EXPERIMENTAL STUDY

The circalunar cycle of salivary testosterone and the visual-spatial performance

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Abstract

Background: **A circalunar cycle is thought to be female sex specific. Results of studies observing the relationship of testosterone and spatial abilities are controversial.**
Objective**: To describe the infradian variations of testosterone and the correlations between salivary testosterone levels and spatial abilities in young healthy volunteers of both sexes.**
Subjects and methods: **Testosterone levels in saliva were determined in 53 young adult male and female subjects (mean age — 20.89±0.91 years). The samples of saliva were collected either once (22 subjects) or daily during a period of 30 days (31 subjects). Salivary testosterone was analyzed by RIA. Both groups of subjects were tested on their visual-spatial performance (mental rotation and spatial visualization). All important data about the menstrual cycle of female subjects were also collected. This data and the results of the visual-spatial tests as well as the circalunar cycle in their relationship to the testosterone levels were analyzed.**
Results: **In this study a circalunar rhythm of testosterone related to the menstrual cycle with a maximum peak in the periovulatory phase was confirmed in women and an analogical circalunar cycle in men was described. A positive correlation of the salivary testosterone levels and the performance in visual-spatial tests in women and a negative dependence in men was found. The outcomes showed a significant statistical difference between the results of the test during the high-testosterone and the low-testosterone phase in both sexes.**
Conclusions: **The levels of testosterone between the high female and low male range seem to be associated with the best spatial ability performance in adults. A male circalunar rhythm similar to the female one is very likely. Recognizing of the infradian fluctuations in women as in men should have many various clinical implications.** ***(Tab. 2, Fig. 7, Ref. 143.)***
Key words: salivary testosterone, circalunar cycle, spatial abilities, steroids, hormones.

Testosterone (TST) is a lipofile C19-steroid that is usually described as the main male sex hormone (Berthold, 1849; Brown-Séquard, 1889; Freeman et al, 2001). The production is localized especially in the smooth endoplasmatic reticulum of the Leydig cells in the testis. In women the ovaries and the suprarenal gland can be seen as the main sources, but about a half of the TST amount in women come from an extraglandular production. Neural tissue, adipocytes and even microbes in gastrointestinal tract are being discussed as the possible places of TST origin (Bilton, 1995; Mensah-Nyagan et al, 1996; Xiong and Maser, 2001). The production regulation is of a very complex character while a set of "intracrinological" relationships plays an important role. Luteinizing hormone and follicle stimulating hormone are, however, still seen as the main regulators (Everett et al, 1949; Risma et al, 1995; Tilbrook and Clarke, 2001; Weinbauer et al, 2001), but the meaning of interactions with dehydroepiandrosterone (Labrie et al, 1997), inhibin B (Andersson et al, 1997; Kubini et al, 2000), growth hormone (Hassan et al, 2001)

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and especially with estradiol (Barlow, 1964; Deyssig and Luger, 1991) are not explained. Similar to other lipofile substances TST in plasma is bound to proteins, to albumin and to a SHBG (Sex Hormone Binding Globulin), that also stands under estradiol regulation (Anderson, 1974). Only a small part of the total TST amount remains unbound — in men about 2 %, in women even only 1 % (Stárka et al, 1997). This free — active fraction is detectable in saliva, where it gets through a mechanism of simple passive diffusion. The recognition of TST by receptive cells is tissue specific while different types of receptors have been found — a cytoplasmatic protein dimmer that can pass the nuclear membrane pores (Fliss et al, 1997; Moilanen et al, 1998), but also different membrane bound G-protein linked receptors (Benten et al, 1999), Na^+-K^+-ATP-ase and even a simple plasmalemma channel (Ramirez and Zheng, 1996). The androgenic and the anabolic effects of TST are mediated especially through dihydrotestosterone after transformation by 5-α- or 5-ß-reductase (Hiipakka and Liao, 1998), the central nervous tissue and the testis, on the other hand, convert TST by aromatase into estradiol (Sharpe, 1998; Simpson et al, 2000; Genissel et al, 2001).

