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The Relation of Cortisol Levels with Pubertal Development in Youth at Clinical High Risk for Psychosis

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Abstract

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Schizophrenia (SZ) and other psychoses are debilitating mental disorders that affect between 1-2 in 100 people. Heightened stress or a defective hypothalamic-pituitary-adrenal (HPA) axis may produce an excess of cortisol. Elevated cortisol has been found in individuals who develop SZ and other psychotic disorders. In order to best study prevention methods, it is important to investigate the period between when an individual begins to show signs of a psychotic disorder to when they develop a full-blown disorder. This phase, the prodromal phase, is often seen during adolescence. The present study examined the relation between cortisol and Tanner stage of pubertal development in healthy youth and youth at clinical high-risk (CHR) for psychosis. It was hypothesized that CHR youth would show a stronger positive relationship between cortisol level and Tanner stage than would controls. Positive correlations were found between stage of puberty and cortisol, and contrary to prediction, were stronger for healthy youth than CHR youth. This study also hypothesized that CHR youth who developed psychosis would have significantly higher baseline levels of cortisol at the first stages of puberty compared to CHR youth who did not develop psychosis. Results from a series of ANCOVAs indicated significant differences in the relation between cortisol and Tanner stage in CHR females who converted and did not convert. These preliminary findings highlight the importance of studying cortisol in relation to pubertal development in order to investigate potential indicators of the development of psychosis.

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The Relation of Cortisol Levels with Pubertal Development in Youth at Clinical High Risk for Psychosis

Individuals experience stress in a multitude of ways. Stress can be defined as a state in which our homeostasis is threatened, and we react with particular behavioral and physiological responses to return to homeostasis (Bradley & Dinan, 2010; Chrousos & Gold, 1992). Acute stressful events stimulate the synthesis and release of glucocorticoids – namely cortisol in primates – from the adrenal gland into the blood. This results from activation of the hypothalamic-pituitary-adrenal (HPA) axis. Cortisol stimulates neurons in the paraventricular nucleus (PVN) of the hypothalamus, causing a secretion of corticotropin-releasing factor (CRF). CRF stimulates the synthesis and release of adrenocorticotropic hormones (ACTH) from the anterior pituitary, which then releases more cortisol from the adrenal cortex (Herman, Flak, & Jankord, 2008). As glucocorticoid levels increase, receptors in the hippocampus and other brain regions detect an excess of cortisol. This negative feedback mechanism responds by turning off the PVN in order to get cortisol levels back to baseline.

Puberty

Our brains are highly influenced by hormones. One of the most important periods of hormonal development and other neurobiological changes is adolescence. The period of adolescence marks the psychological and social transition between childhood and adulthood. During this dynamic period of development, sex hormones have a plethora of organizational effects on brain size, structure and wiring (Lenroot & Giedd, 2010).

Glucocorticoid levels appear to heighten during adolescence in humans, possibly resulting in an increased effect of cortisol on brain maturation and functioning (Sinclair, Purves-Tyson, Allen, & Weickert, 2014). Stress hormones also play a great role in influencing neurotransmitter systems during puberty.

Puberty can be defined as the process of bodily changes by which adolescents reach sexual maturity. Pubertal development tends to begin earlier in females than in males, commencing anywhere from ages 8.0 to 14.9 in females and ages 9.7 to 14.1 in males (Lee, 1980). Puberty is typically complete by ages 12.4 to 16.8 in females and by ages 13.7 to 17.9 in males, although sexual maturation continues through adolescence. Shirtcliff et al. (2012) found a fluctuation of cortisol during puberty. Fifteen-year-olds had lower morning cortisol than when they were nine, although morning cortisol levels appeared lowest at age 11. This work is consistent with other findings indicating that there may be a cortisol "U-shaped" curve, with cortisol levels decreasing from ages nine to 11, then increasing as adolescents develop (Schreiber et al., 2006).

Other findings have shown increased cortisol production during the course of adolescence compared with prepubertal children (Kiess et al., 1995; Linder et al., 1990). Consistent with these findings, Matchock, Dorn and Susman (2007) found cortisol levels were lower during middle stages of pubertal development, using the self-report Tanner scale (Peterson, Crockett, Richards & Boxer, 1988). The Tanner scale is a reliable, self-reported measure of puberty that uses line drawings of Tanner standard photographs illustrating breast and genital development (Tanner, 1962). Cortisol levels in Tanner pubertal stage 3 were lower than in Tanner stages 1, 2 or 4 in boys and girls. These "U-

shaped" curve findings support literature that has suggested that important neurobiological changes occur as individuals enter puberty. One potential explanation for the early rise in cortisol levels that precede a mid-puberty dip in cortisol is due to adrenarche – an early sexual maturation stage that typically occurs around ages 10 to 11. Sontag-Padilla et al. (2012) found that the major physical consequences of adrenarche are androgen effects, such as pubic hair and gonadal development, seen around Tanner stages 2 and 3. To date, researchers have not reached a consensus about the relationship between cortisol and stages of pubertal development.

Stress and Psychopathology

While our stress mechanisms are vital to our survival, chronic stress can be detrimental. On one hand, our hippocampi serve as negative feedback to shut off our HPA axes if exposed to too much cortisol. On the other hand, prolonged cortisol exposure may end up damaging hippocampi, which would then no longer be able to suppress the system as well (Zhu et al., 2014). If the HPA axis can no longer shut off its stress system, an individual would not be able to appropriately respond to stress. This could result in the emergence of other negative effects, such as altered metabolism, suppressed immunity, and the development of psychiatric disorders (Herbert et al., 2006). Important neurobiological changes that take place as individuals enter puberty, such as the normative increase in cortisol during adolescence, may lead to increased susceptibility in certain individuals for psychological disorders (Dahl & Gunnar, 2009; Sontag-Padilla et al., 2012). Researchers have also discussed the possibility that the

increase in cortisol in early adolescence, in combination with premature adrenarche, may in fact be what increases susceptibility for individuals to develop disorders like depression, anxiety, post-traumatic stress disorder (PTSD) and even schizophrenia (SZ) (Gunnar, Wewerka, Frenn, Long, & Griggs, 2009). A 2008 review highlighted five general conclusions about HPA axis function in SZ and other psychotic disorders: 1) evidence of elevated cortisol in patients with psychoses; 2) antipsychotic medications usually reduce cortisol; 3) drugs that increase dopamine levels and/or increase psychotic symptoms also elevate HPA activity; 4) glucocorticoid receptors seem to appear downregulated in patients with psychosis; and 5) reduced hippocampal volume is consistently found in psychotic patients (Walker, Mittal, & Tessner, 2008).

