

Evaluation of Relationships among Cortisol , Stress, Autism and Exercise

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Abstract: The aim of this study was to investigate the relationship among cortisol, stress and the exercise. Autism is a severe neurodevelopmental disorder characterized by a triad of complications. Autistic individuals display significant disturbances in language and reciprocal social interactions, combined with repetitive and stereotypic behaviors. Previously, It was reported that children with autism show significant variability in cortisol. The current investigation was planned to extend these findings by exploring plausible relationships among, cortisol, exercise, stress and autism. Cortisol values for diurnal rhythms and response to stress in children with and without autism were compared to parent-report measures of child stress. Cortisol responses in the exercise and autism were reviewed in this article.

Key words : Cortisol, stress, autism, exercise.

INTRODUCTION

Glucocorticoids are an important class of steroid hormones that modulate a diverse range of physiological effects. They are produced primarily by the adrenal cortex in response to the pituitary hormone ACTH which is in turn regulated by the hypothalamic peptide CRH. Together, these processes form the hypothalamic-pituitary-adrenal axis (HPA). While cortisol is the major glucocorticoid in humans and was first recognized for its role in glucose homeostasis, it is now known to have important anti-inflammatory and immunosuppressive effects on all tissues including the skin. As well as recent reports of cortisol synthesis within skin cells, there have been a number of studies showing that steroid hormones including cortisol can be identified in hair samples from humans. Cortisol is produced in the cortex of the adrenal glands. The lipophilic steroid hormone is released into the circulation and bound to proteins: 90 % to corticoid binding globulin (CBG) and 8 % to albumin. Only 1 –2 % of the total cortisol in the blood is free. Only this part of the cortisol in blood is active on the target cells. The mentioned facts have to be taken into account by regarding correlation studies of cortisol in blood and in saliva. At 145 – 180 ng/ml (400 – 500 nmol/l) of total cortisol plasma level the CBG is saturated. Above this concentration the percentage of free cortisol increases. Therefore the plasma level of total cortisol depends on the CBG concentration. The increased CBG level leads to an elevated cortisol plasma level, but to a normal free cortisol concentration in plasma and saliva. The CBG concentration is affected by various conditions like pregnancy, liver diseases, inflammation, polycystic ovary syndrome and application of different drugs. People can react to a stressor in different ways. For instance, if an individual perceives the stressor as a challenge to his/her control of a situation, norepinephrine, the “fight” hormone is predominantly released. And, if the stress arousal increases and a possible loss of control is felt by the individual, then epinephrine, another “flight/anxiety” hormone is released. When the stress is prolonged and seen as hopeless, the individual becomes more distressed and feels defeated. This activates the hypothalamus in the brain. What follows is a cascade of hormonal pathways resulting in the final release of cortisol from the adrenal cortex (Eek, *et.al.*, 2006; Garde, *et.al.*, 2003a;Garde, *et.al.*, 2005;Griefahn, *et.al.*, 2006;Hansen, *et.al.*, 2001; Hansen,*et.al.*, 2003;Hansen, *et.al.*,2006;Hansen, *et.al.*, 2007;Laudat, *et al.*, 1998;Lewis, 2006; McEwen, *et.al.*, 1999;Matchock, *et.al.*, 2007;Munck, *et.al.*, 2001;Paccotti,*et.al.*, 2005;Theorell,2003;Touitou, *et.al.*, 1997;Touitou, *et.al.*, 2006).

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MATERIALS AND METHODS

This study was arranged by inquiring the investigations related to the cortisol, stress, autism and exercise. The available informations were prepared from the recent references.

Autism and Cortisol:

