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Increased serum androstenedione in adults with autism spectrum conditions

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Summary Molecular and behavioural evidence points to an association between sex-steroid hormones and autism spectrum conditions (ASC) and/or autistic traits. Prenatal androgen levels are associated with autistic traits, and several genes involved in steroidogenesis are associated with autism, Asperger Syndrome and/or autistic traits. Furthermore, higher rates of androgen-related conditions (such as Polycystic Ovary Syndrome, hirsutism, acne and hormone-related cancers) are reported in women with autism spectrum conditions. A key question therefore is if serum levels of gonadal and adrenal sex-steroids (particularly testosterone, estradiol, dehydro-epiandrosterone sulfate and androstenedione) are elevated in individuals with ASC. This was tested in a total sample of $n = 166$ participants. The final eligible sample for hormone analysis comprised $n = 128$ participants, $n = 58$ of whom had a diagnosis of Asperger Syndrome or high functioning autism (33 males and 25 females) and $n = 70$ of whom were age- and IQ-matched typical controls (39 males and 31 females). ASC diagnosis (without any interaction with sex) strongly predicted androstenedione levels ($p < 0.01$), and serum androstenedione levels were significantly elevated in the ASC group (Mann—Whitney $W = 2677$, $p = 0.002$), a result confirmed by permutation testing in females (permutation-corrected $p = 0.02$). This result is discussed in terms of androstenedione being the immediate precursor of, and being converted into, testosterone, dihydrotestosterone, or estrogens in hormone-sensitive tissues and organs.

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1. Introduction

Autism spectrum conditions (ASC) are neurodevelopmental and are characterized by difficulties in social interaction and communication skills, alongside restricted interests and stereotyped behaviours (APA, 1994). The foetal androgen theory of ASC proposes that foetal testosterone (fT) is one influence in the development of psychological and neural sex differences in the general population, and in the development of autistic traits (Baron-Cohen et al., 2004). Evidence in support of this theory comes from the Cambridge Foetal Androgen Study, in which typically developing children whose amniotic fT levels were measured in utero have been followed up at different points in development. Since amniotic testosterone levels measured are the product of renal clearance of hormones produced by the foetus (Finegan et al., 1991), fT can be regarded as a proxy measure of circulating testosterone in the foetus. Results from that longitudinal study reveal that fT levels are *inversely* correlated with social and language development, including measures of eye contact at 12 months, vocabulary size at 18 and 24 months, social skills at 48 months and empathy at 6—9 years (Lutchmaya et al., 2002a,b; Knickmeyer et al., 2005, 2006a; Chapman et al., 2006). In contrast, fT levels are *positively* correlated with autism-related behaviours such as autistic traits at 18—24 months and at 6—9 years (Auyeung et al., 2009, 2010), restricted interests at 48 months (Knickmeyer et al., 2005), and systemizing at 6—9 years (Auyeung et al., 2006). Consistent with these results, females with Congenital Adrenal Hyperplasia (CAH) (whose androgens are elevated prenatally due to a reduced efficiency of cortisol synthesis in the adrenal gland) have a higher than average numbers of autistic traits (Knickmeyer et al., 2006b).

To our knowledge, *postnatal* sex-hormone profiles at different stages of development and in adult life have not yet been systematically studied in relation to ASC and/or autistic traits in the general population. Schmidtova et al. (2010) found that children with autism (both prepubertal and pubertal) and (pubertal) children with Asperger Syndrome (AS) had significantly increased levels of salivary testosterone compared with control children. A further study from Schwarz et al. (2010) found elevated levels of free-testosterone and LH in females with AS compared with control females in a multi-analyte profiling of blood serum. In contrast, Croonenberghs et al. (2010) found lower concentration of serum testosterone in male adolescents with ASC, compared to matched controls. Some indirect evidence that circulating androgens may be dysregulated in ASC also comes from the atypical timing of puberty reported in ASC (Tordjman et al., 1997; Yoshimura et al., 2005; Knickmeyer et al., 2006c). In addition, it has been found that, in adulthood, a number of androgen-related medical conditions (such as Polycystic Ovary Syndrome (PCOS), hirsutism, acne, breast and ovarian cancers) and androgen-related characteristics (such as tomboyism, bisexualism and asexualism) are more common in women with ASC, and in their mothers (Ingudomnukul et al., 2007). This suggests that genetic factors might account for higher levels of androgen synthesis and/or increased local tissue sensitivity to circulating androgens in ASC.