Psychotic disorders, especially SZ, are characterized by severe cognitive distortions, delusions and hallucinations. Individuals with SZ and other psychotic disorders tend to not be able to hold jobs, have close relationships, or maintain a stable life. Research thus far has indicated that psychotic disorders are complex to study due to the fact that they are considered to be heterogeneous in etiology. While heritability accounts for a significant proportion of the risk for development of a psychotic disorder, epigenetic mechanisms appear to play a great role in mediating the expression of genes (Dempster et al., 2011). This means that while an individual may have the underlying genes to develop SZ, there are certain additional internal or external factors that determine whether those traits will be expressed. For example, external events such as more negative life events or early trauma appear to also play a role in increased likelihood of developing a psychotic disorder (Dvir, Denietolis, & Frazier, 2013). More

stressful life events appear to be associated with elevated cortisol levels (van Eck, Berkhof, Nicolson, & Sulon, 1996). Trotman et al. (2014) found stress-sensitization over time with clinical high-risk (CHR) individuals who experienced more stressful life events also rating daily hassles as more stressful.

In a meta-analysis, Varese et al. (2012) found evidence that childhood adversity and trauma significantly increase the risk of developing SZ, and those suffering from psychosis are 2.7 times more likely than controls to have been exposed to adversity in childhood. Trotman et al. (2014) found that CHR individuals rated events as more stressful, and those who converted to psychosis reported experiencing more stressful life events overall. Additionally, Chan, Di, McAlonan, and Gong (2011) suggested that an early disruption of neuronal architecture in combination with epigenetic changes could result in an abnormal inflammatory response, leading to increased susceptibility to psychological disorders. Research has shown that SZ shows indicators of a neuroprogressive illness where treatment efficacy and outcomes are negatively correlated with early disease onset and more severe episodes (Davis, Moylan, Harvey, Maes, & Michael, 2014). Because of cortisol's major psychological impact on the brain in a variety of disorders, many SZ studies have been investigating the relation between elevated cortisol and individuals who develop SZ (Collip et al., 2011; Mondelli et al., 2010; Walker et al., 2013). On account of how debilitating SZ and other psychotic disorders are to an individual and his or her family, it is a critical disorder to study. Through this type of research, our hope is that one day we will be able to not only create adequate treatment options, but also develop effective prevention methods.

From Prodrome to Psychosis

The focus in psychotic disorder research has recently shifted away from just studying diagnosed patients towards looking at the period before psychosis becomes evident, known as the prodromal phase. Individuals who meet standardized criteria for the prodrome are designated as being at clinical high-risk (CHR). The prodromal phase describes the period beginning with the onset of psychotic symptoms up to the development of full-blown psychosis. This phase typically occurs during adolescence, likely due to the neurobiological changes that occur during this period. Eaton, Badawi, and Melton (1995) described the prodromal phase as "precursor signs and symptoms from a diagnostic cluster that precede disorder but do not predict onset with certainty." Yung et al. (1996) described this phase as an "at-risk mental state," which coincides with the idea that SZ is an epigenetic disorder. Studies have found that alterations in brain structure occur at some point in the transition from prodromal state to full-blown psychotic disorder (Pantelis et al., 2003). Walker et al. (2013) found evidence of heightened cortisol secretion in CHR individuals, and reported that cortisol levels were correlated with symptom severity ratings at baseline and with progression of prodromal syndromes. Addington and Heinssen (2012) found that the prodromal period is a time during which positive symptoms may be more responsive to treatment (Addington & Heinssen, 2012). Additionally, the conversion rate of CHR subjects who end up developing a psychotic disorder ranges from 20-40% (Larson, Walker, & Compton, 2010). On account of these findings, the present study is focused on comparing healthy individuals to CHR subjects during the typical prodromal period.

CHR studies focus on adolescent/young adult subjects, because it is assumed that neurodevelopmental changes during this period may play a role in emergence of psychosis (Tanner, 1962; Walker et al., 2009). It has become even more imperative to look at CHR individuals early on in their prodromal phase, in order to provide preventative measures (Walker et al., 2008). Since pubertal development is associated with significant shifts in stress reactivity, in addition to findings suggesting that exposure to high stress during puberty may alter HPA axis function **(**Reardon, Leen-Feldner, & Hayward, 2009), it is important to study stress levels during pubertal development. It is additionally important to study this developmental period, because any altering of the HPA axis may lead to the development of psychological disorders. This makes sense in light of recent findings that individuals with SZ and other psychotic disorders have heightened levels of cortisol (Walker et al., 2009). Thus it follows that CHR individuals would likely show different cortisol levels than would healthy controls.

As SZ appears to differ in age-at-onset and course by sex, it is important to look at males and females separately. On average, males develop SZ at an earlier age than females. This is interesting in light of the fact that females tend to go through puberty earlier than males. Cohen, Seeman, Gotowiec, and Kopala (1999) suggest that women predisposed to SZ with an early puberty develop SZ later than women who have a late maturation. It has been suggested that the later onset and more favorable prognosis for SZ and other psychoses in females is due to the protective effects of estrogens, possibly by dampening dopamine activity (Seeman & Lang, 1990). There are numerous examples of normal brain processes that are modulated differently across the sexes. For example,

during early development, males have a slower cerebral development than do females (Kretschmann, Schleicher, Wingert, Zilles, & Loblich, 1979). During the transition from early childhood to adolescence, ventricle volumes increase significantly over time only for males (Giedd et al., 1996). Finally, even in late maturational development, myelination in the hippocampus differs between sexes, with females having a significantly greater extent of myelin staining than males (Benes, Turtle, Khan, & Farol, 1994).

In terms of psychosis precursors and course, males have poorer premorbid adjustment, more maternal obstetric complications, more negative symptom, and poorer treatment response (Nopoulos et al., 1997). Some research has even postulated that sex differences in the phenomenology and brain morphology of SZ are indicative of separate illnesses among males and females (Castle & Murray, 1991). However, most research indicates that SZ is the same syndrome in males and females, but is modulated in its expression by sex. Differences in sex hormones are known to play an important role in brain development, leading to differences in brain function between the two sexes and resulting in differences in how SZ develops and manifests as an illness. On account of vast sex differences leading to differential development and prognosis of SZ, the present study will examine males and females separately.

While many studies have examined cortisol levels in CHR subjects and individuals with SZ, no study to date has specifically looked at cortisol levels in relation to stages of pubertal development in CHR male and female youth. The main goal of this study is to examine the relation between cortisol levels and stage of pubertal development

in healthy and CHR youth. It is hypothesized that CHR youth will show a stronger, positive relationship between cortisol level and Tanner stage than will the healthy controls. It is also hypothesized that CHR individuals who develop psychosis will have significantly higher baseline levels of cortisol at the first stages of puberty when compared to CHR subjects who do not develop psychosis.

