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19-Norandrostosterone in Pregnant Women
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19-Norandrosterone in Pregnant Women

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Abstract

It is well known that pregnancy may lead to urinary excretion of 19-norandrosterone. The excretion of 19-norandrosterone is probably due to a large increase of the excretion of estrogens during pregnancy. The details regarding its formation are not yet completely understood.

Urinary concentrations of 19-norandrosterone during pregnancy are investigated. Analyzed were urine samples collected from five pregnant women once a week during the whole course of pregnancy as well as 50 spot urine samples from different pregnant women collected one time during the second part of pregnancy. The analysis was performed by gas chromatography/mass spectrometry (GC/MS).

The detection of 19-norandrosterone (detection limit 0.2 ng/ml) was possible from the 14th week of pregnancy.

Statistical evaluation of the urine samples collected systematically by the five volunteers was proceeded by a non parametric test for monotonous trends.

The concentrations of 19-norandrosterone in healthy pregnant women shows an increase during the course of pregnancy.

One volunteer was suffering from placenta malfunction. The distribution of the concentrations of 19-norandrosterone for this volunteer was irregular, no increase during the course of pregnancy was detectable.

The analyzed spot urine samples show concentrations of 19-norandrosterone situated approximately within the range which was shown before.

The distribution of the concentration of 19-norandrosterone in all analyzed urine samples shows for most urine samples values less than 5 ng/ml. 7% show higher concentrations, as max value 16 ng/ml was regarded.
Introduction

In August 1998 the IOC has given advice to the accredited laboratories regarding reporting thresholds for 19-norandrosterone. These cut-off-limits are 2 ng/ml for male and 5 ng/ml for female athletes to avoid false positives due to meat consumption of nandrolone treated animals or endogenous production of 19-norandrosterone. The international cycling federation (UCI) has fixed only one cut-off-limit (5 ng/ml) for both, male and female athletes.

In the last two years low amounts of 19-norandrosterone were detected as endogenous origin in several publications. 1999 Saugy (Lausanne) observed an increase of the excretion of 19-norandrosterone in male football players during exercise (1).

Van Eeemo (Gent) described a case study of a female cyclist where it was possible to detect traces of 19-norandrosterone (2).

Ciardi (Rome), Bizec (France) and Dehennin (France) showed also low amounts of 19-norandrosterone in "normal" urine samples (3,4,5,6).

Ueki (Tokyo) presented at the Manfred Donike Workshop – 18th Cologne Workshop on Dope Analysis (20th till 25th February 2000) a lecture titled “Low Concentrations of Nandrolone Metabolite in Urine of Olympic Athletes.”

It is known that pregnancy may lead to excretion of 19-norandrosterone (7,8).

The IOC recommendations already consider pregnancy as a reason not to report a positive for nandrolone. The same reference to pregnancy was included in the data collected by UK Sports (9).

The formation of 19-norandrosterone is not yet completely understood. Fig.1 shows the biosynthesis of estrogens and proposed pathway to the urinary 19-nortestosterone. Probably oxidation and decarboxylation leads to formation of 19-nortestosterone from 19-oxotestosterone. 19-norandrosterone is the main urinary metabolite of 19-nortestosterone. Less information about urinary ranges and concentrations of 19-norandrosterone in pregnant women does exist.

It is possible that doping control urine samples of pregnant athletes are collected and analyzed. In this context knowledge about urinary concentrations of 19-norandrosterone during pregnancy should be of interest.
Experimental

Sample preparation
The urine samples were prepared and analyzed according to the standard operating procedure for anabolic steroids (10,11).

Derivatisation
The dry residue was derivatised with 100 µl of MSTFA/NH₄I/ethanethiol 1000:2:3 (v:w:v) and heated for 20 min at 60°C.
3 µl of the solution were injected into the GC/MS.

GC/MS parameters
GC/MS: HP 6890/HP 5973 (Hewlett Packard)
electron impact: 70 eV
column: HP Ultra I (OV-1), 17m, 0.2mm i.d., 0.11 µm film thickness
carrier gas: 1ml helium at 180°C, split 1:10
temperature program: 180°C, 3°C per min, 229°C, 40°C per min, 320°C

GC/MS quantitation (12,13)
The calculation of 19-norandrosterone was proceeded with a positive control urine which was prepared by adding sufficient substance to a negative control urine to produce a positive control urine with a nominal concentration of 2 ng/ml.

Urine samples
Five pregnant women collected morning urine samples once a week during the whole pregnancy until delivery.
Spot urine samples from different pregnant women collected once during the second part of pregnancy were made available from a gynecologist facilities.

Statistical evaluation (Fishers exact randomization test)
To check for increase of urinary 19-norandrostosterone concentration during pregnancy the data was analyzed according to the following procedure: Both variables – concentration and week of pregnancy – were transformed into binary data, where the criterion was exceeding or not the median. Testing for any kind of trend now was possible with any test suited for two way contingency tables. Fishers exact randomization test was used (14).

