

# Salivary testosterone levels and autism-spectrum quotient in adults

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Submitted: 2010-01-08 Accepted: 2010-10-05 Published online: 2011-01-09

Key words: testosterone; autism; sex differences

Neuroendocrinol Lett 2010;31(6):837-841 PMID: 21196912 NEL310610A18 ©2010 Neuroendocrinology Letters • www.nel.edu

## Abstract

**OBJECTIVES:** The purpose of this study is to examine the relationship between salivary testosterone levels and autistic traits in adults.

**METHODS:** A total of 92 male and female adults participated in the present study. Their salivary testosterone level (T) and score of Japanese version of Autism-spectrum Quotient (AQ) were assessed to examine the relationship between salivary testosterone level and autistic traits in adults.

**RESULTS:** We observed a positive correlation between T and AQ in a group of both sexes. The correlation disappeared when we conducted correlation analysis by sex. However, although there was no sex difference in the score of the subscale of attention switching, attention switching was related to T.

**CONCLUSIONS:** Although the relationship between T and AQ may mainly result from sex differences, the subscale of attention switching may be modulated by testosterone.

## INTRODUCTION

Autism is the one of the developmental disorders characterized by deficits in empathy, and the ability of communication and building good relationships between peers and behavioral traits such as narrow interest and repetitive behavior (APA 2000). Autism was first reported in mid 20th century (Kanner 1943; Asperger 1944). Today, not only autism, but also high-functioning autism, asperger syndrome, and pervasive developmental disorder are collectively called autism spectrum disorders (ASD). One of the most influential

theories is the “extreme male brain” (EMB) theory proposed by Simon Baron-Cohen (Baron-Cohen 2002; 2003; 2009). EMB theory was based on the fact that ASD has predominantly been observed in males: the incidence of ASD in males is four times higher than in females. The EMB theory pointed that ASD is related to the degree of prenatal exposure to testosterone. Between 8 and 24 weeks of gestation, fetuses are exposed to testosterone in the womb and sex differentiation of brain was occurred (Baron-Cohen *et al.* 2004). The key point

of EMB theory is that the higher fetuses were exposed to testosterone in the womb, the stronger fetuses show autistic trait, e.g., low empathizing and high systemizing tendencies (Baron-Cohen 2002; 2003).

To test EMB theory, Auyeung and colleagues conducted a longitudinal study to examine the relationship between the fetal testosterone level in amniotic fluid and autistic traits of children measured by the psychological scales (the Child Autism Spectrum Quotient and the Childhood Autism Spectrum Test) (Auyeung *et al.* 2009). Their results showed that fetal testosterone levels were positively correlated with the children's autistic traits. Furthermore, Manning and his colleagues (2001) showed that 2D:4D ratio which is the ratio between the length of the second and fourth digit (2D/4D) was negatively correlated with autistic traits. Their study further showed that 2D:4D ratio reflected fetal testosterone levels and males had lower 2D:4D ratio than females (Manning *et al.* 1998). Thus these studies showed that fetal testosterone levels were strongly related to autistic traits.

Psychoneuroendocrinological studies have reported that salivary testosterone level is strongly associated with social cognition (van Honk *et al.* 1999; van Honk *et al.* 2000). Therefore, it can be conceived that salivary testosterone level in adults is also related to their autistic tendency, which is also related to cognitive styles (e.g., empathizing and systemizing) in social situations. However, to date no study examined the relationship between salivary testosterone level and autistic trait in healthy adults. Because (i) we have previously observed sex differences in the relationship between salivary cortisol levels and Baron-Cohen's Empathy and Systemizing Quotients in young adults (Nakayama *et al.* 2007), (ii) testosterone administration reduced empathic behavior in young adults (Hermans *et al.* 2006), (iii) salivary testosterone level reflects the level of bioavailable testosterone which can affect neural activities in the brain mediating social behavior and cognition (Sakaguchi 2007), and (iv) salivary testosterone level in adults cannot be estimated from their 2D:4D ratio (Yang *et al.* 2008), it is important to examine the relationship between salivary testosterone levels and Autism-spectrum Quotient (AQ), by the direct measurement of testosterone concentration in saliva with high precision. In summary, the purpose of this study is to examine the relationship between salivary testosterone levels and autistic traits in adults. We collected saliva in adults to measure the level of salivary testosterone with LC/MS (liquid chromatography/mass spectroscopy) and utilized the Japanese version of AQ (Wakabayashi *et al.* 2004) to measure the autistic traits in adults. We hypothesized that the higher salivary testosterone levels, the higher the AQ scores.

