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A Review of Rhythm and Responsiveness of Cortisol in Individuals with Autism Spectrum Disorders

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Abstract

Examination of the hypothalamic-pituitary-adrenal (HPA) axis via cortisol among individuals with Autism Spectrum Disorder (ASD) has been a growing area of research interest. The following review includes investigations of cortisol conducted with cohorts of individuals with ASD across the lifespan over the past four decades. In general, studies find dysregulation when examining the diurnal rhythm as a whole in lower functioning children with ASD; however, limited evidence exists for alterations in higher functioning individuals and in specific aspects of the diurnal cycle (cortisol awakening response, daily decline, variability) relative to typically developing individuals. Studies examining the responsiveness of cortisol in ASD suggest an overall sluggishness of the HPA axis in responding to physiological or physical manipulation. Hypo-responsiveness was observed in stressors that involve social evaluative threat, however, hyper-responsiveness of the HPA axis was observed in situations involving unpleasant stimuli or relatively benign social situations. A number of important considerations when conducting studies of cortisol in ASD cohorts are discussed.

Cortisol, the primary glucocorticoid in humans, is released from the adrenal cortices of the hypothalamic-pituitary-adrenal (HPA) axis. It produces a variety of effects throughout the body including influences on cardiovascular function, immunity, metabolism, and neurobiology

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Julie Lounds Taylor, Ph.D. read the extant literature, synthesized the results across the selected studies and wrote the initial draft of the manuscript.
Blythe A. Corbett, Ph.D. outlined the organization of the manuscript, guided the literature search, provided interpretation to mixed and consistent findings from the selected studies, and contributed to the final manuscript.

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(Sapolsky et al., 2000), which collectively allow optimal adaptation to changing environmental demands. However, prolonged activation can have deleterious effects as seen in chronic stress resulting in suppression of the immune system (e.g., Munck and Guyre, 1991; Derijk and Sternberg, 1994). In addition to being involved in several vital biological processes and interactions, cortisol is central to the physiological response to physical or perceived psychological stress (Hennessey and Levine, 1979; Herman and Cullinan, 1997).

The diurnal rhythm and responsiveness of cortisol has been evaluated in autism spectrum disorder (ASD), a neurodevelopmental disorder characterized by impairment in social communication, restricted interests, and repetitive behaviors (American Psychiatric Association, 2013). Presumably as a result of these challenges, individuals with ASD often experience poor adaptation to change. Therefore, examination of the HPA axis via cortisol has been a growing area of research interest. The following review includes investigations of cortisol conducted with cohorts of individuals with ASD across the lifespan over the past four decades. While we collectively refer to the population as ASD, the review will use the appropriate nomenclature of the studies (e.g., autistic disorder, Asperger syndrome) whenever possible. Historically, children who currently meet criteria for ASD would have been diagnosed with autistic disorder (significant impairment in social functioning, communication, and restricted, repetitive behavior), pervasive developmental disorder-not otherwise specified (deficits in all three domains to a lesser degree) or Asperger syndrome (deficits in social functioning and restricted behavior but an absence of delays in language) (American Psychiatric Association, 2000).

Circulating cortisol can be measured in blood, saliva or urine, and they are correlated with each other (Goodyer et al., 1996), with high agreement between blood and saliva (Kirschbaum & Hellhamer, 2000). However, there are distinctions in the extent to which it may be bound versus free, as well as the timing to detect differences in response to stressors. Free or unbound cortisol is the portion that is not bound to circulating proteins, such as corticosteroid binding globulin (CBG), and according to the Free Hormone Hypothesis, it is the biologically active fraction of cortisol that is relevant (Mendel, 1989; see Levine et al., 2007, for a critical review). Cortisol in serum is 80% bound to CBG and 10% to serum albumin (Heyns et al., 1967). Serum cortisol requires phlebotomy, which may be stressful; therefore, less invasive methods, such as salivary collection are often employed especially in children. Salivary cortisol levels reflect 70% of the serum free cortisol levels (Vining et al., 1983). Urinary cortisol is non-invasive, collected over a 24 hour period. It serves as a direct assessment of free circulating cortisol and is not impacted by factors that affect CBG levels (Newell-Price et al., 2006). However, cortisol in urine is a relatively minor proportion as it consists primarily of metabolite breakdown products. The detection of cortisol in the periphery lags by 5–20 minutes with the transfer of cortisol from plasma to saliva occurring within less than a minute (Kirschbaum & Hellhamer, 2000). Changes in urinary free cortisol levels occur with a lag of approximately 4 hours (Morineau et al., 1997). All of these methods have been employed in the study of individuals with ASD.

Rhythm of Cortisol in ASD

Overall Rhythm

Table 1 summarizes studies that have examined the rhythm of cortisol in ASD samples. The earliest studies of basal cortisol in ASD focused on examination of the overall diurnal (daily waking hours) or circadian (24-hour cycle) rhythm. The normal circadian pattern of cortisol is a sharp increase in the morning hours, with a gradual decline throughout the day until it reaches its nadir during nighttime sleep (Smyth et al., 1997); deviation from this pattern is suggestive of HPA-axis dysregulation. In contrast to other studies (described later) that have focused on specific aspects of this pattern (e.g., cortisol awakening response, daily decline, variability), these studies examined global regulation or dysregulation.

Using plasma cortisol collected at 6-hour intervals from children with autistic disorder, Yamazaki and colleagues (1975) found that only two of seven children in their sample had the expected diurnal pattern. In another earlier study, Hill and colleagues (1977) reported greater abnormality in circadian patterns of cortisol, assayed from plasma collected over 24 hours, for children with autistic disorder relative to typically developing (TD) children. Similarly, Hoshino and colleagues (1987) reported that compared to TD adults (all of whom showed a normal diurnal rhythm) and children (of whom 96% showed a normal diurnal rhythm), children with ASD were more likely to have abnormalities in the circadian pattern of cortisol – particularly those children whom they defined as lower functioning. More recent studies of children and adolescents with autistic disorder (Corbett et al., 2009; Richdale and Prior, 1992) also suggest greater circadian dysregularity in ASD groups relative to age-matched TD controls.

There has only been one study that measured overall level of circulating cortisol for individuals with ASD. Using 24-hour urine, Marinovic-Curin and colleagues (2008) found no significant differences in total daily cortisol secretion between their sample of children/adolescents with autistic disorder, and age-matched TD controls. Thus, although patterns of diurnal rhythm appear to be abnormal for individuals with ASD, this study suggests that overall cortisol output in the system is similar to controls.

More recent investigations of basal cortisol in individuals with ASD have focused on specific aspects of the diurnal cycle, namely, the cortisol awakening response (CAR), the daily decline, and variability both within-person and within-group. Findings related to each of these aspects of the diurnal cycle are described below.

Cortisol Awakening Response (CAR)

The CAR is a sharp increase of cortisol approximately 30-minutes after waking that is distinct from the normal circadian rhythm and occurs in roughly 77% of individuals (Pruessner et al., 1997; Wust et al., 2000). It is postulated that the CAR is a measure of the reactive capacity of the HPA axis (Schmidt-Reinwald et al., 1999) and may serve a preparatory role in assisting the organism to prepare the body for upcoming challenges during the day (Fries et al., 2009). Developmental factors may influence the presence and magnitude of the CAR, which has resulted in some investigators establishing different child and adult criteria (Gunnar et al., 2009b; Kudielka and Kirschbaum, 2003). In adults, the CAR has been defined as a significant

rise in cortisol of 2.49 nmol or greater (Wust et al., 2000). Since children often do not reach this level, any rise in cortisol suggests the presence of a CAR (Rosmalen et al., 2005).