Methods

The North American Prodrome Longitudinal Study

Participants included in this study were drawn from the subject pool of those participating in the North American Prodrome Longitudinal Study 2 (NAPLS-2). The NAPLS is a consortium of eight research centers, whose aim is to enhance clinical prediction of psychosis risk, elucidate the neural mechanisms associated with conversion to psychosis, and determine the impact of early treatment for prodromal symptoms (Addington et al., 2007). After the initial NAPLS-1 was completed, the NAPLS-2 was launched as a prospective study with support from the National Institute of Mental Health. The NAPLS-2 incorporated more comprehensive biological assessment approaches into the multi-site longitudinal study. The principle investigators hope that by looking at clinical and biological abnormalities that precede psychosis, we will have better insight to the development of psychosis and can combine these predictors with clinical algorithms to enhance predictive power. The total NAPLS-2 sample now consists of over 700 CHR subjects and 240 matched healthy controls.

Participant Characteristics

The samples utilized in the present study were CHR youth and healthy control subgroups of the NAPLS-2 participants who were between the ages of 13 and 20, and for whom data on pubertal development stage was obtained using the self-report Tanner scale. The healthy control sample of 94 subjects consisted of 61 males and 33 females. There were 351 subjects in the CHR sample. Of these, 199 were males with 24 of these males subsequently converting to a psychotic disorder. There were 152 female CHR subjects, of which 17 converted to a psychotic disorder. Demographic data on the study sample is presented in Table 1.

Recruitment Procedure

Again, because this study was specifically examining pubertal stage in relation to cortisol level, the present sample was a subset of the larger sample, including only those who were in the age range that had completed the Tanner scale study measure. The Tanner scales were administered to those to those ages 20 and under. Therefore, this study included information from 351 CHR subjects and 94 controls.

As described in previous reports, the NAPLS-2 project had the following exclusion and inclusion criteria. Both CHR subjects and controls were excluded if they met DSM-IV criteria for an Axis I psychotic disorder, and controls were also excluded if they had a first-degree relative with psychosis or met prodromal criteria. Additionally, individuals with substance dependence, neurological disorder, or IQ less than 70 were

excluded. More detailed information about study procedures is presented in Addington et al. (2012).

Study Procedure and Measures

Each NAPLS-2 site recruited participants and screened individuals using the Structured Interview for Prodromal Syndromes (SIPS) at both initial assessment and follow-ups (Miller et al., 2003). The protocol was approved by Institutional Review Boards at all NAPLS sites where data were collected, and every participant gave either direct informed consent or had parental informed consent if under 18 years of age (Walker et al., 2013).

The goal of the SIPS is to identify CHR individuals. The SIPS contains the Scale of Prodromal Symptoms (SOPS), which rates symptom severity in terms of positive symptoms (hallucinations or delusions), negative symptoms (social deficits, decreased emotional expression), disorganized behavior and other general psychiatric symptoms. Subjects were labeled prodromal/CHR if they met at least one of the following four criteria: 1) brief psychotic syndrome if rated as severe on positive symptom over the past three months; 2) decreased positive symptom syndrome if rated moderately on a positive symptom; 3) genetic risk if a first-degree relative has shown functional decline over the past year; or 4) the individual is 18 or younger and meets criteria for schizotypal personality disorder (Walker et al., 2013).

Throughout the two-year period, the Structured Clinical Interview for Axis I DSM-IV Disorders was administered to subjects in order to diagnose Axis I disorders

(First et al., 2002). Clinical assessments continued to be conducted as well, in addition to information on developmental and family history and any previous psychiatric history. More detailed assessment information can be found in Walker et al., 2013.

Self-Report Measures of Stress

The Psychiatric Epidemiology Research Interview Life Events Scale (PERI-LES), a common stress measure, was used. For this study, an abbreviated version of the PERI Life Events Scale (Dohrenwend, Askenasy, Krasnoff, & Dohrenwend, 1978) consisted of 59 items selected from the original 102 to be appropriate for age levels ranging from adolescence through early adulthood. Participants were provided with a list of major and minor stressful life events (e.g. “lost a job” or “took a vacation”) and asked to indicate whether they had experienced any of the events over the course of their lifetime. The examiner then asked follow-up questions regarding each event endorsed by the participant to determine on a scale of 1 (“no stress”) to 7 (“caused me to panic”) the participants’ perceptions of the stressfulness of the event. These rating were summed to derive an overall index of life event stress exposure.

Saliva Sampling and Cortisol Assay

Participants were given dietary instructions prior to saliva sampling. Each assessment consisted of at least three morning saliva samples at one-hour intervals, which were then averaged. Multiple saliva samples (n=3) were obtained in order to increase the

reliability of the samples. Saliva samples were also taken at follow-up months 6, 12, 18 and 24.

Saliva was stored in a freezer and assayed for salivary cortisol (μg/dL). Samples were rapidly thawed and centrifuged, prior to assay. Each sample was then assayed for salivary cortisol using a highly sensitive enzyme immunoassay. The test used – Salimetrics – uses about 25 μL of saliva, ranges from 0.007-1.8 mg/dL in sensitivity, and has an average intra-assay and interassay coefficients of variation less than 10% and 15%. Each sample was assayed in duplicate.

Tanner Stage Measurement

The Tanner scale was used as a self-assessment measurement of stage of pubertal development for all subjects ages 20 and under (Peterson et al., 1988). The Tanner scale is a reliable self-report measure of puberty. It is a gender-specific, one-page, self-assessment that uses line drawings of Tanner standard photographs illustrating breast and genital development (Netherton, Goodyer, Tamplin, & Herbert, 2004). Previous research indicates this quick measure is comprehensible to youth, and shows good correspondence with direct physical examination. Levels of development are grouped into stages from 1-5, with 1 indicating the beginning of puberty and 5 indicating the end. Further, the Tanner scale is less invasive than a direct physical examination, enhancing compliance (Forbes et al., 2010). In this study, four Tanner scale scores were used for analysis: 1) Tanner male pubic score; 2) Tanner male penis score; 3) Tanner female pubic score; 4) Tanner female breasts score.

