

Serum testosterone concentration in male autistic youngsters

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Submitted: 2009-08-26 *Accepted:* 2009-10-11 *Published online:* 2010-08-27

Key words: **autism; morning concentrations of testosterone; puberty; neurodevelopment**

Neuroendocrinol Lett 2010; **31**(4):483–488 PMID: 20802443 NEL310410A04 © 2010 Neuroendocrinology Letters • www.nel.edu

Abstract

OBJECTIVE: Research on the biological pathophysiology of autism has found some evidence that alterations in androgenic hormones may play a role in the pathophysiology of that disorder. We studied morning concentrations of serum testosterone in a very homogenic group of postpubertal youngsters with autism and a group of normal controls.

METHODS: This study examines the serum testosterone concentration on 9 consecutive time points between 08.00 AM and 12.00 AM in 18 high- functioning male youngsters with autism (age 12–18) and 22 healthy volunteers participated in this study. All subjects passed the onset of puberty (Tanner-stage III–IV) and were of the Caucasian race.

RESULTS: Repeated measures ANOVA revealed a significant time effect, with a decline in the testosterone concentration during the test and time X diagnosis interaction. The total testosterone concentration was significantly lower in the autism group compared to the group of normal controls.

CONCLUSIONS: The significant decrease in serum testosterone concentration in male youngsters with autism suggest that the turnover of testosterone may take part in the pathophysiology of autism. Suggestions for further research are discussed.

INTRODUCTION

Testosterone has an important role in growth and differentiation of genital and extragenital organs (Henneman, 1989; Moir & Jessel, 1989). The hormone induces masculinisation, influences the early development of the central nervous system (CNS) and behaviour, and induces secondary sex characteristics and sexual development (Henneman, 1989; Moir & Jessel, 1989; Nieschlag & Behre, 1990). Prenatal androgens are correlated

to male typical behaviour after birth and are negatively correlated to female typical behaviour (Moir & Jessel, 1989). Because of its role as a morphogenetic agent and differentiation factor in the developing brain, testosterone may play an important role in developmental disorders.

Autism is a psychiatric disturbance characterized by qualitative impairment in communication and social interactions, and repetitive and stereo-

types patterns of behaviour or interests (American Psychiatric Association, 1994; Kanner, 1943).

There is some evidence that testosterone may play a role in the pathophysiology of autism. There is a significant difference between boys and girls in social interaction after birth (Higley *et al.*, 1996; Sanchez-Martin *et al.*, 2000). Girls make more eye contact than do boys, they learn to speak and read earlier and develop a greater vocabulary (Lutchmaya *et al.*, 2002), both part of the core symptoms of autism. A negative correlation has been found between prenatal testosterone levels and making eye contact and language development (Lutchmaya *et al.*, 2002).

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 (Geier & Geier, 2006; Geier & Geier 2007; Tordjman *et al.*, 1995). In boys with autism pubertas praecox is seen and this goes together with increased testosterone levels (Baron-Cohen, 2002; Tordjman *et al.*, 1995). In people with autism low 2D–4D ratios have been found and this is influenced by testosterone (Ingudomnukul *et al.*, 2007; Manning *et al.*, 2001). In autism expression of symptoms often changes after puberty. Geier & Geier (2006) found significantly increased levels of serum/plasma dehydroepiandrosterone and serum total testosterone relative to age- and sex-specific normal laboratory reference ranges in prepubertal children with autism.

Levels of testosterone differ with age, pubertal stadium, medication, certain diseases and stress. Over 90% of postpubertal testosterone is produced in the testes (Henneman, 1989; Moir & Jessel, 1989; Nieschlag & Behre, 1990). The production of testosterone takes place under the influence of luteinizing hormone (LH), and also but less by follicle stimulating hormone (FSH) and prolactin. Circulating LH is governed by gonadotrophin releasing hormone (GnRH), which is released under a pulsatile way by the preoptic nucleus of the hypothalamus (Oomura *et al.*, 1988). Ten percent of testosterone is produced in the adrenal cortex under the influence of the Hypothalamic-Pituitary-Adrenal (HPA) axis. There is a daily variation in levels of testosterone of 30%. Levels are usually higher in the morning and gradually decrease during the day.