To date, only a few investigations have been conducted to assess the frequency and magnitude of the CAR in children and adolescents with ASD. These studies are described in Table 1. Brosnan and colleagues examined the frequency of the CAR in a group of 20 adolescent males 11–16 years with Asperger Syndrome (AS) from an institutional setting compared to 18 TD youth from the community (Brosnan et al., 2009). Using the child criterion of any rise in cortisol, the results showed that frequencies of participants who had a CAR were comparable between the TD and AS group. However, using the *adult* criterion, the AS group was less likely to have a CAR (Brosnan et al., 2009). These investigators also found that the magnitude of the CAR was greater for the TD group relative to the AS group. Zinke and colleagues (2010) investigated the CAR in 15 children between 6 to 12 years of age with high-functioning autism compared to 25 TD children. There were no differences in the frequency of the CAR between children with and without autism whether using the child or adult criterion. Recently, Corbett and Schupp (2014) investigated the CAR in a large sample of 94 pre-pubertal male children 8-to-12 years of age with ASD (n=46) and TD (n=48). Again, the results showed no significant differences on the CAR between the groups. Moreover, there were no significant differences in the proportion of children exhibiting a CAR across the groups regardless of whether using the child or adult criterion. Finally, although not examining CAR directly, Marinovic-Curin and colleagues (2008) compared cortisol values at waking and 30 minutes after waking for 9 children and youth with ASD (mean age = 11.9) to age-matched TD controls. No significant group differences emerged.

A number of other investigations (see Table 1) have examined morning cortisol levels for children through adults with ASD, using saliva (Corbett et al., 2006; Corbett et al., 2008; Kidd et al., 2012), plasma (Ćurin et al., 2003; Hamza et al., 2010; Nir et al., 1995; Sandman et al., 1991; Strous et al., 2005; Tani et al., 2005; Tordjman et al., 1997), and urine (Richdale and Prior, 1992). The majority of these investigations found no differences in morning values between individuals with ASD and typically developing controls, with the exception of two studies (Ćurin et al., 2003; Hamza et al., 2010) finding lower levels of cortisol in individuals with ASD. In addition, Corbett and colleagues (2008) showed a gradual decline in cortisol when measured over six days, which was hypothesized to reflect a possible sensitivity to changes in environmental factors (zeitgebers) that can impact cortisol secretion. Thus, although there is some evidence for a possible hypo-activation in the HPA axis during the morning for individuals with ASD (Brosnan et al., 2009; Ćurin et al., 2003; Hamza et al., 2010), the majority of studies find no irregularities.

Daily Decline

After the peak of the CAR, cortisol declines across the day until it reaches its nadir during sleeping hours (Smyth et al., 1997). Failure to deactivate the HPA axis in the evening, as evidenced by higher evening cortisol values or a flatter slope across the day, indicates difficulties in disengaging from stressors or external demands (Sapolsky et al., 1986; Seltzer et al., 2009).

A few studies (presented in Table 1) have examined the rate of cortisol decline over the course of a day for individuals with ASD. In their study of adolescents with AS, Brosnan and colleagues (2009) found no significant differences between these adolescents and TD controls in the rate of decline from their first morning sample to evening sample, nor in their evening cortisol values. Alternatively, Corbett and colleagues (2009) compared the peak-to-trough (within 30 minutes of waking to within 30 minutes of bedtime) ratio of cortisol values for children with autistic disorder ages 6 to 12 years to aged-matched, TD children. Results suggested that children with ASD had a flatter slope of cortisol over the day relative to controls. A similar pattern (higher evening cortisol values resulting in a flatter slope for the autism sample) was also observed in an earlier study by Corbett and colleagues (2006), however the sample size was considerably smaller (12 children with ASD compared to 22 in the later paper) and thereby the differences between the autism and TD groups did not reach statistical significance.

Other studies (see Table 1) have examined cortisol values measured during discrete periods of time including in the morning, afternoon, evening, or just before bed for individuals with ASD (Corbett et al., 2008; Kidd et al., 2012; Marinović-Ćurin et al., 2008; Nir et al., 1995; Richdale and Prior, 1992; Sandman et al., 1991). The majority of these studies did not find differences between individuals with ASD and TD controls in indicators of afternoon, evening, or bedtime cortisol levels. The exception to this pattern has been found in a few studies by Corbett and colleagues, one of which found that pre-pubertal children with autistic disorder had similar morning cortisol levels relative to TD controls, but higher evening cortisol values (Corbett et al., 2008). Moreover, in a follow-up study, higher evening cortisol levels were correlated with poor response to changes throughout the day (Corbett et al., 2009). Thus, afternoon cortisol secretion in ASD children and adults appears comparable to control groups; however, evening levels may be elevated in some children with autism perhaps consequent to daily challenges.

While depression was not directly examined in the aforementioned studies, lower evening cortisol levels have also been reported in youth (Angold, 2003; Dahl et al., 1991; Van den Bergh, et al., 2008) and adults (Engert et al., 2011; Muhtz et al., 2009) with symptoms of depression. Importantly, depression is a common comorbid condition in persons with ASD (Simonoff et al., 2008) suggesting that future studies should explore a possible association between the cortisol profile and depression symptomology.

Intra-individual and Inter-individual Variability

Another important approach for examining the regulation of the HPA axis is to measure the stability of cortisol levels. Across several studies there is clear evidence for greater intra-individual (i.e., within-person) and inter-individual (i.e., between-person, within-group) variability in ASD samples relative to TD controls. Four studies, described in Table 1, have examined variability in basal cortisol (Corbett et al., 2006; Corbett et al., 2008; Corbett et al., 2009; Kidd et al., 2012); three of these studies focused on pre-pubertal children with autistic disorder ages 6 to 12 years, and one study focused on younger children with autistic disorder ages 2 to 5 years. All three studies that examined intra-individual variability found that children with ASD (both in early childhood and middle childhood) had greater day-to-day variability in their diurnal rhythm than TD controls (Corbett et al., 2008; Corbett et al., 2009; Kidd et al.,

2012). All of the studies focused on older children found significantly greater inter-individual variability in the ASD group relative to age-matched, TD controls (Corbett et al., 2006; Corbett et al., 2008; Corbett et al., 2009). The study focused on younger children did not find significant differences in inter-individual variability between groups; however, the control group was not matched to the ASD group in age, and was slightly younger than the ASD group with greater age variability (Kidd et al., 2012). Thus, there is evidence to suggest that children with ASD experience greater day-to-day variability in basal cortisol relative to TD children, as well as greater within-group variability for school-aged children. At this point, it is unclear whether there is more inter-individual variability in basal cortisol among younger children with ASD.

Given that differences exist in aspects of the diurnal rhythm in persons with ASD, it is reasonable to suspect that distinctions in cortisol response to stimuli would emerge.

Responsiveness of Cortisol in ASD

A number of studies have examined how the HPA axis, and cortisol specifically, responds to acute stressors among individuals with ASD. These studies are summarized in Table 2. For the current review, the stressors have been divided into three types. The first category of stressors focuses on basic regulatory processes. Next, we discuss environmental stressors defined as those that occur in the environment but without an explicit social component (e.g., rigorous physical exercise, medical procedures). Lastly, we summarize studies that have examined the effects of social or psychosocial stressors. As described below, the cortisol response to each of these types of stressors tells us something unique about the responsivity of the HPA axis among individuals with ASD.

Basic Regulatory Processes

Some of the earliest studies of cortisol response to an acute stressor among individuals with ASD focused on the basic regulatory processes of the HPA axis. More specifically, these studies examined cortisol response to biological manipulation, such as to the administration of substances that stimulate or inhibit the release of cortisol. The two major types of stressors in this category that have been examined in ASD samples are the adrenocorticotropic hormone (ACTH) stimulation test and the dexamethasone suppression test (DST). Studies of basic regulatory processes of the HPA axis in ASD are presented in Table 2.

The ACTH stimulation test measures the strength and speed of the HPA axis in responding to stimulation. In this procedure, synthetic ACTH is injected either intravenously or intramuscularly. Because ACTH is a hormone that stimulates the adrenal glands to release cortisol, the expected result from the ACTH test is a rise in cortisol. Alternatively, the DST measures how quickly and to what extent the HPA axis is able to “shut off” the stress response. When dexamethasone is administered in the DST, it binds to glucocorticoid receptors in the pituitary gland, which suppresses the secretion of ACTH and subsequently of cortisol. Thus, the normative response to the DST is suppression of a cortisol response. When cortisol suppression does not occur, this is interpreted as an abnormality in the feedback mechanism of the HPA axis (Hoshino et al., 1987; Kalin et al., 1981).

Overall, studies examining basic regulatory processes have found that the HPA axis tends to respond and recover slower among individuals with ASD. Relative to age-matched TD controls, lower cortisol concentrations have been observed among participants with autistic disorder at 60 minutes (Hamza et al., 2010) and 90 minutes (Marinović-Ćurin et al., 2008) after administration of ACTH, but no differences were observed between groups by 120 minutes after administration (Marinović-Ćurin et al., 2008). This suggests that cortisol response to ACTH stimulation was sluggish, in that it was initially low among individuals with autistic disorder, but eventually caught up to controls.