Data Analysis

Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS). Sample characteristics were evaluated through computation of means and standard deviations of variables (see Table 1). Pearson's correlations were run in order to test the relationship of baseline cortisol with other variables. Using the SPSS General Linear Model, univariate ANCOVAs were performed using Tanner scale category and diagnostic group (healthy controls and CHR) as the independent variables and cortisol as the dependent variable. The covariates were saliva collection time and number of stressful life events. Finally, ANCOVAs were conducted for CHR individuals to test the relationship between Tanner stage and baseline cortisol levels in those who did and did not convert.

Results

Pearson's correlations were derived to test the relations among baseline cortisol, the stress measure, Tanner stage ratings and subject age at assessment. These analyses were conducted for all subjects who had at least one measure of Tanner stage. The correlations for CHR youth and controls are presented in Table 2. As would be expected, subject age was significantly and positively correlated with each of the Tanner measures ($p = 0.00$). This was congruent with previous findings from the larger sample, where age was positively correlated with baseline cortisol level (Walker et. al., 2013) and life event stress (Trotman et al., 2014). Although positive, the correlation between baseline cortisol and life event stress score only approached significance ($p = 0.06$) in the present sample.

Correlations were significant between male penis and pubic scores ($p = 0.00$) and female pubic and breasts scores ($p = 0.00$). With the exception of Tanner female pubic scores, the correlations between life event stress and Tanner scores were all significant and positive ($p < 0.05$). Baseline cortisol was only significantly positively related to Tanner male pubic scores. ($p = 0.03$).

However, when subjects were seprated by diagnostic group (CHR youth versus controls), the pattern of relationships was different. As shown in Table 3 for controls, correlations between Tanner stage measures and cortisol were positive and significant for males ($p < 0.05$) and approached significance for females. A similar pattern was observed for the relation between life event stress and Tanner stages, with significance only in male Tanner scores ($p < 0.05$). In contrast, for CHR subjects, the correlations between cortisol and Tanner scores did not reach significance and the relations between life event stress and Tanner stage were lower in magnitude although significant for males ($p < 0.01$).

Examination of the distribution of scores on the Tanner stage measure indicated that only a small number of subjects had scores of "1" (for males, n=2 and for females n=3). Thus, for subsequent anlyses the first two Tanner stages from the measures were combined in category "2" and the scales ranged from 2 to 5 in these subsequent analyses.

Testing Hypothesis 1

In order to test the hypothesis that CHR youth will show a stronger positive relationship between cortisol level and Tanner stage than will healthy controls,

ANCOVAs were conducted with cortisol levels as the dependent variable, diagnostic group (control versus CHR) and Tanner stage as the independent variables, and saliva collection time and number of stressful life events as covariates.

In the analysis with Tanner male pubic score and diagnostic group as independent variables, sample time and stressful life events as the covariates, and cortisol as the dependent variable, there was no significant main effect of Tanner stage, F (3, 172) = 0.55, p = 0.65, and no significant main effect of diagnostic group, F (1, 172) = 2.65, p = 0.11. There was no significant interaction of diagnostic group by Tanner stage, F (3, 172) = 0.05, p = 0.99. Table 4 shows mean cortisol level by Tanner male pubic score. Similarly, no significant main effect was seen for Tanner male penis score, F (3, 171) = 0.77, p = 0.51, and no significant main effect of diagnostic group, F (3, 171) = 2.27, p = 0.13. There was no significant interaction of diagnostic group by Tanner stage, F (3, 171) = 0.30, p = 0.83. Mean cortisol level by Tanner male penis score can be found in Table 5.

When ANCOVAs were run with Tanner female pubic score as the independent variable, no significant main effect was found for female pubic score on baseline cortisol level, F (3, 117) = 0.65, p = 0.58, and no significant main effect of diagnostic group, F (3, 117) = 0.17, p = 0.68. There was no significant diagnostic group by Tanner stage interaction, F (3, 117) = 0.82, p = 0.49. Table 6 displays mean cortisol levels by Tanner female pubic score. Descriptive statistics for cortisol by Tanner female breasts score are displayed in Table 7. Similarly, no significant effect was found for Tanner female breasts score on cortisol, F (3, 117) = 0.09, p = 0.97, and no significant main effect of diagnostic group, F (3, 117) = 0.24, p = 0.62. The interaction of diagnostic group by Tanner female

breasts score was not significant, F (3, 117) = 0.41, p = 0.74.

Testing Hypothesis 2

Next, in order to test the second hypothesis, that CHR individuals who develop psychosis will have significantly higher baseline levels of cortisol at the first stages of puberty when compared to CHR subjects who do not develop psychosis, two-way ANCOVAs were conducted using CHR youth data. The independent variables were Tanner stage and conversion status (converted versus did not convert), and the covariates were saliva sample time and life event stress.

In CHR males, there was no significant main effect of Tanner male pubic score, F (3, 130) = 0.68, p = 0.57, and no significant main effect of conversion status, F (3, 130) = 1.22, p = 0.27. No significant Tanner stage by conversion status interaction was found, F (3, 130) = .92, p = 0.43 (see Table 8). ANCOVA was also conducted to test the relation of Tanner male penis score and conversion status on baseline cortisol in CHR male subjects (see Table 9). No significant main effect of Tanner stage, F (3, 129) =0.15, p = 0.93, and no significant main effect of conversion status, F (3, 129) = 0.20, p = 0.66. No interaction between Tanner stage and conversion status was found, F (3, 19) = 0.54, p = 0.66. In Fig. 1, we observed slightly elevated cortisol in CHR males who converted, with greatest cortisol differences appearing in the earliest stages.

The same ANCOVAs were conducted for each of the female Tanner scores. There was a significant main effect found for Tanner female pubic score, F (3, 94) = 5.96, p = 0.00, and a significant main effect of conversion status, F (3, 94) = 5.49, p =

0.02. There was also a significant interaction between Tanner female pubic score and conversion status, F (3, 94) = 7.31, p = 0.00. (see Table 10). In Fig. 2, stage 5 in CHR youth who converted stands out as having higher cortisol levels than stage 5 in CHR youth who did not convert.

ANCOVA to test Tanner female breasts (see Table 11) revealed no significant main effect of Tanner score, F (3, 94) = 1.90, p = 0.14, and no significant main effect of conversion status, F (3, 94) = 0.00, p = 0.95. However, there was a marginally significant interaction of Tanner score and conversion status, F (3, 94) = 2.66, p = 0.05. Although not significant, stage 4 appears to be elevated for CHR youth who convert compared to CHR youth who do not convert (see Figure 3).