Related to this, two sex-steroid hormone related genes (*SRD5A1* and *AR*) have been found to be associated with autism (Henningsson et al., 2009; Hu et al., 2009) and polymorphisms in genes involved in testosterone metabolism (*AR* and *SRD5A2*) have also shown association with ASC (Schmidtova et al., 2010). In another study, 10 other genes related to synthesis, metabolism, or transport of sex steroids (*HSD11B1*, *LHCGR*, *CYP17A1*, *CYP19A1*, *SCP2*, *CYP11B1*, *ESR1*, *ESR2*, *HSD17B4*, *HSD17B2*) showed nominal association with Asperger Syndrome and/or autistic traits in the general population. Of these, *CYP11B1*, *CYP17A1*, and *ESR2* survived familywise error rate correction by permutation testing (Chakrabarti et al., 2009). Given these converging lines of evidence, we tested if serum levels of gonadal and adrenal sex-steroids (specifically, testosterone, estradiol, dehydroepiandrosterone sulfate (DHEA-S) and androstenedione) are elevated in adults with ASC.

While testosterone and estradiol are the main sex hormones produced in the male and female gonads respectively, androstenedione is synthesized in both gonads and the adrenal cortex, and DHEA-S is the main androgen synthesized in the adrenal gland. Androstenedione and DHEA-S are both released in the peripheral blood circulation and are converted into testosterone, dihydrotestosterone or estrogens in hormone-sensitive tissues and organs (such as the skin, the pilosebaceous unit, adipose tissue, and most relevant, the brain) via intracrine mechanisms. Consequently, they contribute to the final pool of active androgens in peripheral target tissues and to the development of androgen-related conditions such as PCOS, hirsutism, and acne (Georgopoulos et al., 2009).

The objective of this study was to investigate if androgen biosynthesis is dysregulated in adult males and females with ASC, leading to increases in testosterone, testosterone to estradiol ratio, DHEA-S, or androstenedione in peripheral blood circulation. Since androgen related medical conditions and characteristics have been reported in females with ASC, we predicted that an increase in serum androgens, if present, would be seen particularly clearly in females.

2. Materials and methods

2.1. Participants and procedure

The study was approved by the Cambridge NHS NRES Research Ethics Committee and all participants signed a consent form to participate. The sample comprised n = 166 participants, 62 of whom had a diagnosis of ASC (33 males and 29 females) and n = 104 of whom were controls (49 males and 55 females). Participants with ASC were recruited via the Cambridge Autism Research Centre database of volunteers (www.autismresearchcentre.com), the National Autistic Society (UK), and local autism support groups in the UK. Controls were recruited from the general population via advertising. ASC diagnosis (Asperger Syndrome or high functioning autism) was made by psychiatrists or clinical psychologists based on Diagnostic and Statistical Manual-IV-Text Review Disorders criteria (DSM-IV-TR, 2000). As a check on diagnosis, a subgroup of 14 participants with ASC with available parental informants were also assessed using the Autism Diagnostic Interview — Revised (Lord et al., 1994), all of whom scored above the diagnostic cut off for autism. The 62 individuals with ASC were also given the Autism Spectrum Quotient (AQ) (Baron-Cohen et al., 2001) as a further check on diagnosis,

and all scored in the expected range (26 or above: Woodbury-Smith et al., 2005).

Controls with a family history of psychiatric or medical conditions (such as type II diabetes, hypertension, cardiovascular or autoimmune diseases) or with an AQ > 25 were excluded. This resulted in exclusion of 10 male and 2 female controls. Educational level (number of years in full-time education), socioeconomic status (parental occupation), diet preferences (vegetarian), smoking and drinking habits (weekly units) were investigated for both groups, and no between-group differences were reported (chi-square tests, all $p > 0.5$). None of the participants had eating disorders. Height and weight were measured in all participants and body mass index (BMI) was calculated. 3 ASC males and 4 ASC females were taking medication (such as risperidone) at the time of the study. This was considered in the analysis (see below).

Also, since oral contraceptives affect serum hormonal levels, females using the contraceptive pill ($n = 4$ in the ASC group; $n = 22$ in the control group) were excluded from the hormone analysis. The final sample analyzed for hormones therefore comprised $n = 33$ ASC males, $n = 39$ control males, $n = 25$ ASC females and $n = 31$ control females. The phase of the menstrual cycle did not differ in females with ASC and control females (chi-square = 0.35 (df, 1), p-value = 0.55).

The Wechsler Abbreviated Scale of Intelligence (WASI) was administered to all participants to measure IQ (Ryan et al., 2003). No between-group differences in Full Scale IQ, Performance IQ and Verbal IQ were found in the final sample ($F(3) = 1.02$, $p = 0.39$, $F(3) = 0.92$, $p = 0.43$, $F(3) = 1.38$, $p = 0.25$ respectively).

