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Salivary Cortisol As A Measure Of Stress Reactivity In Adolescents With Psychiatric Disorders

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Salivary Cortisol as a Measure of Stress Reactivity in Adolescents with Psychiatric Disorders

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SALIVARY CORTISOL AS A MEASURE OF STRESS REACTIVITY IN ADOLESCENTS WITH PSYCHIATRIC DISORDERS. CORTLYN BROWN AND KATHLEEN MERIKANGAS. NATIONAL INSTITUTES OF MENTAL HEALTH, BETHESDA, MD (SPONSORED BY ANDRES MARTIN, YALE CHILD STUDY CENTER).

ABSTRACT

Objective: The aims of this study were to 1) examine salivary cortisol distributions in a nationally representative sample of U.S. adolescents; and to 2) evaluate whether youth with mood and anxiety disorders are associated with differential salivary cortisol response to an acute mild stressor.

Background: An association between mood disorders and hypothalamic-pituitary-adrenal (HPA) axis dysfunction, as indicated by elevated levels of cortisol, has been well established [1, 2]. Although studies have suggested that these differences emerge early in the development of mood disorders in children and adolescents [3] the samples have generally been very small and primarily derived from clinical settings [3-5].

Methods: The National Comorbidity Survey-Adolescent Supplement (NCS-A) is a nationally representative face-to-face interview of adolescents aged 13-18 years in the continental U.S. [6]. Adolescents were administered a modified version of the World Health Organization Composite International Diagnostic Interview Version 3.0 and saliva was collected pre- and post- interview on a subset of 1,755 adolescents. Quantile regression models were used to assess pre- interview cortisol and rate of change in salivary cortisol level across entire quantile distributions adjusted for time of day cortisol

sample collected, sex, age, and additionally pre-interview cortisol level when rate of change in cortisol was used as the outcome variable. For descriptive purpose, traditional linear regression analyses were performed in parallel.

Results: Pre-interview cortisol levels were higher in the morning and decreased over the time of day. The magnitude of rate of change in cortisol measures was greater in the earlier time categories than that in the later time categories. After adjustment for time of cortisol sample collected, pre-interview cortisol levels were significantly lower across quantile distributions in the younger age groups (13-14y, 15-16y) compared to the oldest (17-18y), and were higher among adolescents with lower family income around the lower quantile range. There were no sex differences in pre-interview cortisol levels, but the rate of change was significantly smaller at the lower end of quantile distribution among females than that in males. Across the majority of quantile distributions, adolescents with earlier weekday bedtime and mid-sleep time were significantly associated with lower pre-interview cortisol level as well as rate of change compared to those with later bedtime and delayed mid-time sleep. Although suicidality was not associated with pre-interview cortisol level, there was a greater rate of change in cortisol among adolescents with suicidal behavior. Anxiety status was not associated with pre-interview cortisol level, while adolescents with lifetime mood disorders had somewhat lower pre-interview cortisol level at the lower end of quantile distribution. With respect to rate of change in cortisol level, adolescents with either lifetime or 12-month anxiety disorders showed a greater rate of change than those without disorders at the lower quantile distributions.

Those with severe anxiety and those with 12-month mood disorders had a greater rate of changes in cortisol levels than controls.

Conclusions: Our findings reveal that adolescents with mood and/or anxiety disorders have a greater change in salivary cortisol before and after a diagnostic interview as an acute mild stressor compared to their non-anxious or depressed counterparts. These findings provide biologic evidence for greater stress reactivity in adolescents with anxiety and depression in non-clinical samples.

Disclosures: Nothing to disclose.

INTRODUCTION

Literature has estimated the lifetime prevalence of adolescent mood disorders to be 14.3% [6]. It is known that adult mood disorders are associated with a stress response physiologically represented by hypothalamic-pituitary-adrenal (HPA) axis abnormalities. When the body encounters stress, the hypothalamus produces corticotropin-releasing hormone (CRH), which stimulates the anterior pituitary to release adrenocorticotropic hormone (ACTH). ACTH then stimulates the adrenal cortex to produce glucocorticoids such as cortisol. The glucocorticoids serve as negative feedback and suppress ACTH production by the hypothalamus.

HPA hyperactivity-induced elevated cortisol, as well as a blunted response to the dexamethasone suppression test (DST), have been observed in adult depression. In this test, dexamethasone, an exogenous hormone that mimics cortisol, stimu[7]lates a hypercortisolic environment and exerts negative feedback on the hypothalamus [8-10]. Other studies found that adult depression was associated with a hypocortisolism state potentially resulting from HPA axis exhaustion secondary to chronic depressive episodes [11, 12]. Similarly, adult bipolar disease has been associated with both relative hypocortisolism and relative hypercortisolism [13].

A consensus has yet to be reached as to the relationship between cortisol and adult anxiety disorders. In a study of elderly individuals, diurnal cortisol was up-regulated in GAD or phobic disorder [5, 14]. It has also been shown that adults with GAD have lower cortisol awakening response [15].

As with adult studies, there is a lack of consensus on the relationship between cortisol and adolescent mood and anxiety disorders. Furthermore, many of these studies

have been limited by small sample sizes [4, 5] and the invasiveness of collecting serum samples to test for cortisol. In addition, most adolescent cortisol studies are not population based and instead draw from clinical samples. As these cohorts are limited to those that present to clinic, moderate to severe clinical presentations are frequently overrepresented. Lastly, the majority of studies have used laboratory stimuli (either administration of corticotropin-releasing hormone or dexamethasone) that are subject to a large number of false-positives and false-negatives [16]. A second shortcoming with laboratory stressors is that the results cannot be extrapolated to stress reactivity in the everyday lives of the children. The present study was motivated the address these disadvantages.

The National Comorbidity Survey-Adolescent Supplement (NCS-A) consists of a nationally representative, ethnically diverse sample of adolescents in the United States. As we utilized salivary cortisol rather than serum, we were able to obtain cortisol measurements for 1,755 of the adolescents who have complete information on the full Composite International Diagnostic Interview, making it the largest sample of cortisol in U.S. children or adolescents.

Salivary cortisol has been shown to be a direct representation of unbound serum cortisol. In addition, salivary cortisol more accurately reflects acute cortisol changes than serum [17]. Of the few studies that exist, elevated levels of cortisol were observed in adolescent depression [3, 18-21]. Prospective longitudinal studies, however, found no association between elevated salivary cortisol and risk of subsequent depression in adolescents [21].

In regards to adolescent bipolar disorder, Cervantes et al. concluded that there was no significant difference in the degree of hypersecretion of cortisol between the depressive and manic phases [22]. Whereas some studies have found no association between cortisol and adolescent anxiety disorders [23], others have found positive associations that were mediated by sex [24].

A further limitation of all previous studies on cortisol and adult or adolescent mood disorders is that they form estimates on the conditional mean of the response variable. This statistical method is poor when considering outliers in the response measurements. Furthermore, these studies are limited by only displaying one central tendency which may not accurately reflect and represent complex interactions. Quantile regression, however, estimates quantiles of the response variable and, therefore, is a better model to consider outliers in response measurements. In addition, quantile regression allows for the analysis of different measures of central tendency for each quantile.

Although quantile regression coefficients are interpreted the same way linear regression coefficients are, while linear regression posits the question, “What is the relation between X and Y?” quantile regression extends this to, “For whom does a relation between X and Y exist?"’ as well as testing, “For whom is the relation stronger or weaker?" By using quantile regression, we have been able to model any point in the distribution of a continuous outcome variable. We present the first study to use quantile regression to analyze biomarkers of mood and anxiety disorders.

This study has important implications for the screening, diagnosis, and treatment of mood and anxiety disorders across the lifespan. This study has the potential to identify

a biomarker that could screen high-risk pre-symptomatic adolescents leading to earlier intervention and preventative care. This is particularly important for bipolar youth as only half of this population receive mental health treatment [25, 26].

The data from this study could also add a biological and laboratory component to the diagnosis of adolescent mood/anxiety disorders and aid in the identification of distinct syndromes within each of the mood/anxiety disorders. In addition, this data could provide information regarding the mechanism of adolescent mood/anxiety disorders. Furthermore, the research allows the study of comorbidity in mood disorders. Comorbid anxiety, for example, has been highly associated with mood disorders [27].

SPECIFIC AIMS

1) To examine the association between demographic and health related variables and adolescent cortisol levels.
2) To determine if/how pre-interview salivary cortisol is altered in adolescents with mood and anxiety disorders when compared to healthy adolescent controls.
3) To determine if/how stress reactivity in response to an acute minor stressor, as measured by change in salivary cortisol response, is altered in adolescents with mood and anxiety disorders when compared to healthy adolescent controls.
4) To examine the effect that severity of mood and anxiety disorders have on adolescent cortisol levels.

CORTISOL AND MAJOR DEPRESSION

Research on the role of HPA axis hyper/hypo-activation and cortisol in adolescent depression has been inconclusive. Differences have particularly emerged when comparing baseline measures compared to challenge studies of HPA axis by administration of dexamethasone or corticotropin releasing factor (CRF). In the dexamethasone suppression test, dexamethasone, an exogenous steroid similar in structure to cortisol, is given. In a hypercortisol (or hyperdexamethasone) environment, these two hormones exert negative feedback to the hypothalamus, which produces corticotrophin-releasing hormone (CRH). There is also negative feedback to the pituitary gland which, when activated, produces adrenocorticotropic hormone (ACTH).

When given dexamethasone, adolescents with depression have less pituitary suppression and, therefore, higher levels of cortisol than healthy controls [18, 28]. This suggests that similar to adults, adolescents with depression have a decreased ability to regulate cortisol levels [18, 29]. Studies that have used an alternative test, CRH infusion, have failed to demonstrate a difference in cortisol response between depressed and healthy adolescents [30-32]. In the basal state, however, it has been shown that adolescents with depression have higher cortisol levels than control adolescents [3, 19-21, 33].

