

PROJECT REVIEW

"Effects of exhaustive exercise on endogenous production of steroid hormones (nandrolone, DHEAs and testosterone) in females"

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The exogenous uptake of nandrolone has been banned by the International Olympic Committee (IOC 2004), due to its anabolic effects. Though maximal authorised concentrations have been published for nandrolone metabolites (19-NE, 19-NA) in urine, some positively controlled athletes claimed that they never used such products and expressed that a natural endogenous secretion is related to their high level of physical training.

A previous study from our laboratory (Schmitt et al. 2002) has shown that the urinary concentrations of 19-NE and 19-NA in male athletes (judo and long-distance specialists) following exhaustive exercises (Wingate and limited-time tests on ergocycle) did not exceed 0.008 ± 0.02 , and 0.07 ± 0.02 ng.ml⁻¹, respectively. A maximal concentration of 2ng.ml⁻¹ for 19-NA is, however, authorised in male athletes by the IOC.

In females, the endogenous origin of nandrolone is a controversial topic in doping control: firstly, low-dose of norethisterone contained in some birth control pills can produce similar urinary nandrolone-like metabolites and secondly endogenous production of nandrolone by aromatase-rich tissues has been reported in the ovarian follicle and during pregnancy by the placenta. The concentration of nandrolone in blood has been shown to be influenced by estrogens, suggesting that nandrolone may significantly vary during menstrual cycle. It seems possible that high androgens concentrations (e.g. DHEA and testosterone) may influence the production of 19-norsteroids in aromatase-rich tissues. Interestingly, intense exercise has also been associated with raised levels of estrogens in females. Therefore, physical exercise could theoretically influence the urinary excretion of nandrolone metabolites in females.

Now, the maximal authorised concentration of 19-NA (IOC 2004) has been set at 2 ng.ml⁻¹ in female athletes without any details concerning the menstrual cycle, the use of oral contraceptive, and physical exercise.

Our aim is therefore to determine the influence of oral contraceptive, menstrual cycle, and exhaustive exercises on urinary concentrations of nandrolone metabolites and blood DHEAs and testosterone in sedentary and athlete females.

Young voluntary sedentary females (n=16) will be recruited, eight taking similar "low-dosed" monophasic oral contraceptives and eight with regular menstrual cycles. These persons will be investigated at rest, every 30 min

during a morning period and at the same time of the day during and after exhaustive exercises. Two kinds of exercises will be studied on separated days: Wingate and limited-time tests on ergocycle.

For the subjects with regular menstrual cycles, the sessions (rest and exercises) will be organised during the early follicular phase (5 days after the start of menses) and mid-luteal phase (18-23 days after the start of menses). Each subject will be investigated during a 3-month period. The chronology of the sessions "rest" and "exercise1", and "exercise2" will be randomised. For the persons under contraception, the sessions will be organised during the weeks of pill ingestion.

Using the same protocol, female judoka (n=16) and long-distance runners (n=16) will be investigated.

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Results and Conclusions

- Our results provide strong evidence that the urinary concentration of 2 ng/mL of 19-NA is fair as the upper acceptable limit in doping control tests for female athletes.
- In young women, neither androsterone nor DHEA and nandrolone metabolite excretions are influenced by menstrual phase and physical training. However, oral contraceptive intake lowered DHEA excretion in urine and androsterone excretion seems to be slightly affected by prolonged exercise.
- Metabolomics is a promising tool in order to gain insight into physiological status and to clarify the changes induced by short-term, intense physical exercise. Further research is needed to determine whether metabolomics could be employed to diagnose specific disorders induced by exercise (such as overreaching and/or overtraining), and if it could be used as a new anti-doping tool).

Publications

- 1: Enea C, Boisseau N, Diaz V, Dugué B: Biological factors and the determination of androgens in female subjects. **Steroids** **2008**; 73:1203-16
- 2: Enea C, Boisseau N, Dugué B : Facteurs biologiques influençant le profil stéroïdien urinaire lors de contrôles anti-dopage. (Biological factors influencing the urinary steroid profile in doping control analysis –Review article in French-). **Science et Sports** **2009**; 24:119–127
- 3: Enea C, Boisseau N, Ottavy M, Mulliez J, Millet C, Ingrand I, Diaz V, Dugué B: Effects of menstrual cycle, oral contraception, and training on exercise-induced changes in circulating DHEA-sulfate and testosterone in young women. **Eur J Appl Physiology** **2009**; 106:365-373
- 4: Bayle ML, Enea C, Goetinck P, Lafay F, Boisseau N, Dugué B, Flament-Waton MM, Grenier-Loustalot MF: Quantitative analysis of DHEA and Androsterone in female urine: application to the evaluation of sport and contraceptive pill intake influences. **Analytical and Bioanalytical Chemistry** **2009**; 393:1315-1325
- 5: Enea C, Boisseau N, Bayle ML, Flament MM., Denjean A, Diaz V, Dugué B: Nandrolone excretion after exhaustive exercises in females: influence of menstrual cycle, oral contraception and training level. **Scand J Med Sci Sports** **2010**, in press
- 6: Enea C, Seguin F, Petitpas-Mulliez J, Boildieu N, Boisseau N, Delpech N, Diaz V, Eugène M, Dugué B : (1)H NMR-based metabolomics approach for exploring urinary

metabolome modifications after acute and chronic physical exercise. **Anal Bioanal Chem**, 2010, in press (available on line).