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Sex differences in morning cortisol in youth at ultra high-risk for psychosis

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Summary

Research suggests abnormalities in hypothalamic-pituitary-adrenal (HPA) axis function play an important role in the pathophysiology of psychosis. However, there is limited research on the biological stress system in young people at ultra high risk (UHR) for psychosis. Morning cortisol levels are particularly relevant to study in this context, as these markers reflect HPA regulation. This is the first examination of sex differences in morning cortisol levels in UHR individuals. Twenty-eight UHR and 22 matched healthy control participants were assessed in respect to symptoms and had home-based collection of salivary cortisol over three time points in the morning. It was predicted that the UHR participants would exhibit lower morning cortisol levels and lower cortisol would be associated with greater symptomatology (i.e. higher positive, negative, and depressive symptoms). Additionally, sex differences in morning cortisol levels were explored based on recent evidence suggesting that sex differences may play an important role in the exacerbation of psychosis. While there were no group differences in morning salivary cortisol secretion, there was a sex by time interaction among UHR individuals, such that only UHR males exhibited flat cortisol levels across two hours after awakening, whereas UHR females had a pattern of cortisol secretion similar to healthy controls, even among medication-free individuals ($F = 6.34$, $p = 0.004$). Cortisol AUC (area under the curve) across the three time points had a trend

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Conflict of Interest

There are no conflicts of interest to report.

Contributors

Dr. Mittal, Dr. Spencer, and Emily Carol conducted analyses, and drafted the manuscript. Dr. Mittal supervised the administration and attained the funding for this study.

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association (medium effect size; $r = 0.34$, $p = 0.08$) with depressive, but not positive or negative, symptom severity. These results stress the importance of considering sex differences in the psychosis-risk period, as they improve understanding of pathogenic processes.

Keywords

Cortisol; HPA Axis; Prodrome; High-Risk; Schizophrenia

1. Introduction

The neural diathesis-stress model for psychosis posits that early neurobiological vulnerability and psychosocial stress are important factors in the etiology and exacerbation of schizophrenia and related disorders (Walker et al., 2008). A growing body of evidence suggests that the hypothalamic-pituitary-adrenal (HPA) axis is dysfunctional in patients with schizophrenia (Belvederi Murri et al., 2012; Walder et al., 2000), yet little is known about the relationship between the HPA system and the development of psychosis. Understanding the stress systems in individuals at ultra high-risk (UHR) for developing psychosis (those deemed to meet clinical criteria for a prodromal syndrome) is integral, as the prodrome is a viable period of intervention in which considerable neuroendocrine development is still underway (Spear, 2004). Evidence suggests that HPA system impairment may be present in the prodromal stage of psychosis (Carol and Mittal, 2015; Walker et al., 2013); however, significant differences are not always observed (Cullen et al., 2014; Day et al., 2014). A better understanding of HPA system dysfunction stands to inform the etiological conceptualization of psychosis risk and refine efforts of early detection for adolescents exhibiting subthreshold psychotic-like symptoms.

The HPA axis is one of the primary biological stress systems that moderate the physiological response to psychological and physiological stressors and is sensitive to gonadal hormones (Chrousos, 2000). HPA axis function is most commonly measured through cortisol levels and cortisol secretion has a consistent circadian rhythm (Nader et al., 2010; Walker et al., 2008). The basal rhythm follows the pattern of very low levels in the late evening/early morning, followed by a steady rise during the few hours preceding habitual time of awakening. Superimposed on this basal rhythm is often a rapid increase in cortisol after awakening (cortisol awakening response), with increases peaking between 30–45 minutes. This awakening response resolves within 1–2 hours after awakening, and then basal cortisol levels steadily decrease throughout the remainder of the day (Stalder et al., 2016). Changes to the circadian regulation of cortisol are interpreted as an indicator of a dysfunctional biological stress response system and these changes are consistently observed in patients with schizophrenia at multiple time points throughout the day (Corcoran et al., 2003; Nader et al., 2010).