Studies have also found evidence of slower response of the feedback mechanism that terminates cortisol release among children and adolescents with autistic disorder. Jensen and colleagues (1985), for example, found that 85% of their participants (11 out of 13) did *not* show the expected cortisol suppression effect after the DST. Similarly, Hoshino and colleagues (1984; 1987) found that relatively few children and adolescents with autistic disorder exhibited cortisol suppression after the DST, with lack of suppression particularly pronounced for those who were lower functioning.

Thus, although there have been few studies that have examined the basic regulatory processes of the HPA axis by direct manipulation using cortisol as a marker, findings are relatively consistent in showing a delayed cortisol response. The lower initial rates of cortisol release in response to ACTH stimulation, which “catch up” as time passes, suggest that the HPA axis might be slower to respond to stress among some individuals with autistic disorder. Higher rates of non-suppression of cortisol in the DST suggest delays in the “feedback mechanism” of the HPA axis, which returns the system to basal levels.

Non-Social Environmental Stressors

The next category of stressors includes those that occur external to the person with ASD and are not designed to have a social component. Because individuals with ASD exhibit both sensory hyper-responsiveness and hypo-responsiveness to their environments (American Psychiatric Association, 2013; Ben-Sasson et al., 2009), these studies allow for the examination of whether perceived reactivity to a stressor might alter HPA axis functioning beyond dysregulation in basic processes. Examples include activity stressors (bike ride) and exposure to medical procedures (mock MRI scan, blood draw). Cognitive/attention tasks also fit into this category of stressor, however the one study to use this type of task in an ASD sample did not elicit HPA axis activation and thus is not discussed (Jansen et al., 1999). Stressors in this category might be interpreted as having a social component; however, they differ from ones that will be described in the next section in that they do not explicitly manipulate the social environment. Studies examining non-social environmental stressors in ASD cohorts are summarized in Table 2.

Depending on the type of stressor, evidence for both hypo- and hyper-responsivity of the HPA axis was found for individuals with ASD. Two studies examined the impact of vigorous physical activity (riding a stationary bike) on cortisol response (Jansen et al., 1999; Jansen et al., 2003) for children with ASD who primarily had a diagnosis of Pervasive Developmental Disorder – Not Otherwise Specified (PDD-NOS). Although both of these studies found an overall rise in cortisol after the stationary bike ride, the earlier study (Jansen et al., 1999) found

that children with PDD-NOS had a lower rise than comparison groups. The later study (Jansen et al., 2003) found no difference in cortisol response between the ASD (one-half of whom had autistic disorder and other half with PDD-NOS) and a TD control group. Thus, there was some limited evidence for hypo-responsiveness of the HPA axis to physical activity among children with ASD. These studies provide further evidence for abnormality in the basic regulatory processes of the HPA axis among these children.

The opposite pattern, hyper-reactivity, was found when examining HPA axis response to medical procedures that might have a potentially threatening or unpleasant component. Specifically, the mock MRI procedure is loud and involves being confined in a small, enclosed space, which might be particularly difficult for individuals with ASD as sensory sensitivities are quite common (Ben-Sasson et al., 2009). An earlier study by Corbett and colleagues (2006), found that exposure to a mock MRI resulted in a significant rise and prolonged response in cortisol for children with autistic disorder but not for TD children. Their later studies did not replicate the finding of overall group differences (Corbett et al., 2008; Corbett et al., 2009), but it is important to note that samples in the later studies included children with autistic disorder that were slightly higher functioning than in the 2006 study. Interestingly, upon re-exposure to the mock MRI, both the autism and TD comparison group showed a significant elevation in cortisol upon arrival to the laboratory, relative to their typical values for that time of day suggesting sensitization to the stressor environment (Corbett et al., 2008).

The blood draw procedure can also be considered an unpleasant stressor, as it involves some degree of pain. Similar to Corbett and colleagues' (2006) findings of HPA axis hyper-reactivity to unpleasant stimuli among children with ASD, Spratt and colleagues (2012) reported that children with autistic disorder had a higher peak in cortisol after the blood draw, relative to TD controls, as well as cortisol values that remained elevated for a longer period of time. Thus, in contrast to perhaps blunted and delayed cortisol response to direct manipulation of the HPA axis, there is evidence to suggest that cortisol response to unpleasant stimuli might be greater among children with ASD. Further, the Spratt and colleagues (2012) study suggested that the feedback mechanism that signals the end of cortisol hyper-secretion might be delayed after negative stressors, similar to what was found after direct HPA axis manipulation (i.e. the DST).

Psychosocial Stressors

The final category of stressors includes those that explicitly manipulate the psychosocial environment. Because difficulties in social functioning are a hallmark of ASD (American Psychiatric Association, 2013), these studies build on those already described by allowing for the exploration of whether manipulating the social environment results in altered HPA axis functioning for individuals with ASD. Studies examining psychosocial stressors in ASD cohorts are summarized in Table 2. The type of psychosocial stressor most commonly used among studies of individuals with ASD (6 out of 9 studies) is a public speaking task, the Trier Social Stress Test for Children (TSST-C; Buske-Kirschbaum et al., 1997), or a modification of this task. The TSST-C is a social stressor that includes all of the components identified by Dickerson and Kemeny (2004) as critical in eliciting a cortisol response in adults, namely, social-evaluative threat, unpredictability, and uncontrollability. The TSST-C consists of participants giving a prepared speech to an explicitly neutral audience of "judges", and then

performing a serial subtraction task. The subtraction task is of sufficient difficulty to cause errors among nearly all participants, and they are asked to start over if they make a mistake (Buske-Kirschbaum et al., 1997). Variations of this task used in ASD samples include asking participants to give the speech to a one-way mirror (instead of to "judges" in the room) and eliminating the subtraction task (Jansen et al., 2003; Jansen et al., 2000; Jansen et al., 2006). The remaining psychosocial stressors are based on peer interactions (3 out of 9 studies) – including playground interactions with unfamiliar peers as well as playing a game with a familiar vs. unfamiliar peer – or brief parental separation for very young children (1 study).

Of all acute stressor paradigms, the TSST-C has been shown to most consistently elicit a cortisol response among typically developing children and adolescents (Gunnar et al., 2009a). However, studies that have examined the cortisol response to public speaking tasks among children with autistic disorder or PDD-NOS tend to show either an absence of response (Corbett et al., 2012; Jansen et al., 2000; Lanni et al., 2012; Levine et al., 2012), or less of a response among children with PDD-NOS relative to TD children (Jansen et al., 2003). The one study that found similar cortisol response between an ASD and control group included adult participants (Jansen et al., 2006). Thus, it appears that individuals with ASD are less responsive to public speaking tasks designed to activate the HPA axis in childhood, but differences might abate in adulthood. It should be noted, however, that this conclusion is tenuous and in need of further support, as their has only been one, small sample study that has examined the cortisol response of adults with ASD to public speaking tasks.

Alternatively, studies that have primarily focused on interactions with others as the stressor suggest a pattern of hyper-reactivity of the HPA axis (compared to a general hypo-reactivity in public speaking tasks). Stressor paradigms focused around interactions likely have greater ecological validity than public speaking tasks, and might be especially appropriate for ASD samples given the difficulties in social interactions inherent in ASD (American Psychiatric Association, 2013). Indeed, Corbett and colleagues found that children with autistic disorder and PDD-NOS had heightened cortisol response to a playground interaction with unfamiliar peers (Corbett et al., 2012; Schupp et al., 2013). In a direct comparison to social evaluation and social interaction, children with ASD showed blunted cortisol response to the TSST-C but elevated cortisol to play with peers whereas children with TD showed the reverse pattern (Corbett et al., 2012). This effect appeared to be influenced by the older children in their 8 to 12 year old sample (Corbett et al., 2012; Corbett et al., 2010; Schupp et al., 2013); younger children with autistic disorder had little or no cortisol response to the playground interaction. These findings were particularly interesting as the playground interaction was designed to be benign, with no overtly stressful elements, yet it activated the HPA axis for many children with autistic disorder.

