Faulty serotonin – DHEA interactions in autism: results of the 5-hydroxytryptophan challenge test

Jan Croonenberghs[1] Kristien Spaas[1], Annick Wauters[2], Robert Verkerk[3], Simon Scharpe[3], Dirk Deboutte[1], and Michael Maes[4]

1. University Center of Child and Adolescent Psychiatry, A.Z. Middelheim, Faculty of Medicine, University of Antwerp, Antwerp, Belgium;
2. Laboratory of Clinical Biology, A.Z. Middelheim, Antwerp, Belgium;
3. Department of Medical Biochemistry, University of Antwerp, Edegem, Belgium;
4. Clinical Research Center for Mental Health, Antwerp, Belgium.

Correspondence to: Jan Croonenberghs, M.D.,
University Center of Child and Adolescent Psychiatry,
A.Z.Middelheim, Lindendreef 1, 2020 Antwerp, Belgium.
FAX: +32-3-280 49 14; EMAIL: jan.croonenberghs@zna.be

Submitted: 2008-03-24 Accepted: 2008-04-15 Published online: 2008-06-24

Key words: **autism; serotonin; cortisol; DHEA; L-5-hydroxytryptophan; neurogenesis; neurodegeneration; neurotoxic; stress**

Neuroendocrinol Lett 2008; **29**(3):385–390 **PMID:** 18580847 NEL290308A08 © 2008 Neuroendocrinology Letters • **www.nel.edu**

Abstract

BACKGROUND: Autism is accompanied by peripheral and central disorders in the metabolism of serotonin (5-HT). The present study examines plasma dehydroepiandrosterone-sulphate (DHEA-S) and the cortisol/DHEA-S ratio following administration of L-5-hydroxytryptophan (5-HTP), the direct precursor of 5-HT, to autistic patients.

METHODS: Plasma DHEA-S levels were determined both before and after administration of 5-HTP or placebo, on two consecutive days in a single blind order in 18 male autistic patients and 22 matched healthy controls.

RESULTS: The 5-HTP-induced DHEA-S responses were significantly higher in autistic patients than in controls. In baseline conditions, the cortisol/DHEA-S ratio was significantly higher in autistic patients than in controls.

Discussion: The results suggest that autism is accompanied by a major disequilibrium in the serotonergic system. The increased Cortisol (neurotoxic) versus DHEA-S (neuroprotective) ratio suggests that an increased neurotoxic potential occurs in autism.

CONCLUSIONS: It is concluded that a disequilibrium in the peripheral and central turnover of serotonin and an increased neurotoxic capacity by glucocorticoids are important pathways in autism.

ORIGINAL ARTICLE

INTRODUCTION

Dehydroepiandrosterone (DHEA) and its sulfate-conjugated form (DHEA-S) are the most abundant steroids in the human circulation [1]. Under normal circumstances, DHEA and Cortisol are secreted synchronously by the adrenal cortex in response to adrenocorticotrophin (ACTH), secreted by the pituitary in response to corticotropine (CRH) [2]. However, independent control mechanisms exists for Cortisol and DHEA-S [3]. For example, unlike Cortisol, concentrations of DHEA-S vary with age [4]. The normal range of DHEA-S shows a broad, mainly genetically determined variation [5]. The conversion of DHEA to DHEA-S by sulfotransferases takes place in the adrenal cor-

tex, and a multitude of other tissues [6]. There is evidence for a production of DHEA as a neurosteroid in the brain [7], and a direct testicular production as well [8]. Plasma and saliva levels of DHEA correlate highly with levels in the ventricular cerebrospinal fluid (CSF) [9]. The diurnal rhythm in saliva DHEA is much less than for Cortisol. Therefore, the cortisol/DHEA ratio changes during the day from about 5–7 in the morning to about 2 at 20.00 h [10].

