

Validation of an Electrochemical Sensor to Detect Cortisol Responses to the Trier Social Stress Test

Guido G. Urizar Jr.^{a,*}, Hugo Sanchez Hernandez^a, Jessica Rayo^a, Shekhar Bhansali^b

^a Department of Psychology, California State University, Long Beach, 1250 Bellflower Blvd, Long Beach, CA, 90840-0901, USA

^b Department of Electrical & Computer Engineering, Florida International University, 10555 West Flagler St. EC 3900, Miami, FL, 33174, USA

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ABSTRACT

Recent advances in sensor technology allow for the detection of salivary cortisol levels in real-time, yet studies are needed to test their reliability in clinically at-risk populations. This study examined whether a new electrochemical sensor reliably detected cortisol patterns, compared to a conventional immunoassay test (i.e., ELISA), among women and men with low and high depressive symptoms who participated in the Trier Social Stress Test (TSST; a laboratory-based stressor). Results demonstrated that women and those with high depressive symptoms showed lower cortisol levels throughout the TSST overall compared to men and those with low depressive symptoms. The cortisol sensor was highly reliable when compared to the ELISA immunoassay in detecting cortisol responses to the TSST for both women and men and for participants with low and high depressive symptoms. These results suggest that the sensor is a promising tool for assessing real-time cortisol responses to laboratory stressors in at-risk populations.

1. Introduction

1.1. Cortisol and the stress response

Cortisol is a glucocorticoid hormone that has been widely studied in relation to the effects of chronic stress on health given that it regulates a variety of metabolic and immune processes in the body through the hypothalamic-pituitary-adrenal (HPA) axis and is released by the adrenal glands in response to stressors in our environment (Adam and Kumari, 2009). The release of cortisol functions to provide the energy resources needed (e.g., stored glucose sent to muscles) to manage the stressor at hand through the fight-or-flight response. Cortisol reactivity refers to the abrupt rise in cortisol in response to a stressor while cortisol recovery refers to cortisol levels returning to normal (i.e., homeostasis) after the stressor ends or is resolved (McEwen, 2007). Short-term activation of the HPA axis (and subsequent release of cortisol) in response to stressors is adaptive and necessary for everyday functioning. However, frequent or chronic release of cortisol in the body (especially when experiencing chronic stress) has been associated with several negative health outcomes including depression, impaired immune functioning, and cardiovascular disease (Adam et al., 2017). The reported changes in cortisol in response to both acute and chronic stress underscore the

importance of assessing cortisol to identify populations at risk for adverse health outcomes.

1.2. Cortisol response to the Trier Social Stress Test

Cortisol is routinely assessed in research studies through saliva given that it has been shown to be a reliable measure of HPA axis activity and psychological stress, is non-invasive, is easier to collect and handle than other bodily fluids (e.g., blood, urine), and is able to show acute changes in cortisol in both naturalistic and laboratory-based settings (Adam and Kumari, 2009). In the laboratory, altered cortisol activity is identified by exposing individuals to well-validated and widely used laboratory stressors, such as the Trier Social Stress Test (TSST), to determine their biological stress response (Kirschbaum et al., 1993). The TSST is a laboratory procedure that has been found to reliably induce stress and produce changes in salivary cortisol across several populations through the use of standardized stress-generating tasks that include elements of public speaking, mental arithmetic, and anticipation (Allen et al., 2017). Results help researchers to identify participants' cortisol reactivity to and recovery from the TSST with salivary cortisol levels typically rising immediately after the TSST and then returning to baseline levels 30–60 min after the TSST.

* Corresponding author. Department of Psychology, California State University, Long Beach, 1250 Bellflower Blvd, Long Beach, CA, 90840-0901, USA.

E-mail addresses: guido.urizar@csulb.edu (G.G. Urizar), hugos1@uci.edu (H.S. Hernandez), jessrayo@umich.edu (J. Rayo), sbhansa@fiu.edu (S. Bhansali).

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Although these cortisol responses to the TSST are expected, studies have shown that certain populations display elevated and blunted cortisol reactivity and recovery in response to the TSST, which have been associated with increased risk for health problems. For example, studies have generally shown women to have lower cortisol reactivity and less cortisol recovery (i.e., blunted cortisol responses) to the TSST compared to men (Liu et al., 2017). In turn, these blunted cortisol responses to the TSST have been associated with major depression among women, but not men (Zorn et al., 2017). These gender differences in cortisol patterns are particularly salient given that women are twice as likely to develop depression compared to men, with the greatest risk for depression occurring during late adolescence/early adulthood (Kessler, 2003). These results demonstrate the need for additional experimental studies and screening methodologies to examine how altered cortisol activity may serve as a biological indicator for gender differences in depression risk.

1.3. Cortisol screening methodologies

The results from TSST studies are promising in helping to identify populations at risk for depression. However, a major limitation of these studies is the long turn-around period in obtaining cortisol results after collecting an individual's saliva samples. This time period can range from a few weeks to several months because of the need to send large volumes of saliva samples to specialists in a diagnostic laboratory for elaborate chemical analyses. These analyses typically involve assessing salivary cortisol through immunoassays, which is the gold standard measure for the presence of cortisol molecules in saliva through very selective and specific antigen-antibody binding. Of the various types of immunoassays that exist, the enzyme-linked immunosorbent assay (ELISA) has been the most widely used because of its sensitivity and versatility in determining protein concentration (Kaushik et al., 2014). However, many challenges exist when using the ELISA. It is complex, requiring multiple assay steps (e.g., extraction and purification of samples) and long incubation times. It also requires several saliva samples and specific expertise to run an assay (Kaushik et al., 2014). Thus, there is a need for ambulatory assessments of cortisol that are reliable, accurate, cost-effective, and non-invasive to help identify populations at risk for stress-related health problems, such as depression, in order to promote optimal health outcomes.

As a result of the challenges with immunoassays, recent technological advances have allowed electrochemical sensors to increasingly be used as alternative cortisol-detecting methods (for a review, see Singh et al., 2014). These sensors are the size of blood glucose strips and provide instantaneous salivary cortisol results by placing a small drop of

saliva on the sensor, which is connected to a monitoring device that records the information at the surface of the sensor and provides cortisol values on-site (Manickam et al., 2018; see Fig. 1). These sensors' ability to accomplish point-of-care cortisol measurement brings many benefits not present when using the ELISA. They can provide quick cortisol results on-site without transportation of cortisol to a laboratory, can produce cortisol values within a shorter timeframe, and can be significantly less expensive (i.e., no large samples will have to be sent out for elaborate analyses). Thus, the sensor detection of cortisol is simple, flexible, and cost-effective, allowing for real-time reporting of salivary cortisol with the precision and accuracy of traditionally labor-intensive and laboratory-based immunoassays. However, before electrochemical sensors can be used as alternative methods to cortisol detection, they first need to be accurately validated as being effective and reliable by comparing their results with those of the ELISA in determining altered cortisol patterns among clinically at-risk populations (Pasha et al., 2014; Singh et al., 2014).

