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MAY TESTOSTERONE LEVELS AND THEIR FLUCTUATIONS INFLUENCE COGNITIVE PERFORMANCE IN HUMANS?

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A b s t r a c t

There are no sex differences in general intelligence but, in specific cognitive abilities, men and women differ. This study was designed to describe infradian variations in testosterone levels in saliva collected during one month and to identify relationships between salivary testosterone and spatial abilities in 53 young healthy volunteers of both sexes. Our results showed the effect of testosterone on spatial performance. In accordance with general knowledge, men with higher average testosterone levels scored better than women. A circalunar rhythm of testosterone related to the menstrual cycle with the highest testosterone concentration in the periovulatory period was confirmed in women and an analogical circalunar cycle was described in men. Negative relationships between testosterone levels and spatial abilities were found in men whereas positive relationships between these parameters were found in women. When spatial perfomance was related to monthly testosterone variations in both sexes, it was found that, in women, the periovulatory testosterone peak was associated with the best spatial performance while, in men, the high testosterone phase was associated with the worst spatial performance. The understanding of infradian variations in testosterone levels and their effects on cognitive abilities in humans may be important in terms of clinical implications.

K e y w o r d s

Salivary testosterone, Spatial ability, Infradian testosterone fluctuations, Sexual dimorphism

INTRODUCTION

Psychoendocrinological research has recently focused on exploring gender differences in certain domains of cognitive functioning. Although there are no sex differences in global intelligence in the general population, some controversy over sex differences in specific cognitive abilities still remains. Men, on the average, differ from women in a number of specific abilities. It is typically claimed that men outperform women in mathematical and spatial abilities and women outperform men in verbal abilities. Gender differences in spatial orientation may also occur in non-human species (*1*) and have been demonstrated across a number of cultures (*2*).

Underlying reasons for gender differences in cognitive abilities are currently debated, with the prevalence of environmental versus biological factors being considered responsible for these differences. Biological explanations have focused on hormonal influences, cerebral dominance and genetic factors. *Geschwind* and *Galaburda* in their well-known theory proposed the intrauterine testosterone concentration to be responsible for alterations in the central nervous system (*3*). One aspect of the theory is the prediction that elevated levels of intrauterine testosterone slow the growth of the left hemisphere, which consequently leads to an enhancement of specific cognitive abilities (spatial, musical or mathematical abilities) hypothesised to be dependent on right hemispheric functioning. Genetic models have also been studied (*4, 5*). Research data on both animals and humans suggest a possible postnatal organising influence of androgens on spatial ability, exerted at or before the time of puberty (*6, 7, 8, 9,1*).

In studying the activational effects of gonadal hormones, researchers have to rely on naturally occurring biorhythms. The menstrual cycle provides a convenient method for studying how fluctuations in hormone levels might influence cognitive functions in women. *Komnenich et al.* found that women performed significantly worse in spatial ability tests during oestrogen surge just prior to ovulation than in other phases of the cycle (*10*). In *Hampson's* study, higher oestrogen levels prior to ovulation were associated with a poorer performance in tests of spatial ability (*11*).

A few studies have so far indicated that there is a curvilinear or inverted U-shape relationship between testosterone levels and spatial abilities. *Shute et al.* found that females with the highest plasma androgen levels performed better in spatial tests than females with the lowest androgen levels, whereas males with the highest androgen levels performed worse than males with the lowest androgen concentrations (*12*). *Gouchie* and *Kimura* confirmed *Shute's* study, finding a non-linear relationship between testosterone levels and spatial performance (*13*). Males with lower testosterone levels performed better than males with higher levels, while females with higher levels performed better in spatial tests than those with lower testosterone levels. It was proposed that testosterone functions at optimal levels that have lower physiological values for men and higher for women. However, not all studies investigating the relationship between testosterone and spatial ability have found a curvilinear effect. *Christiansen* and *Knussman* reported a positive relationship between testosterone and spatial ability in men (*14*). This was confirmed by findings made by *Christiansen* in Namibia young men (*15*). *Mc.Keever et al.* failed to find any relationship between plasma testosterone levels and spatial visualisation tests in both men and female students.

