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CLINICAL STUDY

Salivary cortisol measurement in normal-weight, obese and anorexic women: comparison with plasma cortisol

Pietro Putignano, Antonella Dubini, Paola Toja, Cecilia Invitti, Simona Bonfanti, Gabriella Redaelli, Daniela Zappulli and Francesco Cavagnini

Department of Endocrinology, University of Milan, Istituto Scientifico Ospedale San Luca, Milan, Italy

(Correspondence should be addressed to Francesco Cavagnini, Chair of Endocrinology, University of Milan, Istituto Scientifico Ospedale San Luca, Istituto Auxologico Italiano, Via Spagnoletto 3 20149 Milano, Italy; Email: cavagnini@auxologico.it)

Abstract

Objective: To compare salivary, plasma and urinary free cortisol (UFC) measurements in patients with anorexia nervosa, in whom an overdrive of the hypothalamic–pituitary–adrenal (HPA) axis is well established but information on salivary cortisol is lacking, in viscerally obese patients in whom subtle abnormalities of cortisol secretion and metabolism are postulated, and in normal-weight healthy women.

Participants and experimental design: Measurement of salivary cortisol offers a convenient way to assess the concentrations of free, biologically active cortisol in plasma in different physiopathological settings. Forty-seven drug-free, newly diagnosed women with active restrictive anorexia nervosa, 30 restrictive anorexic women undergoing chronic psychopharmacological treatment, 47 women with mild-to-moderate visceral obesity, 103 women with severe central obesity and 63 normal-weight healthy women entered the study. Salivary and blood samples were collected at 0800 h, 1700 h and 2400 h, together with three consecutive 24-h urine specimens for UFC determination. In controls and patients with anorexia nervosa ($n = 83$), salivary and plasma cortisol were also measured after a 1-mg overnight dexamethasone suppression test (DST). In patients with anorexia nervosa, mood was rated by the Hamilton scale for anxiety and depression.

Results: Untreated patients with anorexia nervosa showed increased plasma and salivary cortisol and UFC concentrations (all $P < 0.001$ compared with controls), and decreased cortisol suppression after DST in plasma and saliva ($P < 0.0001$ and $P < 0.005$ respectively compared with controls). These alterations were less pronounced, although still statistically significant, in treated patients with anorexia nervosa. Salivary cortisol was highly correlated with paired plasma cortisol in the whole population and after splitting the participants by group ($P < 0.0001$). However, for plasma cortisol values greater than 500 nmol/l (the corticosteroid-binding globulin saturation point), this parallelism was lost. Taking plasma cortisol as a reference, the level of agreement for post-dexamethasone salivary and plasma cortisol was 58.9% among suppressors and 77.8% among non-suppressors (χ^2 test: $P < 0.01$). Decreased 0800 h/2400 h cortisol ratios were observed in plasma and saliva in drug-free patients with anorexia nervosa ($P < 0.005$ and $P < 0.05$ respectively compared with controls), and in saliva in severely obese patients ($P < 0.05$ compared with controls). Depression and anxiety scores were unrelated to cortisol concentrations in any compartment.

Conclusions: Salivary cortisol measurement is a valuable and convenient alternative to plasma cortisol measurement. It enables demonstration of the overdrive of the HPA axis in anorexia nervosa and subtle perturbations of the cortisol diurnal rhythm in women with visceral obesity. With the establishment of more specific and widely acceptable cut-off values for dynamic testing, measurement of salivary cortisol could largely replace plasma cortisol measurement.

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Introduction

In spite of its convenience and inexpensive nature, measurement of salivary cortisol has not gained widespread popularity. Indeed, even though its usefulness in the assessment of the activity of the hypothalamic–pituitary–adrenal (HPA) axis has been reported in various clinical conditions (1–3), several centres are still resistant to adopting this procedure. To date, few large-scale studies have been carried out to compare paired plasma and salivary cortisol concentrations in different physiopathological settings.

An overdrive of the HPA axis with abnormalities of its regulation is well documented in patients with anorexia nervosa (4–12). However, only one study of salivary cortisol in a small series of these patients has

been published (13). On the basis of some experimental observations, an activation of the HPA axis has also been hypothesized in obesity, chiefly of the visceral type (14–17), where it appears to be associated with an increased risk for cardiovascular and metabolic complications (18). Recent findings would also indicate that plasma cortisol may be an independent predictor of metabolic and haemodynamic morbidity in normal-weight individuals (19).