1.4. Current study

The purpose of the current study was to examine whether a new electrochemical sensor would reliably detect cortisol patterns by comparing its results to that of the ELISA among young women and men with low and high symptoms of depression who participated in the TSST. Based on findings from previous studies, we hypothesized that women, particularly those with high depressive symptoms, would demonstrate more blunted cortisol reactivity and recovery in response to the TSST compared to men. We also hypothesized that there would be a strong relationship (i.e., high association, consistency, and agreement) between the cortisol values obtained from the electrochemical sensor and those from the ELISA when examined by gender and level of depressive symptoms.

2. Method

2.1. Participants

Our sample consisted of 110 women ($n = 82$) and men ($n = 28$), who participated in a study examining depression risk and health outcomes among young adults. Participants were recruited from an undergraduate psychology research subject pool and flyers posted around campus of a public university in southern California. To be eligible for the study, participants had to be 18 years of age or older, fluent in English, and be willing to attend a 2-h laboratory session to assess their stress response. Participants were excluded from this study if they had smoked any

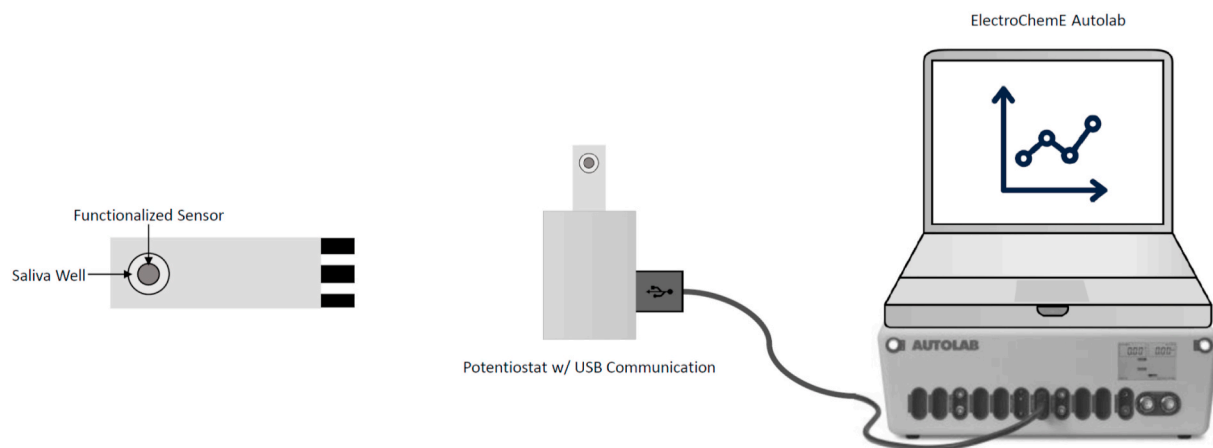


Fig. 1. Electrochemical cortisol sensor system comprising of disposable collector strip and a potentiostat/galvanostat reader with digital display. A drop of saliva is placed on the well of the collector strip (carbon electrode) which is connected to an autolab potentiostat/galvanostat to record cortisol values using scanning electron microscopy in approximately 1 min.

tobacco or nicotine products during the past six months (e.g., cigarettes, tobacco, or vaporizers), had Type 1 or Type 2 diabetes, had any currently diagnosed mental health condition (e.g., major depressive disorder, anxiety disorder), and used any medication containing corticosteroids (e.g., asthma inhalers; cortisone, prednisone, or prednisolone medications; and/or corticosteroid creams) during the past six months.

2.2. Procedures

Eligible participants who were interested in the study signed an informed consent form and took part in the 2-h Trier Social Stress Test (TSST), which has been found to reliably induce changes in salivary cortisol in young adults (Kirschbaum et al., 1993). The TSST took place between 12pm and 6pm to minimize the effects of the pronounced diurnal variation found in cortisol. Participants were instructed to refrain from engaging in certain behaviors (e.g., eating, drinking, exercising) known to interfere with salivary cortisol samples for at least 30 min before their appointment time (Gröschl et al., 2001).

Upon arrival to their appointment, participants were brought to an assessment room by a lead research staff member and asked to provide the first of five saliva samples to serve as a baseline prior to participating in the TSST. The first saliva sample was collected during an initial 30-min rest and acclimation period. Participants provided these samples using a passive drool method by allowing saliva to pool in their mouths, and then transferring their saliva into a collection tube using a saliva collection aid (similar to a straw) by tilting their head forward and carefully spitting into the collection tube (Granger et al., 2007). Participants were then led to a second room to perform public speaking (simulated job interview) and mental arithmetic (serial subtraction) tasks in front of two judges (i.e., research assistants) who were videotaping their performance. These tasks lasted a total of 15 min. After participants exited the room, a second saliva sample was immediately taken. Throughout the 45-min recovery period, three additional saliva samples were collected at 15, 30, and 45 min after the TSST. During the recovery period, participants were debriefed on the TSST and asked to complete a questionnaire packet, which included demographic questions and a depression measure. At the conclusion of the study, participants were compensated for their time and effort with course credit for their introductory psychology course and/or a gift card (up to \$10). All study procedures were approved by the Institutional Review Board at California State University, Long Beach.

2.3. Measures

Sociodemographics. A sociodemographic questionnaire assessed participant characteristics including gender (female, male), ethnicity, age (in years), level of education, living situation (living with parents, living alone), and source of financial support (financial aid, family support, independent support).

Depressive symptoms. Depressive symptoms were assessed using the second edition of the Beck Depression Inventory (BDI-II; Beck et al., 1996). The BDI-II is a 21-item, self-report measure assessing symptoms of depression during the past two weeks. Each item is rated on a 4-point Likert scale ranging from 0 to 3, with higher scores (range = 0 to 63) reflecting greater depressive symptoms. Previous studies have used a cut-off score of 17 to distinguish mild from moderate/severe depressive symptoms (Huffman et al., 2010). Therefore, this cut-off score was used in the current study to identify participants with lower (<17 on the BDI-II) or higher (≥ 17 on the BDI-II) levels of depressive symptoms. The BDI-II has been validated for use with young adults and has shown high internal consistency (Beck et al., 1996). The internal consistency for the present study was also high ($\alpha = 0.88$).

