PROJECT REVIEW

"Human Andogen Metabolism, Kinetics and Excretion – Genetic and Ethnic Determinants of Variation"

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Administration of testosterone (T) or testosterone derivatives is one of the mainstays in human sports doping. Our knowledge about potential risks and the alleged advantage is limited as most doping is concealed. Very often the structure and dose of the used agents is unknown. In addition, doping tests are generally designed for qualitative identification of the agents with use of predetermined cut-off levels.

There are many pitfalls with the current routines for interpretation of analytical results. As an example, the interindividual variations in turnover and metabolic patterns of endogenous and exogenous androgens are not considered in the antidoping laboratory tests, In addition, dose — sample collection interval is generally unknown which confers an uncertainty about the interpretation of the results.

Genetic and constitutional factors are the major causes of variations in androgen metabolism and effects. Research carried out over the past decades has revealed important genetic differences in the capacity of enzymes in drug metabolism, particularly the members of the cytochrome P450 (CYP) family. Some of these enzymes, albeit relatively few, are inherited in a polymorphic way which may confer a 100 — 1000-fold interindividual metabolic variation in the population (1). The function of the majority of these enzymes is however governed by many genes polygenic inheritance. Nevertheless, their interindividual variation is often 10- 50-fold and it has been estimated that 50-60 % of the variation may be ascribed to genetic variation.

As many androgens and androgen precursors are metabolised by the same or related enzyme members of the *CYP* family, there are reasons to believe that a similar genetic variability exists in the metabolism of anabolic androgenic doping agents. This would have conspicuous influence on turnover and excretion of such agents, which should be considered in the anti-doping test. The ethnic differences in the epimerisation of testosterone is only one example (2) which is probably based on genetic grounds.

The mapping of the human genome (3, 4) has now paved the way to identify important sites of variation such as single nucleotide polymorphisms (SNPs) in genes of relevance for the synthesis, metabolism, and receptors of androgens

(5). These genes encode several enzymes in the androgen and estrogen metabolism (see Appendix 2), as well as androgen and estrogen receptors.

Many of the problems in anti-doping tests are associated with the identification of testosterone doping: assessment of the T/epitestosterone *(TIE)* ratio is probably affected by interindividual and ethnic genetic differences and variation, as well as interaction with concomitantly used agents or drugs. The various methods to check the validity of the *TIE* ratio includes repeated measurement of 17 OH-progesterone in serum after presumed doping. (6, 7). Another method is to determine ^{12}C-T/^{13}C-T ratio which is changed in testosterone doping due to different relative concentrations of ^{12}C and ^{13}C in natural sterol precursors in the testosterone synthesis. Naturally elevated *TIE* ratios may also be due to inherently low epitestosterone concentration.

In identification of testosterone doping one must consider the following:

1. The possibility of a naturally high T/E ratio must be excluded.
2. In males: monitoring of *TIE* ratios over three months is necessary in order to ascertain that the ratio is stable in absence of doping.
3. In females: low absolute *TIE* values may be influenced by ovulation. There is no information about the effect of contraceptives on the TIE ratio.

Results and Conclusions

"Human Androgen Metabolism, Kinetics, and Excretion – Genetic and Ethnic Determinants of Variation"

Our research program was aimed to study the large inter-individual variation in the disposition of exogenous and endogenous doping agents, such as anabolic androgenic steroids. There are reasons to believe that genetic variation is the single most important cause of variation in disposition of many androgenic compounds as it is for drugs. The conventional way to detect testosterone abuse is to measure the urinary ratio between testosterone glucuronide and epitestosterone glucuronide, (T/E). However, the large differences in this ratio observed within and between ethnic populations make it difficult to disclose all testosterone abusers.

We have shown that the distribution of urinary testosterone excretion showed a distinct bimodal pattern, both in a Caucasian and a Korean population. However, the distribution into the *low* and *high* excretion mode differed markedly in that 74 % of Koreans, but only 7 % of the Caucasians belonged to the *low* excretion group. Our research group has identified the genetic explanation for this inter-individual and inter-ethnic discrepancy. Individuals avoid of the uridine diphospho (UDP) glucuronosyl transferase 2B17 (UGT2B17) gene all belong to the *low* excretion group, and hence also the low T/E-ratio group [1] [2].

We have continued our work to evaluate the sensitivity and specificity of the T/E test in different UGT2B17 genotype panels of 55 healthy volunteers. Our results show that the excretion of administered testosterone is highly dependent on the UGT2B17 genotype. In fact, 40 % of the subjects devoid of the UGT2B17 gene never reached the cut-off T/E ratio on any of the days investigated [3]. Our results strongly suggest that urine analysis should be combined with a genetic test of the UGT2B17 deletion polymorphism in order to refine and improve the testosterone doping test.

Additionally we have identified polymorphisms in other androgen metabolising enzyme genes, e.g. in the aldo-keto reductase (AKR) 1C3 gene [4] and the P450 cytochrome (CYP) 7B1 gene [5]. An AKR1C3 single nucleotide polymorphism (SNP) was located in the promoter region and was 50 times more common in Caucasians compared to Oriental subjects. Another SNP, which conferred a Glu77Gly exchange in the protein was completely absent in the Oriental population, but occurred in 4.8 % of the Caucasians. Interestingly, subjects with this polymorphism had significantly lower levels of testosterone in serum and in urine.

The results will pave the way towards personalised test strategies where the genetic factor will be considered in the assessment of the individual's androgen excretion profile in the current test program. A better sensitivity and specificity of the test is mandatory for the fairness in sports and to the concerned individuals.

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