CORTISOL AND DYSTHYMIA

Dysthymia is a mood disorder with behavioral and physical problems similar to depression. In dysthymia, however, the disease course is more chronic and symptoms are less severe; still, rates of suicide are comparable to major depressive disorder (MDD). Compared to major depression, there is even less known about the relationship between

cortisol and dysthymic adolescents. Of the few studies, Steward showed that early onset chronic atypical depression, similar to dysthymia, has lower afternoon cortisol and greater response to dexamethasone than late onset acute atypical depression [34]. In addition, Jansen *et al* concluded that dysthymic adolescents have a lower cortisol response to exercise induced stress than control subjects [35]. There is much more work that needs to be done with this subset of adolescent mood disorders.

CORTISOL AND BIPOLAR

Bipolar disorder is a mental health problem that ranges from extreme mood elevation during the manic states to severe sadness in the depressive periods. The role of cortisol in bipolar disorder is complex. Maripuu has shown that both relative hypocortisolism and relative hypercortisolism increase disease burden in bipolar adults [13]. Further understanding of the role of cortisol could lead to targeted therapies. Potential targets include leptin, lithium, and valproic acid. Each of these has been shown to decrease the effect of cortisol at the glucocorticoid receptor [36]. Roxanas published similar results and concluded that sodium valproate was able to reverse glucocorticoid induced mania [37].

Another interesting area of research regarding bipolar disorder and cortisol is age of onset. It has been shown that cortisol is higher in subjects where age of onset is ≥ 30 years than in those where age of onset is ≤ 30 years [38]. It is important to study this age relationship in adolescents as well. In general there is little cortisol research focused on adolescents with bipolar disorder. Of the adolescent research that exists, it has been

shown that offspring of bipolar parents have higher cortisol levels than those with non-bipolar parents [39].

CORTISOL AND ANXIETY

Similar to mood disorders, the research on the relationship between cortisol and anxiety disorders has been inconclusive. Female adolescents with generalized anxiety disorder (GAD) have been found to have elevated cortisol whereas no relationship was found between male adolescents with GAD and cortisol [24]. Other studies, however, have found associations between decreased hair cortisol and GAD but no association between salivary cortisol and GAD [40]. Panic disorder, another anxiety disorder, has been found to be associated with absent cortisol reactivity in response to the Trier Social Stress Test [41]. Studies examining a relationship between baseline plasma cortisol and panic disorder have been mixed with some showing a relationship between panic disorder and elevated cortisol [42] and others finding no such association [43].

METHODS

Please note, the author of this thesis came up with the study question, performed a complete literature review, conducted interviews, worked directly with a statistician to perform the data analysis, and interpreted all results presented.

SAMPLE

The National Comorbidity Survey-Adolescent Supplement (NCS-A) is a nationally representative face-to-face interview of adolescents aged 13-18 years in the continental U.S. The survey used was very similar to the World Health Organization

(WHO) Composite International Diagnostic Interview Version 3.0 (CIDI) used in NCS-R (replication survey) modified for adolescents. Disorders with low prevalence in the adolescent population such as pathological gambling were removed. In addition, the language in the interview was made more kid friendly and contexts were changed to relate to the adolescent experience (e.g. work life to school life). The survey was carried out in a dual-frame sample that included a household and school subsample. The interview was administered face-to-face using laptop computer-assisted personal interviews (CAPI) in the adolescents' homes by professional interviews based at the Survey Research Center of the Institute for Social Research at the University of Michigan. CAPI was used rather than paper-and-pencil because the interview contained many complex skips. In addition, CAPI is cost-effective with large sample sizes as programming costs are less than labor costs associated with reading the keypunch paper-and-pencil responses.

The adolescent response rate of the combined sub-samples was 82.9%. Post-stratification weighing corrected the minor differences in sample and population distributions on census sociodemographic variables and the school sample on school characteristics.

One parent or guardian was asked to complete a self-administered questionnaire (PSAQ) about the participating adolescent's developmental history and mental health. The professional staff of the Institute for Social Research at the University of Michigan administered the survey. The Human Subjects Committees of Harvard Medical School and the University of Michigan approved the recruitment and consent procedures.

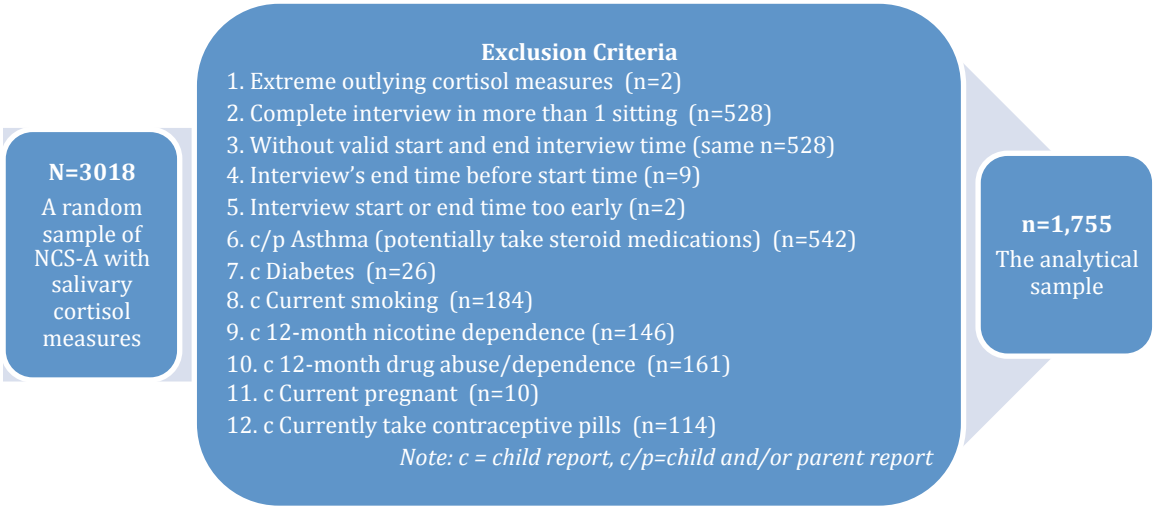
6,491 parents completed the full SAQ. Cases were then weighted for within-household probability of selection variation and for any remaining differences between the sample and the US population on sociodemographic variables, including age, sex, race/ethnicity, parent education, urbanicity, poverty index ratio, marital status of parents and geography. The poverty index ratio was based on family size and the ratio of family income to the family's poverty threshold level.

It is important to note that although no prior study has used our clinical interview as an acute mild stressor, it is known that social stressors (e.g. having subjects give short speeches to an interviewer, background counting in public, etc.) are associated with an acute stress reaction and a related change in salivary cortisol [44]. Although anecdotal, many of our participants have had strong emotional reactions to the interview that have included crying, yelling, and leaving the room. Interview questions may be invasive and often prompt the respondent to relive traumatic events or think about triggers (e.g. specific phobias). In addition, the face-to-face nature of the interview, particularly with a stranger, is likely to promote social anxiety. Studies have shown that subjects feel more comfortable reporting embarrassing behaviors, such as those asked in NCS-A, to audio computer-assisted self-administered interviews where there is no face-to-face contact with a stranger [45]. While we do not have ratings of our interview experience or anxiety, it follows that the novel situation of talking to the interviewer as well as the personal, and sometimes embarrassing, nature of the interview questions will induce a stress response particularly in adolescents.

A random sample of 3,018 adolescents was selected from the full NCS-A set for earlier batches of salivary cortisol assay. Our exclusion criteria included 1) extreme

outlying cortisol measures (n=2) 2) complete interview in more than 1 sitting (n=528); 3) no valid start and end interview time (same n=528); 4) interviews end time occurred before start time (n=9); 5) Interview start or end time too early (n=2); 6) diagnosis of asthma (potential for steroid medication) per child and/or parent report (n=542); 7) diagnosis of diabetes (n=26) per child report; 8) current smoking (n=184) per child report; 9) 12-month nicotine dependence (n=146) per child report; 10) 12-month drug abuse/dependence (n=161) per child report; 11) current pregnancy (n=10) per child report; 12) current prescription for contraceptive pills (n=114) per child report.

After applying the exclusion criteria, the analytical sample contained 1,755 adolescents who had both pre- and post- interview cortisol measurements as well as full data from CIDI interview by adolescent reports. Of these, 1,097 had additional information from PSAQ by parent reports.



SALIVARY CORTISOL MEASURES – OUTCOME VARIABLES

Cortisol levels in ng/mL were measured using saliva samples. Saliva samples were collected in a salivette by passive drool after the participant chewed on a piece of

sugarless gum immediately before and after the interview while the interviewer was present. The interviewer's laptop automatically recorded the time and date of each sample collection. The interviews were conducted throughout the day from 8:00 AM to 11:00 PM. The mean duration of the interview was 2.4 hours (SD =0.6). Salivettes were treated with sodium azide at Harvard University to retard bacterial growth, centrifuged, and pre-labeled with participant identification numbers and study information prior to sample collection.

After collection, samples were mailed to NIH where they were stored at -80 Celsius until testing. Quantification of cortisol levels was done by a radioimmunoassay (SiemensDiagnostic). The sensitivity of the assay was 0.0165 ng/mL. Intra-and inter-assay coefficients of variation were 5.4% and 26.0%, respectively. Similar coefficients of variation for this method have been reported previously [46].

We examined three outcome variables in the present study: 1) point-in-time cortisol at pre-interview, 2) point-in-time cortisol at post-interview, and 3) rate of change in cortisol (post-interview minus pre-interview cortisol divided by interview length in hours). Since patterns revealed for post-interview cortisol were very similar to pre-interview cortisol, their results were omitted in this report.

DSM-IV DISORDER ASCERTAINMENT

The current analyses are based on the adolescent interview to assess diagnostic criteria for mood and anxiety disorders. Mood disorders (major depressive disorder [MDD], dysthymic disorder, mania, and hypomania) and anxiety disorders (agoraphobia, generalized anxiety disorder, panic disorder, separation anxiety disorder, social phobia,

specific phobia, and posttraumatic stress disorder) were assessed in the CIDI. Definitions of all psychiatric disorders adhered to DSM-IV criteria. There was good concordance between psychiatric diagnosis from the modified CIDI and a clinical reappraisal sub-sample [47].

