Oxandrolone excretion: effect of caffeine dosing

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Introduction

Caffeine was included as a doping agent by the IOC in 1984. Initially a cut-off of 15 µg/mL was established and this value was lowered to 12 µg/mL in 1985. In 2004 the World Anti-Doping Agency (WADA) did not include caffeine on the list of prohibited substances. Although some performance-enhancing effects of caffeine as stimulant and diuretic have been well described, it is problematic to control abuse of caffeine and to distinguish it from normal dietary intake.

Oxandrolone (17β-hydroxy-17α-methyl-2-oxa-5α-androstane-3-one) has been described as enhancing protein synthesis and lean muscle mass, advantageous effects which would prompt athletes to abuse it. This synthetic 2-oxasteroid derived from testosterone was first prepared in 1962. It showed a very strong anabolic effect, yet apparently with much lower androgenic activity, thereby significantly reducing virilization effects.

Recent information obtained as a result of law enforcement activities in Portugal indicated that athletes may be using caffeine in conjunction with oxandrolone. In order to investigate the potential implications in doping analysis, excretion studies were undertaken. In these studies a single dose of oxandrolone was given, with and without the simultaneous application of caffeine.

Experimental

Oxandrolone Administration

Oxandrolone (400µg) was orally administered to a regular drinker of caffeine (the equivalent of 3 expresso coffees per day). A blank urine and all urines for 70 hours post administration were collected. The aim of this first study was to establish what was the excretion profile of oxandrolone for this subject.

The second study took place 4 days after the first study. The same dose of oxandrolone was given orally to the same subject combined with 300 mg of caffeine. All urines were collected for 70 hours.

Quantification of oxandrolone, epioxandrolone and caffeine was done based upon calibration curves.

Sample Preparation

• Caffeine quantification

Caffeine quantification was performed using Screening I (Stimulants and Narcotics by GC-NPD). Diphenylamine was used as internal standard (50 µL of a 250µg/mL solution). To 5mL of urine were added 500 µL KOH 5N, 2mL TBME and 3g Na_2SO_4. The organic phase was extracted and analyzed by GC-NPD.

• Oxandrolone and Epioxandrolone quantification

The preparation of the samples for the quantification of oxandrolone and epioxandrolone was done using Screening III (Glucocorticoids and Steroids by LC-MS) procedure. Methyltestosterone was used as internal standard. Sodium acetate buffer 0.2M (pH 5.2) was added to 3 mL of urine. No hydrolysis was performed since oxandrolone and epioxandrolone are excreted in the unconjugated form. The extraction was done by solid phase extraction (SPE, HLB). A mixture of TBME and methanol (9:1, v/v) was used to extract the urines.

GC-NPD Analysis

Agilent 6890N/5973inert GC-MSD/NPD.
Column: Phenomenex Zebron-ZB5, length 15 m, i.d. 0.25 mm, film thickness 0.25 µm. Carrier gas: helium, 11.5 psi. Injector: 250 ºC, split 1:10, injection volume 3 µL. Temp. Program: 178 ºC (0 min), 15 ºC/min to 230 ºC (0 min), 40 ºC/min to 305 ºC (2 min). NPD parameters: temperature 310 ºC; air flow 60 mL/min; hydrogen flow 3 ml/min; make-up gas flow 5 mL/min.

LCMSMS Analysis

LC (Waters Alliance 2795), detector MS (Micromass Quattro micro TM API).
Column: XTerra® MS C18, length 150 mm; i.d. 2.1 mm; particle size 5 µm; flow rate 0.3 mL/min. Solvents: (A) Formic acid 0.1% and acetonitrile (95:5, v/v) and (B) Formic acid 0.1% and acetonitrile (5:95, v/v). Gradient: 25% A (1 min), 25% A to 40% A in 8 min, 40% A to 80% A in 5 min, 80% A (2 min), 80% A to 25% A in 0.1 min, 25% A (4.9 min). Ionization mode: ESI positive.