Rhythmic changes in hormonal levels are partly accepted and recognized in various clinical situations. Nowadays, to the most relevant belong the menstrual circalunar cycle of female sex hormones, but also the circadian rhythms of glucocorticoids and the growth hormone. The best-described cycle of TST levels is due to methodological reasons of a circadian period. The highest TST levels are reached in the early morning hours in both sexes, what can be altered by the individual health status. A circannual rhythm with maximum levels found in autumn is also known (Ostatníková et al, 1995; Valero and Fuentes, 1998). Studies searching for other infradian rhythms are surprisingly rare (Exley and Corker, 1966; Doering et al, 1975). Nevertheless, periodic circalunar changes of TST levels are at least in women very probable (Ostatníková et al, 1996) while the main hypothalamic (GNRH) and hypophyseal (LH and FSH) regulators undergo circalunar rhythms related to the menstrual cycle (Carandente et al, 1989; Buffet et al, 1998). The male hormonal system was thought to be axiomatic acyclic for more than a century in the majority of researchers, although some have proposed chronobiological changes in man (for review see Halberg, 1969). Daily and annual rhythms are accepted while a male circalunar cycle is not. The reason was looked for in the high perinatal TST levels in boys that could influence the central regulator in some way (Harris, 1964). However, no molecular, genetic, mechanistic or morphological correlate of this possible interaction was found.

Cognition is a general term covering various modes of knowing. Psychoneuroendocrinological research of recent years has focused on exploring gender differences on particular domains of cognitive functioning. Although there are no sex differences in the general population in global intelligence, the controversy over sex differences in specific cognitive abilities still remains. Men on 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 abilities may also occur in other species (Williams et al, 1990) and have been demonstrated across a number of cultures (Witkin and Berry, 1975). The underlying reasons for gender differences in cognitive abilities are hotly debated with prevalence of environmental versus biological factors. Biological explanations have focused on hormonal influences, cerebral dominance and genetic factors. Geschwind and Galaburda (1985) proposed in their well-known theory the concentration of TST in utero to be responsible for alterations in central nervous system leading to anomalous dominance, hand preference and immune system disorders. One aspect of the theory is the prediction that elevated levels of TST in utero slow the growth of the left hemisphere, which consequently leads to the enhancement of specific cognitive abilities hypothesized to be dependent on right hemispheric functioning (spatial abilities, music, mathematics). The theories that sex differences in spatial ability are due to an X-chromosome linked recessive gene and other genetic models (Annett, 1975) have been studied (Blatter, 1982) but the results remain unsatisfying.

Concluding from animal research (Williams et al, 1990; McEwen, 1991) and clinical studies of individuals exposed prenatally to unusual hormone environments authors reported of modifications in spatial abilities. Congenital adrenal hyperplasia results in women in an enhance of their spatial performance (Resnick et al, 1986), as does a lifelong deficiency in GNRH in men (Hier and Crowley, 1982). The disorder typically becomes manifest as a failure of pubertal development, but these males are believed to undergo normal masculinization in utero, thanks to the presence of maternal gonadotropins. Administration of gonadotropins in the adulthood does not improve the spatial ability. Interestingly, men with similar disorder, which was acquired after a normal puberty, do not show any spatial deficit despite having severely low androgen levels. These data suggest a possible postnatal organizing influence of androgens on spatial ability exerted at or before the time of puberty (Hampson and Kimura, 1992). As in studies searching for prenatal effect of androgens, also for studying the activation effects of gonadal hormones, researchers have to rely on naturally occurring biorhythms. The menstrual cycle provides convenient method for studying how fluctuations in hormone levels might influence cognitive functions in women. Women perform more poorly in spatial ability test during estrogen surge just prior to ovulation than at other points in the cycle (Komnenich et al, 1978; Hampson, 1990).

Investigating the correlation between TST and spatial ability, few studies indicated that there exists a curvilinear or an inverted U shaped relationship. Females with the highest androgen levels performed better on spatial test than females with the lowest androgen levels, whereas the highest androgen males performed more poorly than males with the lowest androgen concentrations (Shute et al, 1983; Gouchie and Kimura, 1991). It was proposed that optimal levels of TST might exist, which are in lower physiological values for men and higher physiological values for women. However, not all studies investigating this

relationship found an inverted U shaped regression curve (Christiansen and Knussman, 1987; McKeever and Deyo, 1990; Christiansen, 1993; Gordon et al, 1995).

The aim of this study was to describe changes of testosterone levels during the menstrual cycle in women, to look for similar variations during a month in men and to find possible relationships between the testosterone levels and the visual-spatial abilities in our probands.