2.2. Hormone measurements

Both gonadal (testosterone and estradiol) hormones and adrenal androgens (DHEA-S and androstenedione) were analyzed in the serum of ASC and controls. Total testosterone was analyzed using the Siemens Coat-A-Count Radioimmunoassay (Siemens Healthcare Diagnostics, Tarrytown, NY) while estradiol was analyzed using the Perkin Elmer Auto-DELFIA Automated Immunoassay System (Perkin Elmer Life and Analytical Sciences, Boston, MA). Androstenedione and DHEA-S were analyzed using the Siemens Immulite 1000 Automated Immunoassay System (Siemens Healthcare Diagnostics, Tarrytown, NY). Sex-Hormone binding globulin (SHBG) was also analyzed using the Siemens Immulite 1000 Automated Immunoassay System (Siemens Healthcare Diagnostics, Tarrytown, NY) to calculate free testosterone levels. Analyses were conducted blind with respect to group membership and the samples were analyzed randomly to avoid any bias due to the presence or absence of diagnosis, participant age, or age of material. Assay sensitivity, number of replicates and intra-assay variability are reported in Table 1.

2.3. Statistical analysis

Statistical analysis was performed using the R software environment for statistical computing and graphics, version 2.12.1 for MacOS. Hormone levels were analyzed separately for each sex, since they can vary considerably between males and females and nonparametric methods of statistical inference were used, since one/more of the assumptions of linear model (Linearity, Homoscedasticity, Uncorrelatedness and Normality) were violated. Effect size (Cohen's d) was calcu-

Table 1 Hormone assay sensitivity, number of replicates and intra-assay variability.

	Intra-assay coefficient of variation		Replicates	Detection limit	Method used
	Conc.	CV (%)			
Total testosterone (nmol/L)	0.7	18.0	2	0.2 nmol/L	Siemens Coat-A-Count RIA
	3.5	7.0			
	27.7	5.0			
SHBG (nmol/L)	4.5	6.9	1	0.2 nmol/L	Siemens Immulite 1000
	11	7.7			
	33	6.1			
	64	4.1			
	121	7.5			
Estradiol (pmol/L)	183	5.2	1	50 pmol/L	Perkin Elmer AutoDELFIA
	855	3.8			
	9340	4.3			
Androstenedione (nmol/L)	2.3	9.1	1	1.0 nmol/L	Siemens Immulite 1000
	5.5	5.1			
	15.2	5.3			
	31.8	6.4			
DHEA-S (μmol/L)	1.2	7.6	1	0.05 μmol/L	Siemens Immulite 1000
	2.4	9.2			
	5.1	9.5			
	11.4	8.1			
	21.2	6.8			

Table 2 Descriptive characteristics (mean ± SD) of the final sample analyzed for hormones, comprising controls with AQ scores <25 and females not taking contraceptives.

	Males		Females	
	ASC (n = 33)	Controls (n = 39)	ASC (n = 25)	Controls (n = 31)
Age (years)	30.15 (±8.40)	29.77 (±7.76)	33.24 (±8.03)	33.52 (±6.58)
Body mass index	24.75 (±3.54)	23.92 (±3.63)	25.72 (±5.34)	23.6 (±4.09)
Height (cm)	176.4 (±6.81)*	179.5 (±6.05)*	163.8 (±5.18)	165.5 (±7.4)
Time of blood collection (hh:mm)	13:36 (±2.1 h)	12:54 (±2 h)	13:18 (±2.4 h)	13:12 (±2.4 h)
IQ total	113.3 (±14.52)	115.9 (±9.28)	111 (±14.22)	112.5 (±8.97)
IQ Performance	112.6 (±12.80)	112.7 (±11.62)	107.6 (±17.20)	110.6 (±10.13)
IQ Verbal	110.6 (±14.94)	115.3 (±7.98)	112 (±12.04)	111.6 (±9.15)
AQ	34.28 (±8.69)[a]	16.44 (±4.94)[a]	40.04 (±7.27)[a]	13 (±5.14)[a]

[a] Significant group and sex differences reported (p-values from pairwise comparisons after Bonferroni correction, <0.01).
* $p < 0.05$.

lated for each hormone. The Kernel density plot (R library: *lattice*) was used as a non-parametric method of estimating the probability density function of hormone levels. Correlations between hormone levels and the time of blood collection were analyzed using Pearson's correlation test. Finally a regression analysis with sex, diagnosis, sex by diagnosis interaction, age and BMI (entered in the model in the reported order) as independent variables and each hormone level as dependent variable was performed.

