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Salivary versus serum cortisol as biomarkers of chronic stress in shift workers: A cross-sectional comparison of agreement and validity

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Abstract

Background: Salivary and serum cortisol are widely used biomarkers of stress, although they represent different physiological fractions and may not provide equivalent estimates of chronic stress. This study compared their agreement and validity in shift workers using hair cortisol as a reference measure of chronic exposure. **Methods:** A cross-sectional study included 160 adults, comprising 80 shift workers and 80 daytime workers. Salivary cortisol, serum total and free cortisol, corticosteroid-binding globulin, hair cortisol, and perceived stress were assessed. Agreement and criterion validity were examined using correlation, concordance, Bland-Altman, regression, and receiver operating characteristic analyses. **Results:** Shift workers showed higher salivary, serum, and hair cortisol concentrations than daytime workers. Salivary and serum free cortisol showed moderate agreement ($r = 0.70$; concordance coefficient = 0.62) with proportional bias. Salivary cortisol correlated more strongly with hair cortisol ($r = 0.592$) than serum total cortisol ($r = 0.438$; $p = 0.006$ for difference). Adjustment for corticosteroid-binding globulin increased the serum correlation to 0.53. Salivary cortisol remained independently associated with hair cortisol and demonstrated greater discrimination of high chronic stress (AUC = 0.801) than serum total cortisol (AUC = 0.716). **Conclusion:** Salivary cortisol showed greater validity than serum total cortisol for assessing chronic stress, partly because serum measurements are influenced by binding-globulin variation.

Keywords: Salivary cortisol, Serum cortisol, Hair cortisol, Chronic stress, Corticosteroid-binding globulin, Biomarker validity

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1. Introduction

Shift work disrupts the circadian organisation of physiology and is associated with a broad range of adverse health outcomes, from sleep disturbance and metabolic disease to cardiovascular disease and impaired psychological wellbeing (Kecklund and Axelsson, 2016; James *et al.*, 2017; Hulsege *et al.*, 2019). Much of this burden is attributed to chronic stress, and cortisol, the principal effector of the hypothalamic-pituitary-adrenal axis, is the biomarker most often used to quantify it. In occupational studies the hormone is measured in

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several matrices, and the choice between them is frequently made on grounds of convenience rather than of validity, on the tacit assumption that they are interchangeable measures of the same underlying quantity (Ritonja *et al.*, 2019; Boivin *et al.*, 2022).

That assumption is questionable on physiological grounds. In the circulation, cortisol exists largely bound to corticosteroid-binding globulin, with only some five to ten percent circulating in the free, biologically active form, and the serum cortisol reported by most clinical laboratories is the total concentration, the sum of the bound and free fractions (El-Farhan *et al.*, 2017; Debono *et al.*, 2016). Saliva, by contrast, contains only the free fraction, since the bound hormone cannot cross into the salivary gland, so that salivary cortisol is a direct measure of the biologically active hormone (Kirschbaum and Hellhammer, 1994; Gozansky *et al.*, 2005). The two matrices therefore measure different things, and they can diverge whenever the binding capacity of the blood varies, as it does with sex, with oral contraceptive use, with pregnancy and with a range of illnesses that alter the concentration of the binding globulin (Perogamvros *et al.*, 2010).

This distinction acquires particular importance when the object of measurement is chronic rather than acute stress. A single measurement of cortisol in saliva or serum captures the hormone at one moment and is heavily influenced by the time of sampling, by the awakening response and by transient events, and in shift workers, whose circadian rhythm is displaced and often flattened, a single time point is especially difficult to interpret (Stalder *et al.*, 2016). For this reason the measurement of cortisol in hair, which integrates secretion over the weeks to months during which the hair has grown, has become established as the reference measure of chronic cortisol exposure, and it is elevated in shift workers and in other chronically stressed groups (Russell *et al.*, 2012; Stalder *et al.*, 2017; Manenschijn *et al.*, 2011). Hair cortisol thus provides a criterion against which the shorter-term salivary and serum measures can be validated.

If salivary cortisol measures the free hormone while serum measures the total, then the two should agree only imperfectly, and salivary cortisol, as the biologically active fraction, might be expected to reflect the chronic stress captured by hair cortisol more faithfully than total serum cortisol, whose relationship to stress is diluted by the variable bound fraction. This reasoning has rarely been tested directly in a working population, with the two blood-based measures, the binding globulin and the hair reference all determined in the same individuals, and the comparison is the more relevant in shift workers, in whom the assessment of chronic stress is both important and difficult (Vanaelst *et al.*, 2012).