Cortisol and DHEA-S play a pivotal role in the regulation of the stress-response caused by external (psychosocial) stressors. Under conditions of acute stress, DHEA-S acts as an anti-glucocorticoid. Its anabolic action antagonizes the catabolic effects of Cortisol [11–13]. In chronic stress, however, DHEA-S levels may decline and thus the ability to counteract the catabolic effects of glucocorticoids becomes impaired [14]. Consequently, the cortisol/DHEA-S ratio is an important index reflecting the integrative role for these two adrenal steroids in neurotoxic processes in the brain. A high cortisol/DHEA-S ratio may reflect an increased neurotoxic risk to the brain [14] and, therefore, may play a role in abnormal psychological processes and psychiatric disturbances [10,15].

Autism is a psychiatric disturbance characterized by a problematic stress-perception [16]. It is characterized by qualitative impairment in communication and social interactions, and repetitive and stereotyped patterns of behavior or interests [17]. Children with autism have been described as experiencing difficulty tolerating novelty and environmental stressors. Stress responses are in part mediated by the hypothalamic-pituitary-adrenal (HPA)-axis. However, little is known regarding the relationship between Cortisol levels and social adaptation of autistic patients [18]. It has been shown that children with autism have significantly elevated androgen levels and that anti-androgen therapy may be of benefit in some autistic patients [19–22].

Multiple studies [23–26] have shown a disturbed turnover of serotonin (5-HT) in autism. We have shown that there is a major disequilibrium in the serotonergic metabolism between the brain and the periphery [25,26]. Thus, in a previous study, we found a blunted Cortisol response to the administration of L-5-hydroxytryptophan (5-HTP) in autistic children compared with normal controls, suggesting a central hyporesponsivity in the serotonergic system [25]. Indeed, the release of the HPA-axis hormones is mediated in part by 5-HT, while the hormonal responses to a challenge with 5-HTP, the direct precursor of 5-HT, offer an index for the functional state of the central serotonergic system [27,28]. On the other hand, administration of 5-HTP induced a greater response in blood serotonin in autism, suggesting an increased peripheral turnover of 5-HT [26]. However, no data are available on the effects of serotonergic agonists on plasma DHEA-S in autism.

The aims of the present study were to examine whether the DHEA-S responses and the cortisol/DHEA-S ratio following 5-HTP administration are significantly different in a group of autistic youngsters as compared with normal controls.

SUBJECTS AND METHODS

Subjects

In the present study, forty male subjects participated; 18 autistic youngsters and 22 normal controls, aged between 13 y.–19 y. and with an I.Q.> 55. One subject in the autistic group had a mild mental retardation (I.Q. between 55–60), all other subjects showed a borderline or normal intellectual functioning (I.Q. between 71–84, resp. 85–120). All subjects passed the onset of puberty (Tanner-stage III-IV) and were of the Caucasian race. Normal volunteers and autistic patients had a normal hematological screening. All subjects were free of any infections, inflammatory or allergic reactions for at least 2 weeks prior to the blood samplings. Exclusion criteria for autistic patients and healthy volunteers were: subjects suffering from a neurological, inflammatory, endocrine or clinically significant chronic disease; immunocompromized subjects; subjects with an active seizure disorder; subjects with tuberous sclerosis, FRAXA or other chromosomal disorders; and subjects receiving drugs with known or potential interaction with immune and endocrine functions. All healthy youngsters had a negative past, present or family history for psychiatric disorders such as autistic-, bipolar-, schizophrenic-, paranoid-, organic mental- and eating disorders and psychoactive substance use. None was a regular drinker and none had ever been taking psychotropic drugs. All were free of any medications and substance abuse for at least one month and had a negative drug-screening in the urine.

The autistic youngsters were recruited from the outpatient clinic of Child and Adolescent Psychiatry in Antwerp, Belgium; the Mental Health agencies of the same city; and from a Residential Treatment Center for Autistic Youngsters in Booischot, Belgium. We employed the DSM-IV [17] criteria to make the diagnosis of autism. The diagnosis was made on the basis of a consensus between, at least three clinicians (psychiatrists and psychologists), working with the autistic subjects in residential, semi-residential or day-care centers. The Autism Diagnostic Interview-Revised (ADI-R) [29], a semi-structured interview, was performed with the parents of youngsters with autism by a trained Master's level clinician (who was blind to clinical status before the interview) or by the primary author. Consensus meetings were held after the structured interview with the clinician. In order to evaluate the associated behaviors frequently seen in autism and the absence of psychopathology in the control-group, all subjects completed the Youth Self Report (YSR) scale [30] during the initial screening. Parents of subjects completed the Child Behavior Checklist (CBCL) [31] and the Aberrant Behavior Checklist (ABC) [32].