Further, a study of the cortisol response to brief caregiver separation among young children with ASD found that children with autistic disorder, but not those with PDD-NOS, had greater cortisol response to separation relative to controls (Naber et al., 2007). Thus, it appears that public speaking tasks may be less likely to elicit HPA activation among children with ASD relative to controls, whereas tasks that are focused on social interaction may be more likely to elicit HPA activation.

Summary of Rhythm and Responsiveness Studies

Studies examining the rhythm of cortisol in ASD generally find dysregulation in the diurnal cycle as a whole in lower functioning children (e.g., Hoshino et al., 1984). However, limited evidence exists for alterations in higher functioning individuals and specific aspects of the diurnal cycle (CAR, daily decline) relative to TD individuals. There are two exceptions to this conclusion that are worth noting. First, Corbett and colleagues (Corbett et al., 2006; Corbett et al., 2008; Corbett et al., 2009) consistently found elevated evening cortisol in their samples of pre-pubertal children with autistic disorder, which appear to be linked to reports of stress during the day (Corbett et al., 2009). Second, studies consistently find greater variability, both intra-individual and inter-individual, among ASD samples relative to TD controls (e.g., Corbett et al., 2008).

Studies examining the responsiveness of cortisol in ASD suggest an overall sluggishness of the HPA axis in responding to physiological or physical manipulation. Hypo-responsiveness was observed in tasks that involve social evaluative threat, such as the TSST-C; however, hyper-responsiveness of the HPA axis was observed in situations involving unpleasant stimuli or relatively benign social situations.

It is important to note that hypercortisol and hypocortisol secretion are symptoms of two relatively rare endocrine disorders; namely, Cushing Syndrome (e.g., Cushing, 1932; Nieman et al., 2008) and Addison's Disease (e.g., Husebye et al., 2013; Ten et al., 2001), respectively. As discussed below, while there is significant variability in the basal and stress response cortisol profiles in ASD, there is no published evidence of a link between these serious medical conditions and ASD. Individuals with ASD rarely present with physical symptoms and distinct facial and body features associated with these conditions (e.g., fragile bones and moon facies seen in Cushing syndrome). Moreover, the differences in cortisol detected in children with ASD are not of the magnitude generally observed in endocrine disorders.

Issues and Considerations in Cortisol Studies in ASD

Developmental Stage

As development is an important determinant of basal HPA axis functioning (Gunnar and Vazquez, 2006; Gunnar et al., 2009b), age of the participants is an important consideration in studies examining cortisol in ASD cohorts. The studies of basic regulatory processes, for example, included both pre- and post-pubertal individuals with ASD, and thus it is unclear to what extent differences between studies might be accounted for by developmental status. It has been shown, however, that older, pre-pubertal children with ASD have a greater stress response to peer interactions than younger, pre-pubertal children (Corbett et al., 2012; Corbett et al., 2010).

Studies using the TSST-C or similar public speaking tasks may have failed to elicit a cortisol response among children with ASD because of normative age effects. For reasons that are not well known, the TSST-C is less effective in producing a stress response for TD children under 13 years of age (Gunnar et al., 2009a). All of the studies that found no or weak cortisol response to the TSST-C or a TSST-like task involved children with ASD in this age range. It may be,

then, that public speaking tasks are more appropriate stressors for individuals with ASD who have already entered adolescence – particularly if the ASD and control groups are not matched on cognitive functioning or pubertal status. This assertion is supported by the only study to examine response to a public speaking task in adolescents or adults with ASD (Jansen et al., 2006), which was also the only study to find a similar cortisol response between the ASD group and TD controls.

Severity of Impairment

Studies suggest that HPA axis dysregulation might be affected by the severity of impairment for individuals with ASD, and this factor should be considered in future research. For example, Hoshino and colleagues (1984) found that none of the lower functioning children and adolescents with autistic disorder demonstrated cortisol suppression; however, suppression rates for the higher functioning individuals with autistic disorder did not differ from other clinical and non-clinical populations. This was confirmed in the authors' later study (Hoshino et al., 1987), in which they found that just over one-half of the higher functioning children with autistic disorder, and only 20% of the lower functioning children had the expected cortisol suppression response. Similarly, less response to ACTH stimulation for more severely impaired individuals with autistic disorder was reported by Hamza and colleagues (2010).

Studies of HPA axis response to non-social environmental stressors also provide some evidence that individuals with ASD who are more severely impaired might experience the greatest HPA axis dysregulation. The Jansen et al. (1999) study that did not exclude participants with ASD with low IQ scores found significant differences between ASD and control groups in response to physical activity, but their study that excluded children with ASD who had an intellectual disability (Jansen et al., 2003) did not find any differences. Furthermore, of the three studies conducted by Corbett and colleagues that examined the effects of the mock MRI on cortisol response, only the study that allowed ASD participants across the full range of IQ found cortisol hyper-reactivity relative to controls (Corbett et al., 2006). The other two studies excluded children whose IQ scores were below 80. Thus, although further research is clearly needed, these studies provide some preliminary evidence to suggest that individuals with ASD who have greater impairments in intellectual functioning might experience more marked hyper-reactivity to non-social environmental stressors.

Further, although many of the studies of basic regulatory processes included participants with autistic disorder who had significant cognitive limitations, the majority of studies of non-social and psychosocial stressors excluded participants with ASD who had co-occurring intellectual disability. It is therefore difficult to tell to what extent different conclusions between types of stressors might be related to differences in cognitive functioning between samples. In order to determine whether differences in HPA axis basic regulatory processes by level of functioning extend to studies of nonsocial or psychosocial stressors, it is necessary to conduct research with samples that include those with ASD both with and without intellectual disability.

Gender

Gender is another important factor that should be considered, particularly in studies examining response to psychosocial stressors. Gender differences across the lifespan are well documented

and report differential effects. For example, adult males often have higher HPA responses than female participants to psychosocial stress (Kudielka et al., 1998; Kirschbaum, et al., 1992). There is some evidence suggesting that age and gender may interact especially during the adolescent years. For example, exposure to psychosocial stress results in significantly higher cortisol in 13-year old adolescent girls, relative to boys (Gunnar et al., 2009b); however, differences are influenced by the type of stressor, pubertal stage and experience (for a review see Gunnar et al., 2009a). Moreover, gonadal steroid hormones and CBG impact the physiology of the HPA axis (Levine et al., 2007; Marceau et al., 2014; Rotenberg et al., 2012).

Examining cortisol response by gender in ASD is difficult, given that most studies include relatively small samples with a much higher proportion of males to females (Centers for Disease Control and Prevention, 2012). Some studies (e.g., Corbett et al., 2010; Lanni et al., 2012) have dealt with this by only including males in their ASD and control groups, whereas other studies have included relatively equal proportions of males to females in both groups (Jansen et al., 2006). However, studies that include primarily males in the ASD group and a greater proportion of females in the control groups (Jansen et al., 2003; Jansen et al., 2000) should be interpreted with caution, as different gender compositions between groups likely confounds differences based on diagnosis.

Comorbidities and Medication Use

Medical and psychiatric comorbidities are common among individuals with ASD – particularly in adolescence and adulthood – and can affect cortisol values. As already mentioned, mood disorders are related to hyper-activation of the HPA axis (Doane et al., 2013; Greaves-Lord et al., 2009) and are estimated to affect up to 50% of adolescents and adults with ASD (Bradley et al., 2004; Eaves and Ho, 2008; Hofvander et al., 2009). Further, psychotropic medication use is extremely common among adolescents and adults with ASD (Esbensen et al., 2009), and can significantly alter their cortisol values (Granger et al., 2009). In order to control for confounding effects of medication, many studies of cortisol in children with ASD exclude those who are on these types of medications (e.g., Corbett et al., 2008; Lanni et al., 2012). Studies that have not excluded for psychotropic medication use tend to have greater use in children with ASD than in TD controls (e.g., Jansen et al., 2000), which might skew the cortisol values used to infer a stress response.

Although excluding participants with ASD based on comorbid psychiatric disorders or psychotropic medication use might be advisable, it becomes less feasible in studies of adolescents and adults with ASD, given the high frequency of comorbid disorders and medication use. This type of exclusion also makes the findings less generalizable to the population of individuals with ASD. Thus, the presence of these medications and disorders should be carefully measured, and considered in the design of studies and in the interpretation of stress response data (for a guideline for medication use, see Granger et al., 2009).