Results and Discussion

The urine samples were prepared and analyzed according to the screening procedure for anabolic steroids (10,11). This method covers free and conjugated 19-norandrostosterone after hydrolysis. The standard operating procedure for norandrosterone confirmation (which includes the separation of the free fraction with ether and extraction with n-pentane) was not used for this study because a comparison to data from routine analysis was intended.

Due to the fact, that the density of the urine samples was not higher than 1.020, the concentrations were not corrected according to the recommendations of the IOC.
In fig.2 the urinary concentrations of 19- norandrosterone during pregnancy are shown for 5 volunteers.
Volunteer UO started collecting urine samples first in the 28th week of pregnancy. Most of her urine samples show concentrations of 19-norandrostosterone higher than 2 ng/ml.
The other volunteers started with the collection of the urine samples between 5\textsuperscript{th} and 10\textsuperscript{th} week of pregnancy.

For three volunteers 19-norandrosterone was detected the first time between the 14\textsuperscript{th} and 16\textsuperscript{th} week of pregnancy.

Volunteer IS showed 19-norandrosterone first in the 21\textsuperscript{st} week of pregnancy.

In the first part of pregnancy the calculated concentrations of 19-norandrosterone for all volunteers are situated between 1 and 2 ng/ml.

Four of five volunteers appear to have an increase of the 19-norandrosterone concentration during their course of pregnancy.

Volunteer KE was suffering from placenta malfunction.

The concentrations of 19-norandrosterone were situated in a range between 1 and 2 ng/ml during the whole course of pregnancy. She delivered in the 34\textsuperscript{th} week of pregnancy.

To check for increase of urinary 19-norandrosterone concentration during pregnancy the data was analyzed with "Fishers exact randomization test".

The Fisher test gives a positive result, where the odds ratio is significantly higher than one.

This gives evidence for an increase of the concentration of 19-norandrosterone during pregnancy.

Exemplarily the data for volunteer SM are shown in fig.3. The median of week of pregnancy and concentration of 19-norandrosterone are selected and the cut off lines marked.

On the left and right side of the diagram as well as above and below the same number of data points exist.

Expecting an irregular distribution (this means: no increase of the 19-norandrosterone concentration during the course of pregnancy) each of the four fields should contain the same number of data points.

However, volunteer SM shows in the first part of pregnancy most data points for low concentrations of 19-norandrosterone (0-2.7 ng/ml) and in the second part most data points for high concentrations of 19-norandrosterone (2.7-7 ng/ml).

This kind of statistical evaluation proves a significant increase of the concentration of 19-norandrosterone during the course of pregnancy.

For all other volunteers – except KE – this statement is also true.

Volunteer KE shows almost the same number of data points in each field (fig.4). Due to her placenta malfunction there may be no increase of the concentration of 19-norandrosterone during the course of pregnancy.

50 anonymous spot urine samples from different pregnant women collected once during the second part of pregnancy were made available from a gynocologist facilities.
The urine samples were collected on different daytime hours. The only known information of the volunteers is the week of pregnancy. Moreover, no individual data exist (e.g. age, weight, number of children, special problems or diseases during pregnancy). The results are shown in fig. 5.

The concentration of 19-norandrosterone in the spot urine samples are situated approximately within the range which was shown by the five volunteers collecting urine samples during the whole course of pregnancy once a week.

In fig. 6 the distribution of the concentration of 19-norandrosterone in all analyzed urine samples is shown. In total 252 urine samples were investigated. In 154 urine samples 19-norandrosterone was detectable. The detection of 19-norandrosterone was possible from the 14th week of pregnancy (detection limit 0.2 ng/ml). Most of the urine samples containing 19-norandrosterone show concentrations less than 5 ng/ml. 7% (= 12 urine samples) show concentrations of 19-norandrosterone higher than 5 ng/ml. The maximum value was 16.5 ng/ml.

For doping control urine samples it is important to know that for pregnant women concentrations higher than 5 ng/ml are possible. However in these cases other parameters of pregnancy are also detectable such as abnormally large hCG concentration and large signals of pregnanediol. According to the recommendations of the IOC these parameters of pregnancy lead to negative results for 19-norandrosterone.

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References


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(14) Bortz, Lienert and Boehnke: Verteilungsfreie Methoden in der Biostatistik; Springer (1990)
Fig. 1: Biosynthesis of estrogens and proposed pathway to 19-norandrosterone
Fig. 2: Urinary concentration of 19-norandrosterone during pregnancy (5 volunteers)
Fig. 3: Distribution of 19-norandrosterone concentration during Pregnancy in urine samples of one volunteer (volunteer SM)
Statistical evaluation with "fisher exact randomization test for cross tables"

Fig. 4: Distribution of 19-norandrosterone concentration during Pregnancy in urine samples of a volunteer with placenta malfunction (volunteer KE)
Statistical evaluation with "fisher exact randomization test for cross tables"
Fig. 5: Urinary concentration of 19-norandrosterone in 50 spot-urines of pregnant women

Fig. 6: Distribution of 19-norandrosterone in 154 urine samples of pregnant women