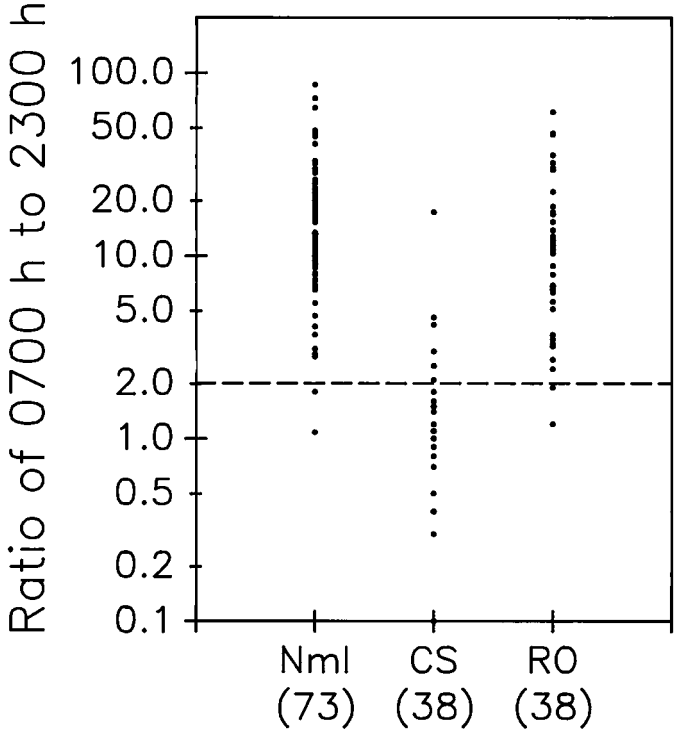

FIG. 2. Individual data for the calculated ratio of 0700-h to 2300-h salivary cortisol (index of diurnal rhythm) for normal subjects (Nml), patients with proven Cushing's syndrome (CS), and patients in whom Cushing's syndrome was excluded or not firmly established (RO). Numbers of data points are shown in *parentheses* and were N = 38, for both CS and RO groups, because 0700-h measurement was not done in 1 patient from each group (see legend for Fig. 1). The *dotted line* indicates the nonparametric lower limit of the reference range of the normal subjects.

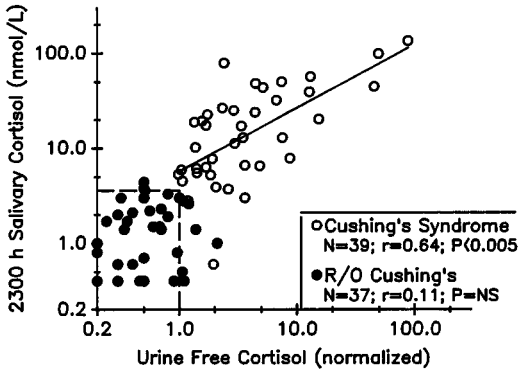

FIG. 3. Correlation of 24-h UFC (normalized by dividing by the upper limit of the assay reference range) with 2300-h salivary cortisol for patients in whom UFC was measured within 1 month of 2300-h salivary cortisol. *Dashed lines* at the *bottom left* represent the upper limit of references ranges. The correlation for patients with proven Cushing's syndrome was highly significant, whereas there was no significant correlation for the patients in whom Cushing's was excluded or not firmly established. NS, Not significant.

TABLE 2. Salivary cortisol *vs.* UFC in patients with Cushing's syndrome

Salivary cortisol	Normal UFC	Elevated UFC
2300-h ≤ 3.6 nmol/L	0	3
2300-h > 3.6 nmol/L	1	35
2300-h ≤ 3.6 nmol/L *and* 0700:2300-h ≥ 2	0	2
2300-h > 3.6 nmol/L *or* 0700:2300-h < 2	1	36

2300-h refers to salivary cortisol sampled at 2300 h; 0700:2300-h is the ratio of salivary cortisol sampled at 0700 h divided by salivary cortisol sampled at 2300 h.

ably not hypersecreting cortisol at the time of saliva sampling (same outlier as in Fig. 1), even though UFC was elevated 1 month earlier. Of the 39 patients with Cushing's syndrome, 3 had normal 2300-h salivary cortisol and elevated UFC, whereas 1 had elevated 2300-h salivary cortisol and normal UFC (Table 2). No patients with proven Cushing's syndrome had both normal 2300-h salivary cortisol and normal UFC.