The aim of this study was take a further step towards validation of the measurement of salivary cortisol and to explore its usefulness in the assessment of the HPA function in obesity and anorexia nervosa. To this end we investigated the correlations between salivary and plasma cortisol in samples obtained during the day and in the late night, in a large series of obese patients, drug-free and pharmacologically treated patients with anorexia nervosa, and normal-weight healthy women. Plasma and salivary cortisol was also determined after an overnight 1-mg dexamethasone suppression test (DST) in a group of anorexic patients and a group of normal women.

Participants and methods

Participants

The study group consisted of 290 women admitted consecutively to the Endocrine Division of our hospital between 1994 and 1997. They were 63 normal-weight women (age 31.2 ± 1.29 years (mean±S.E.M.), body mass index (BMI) 20.8 ± 0.27 kg/m^2, waist to hip ratio (WHR) 0.77 ± 0.04), 47 drug-free newly diagnosed patients with active restrictive anorexia nervosa (23.0 ± 0.92 years, BMI 13.7 ± 0.33 kg/m^2), 30 patients with active restrictive anorexia nervosa (22.9 ± 0.91 years, BMI 14.7 ± 0.48 kg/m^2) receiving psychopharmacological therapy (citalopram 10–40 mg/day or alprazolam 0.5–3 mg/day, or both) for more than 3 months, and 150 unselected obese women on a free diet with central fat distribution. Of the last group, 47 had mild obesity (BMI 31.1 ± 0.40 kg/m^2, WHR 0.92 ± 0.03, age 35.5 ± 2.29 years) and 103 were severely obese (BMI 41.8 ± 0.55 kg/m^2, WHR 0.93 ± 0.04, age 38.2 ± 1.47 years). All the women gave informed consent to participate in this study, which was approved by the ethics committee of our Institution. Normal-weight women were recruited from patients admitted to hospital for suspected endocrine/metabolic disorders that were excluded by subsequent investigations. All had no history of endocrine or psychiatric disorders, were drug-free and had not been taking medications for the previous 6 months. In-patient assessment was preferred in order to perform paired plasma and salivary sampling throughout the 24 h. A 0800 h/2400 h cortisol ratio in plasma and saliva was measured as an index of the circadian rhythm of cortisol secretion. Diagnosis of restrictive anorexia nervosa was established according the criteria of the American Psychiatric Association (20). All women with anorexia nervosa exhibited severe food restriction without bingeing, purging or vomiting behaviour, and were amenorrhoeic. To evaluate whether psychological perturbations were associated with hypercortisolism in newly diagnosed patients with anorexia nervosa, plasma, salivary and urinary cortisol were correlated with the degree of depression (HAM-D) and anxiety (HAM-A) assessed by the Hamilton Depression and Anxiety Inventory Score (21). Anxio-depressive symptoms in general were present in 100% of untreated patients with anorexia nervosa. HAM-D scores were 18–30, 31–40 and more than 40 in 23%, 34% and 43% of the patients respectively; mild, moderate and severe anxiety (HAM-A 2, 3 and 4) were found in 54%, 34% and 12% of the patients respectively.

Anthropometry

Body weight was measured to the nearest 0.1 kg with the woman wearing underwear, and height was recorded to the nearest centimetre. BMI was calculated as weight in kilograms divided by height squared in metres. Waist circumference was measured midway between the lowest rib and the iliac crest, hip circumference at the level of great trochanters and the ratio between the two measures (WHR) was calculated (22). A WHR value greater than 0.85 was taken as an index of visceral obesity.

Study design

After an overnight fast, and at least 2 days after admission to hospital, salivary and blood samples were taken at 0800 h, 1700 h and 2400 h. In each woman, three 24-h urinary free cortisol (UFC) collections were obtained during the week preceding or following the admission. Two to three days after the completion of these procedures, in 83 women (45 normal volunteers, 21 untreated anorexic patients and 17 anorexic patients receiving antidepressant treatment) blood and salivary samples were collected at 0800 h for estimation of cortisol after the administration of an oral dose of 1 mg dexamethasone at 2300 h.