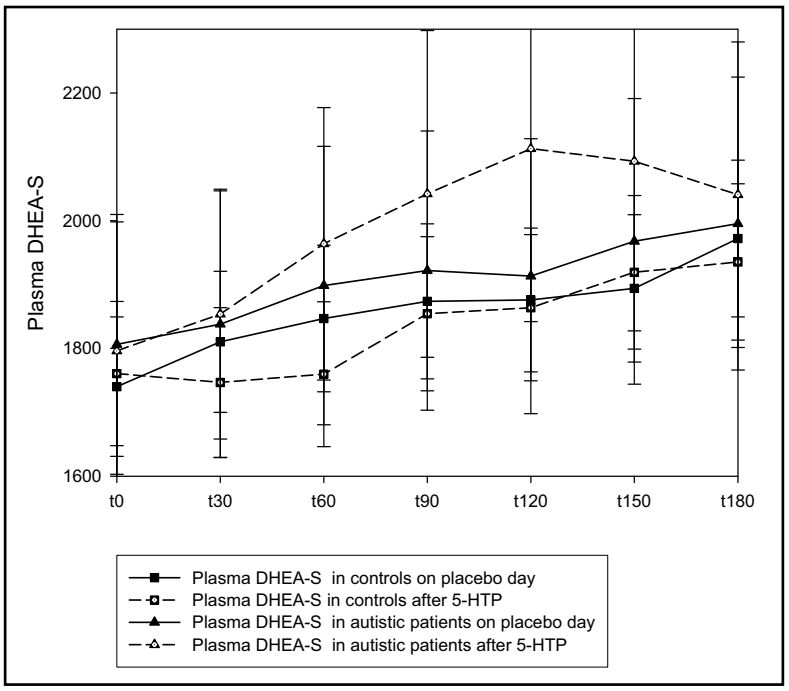

Figure 1. This figure shows the plasma DHEA-S (+/– SEM) concentrations (µg/dl) at t0 and 30 (t30), 60 (t60), 90 (t90), 120 (t120) and 180 (t180) minutes after administration of 5-hydroxytryptophane (4 mg/kg in non enteric-coated tablets) or after placebo in autistic patients and matched controls.

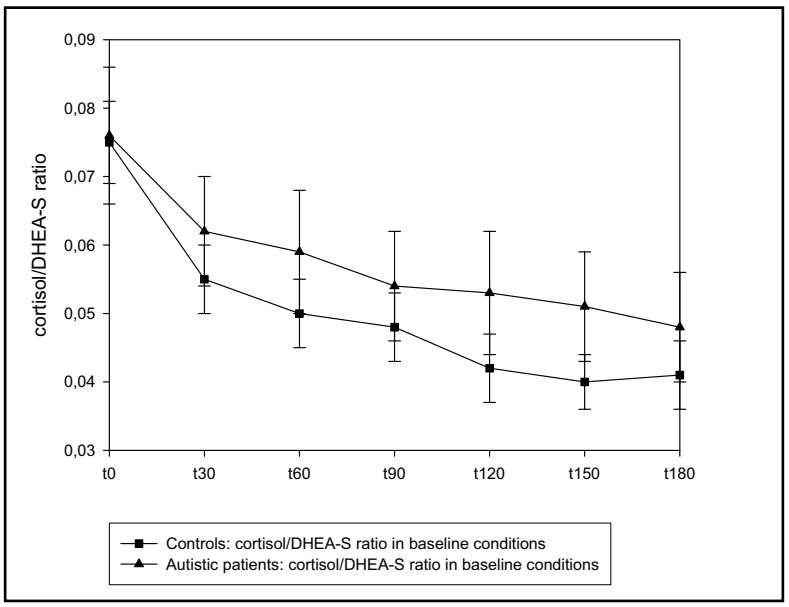

Figure 2. This figure shows the ratio cortisol/DHEA-S in baseline conditions at t0 and 30 (t30), 60 (t60), 90 (t90), 120 (t120), 150 (t150) and 180 (t180) minutes after placebo, in autistic patients and matched controls.