The patients in whom Cushing's syndrome was excluded or not firmly established showed no significant correlation of UFC and 2300-h salivary cortisol (N = 37; Fig. 3). Thirty of 37 of these patients had normal UFC. Seven had normal 2300-h salivary cortisol but had UFC levels that were initially elevated. Of these 7, 2 had normal subsequent UFC measurements, 1 was found to have alcohol-induced pseudo-Cushing's, and 2 were obese and hirsute females with normal suppression of cortisol after overnight 1-mg dexamethasone. The etiology of the elevated UFC in the remaining two subjects, with normal salivary cortisol but elevated UFC, has not been explained.

Discussion

The measurement of late-night salivary cortisol is a simple, convenient, and reliable way to screen patients for Cushing's syndrome. A late-night salivary cortisol level greater than the upper limit of the reference range calculated from normal subjects (3.6 nmol/L) yielded a diagnostic sensitivity of 92% and, using a cutoff point of greater than 2.7 nmol/L, yielded a sensitivity of 97%. An elevated late-night salivary cortisol (>3.6 nmol/L) and/or an elevated 24-h UFC yielded a sensitivity of 100% for spontaneous Cushing's syndrome. Cortisol circulates in the plasma approximately 95% bound to carrier proteins (primarily corticosteroid binding globulin) and approximately 5% in the free form (15). It is the free form that is biologically active. The concentration of cortisol in the saliva is an equilibrium with free plasma cortisol and is independent of the rate of saliva production (5–7). Despite previous studies demonstrating the usefulness of the measurement of salivary cortisol to assess hypothalamic-pituitary-adrenal secretory activity and rhythm and to diagnose hypercortisolism (5–7, 16–19), it is surprising that this technique has not been more widely used. The collection of a salivary specimen is simple (using a Salivette device), involves only chewing on a cotton tube for 2–3 min at home, and therefore, is not stressful. Furthermore, salivary cortisol samples collected in this fashion are stable at room temperature for 1 week and can be transported to the laboratory by mail or express carrier without any loss of cortisol activity (8). The actual measurement of cortisol in the saliva can be easily performed with simple modifications of a widely available RIA for cortisol (12).

Reference ranges for 2300-h and 0700-h salivary cortisol in normal subjects were similar to those previously described using different assay methods (16–19). However, we did not find a significant difference between male and female normal subjects, as has been previously described (16). We also demonstrated that the concentration of cortisol in the saliva is stimulated by administration of cosyntropin, consistent with a previous study (5), and that there was excellent correlation between salivary (free) cortisol and total serum cortisol measurements (7). Finally, we also raise the possibility that a stressful event in the evening of sampling has the potential to confound the measurement.

We clearly demonstrated that patients with spontaneous Cushing's syndrome have a markedly elevated late-night salivary cortisol, as well as disrupted circadian rhythm (decreased ratio of 0700-h to 2300-h salivary cortisol). However, 3 of 39 patients with Cushing's syndrome had late-night salivary cortisol within the normal reference range and, of these, 2 had normal 0700-h to 2300-h ratios. Laudat *et al.* (16) found no overlap in 2000-h salivary cortisol between normal subjects and patients with Cushing's syndrome. This difference from our study may be caused by the timing of the evening sample, the degree of hypercortisolism, or the fact that we studied a larger sample size. If a late-night salivary cortisol level of 2.7 nmol/L was used as the upper limit of normal, we were able to identify all patients with Cushing's syndrome, with the exception of a single patient who had intermittent hypercortisolism and apparently normal cortisol secretory dynamics at the time of measurement. In addition, six patients with Cushing's disease had normal 0700-h to 2300-h ratios, suggesting intact diurnal rhythmicity. This confirms other studies that suggest that some patients with spontaneous Cushing's syndrome actually retain normal diurnal rhythm (albeit at a higher secretory rate) (20, 21).