Two most common categories of spatial abilities were tested in our study, i.e., mental rotation and spatial visualisation. Our presumption was that the scores of both mental rotation and spatial visualisation would be higher in men than in women. Performances in these two specific cognitive abilities were related to average salivary testosterone levels and to testosterone daily levels during the month cycle in every subject investigated. Salivary testosterone reflects a free, bioavailable fraction of this hormone, permitting prompt responses of receptive tissues including the brain. The aim of this study was to find new facts about the activational effect of salivary testosterone on spatial ability. We hypothesised that, in both sexes, infradian, short-term changes in testosterone levels might be associated with changes in spatial performance.

MATERIALS AND METHODS

Subjects

Fifty-three young healthy volunteers, between 19 and 23 years of age, participated in the study. In October 2000, 31 subjects collected saliva every day and 22 subjects collected them only on one occasion during that month. Because the circadian rhythm may interfere with the data, saliva samples were collected between 7.30 a.m. and 8.30 a.m. before eating or cleaning the teeth. No saliva stimulants were used. The subjects were instructed to reduce physical and irregular sport activities and to abstain from drinking alcohol and from sexual activities at the time of sampling in order to diminish the risk of any artificial influence on the testosterone level as much as possible. They were also asked to keep a diary and record all activities that could affect the hormone concentration. None of the subjects used any hormonal replacement therapy, contraception or other hormonal or non-hormonal drugs known to affect testosterone levels (*17*). The saliva samples in 4 ml amounts were deep frozen until assayed within three months of sampling.

Radioimmunoassay

Saliva samples (1.0 ml), a tritiated testosterone recovery standard (1200 d.p.m.), a water blank (1.0 ml redistilled water) and a control pool saliva sample were all extracted on a PRESEP plastic column with the packing sorbent SEPARON SGX C18 60 μm (Laboratory Instruments Prague). After washing with 5 ml distilled water, steroids were eluted with 3 ml methanol that was subsequently evaporated under a stream of nitrogen at 37°C. The efficiency of extraction was assessed by reconstituting the extracts in 500 μl ethanol, 100 μl of which was removed, dried and counted in a toluene scintillant for 10 min. A standard curve consisting of 0, 0.1, 0.2, 0.4, 0.8 and 1.6 nmol amounts of testosterone per litre (MRC England) was prepared in duplicate. Then anti-testosterone-3-BSA antibody (diluted 1:100 000) was added to all tubes. Subsequently, (125 I)-testosterone was added to each tube, which was then equilibrated at room temperature for 1 h or overnight at 4°C. After incubation, a dextran-coated, charcoal suspension (1 ml) was used to separate the free from the bound fraction and the free fraction was counted for 1 min.

The results were calculated from the standard curve using a log-logit transformation, corrected for recovery and expressed as nmol testosterone per litre of sample.

Assessment of Spatial abilities

Spatial abilities enable a person to locate an object in space, mentally rearrange objects, reorganise shapes and so on (*18*). Mental rotation requires from the subject to mentally rotate a defined structure in space. Tests of spatial visualisation often require stimulus transformations of dynamic serial operations. For the psychological evaluation of spatial ability, standard psychological tests were used. Mental rotation scores of our subjects were assessed with the use of a non-verbal subtest of the general standardised test on intelligence (*19*). The assessment involved 20 tasks and 9 min were allowed for each of the tasks. Spatial visualisation was assessed on the basis of a subtest of the non-verbal intelligence test developed by *Smith* and

Whetton (*20*). This also consisted of 20 tasks and 20 minutes were allowed to complete the whole set of them.

For either test, coefficient K was calculated according to the formula:

$$K = \frac{\psi^2}{\rho\omega}$$

where Ψ is the number of correct answers, ρ is the number of trials and ω is the total number of tasks.

Each of these tests had two forms of presentation (A and B) in order to prevent the subjects from remembering the answers in case the test was repeated within a short period of time.

