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Is late-night salivary cortisol a better screening test for possible cortisol excess than standard screening tests in obese patients with Type 2 diabetes?

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Abstract

Aim To compare the performance, in terms of specificity for cortisol excess, of late-night salivary cortisol with 24-hour urine-free cortisol (24hr UFC) and overnight 1mg dexamethasone suppression test (1mg DST) in a group of obese T2DM patients.

Methods Forty obese patients with T2DM without clinical features of Cushing's syndrome were recruited. Plasma, urinary and salivary cortisol were measured directly by an enzyme-linked immunosorbent assay using monoclonal antibodies. The specificities of the three tests using various cutoffs were calculated and compared, employing the assumption that none of the patients had hypercortisolism.

Results The patients had a mean age and BMI of 56 years (range 31–75) and 37kg/m^2 (31–56) respectively. All 40 provided late-night salivary cortisol samples. Thirty-eight patients completed all three tests. Two patients only completed two screening tests. The specificities of late-night salivary cortisol (cutoff 10nmol/L), 24hr UFC (400nmol) and 1mg DST (50nmol/L) were 70% (95% CI 53–83%), 90% (76–97%) and 72% (55–85%) respectively. The specificity of late-night salivary cortisol was significantly less than 24hr UFC (P=0.039) but not 1mg DST (P>0.99).

Conclusion Late-night salivary cortisol has a poor specificity for cortisol excess in obese patients with T2DM with 24hr UFC showing significantly better specificity in our population.

Overt Cushing's syndrome is associated with a high prevalence of impaired glucose tolerance (60%) and Type 2 diabetes (15–20%), reflecting multiple adverse effects of glucocorticoids on glucose homeostasis.[1] These include increased hepatic gluconeogenesis, peripheral insulin resistance and finally a suppressive effect of glucocorticoids on beta-cell function. Furthermore, patients with Type 2 diabetes (T2DM) often have several clinical features of Cushing's syndrome, including central weight excess, hypertension and hyperglycaemia, raising the question of whether cortisol excess is more common in patients with T2DM than in a control population.[2]

An early prospective study by Catargi et al of 200 overweight inpatients with poorly controlled T2DM (HbA1C>8%) reported a 2% prevalence of occult Cushing's syndrome and concluded that systematic screening for cortisol excess might be warranted in this group.[3] Apart from a 9.4% prevalence of subclinical Cushing's syndrome later reported by Chiodini et al,[4] subsequent studies have generally found a low prevalence of Cushing's syndrome in patients with T2DM (0–1%), arguing against routine screening.[5–8]

Salivary cortisol is in equilibrium with biologically-active-free plasma cortisol and has a circadian rhythm which mirrors plasma cortisol. The measurement of late night salivary cortisol has been promoted as a non-invasive screening test for cortisol excess with 92–100% sensitivity and 93–100% specificity for the diagnosis of Cushing's syndrome.[9–12] However recent Endocrine Society guidelines caution that 'the influence of gender, age and coexisting medical conditions on late night salivary cortisol concentrations has not been fully characterised.'[13]

Previous studies utilising measurement of plasma and 24-hour urine cortisol (24hr UFC) have suggested that patients with T2DM, particularly those with microvascular complications, have activation of the hypothalamic-pituitary-adrenal (HPA) axis.[14–16] There is however limited and conflicting data regarding the utility of late night salivary cortisol to screen for cortisol excess in the setting of T2DM.

A study by Liu et al suggested that a raised bedtime salivary cortisol (>10nmol/L) was relatively uncommon in 141 patients with T2DM without Cushing's syndrome (3%).[17] Conversely, Mullan et al recently reported that 23% of 201 consecutive T2DM patients without evidence of Cushing's syndrome had a raised bedtime salivary cortisol (>10nmol/L).[8]

We hypothesised that bedtime salivary cortisol would be more specific than conventional tests, namely the overnight 1mg dexamethasone suppression test (1mg DST) and 24hr UFC, in screening for Cushing's syndrome in patients with T2DM. Thus we aimed to clarify the diagnostic performance of bedtime salivary cortisol compared with the 1mg DST and 24hr UFC in this patient group.

Since we were interested in examining the specificity of the various screening tests in the context of T2DM, we systematically excluded patients who had clinical features suspicious for cortisol excess.

