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Oxandrolone excretion: effect of caffeine dosing

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Abstract

Recent information obtained as a result of law enforcement activities in Portugal has indicated that a particular group of athletes is likely using caffeine in conjunction with oxandrolone. In this paper we present our work to investigate the possible effect of this co-administration. Therefore excretion studies were undertaken to determine what effects were produced and what was their extent. In these studies a single dose of oxandrolone was given, with and without the application of caffeine. A noticeable effect was observed upon both the clearance of oxandrolone and the metabolism to epioxandrolone. The concentration of both oxandrolone and epioxandrolone excreted in the urine increases substantially with caffeine intake. The results are discussed and assessed.

Introduction

Caffeine use was prohibited by the IOC in 1984, initially with a 15 µg/mL cut-off. In 1985 this was reduced to 12 µg/mL. In 2004 the World Anti-Doping Agency (WADA) did not include caffeine on the list of prohibited substances. Some performance-enhancing effects of caffeine as a stimulant and diuretic have been well described.⁵ Although Flenker U. described an IRMS method which differentiates caffeine from synthetic or natural origin⁶, it is problematic for the laboratories in their usual screening methods to control the abuse of caffeine and to distinguish it from normal intake.

Oxandrolone (17β-hydroxy-17α-methyl-2-oxa-5α-androstane-3-one) has been described as enhancing protein synthesis and lean muscle mass⁷, advantageous effects which would prompt athletes to abuse it. This synthetic 2-oxasteroid pharmacologically related to testosterone was first prepared in 1962. It is purported to have a pronounced anabolic effect yet, apparently, with much lower androgenic activity, thereby significantly reducing virilization effects.⁷

Experimental

Oxandrolone (400 µg) was orally administered to a regular drinker of caffeine (the equivalent of 3 espresso coffees per day). A blank and all urines post-administration were collected for 70 hours. 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.

Oxandrolone, epioxandrolone and caffeine were quantified. Caffeine quantification was performed using our screen for Stimulants and Narcotics by GC-NPD. Diphenylamine was used as internal standard. To 5 mL of urine were added KOH 5 N (500 µL), TBME (2 mL) and Na₂SO₄ (3 g). Samples for the quantification of oxandrolone and epioxandrolone were prepared using our screen for Glucocorticoids and Steroids by LC-MS with methyltestosterone as internal standard. Sodium acetate buffer 0.2 M (pH 5.2) was added to 3 mL of urine. No hydrolysis was performed (oxandrolone and epioxandrolone are excreted unconjugated). The extraction was done by solid phase extraction (SPE, Oasis® HLB). A mixture of TBME and methanol (9:1, v/v) was used to extract the urines.

GC-NPD Analysis: Agilent 6890N/5973 inert 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 °C, split 1:10, injection volume 3 µL. Temp. Program: 178 °C (0 min), 15 °C/min to 230 °C (0 min), 40 °C/min to 305 °C (2 min). NPD parameters: temperature 310 °C; air flow 60 mL/min; hydrogen flow 3 ml/min; make-up gas flow 5 mL/min.

LC-MSMS 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.

Results

Figures 1 and 2 show the excretion profiles of oxandrolone and epioxandrolone, with and without caffeine dosing. The maximum excretion rate of oxandrolone was 10 ng/min; but with caffeine this increased to 150 ng/min. Similarly, for epioxandrolone the maximum rate was 0.9 ng/min and 19 ng/min with caffeine. The maximal excretion rates occurred at less than 4 h and less than 6 h, respectively.

The caffeine excretion shows a maximum concentration of ~18 µg/mL at 4.7 h (Fig. 3).