3. Results

Cases and controls did not differ in age ($F(3) = 1.63$, $p = 0.18$) or BMI ($F(3) = 1.61$, $p = 0.21$) although ASC males were shorter than control males ($t(64.38) = 2.08$, $p = 0.041$). As expected, on the AQ the ASC group scored significantly higher than controls, and control males scored significantly higher than control females (p-values from pairwise comparisons after Bonferroni correction, all $p < 0.01$). The time of blood collection was recorded. No main effects of group (ASC versus controls) or sex (males versus females) were found for the time of blood collection (group: $F(\text{df}, 1) = 0.46$, p-value = 0.5; sex: $F(\text{df}, 1) = 0.08$, p-value = 0.78), and nor was there any group by sex interaction ($F(\text{df}, 1) = 0.42$, p-value = 0.52). The descriptive characteristics of the sample and the time of blood collection are reported in Table 2.

The correlation between hormone levels and the time of blood collection was also analyzed in the whole sample, and in relation to diagnosis and sex. A significant inverse correlation with time of blood collection was found for free testosterone levels in the male sample. This did not survive a correction for multiple comparisons, and hence could have arisen by chance. No other significant correlations were found for any of the other hormones with time of blood collection. Table 3 reports the correlation coefficients and p-values for each hormone in ASC, controls, males and females.

To explore if different factors (sex, diagnosis, age and BMI) predicted hormone levels, we performed a regression analysis with sex, diagnosis, sex by diagnosis interaction, age and BMI (entered in the model in the reported order) as independent variables and each hormone level as the dependent variable. Diagnosis (without any interaction with sex) was found to predict the androstenedione levels in our sample. The results are shown in Table 4.

The between-group analysis showed that the ASC group had significantly higher levels of androstenedione than controls (Mann–Whitney $W = 2677$, $p = 0.002$). Since sex hormones such as androgens (including androstenedione) and estrogens have a different biology in males and females (e.g., in terms of final pathways, clinical effects and metabolism), we analyzed hormone levels separately in males and females. Between-group differences in androstenedione levels were also found within each sex (Mann–Whitney $W = 466$, $p = 0.045$ and $W = 247.5$, $p = 0.021$, in males and females respectively) and the familywise error rate corrected p-value was estimated using 10,000 permutations in females (corrected $p = 0.022$). The effect size was medium for androstenedione in females (Cohen's $d = 0.62$) and small for all the other hormones in both sexes (see Table 3). The data was

Table 3 Correlation between hormone levels and the time of blood collection according to diagnosis and gender.

Time of blood collection								
	ASC group		Control group		Males		Females	
	Pearson's r	p	Pearson's r	p	Pearson's r	p	Pearson's r	p
Androstenedione (nmol/L)	−0.08	0.52	−0.03	0.8	0.03	0.78	−0.08	0.44
DHEA-S (μmol/L)	0.14	0.26	0.15	0.12	0.2	0.07	0.13	0.23
Total testosterone (nmol/L)	−0.03	0.8	−0.06	0.55	−0.2	0.06	−0.06	0.58
Free testosterone (nmol/L)	−0.02	0.89	−0.05	0.58	−0.24	0.03[a]	−0.04	0.73
Estradiol (pmol/L)	−2.2	0.08	0.13	0.2	−0.03	0.81	−0.06	0.6

[a] Not significant at $p < 0.05$ after Bonferroni correction.

Table 4 Regression analysis with gender, diagnosis, gender by diagnosis interaction, age and BMI (entered in the model in the reported order) as independent variables and each hormone level as dependent variable.

	Coefficient	Standard error	t	$p > \|t\|$
Androstenedione (nmol/L)				
Gender (male)	0.21	0.51	−0.4	0.68
Diagnosis (ASC)	1.54	0.58	2.65	0.009**
Gender × diagnosis	−0.76	0.76	−1.003	0.32
Age	−0.09	0.02	−3.5	<0.00***
BMI	−0.03	0.05	−0.59	0.56
DHEA-S (μmol/L)				
Gender (male)	2.38	0.78	3.1	0.003**
Diagnosis (ASC)	0.17	0.88	0.2	0.84
Gender × diagnosis	1.05	1.15	0.92	0.36
Age	−0.23	0.04	−6	<0.00***
BMI	0.006	0.07	0.08	0.94
Total testosterone (nmol/L)				
Gender (male)	15.7	1.08	14.53	<0.00***
Diagnosis (ASC)	0.48	1.22	0.4	0.69
Gender × diagnosis	0.84	1.59	0.53	0.6
Age	0.01	0.05	0.24	0.8
BMI	−0.19	0.1	−1.9	0.06
Free testosterone (nmol/L)				
Gender (male)	0.35	0.02	19.6	<0.00***
Diagnosis (ASC)	−0.001	0.02	−0.04	0.96
Gender × diagnosis	0.003	0.03	0.12	0.9
Age	−0.003	0.001	−3.2	0.002**
BMI	0.001	0.002	0.5	0.63
Estradiol (pmol/L)				
Gender (male)	−164.6	32.72	−5.03	<0.00***
Diagnosis (ASC)	41.55	36.84	1.13	0.26
Gender × diagnosis	−44.89	48.27	−0.93	0.35
Age	1.45	1.6	0.9	0.4
BMI	3.1	2.98	1.03	0.3