Assays

Saliva was collected into a commercially available device (Salivette) using a cotton swab chewed on for 2–3 min, and inserted into a double-chamber plastic test tube. Blood and saliva samples were centrifuged at 4 °C and stored at −20 °C until required for assay. Plasma, salivary and urinary cortisol were measured by radio-immunoassay (Byk-Sangtec Diagnostica, Dietzenbach, Germany for plasma and salivary cortisol; DPC, Los Angeles, CA, USA, for urinary cortisol). UFC was

assayed after urine extraction with dichloromethane. UFC values are the mean of three measurements on three separate urine collections. Sensitivities of the methods were 0.28 nmol/l for salivary cortisol and 13.5 nmol/l for plasma and urinary cortisol. Intra- and interassay coefficients of variations were 3.0 and 4.7% for plasma cortisol, 4.5 and 5.8% for salivary cortisol and 3.5 and 6.2% for UFC respectively.

Statistical analyses

Data were analysed using StatView 4.5 software (Abacus Concepts, Inc., Berkeley, CA, USA). Results are expressed as mean±S.E.M. Intergroup differences were evaluated by ANOVA, followed by the Bonferroni *post hoc* test. Correlations between plasma, salivary and urinary cortisol were performed by linear regression. The concordance of salivary with plasma cortisol regarding the dexamethasone suppressibility or non-suppressibility was established by a 2×2 contingency table. A P value less than 0.05 was considered statistically significant.

Results

Plasma and salivary cortisol: circadian rhythm and overnight suppression test

Concentrations of plasma, salivary and urinary cortisol are shown in Table 1. Compared with normal controls, untreated patients with anorexia nervosa showed significantly greater plasma and salivary cortisol concentrations at each time of day sampled and after dexamethasone testing, in addition to decreased 0800 h/2400 h plasma and salivary cortisol ratios. These alterations were also present, though to a lesser extent, in patients with anorexia nervosa who were receiving stable psychopharmacological therapy. In particular, these patients were found to have lower salivary cortisol values at midnight and lower UFC concentrations ($P < 0.05$), and greater cortisol suppression after DST in plasma and saliva compared with untreated patients with anorexia nervosa ($P < 0.05$ and $P < 0.005$ respectively). Conversely, severely obese patients displayed diminished 0800 h and 1700 h plasma cortisol values ($P < 0.005$ and $P < 0.05$ respectively) and decreased 0800 h/2400 h salivary cortisol ratios ($P < 0.05$) compared with controls. In each group of women, both plasma and salivary cortisol values were unrelated to BMI. Those who had undergone dexamethasone testing were classified as 'suppressors' and 'non-suppressors' by a cut-off value for plasma cortisol of 50 nmol/l (23). As corresponding cut-off values for salivary cortisol are not firmly established, on the basis of the mean saliva/plasma cortisol ratio of 6.8 ± 0.3% observed in our population, we fixed a cut-off value of 3.4 nmol/l, corresponding to 50 nmol/l for plasma cortisol (24). By these criteria,

Table 1 Plasma, salivary and urinary free cortisol concentrations in the study participants.

	Plasma cortisol (nmol/l)				**Salivary cortisol** (nmol/l)				**Cortisol after DST** (nmol/l)		**UFC** (nmol/24 h)
	0800 h	1700 h	2400 h	0800 h/2400 h	0800 h	1700 h	2400 h	0800 h/2400 h	Plasma	Saliva	
Controls	419.5±29.8	248.4±36.4	88.3±16.6	7.8±2.2	13.5±0.8	7.2±0.5	5.0±0.3	3.5±0.3	23.7±2.8	3.9±0.8	131.1±10.2
Untreated AN	546.5±24.6[c]	394.7±21.5[a]	281.5±28.4[a]	2.8±0.3[b]	26.2±3.0[a]	13.8±1.1[a]	15.2±3.0[a]	2.6±0.3[c]	218.9±40.0[a]	12.4±3.9[b]	260.8±30.0[a]
Treated AN	535.4±56.6[c]	—	—	—	20.7±1.9[be]	11.6±1.1[a]	9.7±1.7[be]	2.7±0.2	110.4±40.3[ce]	5.0±0.5[d]	208.7±29.0[be]
Moderate OB	383.6±19.9[c]	207.0±16.8	121.4±18.8	6.4±0.9	13.5±1.1	7.7±0.5	6.3±0.5	2.8±0.3	—	—	120.1±10.9
Severe OB	336.7±15.7[b]	184.9±10.8[c]	93.8±7.7	6.6±0.6	11.9±0.5	6.3±0.3	5.2±0.3	2.5±0.1[c]	—	—	104.1±5.7