Subjects and method

Subjects and sampling. 53 young healthy probands (26 males and 27 females) with an age between 19 and 23 years were divided into 2 groups. Saliva samples were collected either once (22 subjects) or daily during 30 days (31 subjects) in autumn 1999. While the circadian rhythm could interfere with the data to reduce its effect saliva samples were collected during the first 30 minutes after waking up before eating or cleaning the teeth. No saliva stimulants were used (Malamud and Tabak, 1993; Granger et al, 1999; Shirtcliff et al, 2001). During the whole time of sampling the probands were instructed to reduce physical and irregular sport activities and to keep an alcoholic and sexual abstinence to diminish as much as possible the risk of an artificial influence on the TST level. Subjects were also asked to keep a diary, which yielded all activities that could affect the hormone concentration. None of the subjects used any hormonal substitution, contraception or other hormonal and non-hormonal pharmacies known for changing the TST levels (Argüelles and Rosner, 1975; Schürmeyer and Nieschlag, 1982). No information concerning the marital status or the partners were collected.

Biochemical analysis. Saliva samples were frozen and stored in a deep-freezer at -20 °C after collection. Salivary testosterone, estradiol and progesterone levels were determined by radioimmunoassay (Putz et al, 1983; Vining and McGinley, 1987; Sinha-Hikim et al, 1998; Tschöp et al, 1998). Saliva sample (1.0 ml), tritiated testosterone recovery standard (1200 d.p.m.), water blank (1.0 ml of double distilled water) and control pool saliva sample were all extracted by a plastic column PRESEP with a packing sorbent SEPARON SGX C18 60 µm (Laboratory Instruments Prague). After washing with 5 ml of distilled water the steroids were eluted with 3 ml of methanol, which was evaporated to dryness 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 were removed, dried and counted in toluene scintillant for 10 min. A standard curve consisting of 0; 0.1; 0.2; 0.4; 0.8 and 1.6 nmol testosterone per l (MRC England) in duplicate was also prepared, after which antibody anti-testosterone-3-BSA (diluted 1:100 000) was added to all tubes. I^{125}-testosterone was added to each tube, which was then equilibrated at room temperature for 1 hour or overnight at 4 °C. After incubation dextran-coated charcoal suspension (1 ml) was used to separate the free and bound fraction and the free fraction counted for 1 minute. Results were calculated from the standard curve using a log-logit transformation, corrected for recovery and expressed as nmol testosterone per liter sample. The antiserum was checked for cross reactivity with 16 steroids likely to be found in plasma. The only compounds that showed a significant cross-reaction were 5-α-dihydrotestosterone (33.0 %), 11-ß-hydroxytestosterone (0.1 %), 17-ß-estradiol (0.1 %) and androstendione (0.1 %). The recovery of known amount (1—5 pg) of testosterone added to saliva was 101.4±9.0 % (n=24) after correction for recovery of ^{3}H-testosterone. The sensitivity of the assay, the precision of the measurement of zero, was 1 pg. The interassay variation calculated from the results of a quality control run in each assay gave a value of 0.220±0.018 nmol/l (CV 8.2 %; n=50). After the assay had been in routine use the results calculated using the recovery measured for each sample were compared with those calculated using the mean overall recovery for all previous assays. No significant differences were observed (regression analysis r=0.99; y=0.996x+0.02; n=300).

Spatial abilities. According to one of various definitions "spatial abilities are those that enable a person to locate an object in space, mentally rearrange objects, reorganize shapes and so on" (Lips et al, 1978). Factor analytic studies have shown that there are at least three different subtypes of spatial ability, all showing a sex difference in favor of males. These sub-abilities are referred to as spatial orientation represented by mental rotation (MR), spatial visualization (SV) and flexibility of closure (Hampson and Kimura, 1992). Spatial orientation (mental rotation) requires the subject to rotate a configuration mentally in space. Tests of spatial visualization often require stimulus transformations of dynamic serial operations. Flexibility of closure refers to the ability to discern a target configuration from a more complex pattern or design.