Autism currently is a worldwide epidemic, with approximately one in 100 children affected. The most successful treatments are naturopathic, focused on treating the cause and acknowledging every part of the body. Starting at the root of this disease, we can see how the branches and leaves of autism stem into all parts of the body. Beginning with toxic deposition of heavy metals or other chemicals, the body reacts to remove the offenders. The deeper the toxins are in the tissue, the stronger the reaction of the young body. Inflammation, altered immune response from vaccinations, altered endocrine response, increased action potentials of the nervous system, increased sympathetic tone and gut inflammation are all homeodynamic responses to removing the toxins buried deep within the tissue. The more severe the autism, the more abnormal the diurnal rhythms (Blythe, *et.al.*, 2008; Del Ponte, *et.al.*, 1984; Garde, *et.al.*, 2003b; Garde, *et.al.*, 2005). The need for cortisol is so great however, that cortisol suppression is resistant to dexamethasone-suppression testing. While there is a huge cortisol dysregulation, DHEA-S and testosterone seem to be similar in children with autism and those without. The main findings of the first part of the study were that autistic children did not differ from control children in their cortisol response to psychosocial stress, while their heart rate response to this test was significantly different from controls. In addition, autistic children showed an increase in saliva cortisol levels during the control test. In contrast, Multiple Complex Developmental Disorder (MCDD) children showed both decreased heart rates and saliva cortisol responses to the psychosocial stress test, while their cortisol levels during the control test did not differ from those of controls. Autistic patients differed from control children in their psychosocial stress response in that they show normal cortisol responses in the face of decreased heart rate responses. During the actual talk, they showed a small but significant heart rate increase, indicating that they perceived the test as stressful in some way. However, they did not show an increase in heart rate in anticipation of the talk, during the preparation period, unlike normal control children (Blythe, *et.al.*, 2008; Del Ponte, *et.al.*, 1984; Garde, *et.al.*, 2003b; Garde, *et.al.*, 2005; Hansen, *et.al.*, 2001; Hansen, *et.al.*, 2006; Hansen, *et.al.*, 2007; King, *et.al.*, 2000; Lucres, *et.al.*, 2003; McEwen, *et.al.*, 1999; Matchock, *et.al.*, 2007; Theorell, 2003; Touitou, *et.al.*, 1997; Touitou, *et.al.*, 2006). The normal cortisol response, given the inability of autistic children to fully perceive the social stress situation, may be interpreted as a hyper-responsivity of the HPA system. Similarly, the increase in cortisol levels during the control test may be interpreted as an HPA hyper-response to a situation that is not perceived as stressful. Regulation of the HPA axis involves 3 interrelated processes: the maintenance of a diurnal rhythm, activation in response to stress or threat and the restoration of basal activity via negative feedback mechanisms. Cortisol is the primary glucocorticoid in humans. Cortisol exhibits diurnal variations peaking in the early morning hours (about 30 minutes after waking), declining rapidly in the morning, with a slower decrease in the afternoon, and reaching its lowest level in the evening. The HPA axis, like most biological systems, is highly regulated and dependent on the ability of the system to maintain, respond and reset itself (homeostasis). One form of dysregulation of the HPA axis is manifested by disruptions in circadian rhythms. Dysregulation of the circadian rhythms may be characterized by a change in the pattern that results in the absence, elevation or suppression of the slope. An example of this would be the flattening of the slope that has been reported in at-risk populations of children. Although the findings are not entirely consistent, some of the early work in children with autism shows alterations in the normal circadian patterns of cortisol. It has been observed no differences in the slope of the circadian decline in cortisol for children with and without autism. If circadian rhythms for autistic children are less predictable, this may represent another, less explored form of dysregulation that has been described as chaotic circadian rhythms. Such variance would not be detected in the slope; instead, it would need to be evaluated through repetitive sampling over several days and comparable times. Systemic stressors are physical, can occur independent of context and conscious awareness and usually involve a life-threatening event. Systemic stimuli that activate the HPA system are relayed to the periventricular nuclei of the hypothalamus via the brain stem. In contrast to systemic stressors, processive stimuli require the comparison of current information with past experience, are context-dependent and are assigned emotional meaning. Processive stimuli are mediated by the frontal lobes and the limbic system structures. Among the processive events that can activate the HPA axis is exposure to novelty or unpredictability. There is, however, little information on habituation and sensitization in children. Insofar as habituation appears to be yet another example of HPA regulation, we exposed a subsample of our population to a second homotypical stress experience to determine

whether children with autism would demonstrate habituation or sensitization to a relatively noninvasive stressor. Autism has often been characterized as a disorder accompanied by increased arousal, stress and sensory sensitivity. In as much as the HPA axis has been shown to reflect increased levels of arousal and stress, it is not surprising that studies have been conducted on the HPA axis and autism (Hansen, *et.al.*, 2001; Hansen, *et.al.*, 2006; Hansen, *et.al.*, 2007; King, *et.al.*, 2000; Lucres, *et.al.*, 2003; McEwen, *et.al.*, 1999; Matchock, *et.al.*, 2007; Theorell, 2003; Touitou, *et.al.*, 1997; Touitou, *et.al.*, 2006).

