

PROJECT REVIEW

“Excretion of 19-Norsteroids in Human from Consumption of Pork Meat and Offal: Combined GC/MS and GC/C/IRMS Analysis of a Double Blind Study”

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Extremely low levels of 19-norandrosterone can be *naturally* excreted in human urine. Sensitive methods being applied for the detection and identification of anabolic agents, the International Olympic Committee safely recommended in 1998, a threshold in males and females for reporting positive results. However, as it is the case with the other androgens, which could be endogenous in human, natural factors are systematically invoked to challenge the positive test results. The inadvertent intake of 19-nortestosterone by eating contaminated meat of animals treated with growth promoters or from animals in which 19-nortestosterone has been suspected to be endogenously formed, is a recurrent argument.

This project is aimed at applying the combination of GC/MS and GC/C/IRMS (isotope ratio mass spectrometry) to the detection, identification and quantification of 19-norsteroids in human urine samples collected following the ingestion of pork meat and offal. Complement of the existing GC/MS methods, the GC/C/IRMS permits the differentiation of the exogenous (from synthetic standards) or endogenous origin of urinary metabolites, by measuring the isotopic content of their carbon atoms. The 13C contents of 19-nortestosterone reported to be present *naturally* in boar offal and of 19-norandrosterone, expected to be excreted following its consumption, could be measured and compared to those of the other urinary reference steroids. There are reports from the laboratories involved in the analysis of drug residues in animal tissues, of the presence of *endogenous* 17 α - or 17 β -nortestosterone, in a few animal species. One paper described last year the excretion of 19-norandrosterone in the urine of male volunteers having eaten offal and meat of an uncastrated pig.

This study should provide the means to estimate and document objectively, the link between the detection of 19-norandrosterone excreted in human urine and the consumption of meat from animals in which 19-nortestosterone has been reported to be endogenous. The importance of this study goes beyond the scope of athletic drug testing. If androgens such as 19-nortestosterone are present in edible tissues of animals, in sufficient amount to detect its metabolites in the urine of humans having eaten that meat, it should be known and the risks for the health of the population properly evaluated.

Excretion of 19-nor-steroids from consumption of pork meat and offal: combined GC/MS and GC/C/IRMS analysis.

Results and Conclusions

Administration of 19-nortestosterone, a well known anabolic steroid, leads mainly to the excretion of 19-norandrosterone, 19-noretiocholanolone and 19-norepiandrosterone. Nortestosterone and the precursors, norandrostenedione and norandrostenediol are listed as prohibited substances by the International Olympic Committee and the presence of 19-norandrosterone in an amount greater than 2 ng/mL in athletes' samples constitutes a doping offence. Excreted in very low amounts in human urine samples, endogenous 19-norandrosterone is not detected by the methods routinely employed in drug testing laboratories. A more sensitive instrumentation, larger volumes of urine and extensive sample clean-up are needed to detect, identify and quantify endogenous 19-norandrosterone. The physiological levels of 19-norandrosterone measured in samples collected from males and females are 0.6 ng/mL and 1 ng/mL, respectively. Recently, the results of the only truly controlled study involving athletes demonstrated that exercise does not influence the excretion of 19-norandrosterone. Again, very low levels ranging from undetectable to a maximum of 0.25 ng/mL were measured.

This work aimed at studying the phase II metabolites originating physiologically during pregnancy or after intake of norsteroids in the three typical following conditions: 1) intake of a "dietary supplement" of 19-norandrost-4-en-3,17-dione; 2) ingestion of edible parts of non-castrated and castrated pig; 3) in several athlete's samples found to be positive during routine doping controls. We have estimated by GC/HRMS the excreted levels of 19-norandrosterone, 19-noretiocholanolone and 19-norepiandrosterone when possible after selective hydrolysis of the glucuro and sulfoconjugated metabolites. The ¹³C content of the metabolites present in sufficient amounts after the ingestion of edible parts of non-castrated pig was measured by isotope ratio mass spectrometry.

Our results indicate that when the norsteroids conjugates are properly measured, 19-norandrosterone and 19-noretiocholanolone glucuronides and sulfates are present in relative amounts which do not enable a distinction between either exogenous or endogenous origin. The use of isotope ratio mass spectrometry is the only way, when the norsteroids are present in a sufficient amount, to prove the origin of the metabolites.

Publications (including in press or submitted) and poster presentations

- 1) Guay C et al., Excretion of norsteroids' phase II metabolites of different origins in human, Steroids, *submitted*.