*Journal of Behavioral and Brain Science*, **2013**, **3**, **518-521** Published Online November 2013 (http://www.scirp.org/journal/jbbs) http://dx.doi.org/10.4236/ibbs.2013.37054



# Sex-Role Orientation in Men Is Related to Salivary Testosterone Levels

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Received September 9, 2013; revised October 8, 2013; accepted October 21, 2013

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### ABSTRACT

Previous research has implicated the involvement of androgens in sex-role orientation in males, from studies of 2<sup>nd</sup> to 4<sup>th</sup> digit ratio (a purported marker of prenatal testosterone). The present pilot study investigates the relationship between salivary testosterone levels and sex-role orientation using Bem Sex Role Inventory (BSRI) scores in adult males. Twenty-one males (aged 18 - 24) completed the BSRI and provided saliva samples for assay. BSRI Femininity scores were significantly negatively correlated with testosterone levels; the higher the Femininity scores, the lower the testosterone levels. There was no relation of BSRI Masculinity scores with testosterone levels. Our preliminary results add to the research suggesting that sex-role orientation in males may be partially related to underlying hormone levels.

Keywords: Testosterone; Androgens; Sex-Role; Masculinity; Femininity; Gender; Personality

## 1. Introduction

The aim of the present pilot study was to explore the relationship between salivary testosterone levels and sex role identity in typically developed adult males, as measured with the Bem Sex Role Inventory (BSRI; [1]). Previous research with typically developed adult females has linked scores on the BSRI with salivary testosterone levels [2]; with higher scorers on the Masculinity scale having significantly higher testosterone levels than those with lower Masculinity scores. Androgenous and masculine-sex-typed females (as classified by the BSRI) have also been found to possess higher salivary testosterone levels than feminine-sex-typed females [3]. In addition, females with a low 2<sup>nd</sup> to 4<sup>th</sup> digit ratio (a purported marker of high prenatal testosterone levels) have been found to show masculinised bias scores in the BSRI [4].

These findings in typically developed females parallel the findings in clinical studies on females with congenital adrenal hyperplasia (CAH), a condition caused by a deficiency in the enzymes necessary for steroidogenesis within the adrenal glands, ultimately resulting in a hypersecretion of adrenal androgens. In addition to physical masculinisation, females with CAH have been shown to demonstrate less interest in infants, less interest in female-typical toys (e.g. dolls) and female playmates, more interest in male-typical toys and male playmates, score lower on measures of nurturant tendencies, and show a masculine bias on a variety of personality inventories compared with control females (see [5] for review).

In contrast to the research in females, no studies to date have looked at how typically developed adult male's sex role orientation relates to their testosterone levels. Many studies have investigated 2<sup>nd</sup> to 4<sup>th</sup> digit ratio (2D:4D) in relation to men's sex role orientation using the BSRI (e.g. [6-8]), however, the results have been equivocal. A recent large-scale study [9] found no associations between 2D:4D and sex-role orientation (including BSRI scores) in men. However, the same authors meta-analysis of 28 studies showed a reliable association of men's 2D:4D and sex-role orientation (the majority of studies used the BSRI) with more masculine scorers having lower (more masculine) 2D:4D, albeit with a very small effect size [9].

Convincing evidence for the involvement of androgens in sex-role behaviour in males comes from genetic studies in males with impaired androgen function. The semi-

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nal studies in this area [10,11] investigated genetic males with either of two autosomal recessive mutations that impair androgen synthesis or metabolism during embryogenesis [17b-hydroxysteroid dehydrogenase 3 (17b-HS-D3) deficiency or steroid 5a-reductase 2 (5a-R2) deficiency] and result in formation of female external genitalia and therefore female sex of rearing. The authors found that these males frequently change gender role behaviour from female to male at or after the time of puberty, whereas genetic males with mutations that permanently impair the function of the androgen receptor similarly show a female phenotype at birth, and are raised as females, yet retain female-gender type behaviour as adults (see [5] for review).

Although no studies have related testosterone levels tosex-role orientation in typically developed adult males, recent research has demonstrated links between testosterone levels and variability in sex-typical behaviours in typically developing male boys. Auyeung *et al.* (2009) found that Prenatal testosterone levels measured in amniotic fluid relate positively to male-typical scores on a parental report questionnaire of sex-typical play in boys (and girls) [12]. Collectively these studies reviewed above, in typically developed males and in clinical populations, suggest that androgens may constitute one of the important determinants of sex-role orientation in males.

The current pilot study investigates how salivary testosterone levels in typically developed adult males relate to sex-role identity as measured by the Bem Sex Role Inventory (BSRI). Based on previous research, it is expected that men with higher salivary testosterone levels will have more male-typical scores on the BSRI.

### 2. Methods

#### 2.1. Participants

Twenty-one healthy male students (age 18 - 24, mean age = 21.00, SD = 1.81) were recruited from the University of Stirling, Scotland. All participants were of European ethnic origin (in order to minimise variance in testosterone levels found between different ethnic groups e.g. [13]). The age range of participants was limited to young adults in order to minimise variation due to agerelated declines in testosterone levels (albeit a slight decline of maximal 1.6% per year; see [14] for review). All participants were undergraduate or postgraduate students and were paid for their participation.

#### 2.2. Measurement of Sex Role Orientation

All subjects completed the Bem Sex Role Inventory (BSRI; [1]). The BSRI was developed in order to measure psychological masculinity and femininity [1]. The BSRI is a widely used instrument, and has been demonstrated to have strong psychometric properties (see [15]

for review). There are 60 items in the BSRI (20 feminine, 20 masculine and 20 non-gender related items). Using a seven-point Likert-type scale (1, never and almost never true; 7, always or almost always true), respondent sindicate how well each characteristic fits their self perception. Scores for the masculine and feminine items are calculated for each scale separately, giving each participant a psychological Masculinity and psychological Femininity score. For both scales, higher scores indicate higher levels of masculine or feminine personality traits, respectively.