Data are expressed as mean±S.E.M. AN, anorexia nervosa; OB, obesity.
[a]$P < 0.0001$, [b]$P < 0.005$, [c]$P < 0.05$, compared with controls; [d]$P < 0.005$, [e]$P < 0.05$, compared with untreated AN.

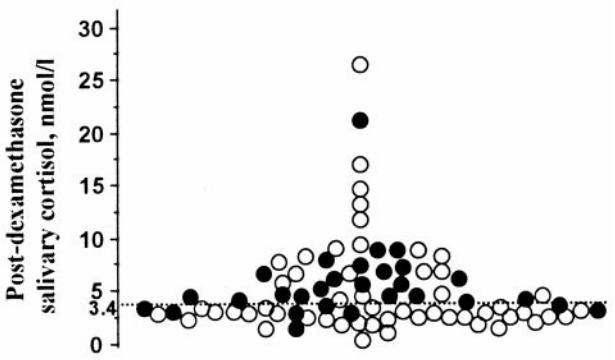

Figure 1 Salivary cortisol concentrations after the dexamethasone suppression test. The dashed line indicates the cut-off level of dexamethasone suppression (3.4 nmol/l). ○, Concordant results; ●, discordant results.

cortisol suppression in plasma and saliva was found respectively in 44 and 29 of the normal women (97.8% and 64.4% respectively), in nine and six of the 17 medicated patients with anorexia nervosa (52.9% and 35.3% respectively) and in three and four of the 21 untreated patients with anorexia nervosa (14.3% and 19.0% respectively). Overall, 54 women had concordant results in both plasma and saliva (33 being suppressors and 21 non-suppressors), whereas 29 had discordant results (23 with cortisol suppression in plasma but not in saliva and six *vice versa*) (Fig. 1). Taking plasma cortisol as the reference parameter, the level of agreement for post-dexamethasone salivary cortisol was 58.9% among suppressors and 77.8% among non-suppressors (χ^2 test: $P < 0.01$).

Anxio-depressive features and cortisol secretion in patients with anorexia nervosa

No correlations were found between plasma, salivary and urinary cortisol concentrations and depression and anxiety scores according to the HAM-A and HAM-D rating scales.

Correlations between plasma, salivary and urinary cortisol

Salivary cortisol correlated with plasma cortisol at 0800 h, 1700 h and 2400 h ($r = 0.502$, $P < 0.0001$; $r = 0.626$, $P < 0.0001$ and $r = 0.643$, $P < 0.0001$ respectively) in the population as a whole (Fig. 2) and after the women were split by group (data not shown). We examined the correlation between the two