The present study was therefore designed to compare salivary and serum cortisol as biomarkers of chronic stress in shift workers. Its objectives were to determine the agreement between salivary and serum cortisol, to establish which of the two more closely reflects chronic stress as indexed by hair cortisol, and to test whether any advantage of salivary cortisol is explained by the confounding of the serum total measure by corticosteroid-binding globulin.

2. Materials and methods

2.1. Design and participants

A cross-sectional comparative study was conducted among 160 working adults, comprising 80 rotating or night-shift workers and 80 regular daytime workers. The groups were comparable in age and sex distribution. Shift workers were required to have followed their current work schedule for at least one year. Participants were excluded if they were pregnant, receiving oral or systemic corticosteroid therapy, had a diagnosed endocrine or major systemic illness, or had coloured or chemically treated hair that could interfere with hair cortisol assessment. The study was conducted and reported in accordance with recommendations for observational studies (Vandenbroucke *et al.*, 2007).

2.2. Cortisol and stress measurements

Cortisol sampling was performed under standardized conditions, with salivary sampling undertaken on awakening to minimize variation attributable to differences in sampling conditions. Salivary cortisol, representing the unbound fraction, was measured by immunoassay. Venous blood was collected shortly after salivary sampling, and serum was analyzed for total cortisol and Corticosteroid-Binding Globulin (CBG). Serum free cortisol concentration was subsequently derived by accounting for CBG. Hair samples were obtained

from the posterior vertex, and cortisol was measured in the proximal 3-cm segment, representing approximately the preceding three months of cortisol exposure (Wester and van Rossum, 2015). Hair cortisol was used as an integrated index of longer-term cortisol exposure. Perceived stress during the preceding month was assessed using the 10-item Perceived Stress Scale (PSS-10) (Cohen *et al.*, 1983).

2.3. Statistical analysis

Values are summarised as mean with standard deviation, or as median with interquartile range where the distribution was skewed, and the shift and day groups were compared by the t test or the Mann-Whitney test. The agreement between salivary and serum free cortisol was quantified by the Pearson correlation, by the Lin concordance correlation coefficient, and by the Bland-Altman method, from which the mean bias, the limits of agreement and any proportional bias were derived (Lin, 1989; Bland and Altman, 1986). The validity of each cortisol measure was assessed by its correlation with hair cortisol, the difference between the salivary and serum correlations being tested for significance by the Steiger method for dependent correlations (Steiger, 1980). The contribution of the binding globulin to the serum measure was examined by partial correlation, and the independent value of each measure by multiple regression of hair cortisol on the salivary and serum concentrations together. The discrimination of high chronic stress, defined by the upper tertile of hair cortisol, was assessed by the area under the receiver operating characteristic curve. All statistical tests were two-sided, and $p < 0.05$ was considered statistically significant.