Salivary Cortisol. Salivary cortisol samples were frozen and stored in a $-20\text{ }^{\circ}\text{C}$ freezer until analysis at Florida International University where they were thawed and analyzed using two different methodologies. One research staff member independently analyzed samples for all 110

participants with the traditional ELISA, using a commercially available cortisol assay kit (Salimetrics, LLC), which involved using a time-resolved immunoassay with fluorescence detection. A second research staff member independently analyzed samples for 20 random participants from the larger participant sample with the electrochemical cortisol sensor.

The 20 participants were chosen using a stratified randomization procedure. They were first stratified by gender (10 women, 10 men) and then stratified by depression level (5 with low depressive symptoms, 5 with high depressive symptoms for both women and men) to ensure equal numbers across these groups when analyzing their cortisol samples using both the ELISA immunoassay and electrochemical cortisol sensor. Cortisol values were obtained for each of the five study time points (baseline, 1 min-post-TSST; 15 min-post TSST; 30 min post-TSST; and 45 min post-TSST). The two research staff members who independently analyzed the saliva samples were blind to participant characteristics of the samples (i.e., gender, depression level) throughout the study and submitted the cortisol values obtained from both the ELISA and the cortisol sensor to separate databases for later comparison by a third research staff member. Higher values (nmol/L) were indicative of higher cortisol levels. Intra- and interassay variability for both the ELISA and cortisol sensor were both under 10%.

The cortisol sensor system consisted of a disposable collector strip and a potentiostat/galvanostat reader with digital display (see Fig. 1). The collector strip was made of a saliva well at its tip along with a planar screen-printed carbon electrode that consisted of a carbon working electrode, carbon counter electrode, and an Ag/AgCl reference electrode that were treated and functionalized to make the cortisol sensor (CH Instruments, Inc., USA). The stepwise sensor fabrication procedure was as follows. Multi-wall Carbon Nanotubes (MWCNT) suspension was prepared by diluting the CNT in the Nafion solution (0.5%, w/v) at the concentration of 10 mg/mL and sonicated for 3 min using an ultrasonic probe. To this, metalloporphyrin (MTPP) solution (1 mg/mL in chloroform) was added and the mixture was stirred for 2 h at room temperature. The resulting mixture was again sonicated for 2 min to achieve a homogeneous dispersion. Ten μL of the composite solution was then drop casted onto the working electrode to completely cover it, followed by drying at $40\text{ }^{\circ}\text{C}$. The same sequence of drop casting and drying was repeated multiple times when necessary to achieve the multiple layers of electrocatalyst coating onto the working electrode. For control studies, CNT nanostructure was obtained by drop casting 10 μL of CNT solutions (without the addition of MTPP) on the working electrode followed by drying at $40\text{ }^{\circ}\text{C}$. Fig. 2 shows the typical electrochemical responses obtained for Copper-MTPP and MWCNTs-Copper MTPP modified SPCE at a scan rate of 50 mV s^{-1} in 0.1 M PBS (pH 7.0). It can be seen that the MWCNTs-CuTPP exhibited a significant increase in current response than the CuTPP-SPCE. This is attributed to the enhancement of electron transfer characteristics of CuTPP by MWCNTs.

The cortisol sensor is based on an electrochemical response of the analyte that is measured using a cyclic voltammetry technique. The cortisol sensors are fabricated in batch and each sensor in a batch shows less than 5% variability in the output. The output of the bare sensor is compared with the test sensor to quantify the cortisol level. The time required to measure cortisol levels using the sensor is about 1 min, the typical shelf life of the sensor is three to four weeks, and the cost for each test is one to two dollars (cost of screen-printed electrode and analytes). In comparison, the ELISA is generally carried out using a 96 well plate. The time required for measuring cortisol levels using the ELISA is in hours and the typical cost for each test is about \$30 (cost of the 96 well plate and ELISA kit).

The as prepared screen-printed cortisol sensors were used for measurement of cortisol. A small drop of saliva was placed on the well of the collector strip, which was connected to an autolab potentiostat/galvanostat (Eco Chemie, Netherlands) to record the cortisol values from the surface of the sensor using field emission scanning electron microscopy (SEM; compositional and topographic) equipped with energy dispersive

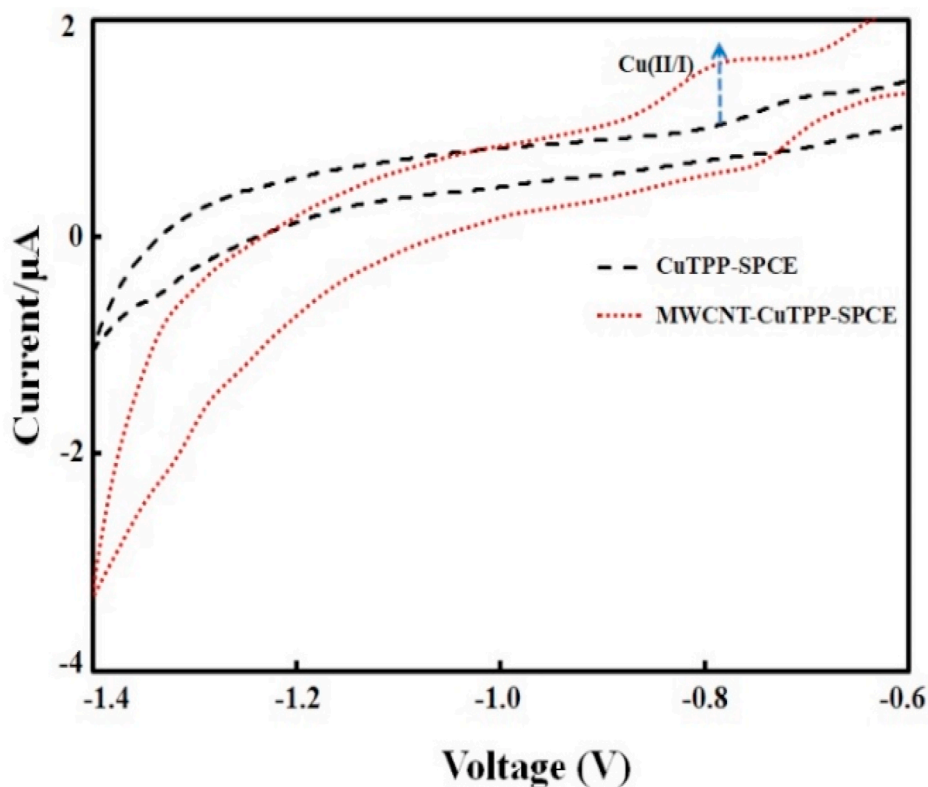


Fig. 2. Electrochemical response of copper metalloporphyrin (Cu MTPP) and multi-wall carbon nanotubes-copper MTPP modified screen-printed carbon electrode (MWCNT-Cu MTPP-SPCE) in 0.1 M PBS (pH 7.0).