Methods

Inclusion and exclusion criteria—We prospectively recruited 40 patients from the Christchurch Diabetes Centre outpatient clinic.

Inclusion criteria for the study were

- T2DM,
- Age 20–75 years, and
- Obesity (body mass index, BMI>30kg/m^2).

Exclusion criteria were

- Clinical features suspicious of Cushing's syndrome—namely proximal muscle weakness, easy bruising or broad (>1cm) violaceous striae,
- Use of oral, inhaled or topical steroids within the last 3 months,
- Oestrogen replacement,
- Current use of enzyme-inducing drugs,
- Shift workers,
- Depression (on antidepressants or under active clinical management for depression),
- Heavy alcohol intake (>14 or 21 standard drinks per week for women and for men respectively), and
- Hospitalisation for an acute condition within the previous month and
- Pregnancy.

Testing protocol—Baseline clinical and demographic data were recorded. Patients performed the three tests in the order listed with supervision by an endocrinology research nurse:

- Five consecutive bedtime salivary cortisol samples (normal <10nmol/L as per our previous report).[18] A minimum of 1ml of saliva was collected into a plastic container (Salivette®) on five consecutive evenings before retiring to bed and prior to brushing/flossing their teeth.
- A 24-hour urine collection for creatinine and cortisol (normal <400nmol).[19]
- A 1mg-DST (normal <50nmol/L).[13]

Steroid assays—Plasma, urinary and salivary cortisol were measured directly by an enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies.[20] For saliva and urine the cortisol was extracted with dichloromethane prior to ELISA whereas plasma cortisol was measured by a direct ELISA. The salivary cortisol assay has a limit of detection (LOD) of 3nmol/L, interassay coefficient of variation (CV) of 12.6% for the "low control" (mean cortisol value 7 nmol/L) and 7.4% for the "high control" (mean cortisol value 22 nmol/L). The urinary cortisol assay has a LOD of 22nmol/L and interassay CV of 8.5–13.3% over the range of 99 to 217nmol/L. The plasma cortisol assay has a LOD of 55nmol/L and interassay CV of 6.9–8.5% over the range of 98 to 1007nmol/L).

Statistical analyses—Test specificity was defined as:

$$Specificity(\%) = \frac{True\ negatives}{True\ negatives + False\ positives} \times 100$$

Specificity was calculated based on the assumption that all included patients did not have Cushing's syndrome.

McNemar's Chi-squared test was used to compare the performance of the screening tests, in terms of specificity for Cushing's syndrome. The 95% confidence interval (95% CI) of each test's specificity was calculated using the binomial distribution.

The relationships between BMI, HbA1C and salivary cortisol, 1mg-DST and 24hr-UFC were examined by Spearman's rank correlation coefficient. GraphPad Prism version 5.04 for Windows (GraphPad Software, San Diego, California USA) was used to generate the graphs.

The study was approved by the New Zealand Upper South B Regional Ethics Committee, and informed written consent was obtained from all patients.