Diagnostic Differences throughout History and Between Samples

Changing diagnostic criteria for ASD likely impacts the conclusions that can be drawn about HPA axis dysregulation. This is particularly true for studies of overall circadian dysregulation and basic regulatory processes, many of which were conducted before the widening of the

autism diagnostic criteria in DSM-IV (American Psychiatric Association, 1994). Although the later studies of basic regulatory processes (Hamza et al., 2010; Marinović-Ćurin et al., 2008) restricted their samples to children with autistic disorder (as opposed to AS or other disorders on the autism spectrum), the earlier studies likely included a narrower range of children and adolescents with autistic disorder who were more severely impaired. More recent studies tend to find nuanced and less pervasive HPA dysfunction, which might be accounted for by greater heterogeneity in the samples.

Accounting for Basal Levels and Anticipatory Stress in Responsivity Studies

Not all of the stress response studies accounted for basal differences in cortisol levels, which greatly compromises the interpretation of study findings. For example, in the Hamza et al. (2010) study, participants with autistic disorder had lower cortisol levels than controls after ACTH stimulation, however their morning basal levels were also lower than controls. Although differences in pre- to post-ACTH stimulation were not tested, the difference between the mean morning basal cortisol and mean cortisol after ACTH was actually greater for the ASD group (11.9 for autism group and 8.07 for control group). Thus, the evidence for hypo-reactivity of the HPA axis in response to ACTH stimulation was inconclusive in this study (although patterns were similar to Marinovic-Curin et al., 2008, which did not observe differences between groups in baseline cortisol). Similarly, in the same study, lower basal cortisol among those who were more severely impaired (versus less severely impaired) was not taken into account in the interpretation of response to ACTH stimulation by autism severity.

It is also important to account for the basal cortisol levels of participants upon arrival but prior to administration of the stressor, as anticipatory stress and participants' expectations can affect their cortisol response (Corbett et al., 2008). For example, Lopata and colleagues (2008) found that children with ASD who had interacted with a familiar peer in a lab setting the day before had a much higher cortisol response when interacting with an unfamiliar peer. Their cortisol response was also higher than that of a group of children with ASD who interacted with an unfamiliar peer, without having interacted with a familiar peer the previous day. The authors suggested that the children with ASD who expected to interact with someone familiar experienced a greater stress response when discovering that they would be interacting with someone they haven't met.

Similarly, Corbett and colleagues (2008) found that after children with autistic disorder had been exposed to a mock MRI scan, they had higher cortisol levels upon arrival for the second mock MRI scan. These same authors found that cortisol values upon arrival for the psychosocial stressor were higher than children's typical values at that time of day (Corbett et al., 2012). Thus, it may be important to take into account the expectations of the children with ASD, as well as any type of anticipatory stress that may be experienced – particularly if anticipatory stress is different for individuals with ASD than controls. If individuals with ASD are evidencing abnormally high cortisol levels before the stressor paradigm begins, this may limit the ability of the researcher to observe a stress response. This concern is relatively easy to address by collecting a sample of the individual's "typical" cortisol value for that time of day, via home sampling (Corbett et al., 2008; Corbett et al., 2012; Corbett et al., 2010; Lanni et al., 2012).

Sample Size

It should also be noted that many of the earlier studies of stress response among individuals with ASD had relatively small sample sizes, with a median sample size of 20 individuals with ASD (range = 9 to 50 people with ASD). Many of the studies compensated for the small sample sizes by using inclusion criteria that restricted the heterogeneity in their ASD samples (e.g., limiting the age range or only including participants who met a threshold of intellectual functioning), however, small samples limit the generalizability of each individual study, making replication of findings across samples of critical importance.

Considering Other Biomarkers

Finally, although a full discussion is beyond the scope of this review, the extent of HPA axis dysregulation among individuals with ASD can likely best be determined in the context of other biological systems and biomarkers. For example, studies by Jansen and colleagues (2003; 2006) demonstrated a normal cortisol response to a public speaking task for some participants with ASD, but without a corresponding increase in heart rate. Alternatively, Levine and colleagues (2012) found that children with ASD experienced autonomic activation to a public speaking task, but not a cortisol response. Having this additional information about autonomic activation or functioning of other regulatory systems might change the interpretation of HPA axis regulation, and provide a more complete picture of arousal and stress response.

Overall Summary

The current review provides an up-to-date summary of the rhythms and responsiveness of cortisol in individuals with ASD. Future studies can contribute to the expanding literature by investigating large, well-characterized cohorts using rigorous methodology while considering potential influential factors such as age, gender, intellectual development, and medication status. Collectively, the findings suggest that there is significant diversity in HPA axis functioning within persons with ASD resulting in subgroups and associations with symptom profile. Most individuals with ASD show normal diurnal rhythms, although significant between- and within-group variability exits (Corbett et al., 2006; Corbett et al., 2008; Kidd et al., 2012). When altered circadian rhythms are observed, it is usually in lower functioning individuals (Hill et al., 1977; Hoshino et al., 1987). Basic regulatory processes may also be slower to respond and recover in ASD, which has also been related to lower level of functioning (Hamza et al., 2010; Hoshino et al., 1984; Hoshino et al., 1987; Jensen et al., 1985). Although morning and afternoon levels of cortisol generally show no difference compared to control groups, heightened evening cortisol has been associated with poor adaptation to daily events (Corbett et al., 2008; Corbett et al., 2009). Next steps may also consider plausible associations between depression and the featural components of cortisol in persons with ASD.

Difference in stress responsivity is more commonly found in ASD such that many individuals show hyper-responsiveness to various benign and novel stimuli (Corbett et al., 2006; Spratt et al., 2012) and natural social conditions (Corbett et al, 2010; Corbett et al., 2012; Lopata et al., 2008; Naber et al., 2007). In contrast, most studies do not find social evaluative threat to activate the HPA axis in children with ASD (Jansen et al., 2000; Lanni et al., 2012; Levine et al., 2012). To explain some of the variability, a neuroendocrine spectrum model has received some support demonstrating a relationship between cortisol levels and social responsiveness (Corbett

et al., 2013; Schupp et al., 2013), and associations have been reported between heightened cortisol levels and self-reported of anxiety (Bitsika et al., 2014; Lopata et al., 2008; Simon and Corbett, 2013). Although cortisol dysregulation is not consistently found in persons with ASD, within group investigations exploring individual differences in their biobehavioral profile may help reduce the notable phenotypic heterogeneity to better inform treatment.

Cortisol is involved in many regulatory processes including immune and metabolic functioning; however, there are no known studies examining whether glucocorticoid receptors or intracellular signaling are abnormal in individuals with ASD. Therefore, an important next step is the exploration into whether cortisol dysregulation observed in a subset of children with ASD may have cascading effects on other biological systems. For example, immune abnormalities have been detected in peripheral blood in some children with ASD (e.g., Ashwood et al., 2011; Corbett et al., 2010). Moreover, future directions may include the examination of defective glucocorticoid hormone receptor signaling (Godavarthi et al., 2012) and prenatal contribution of intrauterine cortisol (Rose'meyer, 2013) in ASD.

In summary, many persons with ASD exhibit marked stress responses in otherwise benign, novel and social situations. The hyper-responsivity may contribute to increased anxiety, neophobia or even chronic stress. Due to the known deleterious effects of frequent and prolonged exposure to cortisol on mental and physical wellbeing, continued study in persons with ASD who evidence such dysregulation of the HPA axis, appears warranted.