spectroscopy (JEOL SEM 6330 F; Manickam et al., 2018). Cortisol values were displayed as a digital readout (in nmol/L) with a date and time stamp. From saliva collection to readout, the duration of the entire test was approximately 1 min.

2.4. Statistical analyses

A mixed effect linear model was used to test for changes in salivary cortisol levels over the five study time points (baseline and 1min, 15min, 30min, and 45min post-TSST), as well as average cortisol differences by gender and depression group. Mixed effect linear models were also performed to test for significant two way (gender by time, depression group by time) and three way interactions (i.e., gender by depression group by time) that could influence gender and depression effects on salivary cortisol responses to the TSST. These mixed effect linear models were tested controlling for oral contraceptive use given that women who use oral contraceptives show reduced cortisol responses to the TSST compared to women who do not use oral contraceptives (Liu et al., 2017). The mixed model was estimated by maximum likelihood using SAS PROC MIXED (SAS Institute, Cary, NC, USA). The effect sizes for these time and group effects were presented as partial eta squared (η_p^2), as is recommended for mixed models (Baguley, 2009).

Second, we examined whether the electrochemical sensor would reliably detect cortisol responses to the TSST by comparing its results to that of the ELISA in 20 individuals from the larger sample. Five analyses were conducted to test the relationship between the cortisol values obtained through the ELISA and those obtained through the cortisol sensor method. First, a Pearson correlation was performed (coefficients range from 0 to 1 or -1), with values closer to 1 representing a stronger and more positive association between the two cortisol analysis methods (correlation coefficients > 0.7 generally considered to be a strong positive association). Second, a Cronbach's alpha test was performed (alpha coefficients range from 0 to 1), with values closer to 1 indicative of a

higher internal consistency between the two methods (alpha coefficients > 0.7 is acceptable; > 0.8 is good; > 0.9 is excellent; George and Mallery, 2003). Two additional correlations, intraclass correlation (ICC) and concordance correlation (CC), were also performed in order to measure how consistently the cortisol sensor method reproduced similar values to the ELISA method. ICC coefficients range from 0 to 1, with values closer to 1 indicating higher agreement between the two methods (ICC coefficients > 0.4 is fair; > 0.6 is good, > 0.75 is excellent; Cicchetti, 1994). The ICC coefficient for absolute agreement was used for the current study. CC coefficients (r_c) range from 0 to 1 or -1, with values closer to 1 indicating higher agreement between the two methods (CC coefficients of 0.9–0.95 is moderate; 0.95–0.99 is substantial; > 0.99 is almost perfect; McBride, 2005).

The Bland-Altman approach (Bland and Altman, 2010) was also used as a secondary analysis to examine the agreement between the cortisol values from the ELISA and those from the cortisol sensor. For this approach, the two sets of values (ELISA values and cortisol sensor values) were plotted along with the line $Y = X$, with values tightly scattered around the line indicating that the values of the two methods are in close agreement. The difference values between the two methods (ELISA – sensor) were also plotted against their mean [(ELISA value + sensor value)/2] along with the line $Y = 0$, with smaller difference values (i.e., centered around $Y = 0$) indicating that the values of the two methods are in close agreement and show no systematic variation with the mean values. Finally, calculation of the limits of agreement was also used as part of the Bland-Altman approach to assess whether the agreement between the two methods was sufficient or not. Limits of agreement were obtained by using the mean (m) and the standard deviation (sd) of the difference values. Bland and Altman (2010) recommend that 95% of the difference values fall within two sds of the m of the difference values ($m - 1.96sd$ and $m + 1.96sd$) to demonstrate good agreement (Giavarina, 2015).

3. Results

3.1. Sample characteristics

A majority of our sample ($n = 110$) were women (75%), Hispanic/Latino (44%) or Asian American/Pacific Islander (24%), and approximately 19 years of age ($SD = 1.3$, range = 18–24 years). Most participants were in their first year in college (72%), living at home with their parents (56%), and receiving financial aid (57%) or financial support from their parents (39%) to support their education. Among women in our sample, 12% used oral contraceptives ($n = 13$). In addition, 32% ($n = 26$ of 82 women) reported higher levels of depressive symptoms compared to 25% of men ($n = 7$ of 28 men), $\chi^2(5) = 13.02$, $p = .02$. As expected based on our depression group definition ($<17 \geq$ on the BDI-II), participants in the high depression group had higher levels of depressive symptoms ($M_{BDI-II} = 21.27$, $SD = 5.74$) compared to those in the low depression group ($M_{BDI-II} = 8.01$, $SD = 4.38$), $t(108) = -13.18$, $p < .001$. For our subsample of 20 participants who were randomly chosen from the larger sample ($n = 110$) to have their salivary cortisol samples analyzed by both the ELISA and cortisol sensor, 10 were women and 10 were men. For both women and men in this subsample, five had low depressive symptoms and five had high depressive symptoms. Participants in the subsample did not significantly differ from the larger sample on any sociodemographic characteristics.

3.2. Response to TSST

Mixed effect linear model analyses of change revealed that the TSST produced a significant biological stress response among participants (quadratic pattern) with the greatest cortisol reactivity occurring from

timepoint 1 (baseline rest period; $M_{CORT} = 5.04$ nmol/L) to timepoint 2 (1 min post TSST; $M_{CORT} = 7.07$ nmol/L). Similarly, the greatest cortisol recovery occurred from timepoint 2 (1 min post TSST; $M_{CORT} = 7.07$ nmol/L) to timepoint 5 (45 min post TSST; $M_{CORT} = 4.89$ nmol/L), $F(1, 105) = 21.96$, $p < .001$, $\eta_p^2 = 0.18$.

