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DETERMINATION OF URINARY NORANDROSTERONE EXCRETION IN FEMALES DURING ONE MENSTRUAL CYCLE BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY

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Extended abstract

Introduction

Conjugated norandrosterone is the main urinary metabolite of anabolic steroids like nandrolone, norandrostenedione and norandrostenediol as well as a minor metabolite of oral contraceptives like norethisterone. Of endogenous origin, nandrolone traces have been identified in human follicular fluid [1,2] and further investigations revealed the urinary excretion of norandrosterone in pregnant [3,4] and non-pregnant females [5] and even males [6,7]. These facts have led to a threshold level of five and two ng/ml in urine for females and males, respectively, when proving the prohibited administration of nandrolone or its precursors in human doping control.

This investigation was undertaken in order to study more systematically the urinary excretion of norandrosterone in females during a whole menstrual cycle.

Materials and methods

One night's urine was collected from 12 female volunteers, who were not pregnant and did not use any contraceptives, every day during a whole menstrual cycle. The collection of the samples started on the first day of the menses, and they were stored at 4°C until analysis.

The samples were analysed for norandrosterone both quantitatively and qualitatively

according to modified procedures described in the literature [7,8]. Sample preparation included solid phase extraction, enzymatic hydrolysis, liquid-liquid extraction with n-pentane and Trimethylsilylation with N-Methyl-N-trimethylsilyltrifluoracetamide (MSTFA). For the identification of norandrosterone as bis-O-trimethylsilyl derivative a HPLC semi-preparative clean-up was applied. For quantitation D3-Noretiocholanolone was used as internal standard and multiple ion mass detection (MID) was performed at a resolution of R=5000. For identification the criteria proposed by the International Olympic Committee (IOC) were applied as well as full scan mass spectra were recorded.
The method achieved a limit of detection and quantitation of 0.02 ng/ml and 0.05 ng/ml, respectively. The precision was determined to 3 – 5% (intra-assay) and 5 – 27% (inter-assay) in a concentration range of 0.1 to 1,5 ng/ml.
All urine samples were also analysed for luteinizing hormone (LH) by a competitive fluoroimmunoassay (DELFIA, Wallac, Finland).

Results

The results show clearly that all the volunteers excrete norandrosterone glucuronide in a characteristic pattern during one menstrual cycle. At the beginning of the follicular and at the end of the luteal phase urine concentrations were considerably lower than midcyclic. Peak concentrations up to 0.8 ng/ml (2.9 nmol/l) were recorded and they were three to four times increased compared to the values at the beginning and end of the cycle. Fig. 1 shows the mean concentrations of norandrosterone – corrected for urine density – on the day with the maximum concentration for each volunteer, where the standard deviation (n=12) is indicated as a vertical line. Additionally the average concentrations including standard deviations for the five preceeding and five following days are shown. Fig. 2 shows one typical concentration profile of one volunteer for norandrosterone and the luteinizing hormone (LH) exemplifying the condensed data in fig. 1.

Discussion

The established quantitative method for the determination of norandrosterone in urine was appropriate in order to detect norandrosterone in almost all female urine samples. This confirms previous observations of the excretion of nandrolone metabolites in females. The systematic approach indicated clearly a consistent pattern of norandrosterone excretion during