**p-value < 0.01, ***p-value < 0.001.

reanalyzed after excluding the 7 participants with ASC who were on medication. This did not significantly change the p-values of the observed effects (androstenedione: $W = 821$, $p = 0.045$ and $W = 478.5$, $p = 0.03$ in males and females respectively). No between-group differences were observed in free testosterone levels ($W = 604.5$, $p = 0.66$ and $W = 332.5$, $p = 0.37$, in males and females respectively) or in testosterone to estradiol ratio ($W = 361$, $p = 0.76$ and $W = 398$, $p = 0.87$, in males and females respectively). Hormone levels (median and interquartile range), between-group comparisons and the effect sizes are shown in Table 5.

Fig. 2 shows the density estimation of androstenedione levels in ASC versus control, in males and females respectively.

Finally, hormones levels in the females were analyzed in terms of the phase of the menstrual cycle (follicular phase, ovulatory phase and luteal phase) that each woman was in at the time of collection of each blood sample. Between-group analyses (females with ASC versus typical females) were also conducted in the follicular and luteal phase using a Mann–Whitney's U-test. Although the hormone values (see median and interquartile range) parallel those displayed in ASC and

Table 5 Hormone levels (median and interquartile range), between-group comparisons and effect sizes, in the final ASC and control samples.

	Males					Females				
	ASC ($n = 33$)	Controls ($n = 39$)	W	p-Value	Cohen's d	ASC ($n = 25$)	Controls ($n = 31$)	W	p-Value	Cohen's d
Androstenedione (nmol/L)	7.53 (1.64)	6.69 (1.92)	466	0.045*	0.39	7.42 (2.52)	6.41 (2.31)	247.5	0.021*	0.61
DHEA-S (μmol/L)	8.71 (5.58)	7.92 (4.01)	537.5	0.23	0.28	4.86 (2.5)	4.72 (2)	370.5	0.79	0.09
Total testosterone (nmol/L)	16.1 (4.1)	15.6 (6.4)	602	0.64	0.2	0.9 (0.6)	0.8 (0.6)	358	0.63	0.16
Free testosterone (nmol/L)	0.37 (0.12)	0.37 (0.12)	604.5	0.66	0.02	0.01 (0.01)	0.009 (0.01)	332.5	0.37	0.19
Estradiol (pmol/L)	90.2 (38.3)	85.3 (31.35)	627.5	0.86	0.01	251 (247)	205 (206)	316	0.24	0.38

W: = Mann–Whitney's U-value.
*p-Value < 0.05.