For psychological evaluation of spatial ability standard psychological tests were used. Mental rotation score of our subjects was assessed by the use of nonverbal subtest of general standardized test on intelligence (Amthauer, 1993). Subjects were supposed to rotate cubes with six different sides and compare it to four differently designed and one identical rotated cubes. The aim was to depict the target cube in rotated position. Each of 20 tasks consisted of a target drawing and five test drawings. Nine minutes were allowed to our subjects. For spatial visualization assessment the subtest of nonverbal intelligence test (Smith and Whetton, 1993) was used. The aim was to fold mentally unfolded geometric figure and find the three-dimensional equivalent of the two-dimensionally unfolded object. Twenty minutes were allowed for the set of 20 tasks.

The coefficient K was evaluated for each test considering not only the number of correct answers, but also the number of trials according to the formula:

$$K = \frac{\Psi^2}{\rho.\omega};$$

Ψ— number of correct answers
ρ— number of trials
ω— total number of tasks

The subjects were tested twice a month, the interval between testing were two weeks. In women one test session was in the menstrual phase of the cycle, the other in the follicular phase. Two forms (A and B) of both tests were used in each testing session. Thus the changes in performance during stages of menstrual cycle in women and during the month in men were obtained. The average salivary TST levels and average coefficient of MR and SV performance were correlated and spatial performance coefficient was related to actual salivary TST levels in every subject. Tests with a result of K=1 were excluded. That enabled us to study the relationships between salivary TST levels in different phases of the circalunar cycle and spatial ability performance in both tests.

Statistical analysis. Analysis of the results were performed with Microsoft Excel® software and with the aid of literature information (Ridgman, 1981; Clauss and Ebner, 1988; Zvára, 1989; Chajdiak, 1995; Borchert, 1996; Šťastný, 1999; Van Dongen et al, 1999). We used standard statistical processes — t-tests, moving averages and least square differences regression method of different polynomic grade. For chronobiological changes only daily sampled subjects during the period of 30 days were analyzed. In the data of TST levels in women smoothing using moving averages and an artificial increase of the dynamic weight using the correction with the difference between the intra- and interindividual average levels were performed to minimize the influence of interindividual differences in testosterone levels in chronobiological evaluation. The data were synchronized with the menstrual cycle of a standardized relative period while the 1st day of menstruation has been chosen as the beginning of the cycle. In men there is no usable natural synchronization point known, so the levels have to be synchronized using mathematical routines. There are various chronobiological methods to detect or determine the probability of cyclic changes in data. Mostly used are the cosinor, spectral and sine wave analysis, Monte Carlo and other methods using the Fourier series expansion, the cross-correlation method and the concordance analysis (Alberola et al, 1999; Rocchi and Ghiandoni, 1999; Ruf, 1999; Hassnaoui et al., 2000). These methods differ in their sensitiveness and in their robustness against noisy data but they do not serve exactly our purposes. Every technique has its own specific problems, so we have tried to apply other statistical processes. We have developed a new routine to synchronize data with an anticipated period-length. The differences between the values smoothed using moving averages (k=5 days) with a time distance of half of the predicted period have been analyzed. This predicted period has been calculated as the sum of the average time distances between the minimum and the maximum value and vice versa in all subjects ($t_{1/2}$=15.23 days). The highest difference is thus an indicator of the zones of extremes, which are in our method of 5 days length. In men the zone of minimum was chosen as the beginning of the predicted cycle, the dynamic weight increase was also performed.

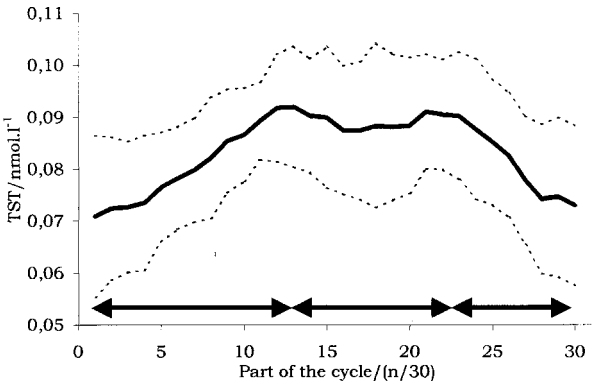

Fig. 1. The circalunar cycle of salivary testosterone in women. The average levels are related to the relative time parts (n=30) of the standardized menstrual cycle. (Dotted curves show A±SD, the arrows show the local extremes.)