Methods

Subjects were kept at rest during the blood collections. Subjects were not allowed to eat or drink during the study period. In order to control for possible seasonal effects in serotonergic measurements [33] all samples were collected in the same season, i.e. between July and September 2000. Each patient and control was tested on two consecutive days with administration of 5-HTP or indistinguishable placebo, orally, in a single blind, randomized order. The subjects arrived at the Clinical Research Center around 7:15 a.m. After insertion of an intravenous cannula between 7:45 a.m. and 8.00 a.m., blood collections were carried out for the assay of plasma Cortisol and DHEA-S and plasma 5-HTP, i.e. 45 (t-45) and 30 (t-30) minutes before administration of placebo or 5-HTP at tO and, thereafter, every 30 minutes

over a 3-hour period, i.e. t30, t60, t90, tl20, tl50 and tl80. At tO, subjects received either a single oral dose of 5-HTP (4 mg/kg; non enteric-coated) or an identical placebo in a single blind order. Plasma Cortisol and DHEA-S were assayed in all samples, whereas plasma 5-HTP was assayed in the tO, t60, tl20, tl50 and tl80 samples only. Blood was stored in plastic tubes at 75 °C until thawed for assay. All assays were done blind to the subject's status. In order to minimize the analytical variability, all blood specimens for the assays of the above parameters were assayed in a single run with a single lot number of reagents and consumables employed by a single operator. Cortisol was measured using the Bayer Immuno 1 system (Bayer, Brussels, Belgium). DHEA-S was determined with a radio-immunoassay method (Coat-A-Count DHEAS-S04, DPC, Belgium). Cortisol and DHEA-S were analyzed in one and the same run by the same technician. The intra-assays coefficients of variation (CV) for Cortisol and DHEA-S are 3,1 % and 6,5 % respectively. A HPLC method (Gilson, Namur, Belgium) was employed to measure plasma 5-HTP. The intra-assay CV values for 5-HTP obtained in our laboratory are 4.1%.

Statistics

Group mean differences were checked by means of analysis of variance (ANOVA). Repeated measure (RM) design ANOVAs or RM design analyses of covariance (ANCOVAs) were employed to examine a) the within-subject variability with the effects of time and drug, i.e. 5-HTP versus placebo; b) the between-subject variability with diagnosis (autism versus controls) as grouping variable; c) the two way interactions between time X drug; drug X diagnosis; and time X diagnosis; and d) the three way interaction term time X drug X diagnosis. Tests on simple effects were carried out in order to examine significant main effects or significant interaction patterns. Relationships between variables were assessed with Pearson's product moment correlation coefficients and through regression analyses. Normality of distribution was assessed with the Kolmogorov-Smirnov test. Transformations (natural In) were used to reach normality of distribution or to adjust for heterogeneity of variance between study groups.

RESULTS

There were no significant differences in age, Tanner stage and body mass index (BMI) between patients with autism and healthy volunteers. On the CBCL, autistic subjects showed significantly more thought problems, social problems, and greater withdrawal, somatic complaints, anxiety and depression and attention problems than controls. The CBCL-total score was significantly higher in the autistic subjects. On the YSR autistic subjects reported significantly more social problems and internalizing symptoms (including greater withdrawal and attention problems subscale measures). Examination of the subscales of the Aberrant Behavior Checklist (results not shown) showed that there are no subjects who suffer from irritability (aggression, self-injurious behavior, temper tantrums, irritability, screaming, extreme mood changes). Ten out of the 18 autistic patients show a high score on the social withdrawal subscale (lethargy, inactivity, few social or emotional reactions); 2 out of the 18 subjects present a high score on the stereotypic behavior subscale (repetitive movements, odd in behavior) and 10 out of the 18 subjects present a high score on the hyperactivity subscale (inattentiveness, hyperactivity, impulsive, uncooperative, disturbing others).