In the current investigation, it has been investigated the neuroendocrine activity of children with high-functioning autism in comparison with typically developing children. It has been obtained significant overall differences in the diurnal variation between the groups. The circadian pattern shown by the neurotypical children has been revealed no significant change over the 6 days of sampling. By contrast, over the course of the sampling, children with autism has showed a gradual decrease in the morning values. Although, several factors have been shown to affect morning cortisol levels, including day of the week, wake-up time and sleep difficulties. None of these factors provide an adequate explanation of the findings. All the children have been provided samples on the same days, and the change occurred on successive days regardless of day of the week. Sample collection times in the morning and evening have not been showed the same gradual change across days and have been determined by each child's own sleep pattern. Finally, there have no discernable between-group differences in sleep patterns or sleep duration. It thus appears that the standard explanations do not account for the trend for morning values to be decreased. It is possible that our methods, which included a timebound sampling regimen, provided an additional zeitgeber and, over days, altered the circadian rhythm for children in the autism group. In other words, children with autism may be more susceptible to the influence of zeitgebers, an interpretation that supports the notion of greater circadian variability and less regulated responses in children with autism. The more unexpected finding was that the evening values for the children with autism tended to be consistently elevated in comparison with the neurotypical group. The decrease over time in the peak morning values in combination with the elevation in the evening results in a diminished peak-to-trough difference. It is important to point out that, in older children and adolescents with depression, reported alterations in hormones have included hypersecretion of evening cortisol. These findings imply a greater degree of individual differences among children with autism, which may reflect the significant variability observed in many areas of metabolic, neurologic and behavioural functioning in these children. Rather it is likely mediated by a host of interrelated psychological and metabolic variables such that, on re-exposure to a stressor, cognitive and emotional factors influence responsiveness. These context-dependent progressive stimuli that have been assigned emotional meaning support the notion that novelty may not be the primary mediator of the HPA response. Still, in children it is unclear what cognitive appraisal domains may be related that contribute to or ameliorate a response to stressful situations.

Multiple Complex Developmental Disorder (MCDD) represents a distinct group within the autistic spectrum based on symptomatology. Unlike autistic children, part of MCDD children develop schizophrenia in adult life. Despite the differences, patients of both disorders are mainly characterized by abnormal reactions to their social environment. Although at a symptomatological level, autistic children indeed differed significantly from MCDD children, this could not explain the differences in cortisol response to the public speaking test. In fact, the only symptom score that was significantly correlated to the cortisol response to psychosocial stress has a higher communication score on the ADI. However, this was a positive correlation, meaning that more impairments have been related to higher cortisol levels. This is in contrast to our expectation that disturbances in social functioning would be related to diminished cortisol responses. Findings of this study are given below Figure 1.

Exercise and Cortisol:

Stress is defined as a physiologic response to events perceived as potentially or actually threatening the integrity of the body. Physical exercise is associated with increases in serum and salivary levels of cortisol. The concomitant increase in serum lactate has been implicated as 1 of the mechanisms responsible for adrenocortical activation. Interindividual differences in the lactate and cortisol responses to an acute bout of high-intensity isokinetic exercise as a function of the type and intensity of training can determine, and the relationships between lactate and cortisol production and between serum and salivary cortisol levels can measure. Responses of serum lactate and serum and salivary cortisol to an acute bout of high-intensity isokinetic exercise have been evaluated in noncompetitive (NCA) and competitive athletes (CA). The CA group has been composed of both endurance-trained and power-trained athletes. The isokinetic exercise test elicit significant cortisol and lactate responses. No apparent differences find in the lactate responses between the NCA and CA groups, but in the CA group the power-trained athletes show a higher response during and after

the exercise compared with that of the endurance-trained athletes (Bjalie, *et.al.*, 1998; Bosco, *et.al.*,2000; Clow, *et.al.*, 2004; Duclos, *et.al.*, 1998; Elizabeth, 2003; Garde, *et.al.*, 2005; Griefahn, *et.al.*, 2006; Hansen, *et.al.*, 2001; Hansen, *et.al.*,2006; Hansen, *et.al.*, 2007; McEwen, *et.al.*, 1999; Matchock, *et.al.*, 2007; Theorell,2003; Touitou, *et.al.*, 1997; Touitou, *et.al.*, 2006).