## METHODS

### Subjects

A total of 92 healthy, Japanese adults (Male = 45, Female = 47) who live around Hokkaido University participated in the study. The mean age of the subject is 47.9 ( $SD = 12.4$ ; age range = 21 to 68). All participants were recruited from the local newspapers. All participants submitted a consent form prior to the research. This study was conducted under a protocol approved by the Ethical Committee of the Center for Experimental Research in Social Sciences at Hokkaido University.

### Procedure

All participants arrived at the Center at 9:00 AM and were informed about the experiment by the experimenters. The saliva sampling procedures were the same as those in our previous study (Takahashi *et al.* 2006). The experimenter collected each participant's saliva by spitting into a 50-ml Bakelite tube in the morning (9:00–9:30 AM). The saliva collection took place in the Center for Experimental Research in Social Sciences. They were also instructed to maintain an interval of at least 1.5 h after eating or drinking any fluid other than water and 30 min after any strenuous exercise prior to collecting the saliva samples. Further, they were requested to refrain from brushing their teeth vigorously and from other dental care procedures that could possibly damage the inside of the mouth (Granger *et al.* 2004).

The saliva samples collected were immediately frozen and kept at  $-20^{\circ}\text{C}$  until hormonal assays. All procedures of measuring salivary testosterone levels with LC/MS (liquid chromatography/mass spectroscopy) were conducted at the ASKA pharmaceutical company (Tokyo, Japan), which has significant experience in hormonal assays (Takahashi *et al.* 2006; Sakaguchi *et al.* 2007). We employed LC/MS for the hormonal assays, rather than a radioimmunoassay (RIA), because RIA is susceptible to the effect of foreign substances (Granger *et al.* 2004). For measuring the testosterone levels, 1 ml of saliva from each sample was used. An analytical curve was created from 5-, 10-, 20-, 65-, 125-, 500-, and 1,000-pg standard testosterone (Sigma-Aldrich, Tokyo, Japan) in 50  $\mu\text{l}$  methanol. These standard analytes were processed in the same manner as the other analytes. The internal standard was 1 ng T-d3 (Sigma-Aldrich) in 50  $\mu\text{l}$  methanol, and it was added to every analyte. The standard testosterone samples were diluted with 1 ml purified water. Two hundred microliters of a 2% dichloromethane solution of 2-Fluoro-N-methylpyridinium p-sulfonate (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan) and 30  $\mu\text{l}$  of a 10% dichloromethane solution of triethylamine were added to the ether-extracted analytes and maintained at the ambient temperature for 1.5 h for derivatization (Quirke *et al.* 1994). The solvent was then removed by evaporation and the analytes were dissolved in a 25% aqueous solution of methanol (1 ml)

**Tab. 1.** AQ scores by sex.

Variables	Male (N = 45)		Female (N = 47)		t
	M	SD	M	SD	
Social skill	3.58	2.54	3.06	2.51	0.98
Attention switching	4.33	2.10	3.85	2.00	1.13
Attention to detail	4.53	2.43	4.72	2.24	0.39
Communication	3.24	2.49	2.19	1.93	2.27 *
Imagination	4.02	1.82	2.83	1.51	3.43 ***
Total AQ	19.71	7.02	16.66	5.73	2.29 *

\*  $p < 0.05$ , \*\*\*  $p < 0.001$ 

and charged on a Bond Elute C18 cartridge (Varian, Palo Alto, CA) conditioned with methanol and deionized water. The cartridge was successively rinsed with 1 ml deionized water, 3 ml ammonia water, 2 ml methanol, and a mixture of 0.01% aqueous solutions of formic acid and methanol (3 ml). The analyte was eluted with a mixture of 10% aqueous solutions of formic acid and acetonitrile (2.5 ml). The analytes were measured with an electrospray mass spectrometer API4000TM (Applied Biosystems/ MDS SCIEX, Tokyo, Japan) in the positive ion mode. This device monitored the  $m/z$  380 to  $m/z$  253 (T) and  $m/z$  383 to  $m/z$  256 (T-d3) transitions. The ion spray voltage was set to 5,000 V.