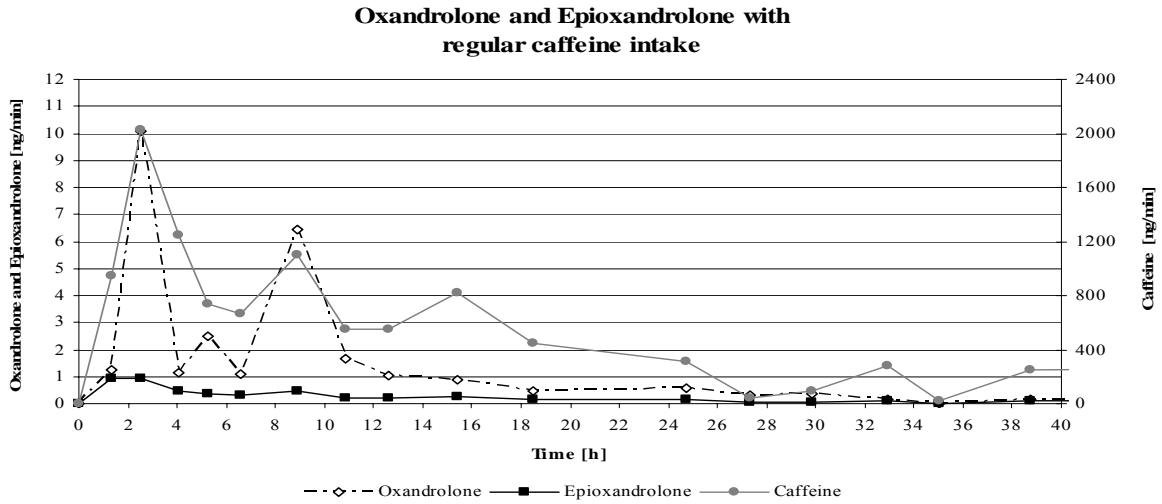


Figure 1: Excretion of oxandrolone and epioxandrolone with regular caffeine intake.

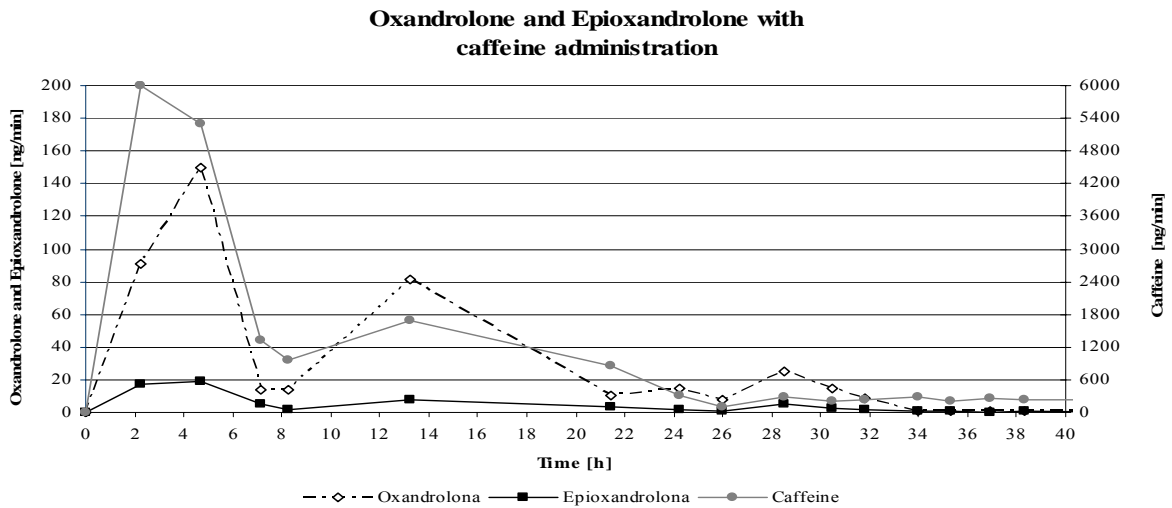


Figure 2: Excretion of oxandrolone and epioxandrolone with 300 mg caffeine oral administration.