Results

Circalunar cycle in women. The dynamic changes of salivary TST synchronized and related to the menstrual cycle after the dynamic weight increase are shown in Fig. 1. The lowest levels were found during the menstruation phase, two local maximum peaks in the predicted ovulation phase. (The significances of the differences between the TST levels reaching local extreme levels on the 1st, 13th, 21st and 30th day were as follows — TST1 vs. TST13 — $p<0.004$; TST13 vs TST21 — $p>0.05$; TST21 vs TST30 — $p<0.01$. For statistical analysis the original data, not smoothed by moving averages, were evaluated by paired t-test).

To prove our results on relevance salivary concentrations of estradiol and progesterone in female subjects were also determined. The variations of these hormones during the menstrual cycle are best described in endocrinological literature. In our subjects we found very similar changes shown on Figs 2 and 3.

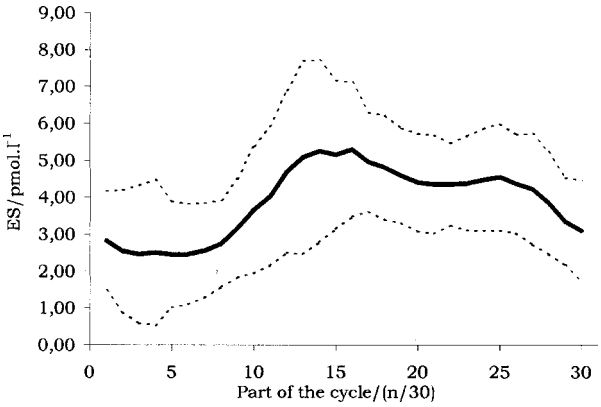

Fig. 2. The circalunar cycle of salivary estradiol in women. The average levels are related to the relative time parts (n=30) of the standardized menstrual cycle. (Dotted curves show A±SD.)

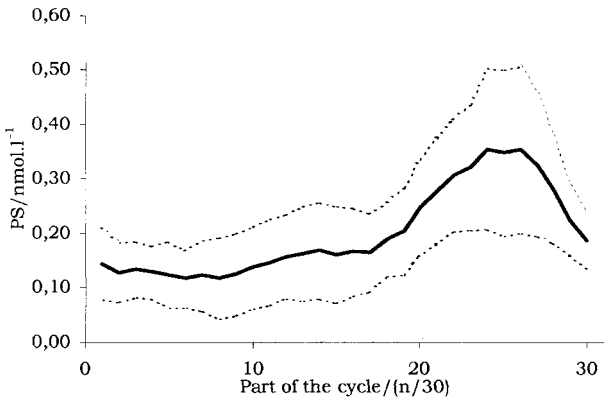

Fig. 3. The circalunar cycle of salivary progesterone in women. The average levels are related to the relative time parts (n=30) of the standardized menstrual cycle. (Dotted curves show A±SD.)

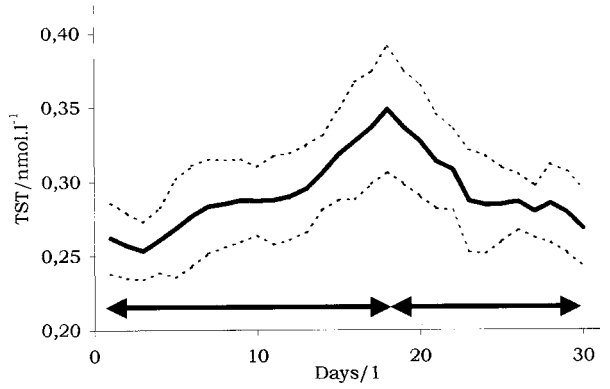

Fig. 4. The predicted circalunar cycle of salivary testosterone in men. The average levels are related to absolute days. (Dotted curves show A±SD, the arrows show the local extremes.)

Circalunar cycle in men. In men after performing of all statistical and mathematical routines described above very interesting and surprising variations shown on Figure 4 were found. A clear maximum peak on the 18th day of the predicted cycle was observed. At the end of the predicted cycle the TST levels got similar to the levels on the beginning. This fact cannot be explained by the Slutsky effect of moving averages while we used them with k=5 days and the artificial synchronization may affect only the average levels during the zones of extremes. Thus, our results indicate a possible male circalunar cycle. (The significances of the differences between the TST levels reaching local extreme levels on the 3rd, 18th and 30th day were as follows — TST3 vs TST18 — $p<0.00006$; TST18 vs TST30 — $p<0.0003$. For statistical analysis the original data, not smoothed by moving averages, were evaluated by paired t-test).

