Drugs of abuse and other illicit drugs: the role of urine analysis

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Abstract
We compared urinary levels of anabolic steroids and their ratios with T/E (Eipol). The ratio was calculated according to the TO and WADA’s International standard for laboratories. The comparison is between three groups - control, people using drugs and overweight people. The testosterone/epitestosterone ratio was significantly decreased in the narcotics group and the control group. The decrease of testosterone/epitestosterone ratio was significantly decreased in the narcotics group and the control group.

Introduction
Determination of the specific identities of drugs is a major concern of doping control specialists for detection and confirmation of endogenous steroid hormones. The urinary steroid profile is composed of concentrations and ratios of various endogenous hormones such as Androsterone, Androsterone, Etiochololone, 5α-Androstan-3α-ol-17β-Diol, 5β-Androstan-3α-ol-17β-diol, and many others. Synthesis of endogenous steroid is shown on figure 1. Sex hormones are often studied as biological factors for sex differences due to their role in the central nervous system regulating activity. The endogenous endocrine system in the pathophysiology of substance use disorders and addictive behaviour. Addictive behaviour is an opioid dependence. The purpose of this study is to examine endocannabinoid damages in opioid addicts using [methadone therapy] and to study the changes in body weight and fat tissue distribution with several abnormalities of steroid balance. Androgens have an important impact on glucose and lipid metabolism and fat homoeostasis. It is likely that an androgen imbalance in obesity may play a role in the pathophysiology of the metabolic syndrome and increase the risk for cardiovascular diseases.

Materials and Methods
All reference standards were obtained from ACHMI (Australia). The GC/MS STANDARD C18-C4, C18 was obtained from Chiron (Norway). The C4 cartridge (200 mg/ml; 500mg/10ml) were from Thermo Scientific (Bremen, Germany), Silicycle (Canada). All organic solvents were of HPLC grade.

All samples were measured on an Agilent 7890A gas chromatograph and 5977A mass spectrometer. The GC system was equipped with a DB-1MS UI, 2 mm x 0.18mm x 0.18µm film thickness. The injection volume was 2 µl, the injections were performed split 1:5 at 280°C. A constant flow of 1.0 ml/min of helium carrier gas was used. The initial oven temperature was 150°C, followed by a ramp from 2°C/min to 280°C held for 4 min. Then a ramp at 5°C/min to 300°C and held for 6 min. After hydride with 5% germanium (0.1 ml) and liquid-phase extraction with TRIMM at pH 5.0, the steroids were analysed by GC/MS. Table 1 shows the screening method validation data.

The method developed for confirmatory analysis consists of solid phase extraction (SPE), liquid−liquid extraction (LLE) and preparative – HPLC purification. A 0.10-2 ml urine sample, depending on the previously measured steroid profile, was prepared by a standard operating procedure for steroids – figure 2. The clean-up was performed on a Dionex Sofam GmbH and analyzing column. A column HPLC C18, dim. (mm) 256 x 4.6, particle size 5 µ, with guard column Hyperil gold 3µm.6mm. The injection volume was 100 µl, flow rate 1.0ml/min and the FAD wavelength 286 nm.

For HPLC cleanup up 1, linear gradient was used increasing from 30% acetone/water to 100% acetone in 25 min; after 3 min at 100% acetone, the column was reequilibrated for 6 min. Four fractions were collected, two of them (A and F4) were dried. Reconstituted in acetone and injected in GC/MS. The other fractions were dried and acetylated (with 50 µl acetic anhydride at 50°C, 20 min). Then after evaporation, they were reconstituted in methanol, water 60:40 and submitted to a second HPLC cleanup.

For HPLC cleanup 2, a linear gradient was used increasing from 70% acetone/water to 100% acetone in 33 min; after 3 min at 100% acetone, the column was reequilibrated for 6 min. All samples were measured on an Agilent 7890A gas chromatograph coupled through GC-isokin and CONFID Sc to a DELTA PLUS isotope ratio mass spectrometer (Thermo Scientific). The GC system was equipped with a DB-1MS U, 2 mm x 0.18mm x 0.18µm film column. The injection volume was 2 µl; the injections were performed splitless at 350°C. A constant flow of 1.0 ml/min of helium carrier gas was used. The initial oven temperature of 150°C was held for 1.5 min, increased at 40°C/min to 240°C, followed by a ramp at 2°C/min to 280°C held for 1 min., followed by a ramp at 5°C/min to 300°C and held for 4 min. The ionization reactor was operated at 940°C and oxidized for 60 min after each sequence of 60-70 injections. The water removal was done by a Nafion membrane. The δ13C value of the CO2 reference gas was calibrated towards the GC/MS STANDARD C1/C2 program.

Results and Discussion
Using the two validated methods we analyzed three groups: control group, overweight group and methadone group. The ratio between different steroids was compared. Results are presented in Figures 3 and 4.

The period of methadone treatment is from 6 months to 100 months. The concentration of methadone is between 0.94 ng/ml and 13.5 ng/ml and depends on the course of treatment. Methadone treatment group had suppressed levels of steroids (A=3.30 ng/ml, E=2.00 ng/ml, T=0.05 ng/ml, SADiol=0.08 ng/ml, SADiol=0.11 ng/ml) compared to controls (A=1.60 ng/ml, E=3.00 ng/ml, T=0.05 ng/ml, SADiol=0.08 ng/ml, SADiol=0.11 ng/ml).

The levels of steroids in overweight group were A=34.14 ng/ml, E=31.81 ng/ml, T=0.07 ng/ml, SADiol=0.25 ng/ml, SADiol=0.24 ng/ml compared to controls A=3.60 ng/ml, E=2.03 ng/ml, T=0.05 ng/ml, SADiol=0.08 ng/ml, SADiol=13.5 ng/ml.

The control group has universal steroid profile genotype (A1, E1, T1, SADiol1.5, 2.5) with the other two groups show a sudden increase in the ratio of SADiol/SADiol. Comparing the control group and the methadone group that show a low increase methadone use had lower levels of testosterone. Samples tested by isotope mass spectrometry for determining the origin of steroids and showed that they have an endogenous character.

The overweight group set higher hematocrit levels. However, the resulting testosterone ratios did not show such large deviations from the control group. Samples were placed on the isotope mass spectrometer and showed that steroids were of an endogenous nature.

In both groups it was observed that when comparing the ratios of SADiol/SADiol, the ratio of control group was lower than the one of the other. It can be concluded that this ratio is not affected vastly by the drug’s concentration and abundances in the metabolic pathway.

Due to the observed lower levels of testosterone the ratio of Androsterone to Testosterone in the methadone group was in higher ratios than in the overweight group. The δ13C value of the following anabolic enogenous steroids were measured: Androsterone, Androstanolone, 5α-Androstan-3α-ol-17β-diol, 5β-Androstan-3α-ol-17β-diol and many others.

The results are shown in Table 2. In figure 5 are shown GC/MS chromatograms for SADiol and SADiol - overweight and control groups.

Conclusions
From the above results, the following conclusions can be drawn:

It can be concluded that the chronic use of methadone leads to a distinct toxic effect on the sex hormone testosterone. Testing of samples in isotopic mass spectrometry shows that their decrease in the concentration is not due to additional intake of prohibited substances. The results have confirmed the suppressive effect of methadone on testosterone and non-steroid treatment. The endogenous testosterone levels in men receiving methadone treatment was significantly lower than controls.

When comparing the two groups, the control and overweight ones, the conclusion can be made that slow metabolism does lead to increased amounts of endogenous steroids. Testing with the isotopic mass spectrometer has confirmed that the higher levels are due to abundances in the metabolic pathway.

References