3. Results

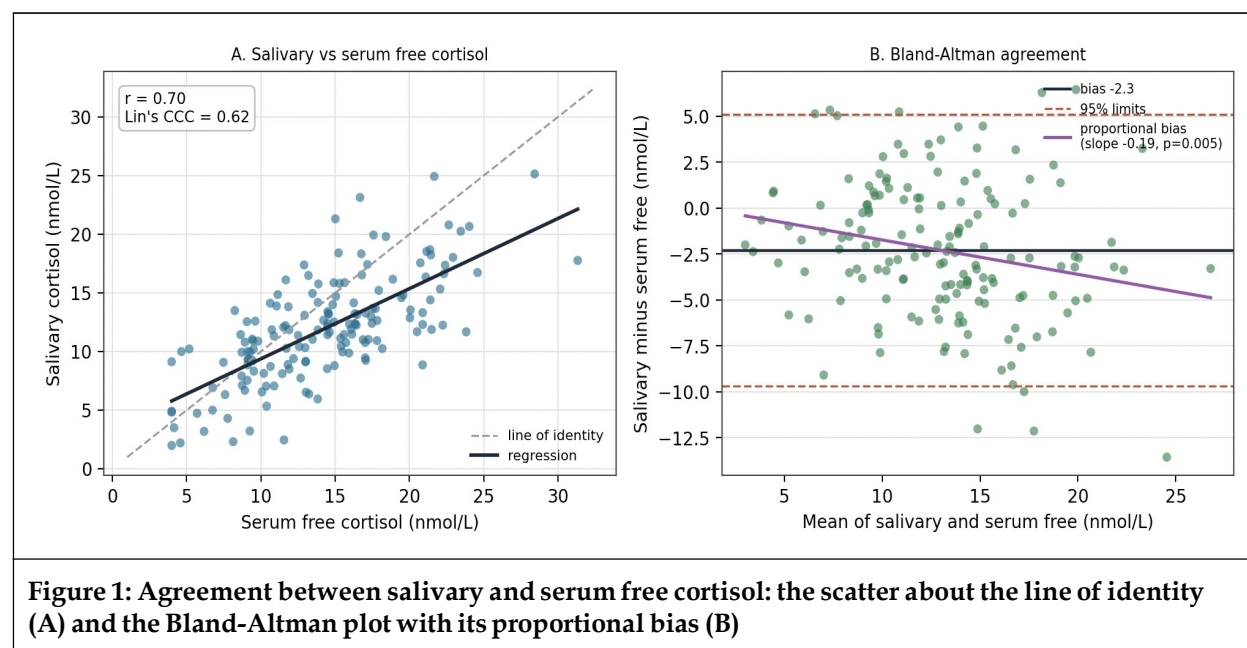
3.1. Characteristics of shift and daytime workers

The shift and daytime worker groups were comparable in age, sex distribution, and body mass index (Table 1). Perceived stress was significantly higher among shift workers, with a mean PSS-10 score of 20.4 ± 3.7 compared with 13.6 ± 4.0 among daytime workers ($p < 0.001$). Median hair cortisol concentration was also higher among shift workers [6.5 (IQR 4.5 – 9.1) pg/mg] than among daytime workers [4.6 (IQR 3.2 – 6.4) pg/mg; $p < 0.001$]. Similarly, salivary cortisol, serum total cortisol, and serum free cortisol concentrations were significantly higher in shift workers (all $p < 0.001$). In contrast, CBG concentrations did not differ significantly between the groups (42.0 ± 8.5 versus 41.3 ± 8.1 mg/L; $p = 0.615$).

Characteristic	Shift workers (n = 80)	Day workers (n = 80)	p
Age (years)	37.5 ± 8.6	38.6 ± 7.9	0.421
Female, n (%)	38 (48)	35 (44)	0.751
Body mass index (kg/m ²)	24.8 ± 2.7	24.8 ± 2.9	0.997
Perceived Stress Scale (PSS-10)	20.4 ± 3.7	13.6 ± 4.0	<0.001
Hair cortisol (pg/mg), median (IQR)	6.5 (4.5-9.1)	4.6 (3.2-6.4)	<0.001
Salivary cortisol (nmol/L)	13.3 ± 4.1	10.4 ± 4.3	<0.001
Serum total cortisol (nmol/L)	392 ± 129	308 ± 133	<0.001
Serum free cortisol (nmol/L)	15.8 ± 4.7	12.6 ± 5.1	<0.001
Corticosteroid-binding globulin (mg/L)	42.0 ± 8.5	41.3 ± 8.1	0.615
Duration of shift work (years)	7.9 ± 4.1	NA	-

3.2. Agreement between salivary and serum cortisol

Salivary cortisol and serum free cortisol were positively correlated ($r = 0.70$), but their agreement was moderate, with a Lin concordance correlation coefficient of 0.62. Bland-Altman analysis demonstrated a mean bias of -2.30 nmol/L together with wide limits of agreement and significant proportional bias, indicating that the



magnitude of the difference between the two measurements varied across the concentration range (Figure 1). Serum total cortisol concentrations were substantially higher than salivary concentrations because total serum cortisol includes both protein-bound and unbound fractions. Collectively, these findings indicate that salivary and serum cortisol measurements should not be considered directly interchangeable.