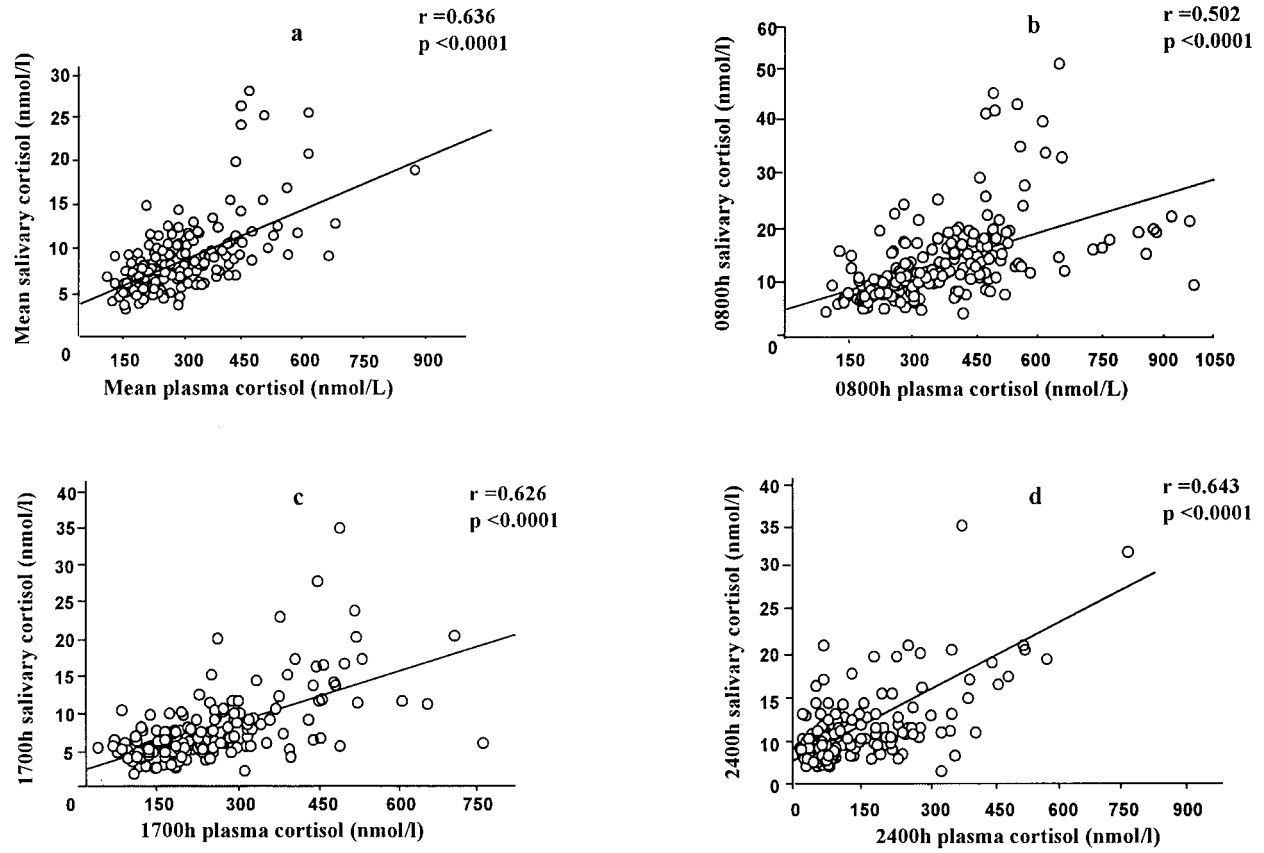

Figure 2 Salivary compared with plasma cortisol concentrations. (a) Mean values; (b) 0800 h values; (c) 1700 h values; (d) 2400 h values.

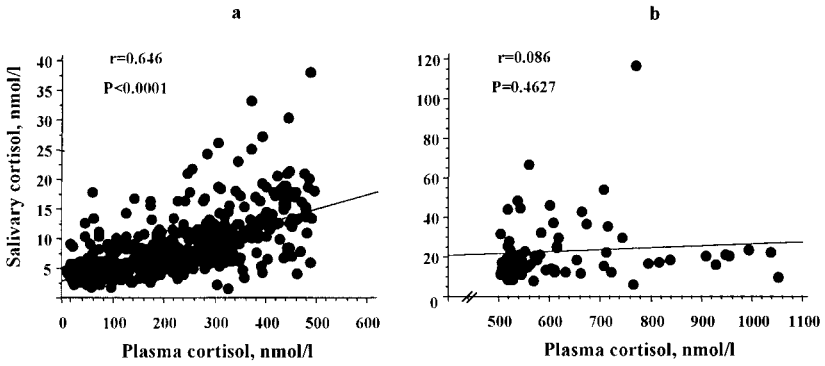

Figure 3 Correlation between spontaneous plasma and salivary cortisol concentrations. (a) Plasma cortisol concentration less than 500 nmol/l; (b) plasma cortisol concentration greater than 500 nmol/l.

parameters after splitting cortisol values by concentration ranges, excluding from the analysis the post-dexamethasone cortisol values, because of the influence of exogenous glucocorticoids on circulating corticosteroid-binding globulin (CBG) concentrations (25). We found that the correlation was lost for plasma cortisol concentrations greater than 500 nmol/l (Fig. 3), which is the approximate CBG binding saturation point (26, 27). In the entire group of women studied, no correlation was found between both the mean of 0800 h, 1700 h and 2400 h plasma and salivary cortisol values and UFC concentrations ($r = 0.02$ and $r = 0.04$, NS, respectively; data not shown).