Discussion

There was no support for the first hypothesis, that CHR youth would show a stronger positive relationship between cortisol level and Tanner stage than would healthy controls. Further, the predicted relations between Tanner stage and cortisol were not observed, and there was no significant interaction between Tanner stage and diagnostic group in baseline cortisol. Contrary to prediction, there were stronger positive relations between cortisol and Tanner score in the control group than in the CHR subject group. The generalized trend toward higher cortisol levels as well as greater stress exposure in CHR youth may have obscured the relation between cortisol levels and Tanner stage observed in the healthy control group. These findings may indicate that factors other than

pubertal maturation are having a greater influence on cortisol levels in CHR individuals when compared to healthy youth.

While there was no evidence of an interactive effect of Tanner stage and conversion status on cortisol in CHR males, there was for CHR females. The ANCOVA for CHR females revealed a significant interaction between Tanner female pubic stage and conversion status on cortisol, and a marginally significant interaction for Tanner female breasts stage and conversion status. Although not significant, the slightly elevated cortisol levels in CHR female youth who converted compared to those who did not convert in the earliest stages were consistent with previous findings (Dahl & Gunnar, 2009). Contrary to prediction, however, the higher scores for converters were in the latter stage of puberty. The significantly higher cortisol levels in stage 5 pubic score for CHR female subjects who converted to psychosis are however consistent with previous reports of elevated cortisol levels in CHR youth who converted to psychosis (Walker et al., 2013).

Cortisol levels also appeared to be elevated overall in the majority of Tanner stages in CHR youth who converted to psychosis compared to CHR youth who did not convert (see Tables 8-11). These results are consistent with prior reports that individuals who develop psychosis tend to have higher baseline cortisol levels. In general, these results confirm prior findings, yet also expand on them, integrating our knowledge on cortisol in regular pubertal development and heightened cortisol levels in development of psychosis.

Advantages of the present study

The present sample is well characterized and the CHR and healthy controls are matched for demographic factors. The baseline age for healthy controls and CHR youth were almost identical, because CHR youth were matched with controls in order to achieve the most accurate, representative results (Addington et al., 2013). The number of stressful life events was found to have a positive relationship with cortisol, meaning that, despite the young age of the present sample, individuals who experienced more stressful events in their life had higher cortisol levels than those who did not. This finding makes sense in relation to other studies that have looked at cortisol and stressful life events (Trotman, et al., 2014; van Eck et al., 1996). Because of this finding, the number of stressful life events was controlled for in the ANCOVAs. The time of day cortisol saliva samples were obtained was also controlled, so this would not be a confounding variable. Additionally, cortisol differed between controls and CHR, which previous studies have found as well and which we based our hypotheses on for this study (Walker et al., 2008).

Further Interpretations

First, the present results suggest that age is more strongly associated with cortisol than Tanner stage, and this is especially true for CHR subjects. There are several potential explanations for this pattern of results. It is reasonable to assume that there is minimal error in the measurement of age, thus affording high predictive power for detecting relations with other variables. In contrast, Tanner staging, which is based on physical characteristics, likely entails more measurement error. There are two reasons for

this. First, the physical manifestations of pubertal stage that are the basis for Tanner staging are assumed to be an index of the underlying hormonal changes that define pubertal status. Thus, the physical manifestations are a 'proxy' measure of the underlying pubertal process, and Tanner staging is not a direct measure of puberty as defined by hormonal factors. Second, the present study utilized a self-report Tanner measure. This is considered to have advantages over direct physical exams by a physician because such exams are costly and can increase attrition due to participant discomfort. Although the use of such measures is the norm in longitudinal research on puberty, they are dependent on the accuracy of the individual's self-report. Thus, self-report bias is another potential source of measurement error in the Tanner scores, and self-report error may be greater in CHR than control youth.

Second, for CHR youth, factors other than pubertal development may be playing a greater role in determining cortisol levels. Previous research has demonstrated that life event stress exposure is higher in CHR subjects than control samples (Trotman et al., 2014). There is also evidence of brain abnormalities in CHR subjects, including a reduction in hippocampal volume (Walker et al., 2008). The hippocampus is the brain region that plays a role in negative feedback to the HPA axis, and it has been suggested that abnormalities in this region may contribute to elevated cortisol levels in individuals at risk for psychotic disorders. Thus, the relation between Tanner stage and cortisol level in CHR youth may be obscured by the influence of these other factors.

Third, one of the chief goals of prodromal research is to improve clinical prediction, as current measures include many false positives (Addington and Hienssen,

2012; Pantelis et al., 2003). It is well established that risk prediction using clinical measures, such as the SIPS, is superior to previous approaches such as focusing on individuals presumed to be at genetic risk due. Nonetheless, psychosis risk prediction must be substantially improved in order to implement preventive interventions. Thus, one of the primary aims of the NAPLS is to develop better prediction algorithms so that preventive interventions can be targeted to those at greatest risk. It is important to keep this in mind in light of this study's findings.

Fourth, although our population consisted of 94 controls and 351 CHR subjects, the NAPLS-2 only encompasses age ranges known to be associated with greatest risk for the onset of the prodrome. Thus, the number of subjects at the lower Tanner stages was small. When subjects were subdivided based on Tanner stage, the numbers were too small at the lower stages to reliably test predictions. Therefore, it is important to keep in mind that some of the results that appear trending may have been significant had the groups been larger. Thus, it will be important to address these research questions again in future studies with larger groups at each stage of pubertal development.

Finally, it is important to note that there was an absence of control over psychotropic medications. For ethical reasons, participants in the NAPLS and other prodromal studies are not withdrawn from medication in order to participate. While prior NAPLS results did not find a relation between psychotropics and cortisol (Walker et al., 2013), other studies have found that atypical antipsychotics tend to reduce cortisol (Bidkova et al., 2011). Therefore, although most of the CHR subjects are not on a

psychotropic medication, it is important to note that medication may be reducing cortisol levels in the subgroup of CHR group that is on an antipsychotic.

Conclusions and future studies

The present study examined the relation of cortisol levels with pubertal development in healthy controls and youth at clinical high-risk (CHR) for developing psychosis. Consistent with expectations, there was a significant positive relation between age and all of the Tanner stage measures. Also, replicating previous reports from this project using the larger sample, there was a significant relation between age (Walker et al., 2013) and cortisol and life event stress (Trotman et al., 2014).

This study provided evidence that pubertal development is associated with increased cortisol levels in healthy subjects. The finding that there was a stronger positive relation between cortisol and healthy controls than cortisol and CHR subjects may shed light on the complexity of the development of psychotic disorders. Perhaps this information can be applied to future studies, in order to determine why there is this difference in relation in cortisol trend.