#### 2.3. Measurement of Testosterone

Saliva was collected at the same time of day for each participant (2 - 4 pm), in order to minimise the impact of diurnal variation in testosterone levels. Saliva methods can measure the "free" portion of testosterone which is not bound to sex-hormone binding globulin, and is therefore free to bind to receptors. Salivary testosterone concentrations are highly correlated with serum testosterone concentrations, and single measurements have been demonstrated to be reliable enough for use in behavioural research (e.g. [16]). Salivary flow was stimulated by having participants chew on a quarter stick of sugar free gum. Typically, participants collected between 3 and 5 mL. Testosterone levels were assayed by the biological sciences laboratory at Queen Margaret University College using an "in-house" enzyme-linked immunosorbant assay (ELISA). The assay procedure is based on the indirect, competitive binding technique [17]. Samples were first extracted using diethyl-ether. Four millilitres of ether was added to 500 µL of sample, vortex mixed for 10 min and then frozen at -80°C until the aqueous phase was frozen. The unfrozen ether was decanted and evaporated with forced nitrogen. Samples were finally reconstituted with 500 µL of assay buffer and vortex mixed prior to assay. Assay sensitivity was 1.24 pg/mL; intraand inter-assay imprecision coefficients of variation, obtained over 50 assay runs, were 5.7% and 8.7%, respectively; cross reactivity with related compounds was minimal and the standard curve was highly reproducible (r = 0.998). Mean testosterone levels were 0.19 ng/mL (S.D. = 0.07) which falls within population norms for males in the laboratory at that time of day (2 - 4 pm).

#### 3. Results

Shapiro-Wilk tests for normality revealed that not all variables were normally distributed, therefore Spearman's Rank correlations were used. Although Pearson's R correlation analyses produced comparable results, the Spearman's Rank correlation co-efficients are reported as they represent a more conservative measure of correlation. One-tailed tests were used as clear directional predictions were made.

Testosterone levels were significantly negatively correlated with BSRI femininity scores (r = -0.415, n = 21, p = 0.031) see **Figure 1**. There was no significant correlation between testosterone levels and BSRI masculinity scores (r = 0.096, n = 21, p = 0.340).

#### 4. Discussion

The results of this pilot study demonstrate a statistically significant negative association between male salivary testosterone levels and scores on the BSRI femininity scale; those men with higher testosterone levels showed lower femininity scores. This result adds support to the idea that sex-role personality traits may be related to underlying androgen levels. Previous research has implicated the involvement of androgens in sex-role behaviour in males with disorders of impaired androgen function, and in sex-role orientation in typically developed males in studies measuring 2D:4D. The current pilot study is the first to suggest there is a relationship between salivary testosterone levels and sex-role orientation in typical males.

The current study found no correlation between maletestosterone levels and BSRI masculinity scores. Although this result might be unexpected, it is comparable to a study investigating the same variables in women [2]. These authors found women with higher testosterone levels had higher BSRI masculinity scores than those with lower testosterone levels, whereas there was no relationship between testosterone levels and BSRI femininity scores [2]. Therefore, in both this previous research [2] and the current study, testosterone was seen to relate to the *opposite*-sex trait scale. Additional research is needed to elucidate the factors producing this result, including replication of the study with a larger sample size. How-

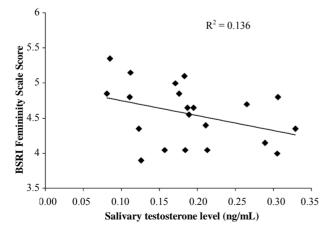


Figure 1. Salivary testosterone levels (ng/mL) and Bem Sex Role Inventory (BSRI) femininity scale scores in 21 men aged 18 - 24 (line of best-fit derived from Pearson's correlation).

ever, we can speculate the results may be due to differing impacts of social versus biological factors on the development of sex-typical and sex-atypical traits. It is possible that sex-typical traits are more heavily influenced by early socialisation, whereas *opposite-sex* typical traits are much less likely to be a result of socialisation, and therefore their development may be more related to underlying biological factors. Much social research has looked at the aspect that how gender-congruent behaviour develops in the context of gender stereotypes via socialisation (social role theory e.g. [18]).

Measuring circulating testosterone levels cannot identify the proximate mechanism by which testosterone may impact on sex-role orientation. It is possible there may be a direct relationship, or alternatively that adult testosterone levels may correlate with testosterone levels at key growth periods (e.g. prenatally, or at puberty), where organisational effects on the central nervous system may have occurred. Thus, current testosterone levels may serve as a proxy for earlier levels of testosterone which have the direct relationship with adult behaviour. Future studies should seek to relate the BSRI to pubertal, and prenatal testosterone levels in males, to elucidate which may have the most direct relationship to adult sex-role orientation.

In summary, the current pilot study is the first to demonstrate a relationship between testosterone levels and sex-role orientation in typically developed adult males. Specifically, testosterone was seen to correlate negatively to the *opposite*-sex trait scale (Femininity scale). Our preliminary results extend the research, which implicates androgens in sex role orientation in males via associations with 2D:4D, by demonstrating a relationship with salivary testosterone in adult males. In doing so, our results lend support to the suggestion that sex-role orientation may have a biological component related to underlying hormone levels.

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