Accumulating evidence suggests that basal cortisol levels during the day are elevated and the dynamic change in cortisol levels in response to awakening is blunted in patients with schizophrenia (Aas et al., 2011; Collip et al., 2011; Mondelli et al., 2010a; Pruessner et al., 2008; Pruessner et al., 2013b). Studies observed that elevated basal cortisol levels during the

day are closely associated with positive symptoms in patients with schizophrenia (Collip et al., 2011; Franzen, 1971; Walder et al., 2000). Additionally, studies posit that the cortisol awakening response is lower in patients with schizophrenia when compared to controls and levels are associated with neurocognitive deficits, environmental stressors, and sex differences (Aas et al., 2011; Mondelli et al., 2010a; Pruessner et al., 2008; Pruessner et al., 2013b). Specifically, repeated studies have observed a blunted cortisol response to awakening in male but not female patients with schizophrenia (Pruessner et al., 2008; Pruessner et al., 2015). In summary, findings illustrate a paradoxical relationship between elevated basal levels and blunted awakening response in patients with schizophrenia; however, there is little understanding of this relationship in UHR individuals.

Recent investigations have observed similar basal cortisol elevations in psychosis risk populations (Carol and Mittal, 2015; Day et al., 2014; Karanikas and Garyfallos, 2014; Walker et al., 2010; Walker et al., 2013). Additionally, it has been shown that cortisol levels are closely tied to the illness progression in the UHR period (Walker et al., 2010; Walker et al., 2013); however, the relationship is nuanced, and group differences are not always observed (Cullen et al., 2014; Day et al., 2014). There is currently little understanding of morning cortisol levels and how subsequent cortisol levels change throughout the day in psychosis risk. Only one previous study has examined the cortisol awakening response in an older sample of UHR young adults (Day et al., 2014). Specifically, Day et al. (2014) reported a blunted cortisol awakening response in medication free UHR participants compared to healthy controls and no daytime group differences. Interestingly, there was no difference in the cortisol awakening response when UHR participants who were taking medication were included (Day et al., 2014). These mixed results highlight the increasing need to understand the relationship between changes in cortisol throughout the morning and the symptomatology of UHR individuals, as it stands to inform our etiological conceptualization and refine efforts of early detection for adolescents exhibiting attenuated psychosis symptoms.

In the present study, including a total of 50 adolescents (28 UHR and 22 matched healthy participants), we evaluated if morning cortisol at three different time points in UHR individuals is different compared to healthy controls and if cortisol is associated with symptomatology. Samples were collected upon waking up and 1 hour and 2 hours post awakening in order to gain an understanding of any abnormalities in the broader morning cortisol pattern. Collection occurred at home to allow for a naturalistic and noninvasive measurement of HPA axis functioning. At-home collection allowed us to follow-up on our previous findings of higher late morning cortisol levels in UHR youth as assessed in a lab setting (Carol and Mittal, 2015). We predicted that the UHR participants would exhibit lower morning cortisol levels when compared to matched healthy controls based on observations from previous findings in high-risk youth (Day et al., 2014). We also predicted that lower cortisol would be associated with greater symptomatology (i.e. higher positive, negative, and depressive symptoms) based on studies reporting a relationship between cortisol abnormalities and psychosis risk symptoms (Mittal and Walker, 2011; Walker et al., 2013). Additionally, in line with sex differences observed in the cortisol awakening response of patients with schizophrenia (Pruessner et al., 2008; Pruessner et al., 2013b) and recent evidence suggesting that sex differences may play an important role in the exacerbation of

psychosis (Leung and Chue, 2000; Pruessner et al., 2013b; Walder et al., 2013), we conducted a secondary analysis examining sex differences in morning cortisol levels.

2. Methods

2.1 Participants

Participants were recruited at the Adolescent Development and Preventative Treatment (ADAPT) research program and a total of 28 UHR adolescents (15 male, 13 female) and 22 control adolescents (11 males, 11 female) participated in a study (see Table 1 for demographic characteristics of this sample). Adolescent UHR and control participants (mean age=17.9) were recruited by Craigslist, e-mail postings, newspaper ads, bus ads, and community professional referrals. Exclusion criteria included history of head injury, the presence of a neurological disorder, and lifetime substance dependence. The presence of an Axis I psychotic disorder (e.g. schizophrenia, schizoaffective disorder, schizophreniform) was an exclusion criterion for UHR participants. Other comorbid Axis I disorders were not exclusion criteria for UHR participants. Rates of current comorbid Axis I disorders in the UHR participants included 11(39%) affective disorders, 2(7%) PTSD, 8(29%) other anxiety disorders, and 3(11%) ADHD. Comorbid Axis I disorders are typical of UHR individuals and the present rates are comparable to other studies (Fusar-Poli et al., 2014). UHR individuals met criteria for a prodromal syndrome including: (a) recent onset or escalation of moderate levels of attenuated positive symptoms (a score of 3–5), and/or (b) a decline in global functioning over the last 12 months accompanying the presence of schizotypal personality disorder (SPD), and/or (c) a decline in global functioning over the last 12 months accompanying the presence of a first-degree relative with a psychotic disorder such as schizophrenia (Miller et al., 1999). Meeting for an Axis I disorder or the presence of a psychotic disorder in a first-degree relative were exclusionary criteria for controls. The protocol and informed consent procedures were approved by the University of Colorado Institutional Review Board.