One of the unique aspects of this study is the reporting of a significant number of patients referred for evaluation of possible Cushing's syndrome based on their clinical signs

and symptoms but in whom the diagnosis was excluded or not firmly established. The diagnosis was excluded in most of these patients by measurement of a normal UFC by high-performance liquid chromatography. In five of the seven remaining patients, either repeated urinary free cortisol measurements were normal or the patients had adequate suppression of serum cortisol after low-dose dexamethasone suppression. All of these patients had either normal 2300-h salivary cortisol (<3.6 nmol/L) or a normal 0700-h to 2300-h ratio (>2). If the 2300-h salivary cortisol cutoff was lowered to 2.7 nmol/L to improve sensitivity for diagnosing Cushing's syndrome (see above), the specificity in these patients decreased (77%). However, specificity in normal subjects, using 2.7 nmol/L as the cutoff, was significantly higher (95%) than in the patients in whom Cushing's syndrome was not established, suggesting that some patients in whom Cushing's could not be firmly established by any approach might have very mild hypercortisolism.

It is well appreciated that there is a large overlap of morning serum cortisol values between patients with Cushing's syndrome and normal subjects; and thus, this sampling time affords poor discrimination. Two studies have attempted to take advantage of the fact that cortisol secretion in the evening in Cushing's syndrome patients is higher than in normal subjects, by means of measuring spot or timed urine cortisol levels in the late evening (22, 23). These studies showed that the ratio of urinary cortisol to creatinine was able to distinguish patients with Cushing's syndrome from normal subjects. However, there was some overlap between patients with hypercortisolism and a group of obese control subjects. Newell-Price *et al.* (3) measured a single sleeping plasma cortisol level at midnight in 150 patients with proven Cushing's syndrome and showed that a plasma (total) cortisol greater than 50 nmol/L (1.8 μg/dL) yielded a diagnostic sensitivity for Cushing's syndrome of 100%. This corresponds well with a salivary (free) cortisol of 2.7 nmol/L (the arbitrary cutoff point that yielded more than 95% sensitivity in our patient population. However, the measurement of an unstressed late-night serum cortisol required inpatient hospitalization for a period of at least 48 h, making it impractical as a screening test (3). Another report suggested that the assessment of midnight cortisol values may allow discrimination between Cushing's syndrome and pseudo-Cushing states (depression, alcoholism, and eating disorders) (24). The level of plasma cortisol at midnight was greater than 7.5 μg/dL (207 nmol/L) in 96% of 234 patients with Cushing's syndrome, whereas it was less than this in all patients with pseudo-Cushing states. Because it is well known that some patients with Cushing's syndrome may have intermittent hypercortisolism (25, 26), normal results of any test of cortisol secretory dynamics may not be adequate in excluding the diagnosis. In the present study, UFC and salivary cortisol were not measured concurrently, and therefore, intermittent hypercortisolism could have led to one or the other being normal. That UFC and salivary cortisol correlated well, except in 1 subject, suggests that significant time between samples may not be a disadvantage.

As a screening test for Cushing's syndrome, a late-night salivary cortisol compares favorably with the traditional overnight 1-mg dexamethasone suppression test and is easier to perform. The reported cutoff values for the suppression of serum cortisol in normal subjects after 1 mg dexamethasone, administered at 2300 h, has ranged from 3.0–7.2 μg/dL (80–195 nmol/L) (27–29). However, some patients with Cushing's syndrome demonstrate unusual sensitivity to dexamethasone suppression; and thus, even cutoffs at this level are likely to result in a significant number of false negative responses (30). To increase the sensitivity of the overnight 1-mg dexamethasone suppression test, a recent extensive review suggested that suppression of the post-dexamethasone serum cortisol to 1.8 μg/dL (50 nmol/L) more or less effectively excludes Cushing's syndrome (30). Using such a low cutoff value will undoubtedly increase the false positive rate and may very well obfuscate its use as a screening test. Some studies have combined the dexamethasone suppression test with salivary cortisol measurement, but this does not eliminate the conceptual problems with using glucocorticoid negative feedback to evaluate hypercortisolism (31–33).

The clinical diagnosis of Cushing's syndrome does not depend on any one specific clinical feature but on a constellation of features. The appreciation of subclinical Cushing's syndrome in some patients with incidentally discovered adrenal masses has provided evidence that mild hypercortisolism is as difficult to appreciate as subclinical hypothyroidism or hyperthyroidism (34). Although spontaneous Cushing's syndrome is considered to be an unusual disorder, a recent study, showing that 3–4% of poorly controlled patients with type 2 diabetes may have unsuspected Cushing's syndrome, provides evidence that this disorder is more common than currently appreciated (35). The diagnosis can only be achieved with a high index of suspicion and the use of simple biochemical screening studies.