This study also provided evidence that pubertal development is associated with increased cortisol levels in CHR female subjects who subsequently develop psychosis. For females, pubertal stage may be one of the factors contributing to the elevated cortisol levels that have been observed in CHR youth, especially those who subsequently develop psychosis (Walker et al., 2013). Perhaps with a larger study, significant findings will be found in males as well. Future longitudinal studies will benefit from looking at larger

samples of youth who are at the early stages of puberty, and follow their development while taking cortisol saliva samples. In fact, the NAPLS has recently completed data collection on an additional 500 subjects; so future studies can test the present hypotheses with larger samples of CHR youth and healthy youth. Additionally, following the idea of this "U-shaped" curve, although our study population was too small to find conclusive results from early stages of puberty, our study did find significantly higher trends in later stages of puberty in females. Clearly, the adolescent and young adult periods are the optimal time to investigate potential indicators of the development of psychosis.

In the meantime, it is imperative that further research continues to look at the prodromal period in order to develop effective preventative measures to hopefully help those who may be at risk for developing a psychotic disorder. Perhaps one day, youth who meet refined CHR criteria could be given an anti-stress regimen to follow, such as psychotherapy to reduce reactions to stress, during the period that individuals are most susceptible to conversion. If we can implement anti-stress measures through medication and/or therapy, perhaps we can reduce the number of individuals who develop such devastating disorders. This study was the first of its kind to look at stages of pubertal development in relation to cortisol and psychosis, and hopefully more studies will continue to follow.

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Table 1

Demographic Characteristics by Diagnostic Group

		Diagnostic Groups		
		Healthy Controls	Prodromal	Total
Gender				
	Male (*n*)	61	199	260
	Converted	0	24	24
	Female (*n*)	33	152	185
	Converted	0	17	17
Baseline Age				
	M(SD)	15.27 (2.05)	15.66 (1.86)	
Ethnicity				
	African American (*n*)	16	44	60
	Caucasian (*n*)	55	205	260
	South/East Asian (*n*)	6	25	31
	Other (*n*)	17	77	94
Marital Status				
	Single/Unmarried (*n*)	94	351	445
	Married (*n*)	0	0	0
Children				
	No children (*n*)	94	350	444
	≥ 1 child (*n*)	0	1	1
Education Years				
	< 12 years (*n*)	80	313	393
	12 years (*n*)	9	31	40
	> 12 years (*n*)	5	7	12
Working				
	Full time (*n*)	1	2	3
	Part time (*n*)	13	38	51
	Not working (*n*)	80	310	390

Table 2

Correlations between Cortisol at Baseline and Other Variables for All Subjects

	Pearson's Correlations						
	BC	LES	Age	TMPu	TMPe	TFP	TFB
BC	1						
LES	0.09	1					
Age	0.17*	0.27*	1				
TMPu	0.13*	0.22*	0.46*	1			
TMPe	0.04	0.23*	0.44*	0.58*	1		
TFP	0.11	-0.04	0.20*			1	
TFB	0.00	0.12	0.29*			0.40*	1

Note. BC = baseline cortisol; LES = life event stress score; TMPu = Tanner male pubic score; TMPe = Tanner male penis score; TFP = Tanner female pubic score; TFB = Tanner female breasts score.

*. Correlation is significant at the 0.05 level (1-tailed).

Table 3

Correlations among the Predictor and Dependent Measures across Groups

	Pearson's Correlations						
	BC	LES	Age	TMPu	TMPe	TFP	TFB
BC	1	**0.06***	**0.15***	**0.11**	**0.01**	**0.10**	**-0.06**
LES	0.10	1	**0.21***	**0.21***	**0.22***	**0.03**	**0.10**
Age	0.32*	0.35*	1	**0.44***	**0.37***	**0.17***	**0.27***
TMPu	0.33*	0.24*	0.55*	1	**0.55***		
TMPe	0.27*	0.28*	0.67*	0.67*	1		
TFP	0.27	-0.03	0.29*			1	**0.35***
TFB	0.33	0.22	0.36*			0.73*	1

Upper part of the table denotes correlations for CHR subjects; bottom part of the table denotes correlations for healthy controls.

Note. BC = baseline cortisol; LES = life event stress score; TMPu = Tanner male pubic score; TMPe = Tanner male penis score; TFP = Tanner female pubic score; TFB = Tanner female breasts score.

*. Correlation significant at 0.05.

Table 4

Mean Baseline Cortisol Levels by Tanner Male Pubic Stage and Diagnostic Group

Subject Group	Tanner Male Pubic Score	Baseline Cortisol	
Healthy Controls		M (SD)	N
	1	0.03 (0)	1
	2	0.11 (0.07)	5
	3	0.09 (0.04)	5
	4	0.12 (0.05)	15
	5	0.16 (0.09)	21
Grand Total		0.13 (0.08)	47
CHR			
	1	0.13 (0.03)	2
	2	0.12 (0.07)	9
	3	0.13 (0.07)	19
	4	0.18 (0.14)	70
	5	0.18 (0.23)	53
Grand Total		0.17 (0.17)	152

Table 5

Mean Baseline Cortisol Levels by Tanner Male Penis Stage and Diagnostic Group

Subject Group	Tanner Male Penis Score	Baseline Cortisol	
Healthy Controls		M (SD)	N
	1	0.17 (0)	1
	2	0.08 (0.02)	5
	3	0.09 (0.07)	5
	4	0.14 (0.04)	10
	5	0.15 (0.09)	26
Grand Total		0.13 (0.08)	47
CHR			
	1	0.17 (0.12)	2
	2	0.10 (0.07)	12
	3	0.17 (0.08)	21
	4	0.21 (0.25)	54
	5	0.15 (0.17)	63
Grand Total		0.17 (0.17)	152

Table 6

Mean Baseline Cortisol Levels by Tanner Female Pubic Stage between Subject Groups

Subject Group	Tanner Female Pubic Score	Baseline Cortisol	
Healthy Controls		M (SD)	N
	1	0.25 (0)	1
	2	0.04 (0)	1
	3	0.10 (0.05)	8
	4	0.14 (0.09)	8
	5	0.18 (0.10)	7
Grand Total		0.14 (0.09)	25
CHR			
	1	0.26 (0)	1
	2	0.13 (0.08)	21
	3	0.16 (0.11)	41
	4	0.15 (0.11)	34
	5	0.19 (0.16)	15
Grand Total		0.16 (0.11)	111

Table 7

Mean Baseline Cortisol Levels by Tanner Female Breasts Stage between Subject Groups