Figure 1 shows the measurements of plasma DHEA-S following 5-HTP or placebo in autistic patients and normal volunteers. A RM design ANOVA performed on the log-transformed DHEA-S data from tO to tl80 showed a significant effect of time (F=13.6 df=6/494, p<10–5) and a significant interaction between 5-HTP X diagnosis (F= 13.1, df=l/494, p=0.0006), but no significant effect of 5-HTP (F=2.3 df=l/494, p=0.11) and no significant interactions between 5-HTP X time (F=0.6, df=6/494, p=0.7), time X diagnosis (F=0.4, df=6/494, p=0.82) and between time X drug X diagnosis (F=0.6, df=6/494, p=0.68).

A RM design ANOVA performed on the cortisol/DHEA-S ratio showed a significant effect of time (F= 11.8, df=6/494, p<10–5) and 5-HTP (F=79.1, df=l/494, p<10–5) and significant interactions between 5-HTP X time (F=10.2, df=6/494, p<10–5) and 5-HTP X diagnosis (F=15.8, df= 1/494, p=0.0002), but no significant interaction patterns between time X diagnosis (F=0.2, df=6/494, p=0.9) and time X drug X diagnosis (F=0.13, df=6/494, p=0.6). Analyses of simple effects performed after ANOVA showed i) significant effects of 5-HTP in controls (F=86.8, df=l/418, p<10–5), and in autistic subjects (F=27.7, df=l/418, p=0.00001). These results show that the significant interaction between drug X diagnosis found in the RM design ANOVA should be explained by 5-HTP increasing the cortisol/DHEA-S ratio significantly less in autistic subjects than in controls. Covarying with t0 in a RM design ANCOVA did not change the above results (F=5.1, df=l/413, p=0.02).

A RM design ANOVA performed on the 5-HTP values obtained at tO, t60, tl20, tl50 and tl80 on the active challenge day showed a significant effect of time (F=58.7, df=4/152, p<10–5) and no significant interaction pattern between time X diagnosis (F=l. 1, df=4/152, p=0.4). A RM design ANOVA performed on the 5-HTP values measured on the 5-HTP challenge day at t60, tl20, tl50 and tl80 (the 4 time points at which 5-HTP concentrations were measurable), did not show significant differences (F=2.0, df=l/38, p=0.2) in plasma 5-HTP levels between autistic patients (mean value of the 4 time points=984 ±547 nM/L) and controls (mean=1227 ±673 nM/L). Regression analyses pooled over the subjects (intra-class correlations) and per-

formed on the data obtained on the 5-HTP challenge day show no significant time-relationships between the changes in plasma 5-HTP on the one hand, and Cortisol or DHEA-S, on the other. In order to exclude possible order effects (5-HTP and identical placebo were administered in a single blind order on two consecutive study days) we have carried out additional RM design ANOVAs considering the within-subject (time) and between-subject (order effect) variability. RM design ANOVA did not show significant interaction patterns between time X order effect either for plasma Cortisol or DHEA-S. There were no significant correlations between age and Cortisol or DHEA-S at the different time points during the challenge test (even without p-correction).

Figure 2 shows the measurements of the cortisol/DHEA-S ratio during the placebo day in autistic patients and normal volunteers. A RM design ANOVA performed on the cortisol/DHEA-S ratio showed that the ratio was significantly higher in autistic subjects than in controls (F=5.3, df=l/228, p=0.02).

DISCUSSION

A first major finding of this study is that the 5-HTP-induced plasma DHEA-S values were significantly higher in autistic patients compared to normal volunteers. At first sight, this is in contrast to earlier research, which showed a blunted Cortisol response to the administration of 5-HTP in autistic children [25]. The latter findings suggested a central serotonergic hyporesponsivity in autism. Moreover, previous findings showed that 5-HTP increased the peripheral 5-HT levels significantly more in autism than in controls [26]. The latter findings indicated an enhanced peripheral metabolism of 5-HT in autism. Thus, autism is characterized by a disequilibrium in the serotonergic metabolism. This disequilibrium could explain the differences in the effects of 5-HTP on plasma Cortisol versus DHEA-S, the former being under the influence of central serotonergic circuits, whereas the latter could be more affected by peripheral circuits. Phrased differently, the impact of the peripheral hyperactivity of 5-HT turnover could explain the higher 5-HTP-induced increases in plasma DHEA-S. This effect is underscored by findings that DHEA-S levels can increase independently from pituitary stimulation by ACTH [12]. These peripheral mechanisms may include the testicular or other production of DHEA-S [8]. Another possible mechanism which may explain the greater DHEA-S than Cortisol response to 5-HTP is that an immature HPA-axis in autism [34] may have caused a compensatory mechanism, which induces greater increases in DHEA-S than in Cortisol. Another hypothesis is that increased levels in omega-3 PUFAs in autism may have induced different changes in the serotonergic receptors and 5-HT turnover [35].