Discussion

This study has shown that cortisol in saliva significantly correlates with paired plasma cortisol concentrations at different times of the day in normal-weight controls, in women with visceral obesity and in patients with either treated or untreated anorexia nervosa. Conversely, no correlation was found between salivary cortisol and UFC, most probably because the former reflects the instant cortisol secretion whereas the latter is an estimation of the integrated daily hormone output. In common with Vining and coworkers (26), we also failed to detect a linear correlation between free and total cortisol concentrations over the entire cortisol concentration range but, at variance with these authors who observed a steeper correlation line for plasma cortisol values greater than 500 nmol/l (the approximate saturation point of CBG binding affinity (26, 27)), we observed in our population that the correlation between the two parameters was lost at plasma cortisol concentrations greater than these values. The marked differences in the experimental conditions of the two studies (sustained and spontaneous cortisol increase compared with acute hypercortisolaemia induced by cortisol succinate infusion or ACTH stimulation) might partly account for this discrepancy. Measurement of cortisol concentrations in saliva has several advantages over its determination in plasma. It closely reflects the concentrations of free – that is, biologically active – cortisol in plasma and is independent of the rate of saliva secretion (1–3). Oestrogens increase circulating CBG concentrations, thus inducing falsely increased cortisol concentrations in plasma, but not in saliva, in pregnancy and in women taking oral contraceptives (28). Furthermore, measuring cortisol in saliva may prove particularly useful when blood sampling is difficult, as in a paediatric population or in massively obese individuals, and when a complicated venepuncture may stimulate cortisol output (29). Lastly, although plasma cortisol concentrations may be affected by inappropriate plasma storage (30), cortisol in saliva is stable at room temperature for several days (31) and saliva samples can be mailed to the laboratory after a collection made at home without medical assistance (32).

In spite of the above mentioned advantages, measurement of salivary cortisol may present some limitations. Salivary sampling may be difficult in dehydrated patients, as occurred in some of our anorexic patients who had to be instructed to chew the cotton swab for a long time in order to collect adequate amounts of saliva. Several assays for the measurement of salivary cortisol, with different performances, are currently available and there is no general agreement regarding the cut-off points for dynamic tests exploring HPA function. In this context, both poor (24) and satisfactory (33) levels of concordance have been reported in classifying the patients as dexamethasone suppressors or non-suppressors on the basis of salivary and plasma cortisol criteria. More specific and widely acceptable criteria for the interpretation of the pattern of salivary cortisol after dexamethasone are clearly needed.

Assessment of the HPA axis is often required in patients with anorexia nervosa, in whom its overdrive is well established (4–12), and in those with visceral obesity, in whom recent experimental findings would suggest an enhanced HPA function (14–17). Studies on salivary cortisol in anorexia nervosa are lacking. In this condition, in which a decreased apparent affinity constant of CBG for cortisol has been reported (34), we found no difference in the correlation pattern

between plasma and salivary cortisol compared with the other groups of women. In untreated anorexic patients, salivary cortisol was a reliable tool with which to explore the HPA axis, confirming the existence of major abnormalities (increased daily cortisol secretion and blunted inhibition by low doses of dexamethasone). In accordance with previous reports (5, 8, 35), these alterations were unrelated to depressive symptoms and BMI values, and were less pronounced in anorexic patients receiving chronic antidepressant drug treatment.

In comparison with the findings in control women, a decreased 0800 h/2400 h salivary cortisol ratio, indicating a flattened diurnal curve of cortisol secretion, was demonstrated in patients with marked abdominal obesity. Conversely, no major differences were found in the concentrations and diurnal rhythm of plasma cortisol between severely obese and controls.

The lower concentrations of salivary cortisol recorded in medicated than in untreated anorexic patients in the absence of significant differences in body weight among the two groups, may suggest that salivary cortisol might be measured to monitor compliance with psychopharmacological treatment in anorexia nervosa. Furthermore, a single late-night salivary cortisol value may be a cost-effective means of screening large populations with visceral obesity for Cushing's syndrome (36, 37). Lastly, as emerging evidence suggests that a hypersensitive HPA axis should be included in the multifactorial web of causation of syndrome X, measurement of cortisol in saliva might also help in the assessment of an individual's cardiovascular risk profile.