Therefore, in this study we want to examine if autism is accompanied by abnormalities in the serum testosterone concentration in a very homogenic group in sex, age, race, IQ and pubertal status (Tanner stage III–IV) at 9 different time points in the morning obviating the effect of possible diurnal differences.

SUBJECTS AND METHODS

Subjects

In the present study, forty male subjects (n=18 with autism and n=22 normal controls) aged between 13 years – 19 years and with an IQ > 55 participated. One

subject in the autistic group had a mild mental retardation (IQ between 55–60); all other subjects showed a borderline intellectual functioning (IQ between 71–84) or normal intellectual functioning (IQ between 85–115). All subjects passed the onset of puberty (Tanner-stage III–IV) and were of the Caucasian race.

The autistic youngsters were recruited from the outpatient clinic of University Child and Adolescent Psychiatry in Antwerp, Belgium; the Mental Health Centres of Antwerp, Belgium; and a Residential Treatment Center for Autistic Youngsters in Booischoot, Belgium. We employed the DSM-IV (American Psychiatric Association, 1994) 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 centres. A trained Master's level clinician (who was blind to clinical status before the interview) or the primary author carried out a semi-structured interview, i.e. the Autism Diagnostic Interview-Revised (ADI-R) (Lord *et al.*, 1994), with the parents of patients. Consensus meetings were held after the structured interview with the clinician. In order to evaluate the associated behaviours and comorbidity frequently seen in autism and the absence of psychopathology in the control-group, all subjects completed the Youth Self Report (YSR) scale (Achenbach, 1991) during the initial screening. Parents of subjects completed the Child Behaviour Checklist (Achenbach & Edelbroch, 1983) and the Aberrant Behaviour Checklist (ABC) (Aman, 1994).

Normal volunteers and autistic patients had a normal haematological screening. All subjects were free of infections, inflammatory or allergic reactions for at least 2 weeks prior to blood samplings. Exclusion criteria for patients and controls were: subjects suffering from a neurological, inflammatory, endocrine or clinically significant chronic disease; immunocompromised subjects; subjects with an active seizure disorder; subjects with tuberous sclerosis, FRAXA (Fragile-X Syndrome) 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 medication for at least one month. None of the subjects suffered from substance abuse. Subjects with a positive drug-screening in the urine were excluded to participate in this study.

Methods

Subjects were kept at rest during the blood collections. All subjects fasted for at least 12 hours. Subjects were not allowed to eat or drink during the study period. In order to control for possible seasonal effects (Maes

et al., 1995), all samples were collected in the same season, i.e. between July and September. All samplings were carried out in the morning to obviate the effect of the marked diurnal variation in testosterone production (Diver, 2009). The subjects arrived at the Clinical Research Centre around 7:15 a.m. After insertion of an intravenous cannula between 7:45 a.m. and 8:00 a.m. (Maes *et al.*, 1995), blood collections were carried out for the assay of baseline serum testosterone, i.e. 45 (t-45), 30 (t-30) minutes before t0, t0, 30 min (t30), 60 min (t60), 90 min (t90), 120 min (t120), 150 min (t150) and 180 min (t180) after t0. Blood was stored in plastic tubes at -70°C until thawed for the assays. All assays were done blind to the subjects status. In order to minimize the analytical variability, all blood specimens for the assays of the above parameters in autistic patients and healthy volunteers were assayed in a single run with a single lot number of reagents and consumables employed by a single operator. Testosterone was measured using the Bayer Immuno 1 System (Bayer, Brussels, Belgium). The inter-assays coefficients of variation (CV) for testosterone range from 5.2% at 15 ng/ml to 13% at 0.11 ng/ml.