Results

Demographic data

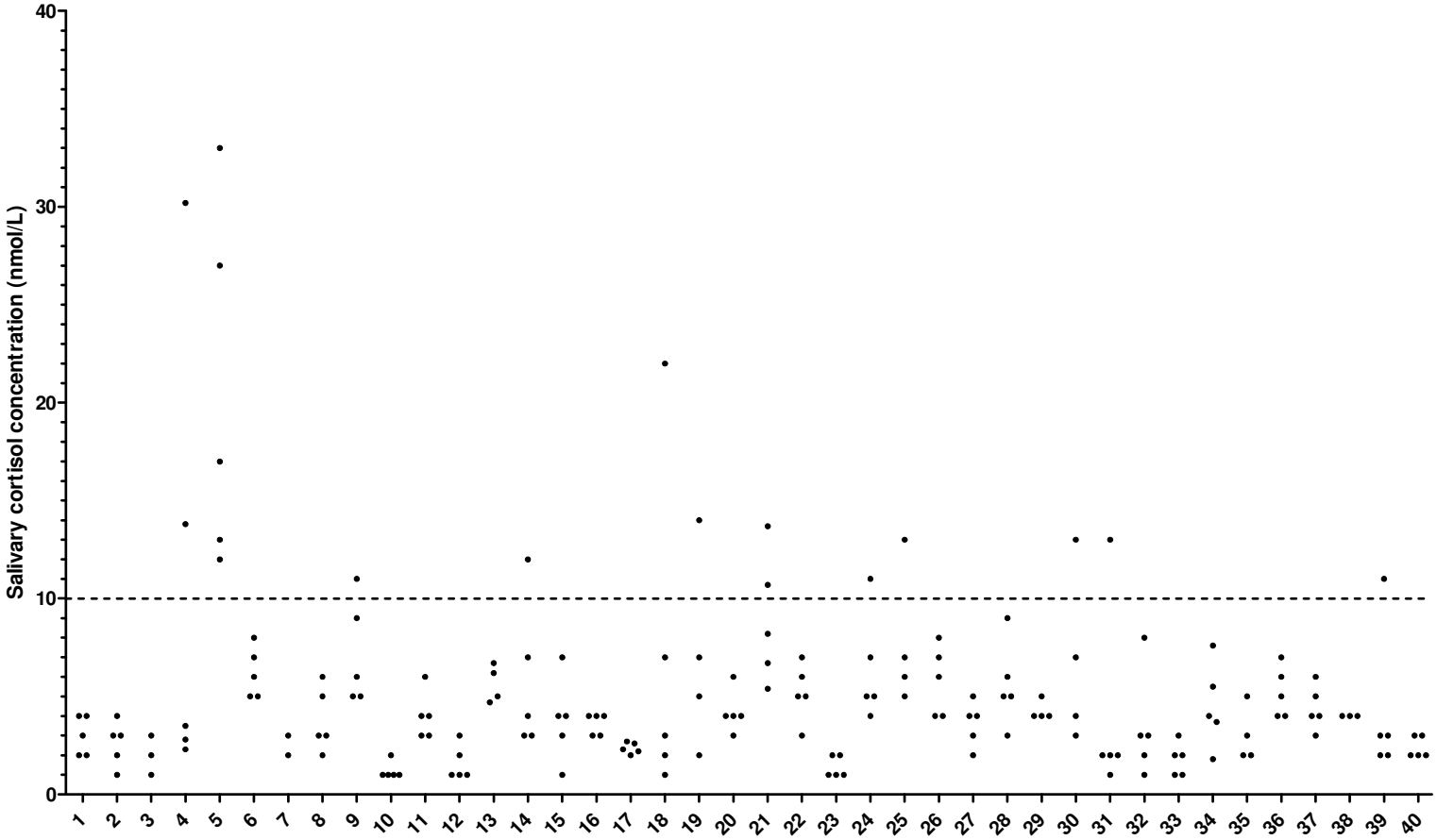
Forty patients with T2DM were studied, 15 male and 25 female, with mean age 56 years (31–75), BMI 37kg/m^2 (31–56) and HbA1C 8.6% (6.2–11.6). Overall glycaemic control was suboptimal with only 6 subjects (15%) having HbA1C ≤7% and 9 (23%) ≤7.5%.

Biochemical data

Salivary cortisol—32 subjects (80%) provided 5 bedtime salivary samples, a further five (12.5%) provided 4 samples, two provided 3 samples (5%) and a single patient provided only 2 samples (2.5%). Applying the 10nmol/L cutoff for bedtime salivary cortisol, 12/40 had at least one salivary cortisol result ≥10nmol/L—a false positive rate of 30% or specificity of 70% (95% CI 53–83%) (see Figure 1).

Seven of the 12 patients with one or more raised salivary cortisol results had only a single salivary cortisol minimally raised in the10–15nmol/L range and had both normal 1mg DST and 24hr UFC. Table 1 details the results of the remaining 5 subjects (with at least one salivary cortisol >15nmol/L or 10–15nmol/L with another abnormal test result).

Figure 1. Salivary cortisol concentrations



Note: Each dot represents one result (n=187 for 40 patients); Broken horizontal line represents cutoff (see text).