3.3. Effect of gender and depressive symptoms on cortisol patterns

A mixed effect linear model analysis of change determined that there was a significant main effect of gender on cortisol levels, controlling for oral contraceptive use, $F(1, 105) = 11.79$, $p = .001$, $\eta_p^2 = 0.10$. Specifically, men showed higher cortisol levels overall than women, who showed more of a blunted cortisol response (see Fig. 3a). There was also a significant main effect of depression group on cortisol levels, controlling for oral contraceptive use, $F(1, 105) = 9.05$, $p = .003$, $\eta_p^2 = 0.08$. Specifically, participants with lower levels of depressive symptoms showed higher cortisol levels overall than participants with higher levels of depressive symptoms who showed a blunted cortisol response (see Fig. 3b). There were no significant two-way interactions found for gender $[F(1, 105) = 0.29$, $p = .591$, $\eta_p^2 = 0.003]$ or depression group on cortisol patterns over time $[F(1, 105) = 0.94$, $p = .335$, $\eta_p^2 = 0.01]$. Similarly, there was no significant three-way interaction effect of gender by depression group on cortisol patterns over time, $F(1, 105) = 2.66$, $p = .106$, $\eta_p^2 = 0.03$ (see Fig. 3c and d). Oral contraceptive use was not associated with cortisol patterns, $F(1, 105) = 1.19$, $p = .308$, $\eta_p^2 = 0.01$.

3.4. Comparison of the ELISA and the cortisol sensor

Data for one of the women in our subsample of 20 participants (high depression group) were not used in subsequent analyses because the

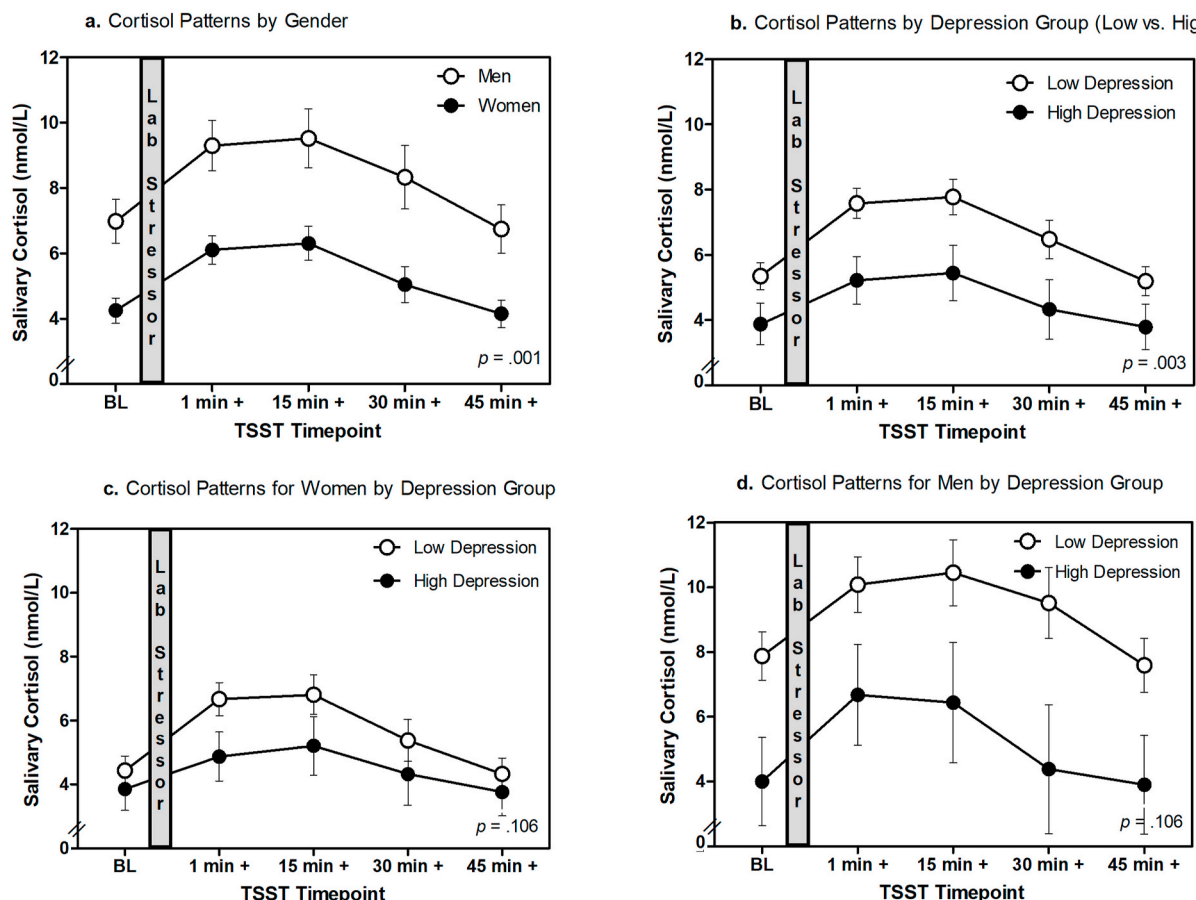


Fig. 3. Changes in salivary cortisol following a laboratory stressor by gender and depression group.

ELISA was not able to detect their cortisol values due to a small amount of saliva collected, although the cortisol sensor was able to provide this data. Therefore, reliability analyses between the ELISA and cortisol sensor were conducted with 19 participants. Pearson correlation analysis showed that the association between the cortisol values from the ELISA and those from the cortisol sensor was strong, $r = 0.98$, $p < .001$. A Cronbach's alpha test showed that internal consistency between the two methods was excellent, $\alpha = 0.97$. Intraclass correlation analysis showed that the cortisol values from the ELISA and those from the cortisol sensor were in excellent agreement, with an ICC of 0.95, $F(94, 94) = 38.52$, $p < .001$, $CI_{.95} = 0.92, 0.97$. Finally, concordance correlation analysis showed that there was substantial agreement between the two methods, $r_c = 0.95$, $CI_{.95} = 0.93, 0.96$.