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Table 1

Studies Examining Basal Cortisol in Autism Spectrum Disorder Cohorts

Authors	ASD sample	Comparison sample	Methods	Summary of findings	Notes
Overall Rhythm					
*Corbett et al., 2009	22 participants with autistic disorder. Age range = 6 to 12 years (M = 8.8 years).	TD children, matched on age (not matched on IQ)	salivary cortisol taken within 30 minutes of waking, in the afternoon, and within 30 minutes of bedtime. 6 days of sampling	• Children with ASD had greater dysregularity in circadian rhythm relative to TD controls	Children were excluded if IQ scores were less than 80.
Hill et al., 1977	6 participants with autistic disorder. Age range = 6 to 9 years (M = 7.8 years).	TD children, not matched on age (age range = 9 – 11 years)	plasma cortisol taken every 4 hours for 24 hours (beginning at 10 am). 1 day of sampling.	• Overall abnormalities in patterns for ASD group were observed relative to TD controls • 4 of 6 in ASD group had lower cortisol than controls at 6 am • 3 of 6 in ASD group had low levels of cortisol across waking hours, relative to controls	ASD Participants were referred to as “having autistic traits” and all were institutionalized
*Hoshino et al, 1987	22 participants with autistic disorder. Mean age = 9 years (range not given).	TD children and TD adults (not matched on IQ)	salivary cortisol taken at 8 am, 10 am, 12 pm, 4 pm, 8 pm, and 10 pm. 1 day of sampling.	• 9 of 12 (75%) in the higher-functioning ASD group had a normal diurnal rhythm • 3 of 5 (60%) in the lower-functioning ASD group had a normal diurnal rhythm • 25 of 26 (96.2%) TD children showed normal diurnal rhythm • All 6 TD adults showed normal diurnal rhythm	
*Marinovic-Curin et al., 2008	9 participants with autistic disorder. Mean age = 11.9 years (range not given).	age-matched, TD controls (not matched on IQ)	24-hour urine	• No group differences in daily cortisol secretion measured by 24-hour urine.	Level of functioning of sample not provided.
*Richdale et al., 1992	18 participants with autistic disorder. Age range = 4 to 14 years. (M = 8.3 years)	TD children, matched on age and IQ	cortisol collected from 24-hour urine (every 2 hours). 2 days of sampling.	• No significant differences between ASD group and TD controls in cortisol values at any time point (although values in ASD group appeared higher). • Older age was related to greater higher cortisol among the ASD and comparison groups • Higher IQ was related to higher cortisol in TD controls, but not ASD group • Abnormal circadian rhythms of cortisol seemed somewhat more pronounced in ASD group.	
Yamazaki et al., 1975	7 participants with autistic disorder. Age	2 children with Heller’s syndrome, ages 4 and 5	plasma cortisol taken 4–5 times over 24 hours (at 6 hour intervals)	• Overall abnormal patterns were observed in circadian cortisol • 2 of the 7 children with ASD showed patterns similar to what would be expected	

Authors	ASD sample	Comparison sample	Methods	Summary of findings	Notes
	range = 6 to 10 years.				
Cortisol Awakening Response					
*Brosnan et al., 2009	20 participants with Asperger syndrome. Age range = 11 to 16 years (M=13.3 years).	TD adolescents, matched on age (not matched on IQ)	salivary cortisol taken at waking, 30 minutes after waking, and 1 hour before sleep. 2 days of sampling	• On average, adolescents with ASD did not have a significant increase in cortisol, whereas the typically developing adolescents did. • 60% of ASD group and 72% of TD group had any increase over the morning • Using adult criteria, 28% of the TD group and 5% of the ASD group had a CAR.	All participants with ASD were "high functioning" (IQ scores not reported). Adult criteria for a CAR is a rise of 2.49 nmol and above.
*Corbett et al., 2006	12 participants with autistic disorder. Age range = 6 to 11 years (M = 8.5 years).	TD children, matched on age and gender (not matched on IQ)	salivary cortisol taken within 30 minutes of waking, in the afternoon, and within 30 minutes of bedtime. 2 days of sampling	• Both groups showed a decline from morning to afternoon. • No differences between groups in the rate of decline from morning to afternoon	
*Corbett et al., 2008	22 participants with autistic disorder. Age range = 6.5 to 12 years (M = 8.8 years).	TD children, matched on age (not matched on IQ)	salivary cortisol taken within 30 minutes of waking, in the afternoon, and within 30 minutes of bedtime. 6 days of sampling.	• No average difference between ASD and TD control group on morning values. • For the ASD group, morning values decreased over the 6 days of sampling	Children were excluded if IQ scores were less than 80. Children with ASD were slightly higher functioning than in their 2006 sample
Corbett & Schupp, 2014	35children with autistic disorder and 5 children with PDD-NOS. Age range = 8 to 12 years (M = 10.3 years)	TD children matched on age and gender (not matched on IQ)	Salivary cortisol taken at waking and 30 minutes after waking for three days	• There were no significant differences in the presence, magnitude or variability of the CAR between the groups. • 69% of TD and 61% of ASD exhibited a CAR. • Using adult criteria, 25% of the TD and 15% of ASD exhibited a CAR.	
Curin et al., 2003	36 participants with autistic disorder. Mean age = 15.6 years (SD = 9.5; range not given)	TD individuals, matched on age (not matched in IQ)	plasma cortisol collected between 8 am and 9 am.	• The ASD group had lower morning cortisol levels relative to TD controls. • Pattern was consistent when dividing sample by gender and age groups (children, adolescents, adults) • IQ did not predict cortisol levels in ASD group	Somewhat wide age range that spans pubertal stages.
*Hamza et al, 2010	50 participants with autistic disorder. Age	TD children, matched on age, sex, and pubertal stage (not matched on IQ)	plasma cortisol taken at 9 am. 1 day of sampling.	• The ASD group had lower levels of morning cortisol than the TD controls. • In ASD group, more severe autism was related to lower morning cortisol, but not IQ.	

Authors	ASD sample	Comparison sample	Methods	Summary of findings	Notes
	range = 3 to 12 years.				
*Kidd et al., 2012	26 participants with autistic disorder. Age range = 2 to 5 years (M = 3.8 years)	TD children. Not matched on age or IQ	salivary cortisol taken within 30 minutes of waking, in the afternoon, and within 30 minutes of bedtime. 6 days of sampling.	• ASD group had somewhat higher morning levels, although this wasn't statistically significant	
*Marinovic-Curin et al., 2008	9 participants with autistic disorder. Mean age = 11.9 years (range not given).	age-matched, TD controls (not matched on IQ)	salivary cortisol taken at waking, 30 minutes after waking, just before lunch, 60 minutes after lunch, at 6 pm, and just before bed. 1 day of sampling	• No significant group differences between the ASD group and TD controls in any of the circadian cortisol values.	Level of functioning of sample not provided.
*Nir et al., 1995	10 participants with autistic disorder. Age range = 16 to 30 years. (M = 23.9 years)	TD controls	plasma cortisol taken every 4 hours for 24 hours (beginning at 4 pm)	• No significant differences in cortisol values at any time point between ASD group and TD controls.	Controls were staff members at the hospital where participants were patients.
*Richdale et al., 1992	18 participants with autistic disorder. Age range = 4 to 14 years. (M = 8.3 years)	TD children, matched on age and IQ	cortisol collected from 24-hour urine (every 2 hours). 2 days of sampling.	• No significant differences in cortisol values at any time point between ASD group and TD controls (although values in ASD group appeared higher).	
*Sandman et al., 1991	8 participants with autistic disorder. Mean age = 26 years (SD = 3 years; range not given)	Institutionalized adult and TD adult groups. Unclear whether matched on age.	plasma cortisol taken at 8 am and 8 pm. 1 day of sampling	• Cortisol values for ASD group did not differ from institutionalized or TD controls at either time point	
Strous et al., 2005	15 participants with autistic disorder. Mean age = 23.6 years (SD =4.2 years; range not given)	TD adults, not matched on age or IQ	plasma cortisol taken between 8 am and 10 am. 1 day of sampling.	• No differences in cortisol levels between ASD group and TD controls	Adults were excluded if IQ scores were less than 75.
Tani et al., 2005	20 participants with Asperger syndrome.	TD adults, matched on age, gender, and IQ.	plasma cortisol taken at 8 am. 1 day of sampling.	• Cortisol values were similar between the ASD and TD control groups.	