Table 1. Biochemical data of five obese patients with Type 2 diabetes, no clinical features of Cushing's syndrome with (1) at least one raised salivary cortisol >15nmol/L, or (2) at least one salivary cortisol between 10–15nmol/L with another abnormal test result (abnormal results in bold)

Age (yr)	BMI (kg/m^2)	HbA1C (%)	Salivary cortisol (nmol/L)†	1mg DST cortisol (nmol/L)‡	24hr UFC (nmol)§ [24h creat (mmol)]	Repeat salivary cortisol† (nmol/L)	Repeat 24hr UFC§ (nmol) [24h creat (mmol)]	48hr DST cortisol* (nmol/L)
46	39	10.1	3.5, 2.8, 2.3, **30.2, 13.8**	**90**	234 [6.9]	1.9, 6.8, 1.7, 1.6, 2	364 [14.7]	
54	39	11.1	**12, 27, 13, 33, 17**	**118**	380 [7.9]			**73**
68	33	7.5	2, 7, 1, 3, **22**	**63**	**485** [10.6]			11
58	30	6.5	8.2, 6.7, **10.7**, 5.4, **13.7**	27	**414** [17.3]		**611** [16.9]	
51	31	11.2	**13**, 6, 5, 7	**59**	253 [9.2]			

† normal salivary cortisol <10nmol/L

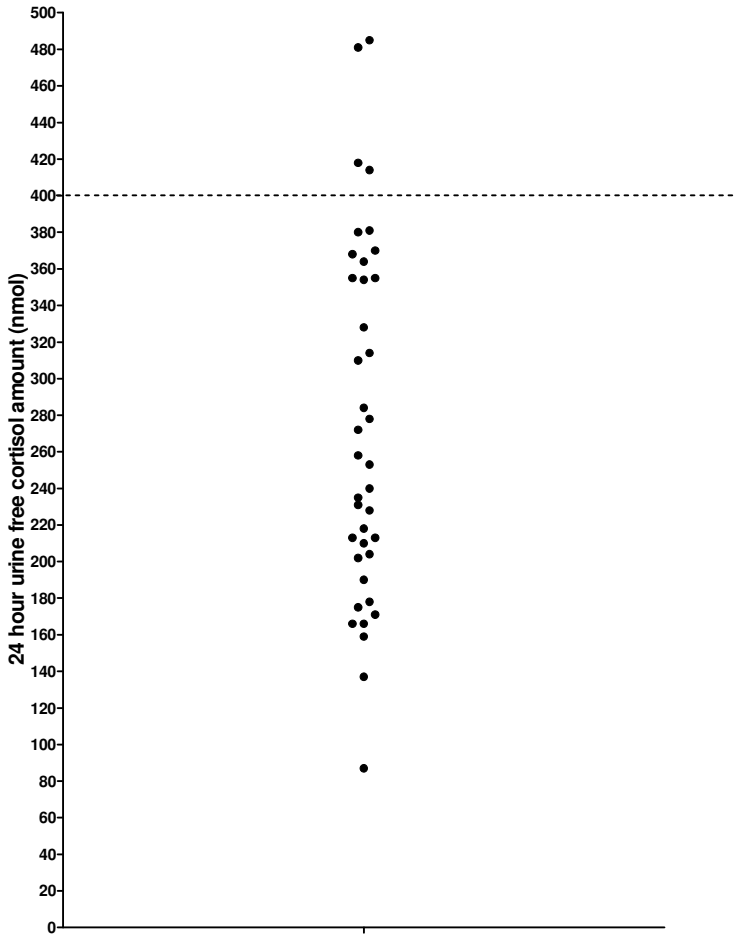
‡ normal morning cortisol after 1mg DST <50nmol/L