Statistics

Transformations (natural ln) were used to reach normality of distribution or to adjust for heterogeneity of variance between study groups. Normality of distribution was assessed with the Kolmogorov-Smirnov test.

Baseline serum testosterone concentrations were defined as the mean values of the 3 time points t-45, t-30 and t0. Group mean differences were checked by one-factor analyses of variance (ANOVA). Repeated measures (RM) ANOVA or RM analysis of covariance (ANCOVA) were employed to examine a) the within-subject variability with the effect of time; b) the between-subject variability with diagnosis (autism versus controls) as grouping variable and c) the two way interactions between time \times diagnosis. Partial eta squared measures were used to assess effect sizes. Post hoc difference tests were used to assess which time group means differ from which others.

RESULTS

Demographic data

There were no significant differences in age, Tanner stage, and Body Mass Index (BMI) between autistic patients and healthy volunteers.

Autistic subjects showed significantly more thought problems, social problems and more internalizing symptoms (including greater withdrawal, somatic complaints, anxiety and depression and attention problems subscale measures) on the CBCL than healthy controls. The CBCL-total score was significantly higher in the autistic subjects. On the YSR, the autistic subjects reported significantly more thought problems, social problems and more internalizing symptoms (includ-

ing greater withdrawal and attention problems subscale measures).

Examination of the associated behaviours by means of the subscales of the Aberrant Behaviour Checklist indicates that there are no subjects who suffer from irritability (aggression, self-injurious behaviour, 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 behaviour subscale (repetitive movements, odd in behaviour) and 10 out of the 18 subjects present a high score on the hyperactivity subscale (inattentiveness, hyperactivity, impulsive, uncooperative, disturbing others) (Croonenberghs *et al.*, 2005; Croonenberghs *et al.*, 2007).

Testosterone in autistic youngsters and controls

Figure 1 shows the measurements on the serum testosterone concentration in autistic patients and normal controls.

Repeated-measures ANOVA revealed a significant effect of time within the total group of subjects with a decline in the testosterone concentration during the test ($F=18.24$; $df=3.20$; $p=0.000$; effect size $F^2=0.349$), a significant effect of diagnosis ($F=9.12$; $df=1$; $p=0.005$; effect size $F^2=0.212$) and a significant time \times diagnosis interaction ($F=0.69$; $df=3.20$; $p=0.56$; effect size $F^2=0.020$) within the total group of subjects. Mean serum testosterone concentrations were significant lower between the autistic children (mean \pm SD=5.33 ng/mL \pm 2.66) (SEM=0.21) and controls (mean \pm SD=6.85 ng/mL \pm 2.21) (SEM=0.16) ($F=33.58$; $df=1$; $p=0.000$). Baseline serum testosterone concentrations were significant lower between the autistic children (mean \pm SD=6.34 ng/mL \pm 3.07) (SEM=0.59) and controls (mean \pm SD=7.50 ng/mL \pm 2.08) (SEM=0.39) ($F=2.72$; $df=1$; $p=0.105$).

RM ANCOVA with t0 as covariate showed the same results: testosterone values remained significantly lower in autistic children than in controls ($F=0.031$; $df=1$; $p=0.862$).

Post hoc pairwise comparisons between the different time groups revealed significance between (t-30)-(t-45); t0-(t-45); (t30)-t0 and (t60)-(t30) (LSD=0.000; 0.002; 0.037; 0.036).

DISCUSSION

The major finding of this study is that the morning serum testosterone (T) concentrations were significantly lower in autistic patients compared to normal volunteers in a population of high-functioning youngsters.

