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Plasma Levels of 19-Norsteroids after Oral and Buccal Administration of Norandrostenedione

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Abstract

A method for the isolation and quantitation of nandrolone, norandrostenedione, norandrosterone and noretiocholanolone in plasma by means of gaschromatography/mass spectrometry (GC/MS) is described. The method was used in a pharmacokinetic pilot study to investigate the metabolic profile of norandrostenedione after oral and buccal application of the steroid. Norandrostenedione given orally or bucally is absorbed in a different manner and as a result the metabolite formation may be different. The knowledge of the method of administration provides a possibility to discriminate the use of lozenges and intra-oral sprays from use of tablets, capsules and contaminated nutritional supplements.

Until now it has not been verified to which extent norandrostenedione is metabolised to its active form nandrolone.

In contrast to urine analysis blood offers a medium in which apart from the main urinary metabolites the parent compound as well as nandrolone are detectable. This preliminary study gives information about the plasma levels of active nandrolone after a single dose of norandrostenedione and can thus contribute to a better understanding of the possible efficacy and mode of action of steroid prohormones.

Design of the pilot study

The project was approved by the Human Ethics Committee of the German Sport University Cologne. The volunteer was advised of the purpose of the study and associated risks and gave his written consent.

A 25 mg norandrostenedione lozenge was administered to a healthy caucasian male via sublingual intake. 10 ml blood samples were withdrawn through the brachial vein using an indwelling catheter according to the time schedule in table 1. Three weeks after the buccal administration the same volunteer took a single dose of a 100 mg norandrostendione capsule by peroral ingestion. Blood collection and sample treatment were the same as for the buccal application study.

Number of Test Persons:	1 (male)
Race:	caucasian
Age:	48 a
Bodyweight:	79 kg
Dosage:	100 mg oral / 25 mg buccal
Taking of Blood Samples:	0`; 10`; 20`; 30`; 45`; 60`; 90`; 3h; 4h; 5h; 6h; 7h; 23h
Withdrawal Volume:	10 ml
Mode of Blood Withdrawal:	Indwelling catheter (Brachial Vein)

Table 1: Design of the pilot study.

Blood sample preparation

a) Combined fraction

The EDTA treated blood specimens were centrifuged and the supernatant plasma was transferred into polypropylene tubes and frozen at -18 °C until analysis. For sample preparation 0,5 ml aliquots of the thawed plasma were applied into glass tubes and processed according to the scheme shown in figure 1.

b) Free fraction

0,5 ml serum were mixed with internal standard (1 µg/ml noretiocholanolone-D3 and 0,1 ng/ml testosterone-D3) and adjusted to pH 12 by addition of 0,5 ml KOH (0,5 M). After extraction with 6 ml TBME all following sample preparation steps were adopted from the method in figure 1.