§ normal 24-hour UFC <400nmol

* normal cortisol after 48hr low dose Dexamethasone <50nmol/L

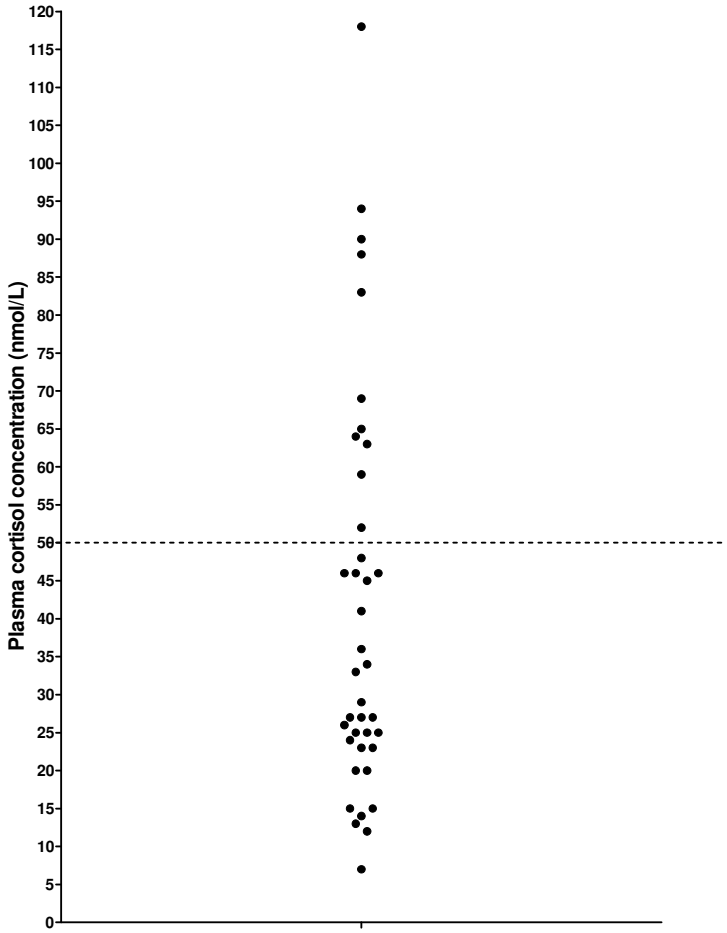
24hr UFC and 1mg DST—39 subjects completed a 24-hour urine collection and the 1mg DST. The specificity of the 1mg DST using the conventional cutoff of <50nmol/L was only 72% (95% CI 55-85%). The 24hr UFC had a specificity of 90% (95% CI 76-97%) using the cutoff <400nmol (see Figures 2 and 3).

Figure 2. 24-hour urine-free cortisol amounts



Note: Each dot represents one result (n=39 for 39 patients); Broken horizontal line represent cutoff (see text).

Figure 3. Plasma cortisol concentrations post-1mg dexamethasone suppression test



Note: Each dot represents one result (n=39 for 39 patients)Broken horizontal line represents cutoff (see text).

Overall, the specificity of salivary cortisol was inferior to 24hr UFC (P=0.039) but not 1mg DST (P>0.99). The difference in specificity between 24hr UFC (cutoff <400nmol) and 1mg DST (cutoff <50nmol/L) was not statistically significant (P=0.146).

Relationship between cortisol results and demographic data

There was no significant relationship between BMI, HbA1C and either the mean salivary cortisol, 1mg DST or 24hr UFC. There was a significant positive correlation between the mean salivary cortisol and 1mg DST cortisol (r=0.36, P=0.02) but not with 24hr UFC (r=0.19, P=0.2).

Discussion

Bedtime salivary cortisol is promoted as an accurate diagnostic test for Cushing's syndrome in view of the close correlation of salivary cortisol with free circulating cortisol, the ease of sample collection and the stability of salivary cortisol at room temperature.[9–12] The reported test sensitivity and specificity are between 95 and 98%, suggesting that bedtime salivary cortisol is an ideal screening test for Cushing's syndrome.[1] However the utility of bedtime salivary cortisol in the setting of T2DM remains contentious with the prevalence of raised salivary cortisol (>10nmol/L) in the two available studies reported as 3% and 23% respectively.[8,17]

Our study was designed to determine the specificity of bedtime salivary cortisol in obese patients with T2DM in the real world setting and aimed to answer the clinical question: how likely is it that a raised bedtime salivary cortisol in an obese patient with T2DM is a false positive result? We thus selected patients who did not have clinical features suspicious of Cushing's syndrome and performed standard screening tests for cortisol excess (bedtime salivary cortisol, 1mg DST and 24hr UFC) to compare the specificity of the tests in this context.

Our results indicate that the specificity of bedtime salivary cortisol using the cutoff of <10nmol/L (70%) was inferior to 24hr UFC using the cutoff of <400nmol (90%, P=0.039) but not 1mg DST using the conventional cutoff of <50nmol/L (72%, P>0.99).