2.2 Symptom Assessment

The Structured Interview for Prodromal Syndromes (SIPS; Miller et al., 1999) was administered to diagnose a prodromal syndrome. As noted, UHR participants in the present study met criteria for a prodromal or high-risk syndrome. The SIPS gauges several distinct categories of prodromal symptom domains including positive and negative dimensions. A sum score for each category is used as an indicator of the respective dimensions of symptomatology. The Structured Clinical Interview for Axis-I DSM-IV Disorders (SCID-IV; First, 1995) was also administered to rule out formal psychosis in UHR participants and an Axis I disorder in the control group (noted exclusionary criterion). This measure has been demonstrated to have excellent inter-rater reliability in adolescent populations (Martin et al., 2000) and has been used in several previous studies focusing on adolescent populations with schizophrenia spectrum disorders (Howes et al., 2009). Training of interviewers (who were advanced doctoral students) was conducted over a 2-month period, and inter-rater reliabilities exceed the minimum study criterion of Kappa .80. The Beck Depression Inventory (BDI-II, Beck et al., 1996) was used to measure self-reported depressive symptoms.

2.3 Morning Cortisol

Saliva collection took place at home and the participants were given clear verbal and written instructions on how and when to collect samples. Based on our prior studies, saliva was collected into 2.0 mL microcentrifuge tubes utilizing a passive-drool method (Carol and Mittal, 2015; Mittal et al., 2007; Mittal and Walker, 2011). Saliva was collected immediately, 60, and 120 minutes after awakening. Sample collection immediately upon awakening and within the first hour after awakening have been used as a measure of morning cortisol in previous work (Goodyer et al., 2009; Halligan et al., 2004; Sheikh et al., 2010), and measurements 1 and 2 hours post awakening were collected to assess for morning changes over time. Participants were instructed to refrain from brushing their teeth and eating or drinking anything other than water prior to collecting the first sample. Participants were also advised to collect the samples on a day when they would be able to wake before 10:30am and instructed to record the exact sample time of each saliva sample. Participants stored the samples in a refrigerator or freezer until returning them to the ADAPT lab, where they were stored in a -20C freezer until ready for assay. Each participant reviewed the data with an ADAPT member to confirm compliance with instructions and accuracy of sample times. The samples were analyzed by Salimetrics and used Salimetrics High Sensitivity Salivary Cortisol Enzyme Immunoassay Kits (Salimetrics, LLC, College Park, PA). Saliva samples (75 µl) were assayed in duplicate within a single assay and an average was taken to yield the raw cortisol value. Participants with missing samples, samples with an extremely low value ($<.007\mu\text{g/dL}$; sensitivity cut-off value recommended by Salimetrics), and samples collected after 10:30am were excluded from the study. No participants were excluded based on deviation from collection time. The mean deviation for the sampling times for the included participants was sample 2 mean = 2.41 minutes, SD = 6.55 minutes and sample 3 mean = .34 minutes, SD = 3.2 minutes, which indicates that the sample was compliant in their collection times.

2.6 Statistical approach

Mixed design ANCOVAs controlling for age were employed to examine cortisol change over time. A three factor mixed design ANCOVA with time (0, 60, 120 min after awakening) and group (UHR vs. control) as independent factors, and cortisol as the dependent variable, was used to examine overall group difference in cortisol levels. To explore sex differences, a series of separate three factor mixed design ANCOVAs were run in the UHR and control groups, with time and sex (men and women) as independent factors and cortisol as the dependent variable. Consistent with previous studies (Pruessner et al., 2008; Pruessner et al., 2015; Pruessner et al., 2013b), raw cortisol values at each time point were used in the ANCOVA. Independent t-tests were used to measure group differences in symptom variables. To obtain a single value of cortisol across the three time points, the area under the curve with respect to increase (AUC_i) was calculated based on an equation provided by Pruessner et al. (2003). Associations between AUC_i and symptoms were investigated using partial correlations, controlling for age, in the UHR group alone. A small portion of UHR individuals (2 male, 7.14%; 0 female) were taking neuroleptic medication and there was a significant difference between cortisol levels at awakening (time 0) between males taking and not taking neuroleptic medication [$t(13)=-2.25, p=0.04$]. Due to this result and differences observed in basal cortisol levels in UHR individuals treated with medication and

medication-free UHR individuals (Sugranyes et al., 2012), cortisol data analysis was performed for both the total sample and the medication-free subsample to avoid any possible effect of medication.