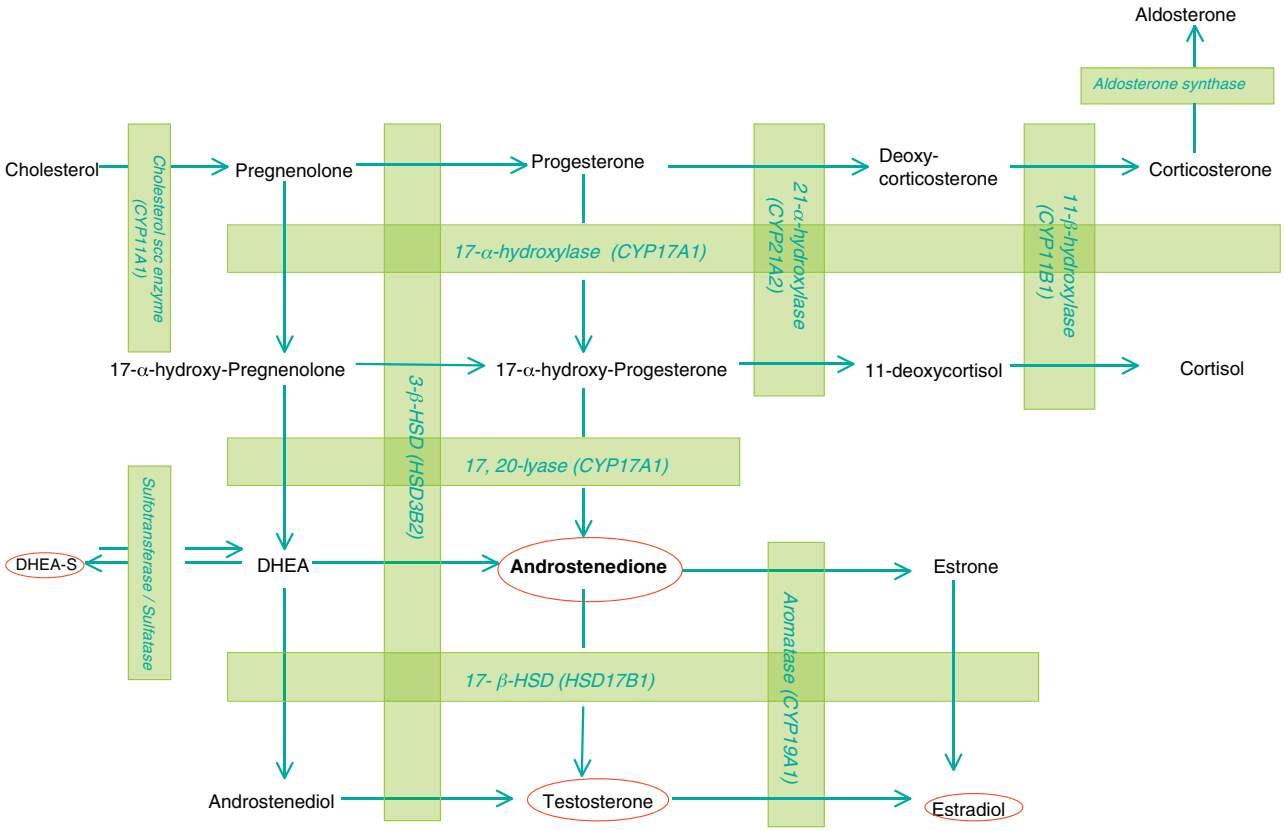

Figure 1 Pathway of steroid synthesis. The names for each enzyme and the correspondent gene in parenthesis are shown by each reaction. Circled in red are the hormones that were analyzed.

control females in Table 5, no significant group differences were found. The number of subjects and the hormone levels in ASC and control females in the different phases of period are shown in Table 6.

4. Discussion

In the present study, we tested if adrenal and gonadal sex hormones (in particular testosterone, estradiol, DHEA-S and

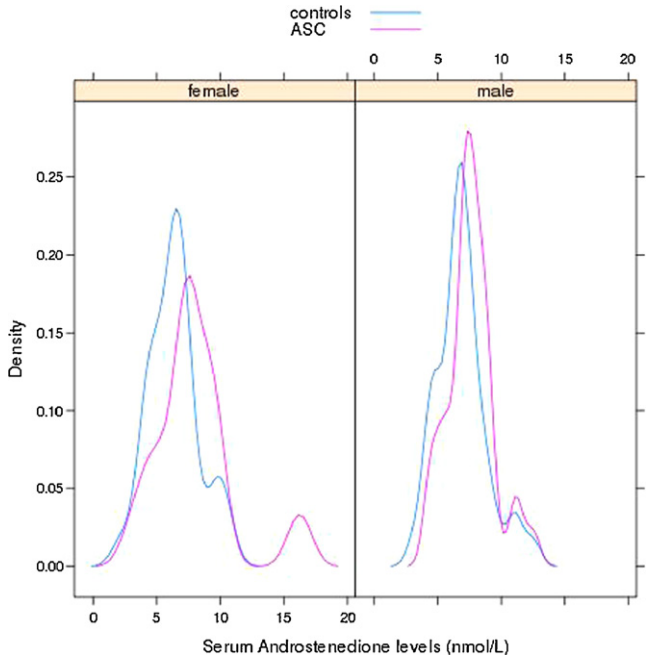

Figure 2 Kernel probability density function of androstenedione levels in males and females, ASC versus controls.

Table 6 Hormone levels (median and interquartile range), in ASC and control females according to the phases of the menstrual cycle.