Spatial abilities. Sex differences. After statistical analysis the observed data have shown significantly higher average TST levels in men than in women ($p<0.0000004$) and better scores

Tab. 1. Pearson coefficients and p-values of the correlations between actual and average TST levels and the results of the spatial ability tests in men.

Men	TST vs K MR	TST vs K SV
Average	r = -0,82; p<0,001	r = -0,49; p<0,07
Actual	r = -0,78; p<0,001	r = -0.75; p<0,001

K MR — Coefficient K of mental rotation
K SV — Coefficient of spatial visualization

Tab. 2. Pearson coefficients and p-values of the correlations between actual and average TST levels and the results of the spatial ability tests in women.

Women	TST vs K MR	TST vs K SV
Average	r = 0,54; p<0,05	r = 0,76; p<0,001
Actual	r = 0,75; p<0,001	r = 0,79; p<0,0001

K MR — Coefficient K of mental rotation
K SV — Coefficient of spatial visualization

on spatial abilities in men in comparison with women (MR — $p<0.005$; SV — $p<0.007$).

Testosterone vs spatial ability. In both tests of spatial abilities negative relationships between average and actual TST levels and spatial tests performances were found in men (Tab. 1). On the other hand positive relationships occurred between average and actual TST levels in saliva and both spatial tests in women (Tab. 2).

We have found the inverted U shaped relationships between TST levels and spatial ability tests performance, which proved the best results in spatial tests for those men with the lowest TST concentrations but for those women with the highest concentrations of TST in saliva. The best spatial performance was observed in most hormonally androgynous subjects in our study (Fig. 5).

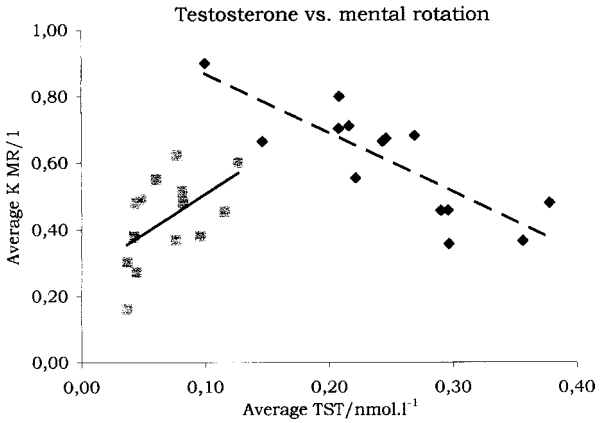

Fig. 5. The relationship between average TST levels and results of mental rotation tests in men (dotted line) and women (full line).

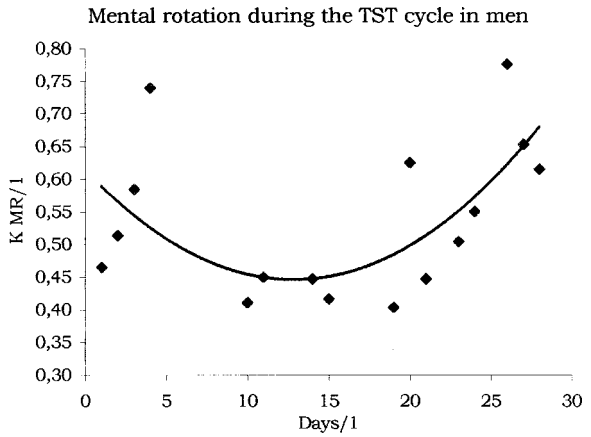

Fig. 6. Average results of mental rotation tests on different days of the predicted cycle in men.

We searched for spatial performance in relation to the month TST variations in women and men separately. The results have shown U shaped curve relating the days of the circalunar cycle and spatial ability coefficient in both tests for spatial orientation in men (Fig. 6). The differences between the results during the high-testosterone and the low-testosterone phase were significant ($p<0.05$ for MR; $p<0.04$ for SV).

The opposite was found in women (Fig. 7). The circalunar cycle was related to the relative parts of the menstrual cycle in women. The relationships between the parts of the cycle and spatial performance have shown an inverted U shape curve. It was apparent that the best results in spatial ability tests were found in the midcycle, when TST levels in saliva were the highest ($p<0.05$ for MR; $p<0.002$ for SV).