3. Results

3.1 Demographic and clinical characteristics

As noted, a total of 50 (28 UHR and 22 control) adolescents participated in a study. There were no significant differences in demographic characteristics such as age [$t(32)=1.99$, $p=0.07$], parental education [$t(47)=-0.50$, $p=0.62$], or sex [$\chi^2(1)=0.06$, $p=0.80$] between the UHR and control groups. As expected, the UHR group showed significantly more positive [$t(48)=10.72$, $p<0.001$], negative [$t(48)=6.34$, $p<0.001$], and depressive symptoms [$t(40)=5.97$, $p<0.001$] when compared with healthy controls (see Table 1). Positive, negative, and depressive symptom severity was not significantly different between those UHR participants on or off medication ([$t(26)=-1.18$, $p=0.25$], [$t(26)=-0.52$, $p=0.61$], [$t(26)=-0.14$, $p=0.89$]), male and female patients in the full sample UHR sample ([$t(26)=-0.64$, $p=0.53$], [$t(26)=-0.90$, $p=0.38$], [$t(26)=1.09$, $p=0.29$]), and medication-free UHR males and females ([$t(24)=-0.35$, $p=0.73$], [$t(24)=-0.84$, $p=0.41$], [$t(24)=1.15$, $p=0.26$]).

3.2 Cortisol group differences

To examine if cortisol levels over two hours after awakening in the morning in UHRs differ from healthy controls, a three factor mixed design ANCOVA with time (0, 60, 120 min after awakening), and group (UHR vs. controls) as independent factors, cortisol as the dependent variable, and age as a covariate, was conducted. Inconsistent with our prediction, the analysis revealed no overall group difference in cortisol levels across the three time points ($F=0.61$; $p=0.51$; sphericity violated), (Figure 1). There continued to be no group differences in the medication-free sample ($F=0.44$; $p=0.60$; sphericity violated).

3.3 Associations between cortisol and symptoms

As noted, the AUC_i was calculated to obtain a single value for the cortisol increase after awakening which includes all three assessment times and measures the area under the curve with respect to increase. AUC_i was not related to positive symptoms ($r=0.08$; $p=0.70$) or negative symptoms ($r=-0.17$; $p=0.40$) which did represent a small effect size; however, there was a trend level correlation between AUC_i and depressive symptoms of medium effect size ($r=0.34$; $p=0.08$).

3.4 Sex differences in morning cortisol

A supplementary analysis was conducted to examine sex differences in morning cortisol through running separate three factor mixed design ANCOVAs in the UHR group and the control group. Interestingly, a significant time by sex interaction emerged within the UHR individuals ($F=7.14$; $p=0.002$). Figure 2 illustrates the interaction showing that male UHR individuals' cortisol levels are the same as UHR females at awakening, lower than UHR females at 60 minutes, and higher than UHR females at 120 minutes. In other words, UHR males' and females' cortisol levels across the morning changed over time in different patterns and the UHR females showed cortisol levels similar to that of controls. This time by

sex effect was consistent in the medication-free UHR group ($F=6.34$; $p=0.004$). No such sex effect was observed in the control group ($F=0.31$; $p=0.74$). AUC_i was not correlated with symptoms when only looking at UHR males and effect sizes were small (positive, $r=0.18$; $p=0.55$; negative, $r=0.16$; $p=0.59$; depressive, $r=0.17$; $p=0.57$).