These results suggest that a decrease of serum T concentration may take part in the pathophysiology of autism. As described in the introduction the hypothesis that T plays a role in the pathophysiology of autism can be supported not only by the male predominance

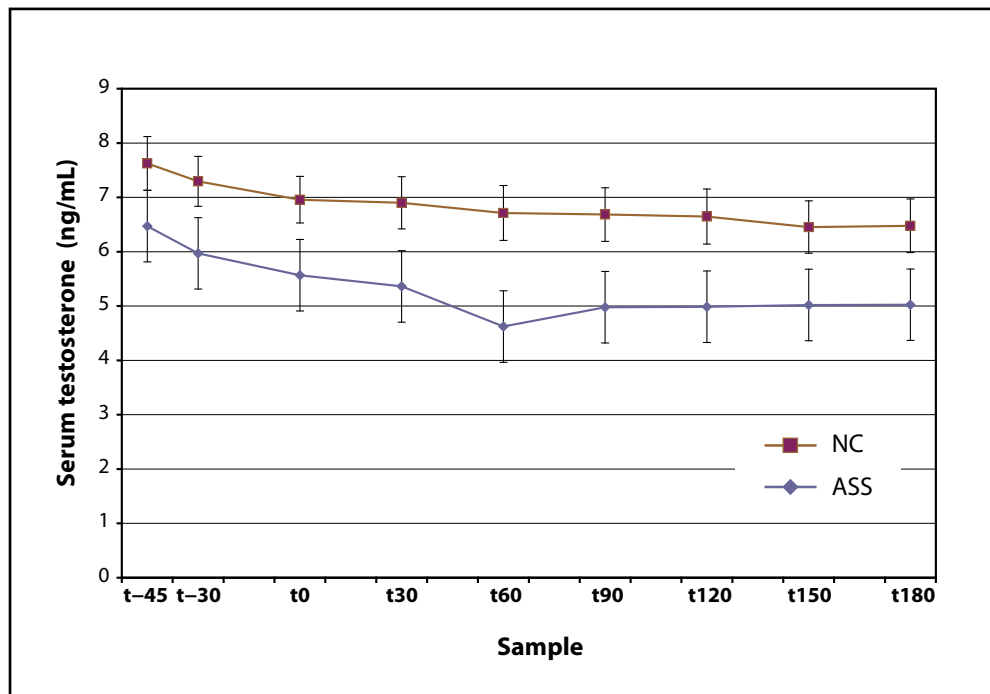


Fig. 1. This figure shows the serum testosterone (\pm SEM) concentrations (ng/mL) at (t-45), (t-30), t0, (t30), (t60), (t90), (t120), (t150), (t180) in autistic children and matched controls.

(autism is four times more common in boys than in girls (Croonenberghs, 2003), but also by the difference in behaviour between boys, girls and the behaviour of high functioning youngsters with autism. Boys are better at systemising than at empathising and girls the opposite. Autistic disorder is characterised by extreme systemising and very low empathising (Baron-Cohen, 2002). Collecting and organising is done more by males and is present more prominent in people with autism. Girls are better at recognising other peoples emotions than boys but youngsters with autism score lower than boys. Females make more eye contact than males and people with autism less than males. Language development goes faster in females and much slower in people with autism (Baron-Cohen, 2002). Females are superior to males in the pragmatics of conversation; people with autism have great difficulty with this. Baron-Cohen *et al.* (2005) have followed 58 children, whose foetal Testosterone (fT) was analysed in amniotic fluid, up at age 4. fT was negatively correlated to quality of social relationships, taking sex-differences into account. fT was also positively correlated with restricted interests in boys. Lutchmaya *et al.* (2002) have found a negative correlation between prenatal T levels and making eye contact and language development. In autism many brain areas are abnormal. The lower T levels found in our study group can be explained by a greater negative feedback at the hypothalamic nucleus. The preoptic region, which is very androgen sensitive, is 2.5 times as large in males and its growth prenatal is strongly determined by the presence of T. Too much T could have

and influence on the number and/or the sensitivity of the androgen receptors of this region, which possibly at a later age, namely during puberty, could cause an increased negative feedback, resulting in lower serum T concentrations. Plasma oxytocin levels have been found significantly lower in autism (Muhle *et al.*, 2004). Low oxytocin possibly alters the sensitivity of hypothalamic receptors for T.