In conclusion, measurement of salivary cortisol appears to be a valuable and convenient alternative to plasma cortisol measurement. It has been shown to be capable of revealing the overdrive of the HPA axis in anorexia nervosa, in addition to the subtle perturbations of the cortisol diurnal rhythm in women with visceral obesity. In the presence of firmly established and widely acceptable cut-off values for dynamic testing, measurement of salivary cortisol could largely replace plasma cortisol measurement in these settings.

References

1 Riad-Fahmy D, Read GF, Walker RF & Griffiths K. Steroids in saliva for assessing endocrine function. *Endocrine Reviews* 1982 **3** 367–395.

2 Vining RF & McGinley RA. The measurement of hormones in saliva: possibilities and pitfalls. *Journal of Steroid Biochemistry* 1987 **27** 81–94.

3 Kirschbaum C & Hellhammer DH. Salivary cortisol in psychobiological research: an overview. *Neuropsychobiology* 1989 **22** 150–169.

4 Boyar RM, Hellman LD, Roffwarg H, Katz J, Zumoff B, O'Connor J *et al.* Cortisol secretion and metabolism in anorexia nervosa. *New England Journal of Medicine* 1977 **296** 190–193.

5 Brambilla F, Cavagnini F, Invitti C, Poterzio F, Lampertico M, Sali L *et al.* Neuroendocrine and psychopathological measures in anorexia nervosa: resemblances to primary affective disorders. *Psychiatry Research* 1985 **16** 165–176.

6 Gold PW, Gwirtsman H, Avgerinos PC, Nieman LK, Gallucci WT, Kaye W *et al.* Abnormal hypothalamic–pituitary–adrenal function in anorexia nervosa. Pathophysiologic mechanisms in underweight and weight-corrected patients. *New England Journal of Medicine* 1986 **314** 1335–1342.

7 Cavagnini F, Invitti C, Passamonti M & Polli EE. Response of ACTH and cortisol to corticotropin-releasing hormone in anorexia nervosa. *New England Journal of Medicine* 1986 **314** 184–185.

8 Kaye WH, Gwirtsman HE, George DT, Ebert HM, Jimerson DC, Tomai TP *et al.* Elevated cerebrospinal fluid levels of immunoreactive corticotropin-releasing hormone in anorexia nervosa: relation to state of nutrition, adrenal function, and intensity of depression. *Journal of Clinical Endocrinology and Metabolism* 1987 **64** 203–208.

9 Estour B, Pugeat M, Lang F, Lejeune H, Broutin F, Pellet J *et al.* Rapid escape of cortisol from suppression in response to i.v. dexamethasone in anorexia nervosa. *Clinical Endocrinology* 1990 **33** 45–52.

10 Ferrari E, Fraschini F & Brambilla F. Circadian rhythms in eating disorders. *Biological Psychiatry* 1990 **27** 1007–1020.

11 Licinio J, Wong ML & Gold PW. The hypothalamic–pituitary–adrenal axis in anorexia nervosa. *Psychiatry Research* 1996 **62** 75–83.

12 Invitti C, Redaelli G, Baldi G & Cavagnini F. Glucocorticoid receptors in anorexia nervosa and Cushing's disease. *Biological Psychiatry* 1999 **45** 1467–1471.

13 Gross MJ, Kahn JP, Laxenaire M, Nicolas JP & Burlet C. Système corticoliberinergique et anorexie mentale. *Annales d'Endocrinologie* 1994 **35** 221–228.

14 Björntorp P. Visceral obesity: a civilization syndrome. *Obesity Research* 1993 **1** 206–222.

15 Pasquali R, Cantobelli S, Casimirri F, Capelli M, Bortoluzzi R, Flamia R *et al.* The hypothalamic–pituitary–adrenal axis in obese women with different patterns of body fat distribution. *Journal of Clinical Endocrinology and Metabolism* 1993 **77** 341–346.

16 Rosmond R, Dallman MF & Björntorp P. Stress-related cortisol secretion in men: relationships with abdominal obesity and endocrine, metabolic and hemodynamic abnormalities. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 1853–1859.

