PROJECT REVIEW

"Studies related to the metabolism of endogenously produced nandrolone and exogenously administered nandrolone precursors"

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Problems related to the detection of 1 9-norsteroid misuse in analytical doping control have been of outstanding importance during the last decade. Both the endogenous production of I 9-nortestosterone (nandrolone) and its metabolites as well as the administration and metabolism of 1 9-nortestosterone precursors have been issues of an ongoing scientific discussion.

In this respect our project should contribute with essential new and supplementary information on the establishment of normal urinary levels as well as the mechanism of endogenous production.
Investigations of 19-norsteroid metabolites by means of IRMS should contribute to methodological evaluation of IRMS and interpretation of metabolic effects. Based on studies on reference steroids, interfering analytical parameters will be considered and standardisation will be proposed. This will permit the investigation of influences of metabolic steps on carbon isotope ratios.

Following up previous publications[1] ,2 and recommendations[3] we would like to investigate further the excretion of nandrolone metabolites as norandrosterone and noretiocholanolone from individuals who have not ingested any nandrolone or nandrolone precursor.

The reporting threshold of 2 ng/ml and 5 ng/ml for the concentration of norandrosterone in urine of male and female athletes, respectively, has been challenged in many doping cases. Although no clear evidence has yet been shown that the excretion of norandrosterone may occur in higher concentrations, it would strenghten the fight against doping to extend the scientific data in this respect. Our investigations shall therefore include:

a) the monitoring of norandrosterone levels in males (at least 100 persons) not having ingested any nandrolone or its precursors.
b) the influence of physical exercise on the excretion of nandrolone metabolites.
c) the influence of alcohol consumption on the excretion of nandrolone metabolites.

In animals the natural production of 19-norsteroids is well documented and their synthesis related to the aromatisation of testosterone/androstenedione to estradiol/estrone[4,5]. Additionally to the observation of 1 9-norsteroids in females

during pregnancy 6,7 metabolites of nandrolone can also be detected in small amounts during the menstrual cycle[8]. The concentrations follow the blood levels of estrogen, supporting that both estrogens and nandrolone are synthesised in the maturing follicle[9]. Because estradiol is mainly synthesised in the dominant follicle's granulosa cells during the time period close to the ovulation, we choose granulosa cells from in vitro fertilizing experiments as model to investigate the mechanism of endogenous nandrolone production. The project intends to study

a) the nandrolone excretion of human granulosa cells,
b) the aromatisation of different amounts of testosterone in this aromatase rich medium with respect to nandrolone or norandrostenedione synthesis,
c) aromatase independent production of nandrolone,
d) the influence of aromatase inhibitors on these reactions,
e) nandrolone as possible substrate for in vitro aromatisation.

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Results and Conclusions

The contractors from the WADA-accredited laboratories in Kreischa/Germany and Oslo/Norway account in this report for their results of the WADA supported project "Studies related to the metabolism of endogenously produced nandrolone and exogenously administered nandrolone precursors". The results are laid down in 9 presentations/publications.
As their main outcome it was shown that:
1. In very few cases certain matrix and storage conditions in urine could lead to a formation of low amounts of 19-norsteroids from androsterone and etiocholanolone. A WADA Technical Note already describes the necessary procedures to take into account this phenomenon.
2. The natural production of nandrolone metabolites can be explained as a side reaction during the aromatisation of testosterone. This explains plausible the occurrence of norandrosterone of natural origin in females during pregnancy and mid-cyclic, while the production in males is considerably less. The threshold for norandrosterone should reflect these circumstances.
3. Investigations of the glucuronide and sulphate fraction of nandrolone metabolites of natural origin and after administration of norandrostenedione show that the distribution between the glucuronide fraction and the sulphate fraction of norandrosterone and noretiocholanolone can not be used as a factual evidence to distinguish between endogenous production and application. The results also show that excretion in the free fraction is of insignificant value.
4. After oral and transdermal administration of the nandrolone precursor 4-estrene-3,17-dione no significant discrimination effect could be observed in relation to a possible influence of the biotransformation reactions on the carbon isotope ratio values of the terminal metabolites norandrosterone and noretiocholanolone.

Publications

1. P. Hemmersbach, A. H. Hågensen Jetne and H. S. Lund. "Determination of Urinary Norandrosterone Excretion in Females during One Menstrual Cycle by Gas Chromatography/Mass Spectrometry" Biomed Chromatogr. 2006;20:710-7.
2. J. Grosse, P. Anielski, D. Thieme, P. Hemmersbach, H. Lund, R.K. Mueller, "Nandrolone and doping - a new aspect in elucidation of questionable results", poster presentation at the 41st International TIAFT meeting, Melbourne, Australia, November 16-20 2003.
3. D. Thieme, P. Anielski, J. Grosse, P. Hemmersbach, H. Lund, C. Rautenberg, "Kinetic of in-situ demethylation of endogenous steroids in urine samples", in W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck (Editors),

Recent Advances in Doping Analysis (12), Sport & Buch Strauß, Köln, 2004, p. 177-188.
4. J. Grosse, P. Anielski, P. Hemmersbach, H. Lund, R.K. Mueller, C. Rautenberg, D. Thieme, „Formation of 19-norsteroids by in-situ demethylation of endogenous steroids in stored urine samples“ Steroids. 2005;70:499-506.
5. C. Rautenberg, J. Grosse, P. Anielski, D. Thieme, P. Hemmersbach, H. S. Lund, R.K. Mueller, Aspects of the in situ formation of 19-norsteroids” in W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck (Editors), Recent Advances in Doping Analysis (13), Sport & Buch Strauß, Köln, 2005, p. 449-52.
6. H. S. Lund, S. Jåthun, P. Fedorcsak, R. Storeng, P. Torjesen, P. Hemmersbach, “Synthesis of nandrolone in the Human Ovary”, in W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck (Editors), Recent Advances in Doping Analysis (10), Sport & Buch Strauß, Köln, 2002, p. 23-34.
7. H. Alstad: “The effect of pathological conditions on endogenous nandrolone production”, (in Norwegian), Master thesis for the degree Cand. Pharm at the School of Pharmacy, University of Oslo, 2004.
8. H. S. Lund, H. Alstad, P. Fedorcsàk, R. Storeng, P. Hemmersbach, “Endogenous nandrolone production: studies in granulosa cells from patients with polycystic overy syndrome (PCOS)“ in W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck (Editors), Recent Advances in Doping Analysis (13), Sport & Buch Strauß, Köln, 2005, p. 483-486.
9. H.S. Lund, I.M. Larsen, P. Hemmersbach, P. Anielski, C. Rautenberg, J. Grosse, D. Thieme, „Inter-laboratory study of low levels f nandrolone metabolites, poster presentation at the 22nd Cologne Workshop on Dope Analysis, Cologne, Germany, March 7-12 2004.
10. H.S. Lund, I.M. Larsen, P. Hemmersbach, P. Anielski, C. Rautenberg, J. Grosse, D. Thieme, “Inter-laboratory study of low levels of nandrolone metabolites”, in W. Schänzer, H. Geyer, A., Gotzmann, U. Mareck (Editors), Recent Advances in Doping Analysis (13), Sport & Buch Strauß, Köln, 2005, p. 485.