4. Discussion

To our knowledge, this is the first study that explores sex differences in morning cortisol in UHR adolescents. Contrary to existing data (Day et al., 2014; Mondelli et al., 2010a; Pruessner et al., 2008), there were no significant group differences in changes in morning cortisol across our three time points between UHR and control participants. Additionally, no significant relationships between morning cortisol and symptomatology were observed, although we did detect a positive trend with depressive symptoms within the UHR group alone. We did observe a significant time by sex interaction in the UHR group in which UHR males' levels were flat compared to UHR females' levels across the morning, while the UHR females' levels were consistent with the controls. This interaction was consistent across the whole UHR sample and the medication-free sample. These noteworthy results suggest that sex has a relationship with HPA axis functioning in the psychosis prodrome such that male adolescents could be either unequally vulnerable to stressors, or their vulnerability is differentially expressed at the level of HPA axis activity. These findings further clarify HPA dysfunction in a psychosis risk sample and in turn, highlight sex differences that are relevant for psychosis development.

In the current study we did not observe significant overall group differences in morning cortisol levels. Studies reporting HPA axis dysregulation in patients with schizophrenia most commonly report elevated resting cortisol levels, blunted cortisol response to acute stress, and blunted cortisol awakening response (Brenner et al., 2009; Pruessner et al., 2013b). A body of evidence suggests that HPA dysfunction may play an important role in the pathophysiology of psychosis (Walker et al., 2008), and examining related biomarkers such as cortisol levels prior to the onset of psychosis may yield important clues. Elevated resting cortisol levels are closely tied to the illness progression in the UHR period (Walker et al., 2010); however, the relationship is nuanced, and other investigations have not detected this effect (Cullen et al., 2014; Day et al., 2014). Only one study has examined the cortisol awakening response in UHR individuals and found that medication-free UHR individuals had an awakening response that was significantly more blunted than healthy controls (Day et al., 2014). Divergent results across the cortisol and psychosis risk literature could be due to differences in cortisol collection times, age of participants, medication, menstrual cycles, time and place of sampling, and sample size. These inconsistencies suggest that HPA dysfunction in UHR individuals is a complex process and continued systematic examination of cortisol levels throughout the whole day is needed.

The present observation did not find a relationship between cortisol levels and positive and negative symptoms in our UHR youth sample; however, a positive trend level associate with depressive symptoms emerged. The relationship between symptoms and cortisol levels in patients with schizophrenia is poorly understood as a number of studies do not report a relationship between the cortisol awakening response and symptom severity (Mondelli et al.,

2010a; Pruessner et al., 2008; Pruessner et al., 2013b; Steen et al., 2011), while others report a positive correlation between elevated resting cortisol levels and positive symptoms (Collip et al., 2011; Walder et al., 2000). This relationship is just beginning to be evaluated during the psychosis risk phase. The current finding is consistent with a previous study in at-risk individuals that did not find a significant relationship between the cortisol awakening response and positive, negative, or disorganized psychosis risk symptoms (Day et al., 2014). Additionally, there is a well-known relationship between HPA axis abnormalities and depression (Goodyer et al., 2009; Heim et al., 2008; Walker et al., 2008) and the current results suggest that this association is present for UHR individuals. Understanding how abnormalities in specific endocrine function relate to symptom severity is important in understanding mechanisms driving the pathogenesis of the disorder. Comorbid Axis I disorders are typical of UHR individuals (Fusar-Poli et al., 2014); therefore, it is possible that comorbid symptoms could add to the diathesis of psychosis. In fact, stress hormone elevations in UHR individuals has also been linked to anxiety (Corcoran et al., 2012), suspiciousness (Corcoran et al., 2012), stressful life events (Labad et al., 2014), and stress intolerance (Pruessner et al., 2013a). Future studies that specifically examine the interplay between comorbid disorders and HPA axis dysfunction in UHR individuals are needed to better understand the relationship between symptomatology, the progression of psychosis risk symptoms, and HPA axis functioning.

The results of the current study suggest that examining sex differences may help resolve apparent inconsistent results involving HPA axis function in UHR individuals and the relationship to symptoms. The accumulation of research depicting sex differences in patients with schizophrenia and UHR individuals is consistent with the current findings, and with recent evidence that suggests that sex differences may play an important role in the exacerbation of schizophrenia spectrum disorders due to the reciprocal relationship between gonadal hormones, neurodevelopmental and psychosocial environment (Leung and Chue, 2000; Walder et al., 2013). Numerous sex differences have been observed in patients with schizophrenia – male patients exhibit a higher rate of treated incidences of psychosis (Aleman et al., 2003), earlier age of onset (Angermeyer and Kühnz, 1988), poorer pre-morbid functioning (Thorup et al., 2007), longer hospital stays, more negative symptoms, and poorer treatment response and long-term outcome (Angermeyer et al., 1990; Szymanski et al., 1995). Similar sex differences have also been observed in UHR individuals. For example, when compared to UHR females, male UHR individuals have higher levels of negative symptoms (Corcoran et al., 2011; Rietschel et al., 2015), worse social and role functioning compared to female UHR youth (Rietschel et al., 2015; Walder et al., 2013), and poorer cognitive functioning (Fusar-Poli et al., 2012). Additionally, sex differences might be an important consideration for early identification and intervention for psychosis risk as positive prodromal symptoms in male but not female UHR individuals significantly predict conversion to psychosis (Walder et al., 2013). Upcoming studies should examine the relationship between HPA functioning and a broad range of sex differences to further understand the mechanisms of these relationships.