Discussion

Circalunar cycle. Though many years of research the origin and the meaning of rhythmic changes in biological systems remains unknown (Glass, 2001). Physical factors like temperature, light, electromagnetic fields and other meteorological and in the end astronomical causes are accepted to influence many of the common biorhythms (Shepard and Shek, 1997). On the other side an internal clock — an endogen Zeitgeber has been postulated. In the mean time its morphological correlates has been found. Nucleus suprachiasmaticus and its anatomical and physiological relations to the pineal and pituitary glands are thought to be responsible for many of the cyclic variations of parameters of the homeostasis (Schlechter, 1996; Swaab et al, 1996). The latest research in molecular biology has even brought evidence for a genetic correlate of the endogen Zeitgeber (Barinaga, 2000; Moore, 1999).

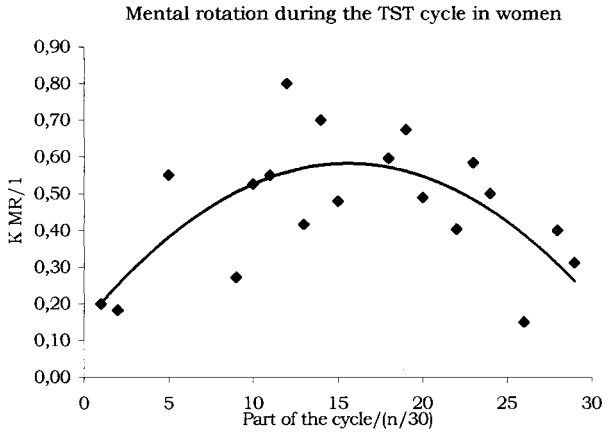

Fig. 7. Average results of mental rotation tests on different parts of the menstrual cycle in women.

The most common and recognized rhythms in nature are probably the circadian cycle of sleep and the reproductive cycle. Both are known to be coupled with changes in the hormonal status but it is unclear whether the variations of melatonin levels and sex hormones are the cause or only the effect of these rhythms. The debate discussing this problem is not only of an academic nature. A plenty of various theoretical and clinical implications are linked more or less to the chronobiological status and characteristics although the pathophysiological mechanisms underlying these relations are not always apparent (Ralph, 1996; Brigagao and Colepicolo, 1998; Gupta et al, 2000; Miliani et al, 2000; Mitamura et al, 2000).

The levels of TST in circulation have a wide spread influence on the risk or the prognosis of different clinical situations. TST plays a crucial role in the gametogenesis and thus it influences the fertility status of both sexes (Takamiya et al, 1998; Zirkin, 1998; Saito et al, 2000). A slowly decrease of both, TST and estradiol levels and the diminishing of their cyclic variations (Tenover et al, 1988; Dabbs, 1990) are tightly connected to the appearance of the menopause (Buckler et al, 1998; Davis, 1999), andropause (Burns and Gingell, 1997; Tenover, 1997; Gauld and Petty, 2000; Gambineri et al, 2001; Harman et al, 2001) and to the whole aging process in both sexes (Lamberts et al, 1997). Affecting the lipid and protein metabolism TST (Gomez and Dallman, 2001) influences also the risk of civilization diseases like the coronary artery disease and atherosclerosis — lowering of the HDL/LDL ratio (Phillips, 1977; Barret-Connor and Goodman-Gruen, 1995; Eckardstein et al, 1997), hypertension — effect on atrial natriuretic peptide (Pandey et al, 1999; Zaugg et al, 2001), NO-synthase (Weiner et al, 1994; Goetz et al, 1999; Singh et al, 2000), electric activity of the heart (Bidoggia et al, 2000), renin-angiotensin system (Leung et al, 2000), but also Alzheimer disease — reduction of amyloid secretion (Gouras et al, 2000; Cunningham et al, 2001; Mong et al, 2001), diabetes mellitus — SHBG mediated increase in insulin sensitiveness (Birkeland et al, 1993; Peiris et al, 1993; Preziosi et al, 1993), depression (Reilly, 2000; Davis and Tran, 2001; Zitzman and Nieschlag, 2001) and kidney dysfunction — anabolic enhance of protein turnover affect the cross linking process of glycated proteins related to renal toxicity (Boor et al, 2001; Dudkowska et al, 2001). Last but not least acting as a growth factor on many types of cells and tissues TST is definitely involved in

the process of cancerogenesis and forming of metastases of various, not only hormone dependent types of tumors (Halberg et al, 1980; Simpson et al, 1989; Cauley et al, 1999; Powles and Hickish, 1995; Umekita et al, 1996; Kmietowicz, 1997; Joly-Pharaboz et al, 2000).