Impairment criteria from DSM-IV were based on an endorsement of some/a lot/extreme level of impairment or moderate/severe/very severe level of symptom severity. We used higher thresholds of impairment to define severity status in the study in order to identify disorders that were clinically significant. The higher thresholds of impairment required endorsement of "a lot" or "extreme" impairment in daily activities, or "severe or very severe" distress. Mood and anxiety disorders required both distress and impairment to be reported by adolescents. The Interuniversity Consortium for Politician and Social Research (ICPSR) at the University of Michigan created an algorithm with SAS that output DSM diagnosis [48]. In addition, ICPSR performed a confidentiality review and consistency checks.

OTHER COVARIATES

The demographic covariates considered in the regression models included age, sex, race/ethnicity, and family income. We also examined health related covariates including sleep and suicidal behaviors. Quantile measurements were created for weekday sleep duration in hours, weekday bed time, and middle sleep time. Middle sleep time served as an indicator of chronotype defined by dividing weekday sleep duration by two and adding it to weekday bedtime. The adolescents with the highest quantile group were used as reference. Suicidality was dichotomous and coded as present if lifetime suicide

ideation, plans or attempts were endorsed. These variables were chosen because they have been associated with psychiatric disorders and cortisol measurements in previous studies [49, 50].

STATISTICAL ANALYSIS

Statistical analyses were completed using the SAS version 9.2 (The SAS Institute Inc. Cary, NC). The mean and standard deviation (SD) for salivary cortisol levels in ng/mL at pre-interview and rate of change are presented by time of day that salivary samples were collected. For descriptive purposes, traditional linear regression models (ordinary least square, OLS) were estimated in parallel with quantile regression (QR) analyses.

The OLS models the relationship between one or more covariates X and conditional mean of the response variable Y given X=x, and hence are limited in that they do not extend to the non-central locations of a distribution. QR is related to OLS but can model any point in the distribution of a continuous outcome variable, such as the 90^{th}, 75^{th}, or 50^{th} percentile (the best-known example of a percentile, being the 50^{th} in a ranked distribution of numbers).

"Quantile" is a general term for what may be referred to as percentiles, deciles, quartiles, etc., in specific cases. QR coefficients are interpreted in the same way as those from OLS, i.e. change in the response variable for each one-unit change in the covariates. QR would allow the impact of the independent variable to vary along the whole range of cortisol values. Thus, estimating a family of conditional quantile functions can provide a more complete picture of covariate effects. The main advantage of QR over OLS is its

flexibility for modeling data with heterogeneous conditional distributions and more robust against outliers in the response measurements.

The equation for QR is given by $y_i=\beta_0^{(p)} + \beta_i^{(p)}x_i +\varepsilon^{(p)}$, where p is a given quantile: β_0 is the constant at the pth quantile point (e.g. cortisol level for the 'no disorder' group at the pth quantile point in the study); β_i is the regression coefficient for a certain covariate x at the pth quantile point (e.g. estimated difference in the cortisol levels at the pth quantile point when comparing the 'mania' vs. 'no mental disorder'); ε is the error term at the pth quantile point [51, 52].

In the current study, a typical OLS regression explaining cortisol level was first estimated to provide a basis for comparison with the QR. Dummy variables were entered in the models for each level of the categorical independent variables. Adolescents who are male, non-Hispanic whites, highest family income group (PIR>6.0) with no disorder served as the reference groups. In the QR, 19 different conditional quantile functions were estimated, starting with the 0.05 (5%) and proceeding in 0.05 increments until the 0.95 (95%) quantile (i.e. 0.05, 0.10, 0.15, …0.95). The QR was estimated using PROC QUANTREG in SAS (SAS Institute, Cary, NC). A Markov chain marginal bootstrap [53] was used to compute confidence intervals for every quantile estimates.

We analyzed data in the following model sequences: First, QR models were employed to describe changes at each of the 19 cortisol quantiles over the time of day that the salivary cortisol sample were collected between 8:00 in the morning (earliest) and 11:00 in the evening (latest). This was done to determine the diurnal rhythm of cortisol levels to examine whether there was any variation in cortisol measurements over the time of day and examine whether the changes were equal across the range of

conditional distribution of cortisol measurements. Second, a set of demographic characteristics (sex, age, race, and family income) was entered into the QR model individually to describe changes at each of the 19 cortisol quantiles while holding the time of day constant. Third, health related variables (weekday sleep duration, weekday bed time, weekday mid-sleep times, suicidality) and fourth, psychiatric disorder variables (mood and anxiety, and number of disorders) were entered into the QR model one at a time to determine if the changes at each of the 19 cortisol quantiles by disorder status were statistically significant independent of time of day and adolescent sex and age (linear and quadratic forms). Pre-interview cortisol level was also entered in the model when the model outcome was rate of change.

Theoretically, estimates could be calculated at any point in the quantile distribution; the selection of these 19 quantile points was arbitrary with intention to provide a full range view of the variation. In result tables we presented estimates at 10, 25, 50, 75, 90^{th} percentiles only due to space consideration.

The QR plots were used to help to reveal whether effects of independent variables were constant across the conditional quantile distribution of the outcome variable. For example, variation in pre-interview cortisol across the presence or absence of a disorder may be larger among individuals with high cortisol levels, as compared to those with low-to-medium pre-interview cortisol in the distribution. In another circumstance, an association linking disorder status with pre-interview cortisol may only be present among individuals at the higher pre-interview cortisol levels.

RESULTS:

DEMOGRAPHIC BREAKDOWN OF THE SAMPLE

In this analytical sample, about half of the sample was male (51.6%) and the mean age was 15.0 years (SD=1.4). Non-Hispanic Whites comprised 56.0% of the sample, non-Hispanic blacks 18.9%, Hispanic 19.3%, and other race adolescents 5.8%.

CORTISOL LEVELS BY TIME OF DAY

OLS displayed that both pre- and post-interview cortisol levels were higher in the earlier time categories than later ones (Table 1, Figure 1). As the post-interview cortisol samples were collected 2.4 hours (SD=0.6) later on average after the pre-interview sample collection, the post-interview cortisol levels was consistently lower at each time of day category. The magnitude of the rate of change was greater in the earlier time categories and diminished over the time of day.

The QR models revealed that both pre-interview and rate of cortisol change were decreased in the later time of day categories than those in the earlier time of day categories. Figure 2 is a QR process graph. In Figure 2a the y-axis depicts the value of quantile regression coefficient; the x-axis values are quantile points. The comparison group is the earliest time of day (8am-11am), for which the regression coefficient is zero across all x-axis values. If and when respondents in later time of day categories have cortisol levels that are not appreciably different from the cortisol level of the earliest time of day category (i.e. reference group), the resulting plot is flat at quantile regression coefficient at zero for all values along the x-axis. The blue highlights the 95% confidence interval thus any point falling above or below the blue highlighting is significant. If the

significant value falls below the absolute zero mark, then the result is a negative association. The opposite is true for significant values that are above the absolute zero mark. The nomenclature for the y-axis is as follows: intercept 8am-<11am serving as reference, zero line, startT4catR1 represents 11am-<2pm, startT4catR2 represents 2pm-<5pm, and startT4catR3 represents >=5pm.

The patterns of associations varied across categories of time of day for pre-interview cortisol levels, i.e. cortisol levels in all three later time of day categories were greater compared to the earliest time of day (Figure 2a). The cortisol levels associated with the latest time of day categories were more pronounced in their departure from zero (i.e. times >5pm). This change was greater in the pre-interview cortisol level than the post-interview cortisol. The effect was downwardly associated with respondent's cortisol level over the full range of quantile distributions of cortisol with a stronger effect among those respondents with higher cortisol values (higher tail in quantile plot, quantile points>50 percentile). However, there was no difference between the rates of cortisol change between the two earlier times of day categories (Figure 2b). The earlier time categories were associated with a greater negative rate of change than the later time of day categories. There was no significant variation across quantile distributions except for the end tail of the quantiles likely due to small n.

Note that patterns of post-interview cortisol are similar to those of pre-interview cortisol but to a lesser extent. The result tables/figures will be presented thereafter for pre-interview cortisol and rate of change only. The results for post-interview cortisol were omitted in the report (available upon request).

CORTISOL LEVELS BY DEMOGRAPHIC CHARACTERISTICS

Table 2 presents the OLS and quantile coefficient estimates for selected quantiles of pre-interview cortisol (Table 2a) and rate of change cortisol measurements (Table 2b) by demographic characteristics (sex, age, race/ethnicity, family income). Based on the OLS regression models, the younger age groups (13-14y, 15-16y) and 'other' race group had significantly lower pre-cortisol level and greater rates of change than oldest age group (17-18y) or non-Hispanic white, respectively.

The QR showed a more detailed view across the quantile distributions for the outcome variables than that in OLS results. The QR revealed there was no significant sex differences in terms of pre-interview, however, rate of change was significantly smaller at the lower end of quantile distribution among females that that in males. The younger age groups (13-14y, 15-16y) were more likely to have significantly lower pre-interview cortisol level across all quantiles than the oldest age group (17-18y) with the exception of the 0.25 quantile point where significance was not reached. The difference was significantly widened especially for 13-14y adolescents in the range of higher than average cortisol level (i.e. quantile>50 percentile) in quantile distributions. There was no age difference for rate of change.

The 'Other' race group was likely to have lower pre-interview cortisol than white at upper tail of the quantile distribution (50-90 percentiles), while the post-interview cortisol level was higher in Hispanic group than White around the upper-middle quantiles (60-85 percentiles). Both Hispanic and black groups showed greater rates of change than that in white especially at the lower tail quantile distribution (<50 percentile).

Compared to adolescents with high family income (PIR>6), those from lower income (PIR<1.5 and PIR <3) families showed greater rates of change that were limited

to the lower tail of quantile distribution. Only at isolated quantiles (at 50 and 75 percentiles), was the pre-interview cortisol level higher in PIR <3.0 group than PIR >6.0 group.

