

## ***"Development of urinary threshold levels for the detection of misuse of glycerol in sports"***

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### **Project Review**

According to the prohibited list 2010, glycerol is specifically named as masking agent. Oral and intravenous application of glycerol is prohibited. Therefore it is necessary to establish threshold values for urinary glycerol concentrations because glycerol may also be of endogenous origin or may be taken up from other exogenous sources.

In sports glycerol is typically used in high doses in combination with excess fluid in order to increase total body water. However, glycerol plasma concentrations can also be elevated during fasting and endurance exercise.

Furthermore, there are natural sources of glycerol such as foodstuff (e.g. wine) and over-the-counter drugs.

The urinary excretion of glycerol following administration has not been studied systematically at rest and during exercise. Also, little is known about urinary glycerol following prolonged fasting, endurance exercise or the intake of glycerol-rich foods or drugs.

A quantification method for urinary glycerol has been published recently but urinary threshold values for the detection of glycerol misuse are lacking.

For the development of urinary threshold levels we intend to perform a) application studies of glycerol at rest and during exercise, b) to assess urinary glycerol excretion due to fasting and exercise and c) to determine reference values for urinary glycerol concentrations in normal doping control samples. Additionally, an IRMS method for glycerol will be implemented.

### **Results and Conclusions**

Glycerol is prohibited by the World Anti-Doping Agency (WADA) as masking agent since 2010. Exogenously administered glycerol is excreted in the urine so that the detection of the misuse of glycerol is possible in theory. However, little is known about the urinary excretion pattern of glycerol. In addition, glycerol may also occur endogenously during increased lipolysis and it can also be ingested from foodstuff or drugs.

In order to contribute to the development of urinary threshold levels for the misuse of glycerol, two placebo-controlled application studies (glycerol dose: 1 g/kg body weight) were performed at rest and in combination with exercise. There was a rapid increase in urinary glycerol and maximum concentrations (50000-60000 µg/mL) were observed 2.5 to 4 h after the administration. Urinary concentrations remained significantly elevated for 12-15 h (exercise) to 15-18 h (rest). Plasma volume expansion (+1.5 to 3%) and the reduction in hemoglobin (-0.2 to

-0.3 g/dL) and haematocrit (-1%) after glycerol administration were rather small when compared to the administration of placebo and statistically significant only after 2.5 h.

After 18 h of food deprivation followed by 90 minutes of endurance exercise, highly elevated plasma glycerol concentrations were observed but urinary glycerol concentrations were only slightly increased (maximum concentration: 50.5 µg/mL).

In 516 randomly selected routine doping control samples, it was confirmed that urinary concentrations are slightly higher in in-competition than in out-of-competition samples, but in all samples urinary glycerol concentrations remained below 200 µg/mL.

Further, a suitable IRMS-method was developed to measure  $\delta^{13}\text{C}$ -values of glycerol in urine. Directly after glycerol administration,  $\delta^{13}\text{C}$ -values were similar to the isotope ratio of the administered glycerol.

In conclusion, the urinary threshold of 200 µg/mL as suggested by Thevis et al. (2008) can be used to identify athletes, who have misused glycerol in relevant amounts. As shown in the study, this threshold is sufficiently high to minimize the risk of false-positive results due to exercise- or fasting induced lipolysis and unintentional intake from other sources.

#### Related publications:

- Thevis, M., Guddat, S., Flenker, U. & Schänzer, W. (2008). Quantitative analysis of urinary glycerol levels for doping control purposes using gas chromatography-mass spectrometry. *European Journal of Mass Spectrometry*, 14, 117-125.