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Inter-Laboratory Study of Low Levels of Nandrolone Metabolites

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Inter-laboratory study of low levels of nandrolone metabolites

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Abstract

An inter-laboratory study with confirmation of findings of low concentrations of nandrolone-metabolites was carried out. The aim of the study was to provide information about the occurrence of norsteroids and their distribution between the free fraction, the glucuroconjugated fraction and the sulphoconjugated fraction. Two laboratories took part in the study. The samples were selected from the routine screening analysis. Additionally, some samples with known endogenous or exogenous origin were analysed. The interlaboratory study gave results in good agreement between the two laboratories. Further the distribution of the different fractions was examined with respect to a possible discrimination between an endogenous origin or an intake of nandrolone or nandrolone precursors. No such correlation could be demonstrated in this study.

Introduction

The identification of norandrosterone (NA) and noretiocholanolone (NE) down to 0.1 ng/ml in urine is possible with high sensitivity screening techniques as the detection by high-resolution mass spectrometry. However, the reporting threshold for these metabolites is 2 ng/ml [1]. An inter-laboratory study was carried out with samples containing low levels (above detection limit but below or close to the reporting threshold) of NA and NE. Findings of NA and NE in such low concentrations occur in approximately 1-2 % of the total number of samples analysed by the two participating laboratories. These samples are not reported as adverse findings, because of the established threshold levels for these analytes. This study provides information about the occurrence of norsteroids and the distribution between the different conjugated forms in these samples. The results are compared to the results from samples with nandrolone of endogenous origin (from pregnant women and women mid-cycle)

and positive samples from late excretion stage after the intake of nandrolone or nandrolone-precursors.

The study

The aim of this study was to compare the results from two laboratories after confirmation of low levels of NA and NE. A total of 85 samples were selected from routine screening results. This amounts to 1-2 % of the total number of samples analysed by the 2 laboratories each year. 46 % of the samples were from women, 47 % from men and in 7 % gender was unknown. When the total number of samples in the 2 laboratories is considered, the percentage of female samples received in Oslo and Kreischa were 27% and 36 % respectively. The fact that the samples represent a diversity of many different sports (handball, athletics, gymnastics, basketball, skating, cycling, biathlon, boxing, wrestling, weightlifting, ice hockey, swimming, cross-country skiing, bowling, football, volleyball, climbing) might indicate that the overall findings are more a result of natural occurrence than administration of doping substances.

Experimental

A harmonised protocol for sample preparation that makes identification and quantification of the analytes in the different fractions (free, glucuronides, sulphates) possible was used. The sample preparation is based on the analytical methods described by Schänzer et al. [2,3] An additional solvolysis procedure was applied for the cleavage of sulphates. [4,5] The method is outlined below:

- 2.5 ml of urine
- Free fraction: collected after extraction with n-pentane at pH 5-7
- Glucuronide fraction: the aqueous phase after removal of free fraction was passed through C₁₈ or XAD solid phase extraction columns, enzymatic hydrolysis was carried out with β -Glucuronidase (E-coli) and extraction with n-pentane at pH 9-10
- Sulphate fraction: 10 N HCl and ethylacetate were added to the aqueous phase after removal of glucuronide fraction. Solvolysis was carried out for 3 h at 50 °C. Extraction with n-pentane at pH 9.
- Derivatization with MSTFA:NH₄I:Ethanethiol
- Analysis by GC-HRMS

Results

Inter-laboratory study

In Table 1 the findings from the inter-laboratory study are presented.

NA is the most frequent occurring metabolite, either as the only metabolite or in combination with NE. NE did not occur as the only metabolite in any of the samples.

Table 1. Results from the inter-laboratory study

| Findings | Oslo | | Kreischka | |
|-----------|------|-----|-----------|-----|
| | n | % | n | % |
| None | 18 | 21 | 15 | 18 |
| NA + NE | 53 | 62 | 57 | 67 |
| NA (only) | 14 | 17 | 13 | 15 |
| NE (only) | 0 | 0 | 0 | 0 |
| Total | 85 | 100 | 85 | 100 |

Distribution of the ratio NA/NE

The distribution of the concentration ratio NA/NE is presented in Figure 1.