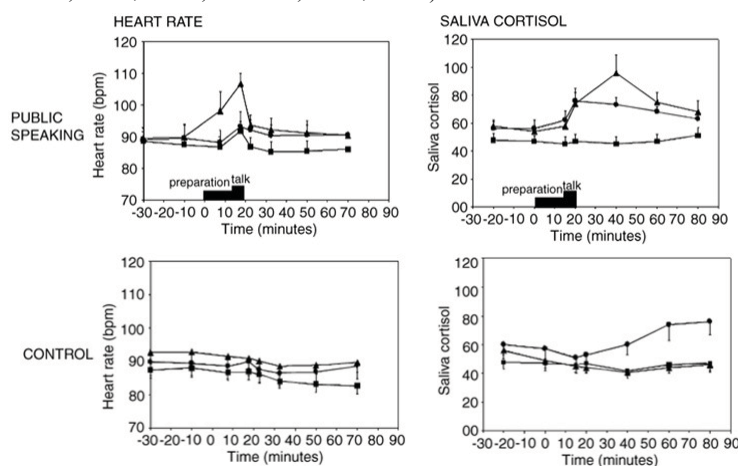


Fig. 1: Mean heart rate and saliva cortisol curves for the psychosocial stress test session and the control test session for autistic, MCDD, and control children (● - , autistic children □ - , MCDD children ■ - , control children).

Serum cortisol is higher in the CA than in the NCA group at 30 and 120 minutes after the end of the exercise, but no differential response is evident between the endurance-trained and power-trained subgroups. Salivary cortisol response is higher in the CA group than in the NCA group immediately after exercise and at 90 and 120 minutes after termination of the exercise and is higher in the power-trained athletes in comparison with the endurance-trained athletes at 60, 90, and 120 minutes after conclusion of the test. No significant correlations have not been noted between serum or salivary cortisol and lactate levels. The relationship between serum and salivary cortisol is markedly nonlinear, and the slope of the serum-saliva regression line has been detected lower for serum cortisol concentrations greater than 500 nmol/L than for serum cortisol concentrations less than 500 nmol/L. These findings support the concept that cortisol and lactate responses to high-intensity isokinetic exercise are independent (Garde, *et.al.*, 2005; Griefahn, *et.al.*, 2006; Hansen, *et.al.*, 2001; Hansen, *et.al.*,2006; Hansen,*et.al.*,2007; McEwen,*et.al.*,1999; Matchock,*et.al.*,2007; Theorell,2003; Touitou, *et.al.*, 1997; Touitou, *et.al.*, 2006).

Physical exercise is associated with increases of serum and salivary levels of cortisol. The concomitant increase in serum lactate has been implicated as one of the mechanisms responsible for adrenocortical activation. Some researches have evaluated the responses of serum lactate and serum and salivary cortisol to an acute bout of high-intensity isokinetic exercise in non-competitive and competitive athletes (NCA and CA, respectively). In this study, the latter group has been composed of endurance- and power-trained athletes (EA and PA, respectively). It has been determined interindividual differences in the lactate and cortisol responses as a function of type and intensity of training and to search for relationships both between lactate and cortisol production and between serum and salivary cortisol levels. The isokinetic exercise test elicited significant cortisol and lactate responses. No difference has been found the evident in the lactate responses between NCA and CA, but the PA has showed a higher response during and after the exercise in comparison to EA (immediately after the exercise: PA 15.0±1.5 mmol/litre and EA 11.1±2.6 mmol/litre, p<0.01). However, serum cortisol has been measured higher in the CA in comparison to the NCA group at 30 and 120 minutes after the termination of the exercise, while no differential response was evident between EA and PA groups. Salivary cortisol response has been evaluated higher in the CA group in comparison to NCA immediately after the exercise and at 90 and 120 minutes after the termination and has been higher in PA in comparison to EA at 60, 90, and 120 minutes after the termination (peak levels at 60 minutes: PA 51.2±18.5 nmol/litre, EA 27.5±20.8 nmol/litre, p<0.05). No significant correlations have been assessed between serum or salivary cortisol and lactate levels. The relationship between serum and salivary cortisol has been markedly non-linear, the slope of the serum-saliva regression line being lower for serum cortisol concentrations over 500 nmol/litre than for concentrations below that value (0.019 and 0.037, respectively, p<0.01). The interindividual differences in