CORTISOL LEVELS BY HEALTH RELATED VARIABLES

Table 3 reports OLS and QR coefficients for pre-interview (Table 3a) and rate of change in cortisol (Table 3b) for sleep and suicidality variables. The OLS showed that earlier weekday bed time (<=22:00) or earlier weekday mid-sleep time (<=27) were significantly associated with lower pre-interview cortisol level than those with bed time after 11pm (>23:00), or delayed mid-sleep times (>27). However, bedtime and mid-sleep times were not associated with rates of change. The OLS displayed no significant change in pre-interview and rate of change in cortisol for sleep duration and suicidality.

The QR results showed that adolescents with shorter weekday sleep duration (the 1^{st}, 2^{nd}, or 3^{rd} quantiles, i.e.<=8.5hrs) had significant higher pre-interview cortisol level only at the mid-quantile distributions (40-50 percentiles) and higher rate of change at the upper middle quantile distribution (50-75 percentiles) than those with more than 8.5 hours sleep duration (the 4^{th} quantile). The earliest bed time group (<=22:00) and mid-sleep times (the 1^{st}, 2^{nd}, 3^{rd} quantiles, i.e. <=27) were significantly associated with lower pre-interview cortisol levels than those with later bed time (4^{th} quantile, i.e. >23:00) or delayed mid-sleep times (4^{th} quantile, i.e. >27).

Dose-response effects were evident for mid-sleep times; specifically, the earlier the mid-sleep times, the lower the level of pre-interview cortisol. Earlier bedtime

(<=22:00) and earlier mid-sleep times (<=22) were significantly associated with lower rate of change in cortisol with little variation across the quantile distribution.

Adolescents who reported suicidal thoughts/plan/attempts had significantly higher rates of change than those without suicidality across the entire quantile distribution except that it was not significant at the upper tail of the distribution likely due to a few outliers. There was no significant difference in pre-interview cortisol level by suicidality.

CORTISOL LEVELS BY DISORDER STATUS

Table 4 displays OLS and QR pre-interview (Table 4a) and rate of change (Table 4b) by disorder status. The OLS analysis did not detect the associations between different status (12-month severe, 12-month, and lifetime) of mood disorder and anxiety disorder and outcome variables (pre-interview cortisol and rate of change) with the exception that levels of pre-interview cortisol were lower among adolescents with lifetime mood disorder compared to those without DSM-IV disorders (i.e. without mood, anxiety, ADHD, and behavior disorders). Comparatively, lifetime mood disorders were associated with a higher pre-interview cortisol than those with no DSM-IV disorder ($p<0.05$). Neither disorder status for anxiety nor mood displayed any significant association with cortisol rate of change. Pre-interview or rate of cortisol change was not associated with number of classes of disorders.

Per QR there is no association between 12-month severe or non-severe mood disorder and pre-interview cortisol level. However, adolescents with lifetime mood disorder had lower pre-interview cortisol level than those without a DSM-IV disorder, especially at lower tail of quantile distributions. With respect to rate of cortisol change,

greater rate of change was observed for adolescents with 12-month severe mood disorder at isolated quantile point (25 percentile) compared to those without DSM-IV disorders. Adolescents with 12-month mood disorder had greater rate of change at a wide range covering entire lower tail of quantile distribution (<75 percentiles). Lifetime mood disorder was not associated with rate of cortisol change.

Regarding anxiety disorders, pre-interview cortisol level was not associated with anxiety disorder status except that adolescents with 12-month (severe and non-severe) anxiety disorder showed an elevated pre-interview cortisol level that were marginally significant ($p<0.10$) compared to those without DSM-IV disorders. Adolescents with anxiety disorder (12-month severe, 12-month, or lifetime) had greater rate of change than those without disorder at the quantile distributions on the lower and middle tail (quantiles <75 percentiles) of quantile distribution.

There were no significant changes in post-interview cortisol by mood or anxiety disorder status nor number of classes of disorder. Pre-interview cortisol was not associated with number of classes of disorders but adolescents with any lifetime diagnosis, one class 12 month diagnosis, two classes 12 month diagnosis, and three or more classes 12 month diagnosis was associated with a increase in cortisol rate of change for the quantiles below average (quantiles<50%) with p values ranging from $p<0.05$ to $p<0.01$

DISCUSSION:

In a large, U.S. population-based sample we found that stress reactivity, in response to an acute minor stressor, was altered with greater magnitude of change in

cortisol before and after the interview in adolescents with mood and anxiety disorders compared to healthy control adolescents. These findings provide biologic evidence for greater stress reactivity in adolescents with anxiety and depression in non-clinical samples.

Studies that have used moderate to intense stressors, such as the Trier Social Stress Test, have not found an association between depression and cortisol reactivity [54]. When one considers our findings in the context of these studies, it is possible that depressed adolescents are more sensitive to the daily mild stressors, such as our interview, than their non-depressed counterparts but not to more intense stressors.

We also found that that cortisol reactivity was influenced by disorder severity and chronicity. Rate of cortisol change was affected by 12-month mood disorder but not lifetime. However, anxiety disorder regardless of severity or chronicity was associated with greater rate of cortisol change. Severe 12-month mood and anxiety disorders were more significantly associated with rate of cortisol change than their non-severe counterparts. In addition, stress reactivity was altered by the number of disease classes an adolescent had. This provides further biological evidence that stress reactivity plays an important role in the relapses and worsening of depression and anxiety. Our findings are in contrast to those of Harkness et al who found that individuals with mild/moderate depression had increased reactivity but those with moderate/severe had blunted cortisol response [55]. Their study, however, focused on the influence of childhood maltreatment, a factor we did not consider, on cortisol response.

While it is known that there is an association between cortisol reactivity and subsequent suicide attempts, very little research has studied cortisol reactivity and past/current suicidal ideation. Beauchaine et al used the dexamethasone suppression test and found that adolescent girls with heightened reactivity to the test had increased suicidal ideation [56].

On the other hand, cortisol non-suppressors (those individuals that reacted less to the test) with the dexamethasone test had increased rates of suicides [57]. We report the first study to examine the link between adolescent cortisol reactivity in response to a mild social stressor and suicidal ideation. Similar to our findings with anxiety and mood disorders, those adolescents with increased cortisol reactivity were more likely to have suicidal behavior (ideation/plans/attempts) indicating an underlying reactivity factor in the pathophysiology.

We also studied the relationship between adolescent cortisol and the circadian clock, sex, age, family income, and sleep. It is known that cortisol displays a strong diurnal rhythm with the highest cortisol levels, known as the cortisol awakening response, occurring within an hour of awaking. Cortisol levels then decline throughout the day and reach a nadir around midnight [58]. The decrease throughout the day is called the diurnal cortisol slope. Our finding of elevated cortisol in the morning and decreases throughout the day is consistent with the literature [58]. Furthermore, our data support the prevailing findings in the literature that the diurnal cortisol slope decreases as the day progresses (i.e. the rate of change of cortisol decreases as the day progresses) [59].

While it is known that women display a greater rate of many anxiety and mood disorders [60], the majority of literature demonstrates that males have a greater cortisol

change in response to a stressor and are, therefore, more stress reactive, than females [61, 62]. However, we found no association between gender and pre-interview or rate of cortisol change in response to an acute mild stressor. This is possibly due to the nature of the stressor that we used, as Stroud et al found that the HPA axis of men vs. females are activated by different types of stressors. Specifically, men had greater cortisol reactivity to achievement-based tasks while women had greater reactivity to interpersonal tasks [63]. Our stressor was neither achievement-based nor involved significant interpersonal challenges.

To better understand and gain a more complete developmentally oriented cortisol picture it is important to consider age differences along with sex differences. Studies examining an association between age and cortisol reactivity have been mixed [64, 65]. The prevailing theory, however, is that cortisol reactivity increases in the transition from childhood to adolescents [66] in accordance with sexual maturation [67]. As our study sample only included adolescents aged 13-18y, the fact that we did not see an association between cortisol reactivity and age is possibly because the majority of our participants were in the latter stages of pubertal development and had already undergone changes in reactivity.

Using the study sample from this report, we have previously shown that adolescents in disadvantaged neighborhoods had higher pre-interview cortisol and steeper rates of change in cortisol over the interview [68]. Neighborhood socioeconomic status was comprised of six U.S. Census Short Form 3 indicators: log median household income, % households with interest, dividend, or rental income, log median value of housing units, % persons over age 25 with high school degree, % persons over 25 with

college degree, and % persons in executive, managerial, or professional specialty operations [69]. The association between neighborhood status and pre-interview cortisol level, as well as cortisol rate of change, remained significant in the unadjusted models as well as those adjusted for household income.

With the present results, family income is associated with higher pre-interview cortisol and a greater rate of change at various quantile points. As with living in a disadvantaged neighborhood, these results perhaps reflect heightened reactivity to and recovery from the stimuli of the interview. As stated in the methods section, our pre- post- and rate of cortisol change cannot be precisely correlated with a specific period in HPA activation. We hypothesize, therefore, that these results are indicative of an increased reactivity to and recovery from the stress of the interview in adolescents from low-income families.

The suprachiasmatic nucleus of the hypothalamus is important in the regulation of cortisol's circadian rhythm. Many adolescents, however, are subject to increasingly demanding academic and extracurricular requirements that impinge on healthy sleep schedules. The prevalence of childhood sleep problems is estimated to range from 20-30% and nearly half of these problems persist into adulthood [70]. Very little research, however, has been done on the connection between HPA functioning and sleep within the adolescent population. HPA axis alterations, however, could serve as the mechanism through which poor sleep influences physical and mental health. The majority of literature on this subject, however, has focused on the physiologic cortisol awakening response and not on cortisol reactivity.

Pesonen et al performed the only study on the associations between cortisol as influenced by the dexamethasone suppression test and sleep in adolescents [58]. They reported that adolescent boys had a decreased cortisol awakening response in associated with short sleep duration and lower sleep quality although the latter result was just below significance. No associations were found with girls.

Other than our study, only two other studies have examined the relationship between adolescent cortisol reactivity and sleep in response to a psychological stimulus and they were each limited by small sample sizes. Raikkonen et al used the Trier Social Stress Test on a sample of 282 8-year-old children undergoing sleep monitoring via actigraphy [71]. They concluded that children with low compared to average-high sleep efficiency had higher peak cortisol and greater rates of change in response to a social stressor.