When examined by gender and depression group, Pearson correlation analyses showed that the association between the cortisol values from the ELISA and cortisol sensor was strong for women ($r = .97$, $p < .001$) and men ($r = 0.98$, $p < .001$), as well as participants with low ($r = 0.98$, $p < .001$) and high levels of depressive symptoms ($r = 0.91$, $p < .001$). Cronbach's alpha tests showed that the internal consistency between the two methods was excellent for women ($\alpha = 0.98$) and men ($\alpha = 0.97$), as well as participants with low ($\alpha = 0.98$) and high levels of depressive symptoms ($\alpha = 0.95$). Intraclass correlation analyses showed that the methods were in excellent agreement when examined among women [ICC = 0.95, $F(44, 44) = 40.41$, $p < .001$, $CI_{.95} = 0.90, 0.97$] and men [ICC = 0.94, $F(49, 49) = 34.97$, $p < .001$, $CI_{.95} = 0.90, 0.97$], as well as participants with low [ICC = 0.95, $F(49, 49) = 42.83$, $p < .001$, $CI_{.95} = 0.91, 0.97$] and high levels of depressive symptoms [ICC = 0.90, $F(44, 44) = 18.55$, $p < .001$, $CI_{.95} = 0.82, 0.94$]. Concordance correlation analyses showed that the two methods were in substantial agreement for women ($r_c = 0.95$, $CI_{.95} = 0.91, 0.97$) and in moderate agreement for men ($r_c = 0.94$, $CI_{.95} = 0.92, 0.96$). Further, these analyses showed that

the two methods were in substantial agreement for participants with low levels of depressive symptoms ($r_c = 0.95$, $CI_{.95} = 0.93, 0.97$) and in moderate agreement for participants with high levels of depressive symptoms ($r_c = 0.90$, $CI_{.95} = 0.83, 0.94$).

When using the Bland-Altman approach to examine the agreement between the two methods' cortisol values for all 19 participants and then by gender and depression, visual inspection of plots showed that cortisol values of the two methods were mostly scattered tightly around the line $Y = X$, indicating mostly close agreement (see Fig. 4). Visual inspection also showed that when plotted against the mean cortisol values between the ELISA and sensor $[(ELISA \text{ value} + \text{sensor value})/2]$, the difference in cortisol values between the ELISA and sensor ($ELISA \text{ value} - \text{sensor value}$) were mostly centered around the line $Y = 0$, indicating that most differences between the two methods were small (see Fig. 5). Limits of agreement were also calculated by using the values representing two standard deviations below and above the mean difference score between the ELISA and sensor ($M = 0.35$, limits of agreement = -3.49 and 4.19) to determine what percentage of cortisol values centered around the line $Y = 0$. Results demonstrated that 94% of cortisol values fell within the limits of agreement (see Fig. 5).

4. Discussion

Given the association found between altered cortisol patterns and certain adverse health outcomes, there is a need for assessments of cortisol that are reliable, accurate, cost-effective, and non-invasive to help identify populations at risk for stress-related health problems. The current study examined whether a new electrochemical sensor would reliably detect cortisol activity patterns by comparing its results to that of the widely used ELISA immunoassay. To test the reliability of the sensor, cortisol patterns were evaluated among young women and men

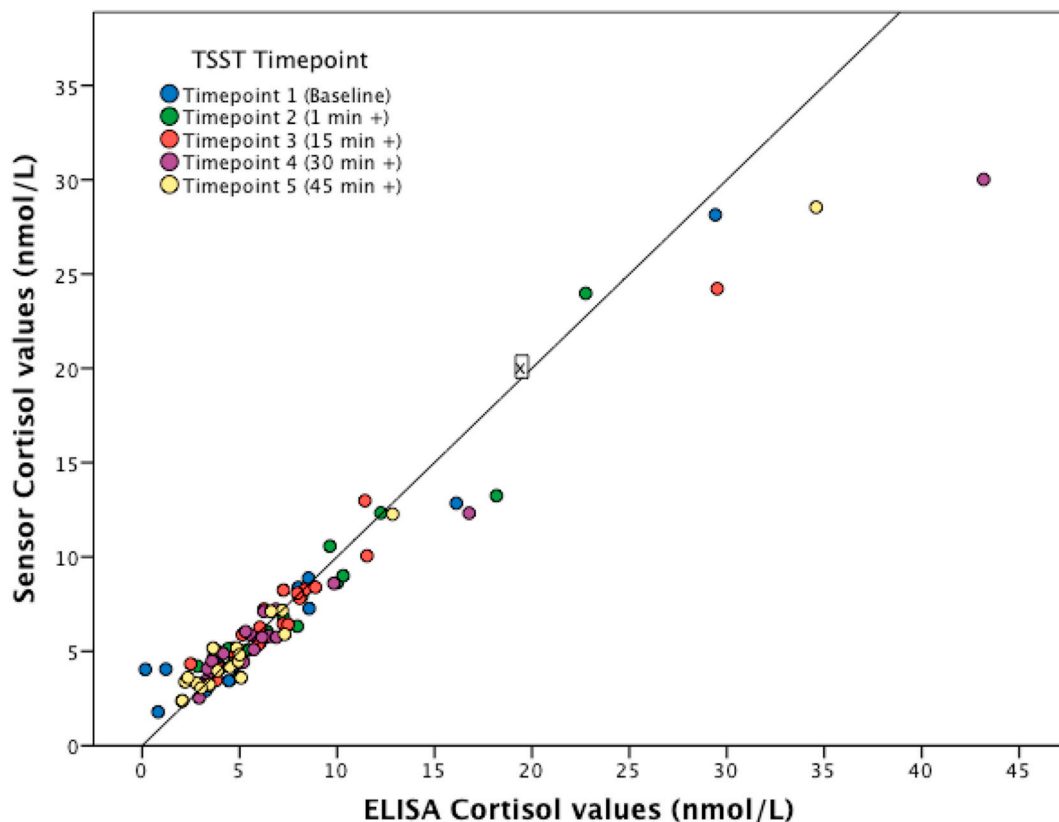


Fig. 4. Degree of agreement between the electrochemical sensor and the ELISA on salivary cortisol values collected from young adults participating in the Trier Social Stress Test ($n = 19$ participants) at each TSST time point (95 total values), Bland-Altman Approach (visual inspection of cortisol values scattered around the line $Y = X$).