cortisol changes are likely to be related to the training status and mode as well as to the correspondence between the evaluation protocol and the discipline individually performed (Bjalie, *et.al.*, 1998; Bosco, *et.al.*,2000; Clow, *et.al.*, 2004; Duclos, *et.al.*, 1998; Elizabeth, 2003; Garde, *et.al.*, 2005; Griefahn, *et.al.*, 2006; Hansen, *et.al.*, 2001; Hansen, *et.al.*,2006; Hansen, *et.al.*, 2007; McEwen, *et.al.*, 1999; Matchock, *et.al.*, 2007; Theorell,2003; Touitou, *et.al.*, 1997; Touitou, *et.al.*, 2006).

In a study, it has been determined the effects of exercise on salivary cortisol levels and the circadian rhythms of resistance-trained individuals. The subjects have been included seven resistance-trained women with the following characteristics [mean (SD)]: age 20.3(0.5) years; height 163.0 (4.0) cm; weight 59.0 (5.8) kg. The testing period has been included two test days per week, incorporating a total of two days of “rest” and two days of “exercise”. Subjects have been randomly selected to participate on the “rest” and “exercise” days. Subjects have been given a mandatory workout regimen to follow on the “exercise” days. The workout regimen consisted of the following exercises: hang pulls, bench press, leg press, upright rows, leg curls, military press, lat pulls, knee extensions, arm curls, and sit-ups. Throughout the testing period, subjects have required to keep a daily food and exercise log. Saliva samples have been collected every two hours for a maximum of 16 hours during each testing day. Results of the testing indicated that cortisol levels have increased immediately following exercise ($p < 0.05$), but has not changed significantly throughout the course of the day. This implies that while exercise have exerted an effect on salivary cortisol production, it has not been exerted a similar effect on the circadian rhythm. Saliva samples have been collected during the acute resistance exercise sessions. Samples have been subsequently stored at -79°C until analysis. Saliva sample one has been collected after 15 minutes rest, after which the acute resistance exercise protocol began on the exercise days. Sample two has been taken after the hang pull exercise has been completed, and then immediately following the completion of the protocol on the exercise days. After the exercise protocol has been completed, the subjects have been allowed to leave and participate in their normal daily activities. Subsequent samples have been taken every two hours for the entire day until 2200 hours by the researchers. Salivary cortisol concentrations have been measured in duplicate by enzyme immunoassay using a diagnostic systems laboratories salivary cortisol enzyme immunoassay kit (Agrimonti, *et.al.*,1982; Bellastella, *et.al.*, 1983, Clow, *et.al.*, 2004, Del Ponte, *et.al.*, 1984; Hansen, *et.al.*, 2001; Hansen, *et.al.*,2006, King, *et.al.*, 2000; Matchock, *et.al.*, 2007; McEwen, *et.al.*, 1999; Piccione , 2003; Refinetti, 2006; Reinberg, *et.al.*, 1978; Touitou, *et.al.*, 1997; Touitou, *et.al.*, 2006; Urban, *et.al.*, 1997; Ulrich, *et.al.*, 2004).

The results of the hourly salivary sampling during the resting and exercise days have been shown in Figure 2.

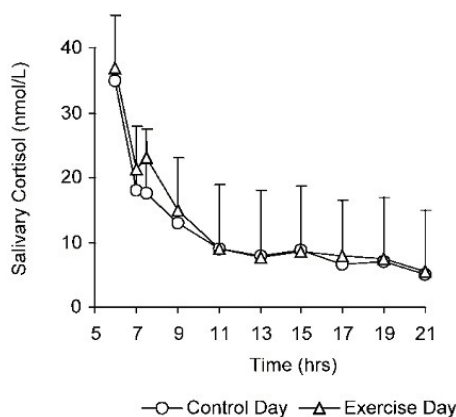


Fig. 2: Circadian rhythm of salivary cortisol (mean ± SD). A significant increase was observed post-exercise (07.30) compared to baseline ($P < 0.05$).