In summary, this study describes the use of a late-night salivary cortisol measurement as a simple and reliable means of screening patients for spontaneous Cushing's syndrome. In addition, late-night salivary cortisol measurements may also help in the evaluation of some patients with suspected intermittent hypercortisolism (25, 26) and may also be useful in facilitating the screening of large high-risk populations (*e.g.* patients with diabetes mellitus).

Acknowledgments

The authors thank Barbara Jankowski and Eric Bruder for their technical expertise, and all of the normal subjects who volunteered by donating their saliva.

References

1. **Yanovski JA, Cutler GB.** 1994 Glucocorticoid action and the clinical features of Cushing's syndrome. Endocrinol Metab Clin North Am. 23:487–509.
2. **Findling JW, Doppman JL.** 1994 Biochemical and radiological diagnosis of Cushing's syndrome. Endocrinol Metab Clin North Am. 23:511–537.
3. **Newell-Price J, Trainer P, Perry L, Wass J, Grossman A, Besser M.** 1995 A single sleeping midnight cortisol has 100% sensitivity for the diagnosis of Cushing's syndrome. Clin Endocrinol (Oxf). 43:545–550.
4. **Jones MT, Gillham B.** 1988 Factors involved in the regulation of adrenocorticotropic hormone/β-lipotropic hormone. Physiol Rev. 68:743–818.
5. **Bolufer P, Gandia A, Rodriguez A, Antonio P.** 1989 Salivary corticosteroids in the study of adrenal function. Clin Chim Acta. 183:217–226.
6. **Read GF, Walker RF, Wilson DW, Griffiths K.** 1990 Steroid analysis in saliva for the assessment of endocrine function. Ann NY Acad Sci. 595:260–274.
7. **Kahn J-P, Rubinow DR, Davis CL, Kling M, Post RM.** 1988 Salivary cortisol: a practical method for evaluation of adrenal function. Biol Psychiatry. 23:335–349.