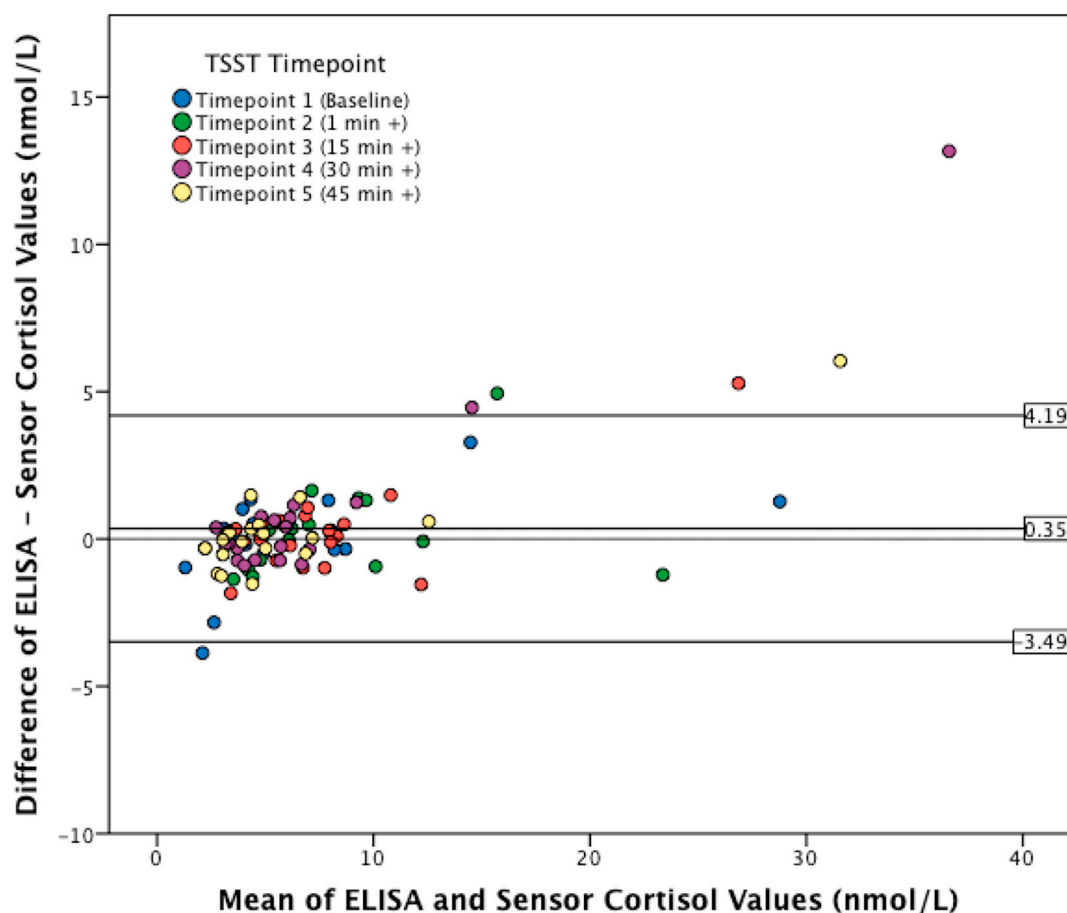


Fig. 5. Degree of agreement between the electrochemical sensor and the ELISA on salivary cortisol values at each TSST time point (95 total values), Bland-Altman Approach (visual inspection of the difference in cortisol values between the ELISA and sensor scattered around the line $Y = 0$) showing that 95% of the difference in cortisol values fall within the limits of agreement (± 2 SD's from the mean).

with low and high symptoms of depression who participated in the TSST, a well-validated method used to induce an acute stress response within a laboratory setting to assess patterns of cortisol reactivity and recovery.

4.1. Effect of gender on cortisol patterns

Our results revealed that women demonstrated more blunted or lower cortisol levels overall in response to the TSST compared to men. These results are consistent with that of a recent meta-analysis showing that men have higher cortisol values at peak and recovery following the TSST compared to women (Liu et al., 2017). Previous studies have suggested that these gender differences may be attributed to procedural variations in the TSST protocol. For example, in studies with a baseline acclimation period of 30 min or more prior to the TSST, no gender differences were observed in cortisol reactivity or recovery (Liu et al., 2017). In contrast, studies with an acclimation period less than 30 min showed gender differences in cortisol levels across all TSST time points, suggesting higher anticipatory salivary cortisol responses to the TSST in men compared to women. Similarly, studies with a longer recovery period after the TSST (>70 min) showed no gender differences in cortisol levels compared to studies with shorter recovery periods (Liu et al., 2017). Additionally, a study by Stroud et al. (2002) demonstrated that the type of stress tasks used in the TSST influenced gender differences in cortisol responses. Specifically, men were found to have greater cortisol responses to achievement-oriented stress tasks traditionally used in the TSST (public speaking, mental arithmetic). In contrast, women showed greater cortisol responses to a social rejection challenge

(i.e., being socially excluded by confederates during a social conversation task) compared to men, suggesting that women may be more physiologically reactive to negative interpersonal events, thereby placing them at greater risk for depression. Therefore, the shorter acclimation (~30 min) and recovery (45 min) periods, as well as the achievement-oriented stress tasks used in our TSST protocol, may help explain the gender differences observed in cortisol reactivity and recovery. Finally, prior studies have found oral contraceptive use in women to result in lower cortisol responses to the TSST compared to women who are not on oral contraceptives. Although we did not find oral contraceptive use to be associated with cortisol responses in the current study, this may be due to the small sample of women who were on oral contraceptives (12%) and, therefore, merits further investigation.

4.2. Effect of depressive symptoms on cortisol patterns

Our results also demonstrated that both women and men with higher levels of depressive symptoms had more blunted cortisol levels overall in response to the TSST compared to those with lower levels of depressive symptoms. These results are somewhat consistent with that of a recent meta-analysis showing that women with major depressive disorder demonstrated more blunted cortisol responses to the TSST compared to women without major depressive disorder (Zorn et al., 2017). In contrast, results from the meta-analysis showed that men with major depressive disorder demonstrated greater cortisol reactivity to the TSST compared to men without major depressive disorder. These gender differences in cortisol responses were more noticeable among those with

current major depressive disorder compared to those with remitted major depressive disorder as these cortisol patterns were not found to differ between women or men with a past history of depression (Zorn et al., 2017). Results also differ for those with subclinical depressive symptoms, as a recent study demonstrated that both women and men with higher non-somatic depressive symptoms (e.g., negative affect) had greater cortisol reactivity to the TSST compared to those with lower depressive symptoms (Fiksdal et al., 2019).