Several previous studies have examined the activity of the HPA axis in patients with T2DM. Early reports found no alteration of the HPA axis in T2DM[21,22] although several more detailed recent studies have consistently described increased HPA axis activity as reflected by an elevation of basal ACTH, basal and post-dexamethasone cortisol, 24-hour urinary and salivary cortisol.[15,17,23–25] Furthermore, in a study of 190 patients with T2DM, Oltmanns et al[26] described a positive relationship between diurnal salivary cortisol concentrations and HbA1C, as well as fasting and postprandial glucose. Oltmanns speculated that the stimulatory effect of cortisol on hepatic gluconeogenesis may exacerbate hyperglycaemia and ultimately promote the development of diabetes-related complications.[26] This hypothesis is supported by cross-sectional studies which revealed a relationship between increased HPA axis activity and several diabetes complications, in particular carotid atherosclerosis, diabetic retinopathy and polyneuropathy.[14,27,28] Although correlations observed in cross-sectional studies do not prove causation, a putative mechanism for HPA axis activation is a reduction in parasympathetic tone, which may result in disproportionate sympathetic activation of the HPA axis.[29]

Our study, similar to recent reports, suggested activation of the HPA axis in a group of generally poorly controlled T2DM patients (mean HbA1C 8.6%) with a relatively high prevalence of false positive screening tests for cortisol excess (30% by bedtime salivary cortisol, 28% by 1mg DST and 10% by 24hr UFC) when applying cutoffs derived from a healthy reference population. The high rate of false positive screening tests for cortisol excess in our patient population, in whom Cushing's syndrome was not felt to be clinically likely, suggests that to confidently diagnose Cushing's syndrome in the setting of T2DM requires the use of normative data derived from a control population of patients with T2DM rather than from healthy controls.

In contrast to the earlier report of a positive association between glycaemic control and cortisol secretion,[26] our study did not reveal any correlation between HbA1C and several measures of cortisol secretion, possibly due to the limited number of subjects enrolled and the relatively narrow range of HbA1C results.

The overlap of clinical features in patients with Cushing's syndrome and centrally obese patients with T2DM has raised the question whether routine screening for cortisol excess is warranted in the context of obese patients with T2DM. This issue has been studied by several groups who have reported a variable prevalence of Cushing's syndrome ranging from 0–9.4% (Table 2).[3-8] Of note, the prevalence of 9.4% reported by Chiodini et al[4] referred to subclinical hypercortisolism, a biochemical diagnosis defined by failure of suppression of cortisol following 1mg DST with either a raised 24hr UFC, suppressed plasma ACTH or raised midnight cortisol. This remarkably high prevalence may be a reflection of the patient population studied (inpatients admitted for poor glycaemic control) and the use of test criteria determined in a healthy control population. On the basis of the relatively low reported prevalence of Cushing's syndrome in most screening studies (0–3%), consensus expert opinion is that systematic screening for Cushing's syndrome in obese patients with T2DM is not warranted.[13]

There are several limitations to our study. Obviously, the results of the study are highly dependent on the cortisol "cutoff" levels used to define an abnormal result. Whilst our cutoff for salivary cortisol was derived from an 'in-house' reference population, the other normal ranges were derived from the published literature rather than our own control population, which may have affected the results. Further, the specificity of the overnight DST in this setting of obesity may have been improved with the use of a higher dose of dexamethasone.[30]

It is also relevant that in our study each participant had several measurements of salivary cortisol compared with only one measurement of urine cortisol excretion and a single 1mg DST, potentially increasing the chance of a spurious salivary result. Additionally, the accuracy of 24-hour urine samples is also questionable because the compliance with urine collection instructions is known to be notoriously variable. Another concern is that we assumed that the included patients did not have Cushing's syndrome, based on the absence of clinical features. However, some of the five subjects with clearly abnormal results (as defined in Table 1) may have had mild Cushing's syndrome at the time of the study. We have not formally re-evaluated the patients but are not aware that any of these five patients have subsequently developed overt Cushing's syndrome 2 to 4 years following study completion. Alternatively, these patients may have had other conditions associated with elevated cortisol that were not part of our exclusion criteria, such as obstructive sleep apnoea.

In conclusion, in a population of obese patients with poorly controlled T2DM selected for the absence of specific features of cortisol excess, bedtime salivary cortisol has a high false positive rate (30%). This suggests that the test has limited specificity in this clinical context and raises questions regarding the utility of bedtime salivary cortisol as a screening test for Cushing's syndrome in patients with T2DM.

Based on our data, 24hr UFC has the lowest rate of false positive results. We cannot comment on test sensitivity, as we did not study a population with proven Cushing's syndrome. Thus, it is important to emphasise that conventional tests for cortisol

excess (1mg DST, 24hr UFC and bedtime salivary cortisol) have low specificity in obese patients with T2DM and such results need to be interpreted with caution.

Competing interests: None declared.

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