8. **Chen Y-M, Cintron NM, Whitson PA.** 1992 Long-term storage of salivary cortisol samples at room temperature. Clin Chem. 38:304–305.
9. **Findling JW, Kehoe ME, Shaker JL, Raff H.** 1991 Routine inferior petrosal sinus sampling in the differential diagnosis of ACTH-dependent Cushing's syndrome: early recognition of the occult ectopic ACTH syndrome. J Clin Endocrinol Metab. 73:408–413.
10. **Graham KE, Raff H, Cook DM, Barnwell TL, Samuels MH.** 1997 Intraoperative adrenocorticotropin levels during transsphenoidal surgery for Cushing's disease does not predict cure. J Clin Endocrinol Metab. 82:1776–1779.
11. **Raff H, Shaker JL, Seifert PE, Werner PH, Hazelrigg SR, Findling JW.** 1995 Intraoperative measurement of adrenocorticotropin (ACTH) during removal of ACTH-secreting bronchial carcinoid tumors. J Clin Endocrinol Metab. 80:1036–1039.
12. **Tunn S, Mollmann H, Barth J, Derendorf H, Krieg M.** 1992 Simultaneous measurement of cortisol in serum and saliva after different forms of cortisol administration. Clin Chem. 38:1491–1494.
13. **Glantz SA.** 1992 Primer of biostatistics software. New York: McGraw-Hill, Inc.
14. **Solberg HE.** 1996 Establishment and use of reference values. In: Burtis CA, Ashwood ER, eds. Tietz fundamentals of clinical chemistry. Philadelphia: Saunders; 182–191.
15. **Mendel CM.** 1989 The free hormone hypothesis: a physiologically based mathematical model. Endocr Rev. 10:232–274.
16. **Laudat MH, Cerdas S, Fournier C, Guiban D, Guilhaume B, Luton JP.** 1988 Salivary cortisol measurements: a practical approach to assess pituitary-adrenal function. J Clin Endocrinol Metab. 66:343–348.
17. **Kirschbaum C, Strasburger CJ, Jammers W, Hellhammer DH.** 1989 Cortisol and behavior: 1. adaptation of a radioimmunoassay kit for reliable and inexpensive salivary cortisol determination. Pharmacol Biochem Behav. 34:747–751.
18. **Silver AC, Landon J, Smith DS, Perry LA.** 1983 Radioimmunoassay of cortisol in saliva with the "GammaCoat" kit. Clin Chem. 29:1869–1870.
19. **Lo MSL, Ng ML, Azmy BS, Khalid BAK.** 1992 Clinical applications of salivary cortisol measurements. Singapore Med J. 33:170–173.
20. **Glass AR, Zavadil AP, Halberg F, Cornelissen G, Schaaf M.** 1984 Circadian rhythm of serum cortisol in Cushing's disease. J Clin Endocrinol Metab. 59:161–165.
21. **Refetoff S, Van Cauter E, Fang VS, Laderman C, Graybeal ML, Landau RL.** 1985 The effect of dexamethasone on the 24-hour profile of adrenocorticotropin and cortisol in Cushing's syndrome. J Clin Endocrinol Metab. 60:527–535.
22. **Laudat MH, Billaud L, Thomopoulos P, Vera O, Yllia A, Luton JP.** 1988 Evening urinary free corticoids: a screening test in Cushing's syndrome and incidentally discovered adrenal tumors. Acta Endocrinol (Copenh). 119:459–464.
23. **Contreras LN, Hane S, Tyrrell JB.** 1986 Urinary cortisol in the assessment of pituitary-adrenal function: utility of 24-hour and spot determinations. J Clin Endocrinol Metab. 62:965–969.
24. **Papanicolaou DA, Yanovski JA, Cutler GB, Chrousos GP, Nieman LK.** 1998 A single midnight cortisol measurement distinguishes Cushing's syndrome from pseudo-Cushing states. J Clin Endocrinol Metab. 83:1163–1167.
25. **Hermus AR, Pieters GF, Borm GF, et al.** 1993 Unpredictable hypersecretion of cortisol in Cushing's disease: detection by daily salivary cortisol measurements. Acta Endocrinol (Copenh). 128:428–432.
26. **Mosnier-Pudar H, Thomopoulos P, Bertagna X, Fournier C, Guiban D, Luton JP.** 1995 Long-distance and long-term follow-up of a patient with intermittent Cushing's disease by salivary cortisol measurements. Eur J Endocrinol. 133:313–316.
27. **Montwill J, Igoe D, McKenna TJ.** 1994 The overnight dexamethasone test is the procedure of choice in screening for Cushing's syndrome. Steroids. 59:296–298.
28. **Blethen SL, Chasalow FI.** 1989 Overnight dexamethasone suppression test: normal responses and the diagnosis of Cushing's syndrome. Steroids. 54:185–193.
29. **Fok ACK, Tan KT, Jacob E, Sum CF.** 1991 Overnight (1 mg) dexamethasone suppression testing reliably distinguishes non-cushingoid obesity from Cushing's syndrome. Steroids. 56:549–551.
30. **Wood PJ, Barth JH, Freedman DB, Perry L, Sheridan B.** 1997 Evidence for the low dose dexamethasone suppression test to screen for Cushing's syndrome - recommendations for a protocol for biochemistry laboratories. Ann Clin Biochem. 34:222–229.
31. **Barrou Z, Guiban D, Maroufi A, et al.** 1996 Overnight dexamethasone suppression test: comparison of plasma and salivary cortisol measurement for the screening of Cushing's syndrome. Eur J Endocrinol. 134:93–96.
32. **Bonnin R, Villabona C, Rivera A, et al.** 1993 Is salivary cortisol a better index than free cortisol in serum or urine for diagnosis of Cushing's syndrome? Clin Chem. 39:1353–1354.
33. **Hagg E, Olsson T, Grankvist K.** 1990 Salivary cortisol during an overnight dexamethasone suppression test using a simple saliva collection device. Horm Metab Res. 22:553–554.
34. **Reincke M, Nieke J, Krestin GP, Saeger W, Allolio B, Winkelman W.** 1992 Preclinical Cushing's syndrome in adrenal "incidentalomas": comparison with adrenal Cushing's syndrome. J Clin Endocrinol Metab. 75:826–832.
35. **Leibowitz G, Tsur A, Chayen SD, et al.** 1996 Pre-clinical Cushing's syndrome: an unexpected frequent cause of poor glycaemic control in obese diabetic subjects. Clin Endocrinol (Oxf). 44:717–722.