Salivary Sex Hormones during the Menstrual Cycle

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Abstract. Infradian rhythms of sex hormones in women are important in several physiological and pathophysiological processes. Detailed analyses of these rhythms are difficult due to problems with sampling. Salivary levels of sex steroids are widely used when repeated sampling is needed. However, a description of variation during the menstrual cycle with daily sampling is lacking. In our study salivary levels of testosterone, estradiol and progesterone were measured in samples collected daily by 17 young healthy women (21.2±0.7 years) during one menstrual cycle. Sex steroid levels were determined using radioimmunoassay. The dynamics of salivary sex steroids in our study resembles the known dynamics in plasma. Similar patterns for estradiol and testosterone confirm the hypothesis that in women testosterone serves as a precursor for estradiol. The primary (peri-ovulatory) peak and secondary (luteal) peak of testosterone are followed by similar peaks of estradiol. Progesterone reaches maximum concentrations during the luteal phase. This study shows that analysis of salivary levels of sex steroids are informative and can be used in neuroendocrine, chronobiological and other research areas, when repeated sampling is needed.

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INFRADIAN variations of sex hormones levels are difficult to study due to problems related to sample collection. Frequency of sampling is one of the most important issues and it cannot be carried out very often. The most convenient solution of this problem is the measurement of hormonal concentrations in other biological fluids that are easier available like urine or saliva. However, urinary values give only partial information. Usually measured metabolites are indirect parameters of the biologically active serum concentrations and represent the average value of an inconstant time period that varies considerably [1]. A clinical study showed that salivary levels of progesterone might be even more informative than serum or urinary concentrations [2]. Although the reason is not clear, it might be suggested that total salivary steroid levels reflect the free – bioactive fraction of serum steroids in contrast to the urinary concentrations. Steroid hormones leak into saliva from plasma. However, only the free unbound fraction is involved. Albumin and SHBG do not allow the bound fraction of the hormones to get into saliva due to their molecular weights. Thus, salivary concentrations represent a valuable marker for the free, unbound and bioactive fraction of the particular steroid [3].

Menstrual cycle is thought to be a female specific characteristic. However, a similar circalunar or circatrigintan hormonal rhythm in men has been reported [4]. Endocrinological changes and the dynamics during the menstrual cycle are described in detail [5]. Yet, this refers to plasma concentrations. Salivary hormone levels and their chronobiological pattern have not been studied in such a manner yet.
The aim of our study was to describe the dynamics of daily measured salivary sex hormone levels in women during the menstrual cycle.

**Subjects & Methods**

Seventeen young healthy women (age 21.2±0.7 years; age at menarche 12.4±1.2 years, BMI between 20 and 25 kg/m²) were enrolled in the study. The average length of their menstrual cycle was 29.3±5.1 days without any disturbances and subjective problems. None of the subjects used hormonal substitution, contraception or other drugs known for affecting sex hormone levels. Saliva samples were collected daily during one period of their menstrual cycle in autumn to avoid methodological problems in relation to circannual fluctuation of sex hormones. While circadian rhythm could also interfere with the outcome, to reduce its effect saliva samples were collected at 7:00-8:00 AM before eating or cleaning the teeth. No saliva stimulants were used. During the whole time of sampling subjects were instructed to reduce physical and irregular sport activities and to keep alcoholic and sexual abstinence. All participants gave an informed consent.

Salivary estradiol (E), progesterone (P) and testosterone (T) levels were determined by radioimmunoassay as described previously [4]. The intra-assay coefficient of variation is under 5% (on average 3.6%). The inter-assay variability is 8.2%. Due to the variability in the length of the menstrual cycle the obtained values were assigned to parts of the cycle and not to days. Paired t-test was used for the comparison of the means of dependent variables, p<0.05 was considered significant. Analysis was performed by Microsoft Excel 2000 and XL Statistics version 5.51 software. Data are presented as mean ± standard deviation.

**Results & Discussion**

The dynamics of salivary E, P and T during one period of menstrual cycle in our subjects are shown on Fig. 1. E reaches the lowest levels during menstrual phase and the highest just around the ovulation and in a secondary peak in the late luteal phase. P produced mainly by corpus luteum is found in highest concentrations during the luteal phase. T peaks are observed just prior to E peaks. The differences between the peak and the nadir values in all followed hormones are highly significant (p<0.001 for E and P; p<0.003 for T). The relationship between E and T dynamics supports the hypothesis that in women T functions mainly as a precursor of E. The conversion is catalyzed by aromatase – an enzyme localized in the adipose tissue but also in the brain [6].

The resulting curves do not differ considerably from those reported for the particular hormones in plasma [7]. However, as we did not measure daily plasma concentrations, the correlation of salivary and
plasma levels can only be supposed according to the studies by others [8, 9]. Our study shows that salivary sex hormone concentrations are valuable for chronobiological and other research where daily levels are needed. The simplicity and availability of saliva sampling makes it suitable for frequent long term hormonal monitoring, which is required for studies on biological rhythms, psychobiological development, interventional experiments, etc. [10].

In summary, according to our knowledge, herein the dynamic pattern of daily measured salivary sex hormone changes in association with the menstrual cycle in young healthy women is presented for the first time.

Our results may have implications in clinical and experimental research.

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