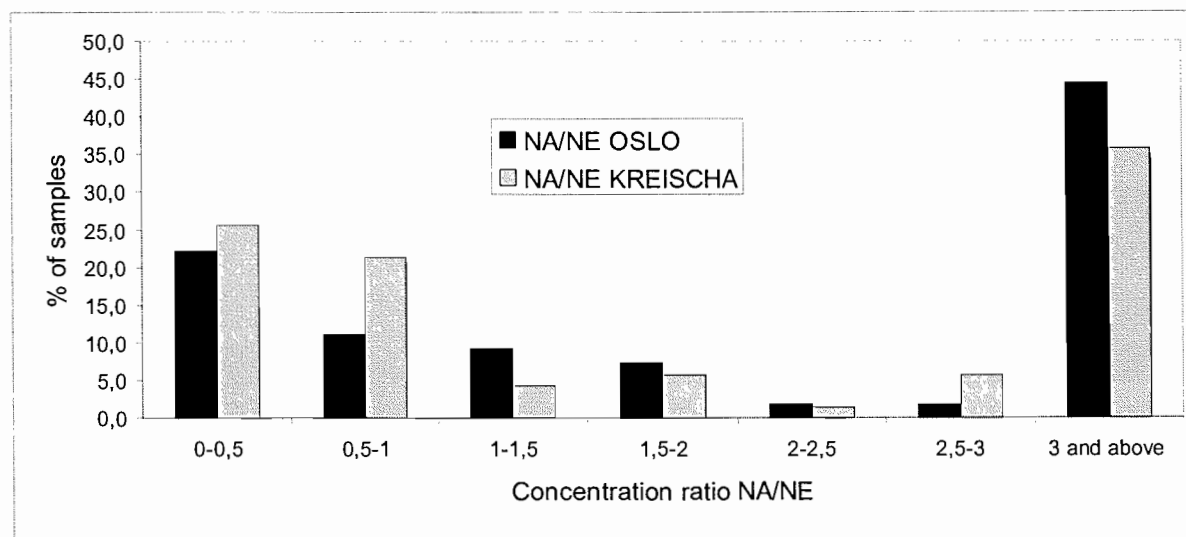


Figure 1. Distribution of the ratio NA/NE

Distribution of NA as conjugates

Figure 2 shows the distribution of NA as glucuronides and sulphates, respectively.

The conjugation occurred predominantly as glucuronidation (70-90 %) in samples where both conjugates were present.

Sulphates were present as well, in few samples as the only conjugate, but usually 10-30 % occurred as sulphates where both conjugates were present.

NE followed the same pattern for distribution among the fractions as NA.

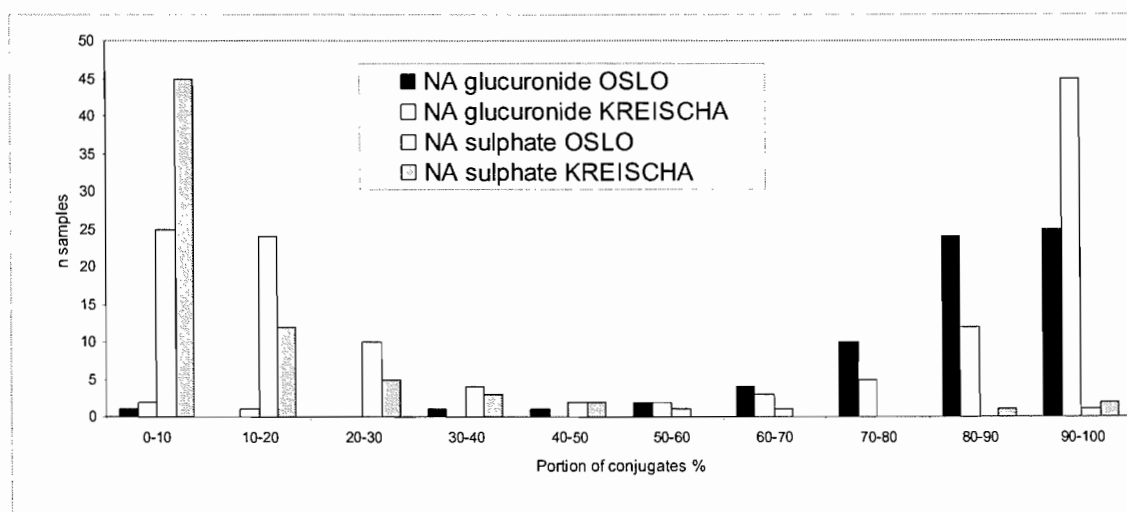


Figure 2. Distribution of NA as conjugates

Analysis of samples that contain endogenous NA

The samples were from pregnant women (n=4) or from women mid-cycle (n=2). The concentration of norandrosterone ranged from 0,3-3,3 ng/ml, noretiocholanolone ranged from 0,2-0,6 ng/ml. The glucuroconjugated metabolites were distributed mainly between 70-90 % and the sulpho-conjugated metabolites were correspondingly distributed from 10-30 %.

Analysis of samples after administration of nandrolone or nandrolone precursors

The samples were collected at late excretion stage after intake of nandrolone or nandrolone precursors. 7 samples were analysed. The concentration of norandrosterone ranged from 0,8-7,4 ng/ml, noretiocholanolone ranged from 0,8-1,9 ng/ml. The conjugated metabolites were distributed in the same pattern as seen for endogenous metabolites, 70-90 % as glucuronides and 10-30 % as sulphates.

Discussion and conclusion

The inter-laboratory comparison gave results with a good agreement between the two laboratories, both with regard to the distribution of NA and NE in the different fractions and to the NA/NE ratio in the samples. One should bear in mind that major contributions to the measurement uncertainty is attributed to the determination of NA and NE at sub-nanogram

levels and to the fact that no commercial standards for NA-sulphate and NE-sulphate are available. The possibility to differentiate between endogenous produced nandrolone and administered nandrolone (precursors) based on the distribution of metabolites in the different fractions was proposed by Le Bizec et al [5]. The findings in this study could not confirm this theory. The distribution of NA/NE ratios may also be seen in relation to the observation of in-situ production of NA and predominantly NE in urine samples [6,7], especially because of the long storage times for the samples analysed in this study.

Acknowledgements

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