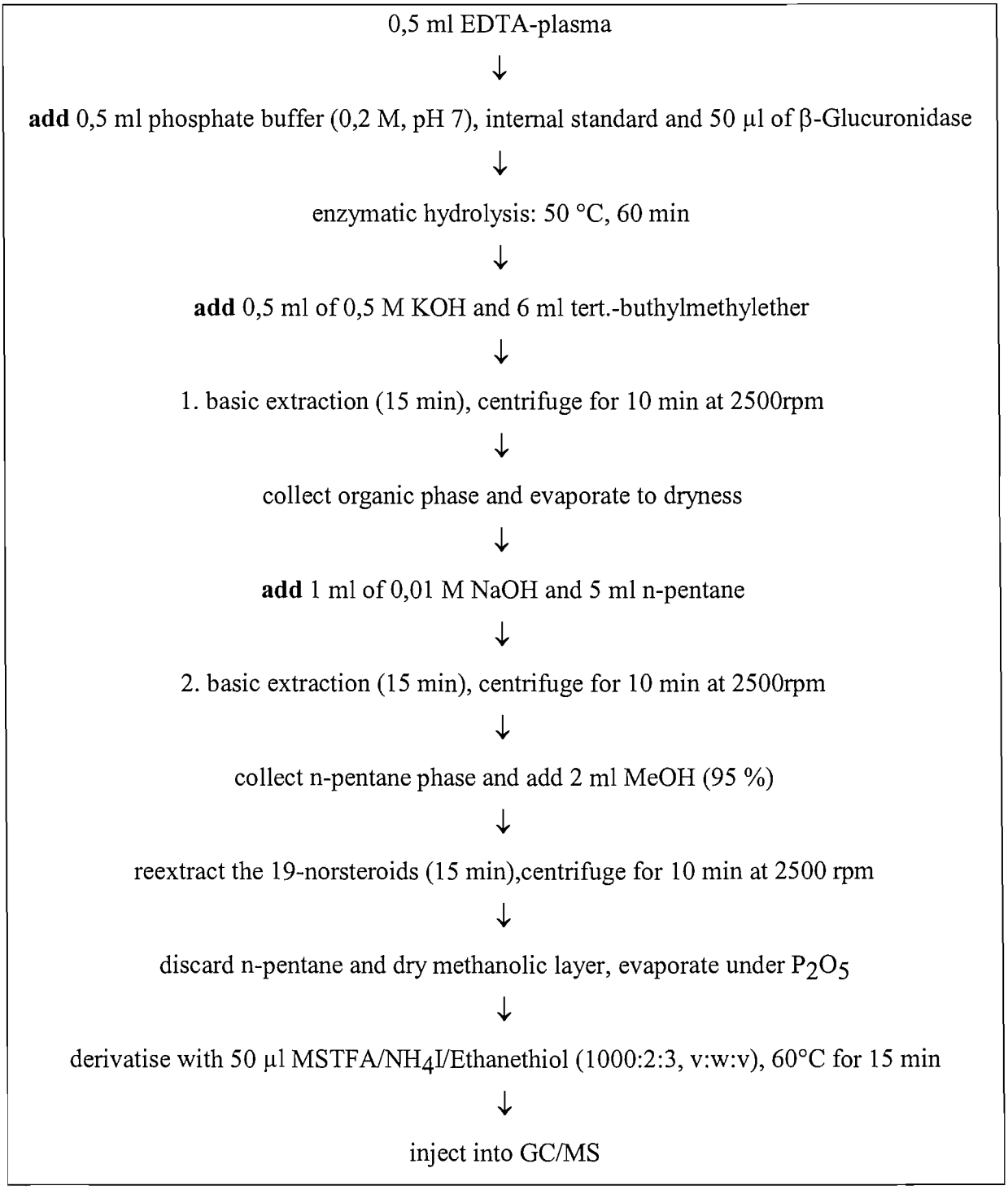

Figure 1: Sample preparation procedure for 19-norsteroids in blood.

Identification and Quantification of the 19-norsteroids

Gaschromatography/mass spectrometry analysis was performed with a quadrupole mass selective detector HP 5973 (Agilent Technologies, Waldbronn, Germany) coupled to an HP 6890 gaschromatograph (Agilent Technologies). A HP 5 crosslinked methyl silicone capillary column (length: 17 m; i.d.: 0,25 mm; film thickness 0,25µm) was employed with helium as the carrier gas at a flow-rate of 0,9 ml/min in the constant pressure mode at 9 psi. A 2 µl aliquot of sample was injected onto the GC column in the splitless mode. The column temperature was programmed from 250 °C at 40 °C/min to 190 °C, then at 5 °C/min to 240 °C, hold for two minutes and finally again at 40 °C/min to 320 °C, final time 3 min. The injection port and transfer line were heated to 300 °C. Ions were formed by 70 eV electron impact (EI) ionization. The ion source was held at 230 °C. Data aquisition was performed in the selected ion monitoring (SIM) mode. The electron multiplier voltage was set to 1600 - 1800 V depending on the actual tune. The following ions were monitored:
m/z 405, 420 for identification of norandrosterone and noretiocholanolone,
m/z 416, 417 for identification of norandrostendione
m/z 403, 418 for identification of nandrolone.
To confirm the linearity within the concentration ranges under study (0,5 - 30 ng/ml for norandrostendion and nandrolone; 4 - 600 ng/ml for norandrosterone and noretiocholanolone) calibration curves were recorded after analysing spiked plasma specimens. Calibration curves were recorded before and following a sequence of samples, so that two values for each concentration were obtained. The steroid concentrations were calculated from the peak area responses of the analyte in relation to the respective responses of the internal standards. Nandrolone and norandrostendione were related to testosterone-D3 (m/z 435), norandrosterone and noretiocholanolone to noretiocholanolone-D3 (m/z 408). The peak areas of the internal standard noretiocholanolone-D3 were corrected by subtracting the isotope signal m/z 408 caused by noretiocholanolone. The isotope ratio of noretiocholanolone m/z 408 to m/z 405 was 0,028.