	Follicular phase (day 1–12)		Ovulatory phase (day 13–15)		Luteal phase (day 16–33)	
	ASC (n = 12)	Controls (n = 9)	ASC (n = 1)	Controls (n = 5)	ASC (n = 11)	Controls (n = 12)
Androstenedione (nmol/L)	6.82 (3.13)	5.32 (2.63)	7.42 (/)	7.21 (0.63)	7.95 (1.64)	6.59 (1.79)
DHEA-S (μmol/L)	4.03 (3.39)	4.04 (3.27)	7.87 (/)	5.16 (0.65)	5.02 (1.28)	4.71 (2.58)
Total testosterone (nmol/L)	0.75 (0.5)	0.7 (0.4)	0.9 (/)	1.1 (0.3)	1 (0.55)	0.9 (0.57)
Free testosterone (nmol/L)	0.01 (0.009)	0.009 (0.005)	0.01 (/)	0.01 (0.003)	0.01 (0.008)	0.009 (0.007)
Estradiol (pmol/L)	274 (241.25)	179 (149)	243 (/)	178 (146)	266 (255.5)	321 (213.5)

1 ASC female and 5 control females deleted due to missing data. Mann–Whitney's U-test for follicular and luteal phase, all p-values > 0.05.

androstenedione) were elevated in an adult sample of male and female individuals with ASC compared with typical matched controls. Regression analysis showed that diagnosis strongly ($p < 0.01$) predicted androstenedione levels. Serum androstenedione levels were also significantly elevated in the ASC group, a result confirmed by permutation testing in females.

This result, of higher androstenedione levels in both ASC males and female, is in line with our previous findings (reviewed in Section 1 above) and is consistent with both the foetal androgen and the extreme male brain (EMB) theories (Baron-Cohen, 2002). Androstenedione is the direct precursor of testosterone, and circulating androstenedione is taken up in hormone-sensitive tissues and converted into the final active androgen metabolites (see Fig. 1). The observed higher serum androstenedione levels in women with ASC are consistent with our earlier findings of elevated rates of androgen-related medical conditions (PCOS, hirsutism, acne, and hormone-sensitive cancers) in females with ASC and their mothers (Ingudomnukul et al., 2007). Indeed, higher androstenedione serum levels, deriving from both adrenal glands and gonads are frequently observed in women with PCOS and in approximately 10% of cases androstenedione is the sole androgen found increased in the peripheral blood (Knochenhauer et al., 1998; Azziz et al., 2006).

The two key enzymes that catalyse the production of androstenedione, 17-alpha-hydroxylase and 17-20 lyase, are both coded by the gene *CYP17A1* and we previously found that SNPs in *CYP17A1* were significantly associated with Asperger Syndrome (Chakrabarti et al., 2009). Furthermore, upregulation of the 17-20 lyase activity, leading to adrenal hyperandrogenism, has been related to early exposure to excess androgen during foetal life in nonhuman primate models of PCOS (Zhou et al., 2005).

To better understand the molecular actions of androstenedione in the target tissues, it is important to define the signalling mechanisms through which it acts. Androstenedione (like the other androgens testosterone and dihydrotestosterone) exerts most of its effects by interacting with the androgen receptor (AR), which activates gene expression at the transcriptional level in the nucleus. However, a novel indirect mechanism has been recognized: it has been found that androstenedione can also react with cell surface receptors and thus activate cytosolic biochemical pathways (Machelon et al., 1998). This mechanism is related to a rapid rise in cytosolic calcium. Interestingly, there is some evidence for altered calcium homeostasis, leading to altered mitochondrial metabolism, in the brains of subjects with autism (Palmieri et al., 2010). Future research in autism should test for such possible alterations in relation to androgens.

In typical individuals, the androstenedione metabolic pathway is differentiated between males and females, so it is of some interest that statistically no sex differences were found in androstenedione levels between males and females with ASC ($p < 0.05$ in both sexes). This is further evidence of dysregulation, in this case defined by the *absence* of the typical sexual dimorphism. In typical males, testes are the main source of testosterone and the mean values of such circulating strong androgens are much higher than in females. Thus in typical males the role of androstenedione in the peripheral production of testosterone is less important: less than 0.3% of serum testosterone derives from the conversion of serum androstenedione to testosterone. In contrast, in typical females, the opposite is the case: 60% of testosterone production comes from the peripheral conversion of androstenedione (about 0.14 mg/24 h), while only tiny amounts of testosterone derive from ovarian (about 0.05 mg/24 h) or adrenal secretion (0.02 mg/24 h) (Horton and Tait, 1966). We must therefore assume that if females with ASC have hyper-masculinized levels of androstenedione, this would lead to hyper-masculinization in different tissues, including the brain.

No significant between-group differences were found for DHEA-S, total and free testosterone, and estradiol. Within females, no significant group differences in the hormone levels were found in the different phases of the menstrual cycle. We cannot rule out that these non-significant results are due to a lack of statistical power, due to the small sample size in each group.