Relationship of test performance to testosterone levels

The subjects with daily saliva collection were tested twice a month, the interval between testing sessions was two weeks. In women, the first test session was in the menstrual phase of their cycle, the second in the follicular phase. It was thus possible to relate changes in performance to different phases of the menstrual cycle in women and to infradian fluctuation in testosterone levels in men. The subjects who collected saliva once in a month were tested on the day of saliva sampling. Pearson's correlation coefficients between testosterone levels and spatial performance were calculated in two steps: First, average salivary testosterone levels and average coefficients of mental rotation and spatial visualisation performance were correlated. Second, the spatial performance coefficient was related to actual salivary testosterone levels in each subject. This enabled us to study the relationship between salivary testosterone levels and spatial ability performance, assessed by both tests, in different phases of the month cycle.

RESULTS

Salivary testosterone levels

After statistical analysis, the data showed significantly higher average testosterone levels in men ($P<0.001$) than in women and better scores on spatial abilities in men in comparison with women (mental rotation, $P<0.005$; spatial visualisation, $P<0.005$). In men, after statistical procedures to synchronise fluctuation in testosterone levels, a clear peak was observed on day 18 of the predicted cycle. At the end of the predicted cycle, testosterone levels were similar to the levels at the beginning of the cycle. These results indicated a possible male circalunar cycle. This assumption, however, needs further investigation.

Correlations between salivary testosterone levels and cognitive test performance

A negative relationship between the average testosterone level and spatial test performance was found in males. A positive, though only marginally significant relationship between these two parameters was observed in women. Pearson's coefficients calculated for each sex are presented in *Table 1*.

The analysis of data showed that the best results in spatial tests were achieved by men with the lowest and by women with the highest levels of salivary testosterone. In other words, the best spatial performance was observed in hormonally androgynous subjects (*Fig. 1*).

The relationships between spatial performances and actual testosterone levels during the month cycle were evaluated for each sex. We observed an inverted U-shaped curve, relating the days of the month cycle and spatial ability, in women in whom the month cycle coincided with the menstrual cycle (*Fig. 2*). The best results in spatial ability were observed in the mid-cycle, when testosterone levels in saliva were the highest. Differences between the results achieved during the high testosterone phase and those from the low testosterone phase in each woman were significant (mental rotation performance, $P<0.05$; spatial visualisation performance, $P<0.002$).

In men, the results showed U-shaped relationships between actual testosterone levels obtained during one month and spatial performance on the day of testing (*Fig.3*). Differences in results between the performance during the high testosterone phase and that during the low testosterone phase were significant (mental rotation performance, $P<0.05$; spatial visualisation performance, $P<0.04$).

Table 1

Pearson's coefficients and *P* values for correlations between salivary testosterone levels in nmol/l and spatial performance in both sexes

	Men		Women	
	Mental rotation	Spatial visualisation	Mental rotation	Spatial visualisation
Average salivary testosterone level	r = -0.82	r = -0.49	r = 0.54	r = 0.76
	P < 0.001	*P* < 0.07	*P* < 0.05	*P* < 0.001
Actual salivary testosterone level	r = -0.78	r = -0.75	r = 0.75	r = 0.79
	P < 0.001	*P* < 0.001	*P* < 0.001	*P* < 0.001

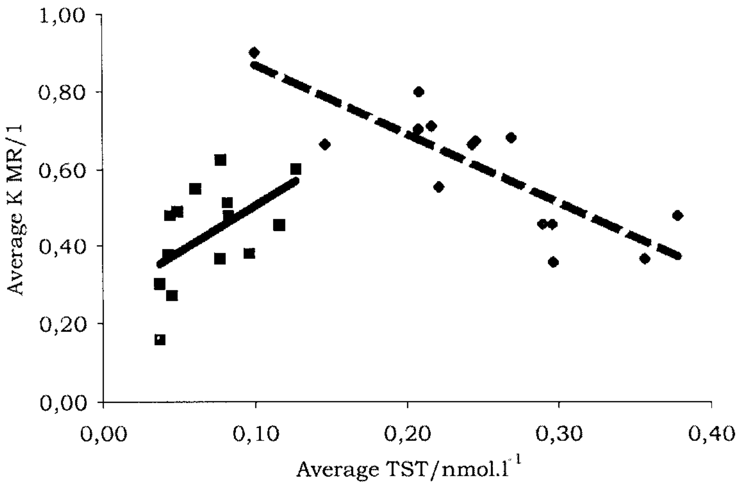

Fig. 1.