Results

a) Plasma levels of the 19-norsteroids in the combined fraction

The obtained plasma levels of nandrolone, norandrostenedione, norandrosterone and noretiocholanolone in the combined fraction after the oral application of a 100 mg norandrostenedione capsule are summarised in figure 2a. A 20 – 30 min latency period was followed by nearly simultaneous peak maxima of all 19-norsteroids after which a relative rapid decrease to pretreatment levels was found.

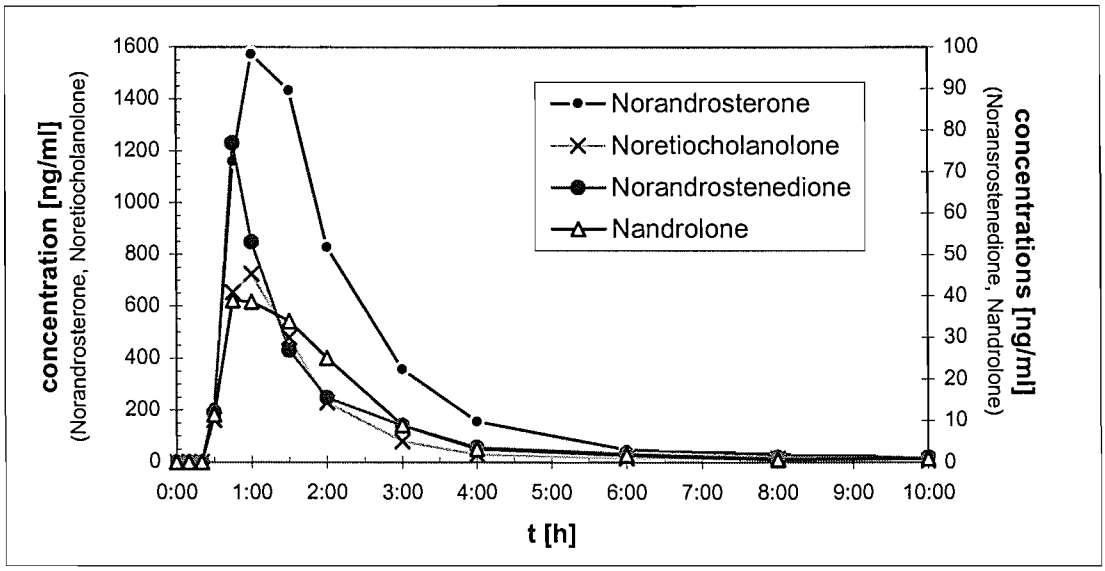

Figure 2a: Plasma levels of 19-norsteroids in the combined fraction after oral application of a 100 mg norandrostenedione capsule.

In the case of buccal administration of a 25 mg lozenge the concentration of norandrostenedione increased immediately to its maximum value whereas the concentration maxima of the metabolites were monitored after a delay.

This led to a shift of the norandrostenedione peak to the left in the concentration over time diagram and therefore to a domination of norandrostenedione in early phases of metabolism (figure 2b).