#### Autism-spectrum Quotient

All participants filled out Autism-spectrum quotient (AQ) (Baron-Cohen *et al.* 2001). We used the Japanese version of AQ (Wakabayashi *et al.* 2004). The AQ is a self-reported questionnaire which measures the tendency of ASD in both autistic and healthy populations. This scale was mostly used to detect ASD and the score of AQ is significantly higher in people with ASD than people without ASD (Baron-Cohen *et al.* 2001). The AQ contains 50 items and is composed of five subscales (social skill, attention switching, attention to detail, communication and imagination).

## RESULTS

The mean of the AQ score was 18.15 ( $SD = 6.54$ ) and this scale had high internal validity (Cronbach's alpha coefficients = 0.83). Sex differences in the mean score of AQ were shown in Table 1. The mean score of male's AQ was significantly higher than that of female's AQ (Male:  $M = 19.71$ ,  $SD = 7.02$ ; Female:  $M = 16.66$ ,  $SD = 5.73$ ;  $t(90) = 2.29$ ,  $p < 0.05$ ), consistent with the previous study employing Japanese adults (Wakabayashi *et al.* 2004). In subscales of the AQ, the mean scores of male's communication and imagination were significantly higher those of female's communication ( $t(90) = 2.27$ ,  $p < 0.05$ ) and imagination ( $t(90) = 3.43$ ,  $p < 0.001$ ).

Salivary testosterone levels in males were significantly higher than that in females (Male:  $M = 54.17$  pg/mL,  $SD = 18.60$ ; Female:  $M = 10.69$  pg/mL,  $SD = 4.47$ ;

$t(90) = 15.57$ ,  $p < 0.0001$ ) and decrease with age but not in females (Male:  $r = -0.38$ ,  $p < 0.01$ ; Female:  $r = 0.13$ ,  $p = 0.40$ ) (Figure 1). This pattern was also observed in the previous study in Japanese men (Sakaguchi *et al.* 2007). To examine the relationship between the score of AQ and salivary testosterone levels in both sexes, we conducted Pearson's correlation analysis. The analysis demonstrated a significant positive correlation between the score of AQ and salivary testosterone levels in the subjects including both males and females ( $r = 0.28$ ,  $p < 0.01$ ) (Table 2). Scatterplot of the score of AQ and salivary testosterone levels is shown in Figure 2. The positive correlation between the score of AQ and salivary testosterone levels remained significant when the effect of age was controlled ( $r = 0.28$ ,  $p < 0.01$ ). In subscales, especially, communication and imagina-

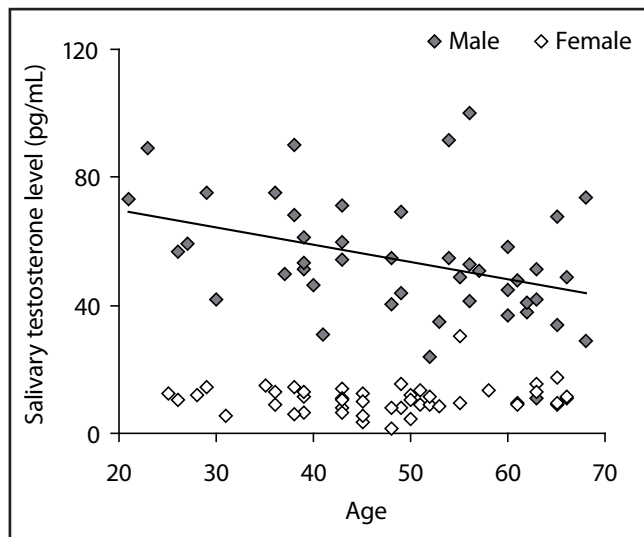
**Tab. 2.** Correlation for testosterone level and AQ scores.