Cumulatively, these study results suggest that the inconsistencies in the associations found between depression and cortisol responses to the TSST may be due to how depression is assessed. For example, the severity and chronicity of depression has been shown to influence the associations found between depressive symptoms and cortisol, with more chronic symptoms of depression being associated with blunted cortisol responses to the TSST compared to more recent-onset, acute depressive symptoms (Zorn et al., 2017). Gender differences in cortisol responses also appear to be more prominent among those with major depressive disorder compared to those with subclinical depressive symptoms (Fiksdal et al., 2019; Zorn et al., 2017). Although participants with major depressive disorder were excluded from the current study, the chronicity of depressive symptoms experienced by women and men in our sample is unclear. Therefore, additional studies and screening methodologies are needed to examine the association of different depression subtypes, by severity and chronicity, on cortisol responses by gender.

4.3. Comparison of the ELISA and the cortisol sensor

The results of these studies highlight the need for new technologies, such as electrochemical sensors, that can be used to reliably detect cortisol responses to the TSST in real-time to help identify cortisol patterns that may be associated with stress-related health outcomes in at-risk populations. Results from the current study demonstrated that our cortisol sensor was highly reliable, showing strong to excellent consistency and agreement with the widely used ELISA immunoassay in detecting cortisol responses to the TSST for both women and men and for participants with low and high depressive symptoms. The correlation between the cortisol sensor and ELISA values confirmed the general accuracy of the sensor with an r value = 0.98. The sensors' precision (average CV < 10%) is comparable to that of the ELISA and falls within the acceptable range for use in research studies. The ICC (ICC = 0.95) and CC ($r_c = 0.95$) analyses that were used to measure how consistently the cortisol sensor reproduced similar cortisol values to the ELISA suggested that there was less than 5% variability in cortisol measurement differences across these assessment methods. Therefore, any measurement error introduced by the sensor is minimal when compared to random measurement variability and when compared to natural variability in cortisol levels observed within a study population.

Our results also established that the sensor reported salivary cortisol values (measured in nmol/L) that were linearly proportional to the cortisol values obtained from the ELISA over a range extending from 0 to 30 nmol/L, with 94% of cortisol values falling within the limits of agreement. For cortisol levels above 30 nmol/L, sensor values showed a deviation from linearity and had a tendency to slightly underestimate cortisol values in comparison to the ELISA, indicating that saturation mechanisms were degrading the signal-to-noise ratio. However, the linear response range of the sensor corresponds well with the range of salivary cortisol values typically found for women and men, with both low and high levels of depressive symptoms, participating in the TSST (i.e., 4–30 nmol/L; Fiksdal et al., 2019; Kudielka et al., 2004; Liu et al., 2017). Future versions of the cortisol sensor would benefit from a wider linear response range capable of reliably capturing salivary cortisol levels beyond 30 nmol/L that may manifest in certain individuals, as well as include standardized calibration strips and embedded software to transform and rectify any estimation errors.

4.4. Study limitations and strengths

The findings presented should be interpreted with some degree of caution given several study limitations. First, given that our sample consisted of undergraduate college students, the results may not be generalizable to other young adult women and men. However, this is one of the few studies to examine cortisol responses to the TSST in a subclinical depression sample during young adulthood. In particular, our results by gender and depression subgroup (i.e., women and men with low and high depressive symptoms) extend research findings on unique cortisol patterns observed in this important community sample (Liu et al., 2017; Zorn et al., 2017). Second, due to limited resources with the number of cortisol sensors available at the time the study was conducted, we were only able to test the reliability of the sensor to the ELISA for 20 individuals from the larger sample ($n = 110$). Participants were randomly selected to ensure equal representation by gender and depression group and the research staff who independently analyzed the saliva samples using the sensor and ELISA were blinded to participant characteristics to reduce experimenter bias. Additional studies in this research area, with larger sample sizes, would aid in supporting the reliability of the cortisol sensor, as well as further our understanding of its application across different research settings (e.g., home-based cortisol collection) and populations. Finally, although salivary cortisol levels could be affected by a number of factors (e.g., eating, drinking, smoking, taking certain medications, time of saliva collection, oral contraceptive use, and menstrual phase; Allen et al., 2017), several precautions were taken in the current study to minimize the impact of these factors on our study results (e.g., asking participants to refrain from engaging in behaviors known to affect salivary cortisol 30 min before their clinic visit; all saliva samples being collected in the afternoon; controlling for oral contraceptive use).

4.5. Conclusions and implications

In summary, our results suggest that women and those with higher levels of depressive symptoms show more blunted cortisol reactivity and recovery in response to the TSST compared to men and those with lower levels of depressive symptoms, respectively. These results have strong implications for the health of women in helping to prevent the onset of health complications, including major depressive disorder, that have been associated with altered cortisol patterns in this population during early adulthood. However, additional studies are needed with larger sample sizes across different depression subgroups, taking into account depression severity and chronicity, as well as comorbid anxiety to determine differences in women's cortisol responses by depression risk status.

Our results also suggest that the cortisol sensor is highly reliable, when compared to the conventional ELISA immunoassay, in detecting cortisol responses to the TSST for both women and men and for participants with low and high depressive symptoms. The strong performance of the cortisol sensor in a laboratory-based setting offers a cost-effective alternative to analyzing salivary cortisol using conventional laboratory-based assay methods (e.g., ELISA) that require multiple steps in freezing, transporting, processing, and assaying samples (e.g., centrifugation and aliquoting) that introduces measurement error and may affect the quality of cortisol values obtained. Furthermore, the small amount of saliva required (<25 μ L; ~1 drop of saliva), the lack of sample preparation steps, and the rapid reporting of results (within 1 min) makes the sensor very useful for providing point-of-care cortisol measurement in both laboratory-based and applied settings that are time-sensitive in nature (e.g., assessing cortisol responses to the TSST; measuring diurnal cortisol patterns). Finally, the cost per sensor is very low (~\$10) due to the reusable collector strips used (good for up to five saliva samples) that are disposable and similar in size to blood glucose strips. Thus, the sensor detection of cortisol is simple, flexible, and cost-effective, allowing for real-time reporting of salivary cortisol with the precision

and accuracy of traditionally labor-intensive and laboratory-based immunoassays.

CRedit authorship contribution statement

Guido G. Urizar: Conceptualization, Methodology, Formal analysis, Resources, Writing - original draft, Visualization, Supervision, Funding acquisition. **Hugo Sanchez Hernandez:** Formal analysis, Investigation, Writing - original draft, Visualization, Project administration. **Jessica Rayo:** Formal analysis, Investigation, Writing - review & editing. **Shekhar Bhansali:** Validation, Resources, Writing - review & editing.

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