Authors	ASD sample	Comparison sample	Methods	Summary of findings	Notes
	Mean age = 27.2 years (SD = 7.3, range not given)				
Tordjman et al., 2007	48 participants with autistic disorder. Mean age = 10.0 years (SD = 7.1, range not given)	Controls with intellectual disability (matched on IQ) and TD controls (not matched on age or IQ).	plasma cortisol taken between 9 am and 11 am. 1 day of sampling.	• Cortisol values for ASD group did not significantly differ from intellectual disability or TD controls • Cortisol values were not associated with age • A trend for higher cortisol levels in prepubertal individuals with ASD than in prepubertal controls • Cortisol values not related to autism severity or IQ	Significant age range (2 to 30 years) spanning pubertal stages
Zinke et al., 2010	9 participants with autistic disorder, 1 with Asperger syndrome, and 5 with PDD-NOS Age range = 6 to 12 years (M = 9.1 years)	TD children matched on age (not matched on IQ)	salivary cortisol taken at waking and 30 minutes after waking. 2 days of sampling	• The difference between groups in rise of cortisol from waking to 30 minutes later was not statistically significant. • 80% of ASD group and 88% of TD group had any increase over the morning. This difference was not significant • Using adult criteria, 73% of the TD group and 84% of the ASD group had a CAR. This difference was not significant.	Children were excluded if IQ scores were less than 78. Adult criteria for a CAR is a rise of 2.49 nmol and above.
Daily Decline					
*Brosnan et al., 2009	20 participants with Asperger syndrome. Age range = 11 to 16 years (M=13.3 years).	TD adolescents, matched on age (not matched on IQ)	salivary cortisol taken at waking, 30 minutes after waking, and 1 hour before sleep. 2 days of sampling	• For both groups, cortisol declined on average over the day • There were no significant differences between groups in the rate of decline, nor in the amount of cortisol in the evening sample.	All participants with ASD were “high functioning” (IQ scores not reported).
*Corbett et al., 2006	12 participants with autistic disorder. Age range = 6 to 11 years (M = 8.5 years).	TD children, matched on age and gender (not matched in IQ)	salivary cortisol taken within 30 minutes of waking, in the afternoon, and within 30 minutes of bedtime. 2 days of sampling	• Both group showed a significant decline in cortisol when comparing an average of morning and afternoon values to evening values • No differences between groups in the rate of decline from morning/afternoon to evening.	
*Corbett et al., 2008	22 participants with autistic disorder. Age range = 6.5 to 12 years (M = 8.8 years).	TD children, matched on age (not matched on IQ)	salivary cortisol taken within 30 minutes of waking, in the afternoon, and within 30 minutes of bedtime. 6 days of sampling.	• Children with ASD had similar morning levels, but higher evening cortisol values relative to TD controls	Children were excluded if IQ scores were less than 80. Children with ASD were slightly higher functioning than in their 2006 sample

Authors	ASD sample	Comparison sample	Methods	Summary of findings	Notes
*Corbett et al., 2009	22 participants with autistic disorder. Age range = 6 to 12 years (M = 8.8 years).	TD children, matched on age (not matched on IQ)	salivary cortisol taken within 30 minutes of waking, in the afternoon, and within 30 minutes of bedtime. 6 days of sampling	• Average peak (morning) to trough (evening) difference in cortisol was smaller for ASD group relative to TD controls	Children were excluded if IQ scores were less than 80.
*Kidd et al., 2012	26 participants with autistic disorder. Age range = 2 to 5 years (M = 3.8 years)	TD children. Not matched on age or IQ	salivary cortisol taken within 30 minutes of waking, in the afternoon, and within 30 minutes of bedtime. 6 days of sampling.	• Average peak (morning) cortisol appeared higher for children with ASD, but difference was not statistically significant. • Average afternoon and evening values were similar between the groups.	
*Marinovic-Curin et al., 2008	9 participants with autistic disorder. Mean age = 11.9 years (range not given).	age-matched, TD controls (not matched on IQ)	salivary cortisol taken at waking, 30 minutes after waking, just before lunch, 60 minutes after lunch, at 6 pm, and just before bed. 1 day of sampling	• No significant group differences between the ASD group and TD controls in any of the circadian cortisol values.	Level of functioning of sample not provided.
*Nir et al., 1995	10 participants with autistic disorder. Age range = 16 to 30 years. (M = 23.9 years)	TD adolescents and adults, matched on age (not matched on IQ)	plasma cortisol taken every 4 hours for 24 hours (beginning at 4 pm)	• No significant differences in cortisol values at any time point between ASD group and TD controls.	Controls were staff members at the hospital where participants were hospitalized.
*Richdale et al., 1992	18 participants with autistic disorder. Age range = 4 to 14 years. (M = 8.3 years)	TD children, matched on age and IQ	cortisol collected from 24-hour urine (every 2 hours). 2 days of sampling.	• No significant differences in cortisol values at any time point between ASD group and TD controls.	
*Sandman et al., 1991	8 participants with autistic disorder. Mean age = 26 years (SD = 3 years; range not given)	Institutionalized adult and TD adult groups. Unclear whether matched on age.	plasma cortisol taken at 8 am and 8 pm. 1 day of sampling	• Cortisol values for ASD group did not differ from institutionalized or TD controls at either time point	
Variability					

Authors	ASD sample	Comparison sample	Methods	Summary of findings	Notes
*Corbett et al., 2006	12 participants with autistic disorder. Age range = 6 to 11 years (M = 8.5 years).	TD children, matched on age and gender (not matched 0n IQ)	salivary cortisol taken within 30 minutes of waking, in the afternoon, and within 30 minutes of bedtime. 2 days of sampling	• Children with ASD had greater between-child variability in circadian rhythm relative to TD controls	
*Corbett et al., 2008	22 participants with autistic disorder. Age range = 6.5 to 12 years (M = 8.8 years).	TD children, matched on age (not matched on IQ)	salivary cortisol taken within 30 minutes of waking, in the afternoon, and within 30 minutes of bedtime. 6 days of sampling.	• Children with ASD had greater between-child and within-child variability in circadian rhythm relative to TD controls	Children were excluded if IQ scores were less than 80. Children with ASD were slightly higher functioning than in their 2006 sample
*Corbett et al., 2009	22 participants with autistic disorder. Age range = 6 to 12 years (M = 8.8 years).	TD children, matched on age (not matched on IQ)	salivary cortisol taken within 30 minutes of waking, in the afternoon, and within 30 minutes of bedtime. 6 days of sampling	• Children with ASD had greater between-child and within-child variability in circadian rhythm relative to TD controls	Children were excluded if IQ scores were less than 80.
*Kidd et al., 2012	26 participants with autistic disorder. Age range = 2 to 5 years (M = 3.8 years)	TD children. Not matched on age or IQ	salivary cortisol taken within 30 minutes of waking, in the afternoon, and within 30 minutes of bedtime. 6 days of sampling.	• Children with ASD had greater within-child variability in circadian rhythm relative to TD controls • There were no differences between groups in between-child variability	

Note. Studies marked with an asterisk appear more than once in Table 1 and/or 2. TD = typically developing; ASD = autism spectrum disorder; PDD-NOS = Pervasive Developmental Disorder – Not Otherwise Specified; CAR = Cortisol Awakening Response

Table 2

Studies Examining Cortisol Responsiveness in Autism Spectrum Disorder Cohorts

Authors	ASD sample	Comparison sample	Methods	Summary of findings	Notes
Basic Regulatory Processes					
*Hamza et al, 2010	50 participants with autistic disorder. Age range = 3 to 12 years.	age-, sex-, and pubertal-stage matched TD controls (not matched on IQ)	Plasma cortisol response to ACTH-S	• Lower cortisol values in ASD group than controls 60 minutes after ACTH-S • Cortisol response to ACTH-S in ASD group depended on level of functioning	Lower basal cortisol levels in ASD group and those who had more severe autism not accounted for.
Hoshino et al., 1984	19 participants with autistic disorder. Age range=5 to 20 years (M = 11.5 years).	4 comparison groups (TD adults, adults with schizophrenia, children with intellectual disability, children with attention deficit disorder)	plasma cortisol response to DST	• Suppression depended on level of functioning of participants	Significant age range spanning pubertal stages
*Hoshino et al, 1987	22 participants with autistic disorder. Mean age = 9 years (range not given).	TD children and TD adults (not matched on IQ)	plasma and salivary cortisol response to DST	• Suppression depended on level of functioning of participants	
Jensen et al., 1985	13 participants with autistic disorder. Age range = 2 to 17 years.	None	plasma cortisol response to DST	• 85% of participants did not show suppression	All but 1 participant had IQ of 40 or less. Large age range
*Marinovic-Curin et al., 2008	9 participants with autistic disorder. Mean age = 11.9 years (range not given).	age-matched, TD controls (not matched on IQ) for DST. TD adults for ACTH-S.	salivary cortisol response to DST and ACTH-S	• No group differences in cortisol values after DST • Lower cortisol values in the ASD group 90 minutes after ACTH-S • Differences between groups were non-significant at 120 minutes	Level of functioning of sample not provided. Comparison sample for ACTH-S was adults.
Non-Social Environmental Stressors					
*Corbett et al., 2006	12 participants with autistic disorder. Age range = 6 to 11 years (M = 8.5 years).	TD children, matched on age and gender (not matched on IQ)	salivary cortisol response to mock MRI	• Children with ASD showed elevated cortisol in response to stressor relative to controls	