Why DHEA-S was not significantly increased in ASC also needs to be clarified, as does the relationship between DHEA-S and DHEA in our sample. To our knowledge, only a few studies have investigated DHEA-S levels in autism, with inconsistent results. Croonenberghs et al. (2008) found that 5-HTP induced plasma DHEA-S levels were significantly higher in ASC than controls, suggesting that a disequilibrium in the peripheral serotonergic metabolism may influence DHEA-S

levels. Tordjman et al. (1995) found no difference in DHEA-S levels in pre- and post-pubertal males with ASC, whereas Strous et al. (2005) found significantly lower levels of DHEA-S, but not of DHEA, in ASC. As shown in Fig. 1, unconjugated DHEA can either proceed via steroidogenesis and be converted into androstenedione, or can be converted into the steroid sulfate form DHEA-S by the steroid sulfatase enzyme (STS). It might be that a dysregulation of this enzyme activity influences the final levels of DHEA-S. Interestingly, X-linked ichthyosis, a genetic condition caused by a deficiency of this enzyme, is associated with cognitive difficulties including autism (Kent et al., 2008).

It may be that we did not find any significant differences in current serum levels of testosterone in our sample with ASC because they were adults, if the hypothesized androgen effects occur prenatally, at an early stage of brain development. However, Schmidtova et al. (2010) in their study on prepubertal and pubertal children did find significantly increased levels of salivary testosterone in ASC children, relative to controls. These results may thus be age-related, since puberty is a crucial period for preserving the sexual dimorphism of the nervous system established pre- and perinatally. Hence, it may be that a higher peak of testosterone occurs in ASC in the pubertal phase. This hypothesis should be further explored. Studies of adolescents and adults with ASC showed contrasting results in males and females. Croonenberghs et al. (2010) found significantly lower concentration of serum testosterone in male postpubertal participants with ASC compared to matched controls, while Schwarz et al. (2010) found elevated levels of free-testosterone in females with Asperger Syndome compared with control females, in a multi-analyte profiling of blood serum. It should be borne in mind that factors such as age, sex, time of blood collection, habits of life (smoke, exercise, diet), stress and medication may affect testosterone peripheral turnover. In addition, peripheral testosterone levels may not reliably correlate with actual levels of testosterone in the brain, since intracrine mechanisms and other intersecting pathways are likely to be involved. In fact, sex steroids can be synthesized *de novo* in the brain (neurosteroids) and/or metabolized in situ (neuroactive steroids) from their precursors (such as androstenedione and DHEA-S) (Compagnone and Mellon, 2000; Stoffel-Wagner, 2001), so that serum levels may not necessarily reflect what happens in nervous system. This may mean an important caveat is required regarding the potential use of steroid serum levels as biomarkers.

Finally, we did not find any difference in estradiol levels between ASC and controls. However, the role of estrogens in the pathogenesis of autism still warrants further investigation. This is because androgen and estrogen pathways are coupled together, since androgen precursors (androstenedione and testosterone) can be converted into estrogens (estrone and estradiol respectively) in the target tissues by the cytochrome P450 aromatase enzyme. Furthermore, evidence from rodent models suggests that estradiol may control prenatal brain and behavioural sexual differentiation by exerting defeminizing actions in male brains. According to this hypothesis, the developing male brain might be exposed prenatally to high levels of estrogens derived from neural aromatization of testosterone (Bakker and Baum, 2008). However, in primate species androgens, not estrogens, seem to be the main hormones that cause brain masculinization (Wallen, 2005). A final reason for continuing to explore the role of estrogens in ASC is that some studies have found strongly significant association with the *ESR2* gene, which is expressed in the brain (Chakrabarti et al., 2009).

We found that ASC males were significantly shorter than control males. This finding could be followed up since androgen excess in childhood is related to early puberty, rapid growth and — perhaps paradoxically — early epiphyseal fusion (mediated by local aromatization of androgens into estrogens), leading to shorter stature in adulthood (Cutler, 1997; Perry et al., 2008). Growth stature is however multiply determined, by genetic, hormonal and environmental factors, so we simply report this interesting finding for further research.

In conclusion, the present study found elevated serum androstenedione levels in ASC. As far as we know this result has not been previously documented. Together with the evidence that several genes involved in steroidogenesis (in particular the *CYP17A1* gene) are associated with ASC, that prenatal androgen levels are associated with autistic traits, and that higher rates of PCOS and androgen related conditions are reported in women with ASC, this new finding points to dysregulation of the androgen pathway in this condition. It may also be very relevant to the foetal androgen theory of ASC. The novel finding of increased androstenedione serum levels in adults with ASC needs to be independently replicated and warrants further research into this important group of hormones — the androgens — in the pathophysiology of autism.

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Conflict of interest

None declared.

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