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Review

Is afternoon cortisol more reliable than waking cortisol in association studies of children with an ASD?

Christopher F. Sharpley [a,b,*], Vicki Bitsika [a], Nicholas M. Andronicos [b], Linda L. Agnew [b]

[a] Centre for Autism Spectrum Disorders, Bond University, Robina, Queensland, Australia
[b] Brain-Behaviour Research Group, University of New England, Armidale, New South Wales, Australia

HIGHLIGHTS

- In children with ASD, cortisol is an indicator of stress and anxiety.
- Waking samples may be influenced by HPA-axis irregularities.
- Early data suggest that afternoon samples have reduced irregularities.
- Afternoon cortisol may provide a more reliable index of stress in ASD children.

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ABSTRACT

Salivary cortisol may be used as a biomarker of stress and anxiety in children with an Autism Spectrum Disorder (ASD) and is particularly valuable in studies of the association between stress-related cortisol concentrations and other factors such as comorbid disorders or aspects of the ASD phenotype. Although protocols for the collection of cortisol shortly after waking are often based on the assumption of the presence of a diurnal rhythm in cortisol, that rhythm may not be as reliable in children with an ASD as in non-ASD children. Alternatively, collecting cortisol during the afternoon may represent a more reliable procedure with less inter-participant variability.

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Autism Spectrum Disorder (ASD) is a neurological disorder that is also often comorbid with elevated stress [20] and anxiety [75]. For example, some recent studies indicate that children with an ASD have a prevalence of between 20.9% [5] and 84% [34] for Generalised Anxiety Disorder (GAD), well over the rates of between 0.6% and 7.1% for non-ASD children [21]. Anxiety detracts from the child with an ASD's ability to carry out a range of tasks [55], with effects that can be so intense as to confound accurate diagnosis of the severity and nature of the ASD experienced by particular individuals and thereby confuse treatment planning [73]. In addition to these effects, high levels of anxiety may

* Corresponding author at: Brain-Behaviour Research Group, University of New England, Armidale, New South Wales 2351, Australia.
E-mail address: csharpl3@une.edu.au (C.F. Sharpley).

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combine with the core symptoms of ASD to significantly impair the ability of these children to interact with others and maintain their educational progression, thus influencing their lifetime goals and achievements [69]. However, due to the difficulties in social communication that characterise ASD [2], the precise degree of stress and anxiety which these children experience can sometimes be obscured. Therefore, it is not surprising that a good deal of attention has been focussed upon the use of biological indicators of overall autonomic arousal as a means of determining the severity and correlates of stress in these children. One of those biological indicators is cortisol.

Cortisol concentrations in blood, saliva and urine are an outcome of several upstream actions in the Hypothalamus–Pituitary–Adrenal (HPA) axis during the early hours of the morning, usually prior to awakening [76] and initiated by the circadian clock within the hypothalamus which then secretes Corticotrophin-Releasing Factor (CRF) to the pituitary, which in turn releases Adrenocorticotropin Hormone (ACTH), stimulating the production and secretion of cortisol from the adrenal cortex into the bloodstream (and thence to saliva and urine) [15]. This flow of cortisol is often observed to reach its maximum concentration in saliva about 30–45 min after waking and then gradually decrease until evening [76], a change that is referred to as the 'diurnal rhythm' (DR) in cortisol.

The presence of severe or chronic stressors can lead to prolonged elevated levels of cortisol or *hypercortisolaemia*, which has been associated with fibromyalgia, over-activation and then depressed activation of the immune system, susceptibility to stress, pain and fatigue [28], muscle wastage and hyperglycaemia [4], increased serum lipids, endothelial damage and resultant increases in coronary heart disease [45,66] and acute respiratory failure [40]. Hypercortisolaemia may also alter the structure and function of brain regions [74] which may contribute to the development of anxiety, depression [30,37,80] and other psychiatric conditions [60].

Prolonged hypercortisolaemia can also induce *hypocortisolaemia* via the feedback process in the hypothalamus that monitors serum cortisol and adjusts production of CRF. In addition, the adrenal cortex may become gradually less sensitive to stimulation from ACTH if that prohormone is consistently elevated [28]. Hypocortisolaemia can lead to physical diseases which have concomitant psychopathological states. About one-quarter of patients with stress-related disorders such as chronic pain, fibromyalgia, irritable bowel syndrome, post-traumatic stress disorder and low back pain also suffer from hypocortisolaemia [28]. Hypocortisolaemia may also have negative effects upon overall health by inhibiting the negative feedback effect of cortisol on catecholamine synthesis and secretion and by over-activating the immune system due to the reduced anti-inflammatory effects of cortisol [28,57].