Authors	ASD sample	Comparison sample	Methods	Summary of findings	Notes
*Corbett et al., 2008	22 participants with autistic disorder. Age range = 6.5 to 12 years (M = 8.8 years).	TD children, matched on age (not matched on IQ)	salivary cortisol response to mock MRI	• Children with ASD did not show elevated cortisol in response to stressor • ASD and TD groups showed elevated cortisol before second exposure to stressor (anticipatory stress) • No significant relations between IQ and cortisol	Children were excluded if IQ scores were less than 80. Children with ASD were slightly higher functioning than in their 2006 sample
*Corbett et al., 2009	22 participants with autistic disorder. Age range = 6 to 12 years (M = 8.8 years).	TD children, matched on age (not matched on IQ)	salivary cortisol response to mock MRI	• Children with ASD and TD groups did not show cortisol response to stressor • Diagnoses (ASD vs. TD) did not differentiate responders from non- responders. Nor did behavioral stress, sensory functioning, or parenting stress.	Children were excluded if IQ scores were less than 80.
Jansen et al., 1999	12 participants with PDD-NOS. Age range = 6 to 12 years.	4 comparison groups (children with dysthymia, oppositional defiant disorder/ conduct disorder, attention deficit hyperactivity disorder, or no psychiatric diagnoses)	salivary cortisol response to attention task and physical stressor (bike ride)	• Attention task did not result in rise in cortisol for ASD or any of the comparison groups • ASD and dysthymia groups had lower cortisol responses to physical stressor (smaller area under the curve) than TD controls	Level of functioning of sample not provided.
*Jansen et al., 2003	10 participants with PDD-NOS (all with MCDD) and 10 with autistic disorder. Mean age = 9.4 years (range not given).	TD children, matched on age (no information about IQ in comparison group)	salivary cortisol response to physical stressor (bike ride)	• Cortisol values rose for all groups after the physical stressor • There were no differences in cortisol response by diagnostic group	Children with intellectual disability were excluded.
Spratt et al., 2012	20 participants with autistic disorder. Age range = 3 to 10 years (M = 7.0 years)	TD children who were younger on average	salivary cortisol response to blood draw	• Both groups had elevated cortisol in response to blood draw • ASD group had a higher peak in cortisol and elevated cortisol lasted longer after blood draw than the comparison group • Area under the curve in response to blood draw was greater for ASD group • Cortisol values were not correlated with autism symptoms	Children with IQ scores less than 55 were excluded. No other information on level of cognitive functioning.
Psychosocial Stressors					
Corbett et al., 2010	21 participants with autistic disorder. Age range = 8 to 12 years (M = 10.0 years)	TD children, matched on age (not matched on IQ)	salivary cortisol response to playground interaction	• On average, no cortisol response to stressor for control group • In ASD group, older children showed cortisol response to stressor; younger children did not.	Children with IQ scores less than 75 were excluded.

Authors	ASD sample	Comparison sample	Methods	Summary of findings	Notes
				• Autism severity did not predict cortisol response	
Corbett et al., 2012	27 participants with autistic disorder (n = 22) or PDD-NOS (n = 5) Age range = 8 to 12 years (M = 10.1 years)	TD children matched on age (not matched on IQ)	salivary cortisol response to TSST and playground interaction	• TSST did not elicit a cortisol response from ASD group, but did elicit a response from the control group. • ASD group had prolonged cortisol reaction to playground interaction, but control group did not • ASD group had greater variability than control group • Age (but not IQ) was related to higher cortisol response to playground interaction in ASD group	Analyses examining responses to both playground and TSST included 14 children with ASD and 8 controls. Children with IQ scores less than 75 were excluded.
Jansen et al., 2000	10 participants with PDD-NOS (all with MCDD), Mean age = 9.8 years. (range not given)	TD children matched on age (no information about IQ in comparison group)	salivary cortisol response to TSST-like task	• Stressor resulted in significant increase in cortisol for controls, but not for ASD group. • Difference in cortisol response between control and ASD group was statistically significant • Antipsychotic medication use was not related to cortisol response	IQ range of ASD group was 69–110. 6 participants in ASD group were on antipsychotic medications
*Jansen et al., 2003	10 participants with PDD-NOS (all with MCDD), Mean age = 9.9 years. 10 participants with autistic disorder, Mean age =9.4 years. (ranges not given).	TD children, matched on age (no information about IQ in comparison group)	salivary cortisol response to TSST-like task	• Children with PDD-NOS had less cortisol response to the stressor than controls. Children with autistic disorder did not differ from controls in response to stressor (both experienced a stress response on average) • Children with autistic disorder had higher cortisol than controls during the control test • More communication impairments in the ASD group were related to greater cortisol response to stressor. IQ was not related to cortisol response.	Children with intellectual disability were excluded.
Jansen et al., 2006	7 participants with autistic disorder and 3 with Asperger syndrome, Mean age = 21.8 years (range not given)	TD adults, matched on age and IQ	salivary cortisol response to TSST-like task	• Both ASD and control groups showed cortisol response to stressor • No significant differences in cortisol response between ASD and control groups	Adults with IQ scores less than 70 were excluded
Lanni et al., 2012	15 participants with autistic disorder Age range = 8 to 12 years (M = 9.9 years)	TD children matched on age (not matched on IQ)	salivary cortisol response to TSST	• Cortisol response to stressor did not significantly differ between groups • However, a one-sample t-test showed a significant response for controls, but not for the children with ASD • Verbal ability did not predict cortisol response to the TSST	Children with IQ scores less than 70 were excluded.

Authors	ASD sample	Comparison sample	Methods	Summary of findings	Notes
Levine et al., 2012	8 participants with PDD-NOS, 9 with Asperger's disorder, and 2 with autistic disorder (total n = 19) Age range = 8 to 12 years (M = 9.7 years)	TD children matched on age. IQ was higher in comparison group but not significantly so.	salivary cortisol response to TSST	• No significant differences between groups in any of the cortisol samples • ASD group did not have cortisol response to stressor • Comparison group had greater change in cortisol in response to stressor than ASD group	Some children in the control group were siblings of the children with ASD. Children with IQ scores less than 70 were excluded.
Lopata et al., 2008	33 children with ASD. Age range = 6 to 13 years (M = 9.8 years)	No control group (within-subjects design)	salivary cortisol response to familiar vs. unfamiliar peer	• Those who played with a familiar peer first had lower cortisol when exposed to the familiar peer, and higher cortisol when exposed to the unfamiliar peer • Children who played with the unfamiliar peer first had similar cortisol levels when exposed second to the familiar peer • Children had a greater stress response to the unfamiliar peer after they had played with the familiar peer. • Moderate relations between self- reported stress and cortisol	Children with IQ scores less than 70 were excluded.
Naber et al., 2007	20 children with autistic disorder and 14 children with PDD-NOS. Mean age = 29.8 months	12 children with intellectual disability, 16 children with language disorder, 18 TD children. Control groups of similar age	salivary cortisol response to Strange Situation	• Children with autistic disorder had greater cortisol response to caregiver separation (stressor) relative to TD control group • More autistic symptoms predicted less cortisol response to stressor.	
Schupp et al., 2013	21 children with autistic disorder and 5 children with PDD-NOS. Age range = 8 to 12 years (M = 10.2 years)	TD children, matched on age and gender (not matched on IQ)	salivary cortisol response to playground interaction	• 38.5% of children with ASD had a cortisol response to the stressor, compared to 19.2% of the control group • In ASD group, older children showed greater cortisol response to stressor relative to younger children	

Note. Studies marked with an asterisk appear more than once in Table 1 and/or 2. ACTH-S = ACTH Stimulation Test; DST = Dexamethasone Suppression Test; TD = typically developing; ASD = autism spectrum disorder; PDD-NOS = Pervasive Developmental Disorder – Not Otherwise Specified; MCDD = Multiple Complex Developmental Disorder; TSST = Trier Social Stress Test