The second major finding of this study is the significantly higher baseline cortisol/DHEA-S ratio in autistic patients compared to normal volunteers. This ratio is an index representing the anabolic/catabolic state with regard to the effects of DHEA-S versus Cortisol. Therefore, the increased cortisol/DHEA-S ratio may represent an increased neurotoxic capacity in autistic children. This finding is of direct importance to the neuropathology of autism since the limbic system and hippocampus are involved in this illness [36] and since both structures are very sensitive to the neurotoxic effects of glucocorticoids [37–41]. Indeed, DHEA and DHEA-S show potent neuroprotective qualities and play an important role in neurodevelopment. During the first year of infant life, the responsiveness of the HPA-axis and hence the production of stress hormones evolves from a highly labile and responsive system to a system characterized by less responsivity to nonspecific general stressors. This buffering of the adrenocortical response to stress during the first year of life is characterized by an adaptation of the stress response to normal life events [34,42]. The occurrence of pre- and perinatal and early life stressors, which are known to play a role in the etiology of autism [43,44], may explain a lowered "buffering" of the adrenocortical response to stress in autism and explain an imbalance in cortisol/DHEA-S ratio.

In our study, we did not find significant differences in plasma 5-HTP concentrations at any of the time points between autistic and normal children, while there were no significant relationships between plasma 5-HTP concentrations and the 5-HTP-induced hormonal responses. Thus, the 5-HTP-induced Cortisol and DHEA-S response in autism are not related to differences in 5-HTP pharmacokinetics between the study groups.

In conclusion, here we report a) increased DHEA-S responses to challenge with 5-HTP in autism, a phenomenon indicating a significant disequilibrium in the serotonergic system; and b) an increased baseline cortisol/DHEA-S ratio, which could have important consequences for neurotoxicity and brain development in autism.

Acknowledgments

The research was supported in part by the Janssen Research Foundation, Beerse, Belgium; and the Clinical Research Center for Mental Health, Antwerp, Belgium. The authors would like to thank 'De Speling' (a treatment Centre for autistic youngsters, Booischot, Belgium), the L.S.A. (an association of parents of children with autism in Limburg, Belgium), and W.Borghys, Pharmacist at the Middelheim Hospital, Antwerp, Belgium.