Although our study design did not permit the evaluation of sleep efficiency, our findings of increased cortisol reactivity to the interview in adolescents with earlier bedtimes and earlier mid-sleep times also suggest that sleep may serve as an important mediator between stress reactivity and the HPA axis. Capaldi et al studied 31 adolescents aged 10-17 and found that greater sleep-wake behavior problems were associated with a decreased cortisol reactivity in response to a performance stressor [72]. Unlike Raikkonen, they did not find an association between sleep quality and cortisol reactivity. Our data add important information to the very scarce literature on HPA involvement and adolescent sleep.

STRENGTHS AND LIMITATIONS

Previous research has been hampered by small sample sizes as well as the invasiveness of obtaining serum to study adolescent cortisol. By using salivary cortisol, a lab value that has been shown to correlate well with serum cortisol [17], we circumvented this previous limitation. An additional benefit of using saliva is that it allowed us to obtain a large number of subjects.

Using quantile regression we were able to acknowledge and address the inter-individual heterogeneity of cortisol. Many previous studies have likely not found significance as they made the assumption that all cortisol quantiles react in a similar way (i.e. somebody with a baseline low cortisol will display similar reactivity to somebody with a basal cortisol level in the medium range).

There were several components of the study specifically designed to assess fieldwork quality. The computerized CIDI had a built-in clock that recorded speed of data entry ensuring the interviewers did not skip or rush through sections. In addition, supervisors contacted 10% of households to review addresses, enumeration, random selection procedures, length of interviews, and responses to random questions [73].

Our results are subject to several limitations. We only collected cortisol pre- and post-interview and did not collect it across the day. In addition, we did not collect cortisol at awakening nor standardize the times of sampling as this was not possible in this large epidemiologic study. There was also a range in the duration of the interview. The variation in interview length was intrinsic to the survey design as each disorder had a stem-branch structure [73]. Despite the two-day General Interview Training course and five-day NCS-A that all interviewers completed, adolescents may still feel more

comfortable talking to certain interviewers and that different interviewers have different tones and delivery.

Cortisol is an extremely variable hormone and its values can vary significantly from minute to minute. As we did not use a laboratory procedure specifically designed to induce anxiety, such as the Trier Social Stress test, our results cannot be neatly correlated to specific phases in the HPA axis activation such as the anticipatory, stress, and recovery stages. We deemed such a test inappropriate with our sample population. Instead, our study reflects different naturalistic settings.

The participant is disrupted from their standard schedule/routine to participate in the interview. Their level of activity prior to sitting down for the interview is not recorded. While the interview is not designed to elicit stress, we hypothesize that the interaction with a stranger and personal nature of the mental health questions will invoke stress in the majority of the adolescents. As the participants remain seated throughout the interview, it is known that the post-interview sample is one collected after the subject has been seated for an average of 2.4 hours. It is not possible to determine to what extent the changes in cortisol are from circadian nature of cortisol, or the low-activity level of the seated interview.

In conclusion, in a U.S. based population study of adolescents we found biological evidence for greater stress reactivity in adolescents with mood and/or anxiety disorders compared to their non-anxious or depressed counterparts. Furthermore, this relationship is influenced by other health related factors such as sleep. These findings suggest that cortisol reactivity might comprise a biological index of major depression and

anxiety in adolescents and may add a component to the diagnosis and monitoring of adolescent mood and anxiety disorders. Furthermore, the biological data has the potential to aid in the identification of distinct syndromes within each of the mood or anxiety disorders. Lastly, these findings may also provide important information on the mechanisms of adolescent mood and anxiety disorders, particularly the role of the HPA axis in stress reactivity that may underlie these disorders.

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TABLES

TABLE 1. MEAN CORTISOL MEASURES (NG/ML) FOR TOTAL AND BY TIME OF DAY CORTISOL SAMPLE COLLECTED

	Time of day Cortisol Sample Collected	N	Mean	SD	Regression Coefficient		T-test of post- & pre- cortisol change	
					β	95% CI	t-stat	p-value
Pre-interview		1755	0.262	0.229				
Post-interview		1755	0.149	0.152				
Change		1755	-0.114	0.234			-20.34	<.0001
Rate of change		1755	-0.049	0.110				
Pre-Interview	1: <11am	313	0.396	0.295	Reference			
	2: 11am-<2pm	333	0.285	0.225	-0.1112****	-0.1448,0.4201		
	3: 2pm-<5pm	597	0.239	0.212	-0.1572****	-0.1870, -0.1274		
	4: 5pm-<8pm	491	0.196	0.155	-0.2034****	-0.2340,-0.1727		
	5: >=8pm	21	0.114	0.109				
Post-Interview	1: <11am	17	0.258	0.195	Reference			
	2: 11am-<2pm	352	0.190	0.183				
	3: 2pm-<5pm	383	0.167	0.151	-0.0265*	-0.0478, -0.0052		
	4: 5pm-<8pm	602	0.134	0.135	-0.0595***	-0.0789, -0.0402		

	5: >=8pm	401	0.113	0.129	-0.0807***	-0.1018, -0.0596		
Change	1: <11am	313	-0.199	0.329	Reference		-10.7	<.0001
	2: 11am-<2pm	333	-0.111	0.249	-0.004	-0.0266, 0.0184	-8.19	<.0001
	3: 2pm-<5pm	597	-0.100	0.205	-0.0303**	-0.0506, -0.0100	-11.86	<.0001
	4: 5pm-<8pm	491	-0.080	0.164	-0.0475***	-0.0687, -0.0263	-10.8	<.0001
	5: >=8pm	21	-0.047	0.109			-1.99	0.0601
Rate of change	1: <11am	313	-0.081	0.149	Reference			
	2: 11am-<2pm	333	-0.048	0.125	-0.0064	-0.0184, 0.0056		
	3: 2pm-<5pm	597	-0.043	0.092	-0.0171*	-0.0280, -0.0063		
	4: 5pm-<8pm	491	-0.037	0.085	-0.0266***	-0.0379, -0.0153		
	5: >=8pm	21	-0.012	0.054				

NOTE: use pre-interview time for pre-Interview cortisol, change, rate of change; use post-interview time for post-interview cortisol sample; Change = post- minus pre- interview cortisol measures; Rate of change = post- minus pre-interview cortisol measures divided by interview length in hours; β = Linear regression unadjusted for pre-interview and post-interview cortisol measures, adjusted pre-interview cortisol level for change and rate of change in cortisol measures. *p<.05, **p<.01, ***p<.001, ****p<.0001

TABLE 2A. QUANTILE COEFFICIENT ESTIMATES (95% CI) FOR SELECTED QUANTILES, PRE-INTERVIEW CORTISOL MEASUREMENTS BY DEMOGRAPHICS

		OLS		Quantile Coefficient (95% CI)									
		β	95% CI	0.1	95% CI	0.25	95% CI	0.5	95% CI	0.75	95% CI	0.9	95% CI
Pre-interview Cortisol level													
	<11am (Reference)	Reference		Reference		Reference		Reference		Reference		Reference	
	11am-<2pm	-0.1112****	-0.1448, -0.4201	-0.0037	-0.0225, 0.0152	-0.059****	-0.0827, -0.0353	-0.101***	-0.1358, -0.0662	-0.1900****	-0.2503, -0.1297	-0.194****	-0.2933, -0.0947
	2pm-<5pm	-0.1572****	-0.1870, -0.1274	-0.031****	-0.0450, -0.0170	-0.086****	-0.1096, -0.0624	-0.134***	-0.1611, -0.1069	-0.2365****	-0.2947, -0.1783	-0.301****	-0.3860, -0.2160
	>=5pm	-0.2034****	-0.2340, -0.1727	-0.051****	-0.0648, -0.0372	-0.113****	-0.1359, -0.0901	-0.172***	-0.1995, -0.1445	-0.2862****	-0.3435, -0.2289	-0.345****	-0.4245, -0.2655
Demographics													
Sex	0. Female	0.0115	-0.009, 0.032	0.0032	-0.0064, 0.0129	0.009[a]	-0.0012, 0.0192	0.0105	-0.0044, 0.0255	0.0026	-0.0309, 0.0362	-0.0130	-0.0645, 0.0385
	1. Male	Reference		Reference		Reference		Reference		Reference		Reference	
Age group	1: 13-14y	-0.05517***	-0.084, -0.026	-0.018**	-0.0289, -0.0071	-0.0140	-0.0311, 0.0031	-0.034**	-0.0558, -0.0122	-0.064**	-0.1053, -0.0227	-0.1061*	-0.1903, -0.0219
	2: 15-16y	-0.03015*	-0.059,	-	-0.0323, -	-0.0070	-0.0251,	-0.0245*	-0.0465, -	-0.0300	-0.0747,	-0.0570	-0.1393,

			-0.001	0.021***	0.0097		0.0111		0.0024		0.0147		0.0253
	3. 17-18y	Reference		Reference		Reference		Reference		Reference		Reference	
Race/Ethnicity	1. Hispanic	0.0132	-0.014, 0.040	-0.0080	-0.0202, 0.0042	0.0020	-0.0166, 0.0206	0.0080	-0.0137, 0.0297	0.0290	-0.0196, 0.0776	0.0380	-0.0244, 0.1004
	2. Non-Hispanic black	-0.0173	-0.044, 0.010	-0.01*	-0.0193, -0.0007	-0.0070	-0.0220, 0.0080	-0.0069	-0.0287, 0.0149	-0.0260	-0.0648, 0.0128	-0.0380	-0.1088, 0.0328
	3. Other	-0.05317*	-0.098, -0.009	-0.0020	-0.0229, 0.0189	-0.0160	-0.0366, 0.0046	-0.0407**	-0.0691, -0.0122	-0.0700**	-0.1186, -0.0214	-0.1010[a]	-0.2085, 0.0065
	4. Non-Hispanic white	Reference		Reference		Reference		Reference		Reference		Reference	
Family Income	1. PIR <=1.5	0.0079	-0.023, 0.039	-0.0074	-0.0221, 0.0073	-0.0060	-0.0225, 0.0105	0.0005	-0.0203, 0.0213	0.0197	-0.0311, 0.0705	0.0538	-0.0305, 0.1380
	2. PIR <=3.0	0.02509 [a]	-0.004, 0.054	0.0016	-0.0117, 0.0149	0.0091	-0.0098, 0.0281	0.0197*	0.0009, 0.0386	0.0507*	0.0083, 0.0930	0.0296	-0.0521, 0.1114
	3. PIR <=6.0	-0.0045	-0.030, 0.022	0.0016	-0.0098, 0.0130	-0.0020	-0.0144, 0.0104	-0.0049	-0.0235, 0.0138	0.0067	-0.0289, 0.0422	0.0008	-0.0625, 0.0640
	4. PIR >6.0	Reference		Reference		Reference		Reference		Reference		Reference	