Subject Group	Tanner Female Breasts Score	Baseline Cortisol	
Healthy Controls		M (SD)	N
	1	0.04 (0)	1
	2	0.09 (0.05)	4
	3	0.16 (0.07)	4
	4	0.14 (0.08)	13
	5	0.19 (0.09)	25
Grand Total		0.14 (0.09)	25
CHR			
	1	0.15 (0.07)	2
	2	0.18 (0.15)	15
	3	0.15 (0.10)	26
	4	0.17 (0.12)	41
	5	0.14 (0.08)	28
Grand Total		0.16 (0.11)	111

Table 8

Mean Baseline Cortisol Levels by Tanner Male Pubic Stage in CHR Youth who Converted or Did Not Convert

Subject Group	Tanner Male Pubic Score	Baseline Cortisol	
Did Not Convert		M (SD)	N
	1	0.13 (0.03)	2
	2	0.10 (0.05)	8
	3	0.13 (0.07)	18
	4	0.19 (0.15)	58
	5	0.17 (0.24)	48
Grand Total		0.17 (0.18)	133
Converted			
	1		0
	2	0.25 (0)	1
	3	0.22 (0)	1
	4	0.15 (0.08)	12
	5	0.25 (0.15)	5
Grand Total		0.18 (0.11)	19

Table 9

CHR Mean Baseline Cortisol Levels by Tanner Male Penis in CHR Youth who Converted or Did Not Convert

Subject Group	Tanner Male Penis Score	Baseline Cortisol	
Did Not Convert		M (SD)	N
	1	0.09 (0)	1
	2	0.09 (0.05)	11
	3	0.17 (0.08)	19
	4	0.21 (0.26)	49
	5	0.14 (0.09)	53
Grand Total		0.17 (0.18)	133
Converted			
	1	0.25 (0)	1
	2	0.25 (0)	1
	3	0.20 (0.15)	2
	4	0.16 (0.08)	5
	5	0.18 (0.13)	10
Grand Total		0.18 (0.11)	19

Table 10

CHR Mean Baseline Cortisol Levels by Tanner Female Pubic Stage in CHR Youth who Converted or Did Not Convert

Subject Group	Tanner Female Pubic Score	Baseline Cortisol	
Did Not Convert		M (SD)	N
	1	0.26 (0)	1
	2	0.13 (0.08)	18
	3	0.17 (0.12)	36
	4	0.15 (0.11)	31
	5	0.14 (0.10)	13
Grand Total		0.15 (0.10)	98
Converted			
	1		0
	2	0.10 (0.05)	3
	3	0.12 (0.07)	5
	4	0.16 (0.04)	3
	5	0.54 (0.17)	2
Grand Total		0.19 (0.17)	13

Table 11

CHR Mean Baseline Cortisol Levels by Tanner Female Breasts Stage in CHR Youth who Converted or Did Not Convert

Subject Group	Tanner Female Breasts Score	Baseline Cortisol	
Did not convert		M (SD)	N
	1	0.20 (0)	1
	2	0.18 (0.16)	14
	3	0.15 (0.10)	20
	4	0.15 (0.10)	37
	5	0.15 (0.08)	27
Grand Total		0.15 (0.10)	98
Converted			
	1	0.10 (0)	1
	2	0.12 (0)	1
	3	0.16 (0.14)	6
	4	0.29 (0.25)	4
	5	0.12 (0)	1
Grand Total		0.19 (0.17)	13

Figure Captions

Figure 1. Mean baseline cortisol levels by Tanner male pubic stage in CHR youth who converted or did not convert.

Figure 2. Mean baseline cortisol levels by Tanner female pubic stage in CHR youth who converted or did not convert.

Figure 3. Mean baseline cortisol levels by Tanner female breasts stage in CHR youth who converted or did not convert.

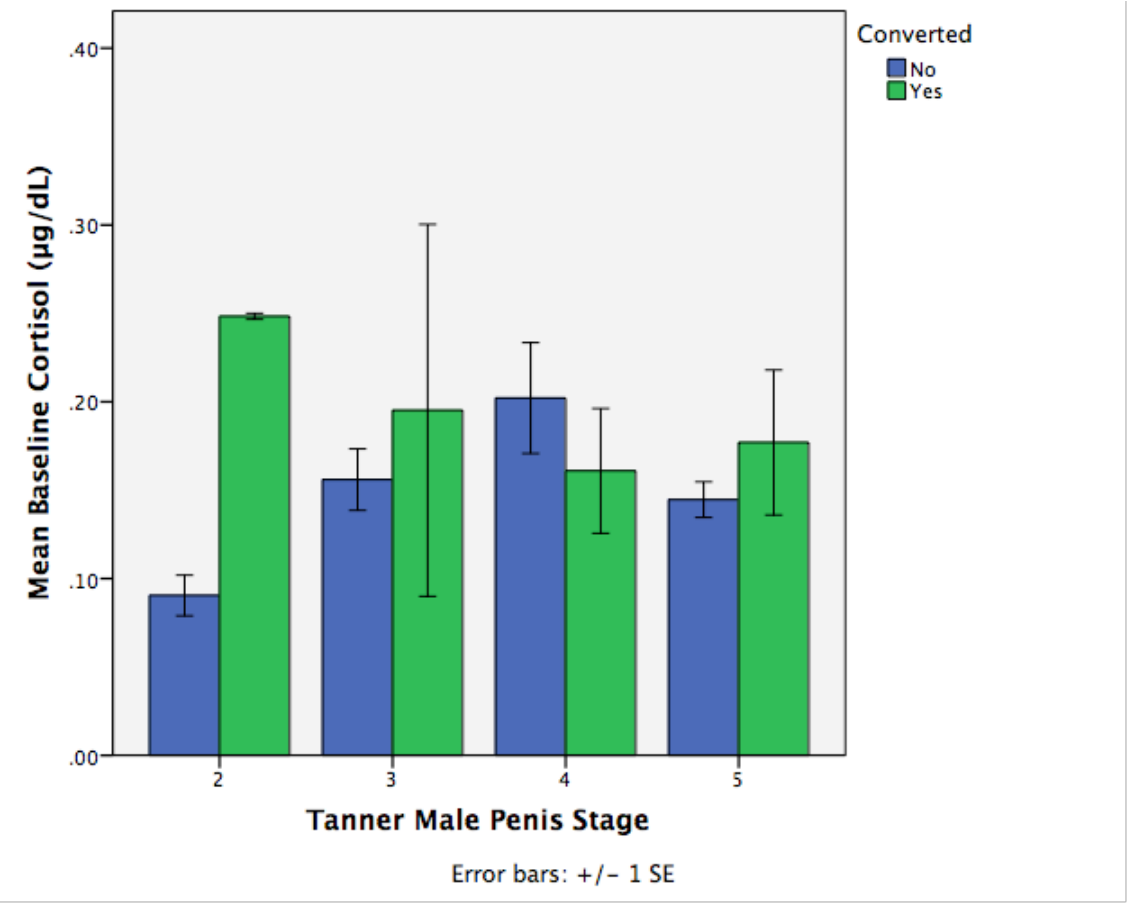

Figure 1. Mean baseline cortisol levels by Tanner male pubic stage in CHR youth who converted or did not convert.