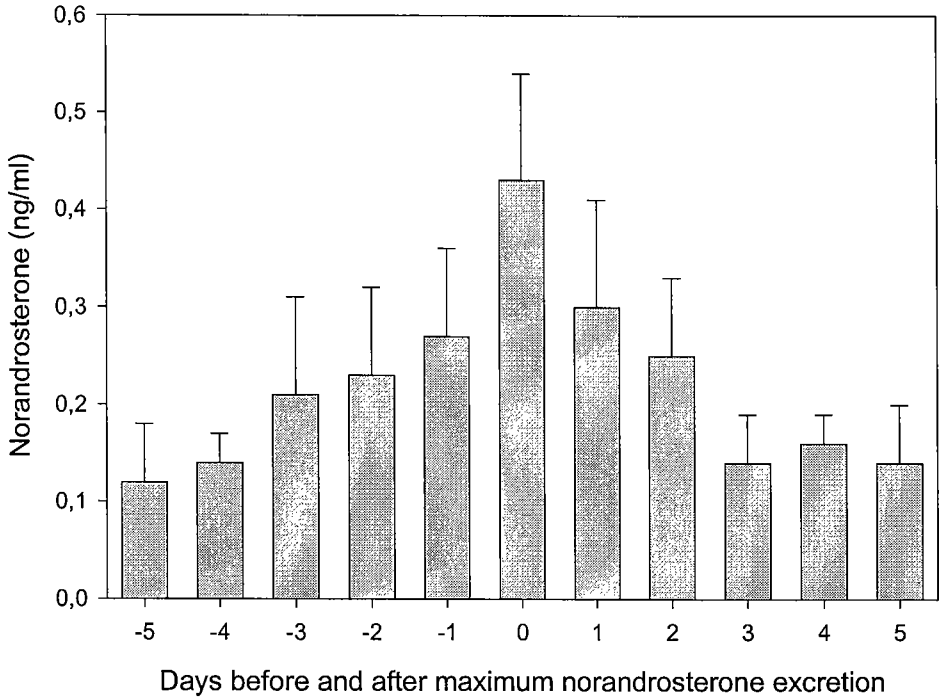

Fig. 1 Urinary norandrosterone concentration (mean and standard deviation, n=12) before and after the midcyclic maximum value.

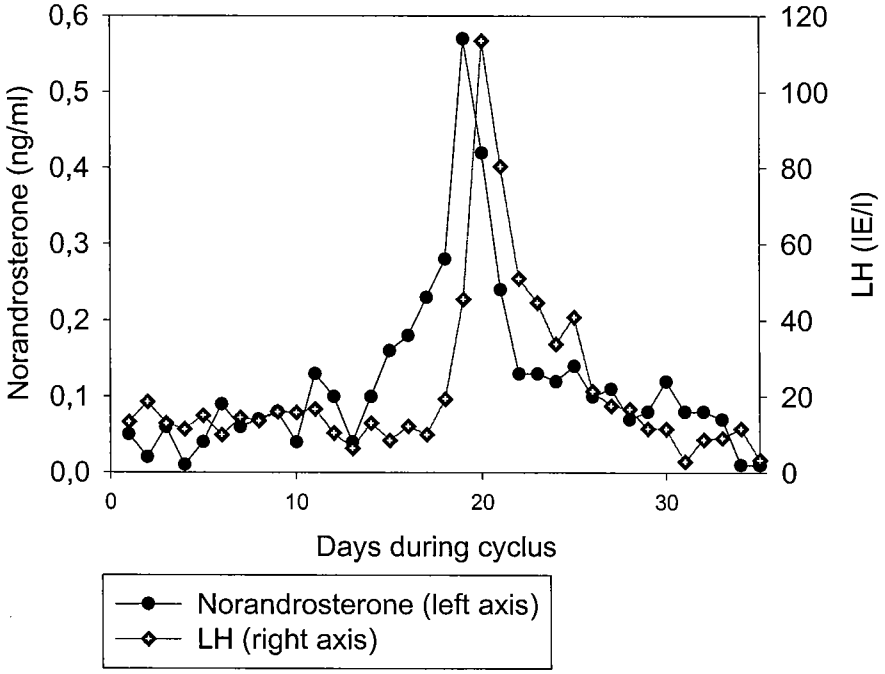

Fig. 2 A typical concentration pattern for urinary norandrosterone and luteinizing hormone concentrations during one menstrual cycle for one volunteer

one menstrual cycle, showing a clear maximum at the time of the anticipated ovulation. Although this study was not planned in order to monitor exactly the time of ovulation, both the LH measurements and the results of two blood samples, taken at the 7^{th} and 20^{th} day confirm these anticipations.

The time of the peak concentration of norandrosterone compared to LH (see fig. 2), which was consistent throughout the group of volunteers, could be coincident with the maximum estradiol concentration. These results support strongly the possibility of an endogenous nandrolone production as a side reaction of the enzymatic aromatisation. An investigation of this mechanism in cell cultures is in progress.

However, when comparing the measured absolute and uncorrected norandrosterone concentrations, ranging from below the limit of quantitation (0.05 ng/ml) to 0.87 ng/ml, all values of 360 samples from 12 female volunteers were well below 5 ng/ml. This threshold value is currently used for reporting adverse findings in doping control of females. The data in this study supports the value of the threshold, because even the observed midcyclic peak concentrations were far below it.

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