3.3. Association with longer-term cortisol exposure

Salivary cortisol showed the strongest correlation with hair cortisol ($r = 0.592$, 95% CI 0.482–0.685; $p < 0.001$), followed by serum free cortisol ($r = 0.520$, 95% CI 0.397–0.625; $p < 0.001$) and serum total cortisol ($r = 0.438$, 95% CI 0.303–0.555; $p < 0.001$). The correlation between salivary cortisol and hair cortisol was significantly stronger than that observed for serum total cortisol (Steiger $Z = 2.75$; $p = 0.006$). After adjustment for CBG, the partial correlation between serum total cortisol and hair cortisol increased from 0.438 to 0.53, approaching the correlation observed for serum free cortisol. Similar positive associations were observed with perceived stress, with correlation coefficients of 0.518 for salivary cortisol, 0.495 for serum free cortisol, and 0.462 for serum total cortisol (Table 2 and Figure 2). These findings indicate that accounting for CBG strengthens the relationship between serum cortisol and longer-term cortisol exposure.

Table 2: Correlation of each cortisol measure with hair cortisol and with perceived stress

Cortisol measure	r with hair cortisol [95% CI]	p	r with PSS
Salivary cortisol	+0.592 [+0.482, +0.685]	<0.001	+0.518
Serum total cortisol	+0.438 [+0.303, +0.555]	<0.001	+0.462
Serum free cortisol	+0.520 [+0.397, +0.625]	<0.001	+0.495

3.4. Independent associations and discrimination

In the multiple regression model adjusted for age, sex, and shift-work status, salivary cortisol remained independently associated with log-transformed hair cortisol (standardized $\beta = 0.263$, 95% CI 0.177–0.348; $p < 0.001$). In contrast, serum total cortisol was not independently associated with hair cortisol after inclusion of salivary cortisol in the same model ($\beta = 0.054$, 95% CI –0.030 to 0.138; $p = 0.204$). The overall model explained 43.6% of the variance in hair cortisol ($R^2 = 0.436$). In ROC analysis, salivary cortisol demonstrated the highest discrimination for high chronic cortisol exposure, defined as the upper tertile of hair cortisol (AUC = 0.801), compared with serum free cortisol (AUC = 0.740) and serum total cortisol (AUC = 0.716) (Table 3 and Figure 3).

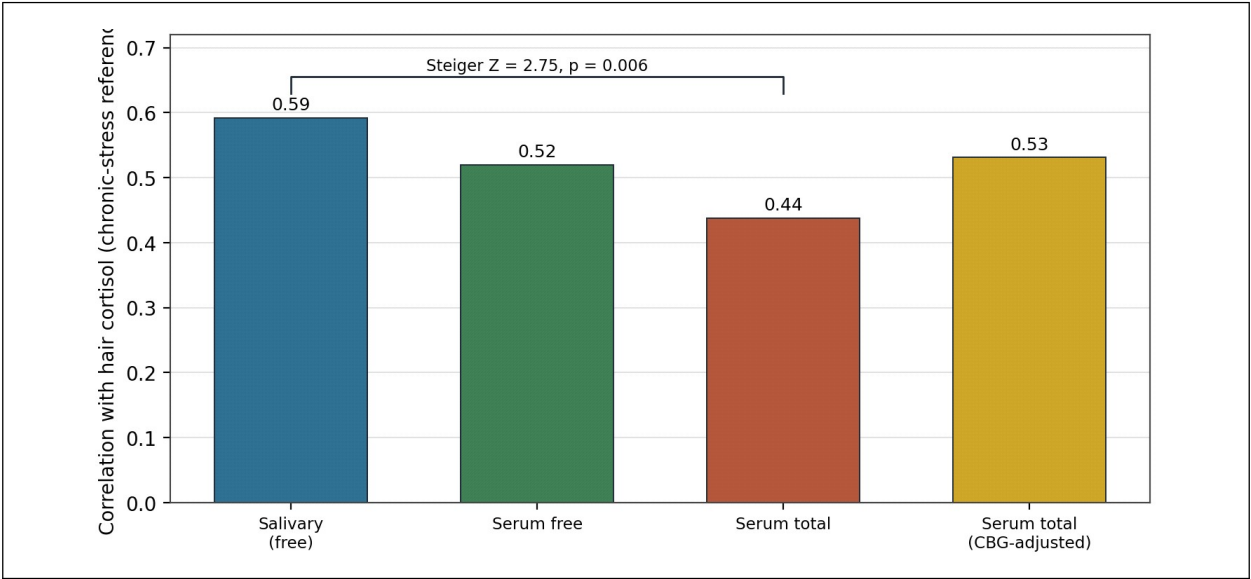


Figure 2: Correlation of each cortisol measure with hair cortisol. Salivary free cortisol tracks the chronic-stress reference most closely, and adjusting the serum total measure for binding globulin removes most of its disadvantage