REFERENCES

1 Baulieu EE, Robel P. Dehydroepiandrosterone and dehydroepiandrosterone sulfate as neuroactive neurosteroids. J Endocrinol. 1996; **150**: S221–S239.
2 Nieschlag E, Loriaux DL, Ruder HJ, Zucker IR, Kirschner MA, Lipsett MB. The secretion of dehydroepiandrosterone and dehydroepiandrosterone sulphate in man. J Clin Endocrinol. 1973; **57**: 123–134.
3 Kroboth PD, Salek FS, Pittenger AL, Fabian TJ, Frye RF. DHEA and DHEA-S: a review. J Clin Pharmacol. 1999; **39**(4): 327–348.
4 Orentreich N, Brin JL, Rizer RL, Volgelman JH. Age changes and sex differences in serum dehydroepiandrosterone sulfate concentrations from puberty through adulthood. J Clin Endocrinol Metabol. 1984; **59**: 551–555.
5 Rotter JI, Wong FL, Lifrak ET, Parker LN. A genetic component to the variation of dehydroepiandrosterone sulfate. Metabolism. 1985; **34**: 731–736.
6 Falany CN. Enzymology of human cytosolic sulfotransferases. Faseb J. 1997; **11**: 206–216.
7 Baulieu EE. Neurosteroids, a novel function of the brain. Psychone uroendocrinology. 1998; **23**(8): 963–987.
8 de Peretti E, Forest MG. Pattern of plasma dehydroepiandrosterone sulfate levels in humans from birth to adulthood: evidence for testicular production. J Clin Endocrinol Metabol. 1978; **47**(3): 572–577.
9 Guazzo EP, Kirkpatrick PJ, Goodyer JJVI, Shiers HM, Herbert J. Cortisol, dehydroepiandrosterone (DHEA), and DHEA sulfate in the cerebrospinal fluid of man: relation to blood levels and the effects of age. J Clin Endocrinol Metabol. 1996; **81**(II): 3951–3960.
10 Goodyer IM, Park RJ, Netherton CM, Herbert J. Possible role of Cortisol and dehydroepiandrosterone in human development and psychopathology. Br J Psychiatry. **201**; 179: 243–249.
11 Hu Y, Cardounel A, Gursoy E, Anderson P, Kalimi M. Anti-stress effects of dehydroepiandrosterone: protection of rats against repeated immobilixation stress-induced weight loss, glucocorticoid receptor production, and lipid peroxidation. Biochem Pharmacol. 2000; **59**: 753–762.
12 Kalimi M, Shafagoj Y, Loria R, Padgett D, Regelson W. Anti-glucocorticoid effects of dehydroepiandrosterone (DHEA). Mol Cell Biochem. 1994; **131**: 99–104.
13 Kimonides VG, Spillantini MG, Sofroniew MV, Fawcett JW, Herbert J. Dehydroepiandrosterone antagonizes the neurotoxic effects of corticosterone and translocation of stress-activated protein kinase 3 in hippocampal primary cultures. Neuroscience. 1999; **89**: 429–436.
14 Bruce S, McEwen B. Protection and Damage from Acute and Chronic Stress. Allostasis and Allostatic Overload and Relevance to the Pathophysiology of Psychiatric Disorders. Ann NYAcadSci. 2004; **1032**: 1–7.
15 Hechter O, Grossman A, Chatterton Jr RT. Relationship of dehydroepiandrosterone ande Cortisol in disease. Med Hypotheses. 1997; **49**: 85–91.
16 Kanner L. Autistic disturbances of affective contact. Nervous Child. 1943; **2**: 217–250.
17 American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders (4th Edition). Washington, DC, 1994.
18 Nakayama Y, Takahashi T, Wakabayashi A, Oono H, Radford MH. Sex differences in the relationship between Cortisol levels and the Empathy and Systemizing Quotients in humans. Neuro Endocrinol Lett. 2007; **28**(4): 445–448.
19 Geier DA, Geier MR. A meta-analysis epidemiological assessment of neurodevelopmental disorders following vaccines administered from 1994 through 2000 in the United States. Neuro Endocinol Lett. 2006; **27**(4): 401–413.
20 Geier DA, Geier MR. A clinical trial of combined anti-androgen and anti-heavy metal therapy in autistic disorders. Neuro Endocinol Lett. 2006; **27**(6): 833–838.
21 Geier DA, Geier MR. A prospective assessment of androgen levels in patients with autistic spectrum disorders: biochemical underpinnings and suggested therapies. Neuro Endocinol Lett. 2007; **28**(5): 565–573.
22 Tordjman S, Anderson GM, McBride PA, Hertzig ME, Snow ME, Hall LM, Ferrari P, Cohen DJ. Plasma androgens in autism. J Autism Dev Disord. 1995; **25**(3): 295–304.