The major finding of this study has been that resistance training significantly increases the amount of cortisol present in saliva immediately following exercise, though it has not affect cortisol patterning of the circadian rhythm. The pattern of the cortisol response appears to be quite reproducible. This implies that while exercise have exerted an effect on salivary cortisol production, it has not exert a similar effect on the circadian rhythm (Bjalie, *et.al.*, 1998; Bosco, *et.al.*,2000; Clow, *et.al.*, 2004; Duclos, *et.al.*, 1998; Elizabeth, 2003; Garde, *et.al.*, 2005;Griefahn,*et.al.*, 2006;Hansen, *et.al.*, 2001; Hansen, *et.al.*,2006; Hansen, *et.al.*, 2007;McEwen, *et.al.*, 1999; Matchock, *et.al.*, 2007;Theorell,2003; Touitou, *et.al.*, 1997; Touitou, *et.al.*, 2006).

It appears that the introduction of exercise to the body activated a hormonal response to the stressor. Once the body had reacted to the stress, the hormonal response has been deactivated. This, in turn, implies that similar responses could have been expected of a situation in which an external stressor is applied to the body. The study reinforces this theory, as well as emphasizes the function of cortisol as a regulator of the body's response to stress. The results obtained in all days clearly have showed a circadian rhythm with the highest concentrations obtained during the morning hours and lower concentrations observed later in the day. The results of the testing have indicated that cortisol levels increased immediately following the resistance exercise ($p < 0.05$), but has not been changed significantly throughout the course of the day.

Discussion:

Cortisol is a steroid hormone produced and released by the adrenal glands. Among its important metabolic functions it exerts a pivotal role in the modulation of blood pressure and cardiovascular function, as well as in the regulation of metabolic substrates and immune system. The secretion of the hormone undergoes diurnal variation, with the highest concentrations present in the early morning, and the lowest around midnight, 3–5 h after the onset of sleep. Cortisol secretion also increases markedly in response to stress, whether physical, that is, illness, trauma, surgery, fever, physical exercise and extreme temperatures) or psychological such as clinical depression, anxiety, strain, fear and pain (Eek, *et.al.*, 2006; Garde, *et.al.*, 2003a; Garde, *et.al.*, 2005; Griefahn, *et.al.*, 2006; Hansen, *et.al.*, 2001; Hansen, *et.al.*, 2003; Hansen, *et.al.*, 2006; Hansen, *et.al.*, 2007).

Altered hypothalamic-pituitary-adrenal function associated with allergic disease has generally been thought to be secondary to the stress of chronic disease. However, recent studies suggest that altered cortisol circadian rhythm and cortisol stress hyper-responsiveness precede the inception of allergic disease and are possible links between preventive factors associated with the hygiene hypothesis and the development of allergies. Elevated endogenous cortisol responses to stressful stimuli could predispose susceptible hosts to atopy and allergic disease by biasing the developing immune system to a T helper 2-predominant immune response, greater total and allergen-specific serum immunoglobulin-E responses, and/or inhibition of peripheral immune tolerance (Lewis, 2006; McEwen, *et.al.*, 1999; Matchock, *et.al.*, 2007; Munck, *et.al.*, 2001; Paccotti, *et.al.*, 2005; Theorell, 2003; Touitou, *et.al.*, 1997; Touitou, *et.al.*, 2006).

Regulation of the HPA axis involves 3 interrelated processes: the maintenance of a diurnal rhythm, activation in response to stress or threat and the restoration of basal activity via negative feedback mechanisms. Cortisol is the primary glucocorticoid in humans. Cortisol exhibits diurnal variations peaking in the early morning hours (about 30 minutes after waking), declining rapidly in the morning, with a slower decrease in the afternoon, and reaching its lowest level in the evening. Steroid analysis in saliva was first introduced more than thirty years ago. Since then, its popularity has enormously increased due to the attractiveness of non-invasive, repeated and simple stress-free sampling, especially in the athletic field. The availability of a simple and reliable assay for assessing saliva cortisol, which is gaining increasing interest as a valuable tool to monitor performance decrements in athletes, would hence be useful for sport physicians and well tolerated by the athletes. Although a variety of limitations have been reported when measuring steroid hormones in saliva, including the collection technique, the variable matrix of saliva, the sensitivity, the stability, the presence of binding proteins and the identification of reliable reference ranges, the results of our study show a high significant correlation between morning saliva and serum cortisol concentrations, as measured with two commercial immunoassays. The high and nearly identical percentage of subjects displaying values above the 95th percentile limit of both saliva and serum cortisol, which is consistent with the high morning peak of the hormone, further attest that salivary measurement might be a suitable approach to detect hypercortisolism in this setting (Eek, *et.al.*, 2006; Garde, *et.al.*, 2003a; Garde, *et.al.*, 2005; Griefahn, *et.al.*, 2006; Hansen, *et.al.*, 2001; Hansen, *et.al.*, 2003; Hansen, *et.al.*, 2006; Hansen, *et.al.*, 2007; Laudat, *et al.*, 1998; Lewis, 2006; McEwen, *et.al.*, 1999; Matchock, *et.al.*, 2007; Munck, *et.al.*, 2001; Paccotti, *et.al.*, 2005; Theorell, 2003; Touitou, *et.al.*, 1997; Touitou, *et.al.*, 2006).