Relationship between average salivary testosterone levels and the results of mental rotation tests in men (dashed line) and women (full line).

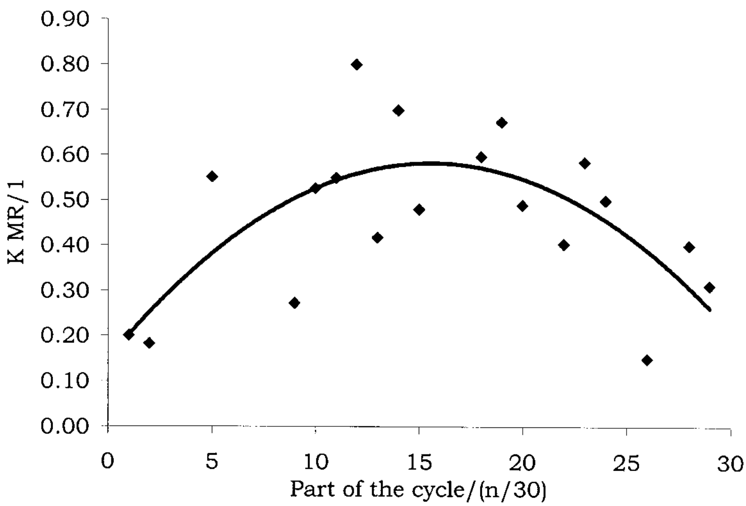

Fig. 2.

Relationship between the average results of mental rotation tests and salivary testosterone levels in different phases of the menstrual cycle in women. Testosterone levels peak in the mid-cycle.

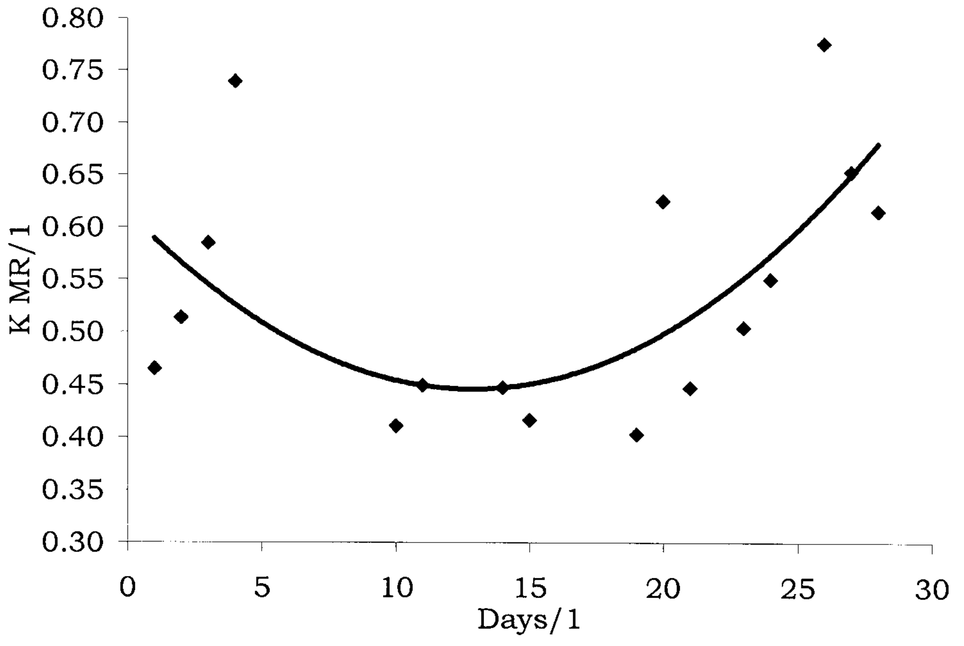

Fig. 3.

Relationship between the average results of mental rotation tests and salivary testosterone levels in men. The peak of salivary testosterone levels are found at the beginning and the end of the predicted period of 30 days.

