

PROJECT REVIEW

“Dose response effect of alcohol ingestion on steroid profile (acronym: profethyl)”

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The imminent introduction of the so called ‘endocrine module’ of the athlete’s biological passport needs to consider the numerous reports showing the effect of ethanol ingestion on the steroid profile. A steroid profile would only be useful for longitudinal monitoring and statistical evaluation if it has not been altered by any uncontrolled circumstance, very particularly alcohol consumption. Ethylglucuronide (EtG) and in a lesser extent Ethylsulfate (EtS) are well established ethanol biomarkers. Recent studies have shown the correlation between their urinary concentrations and changes in T/E ratios and other parameters. Studies are needed to develop cut-off values for biomarkers able to ensure that no alteration of the steroid profile can be claimed so that data can be incorporated in a longitudinal intra-individual passport. We have already performed a number of placebo controlled cross-over studies in which male volunteers have received different doses of alcohol and for which urine samples have been collected. This is a precious material that can be used to monitor the steroid profile before and after the ethanol ingestion and determine the intra-individual effect and correlation between the dose, the variation of the parameters monitored as well as the biomarkers chosen (EtG and EtS).

The main objectives of the project are:

- 1.- study the intra-individual variation of steroid profile parameters as a result of the ingestion of different doses of ethanol using urine samples already available from previous ethanol administration studies performed by our group.
- 2.- Develop cut-off values for the selected biomarkers (EtG and EtS) granting that the steroid profile in a particular urine sample could not have been altered by ethanol consumption.
- 3.- Perform additional clinical studies incorporating Asian volunteers in order to extend the validity of those markers to other populations, particularly those with the well known UGT2B17 gene deletion (e.g. Asians).

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Result and Conclusions:

Background: the introduction of the so called 'endocrine module' of the athlete's biological passport needs to consider the numerous reports showing the effect of ethanol ingestion on the steroid profile. A steroid profile would only be useful for longitudinal monitoring and statistical evaluation if it has not been altered by any uncontrolled circumstance, very particularly alcohol consumption. Ethylglucuronide (EtG) and Ethylsulfate (EtS) are well established ethanol biomarkers and have been proposed in doping control for the detection of its consumption. Large intakes of alcohol produce increases of the T/E ratio, probably because alcohol, excreted in large amounts as glucuronide, compete and disturb the glucuronidation of steroids. Studies had been performed showing those variations and how EtG determination in urine, grossly correlated with the variation in T/E ratios. Those studies led to the impression that a cut-off value for EtG could be developed to justify changes in T/E ratios. Values below EtG would show a consumption of alcohol sufficiently low to not justify variations of T/E.

Study aims included: the evaluation of intra-individual variations of steroid profile parameters as a result of the ingestion of moderate alcohol consumption (15g, 18g, 30g and 42 g of ethanol, n=6-12 per dose). Also the potential modifications of those variations depending on the kind of alcohol source (i.e. vodka or wine, 30g of ethanol, n=6) were also considered.

Results: Doses as low as 15 g ethanol (equivalent to 'one drink') and up to 42 g (the starting dose where subjects display symptoms of acute intoxication) were studied here. T/E values were found to be altered very fast, in parallel with the absorption of alcohol, in the period 0-2h after ingestion. Variations, even with low doses, accounted for more than 70% change in T/E value that would have triggered a confirmation and IRMS analysis. EtG concentrations in urine had a delayed profile, reaching their peak in the period 2-4h. The implication is that while EtG concentrations were quite low in the period 0-2h, T/E values had already varied significantly or even reached their maximum variation. However, when EtG concentrations were maximal, T/E values had already begun to decline or had already returned to normal. Before 12h all T/E values had returned to normal. A linear correlation was found between the alcohol dose and the T/E variation, clearly showing their linkage. Both EtG and EtS have shown a very good correlation. But both have shown to be badly correlated kinetically with the variations produced in T/E ratios with the consequent risk of leading to wrong conclusions regarding the reasons for T/E changes. The ratio T/A (expressed as 100T/A for numerical reasons) kinetically fits much better with the EtG or EtS concentrations. Alterations in the steroid profile observed were independent of the kind of alcohol source.

Future developments: (i) studies need to be performed in women (milder first pass metabolism and lower T and E concentrations), (ii) the effect of

repeated doses of alcohol along several hours and studying particularly the terminal elimination phase with multiple sampling after 8h of the last dose) resembling usual alcohol consumption behavior, and 'next morning' doping control, need to be evaluated and (iii) the performance of studies in ethnicities other than Caucasian and the evaluation of the impact of the UGT2B17 polymorphism (low T/E ratios) and its influence on alcohol effects.