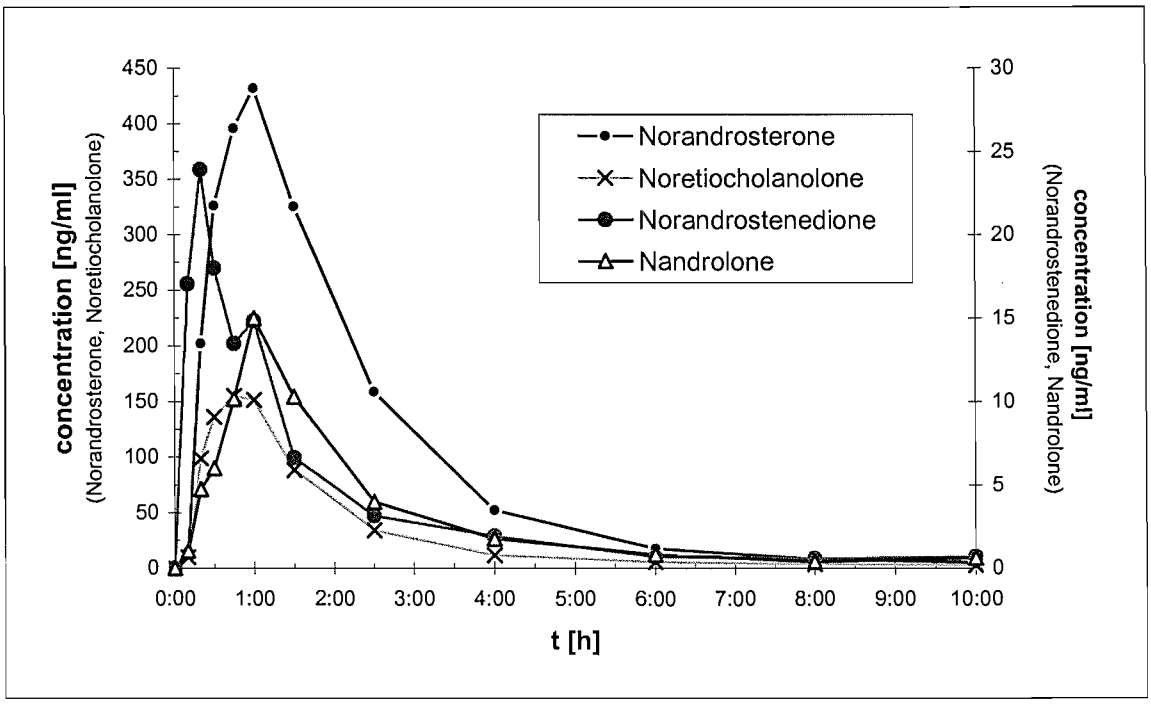

Figure 2b: Plasma levels of 19-norsteroids in the combined fraction after buccal application of a 25 mg norandrostenedione lozenge.

b) Plasma levels of free nandrolone

Constant plasma levels of free nandrolone in the range of 0.5 to 1.0 ng/ml have been considered as the minimum levels that are of physiological relevance in adult man [1,2]. In this concern it is important to know wether the threshold concentration can be exceeded by consumption of nandrolone precursors which are freely available as nutritional supplements. The concentration over time curves in figure 3 were monitored after oral application of the 100 mg norandrostenedione capsule and after sublingual administration of the 25 mg norandrostenedione lozenge, respectively. In both cases the proclaimed physiological levels of free nandrolone were achieved. Under this view the regular use of steroid precursors is expected to provoke effects and adverse effects similar to steroid anabolics.

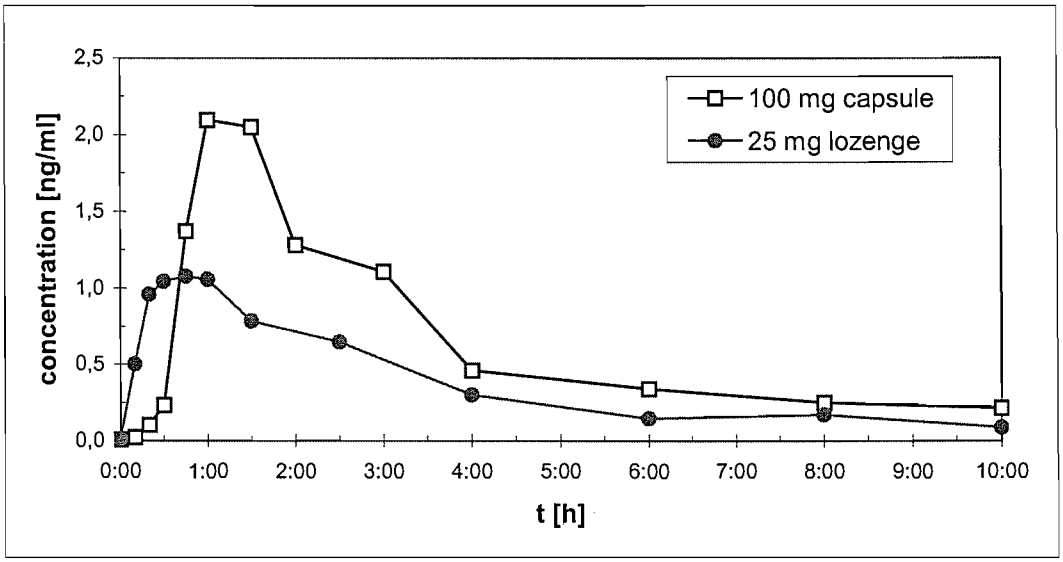

Figure 3: Plasma levels of free nandrolone after administration of nutritional supplements containing norandrostenedione.

This represents the first report in which the plasma concentration of the actually effective free steroid hormone is measured after application of one of its precursors.
Follow up studies are in preparation and the results will be published elsewhere.

References

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[2] U.A. Knuth, H. Behre, L. Belkien, H. Bents and E. Nieschlag: Clinical Trial of 19-Nortestosterone-hexoxyhpenylpropionate (Anadur) for Male Fertility Regulation, Fertil. Steril., 44 (1985) 814 – 821.