Future research examining neurobiological sex differences in UHR individuals and mechanistic properties should specifically consider exploring neurochemical processes and brain regions involved in HPA axis functioning. The release of cortisol from the HPA system

has downstream effects on both glutamate and dopamine (Wand et al., 2007; Weiss, 2007), and high levels of these neurotransmitters can have negative effects on hippocampal structure and function (Corcoran et al., 2003), and can play important roles in psychosis and stress response systems (Soliman et al., 2008; Stone et al., 2007). Specifically, smaller hippocampal volume is associated with abnormal regulation of the HPA axis and is a common finding in patients with schizophrenia (Sapolsky, 2000; Steen et al., 2006), and sensitized dopaminergic response to stress has also been observed in patients with schizophrenia (Mizrahi et al., 2012). Interestingly, sex differences in hippocampal structure and function have been observed in patients with schizophrenia; male patients with schizophrenia have significantly smaller hippocampal volume than female patients, and smaller hippocampal volume is significantly associated with a lower cortisol awakening response in only male patients (Pruessner et al., 2015). Hippocampal shape abnormalities also predict symptom progression in UHR individuals (Dean et al., 2015); however, it remains unclear whether there are shape sex differences present in UHR individuals. Our results document the first observation of sex difference in HPA activity in UHR individuals and the results speak to how abnormalities in endocrine function might be specific to sex and could in part be driving the pathogenesis of the disorder. Our sex by time interaction also highlights that timing during the day is important when examining HPA axis functioning and psychosis risk. The importance of timing is also supported by studies examining hippocampal volume in patients with schizophrenia, as smaller hippocampal volume is associated with blunted awakening levels (Pruessner et al., 2015) and higher day time levels (Mondelli et al., 2010b).

The current results support a neural diathesis-stress model of psychosis and suggest that there might be meaningful sex difference in the early biological vulnerability of the HPA system. Research posits that males and females might respond to stress differently, as life events are associated with more subsequent health problems in men compared to women (Kivimäki et al., 2002). Future longitudinal studies examining HPA axis functioning should also incorporate gonadal steroids, menstrual cycle phase, stress sensitivity, and social stressors to better understand the causal relationships and to help the field understand if sex differences in neuroendocrine abnormalities are in part driving the disorder. Little is known about why males' sensitivity to stress might be more expressed in HPA axis dysfunction relative to females; however, research indicates that gonadal steroids play a role in HPA functioning, sexual differentiation of the brain, and the biological stress response (Abel et al., 2010). Research suggests that UHR individuals may also be more sensitive to stressors (Pruessner et al., 2011), although no research has examined sex differences in both psychological and biological stress response systems in UHR individuals. The present study depicting sex differences in the morning cortisol of UHR individuals indicates that sex might play an important role in symptom development. Further understanding these relationships prior to the onset of psychotic disorders is particularly vital as previous work has acknowledged the need to identify sexually differentiated strategies of identifying psychosis risk and intervention (Walder et al., 2013). Through clarifying our understanding of sex differences and stress response in this critical population, we may be able to target treatment interventions more effectively based on individual presentation with specialized therapy,

such as cognitive behavioral therapy (Addington and Mancuso, 2009), and non-antipsychotic medications (Cornblatt et al., 2007).