Thus, as well as cortisol being useful as a biomarker of anxiety, hyper- and hypocortisolaemia are associated with adverse consequences that are relevant to studies of overall health. It is of note that both hypercortisolaemia [32,50] and hypocortisolaemia [8] have been significantly associated with chronic stress and clinical anxiety in children with an ASD. The justification for studying cortisol in these children is clear.

1. Measuring cortisol in children with an ASD

Two different kinds of research studies of cortisol in children with an ASD are (i) those which measure the immediate *reaction* in cortisol concentration as an index of HPA arousal in response to laboratory- or field-based stressors, sometimes comparing those alterations in cortisol concentrations in children with an ASD to those in non-ASD children, and (ii) those which focus upon the overall level of cortisol concentrations in children with an ASD and seek to either make *comparisons* about those levels with non-ASD children, or investigate the *association* between cortisol concentrations and some other variable, such as anxiety, depression or other comorbid disorders, usually within ASD samples only. Each of these two types of study has different aims and requires different methodologies but only the latter is discussed here because the methodology for the former is relatively straight-forward. Therefore, for the purposes of this discussion, *when* to sample cortisol in association/comparison studies will be defined as referring to the time of day that saliva sampling should occur.

2. *Comparison* and *association* studies using cortisol in ASD

Because ASD is a neurological disorder with behavioural correlates that form the ASD phenotype, investigations of the neurobiology of ASD are consistent with gaining a further understanding of the disorder. In their early review of the neurochemical correlates of ASD, Lam et al. [49] noted that, because of its greater half-life than serum cortisol, salivary cortisol could be used as an indicator of chronic or prolonged stress that might be investigated to "assess the theory that some of the related behavioural disturbances (in ASD) could be due to a chronic heightened level of activation and hyperarousal" (p. 276). Since that comment, and consistent with the greater use of salivary sampling procedures that do not carry the same stressor potential as blood sampling via venepuncture, comparison of the HPA-axis profiles of children with and without an ASD has largely utilised saliva-based measures of cortisol.

Some of these studies compared the HPA-axis (cortisol) profile of children with an ASD and those without an ASD, principally focussed upon the aforementioned Diurnal Rhythm (DR) or some aspect of it. Although it has been reported that the majority of these comparison studies found no significant differences in the DR of salivary cortisol concentrations between ASD and non-ASD young people [71], that finding has not been consistent, with several studies also reporting greater between-child variability in the DR among children and adolescents with an ASD than among non-ASD children [20]. This greater variation in the DR in salivary cortisol concentrations among young people with an ASD is suggestive of some imbalance in the normal HPA-axis function, which may be related to the association between the upstream HPA-axis prohormones (principally CRF) that initiate cortisol secretion from the adrenal glands and are significantly associated with the development of anxiety and depression [3,46]. The assumption of a 'standard' DR is therefore relevant to a discussion of the appropriate timing and frequency of salivary cortisol measures in studies of the HPA-axis in children with an ASD versus those who do not have an ASD (i.e., *comparison* studies) and studies of the *association* between salivary cortisol and some of the phenotypes of ASD (e.g., ASD symptomatology, anxiety, depression).

3. Is the DR always present?

The presence of a DR is commonly assumed in the literature [76] but that assumption is often based upon data from participants without major psychiatric diagnoses or disorders. Typically, studies of the DR utilise healthy human participants who are often young adults (e.g., between 20 and 30 years of age). One such study of 31 of these non-diagnosed young adults found consistency in the serum cortisol DR from samples collected two and four weeks apart [64], and these findings have been replicated in various studies for some time (e.g., [44,56]). Some recent data have distinguished between the short-term fluctuations in cortisol and a "stable, trait-like component" of the cortisol DR ([65], p. 493), further suggestive of an underlying consistent DR in cortisol.

The data regarding the cortisol DR are more variable for participants who are not healthy young males and females. For example, women who suffer from anorexia nervosa do not exhibit the DR in their salivary cortisol [25], and even healthy men and women who undertake intense physical exercise show evidence of the salivary cortisol DR having been "abolished" ([29], p. 353). These findings suggest that it is relatively easy for the cortisol DR to become varied when participants depart from the 'normal, healthy' category.