	Male (N = 45)	Female (N = 47)	All (N = 92)
Social skill	0.10	0.17	0.14
Attention switching	0.30 *	0.07	0.22 *
Attention to detail	-0.16	-0.19	-0.11
Communication	0.22	0.25 †	0.30 **
Imagination	0.13	0.15	0.35 **
Total AQ	0.18	0.15	0.28 **

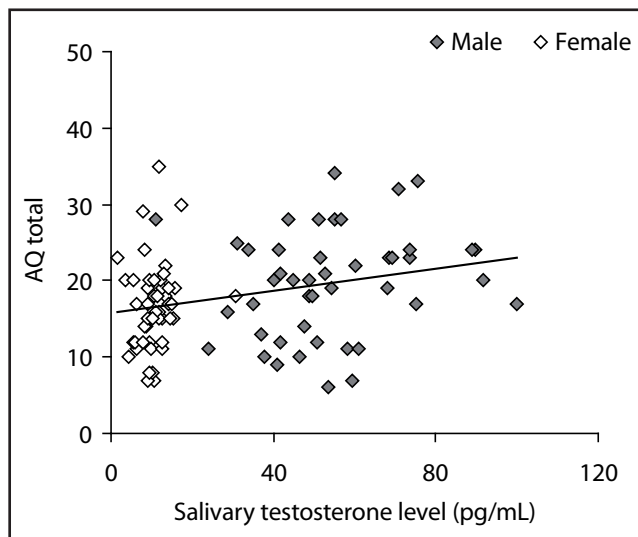
†  $p < 0.10$ , \*  $p < 0.05$ , \*\*  $p < 0.01$ **Tab. 3.** Partial correlation for testosterone level and AQ scores (age was controlled).

	Male (N = 45)	Female (N = 47)	All (N = 92)
Social skill	0.04	0.17	0.13
Attention switching	0.25 †	0.12	0.21 *
Attention to detail	-0.12	-0.17	-0.11
Communication	0.19	0.26 †	0.30 **
Imagination	0.02	0.14	0.35 ***
Total AQ	0.12	0.17	0.28 **

†  $p < 0.10$ , \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$



**Fig. 1.** Relationship between age and salivary testosterone level. Horizontal axis indicates age and vertical axis indicates salivary testosterone level. The regressed line is for males.



**Fig. 2.** Relationship between salivary testosterone level and AQ score. Horizontal axis indicates salivary testosterone level and vertical axis indicates AQ (Autism-spectrum Quotient). The regressed line is for male and female data..

tion were significantly correlated with the score of AQ (Attention switching:  $r=0.22$ ,  $p<0.05$ ; communication:  $r=0.30$ ,  $p<0.01$ ; imagination:  $r=0.35$ ,  $p<0.001$ ). These results showed that the higher the salivary testosterone level, the higher the participants scored on subscales of AQ. However, positive correlation between the total score of AQ and the salivary testosterone level disappeared when we conducted Pearson's correlation analysis by sex (Male:  $r=0.18$ ,  $p=0.22$ ; Female:  $r=0.15$ ,  $p=0.32$ ) (Table 2).

## DISCUSSION

This study is the first challenge to examine between salivary testosterone levels and the score of AQ in adults. Our results showed that salivary testosterone levels positively correlated with the scores of AQ in adults including both men and women, when the effect of age was controlled. This finding is similar to the observed pattern in Auyeung and colleagues' study (2009) regarding the relationship between fetal testosterone levels and autistic trait in male and female children. However, the positive correlation disappeared when we conducted the correlation analysis by sex. There are, at least, two possible interpretations for these findings. First, the concentration range of salivary testosterone level within each sex was too small to detect the relationship between salivary testosterone level and AQ within each sex. Second, the observed positive correlation between salivary testosterone levels and the scores of AQ in a group consisting of both sexes may have been derived from sex differences. As presented in the result section, there were significant sex differences in total AQ and the subscales of imagination and communication. We also observed significant effects of testosterone on these

three types of scores. This consideration implies that sex differences might have produced the observed relationship between testosterone and the three types of scores (i.e., total AQ, imagination, and communication) in the present group consisting of male and female subjects. Regarding the subscale of attention switching, however, although there was no significant sex difference in the subscale, we observed the significant relationship between salivary testosterone level and attention switching in the group of male and female subjects (attention switching also tended to correlate with salivary testosterone in males). Therefore, it is possible that testosterone has effect on this subscale of attention switching. Future studies should examine whether these considerations are correct or not, by employing a larger population including autistic patients.

## ACKNOWLEDGEMENT

This study was supported by Grant-in-Aid for Scientific Research on Priority Area (1904600508).

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