References:

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• Geyer H. *et al.* Oxandrolone and high doses of metandienone found in nutritional supplements. Recent Advannces in Doping Analysis, 2003; 11: 77-84.
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Results

The excretion profiles of oxandrolone and epioxandrolone, with and without caffeine dosing, are shown in Figure 1. For oxandrolone the maximum excretion rate is 10ng/min undosed; with the caffeine that rate increases to 150 ng/min. Similarly, for epioxandrolone the maximum undosed rate is 0.9 ng/min and 19 ng/min dosed. The maximal rates occur at less than 4 h undosed, less than 6 h for the dosed study.

The oxandrolone excretion data plotted together with the caffeine data are shown in Figure 2. The same data for epioxandrolone are in Figure 3.

The caffeine excretion shows a maximum concentration of ~18 µg/mL at 4.7 h (Fig. 4).

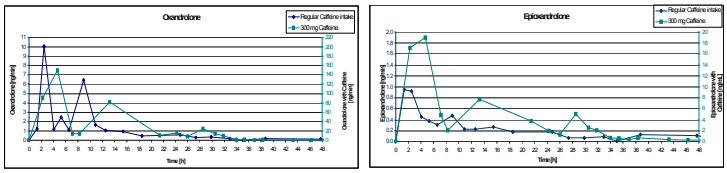

Figure 1: Excretion of oxandrolone and epioxandrolone: 300 mg caffeine oral administration and regular caffeine intake.

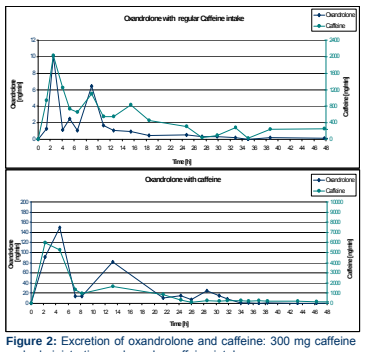

Figure 2: Excretion of oxandrolone and caffeine: 300 mg caffeine oral administration and regular caffeine intake.

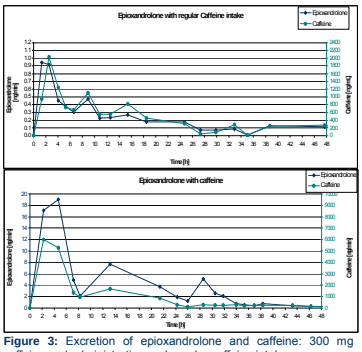

Figure 3: Excretion of epioxandrolone and caffeine: 300 mg caffeine oral administration and regular caffeine intake.

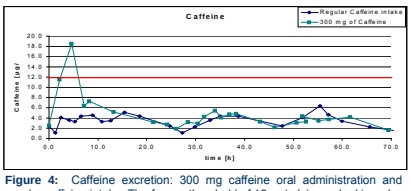

Figure 4: Caffeine excretion: 300 mg caffeine oral administration and regular caffeine intake. The former threshold of 12 µg/mL is marked in red.

Discussion and Conclusion

In comparing the data from the two studies it seems clear that caffeine does, in fact, alter the excretion profile of oxandrolone.

With the 300 mg of caffeine, there were very large increases in excretion amount and rate observed for both oxandrolone and epioxandrolone. Overall the total amount excreted for these two steroids is about 20 times higher compared to the administration of oxandrolone alone (with dietary intake of caffeine). There was however a differential in the increases: total oxandrolone increased more than 20-fold, while total epioxandrolone increased only about 15-fold. It also appears that the patterns of oxandrolone and epioxandrolone excretion follows the caffeine excretion pattern.

The hypothesis is that caffeine is increasing both the absorption and bioavailability of oxandrolone, probably by increasing the gut emptying. From a practical point of view this means that similar concentrations/effects may be achieved using lower dosages.

There are studies which report that caffeine accelerates absorption of some drugs (e.g., paracetamol). To better understand if there is an additional metabolic effect, alternative routes of administration with/without simultaneous caffeine dosing should be tested; in addition blood samples should be taken. For these studies it would be advisable to test other anabolic steroids.

One final observation. With the 300 mg of caffeine there is only one sample in which the concentration rises above the former 12 µg/mL threshold.