NOTE: OLS and QR models for demographics adjusted for time of day pre-interview sample collected only

[a] p<0.10; * p<.05; ** p<.01; *** p<.001; **** p<.0001

TABLE 2B. QUANTILE COEFFICIENT ESTIMATES (95% CI) FOR SELECTED QUANTILES, RATE OF CHANGE IN CORTISOL MEASUREMENTS BY DEMOGRAPHICS

		OLS		Quantile Coefficient (95% CI)									
		β	95% CI	0.1	95% CI	0.25	95% CI	0.5	95% CI	0.75	95% CI	0.9	95% CI
Rate of change in cortisol													
	8am-11am (Reference)	Reference		Reference		Reference		Reference		Reference		Reference	
	11am-<2pm	-0.0064	-0.0184, 0.0056	0.0020	-0.0056, 0.0096	0.0012	-0.0072, 0.0095	0.0010	-0.0096, 0.0115	0.0028	-0.0064, 0.0119	-0.0078	-0.0318, 0.0161
	2pm-<5pm	-0.0171*	-0.0280, -0.0063	-0.0054	-0.0134, 0.0025	-0.0061[a]	-0.0131, 0.001	-0.0127***	-0.0199, -0.0055	-0.0134***	-0.0221, -0.0047	-0.0161	-0.0362, 0.0039
	>=5pm	-0.0266***	-0.0379, -0.0153	-0.0114**	-0.0198, -0.003	-0.0127***	-0.0201, -0.0054	-0.0179***	-0.0258, -0.01	-0.0249***	-0.0332, -0.0166	-0.0384***	-0.0594, -0.0174
Demographics													
Sex	1. Female	-0.0076	-0.018, 0.003	-0.0036***	-0.0084, 0.0012	-0.0029	-0.0067, 0.0008	-0.0021	-0.0064, 0.0022	-0.0041	-0.0092, 0.0010	-0.0038	-0.0147, 0.0072
	0. Male	Reference		Reference		Reference		Reference		Reference		Reference	
Age group	1: 13-14y	0.02339**	0.009, 0.038	0.0001	-0.0065, 0.0067	-0.0013	-0.0064, 0.0039	-0.0037	-0.0095, 0.0020	-0.0060	-0.0133, 0.0013	-0.0097	-0.0244, 0.0049
	2: 15-16y	0.01378[a]	-0.001, 0.028	-0.0043	-0.0121, 0.0034	-0.0029	-0.0082, 0.0024	-0.0047	-0.0105, 0.0011	-0.0026	-0.0099, 0.0048	-0.0017	-0.0186, 0.0152

	3. 17-18y	Reference		Reference		Reference		Reference		Reference		Reference	
Race/Ethnicity	1. Hispanic	-0.0026	-0.016, 0.011	0.0094***	0.0043, 0.0145	0.0072***	0.0030, 0.0115	0.0063*	0.0003, 0.0123	0.0048	-0.0015 , 0.0111	-0.0041	-0.0203, 0.0121
	2. Non-Hispanic black	0.0061	-0.008, 0.020	0.0075**	0.0024, 0.0125	0.0084***	0.0041, 0.0126	0.0077***	0.0033, 0.0122	0.0008	-0.0052, 0.0067	-0.0102	-0.0222, 0.0017
	3. Other	0.03143**	0.009, 0.054	0.0091	-0.0018, 0.0199	0.0055	-0.0014, 0.0124	0.0079[a]	-0.0012, 0.0171	0.0074	-0.0082, 0.0229	0.0036[a]	-0.0212, 0.0283
	4. Non-Hispanic white	Reference		Reference		Reference		Reference		Reference		Reference	
Family Income	1. PIR <=1.5	0.0047	-0.011, 0.020	0.0078*	0.0009, 0.0148	0.0059**	0.0014, 0.0104	0.0013	-0.0042, 0.0069	0.0026***	-0.0051, 0.0102	0.0002	-0.0158, 0.0163
	2. PIR <=3.0	0.0003	-0.014, 0.015	0.0095***	0.0039, 0.0151	0.0036	-0.0016, 0.0087	0.0035	-0.0023, 0.0092	-0.0001	-0.0074, 0.0073	-0.0021	-0.0222, 0.0181
	3. PIR <=6.0	0.0046	-0.008, 0.018	0.0022	-0.0043, 0.0087	-0.0006	-0.0052, 0.0040	-0.0018	-0.0076, 0.0040	0.0003	-0.0067, 0.0072	-0.0019	-0.0165, 0.0127
	4. PIR >6.0	Reference		Reference		Reference		Reference		Reference		Reference	

NOTE: OLS and QR models adjusted for pre-interview cortisol level, for demographics additionally adjusted for time of day pre-interview sample collected

[a] p<0.10; * p<.05; ** p<.01; *** p<.001; **** p<.0001

TABLE 3A. QUANTILE COEFFICIENT ESTIMATES (95% CI) FOR SELECTED QUANTILES, PRE-INTERVIEW CORTISOL MEASUREMENTS BY HEALTH RELATED VARIABLES

		OLS		Quantile Coefficient (95% CI)									
		β	**95% CI**	**0.1**	**95% CI**	**0.25**	**95% CI**	**0.5**	**95% CI**	**0.75**	**95% CI**	**0.9**	**95% CI**
Suicidality	1: Yes	-0.00218	-0.040, 0.036	-0.0043	-0.0189, 0.0102	-0.0089	-0.0294, 0.0116	-0.0102	-0.0425, 0.0222	0.0466	-0.0155, 0.1087	0.0259	-0.0703, 0.1221
	2: No	Reference		Reference		Reference		Reference		Reference		Reference	
Weekday sleep duration	1st Quartile: <=7hrs	0.0118	-0.017, 0.041	0.007	-0.0054, 0.0194	0.005	-0.0113, 0.0213	0.0023	-0.0203, 0.0249	0.0197	-0.0240, 0.0633	0.015	-0.0574, 0.0874
	2nd Quartile: <=8hrs	0.00239	-0.025, 0.030	0.003	-0.0080, 0.0140	0.0052	-0.0080, 0.0185	-0.0054	-0.0263, 0.0156	0.0028	-0.0339, 0.0396	-0.001	-0.0748, 0.0728
	3rd Quartile: <=8.5hrs	0.02833	-0.018, 0.075	0.0052	-0.0245, 0.0350	0.0225	-0.0117, 0.0567	0.0384*	0.0030, 0.0738	0.0408	-0.0289, 0.1106	0.044	-0.0777, 0.1657
	4th Quartile: > 8.5hrs	Reference		Reference		Reference		Reference		Reference		Reference	
Weekday bed time	1st Quartile: <=22	-0.04669**	-0.075, -0.018	-0.0033	-0.0159, 0.0093	-0.0202*	-0.0359, -0.0046	-0.0236*	-0.0469, -0.0003	-0.0638**	-0.1046, -0.0230	-0.0834*	-0.1550, -0.0118
	2nd Quartile: <=22.5	-0.02579	-0.062, 0.010	-0.0003	-0.0163, 0.0157	0.0005	-0.0224, 0.0234	0.0092	-0.0212, 0.0396	-0.0403	-0.0937, 0.0131	-0.047	-0.1210, 0.0270
	3rd Quartile: <=23	-0.00603	-0.040, 0.028	0.009	-0.0073, 0.0253	0.0056	-0.0163, 0.0276	-0.003	-0.0305, 0.0245	-0.0204	-0.0729, 0.0320	-0.0565	-0.1478, 0.0347

	4th Quartile: > 23	Reference		Reference		Reference		Reference		Reference		Reference	
Weekday mid-sleep times*	1st Quartile: <=26	-0.06339 **	-0.095, -0.032	-0.006	-0.0198, 0.0078	-0.032***	-0.0494, -0.0146	-0.0412**	-0.0679, -0.0145	-0.0733**	-0.1198, -0.0268	-0.0983*	-0.1807, -0.0159
	2nd Quartile: <=26.25	-0.06321 *	-0.116, -0.010	0.0155	-0.0139, 0.0449	-0.0108	-0.0393, 0.0177	-0.0243	-0.0593, 0.0108	-0.0897	-0.1449, -0.0346	-0.1377[a]	-0.2795, 0.0041
	3rd Quartile: <=27	-0.03812 *	-0.070, -0.006	-0.0016	-0.0183, 0.0150	-0.014	-0.0323, 0.0043	-0.0272[a]	-0.0585, 0.0041	-0.0612**	-0.1068, -0.0156	-0.0513	-0.1390, 0.0363
	4th Quartile: > 27	Reference		Reference		Reference		Reference		Reference		Reference	

Note: weekday mid-sleep time is calculate by dividing weekday sleep duration by two AND adding it to weekday bedtime; OLS and QR models adjusted for time of day pre-interview sample collected, sex, age (linear, quadratic)

[a] p<0.10; * p<.05; ** p<.01; *** p<.001; **** p<.0001

TABLE 3B. QUANTILE COEFFICIENT ESTIMATES (95% CI) FOR SELECTED QUANTILES, RATE OF CHANGE IN CORTISOL MEASUREMENTS BY HEALTH RELATED VARIABLES

