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LETTER TO THE EDITOR

Practical evaluation of late-night salivary cortisol: a real-life approach

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We were very pleased to read the comments made by Professor Raff [1] regarding our article [2].

We agree, the theory behind late-night salivary cortisol (LNSC) has been exhaustively studied and reviewed with one of the largest inputs in the development of LNSC measurement having been done by professor Raff [3–5]. However, practical implementation remains challenging because of discrepancies in cut-off values [6]. Recently professors Wartofsky and Handelsman [7] mentioned the necessity for LNSC standardization along with some other hormonal assays. Our study, [2] and the study by Carrasco et al. [8] is a good example of this problem, with a twice different cut-off value (4.2 [8]; 9.4 [2] nmol/l) for the same automated electrochemiluminescence immunoassay (ECLIA) method.

Professor Raff [1] suggested that among the obese patients in our study could have been some with subclinical Cushing's syndrome (CS). Subclinical CS is still an ill-defined endocrine condition. According to a recent review, ascertainment should stand on three criteria: adrenal adenoma; an unclear Cushingoid phenotype; autonomous cortisol secretion [9]. We state categorically that all patients with clinical findings suspicious of CS were thoroughly evaluated (including adrenal gland CT) and among 76 patients with constitutional obesity were no overt or subclinical CS. Patients who remained uncertain by the end of the study were excluded from the final analysis.

We provided an alternative explanation for the relatively high LNSC in obese patients, which was defined as functional hypercortisolism (cortisol excess not caused by tumors). Numerous papers describe hypercortisolism as one feature of obesity [10, 11]. Baid et al. measured LNSC by radioimmunoassay and tandem mass spectrometry ((LC–MS/MS) in 261 [12] and 369 [13] obese and overweight subjects did not confirm any cases of CS. They concluded that LNSC is frequently falsely abnormal in obese and overweight patients if laboratory-provided reference ranges are used for diagnostic interpretation [12, 13].

Further exploring this, we invited healthy individuals and a referred population of obese and overweight subjects. Cut-off value calculated to maximize specificity and sensitivity was higher in the referred population compared to healthy volunteers, providing one explanation for cut-off differences.

Carrasco et al. [8] included 35 patients with some clinical features of CS, restricted to having serum cortisol suppression after 1 mg of dexamethasone (DST) below 50 nmol/l and two normal levels of 24 h urinary free cortisol, as a comparator to 26 patients with CS including 4 with suspicion of recurrence.

It is clear the comparator group consisted of subjects who did not differ markedly from healthy volunteers and cut-off values were the same as in the study of CS versus healthy subjects [14].

Nevertheless, Carrasco et al. [8] suggested that the highest LNSC out of two let us to improve diagnostic performance.

We did not find any statistically significant difference between area under the curve (AUC) of single LNSC [0.953 (95 % CI 0.918–0.987)] or mean out of two LNSC [0.962 (95 % CI 0.930–0.993)] [2]. Using the highest LNSC out of two was also non-beneficial—AUC 0.960 (95 % CI 0.927–0.994) in our population. Similarly to Carrasco et al. [8] variability of LNSC was higher in patients with CS, but caused by high figures of cortisol (e.g., 43.8, 109.3 nmol/l;

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59.7, 26.4 nmol/l). However, patients with CS having lower LNSC were quite persistent (3.95, 4.90 nmol/l; DST—200 nmol/l, pituitary adenoma; 5.80, 4.30 nmol/l; DST—573 nmol/l pituitary adenoma; 5.60, 4.30 nmol/l; DST—73 nmol/l adrenal adenoma) [2].

Our data proves that single LNSC and DST are better than two LNSC, which is not different from single LNSC [2]. Consequently time and money can be saved for future evaluation.

Professor Raff expressed some concern about contamination of saliva samples with cross-reacting topical corticosteroids [1]. We emphasized that patients should not eat or brush their teeth at least 30 min before sampling [2].

We are truly blessed with patients, but quite restricted with funding. Happily some countries can afford not only to collect two samples of LNSC for logistical reasons, but also to measure it by LC–MS/MS. [4, 15] Although recent study on diagnostic utility of LNSC by LC–MS/MS (249 patients with 47 confirmed CS) reported similar sensitivity of 83.0 % and specificity of 84.2 % [15], only a "head to head" study for ECLIA and LC–MS/MS let us ascertain whether this more expensive method has diagnostic benefit over the cheaper and easier ECLIA.

To sum up, some cases of CS require a state of the art approach in their diagnostic evaluation. However, first line screening should be easy to perform and interpret in non-specialized centers. We hope that cut-off values for LNSC will be standardized and our data will be useful for this development.

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Conflict of interest The authors have nothing to disclose.

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