23 Cohen DJ, Shaywitz, BA, Johnson WT. Biogenic amines in autistic and atypical children. Arch Gen Psychiatry. 1974; **31**: 845–853.
24 CookE, Leventhal BL. The 5-HT system in Autism. Curr Opin Pediatr. 1996; **8**: 348–354.
25 Croonenberhgs J, Verkerk R, Scharpe S, Deboutte D, Maes M. Serotonergic disturbances in autistic disorder: L-5-hydroxytryptophan administration to autistic youngsters increases the blood concentrations of serotonin in patients but not in controls. Life Sci. 2005; **76**: 2171–2183.
26 Croonenberghs J, Wauters A, Deboutte D, Verkerk R, Scharpe S, Maes M. Central serotonergic hypofunction in autism: results of the 5-hydroxy-tryptophan challenge test. Neuro Endocrinol Lett. 2007; **28**(4): 449–455.
27 Meltzer HY, Maes M. Effect of pindolol on the L-5-HTP-induced increase in plasma prolactin and Cortisol concentrations in man. Psychopharmacology. 1994; **114**: 635–643.
28 Maes M, D'Hondt P, Meltzer HY, Cosyns P, Blockx P. Effects of serotonin agonists on the negative feedback by glucocorticoids on the hypothalamic-pituitary-adrenal axis in depression. Psychoneuroendocrinol. 1995; **20**: 149–167.
29 Lord C, Rutter M, Le Couteur A. Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorder. J Autism Devel Disord. 1994; **24**: 659–685.
30 Achenbach TM. Manual for the Youth Self-report and 1991 Profile. University of Vermont, Department of Psychiatry, Burlington, VT, 1991.
31 Achenbach TM, Edelbrock CS. Manual for the Child Behavior Checklist. University of Vermont, Department of Psychiatry, Burlington, VT. Queen City Printers.
32 Aman MG. Instruments for assessing treatment effects in devellopmentaly disabled populations. Assessm Rehab Expt 1994; **1**: 1–8.
33 Maes M, Scharpe S, Cosyns P, Neels H, Stevens W, Bridts C, et al. Components of biological variation, including seasonality in plasma L-tryptophan and competing aminoacids in man: relationships to serum total protein, climatic variables and violent suicide occurrence. Arch Gen Psychiatry. 1995; **52**: 937–945.
34 Gunnar MR, Krueger K, et al. Dampening of adrenocortical responses during infancy: normative changes and individual differences. Child Dev 1996; **67**: 877–889.
35 Sliwinski S, Croonenberghs J, Christophe A, Deboutte D, Maes M. Polyunsaturated fatty acids: do they have a role in the pathophysiology of autism? Neuro Endocrinol Lett. 2006; **27**(4): 465–471.
36 Kern JK, Jones AM. Evidence of toxicity, oxidative stress, and neuronal insult in autism. J Toxicol Environ Health B Crit Rev. 2006; **9**(6): 485–499.
37 Bastianetto S, Ramassamy C, Poirier J, Quirion R. Dehydroepiandrosterone (DHEA) protects hippocampal cells from oxidative stress-induced damage. Brain Res Mol Brain Res. 1999; **66**: 35–41.
38 Diamond DM, Branch BJ, Fleshner M, Rose GM. Effects of dehydroepiandrosterone sulfate and stress on hippocampal electrophysiological plasticity. Ann N Y Acad Sci 1995; **774**: 304–307.
39 Kimonides VG, Khatibi NH, Svendsen CN, Sofroniew MV, Herbert J. Dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEA-S) protect hippocampal neurons against excitatory amino acid-induced neurotoxicity. Proc Natl Acad Sci U.S.A. 1998; **95**: 1852–1857.
40 Cardounel A, Regelson W, Kalimi M. Dehydroepiandrosterone protects hippocampal neurons against neurotoxin-induced cell death: mechanism of action. Proc Soc Exp Biol Med. 1999; **222**(2): 145–149.
41 Compagnone NA, Mellon SH. Dehydroepiandrosterone: a potential signalling molecule for neocortical organization during development. Proc Natl Acad Sci USA. 1998; 14; **95**(8): 4678–83.
42 Lewis M, Ramsay D. Developmental change in infant's response to stress. Child Dev 1995; **66**: 657–670.
43 Hultman CM, Sparen P, Cnattingius S. Perinatal risk factors for infantile autism. Epidemiology 2002; **13**(4): 417–423.
44 Kolevzon A, Gross R, Reichenberg A. Prenatal and perinatal risk factors for autism: a review and integration of findings. Arch Pediatr Adolesc Med. 2007; **161**(4): 326–333.