17 Duclos M, Corcuff JB, Etcheverry N, Rashedi M, Tabarin A & Roger P. Abdominal obesity increases overnight cortisol excretion. *Journal of Endocrinological Investigation* 1999 **22** 465–471.

18 Walker BR, Phillips DI, Noon JP, Panarelli M, Andrew R, Edwards H *et al.* Increased glucocorticoid activity in men with cardiovascular risk factors. *Hypertension* 1998 **1** 891–895.

19 Fraser R, Ingram MC, Anderson NH, Morrison C, Davies E & Connell JM. Cortisol effects on body mass, blood pressure, and cholesterol in the general population. *Hypertension* 1999 **33** 1364–1368.

20 American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*, 4th edn. Washington, DC: American Psychiatric Press, 1994.

21 Hamilton M. A rating scale for depression. *Journal of Neurology and Neurosurgical Psychiatry* 1960 **23** 56–59.

22 WHO Expert Committee on Physical Status. *The Use and Interpretation of Anthropometry: Report of a WHO Expert Committee*, Geneva: World Health Organisation, 1995.

23 Wood PJ, Barth JH, Freedman DB, Perry L & Sheridan B. Evidence for the low dose dexamethasone suppression test to screen for Cushing's syndrome – recommendations for a protocol for biochemistry laboratories. *Annals of Clinical Biochemistry* 1997 **34** 222–229.

24 Kahn JP, Rubinow DR, Davis CL, Kling MK & Post RM. Salivary cortisol: a practical method for evaluation of adrenal function. *Biological Psychiatry* 1988 **23** 335–349.

25 Schlecthe JA & Hamilton D. The effect of glucorticoids on corticosteroid binding globulin. *Clinical Endocrinology* 1987 **27** 197–203.
26 Vining RF, McGinley RA, Maksvytis JJ & Ho KY. Salivary cortisol: a better measure of adrenal cortical function than serum cortisol. *Annals of Clinical Biochemistry* 1983 **20** 329–335.
27 Tunn S, Möllmann H, Barth J, Derendorf H & Krieg M. Simultaneous measurement of cortisol in serum and saliva after different forms of cortisol administration. *Clinical Chemistry* 1992 **38** 1491–1494.
28 Meulenberg PM & Hoffman JA. The effect of oral contraceptive use and pregnancy on the daily rhythm of cortisol and cortisone. *Clinica Chimica Acta* 1990 **15** 211–221.
29 Meeran K, Hattersley A, Mould G & Bloom SR. Venepuncture causes rapid rise in plasma ACTH. *British Journal of Clinical Practice* 1993 **47** 246–247.
30 Beherend EN, Kemppainen RJ & Young DW. Effect of storage conditions on cortisol, total thyroxine, and free thyroxine concentrations in serum and plasma of dogs. *Journal of the American Veterinary Association* 1998 **212** 1564–1568.
31 Chen YM, Cintron NM & Whitson PA. Long-term storage of salivary cortisol samples at room temperature. *Clinical Chemistry* 1992 **38** 304–305.
32 Clemens AD & Parker CR. The relationship between salivary cortisol concentrations in frozen versus mailed samples. *Psychoneuroendocrinology* 1998 **23** 613–616.
33 Cook N, Harris B, Walker R, Hailwood R, Jones E, Johns S *et al.* Clinical utility of the Dexamethasone Suppression Test assessed by plasma and salivary cortisol determinations. *Psychiatry Research* 1986 **18** 143–150.
34 Casper RC, Chatterton RT & Davis JM. Alterations in serum cortisol and its binding characteristics in anorexia nervosa. *Journal of Clinical Endocrinology and Metabolism* 1979 **49** 406–411.
35 Kennedy SH, Garfinkel PE, Parienti V, Costa D & Brown GM. Changes in melatonin levels but not cortisol levels are associated with depression in patients with eating disorders. *Archives of General Psychiatry* 1989 **46** 73–78.
36 Raff H, Raff JL & Findling JW. Late-night salivary cortisol as a screening test for Cushing's syndrome. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 2681–2686.
37 Toja P, Putignano P, Dubini A, Maraschini C, Invitti C & Cavagnini F. Midnight salivary cortisol for the diagnosis of Cushing's syndrome. *Journal of Endocrinological Investigation* **22** (Suppl 4) 1999 18–40 (Abstract).

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