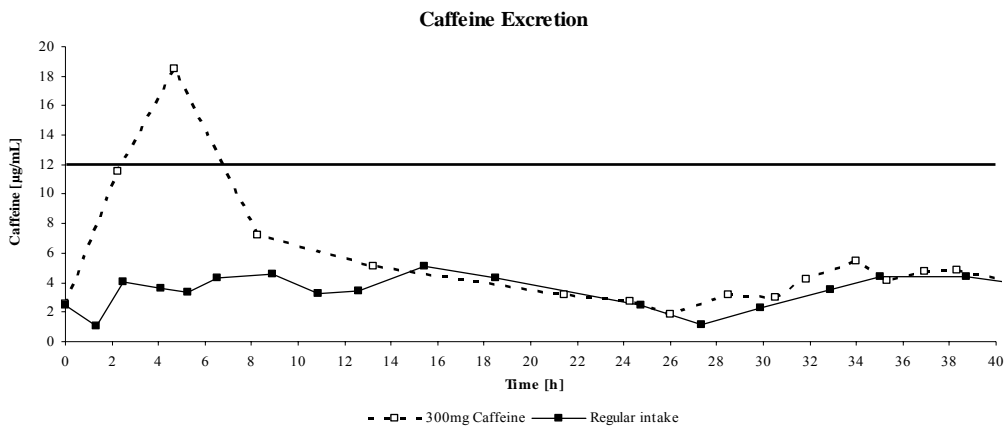


Figure 3: Caffeine excretion: 300 mg caffeine oral administration and regular caffeine intake. The former threshold of 12 µg/mL is marked.

Discussion and Conclusion

Our data show that caffeine alters the excretion profile of oxandrolone.

With 300 mg of caffeine, there were very large increases (about 20 times) in the amount and rate of excretion for both oxandrolone and epioxandrolone. However whereas total oxandrolone increased more than 20-fold, total epioxandrolone increased only about 15-fold. The patterns of oxandrolone and epioxandrolone excretion appear to follow the caffeine excretion pattern.

We postulate that caffeine increases the bioavailability of oxandrolone, probably by increasing gut emptying. Practically this means that similar concentrations/effects may be achieved using lower dosages.

Caffeine is reported to accelerate absorption of some drugs (e.g. paracetamol).³ To determine if there is an additional metabolic effect, alternative routes of administration with/without caffeine dosing should be tested and both blood and urine collected. Other anabolic steroids should also be investigated.

Finally, only one sample (300 mg caffeine dose) exceeded the former 12 µg/mL threshold.

References

1. Massé R, Bi HG, Ayotte C, Dugal R. (1989) Studies on anabolic steroids. II-Gas Chromatographic/mass spectrometric characterization of oxandrolone urinary metabolites in man. *Biomed Environ Mass Spectrom.* 18 (6), 429-438.
2. Geyer H, Bredehoft M, Mareck U, Parr M, Reinhart U, Schanzer W. (2003) Oxandrolone and high doses of metandienone found in nutritional supplements. In: Schänzer W, Geyer H, Gotzmann A, Mareck U. (eds.) *Recent Advances in Doping Analysis* (11), Köln, pp 77-84.
3. Renner B, Clarke G, Grattan T, Beisel A, Mueller C, Werner U, Kobal G, Brune K. (2007) Caffeine accelerates absorption and enhances the analgesic effect of acetaminophen. *J Clin Pharmacol.* 47(6), 715-726.
4. Van Nieuwenhoven M, Brummer M, Brouns F. (2000) Gastrointestinal function during exercise: comparison of water, sports drink, and sports drink with caffeine. *J Appl Physiol.* 89, 1079-1085.
5. Schanzer W, Gotzmann A. (1997) The Cologne Protocol to Follow-up Positive Caffeine Cases. In: Schänzer W, Geyer H, Gotzmann A, Mareck U. (eds.) *Recent Advances in Doping Analysis* (5), Köln, pp 259-268.
6. Flenker U, Schänzer W. (2001) 13C/12C-Ratios in Caffeine from Different Sources and in Urinary Excreted Caffeine. In: Schänzer W, Geyer H, Gotzmann A, Mareck U. (eds.) *Recent Advances in Doping Analysis* (9), Köln, pp 187-190.
7. Sheffield-Moore M, Urban R, Wolf S, Jiang J, Catlin H, Herndon D, Wolfe R, Fernando A. (1999) Short-Term Oxandrolone Administration Stimulates Net Muscle Protein Synthesis in Young Men. *J Clin Endocrinol Metab.* 84 (8), 2705-2711.