		OLS		Quantile Coefficient (95% CI)									
		β	95% CI	0.1	95% CI	0.25	95% CI	0.5	95% CI	0.75	95% CI	0.9	95% CI
Suicidality	1: Yes	0.0193	0.000, 0.038	0.0115***	0.0050, 0.0180	0.0083**	0.0022, 0.0144	0.0135**	0.0043, 0.0227	0.0158**	0.0043, 0.0272	0.0193	-0.0100, 0.0485
	2: No	Reference		Reference		Reference		Reference		Reference		Reference	
Weekday sleep duration	1st Quartile: <=7hrs	-0.0023	-0.017, 0.012	0.0056[a]	-0.0002, 0.0114	0.0037	-0.0011, 0.0085	0.0059*	0.0003, 0.0114	0.0085*	0.0019, 0.0151	0.0066	-0.0077, 0.0203
	2nd Quartile: <=8hrs	-0.0025	-0.016, 0.011	0.0005	-0.0050, 0.0061	-0.0009	-0.0056, 0.0039	0	-0.0052, 0.0052	0.0034	-0.0033, 0.0100	0.0097	-0.0043, 0.0237
	3rd Quartile: <=8.5hrs	-0.0071	-0.031, 0.016	-0.0010	-0.0130, 0.0110	0.0009	-0.0092, 0.0110	0.0027	-0.0078, 0.0132	0.0099	-0.0025, 0.0222	0.0193	-0.0185, 0.0572
	4th Quartile: > 8.5hrs	Reference		Reference		Reference		Reference		Reference		Reference	
Weekday bed time	1st Quartile: <=22	0.0065	-0.008, 0.021	-0.0078**	-0.0135, -0.0020	-0.0077**	-0.0133, -0.0020	-0.0115***	-0.0171, -0.0058	-0.0098*	-0.0172, -0.0023	-0.0074	-0.0260, 0.0111
	2nd Quartile: <=22.5	0.0095	-0.009, 0.028	-0.0046	-0.0132, 0.0040	-0.0039	-0.0111, 0.0033	-0.0074*	-0.0146, -0.0002	-0.0031	-0.0133, 0.0070	0.0057	-0.0168, 0.0282
	3rd Quartile: <=23	-0.0061	-0.023, 0.011	-0.0014	-0.0089, 0.0061	-0.0024	-0.0093, 0.0046	-0.006	-0.0132, 0.0012	-0.009*	-0.0167, -0.0013	-0.0094	-0.0276, 0.0088

	4th Quartile: > 23	Reference		Reference		Reference		Reference		Reference		Reference	
Weekday mid-sleep times	1st Quartile: <=26	0.0129	-0.003, 0.029	-0.0071*	-0.0134, -0.0008	-0.0067*	-0.0133, -0.0001	-0.0089**	-0.0152, -0.0027	-0.0109*	-0.0201, -0.0018	-0.0075	-0.0267, 0.0118
	2nd Quartile: <=26.25	0.0139	-0.013, 0.041	-0.0118*	-0.0229, -0.0006	-0.0054	-0.0175, 0.0067	-0.0008	-0.0150, 0.0135	-0.0025	-0.0180, 0.0130	0.0054	-0.0339, 0.0447
	3rd Quartile: <=27	0.0086	-0.007, 0.025	-0.001	-0.0081, 0.0061	-0.0036	-0.0101, 0.0029	-0.0053	-0.0117, 0.0012	-0.0081[a]	-0.0162, 0.0001	-0.0065	-0.0261, 0.0131
	4th Quartile: > 27	Reference		Reference		Reference		Reference		Reference		Reference	

Note: weekday mid-sleep time is calculate by dividing weekday sleep duration by two AND adding it to weekday bedtime; OLS and QR models adjusted for pre-interview sample collected, sex, age (linear, quadratic) and pre-interview cortisol level

[a] p<0.10; * p<.05; ** p<.01; *** p<.001; **** p<.0001

TABLE 4A. QUANTILE COEFFICIENT ESTIMATES (95% CI) FOR SELECTED QUANTILES, PRE-INTERVIEW CORTISOL MEASUREMENTS BY DISORDER STATUS

		OLS		Quantile Coefficient (95% CI)									
		β	95% CI	0.1	95% CI	0.25	95% CI	0.5	95% CI	0.75	95% CI	0.9	95% CI
Mood													
	1: 12-m severe dx	0.0190	-0.055, 0.093	0.0053	-0.03995, 0.0505	-0.0014	-0.0536, 0.0508	0.0188	-0.1010, 0.1385	0.0703	-0.0415, 0.1822	0.0969	-0.1155, 0.3092
	2: 12-m dx	0.0325	-0.015, 0.080	0.0169	-0.0054, 0.0392	0.0162	-0.0070, 0.0395	-0.0196	-0.0509, 0.0117	0.0571	-0.0359, 0.1502	0.0703	-0.0568, 0.1973
	3: Lifetime dx	-0.05884*	-0.116, -0.002	-0.0328**	-0.0558, -0.0098	-0.0353*	-0.0675, -0.0032	-0.045[a]	-0.0902, 0.0002	-0.0497	-0.1308, 0.0314	-0.0231	-0.1332, 0.0870
	4: Lifetime other dx	0.02114[a]	-0.002, 0.045	-0.0007	-0.0098, 0.0084	-0.0057	-0.0170, 0.0057	0.0100	-0.0070, 0.0270	0.0310	-0.0104, 0.0724	0.0513	-0.0156, 0.1183
	5: No dx	Reference		Reference		Reference		Reference		Reference		Reference	
Anxiety													
	1: 12-m severe dx	0.0251	-0.024, 0.074	-0.0046	-0.0265, 0.0174	-0.0096	-0.0346, 0.0154	-0.0255	-0.0733, 0.0223	0.0707	-0.0457, 0.1871	0.1278[a]	-0.0140, 0.2695
	2: 12-m dx	0.0268	-0.006, 0.060	-0.0022	-0.0165, 0.0122	-0.0092	-0.0265, 0.0081	-0.0002	-0.0216, 0.0212	0.0052	-0.0660, 0.0764	0.1029[a]	-0.0118, 0.2176
	3: Lifetime dx	0.04268[a]	-0.002, 0.087	0.0084	-0.0176, 0.0344	0.0150	-0.0110, 0.0410	0.0270	-0.0188, 0.0728	0.0550	-0.0180, 0.1280	0.0499	-0.0743, 0.1742

	4: Lifetime other dx	-0.00575	-0.034, 0.023	0.0008	-0.0125, 0.0141	-0.0098	-0.0240, 0.0045	0.0038	-0.0217, 0.0293	0.0058	-0.0419, 0.0536	-0.0059	-0.0688, 0.0569
	5: No dx	Reference		Reference		Reference		Reference		Reference		Reference	

Note: OLS and Quantile regression models were adjusted for time of day cortisol sample collected, sex, age (linear, quadratic)

[a] p<0.10; * p<.05; ** p<.01; *** p<.001; **** p<.0001

TABLE 4B. QUANTILE COEFFICIENT ESTIMATES (95% CI) FOR SELECTED QUANTILES, RATE OF CHANGE IN CORTISOL MEASUREMENTS BY DISORDER STATUS

		OLS		Quantile Coefficient (95% CI)									
		β	95% CI	0.1	95% CI	0.25	95% CI	0.5	95% CI	0.75	95% CI	0.9	95% CI
Mood													
	1: 12-m severe dx	-0.0020	-0.039, 0.035	0.0207[a]	-0.0009, 0.0423	0.0119*	0.0025, 0.0212	0.0052	-0.0083, 0.0188	0.0021	-0.0161, 0.0204	-0.0116	-0.0491, 0.0258
	2: 12-m dx	-0.0038	-0.028, 0.020	0.0138**	0.0040, 0.0237	0.0171****	0.0091, 0.0251	0.0138**	0.0039, 0.0237	0.0105[a]	-0.0011, 0.0221	-0.0085	-0.0466, 0.0296
	3: Lifetime dx	0.0276	-0.001, 0.056	0.0030	-0.0059, 0.0119	0.0083	-0.0017, 0.0184	0.0070	-0.0020, 0.0160	-0.0024	-0.0213, 0.0166	0.0079	-0.0285, 0.0443
	4: Lifetime other dx	-0.0065	-0.018, 0.005	0.0064**	0.0017, 0.0112	0.0088****	0.0050, 0.0126	0.0094*** *	0.0051, 0.0137	0.0022	-0.0032, 0.0076	-0.0071	-0.0186, 0.0044
	5: No dx	Reference		Reference		Reference		Reference		Reference		Reference	
Anxiety													
	1: 12-m severe dx	0.0025	-0.022, 0.027	0.0115[a]	-0.0021, 0.0250	0.0094[a]	-0.0002, 0.0190	0.0164**	0.0048, 0.0279	0.012*	0.0009, 0.0231	0.0034	-0.0312, 0.0380
	2: 12-m dx	-0.0090	-0.026, 0.008	0.0072*	0.0006, 0.0137	0.0107***	0.0049, 0.0164	0.0087**	0.0032, 0.0142	0.0017	-0.0056, 0.0091	-0.0088	-0.0232, 0.0056
	3: Lifetime dx	-0.0061	-0.028, 0.016	0.0103*	0.0011, 0.0194	0.0097*	0.0023, 0.0171	0.0111**	0.0031, 0.0191	0.0056	-0.0083, 0.0194	0.0031	-0.0297, 0.0359