Table 3: Multiple regression of log hair cortisol on salivary and serum total cortisol, adjusted for age, sex and shift status ($R^2 = 0.436$)

Predictor	Standardized β	95% CI	p
Salivary	+0.263	+0.177 to +0.348	<0.001
Serum total	+0.054	-0.030 to +0.138	0.204
Age	+0.131	+0.066 to +0.195	<0.001
Female	-0.057	-0.187 to +0.072	0.383
Shift	+0.158	+0.021 to +0.295	0.024

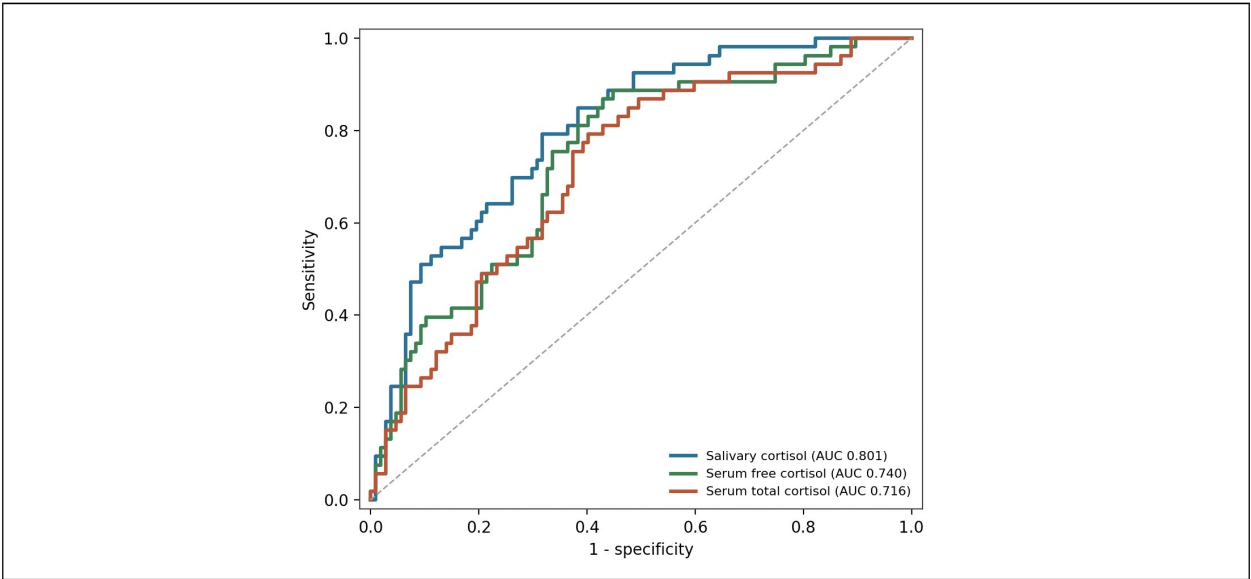


Figure 3: Discrimination of high chronic stress, defined by the upper tertile of hair cortisol, by each cortisol measure

4. Discussion

This study compared salivary and serum cortisol as indicators of chronic stress-related cortisol exposure in shift workers using hair cortisol as an integrated measure of longer-term exposure. Three principal findings emerged. First, salivary and serum free cortisol were correlated but demonstrated only moderate agreement and significant proportional bias, indicating that the two matrices are not directly interchangeable. Second, salivary cortisol showed a stronger association with hair cortisol than serum total cortisol and remained independently associated with hair cortisol when both measures were included in the same multivariable model. Third, accounting for CBG strengthened the association between serum cortisol and hair cortisol, suggesting that interindividual variation in cortisol binding may partly explain the weaker performance of serum total cortisol. Together, these findings support salivary cortisol as the stronger single-morning measure of longer-term cortisol exposure among the cortisol measures evaluated in this study.