For the monitoring of TST levels in our study did not exceed one period of the predicted cycle our results could not bring a clear undeniably biological evidence for the existence of a male circalunar cycle. Nevertheless, some important indices are shown. We have found a singular maximum peak near to the middle of the acrophase, the relative variations throughout the time period are highly significant and the levels at the end of the predicted cycle are very similar to those at the beginning.

In various species a synchronized rhythm of sex hormones related to the reproductive cycle of both sexes has been found (Kihlström and Hornstein, 1964; Degani et al, 1997; Guillette et al, 1997; Yang et al, 1997; Sohn et al, 1999; Tigar and Osborne, 1999; Ott et al, 2000; Rahman et al, 2000; Rhen et al, 2000). There is no relevant reason why the human species should have developed in the phylogenesis an intersexual difference concerning the infradian biorhythms of sex hormones. However, it is possible that the changing living conditions, the permanent stress hormone axis activation and other "civilization factors" could have contributed to the noisiness of the data and to the problems with searching, detecting and identifying of these and other biorhythms in human.

Spatial abilities. The data obtained in our study revealed also the effect of TST on spatial ability in both sexes. In accordance with general knowledge men scored better in both spatial tests (mental rotation and spatial visualization) than women. As for salivary TST levels sexual dimorphism was proved, men having significantly higher salivary TST levels than women. Women with the highest average salivary TST have reached the highest scores in spatial orientation tests. The opposite was found in men, those with the lowest levels of TST achieved the best results in spatial tests. These findings indicate a non-linear relationship between TST and spatial performance, with moderate levels of androgens being associated with better spatial ability. Our results support the data reported by previous studies (Shute et al, 1983; Gouchie and Kimura, 1991; Neave et al, 1999) where significant non-linear associations between TST and spatial performance were found. It can be supposed that hormonal bipotenciality favors spatial orientation as was proposed by Petersen (1976) and later confirmed by Moffat and Hampson (1996). Further results of our study suggest that changes in spatial performance may depend on salivary TST fluctuations in both male and female subjects throughout a circalunar cycle, which would support the activational effects of currently circulating TST levels reported in works studying circannual (Kimura and Hampson, 1994) and menstrual (Hampson, 1990) variations and the effect of a hormone replacement therapy in men on the circulating steroid levels (Janowsky et al, 1994).

However, as stated previously, not all studies investigating the relationship between spatial scores and androgen concentrations in humans have found evidence for non-linear effects. The lack of consensus among published studies of the relationship between TST and spatial ability might be the result of methodological problems in previous studies, which generally have not considered the circadian and seasonal fluctuations of TST levels. There also has been little consensus with regard to the type of TST measured (plasma, total, free, salivary). Many previous studies failed to measure sex steroid hormones and deduced hormonal levels from the stage of menstrual cycle in women, thus leaving the interpretation of their results unclear. We have considered circadian as well as infradian rhythms of TST release in present study. Our findings support the role of salivary TST in influencing spatial orientation in healthy young men and women. This is but not to say, that TST is the determinant of this special intellectual ability. As it is clear, that TST 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 as well (Nyborg, 1983). By determining TST and its active metabolite estradiol in the same sample of investigated subjects, it should be possible to investigate the option of interaction of both sex steroids on cognition.

We can say that we have described the relationship between TST and the spatial abilities, which are as a part of the cognitive performance definitely determined by both, genetic and epigenetic factors. Our study shows the importance of TST as one of these factors. We also described the variations of the levels of TST in women in relation to their menstrual cycle and the results strongly indicate the possibility of a similar circalunar cycle of TST in men. Our chronobiological outcomes have implications in the prevention and treatment of infertility, cancer and diseases of cardiovascular, central nervous and endocrinological systems. Our hypothesis of a male circalunar cycle of TST will be proved in a follow up study.

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