There are several technical and biological advantages that make salivary samples as an easy and suitable biological approach for monitoring cortisol in athletes. Saliva samples offer a more practical alternative in a field-based setting than collecting blood specimens. Then, collection of salivary specimens is non-invasive and well tolerated by the athletes, since it does not require a venipuncture. Finally, saliva cortisol accurately reflects the biologically active free form of cortisol, thereby providing a more reliable measure than serum cortisol when testing the adrenocortical function. To achieve performance gains, training schedules balance challenging workouts and short-term fatigue with adequate rest to enable adaptation. When exercise intensity and volume are increased too rapidly and recovery is persistently inadequate, athletes are at risk of developing overtraining syndrome (OTS), defined as fatigue and suboptimal athletic performance persisting for more than two weeks

despite complete rest. Associated findings include altered mood, increased risk of infection, and alterations in several biochemical and immunologic markers. Muscle fatigue alone does not account for the clinical findings in OTS. Appropriate hormonal modulation is essential to adaptation in training. In a study of male triathletes, overtrained athletes demonstrated an elevated cortisol/ cortisone ratio, possibly indicating a failure to inactivate increased cortisol levels following exercise. Persistently elevated cortisol may contribute to OTS by inhibiting cellular recovery pathways. Complementing this theory, exercise stimulates the release of cytokines such as IL-6, which have peripheral metabolic effects inhibiting healing, as well as central receptors in the hypothalamus, potentially contributing to the mood and motivational disturbances seen in OTS. Leptin and IGF-1, hormones with roles in signaling energy balance and regulating cell growth and repair, are also believed to be dysregulated in athletes with OTS. OTS likely represents a combination of muscle fatigue and metabolic alterations opposing repair and adaptation to physical stress. Biochemical findings in OTS include decreased hemoglobin, serum iron, and ferritin; negative nitrogen balance; increased blood urea; increased uric acid production; decreased glutamine production; low free testosterone; decreased free testosterone to cortisol ratio of more than 30%; and depletion of minerals Zn, Co, Al, Mn, Se, Cu, and other micronutrients. Attempts to use these alterations for prediction or diagnosis have not proved useful, however, due to inter-athlete variations. Immune system abnormalities include increased susceptibility to and severity of bacterial and viral infections as well as decreased functional neutrophil activity, total lymphocyte counts, and production of immunoglobulins. Another frequent finding in OTS is increased or decreased resting heart rate (Blythe, *et.al.*, 2008; Del Ponte, *et.al.*, 1984; Garde, *et.al.*, 2003b; Garde, *et.al.*, 2005; Hansen, *et.al.*, 2001; Hansen, *et.al.*, 2006; Hansen, *et.al.*, 2007). The HPA axis, like most biological systems, is highly regulated and dependent on the ability of the system to maintain, respond and reset itself (homeostasis). One form of dysregulation of the HPA axis is manifested by disruptions in circadian rhythms. Dysregulation of the circadian rhythms may be characterized by a change in the pattern that results in the absence, elevation or suppression of the slope. An example of this would be the flattening of the slope that has been reported in at-risk populations of children. Although the findings are not entirely consistent, some of the early work in children with autism shows alterations in the normal circadian patterns of cortisol. In this study, it has been observed no differences in the slope of the circadian decline in cortisol for children with and without autism. There were marked individual differences within the autism group. In summary, the finding that children with autism and typical development demonstrate an increase in endocrine activity, ostensibly in anticipation of re-exposure to a noxious event, warrants additional investigation of factors of expectancy that must be considered in developmental models of stress. Most notably, the current study reveals clear dysregulation of the circadian rhythm in autism characterized by gradual decrease over the course of the sampling in the morning and by elevated evening values. The greater within-child variation suggests clear disturbances in the limbic HPA axis that cannot be accounted for by mere between-child heterogeneity but points, rather, to fundamental dysregulation and increased susceptibility to external factors such as zeitgebers (Blythe, *et.al.*, 2008; Del Ponte, *et.al.*, 1984; Garde, *et.al.*, 2003b; Garde, *et.al.*, 2005; Hansen, *et.al.*, 2001; Hansen, *et.al.*, 2006; Hansen, *et.al.*, 2007; King, *et.al.*, 2000; Lucre, *et.al.*, 2003; McEwen, *et.al.*, 1999; Matchock, *et.al.*, 2007; Theorell, 2003; Touitou, *et.al.*, 1997; Touitou, *et.al.*, 2006).