The moderate agreement between salivary and serum free cortisol is physiologically plausible because saliva primarily reflects the unbound fraction of circulating cortisol, whereas routine serum total cortisol includes both free and protein-bound hormone. Although serum free cortisol was derived to provide a more comparable measure, the concordance coefficient of 0.62 and the presence of proportional bias indicate meaningful differences between the two matrices. These findings emphasize that correlation alone does not establish agreement and that salivary and serum cortisol values should not be used interchangeably when monitoring individuals or comparing results across studies.

The comparison with hair cortisol provides additional evidence regarding the relative validity of the different measurements. Salivary cortisol showed a significantly stronger correlation with hair cortisol than serum total cortisol. Furthermore, adjustment for CBG increased the serum total cortisol correlation from 0.438 to 0.53, while derived serum free cortisol showed an intermediate correlation of 0.520. These observations are consistent with the hypothesis that variability in cortisol binding influences the relationship between total serum cortisol and longer-term cortisol exposure. The multivariable analysis supports this interpretation: salivary cortisol remained independently associated with hair cortisol, whereas serum total cortisol was no longer significant when both measures were considered simultaneously ([Short et al., 2013](#)).

This account fits the established physiology of the hormone and the existing literature. It is the free cortisol that is biologically active and that enters tissues, including the salivary gland and the growing hair, so that salivary and hair cortisol both sample the same free compartment, whereas the serum total measure sums the free hormone with a bound reservoir that is regulated separately ([Qian et al., 2012](#)). The observation, reported previously, that hair cortisol correlates with salivary cortisol but not with single serum samples is explained on exactly these grounds, and the present study extends it by showing that the serum measure is not intrinsically invalid but is confounded by binding, and can be substantially rescued by accounting for it.

From a practical perspective, these findings favour salivary cortisol when a single, minimally invasive cortisol measurement is required in occupational stress research. Salivary sampling avoids venepuncture, can be performed close to awakening, and predominantly reflects unbound cortisol. However, a single salivary measurement remains sensitive to circadian timing and short-term variability and should not be interpreted as equivalent to an integrated measure of chronic exposure. When serum total cortisol is used, concurrent consideration of CBG may improve interpretation, particularly when comparing individuals with potentially different cortisol-binding characteristics. Hair cortisol remains useful as a complementary measure because it integrates cortisol exposure over a substantially longer period than a single saliva or serum sample.

5. Strengths and limitations

This study has several strengths, including the simultaneous assessment of salivary cortisol, serum total and derived free cortisol, CBG, hair cortisol, and perceived stress within the same participants. The inclusion of both agreement and validity analyses allowed the relationship between salivary and serum measurements to be evaluated beyond simple correlation, while the inclusion of CBG provided a physiological basis for examining differences between total and free cortisol measures. Several limitations should also be considered. First, the cross-sectional design does not establish temporal or causal relationships between shift work, perceived stress, and cortisol exposure. Second, salivary and serum cortisol were assessed at a single sampling occasion and therefore cannot characterize diurnal cortisol profiles or day-to-day variability. Third, hair cortisol, although

useful as an integrated measure of longer-term exposure, may be influenced by individual differences in hair characteristics, washing practices, and growth rate. Fourth, serum free cortisol was derived rather than measured directly. Finally, the sample size may have limited subgroup analyses, particularly those examining sex-specific or shift-pattern-specific differences. Future longitudinal studies incorporating repeated cortisol sampling would strengthen these findings.

6. Conclusion

Salivary and serum cortisol were not interchangeable measures of cortisol exposure in this cross-sectional study of shift and daytime workers. Although salivary and derived serum free cortisol were positively correlated, their agreement was only moderate and proportional bias was present. Salivary cortisol showed a stronger association with hair cortisol than serum total cortisol and remained independently associated with hair cortisol in multivariable analysis. Accounting for CBG strengthened the relationship between serum cortisol and hair cortisol, indicating that variation in cortisol binding may partly influence the performance of serum total cortisol as a stress-related biomarker. Salivary cortisol also demonstrated better discrimination of high longer-term cortisol exposure than either serum total or derived free cortisol. These findings support salivary cortisol as the more informative single-morning cortisol measure among the matrices examined, while emphasizing that a single salivary or serum measurement cannot replace an integrated measure of longer-term cortisol exposure. Consideration of CBG may improve the interpretation of serum cortisol when serum-based assessment is required.

Conflicts of interest

The authors declare that no conflict of interest exists in relation to this work.

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