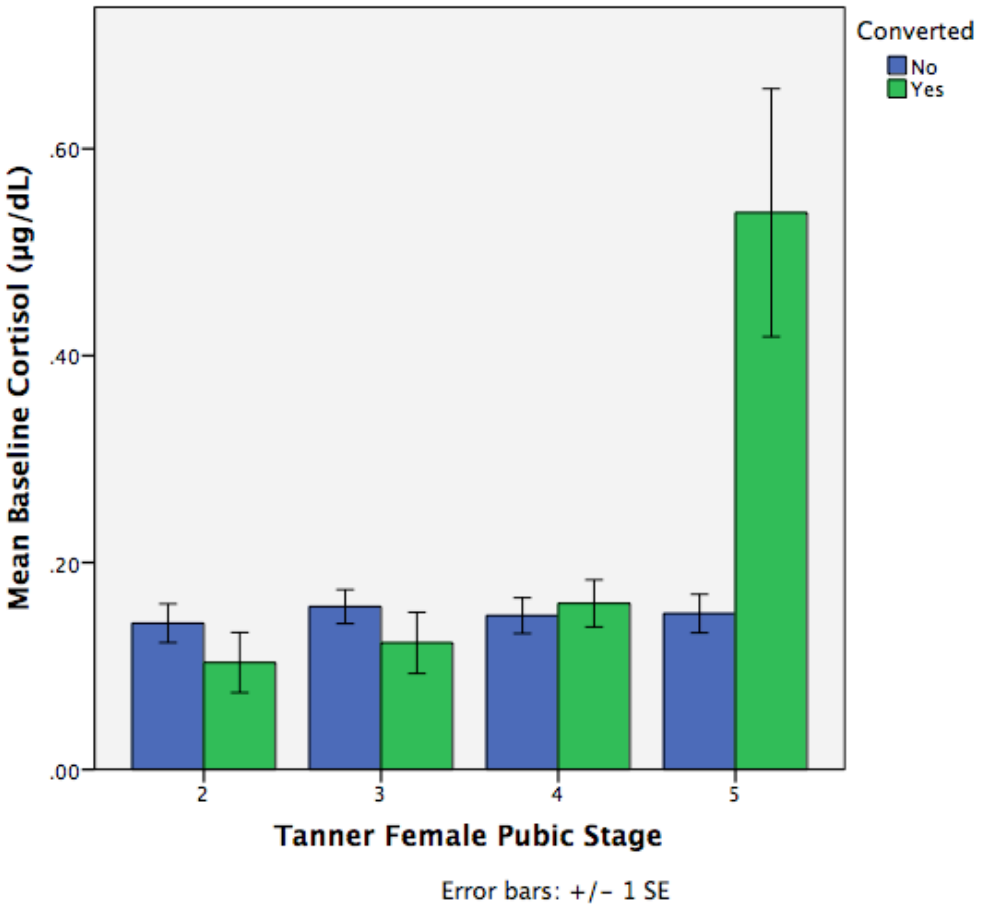

Figure 2. Mean baseline cortisol levels by Tanner female pubic stage in CHR youth who converted or did not convert.

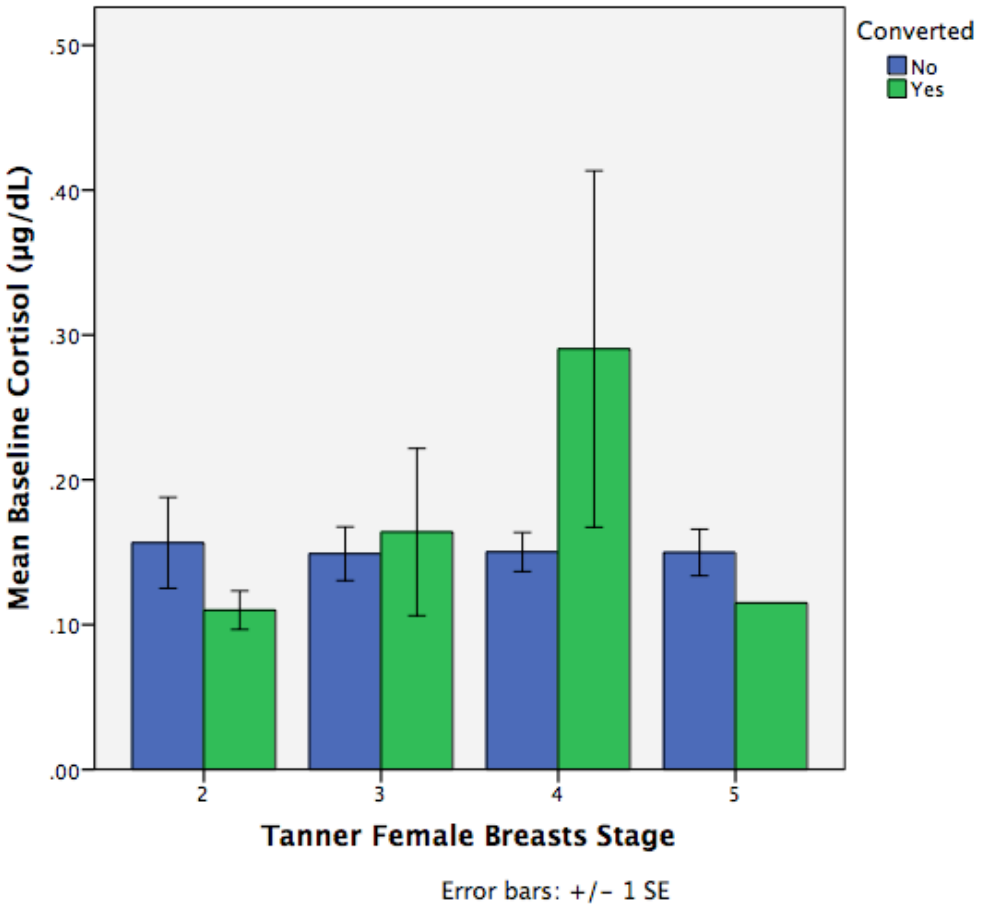

Figure 3. Mean baseline cortisol levels by Tanner female breasts stage in CHR youth who converted or did not convert.