The circadian clock, an endogenous timing system, generates biochemical, physiological and behavioural rhythms. To be useful, these clocks must be synchronized (entrained) to environmental time cues (zeitgebers). The primary environmental zeitgeber is light, and the regular daily change in light intensity at dawn or dusk seems to determine the circadian photo entrainment (Agrimonti, *et.al.*, 1982; Bellastella, *et.al.*, 1983; Clow, *et.al.*, 2004; Del Ponte, *et.al.*, 1984; Hansen, *et.al.*, 2001; Hansen, *et.al.*, 2006; King, *et.al.*, 2000; Matchock, *et.al.*, 2007; McEwen, *et.al.*, 1999; Piccione, 2003; Refinetti, 2006; Reinberg, *et.al.*, 1978; Touitou, *et.al.*, 1997; Touitou, *et.al.*, 2006; Urban, *et.al.*, 1997; Ulrich, *et.al.*, 2004). Obviously, the processes behind abnormal reactions to psychosocial stress are far more complicated and are not easily explained by differences in symptomatology or overt behavior. Instead, the difference between autistic and MCDD children in their responses to psychosocial stress may indicate that the abnormal reactions to the social environment and stress as described clinically in autism and MCDD may have different biological backgrounds. This measurement of only the end product of the biological stress response, cortisol release, is not sufficient to be able to say anything about the central mechanisms involved in the psychosocial stress response. It is, therefore, extremely difficult to find proper explanations for the presently found differences between autism and MCDD, having used a neuroendocrine strategy only. Furthermore, the study of the neuropeptides arginin-vasopressin (AVP) and oxytocin (OT) may be of interest. Both neuropeptides are involved in the regulation of the HPA response in reaction to stress, and psychological stress in particular. AVP stimulates the HPA response, while OT is thought to inhibit the HPA response. Moreover, AVP and OT both play an essential role in the stimulation

of social behaviors that are genetically determined. Thus, it has been hypothesized that abnormalities in AVP and OT may play an important role in disorders like autism, in which disturbances in social behavior are the main characteristics. Indeed abnormal basal concentrations of AVP and OT have been found in autistic children: basal AVP concentrations were found to be increased and OT concentrations were found to be decreased. Thus, the elevated cortisol response to psychosocial stress might be explained by increased stimulation by AVP and decreased inhibition by OT. Similarly, the reduced cortisol response in MCDD children may be related to decreased stimulation by AVP and/or increased inhibition by OT. Finally, the selective impairment in the response to psychosocial stress that is found in MCDD children has also been found in schizophrenic patients. Part of MCDD children develop schizophrenia in adult life. Therefore, the impaired response to psychosocial stress in MCDD children may be a factor in the vulnerability to develop schizophrenia in later life, which is not present in autistic children (Blythe, *et.al.*, 2008; Del Ponte, *et.al.*, 1984; Garde, *et.al.*, 2003b; Garde, *et.al.*, 2005; Hansen, *et.al.*, 2001; Hansen, *et.al.*, 2006; Hansen, *et.al.*, 2007; King, *et.al.*, 2000; Lucres, *et.al.*, 2003; McEwen, *et.al.*, 1999; Matchock, *et.al.*, 2007; Theorell, 2003; Touitou, *et.al.*, 1997; Touitou, *et.al.*, 2006).

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