4.1 Limitations

The present study highlights novel findings, and current results are consistent with the broader literature on sex differences and biological risk factors for developing psychosis. The current approach has several strengths including at-home cortisol collection, use of a conservative assessment of cortisol (i.e., controlling for time/diet/exercise/contraceptives), controlling for medication, using a control group, and screening for formal psychosis. However, there were also several limitations that should be noted. This is the first study conducted in a UHR population to implement cortisol collection at home outside of the lab environment at times of 0, 60, and 120 minutes post awakening. The strength of this method allowed for a naturalistic measurement of cortisol levels across the morning and at time increments that would impart ease of compliance and administration. However, these collection times did not allow for the specific measurement of the cortisol awakening response that peaks within 30–45 minutes after awakening. As noted, the cortisol awakening response is blunted in medication-free UHR individuals and specific sex differences have been observed in the cortisol awakening response in patients with schizophrenia (Day et al., 2014; Pruessner et al., 2008). Our collection times could have implications on the study's capacity to find overall group differences between the UHR and control groups observed in previous studies. The current study is the first step in identifying sex differences in morning cortisol; however, collections times did not allow for an assessment of the cortisol awakening response. Future studies with more collection times within the first hour of awakening are needed to further understand the timing of sex differences and assess if they are present during the cortisol awakening response in UHR individuals.

Additionally, cortisol abnormalities are also often attributed to neuroleptic medication as medication influences both HPA functioning and symptoms (Mondelli et al., 2010a; Sugranyes et al., 2012). While the present study had only two participants taking neuroleptic medication and no differences in morning cortisol or symptoms were observed, no definitive conclusion can be made about the influence of medication based on the current study. Further, a number of medications (outside of neuroleptic medication) may impact cortisol levels (Stalder et al., 2016). The current study did not control for medications beyond neuroleptic medication, which could have influenced the results; future studies should make sure to examine these implications further.

While causation could not be determined in the current study, this is the first step in evaluating sex differences in morning cortisol levels in the psychosis prodrome and future longitudinal studies are also needed to determine if sex differences moderate symptom and HPA dysfunction progression. Although our sample size is comparable to other studies conducted in the UHR population (Corcoran et al., 2012; Pruessner et al., 2013a), future studies with larger samples should be conducted before any definitive conclusions are made. However, it should be noted that normative sex differences have been observed in HPA axis functioning (Pruessner et al., 1997) and sex differences in our control group could have been missed due to reduced statistical power and smaller sample size. Studies with greater power,

from consortia such as the North American Prodromal Longitudinal Study (NAPLS) project (which include hundreds of UHR participants) would be necessary to replicate the non-significant blunting in the UHR males and to definitively determine relationships between abnormalities in morning cortisol and symptoms. Taken together, the present study is the first to illustrate sex difference in the morning cortisol of UHR youth and the present results are an important first step in understanding of the role of sex differences in early serious mental illness.

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Highlights

- Morning cortisol was examined in youth at ultra high-risk (UHR) for psychosis.
- The cortisol in UHR youth did not differ from healthy controls.
- UHR males had significantly different cortisol levels across points compared to females.
- The sex effect was also present in the neuroleptic-free portion of the sample.

FIGURE 1. Morning Cortisol in UHRs and HC Individuals

Note: The morning cortisol in the UHR group ($N=28$) and healthy control group ($N=22$). There is no significant time by group effect ($F=.610$; $p=.514$).

FIGURE 2. Sex Differences in Morning Cortisol

Note: The morning cortisol levels in UHR men ($N=15$), and UHR women ($N=13$) and healthy control men ($N=11$), and healthy control women ($N=11$). There is a significant time by sex interaction within the UHR group ($F=7.14$; $p=.002$).

TABLE 1**Participant Demographics and Symptoms**

Group	Ultra High Risk	Healthy Control	<i>p</i>-value
<u>Sex</u>			
Male	15	11	
Female	13	11	
Total	28	22	<i>p</i> = .802
<u>Age</u>			
Mean years (SD)	18.57(2.01)	17.05(3.37)	<i>p</i> = .070
<u>Parent Education</u>			
Mean years (SD)	15.8(2.00)	16(1.89)	<i>p</i> = .619
<u>SIPS Symptoms</u>			
Positive mean (SD)	12.04(4.96)	0.45(1.10)	<i>p</i> < .001
Negative mean (SD)	9.93(7.14)	0.23(0.53)	<i>p</i> < .001
<u>BDI</u>			
Depression mean (SD)	19.75(12.62)	3.73(5.75)	<i>p</i> < .001

Note: Positive and negative symptoms reflect the total sums from domains from the Structured Interview for Prodromal Syndromes (SIPS); The Depression mean reflects the total sum from the depression domain of the Beck Depression Inventory (BDI).