

# Detection of stanozolol in the urine of athletes at a pg level: The possibility of passive exposure

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Received July 7, 2016; Accepted September 29, 2016

DOI: 10.3892/br.2016.794

**Abstract.** Stanozolol is a synthetic heterocyclic steroid with anabolic and androgenic properties, which has been abused by several high-profile professional athletes. Stanozolol is also used in veterinary medicine to increase appetite, cause weight gain and treat certain types of anemia. The detection of stanozolol metabolites in human urine for doping control purposes depends on the analytical method applied. The most commonly applied methods in the World Anti-Doping Agency (WADA)-accredited doping control laboratories are gas chromatography/high-resolution mass spectrometry (GC/HRMS) or gas chromatography-mass spectrometry (GC/MS/MS). Recently, a new method has been published and validated that makes the detection of 3'-hydroxystanozolol glucuronide in urine possible in a concentration >50-fold less compared to the above-mentioned commonly used methods. It is common practice to administer breeding animals with steroid hormones in order to enhance their growth. Athletes who consume meat containing such hormone residues may be at risk of failing a sports drug test. A randomized study in the general population consuming meat should be conducted, monitoring the levels of 3'-OH-stanozolol glucuronide in human urine, in order to determine the threshold levels of passive exposure, if any, and therefore guarantee that any adverse analytical findings reported in the urine of athlete at a pg level correspond to stanozolol abuse for enhancing performance.

## Introduction

Stanozolol is a synthetic heterocyclic steroid with anabolic and androgenic properties, which has been abused by several high-profile professional athletes, and is also used in veterinary

medicine to increase appetite, cause weight gain and treat certain types of anemia. Chemically, it is a derivative of dihydrotestosterone (DHT), and it contains a 3-2 pyrazol group attached to the first cycloalkane ring (known as the A-ring) of the anabolic steroid structure. The attachment of the pyrazol group to the A-ring actually replaces the 3-keto group that normally sits in the same location.

Stanozolol undergoes a rapid metabolization, leading to low concentration levels of the parent compound in urine, with the level of the urinary excretion also being low enough, i.e., 3-5% out of the total administered amount. Stanozolol is mainly excreted as hydroxylated metabolites, which are detectable at low ng/ml levels up to 3-4 days following the cessation of administration (single-dose administration). The maximum excretion rate of its monohydroxylated metabolites can be detected between 8 and 17 h following oral administration, depending on the applied dose. The most abundant metabolites identified in the human urine are 16 $\beta$ -hydroxystanozolol, 3'-hydroxystanozolol and 4 $\beta$ -hydroxystanozolol (1-3).

The detection of stanozolol metabolites in human urine for doping control purposes depends on the analytical method applied. The most commonly applied methods in the World Anti-Doping Agency (WADA)-accredited doping control laboratories are gas chromatography/high-resolution mass spectrometry (GC/HRMS) or gas chromatography-mass spectrometry (GC/MS/MS). In the WADA technical document TD2015MRPL, the minimum required performance level (MRPL) for 3'-hydroxystanozolol is set at 2 ng/ml, taking into consideration the metabolism, stability, pharmacokinetics and pharmacodynamics of stanozolol. This means that in order for a WADA-accredited laboratory to effectively perform its tasks, 3'-hydroxystanozolol has to be detected at concentrations of  $\leq 2$  ng/ml, as this is the area of concentrations in the urine samples of athletes that stanozolol metabolites vary, when stanozolol is administered to enhance performance with its long-lasting doping effect.

## Current developments

Recently, a new method has been published and validated that makes the detection of 3'-hydroxystanozolol glucuronide in

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**Key words:** stanozolol, doping, passive exposure, animal breeding, 3'-OH-stanozolol glucuronide

urine possible in a concentration >50-fold less compared to the above-mentioned commonly used methods (4). This new state-of-the-art method has been contemporaneously and retrospectively applied to the analysis for doping control purposes of urine samples of athletes, rendering several adverse analytical findings at the level of several pgs.

It is common practice to administer breeding animals with steroid hormones in order to enhance their growth. Stanozolol is an anabolic steroid that is often found in injection sites and cocktails for animals. However, it has never been detected in sample tissues (kidney fat, meat) or excreta (urine, faeces) obtained during regular inspection. The difference between the structure of stanozolol and the other steroids (a pyrazole ring fused to the androstane ring system) is probably the cause of this phenomenon (5). The European Union banned the use of anabolic steroids in general for cattle fattening in 1988. However, traces of various anabolic steroids can still be detected in tissues (kidney fat, meat) or excreta (urine, faeces) obtained during regular inspection (6-8).

Athletes who consume meat containing such hormone residues may be at risk of failing a sports drug test. WADA has issued a warning for meat consumption in Mexico and China for clenbuterol, a  $\beta$ 2-agonist with anabolic effects, while reports from the custom authorities in Russia confirm contaminated meat with anabolic steroids originating from Australia. The concentrations of the metabolites detected in the urine of athletes following the consumption of contaminated meat may vary, and are usually expected well below 1 ng/ml, depending on the amount of meat consumed, the original concentration of the steroid hormone in the meat, and the time elapsed between the consumption of contaminated meat and urination. Hence, the consumption of meat containing small amounts of injected hormones may constitute a serious liability to the athlete (5,7-9).

It is, therefore, possible that the detection of 3'-hydroxystanozolol in the urine of athletes at a pg level could correspond to an inadvertent doping case without the intentional use of stanozolol by the athlete and through an administration route, such as meat consumption for everyday dietary needs, that could not have been prevented by the athlete. In such a case, any advantage over other co-athletes while competing and any intention to enhance performance by steroids also becomes questionable.

In conclusion, a randomized study in the general population consuming meat for dietary purposes should be conducted, monitoring the levels of 3'-OH-stanozolol glucuronide in human urine, in order to determine the threshold levels of passive exposure, if any, and therefore guarantee that any adverse analytical findings reported in the urine of athletes at a pg level correspond to stanozolol abuse for enhancing performance.

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