DISCUSSION

The present study was designed to test the hypothesis that short-term changes in salivary testosterone levels have an effect on spatial cognition in humans of both sexes. The results obtained were consistent with this hypothesis and revealed new findings about the effect of testosterone on spatial ability. In accordance with general knowledge, men scored better in two spatial tests (mental rotation and spatial visualisation) than women. As for salivary testosterone levels, sexual dimorphism was proved because men had significantly higher salivary testosterone levels than women. Women with the highest average salivary testosterone reached the highest scores in spatial orientation tests. The opposite was found in men; those with the lowest levels of salivary testosterone scored best in spatial tests. The same results were obtained in our previous study on adolescent subjects (*21*). These findings indicate a non-linear relationship between testosterone levels and spatial performance, with moderate levels of androgens being associated with a better spatial ability. The results support the data reported by *Shute et al.* (*12*), *Gouchie* and *Kimura* (*13*) and *Neave et al.* (*22*) who found significant non-linear associations between the total plasma testosterone and spatial performance. It can be assumed that hormonal bipotenciality favours spatial orientation, as proposed by *Petersen* (*23*) and further confirmed by *Moffat* and *Hampson* (*24*). The results of our study further suggest that changes in spatial performance may depend on salivary testosterone fluctuations throughout the month in both male and female subjects, which supports the views on activational effects of circulating testosterone levels reported in the studies on circannual (*25*) and menstrual (*6*) variations in circulating steroid levels. In line with our present findings are the results reported by *Janowski et al*. (*26*), who found a better spatial performance in older men after testosterone substitution therapy. Others have not found any relationship between phases of the menstrual cycle and spatial abilities (*27*).

The lack of consensus among studies published on the relationship between testosterone levels and spatial ability might be due to methodological problems in these studies, in which circadian and seasonal fluctuations in testosterone levels have generally not been considered. There has also been little consensus with regard to the type of testosterone measured (plasma, total, free, salivary). Many previous studies failed to measure sex steroid hormones and, in women, deduced hormonal levels from the stage of the menstrual cycle, thus leaving the interpretation of their results unclear.

In women, we confirmed infradian fluctuations in salivary testosterone levels and their relation to temporal changes in spatial abilities. The results reported here on men are original findings. However, further investigation is needed to confirm this infradian testosterone fluctuation in human males.

Our observations support the role of salivary testosterone in influencing spatial orientation in healthy young men and women. But this is not to say that testosterone determines the visual-spatial performance. As it is clear that testosterone exerts many of its physiological effects after conversion to its derivatives (estradiol and dihydrotestosterone), at least the roles of ovarian steroids have to be considered. By determining testosterone and its active metabolite estradiol in the same sample of a subject investigated, it will be possible to calculate the ratio between them and to investigate their interactions on cognition.

A c k n o w l e d g e m e n t s

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MÔŽE HLADINA TESTOSTERÓNU A JEJ KOLÍSANIE OVPLYVNIŤ KOGNITÍVNE SCHOPNOSTI ČLOVEKA?

S o u h r n

Vo všeobecnej inteligencii nejestvujú rozdiely medzi mužmi a ženami, v špecifických kognitívnych schopnostiach sa však výkony mužov a žien odlišujú. V tejto štúdii sme opísali infradiánne kolísanie hladín slinného testosterónu a závislosti medzi slinným testosterónom a priestorovými schopnosťami u 53 mladých dobrovoľníkov oboch pohlaví.

Získané údaje potvrdili vplyv testosterónu na priestorové schopnosti. V súhlase so všeobecným poznaním muži dosahovali lepšie výsledky v priestorových testoch ako ženy. Potvrdili sme cirkalunárny rytmus kolísania testosterónu u žien s najvyššími koncentráciami periovulačne. Analogický cirkalunárny cyklus sme opísali u mužov, u ktorých sme zistili negatívny vzťah medzi hladinou testosterónu a úspešnosťou v testoch na priestorové schopnosti. Pozitívny vzťah medzi oboma parametrami sme zistili u žien. Periovulačný peak slinného testosterónu u žien sa spájal s najlepším priestorovými schopnosťami, kým vysokotestoterónová fáza u mužov sprevádzala najslabšie výsledky v testoch na priestorové schopnosti.

Zistenie infradiánnych variácií testosterónu a ich účinky na kogníciu má mnoho klinických implikácií.

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