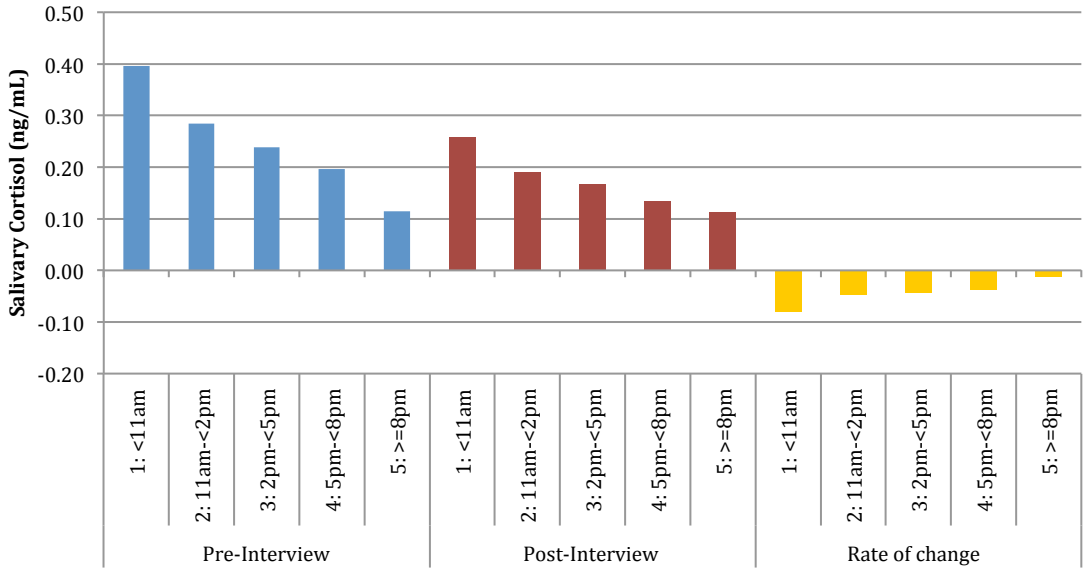
	4: Lifetime other dx	0.0011	-0.013, 0.016	0.0062*	0.0002, 0.0122	0.0095****	0.0047, 0.0143	0.0081**	0.0022, 0.0139	-0.0004	-0.0072, 0.0064	-0.0106[a]	-0.0230, 0.0019
	5: No dx	Reference		Reference		Reference		Reference		Reference		Reference	

NOTE: Rate of change in cortisol = post-interview cortisol measure minus post-interview cortisol measure divided by interview length in hours; OLS and Quantile regression models were adjusted for time of day pre-interview cortisol sample collected, sex, age (linear, quadratic), and pre-interview cortisol measurements

[a] p<0.10; * p<.05; ** p<.01; *** p<.001; **** p<.0001

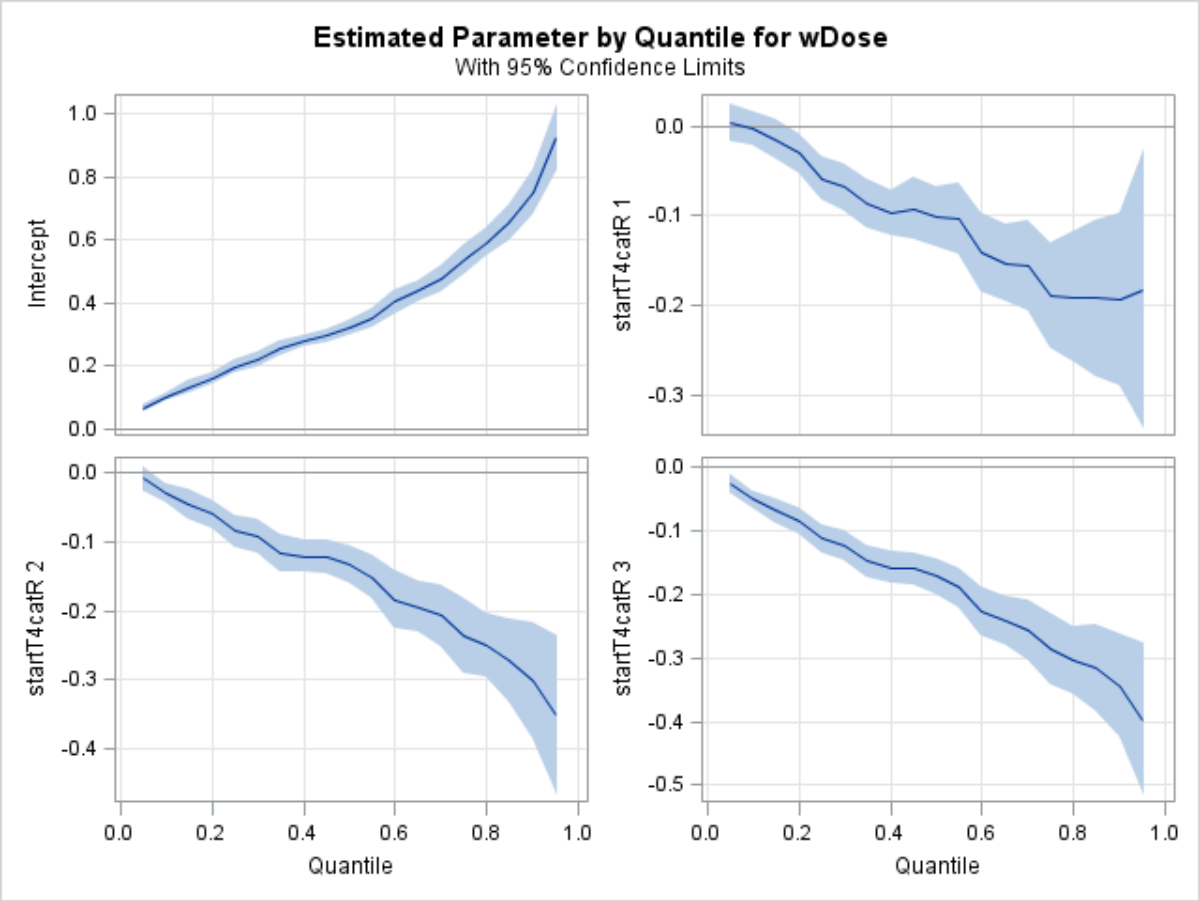
FIGURES

FIGURE 1. MEAN SALIVARY CORTISOL MEASURES BY TIME OF DAY SAMPLE COLLECTED



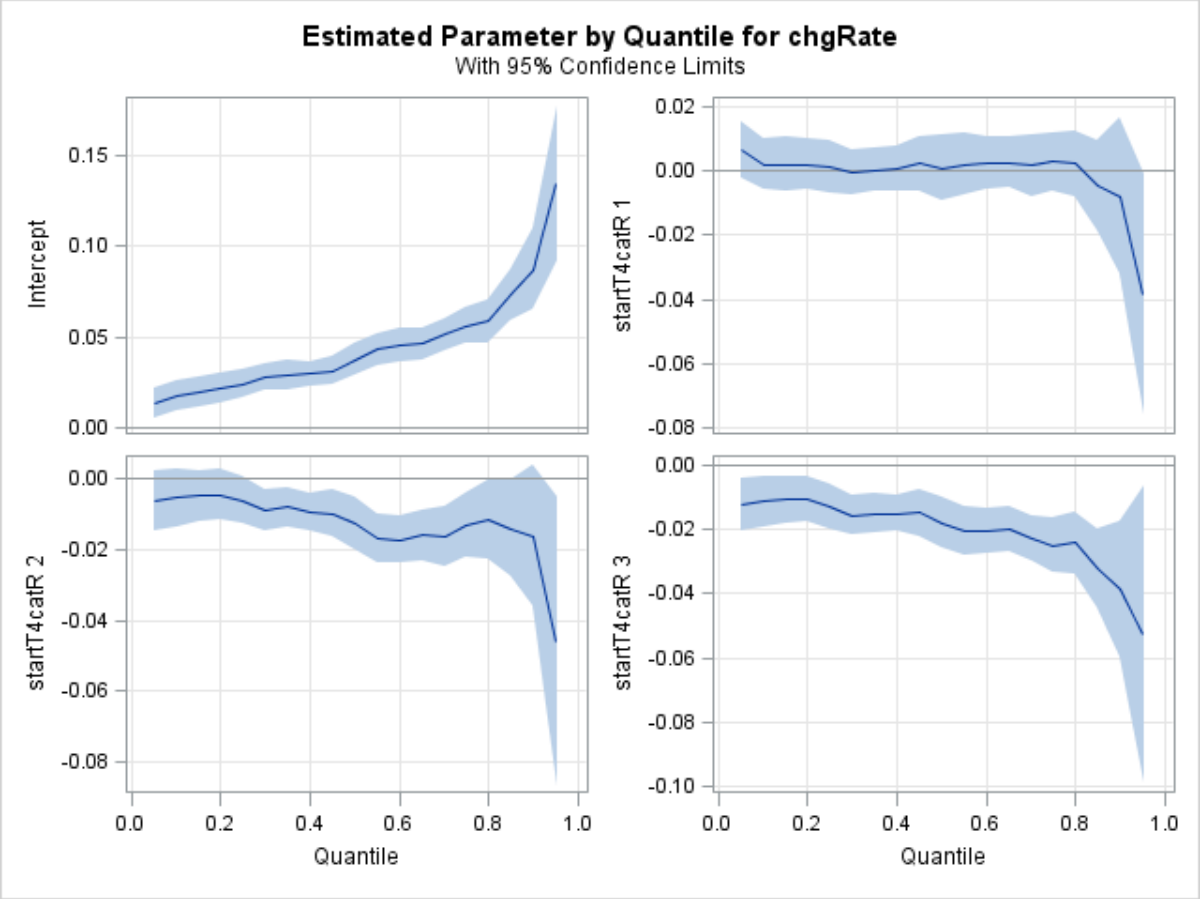
* Rate of change was calculated using post-interview cortisol level minus pre-interview cortisol level divided by interview length in hours. The time of day for pre-interview salivary sample collection was also used for rate of change. The mean difference of time of day between pre- and post- interview salivary sample collections was 2.4 hours (SD=0.6)

Figure 2a. Pre-interview cortisol quantile values by time of day



*__8am-<11am__ (Reference, zero line), *startT4catR 1*: __11am-<2pm__, *startT4catR 2:* __2pm-<5pm__, *startT4catR 3*: __>=5pm__)

FIGURE 2B: RATE OF CHANGE IN CORTISOL QUANTILE BY TIME OF DAY



*(Rate of change = post minus pre-interview cortisol divided by length of interview in hours) QR processing plot adjusting for pre-interview cortisol level: **8am-<11am** (Reference**,** zero line)**,** *startT4catR 1*: **11am-<2pm,** *startT4catR 2:* **2pm-<5pm,** *startT4catR 3*: **>=5pm**)