In a previous study Tordjmann *et al.* (1995) has not found significant differences in T levels in autism. However in this study the group with autism and control group were formed with a very heterogenic population, including pre-, and postpubertal subjects in both groups. Also Lutchmaya *et al.* (2004) have found no significant difference of T levels in autism compared to a normal control population.

Geier and Geier (2006) suggested that the biochemical basis for abnormalities in the androgen synthesis pathway in those with autism may involve the regulator metabolite DHEA. In patients with autism they found decreases in transsulfuration metabolites (Geier & Geier, 2006; Geier & Geier, 2007; James *et al.*, 2004; James *et al.*, 2006; Waring *et al.*, 1997; Waring & Klovzra 2000), impaired sulfation (Alberti *et al.*, 1999) as well as significant increases in common polymorphic variants known to modulate the transsulfuration pathway (James *et al.*, 2006). In a group of 70 autistic subjects they found overall significantly increased levels of serum T, serum free T, % free T, DHEA and androstenedione in comparison to laboratory age –and sex-specific reference ranges (Geier & Geier, 2007). The mean age of

the patients was 10.8 years old, therefore it is not clear whether pubertal state could have an influence.

In our study about the turnover of DHEA in autism, we found that in baseline conditions, the cortisol/DHEA-S ratio was significantly higher in autistic patients than in controls and that the 5-HTP-induced DHEA-S responses were significantly higher in autistic patients than in controls. The results show that the serotonergic function may play a role in the turnover of this hypothalamic-pituitary-adrenal (HPA)-axis hormone (Croonenberghs *et al.*, 2008). Multiple studies have shown a disturbed turnover of serotonin (5-HT) in autism (Cohen *et al.*, 1974; Cook & Leventhal, 1996; Croonenberghs *et al.*, 2005; Croonenberghs *et al.*, 2007). Further study should be made on the effect of the serotonergic system on the HPA- and hypothalamic-pituitary-gonadal (HPG)-axis and hence the turnover of T. Challenge tests with pregnyl or 5-HT-agonists could provide better insight in the dynamic aspects of the androgenic system in subjects with autism and normal control subjects.

T is detectable in plasma as free T (1–2%) and total T, the latter containing as well the binding fraction to sex hormone binding protein (SHBG) (60–70%) and albumin (30–35%), both possibly having an influence on the concentrations of T. Further studies should be directed to measure T concentrations correlated to concentrations of albumin and SHBG.

Correlations between T concentrations intra-uterine and T concentrations in serum and behaviour during puberty has to our knowledge not been made yet. Measurements of T concentrations in umbilical cord blood and in saliva during the weeks after birth and prospective follow-up studies should be done with a regular evaluation of the androgenic system and special environmental, behavioural and developmental items in order to discover etiologic factors, protective factors and factors responsible for the persistence of the disorder and to highlight correlations between bio-psychosocial factors.

In this study we have controlled for or otherwise may dismiss the intervening effects of variables such as age, sex, race and I.Q., drug state, seasonal effects or autistic symptomatology. Moreover we minimized the analytic variability in the biological variables since all the assays were run at the same time using the same chemicals. Given the fact that all but one of the autistic subjects of our study group belong to the high-functioning group we cannot exclude whether the findings are specific for the entire autistic population or might be confined to the higher 30% of the overall population of autistic subjects. It remains unclear if or to what degree disturbances in the androgenic system may contribute to the overall phenotype of autism or only to one or more specific behaviours of autism and/or the presence and severity of associated features. Also from the genetic point of view recent evidence shows that the disorder is genetically heterogeneous (Szatmari, 1999). Higher

functioning individuals with autism may have another genetic background than lower functioning ones.

Limitations of the present study, however, are the small number of autistic subjects and controls (n=40) and the presence of autistic patients with different psychopathological dimensions, e.g. 10 out of the 18 subjects present a high score on the social withdrawal subscale, 2 out of the 18 subjects present a high score on the stereotypic behaviour subscale and 10 out of the 18 subjects present a high score on the hyperactivity subscale.

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.

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