PROJECT REVIEW

"Understanding of microbiological processes in doping control"
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In doping control, abuse of drugs is normally monitored from urine samples using analytical methods such as chromatography combined to mass spectrometry. In interpretation of analytical results, possible influence of microbiological processes should be considered, as microbes can affect drug monitoring in many ways. For instance, intestinal microbes in the gut can metabolize exogenous and endogenous substances, which are then absorbed back to bloodstream and processed further in the body prior their final excretion in urine. Microbes are also almost always present in delivered urine – the most used sample matrix in doping analysis. It is possible that in some cases microbial activity in urine may decrease the stability of the sample and thus complicates analytical measurement and interpretation of test results.

The aim of this project is to improve understanding of microbiological processes related to doping control in general, and to design detection and prevention methods for microbes which may have influence in doping analysis. This all will increase reliability of analytical methods and data interpretation, as well as will prevent chances to adulterate samples for doping analysis.

The research project is divided in two sections: study of the influence of digestive tract microbes in metabolism of doping agents and study of the influence of microbial activity in urine samples. Traditional incubation procedures as well sophisticated methods used in modern microbiology will be applied in this project. For instance, DNA based methods will be used for the analysis of microbial communities and laboratory simulations for studying of metabolic functions of microbes. Tools to be developed to detect possible microbial activity of urine samples will be based on DNA techniques and determination of microbial metabolite profiles.

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Results and Conclusions

In the study of the influence of digestive tract microbes in the metabolism and production of doping agents, a human large intestinal simulation model using testosterone (T) and androst-4-ene-3,17-dione (AED) as substrates was used to investigate the frequency of boldenone producing trait among humans, and the intestinal microbial fermentation conditions that favor formation of boldenone precursor androsta-1,4-diene-3,17-dione (ADD). The model yielded the following results: Under authentic large intestinal conditions, characterized by total anaerobiosis, the fecal sample inoculants failed to show detectable ADD formation. Under highly reductive conditions ADD was produced in large quantities by fecal bacteria from all human subjects. Many ingested food products and the compartments of the upper digestive tract are rich in microbes, and, are habitats with higher redox potential than the large intestine or feces. Therefore, it is possible that the formation of “endogenous boldenone” takes place in these habitats instead of the large intestine. Similarly, some abnormal conditions, such as diarrhea or broad spectrum antibiotic treatment may decrease microbial activity in the large intestine and shift the conditions to favor endogenous microbial boldenone formation.

In the study of the influence of microbial contamination in urine, authentic or artificial pooled urine samples were inoculated as such or spiked with selected steroids with various potential contaminating microbes in different incubation conditions. It was observed that microbial contaminants can cause adverse reactions for endogenous and exogenous steroids during transportation, storage or analytical sample pretreatment process. However, most reactions required high contamination and extreme incubation conditions. The most effective contaminants were fecal and saliva source and Eschericia coli. The contaminants expressed mainly b-glucuronidase activity and oxido-reductive activities, whereas neither sulphatase nor demethylation activity was found. Monitoring the free form of highly glucuronide conjugated steroids, such as androsterone and etiocholanolone, could be used as a sensitive indicator for microbial degradation, but pH and 5a- and 5b-androstan-3,17-dione were specific only for certain microbial contaminants. When urine sample is badly contaminated by microbes, steroids can be converted even during incubation steps of sample pretreatment to other steroid. Therefore, potential microbial contaminants should be destroyed or removed prior to direct enzymatic hydrolysis of urine samples, especially in confirmation analysis.