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Strategies that athletes use to avoid detection of androgenic-anabolic steroid doping and sanctions

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ABSTRACT

Androgenic-anabolic steroids (AAS) are potent and widely used performance-enhancing substances (PES). Since the International Olympic Committee (IOC) began testing athletes for AAS in the 1970s, athletes and their teams have endeavored to beat the system to avoid doping violations and/or sanctions derived from positive test results. This review will discuss the strategies used to avoid detection based on the pharmacology, biochemistry, and genetics of AAS metabolism and testing principles. Another strategy used is to dope with testosterone under the guise that the athlete has a true medical condition that requires testosterone treatment, using the therapeutic use exemption (TUE) mechanism. Misrepresentation in TUE applications is extending to amateur athletes, as testosterone prescription outside of FDA guidance increases and sport organizations broaden their efforts to police doping at all levels of competition. Strict criteria are enforced under which a TUE for testosterone use may be granted, to maintain the integrity of sport. The challenge of upholding a zero-tolerance policy for AAS abuse, despite popular misconceptions of androgen physiology and pervasive attempts to dope among athletes and physicians, remains a daunting and evolving task for the anti-doping community.

1. Introduction

1.1. Androgenic anabolic steroids abuse

The use of performance-enhancing substances (PES), particularly androgenic-anabolic steroids (AAS), has been recognized as a common practice and a detriment to fair competition in elite sport since the 1960's. More importantly, AAS abuse now extends well beyond elite athletes and has become a serious global public health issue (Pope et al., 2014). In-competition testing for PES was introduced in the 1970's and has progressively evolved in frequency and sophistication to compensate for the elaborate schemes athletes use to avoid detection. AAS are the most frequently identified PES, representing 48% of violations according to the World Anti-Doping Agency (WADA) (World Anti-doping Agency, 2014). AAS are relatively inexpensive, easily obtained from legal and illegal sources, easily administered, and highly effective. In the absence of proper controlled studies, the medical profession questioned the efficacy of AAS as PES for a long time, but even the endogenous AAS testosterone (T) has been shown to increase muscle size and strength when administered in supraphysiologic doses (Bhasin et al., 1996). The World Anti Doping Agency (WADA) maintains a list of prohibited substances for sports, which includes both PES and other drugs that are used to mask the administration of PES (WADA, 2015). In this review, we discuss different strategies used by athletes to avoid detection and sanctions derived from positive test results.

2. AAS metabolism and testing

2.1. Steroid metabolism

Conceptually, the metabolism of endogenous steroids consists of 2 components: the steroid-specific enzymatic conversion and drug/xenobiotic transformations. Consider T metabolism first as an example. In general, steroids from the circulation can undergo “pre-receptor” metabolism in target cells, either activation or inactivation (Miller and Auchus, 2011). For T, oxidation of the 17β-hydroxyl group to androstenedione, a reaction catalyzed by 17β-hydroxysteroid dehydrogenase type 2, ablates the androgenic activity and

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represents a step in preparing T for elimination. In contrast, specific tissues such as genital skin and prostate contain the enzyme 5α-reductase type 2 and further activate T to the more potent androgen 5α-dihydrotestosterone (DHT). A second isoenzyme, 5α-reductase type 1, also converts T to DHT in other tissues, primarily liver and skin. Paradoxically, the hepatic 5α-reduction poises the steroid for further conversions towards elimination. For T, yet a third pathway exists that changes the biological activity from androgen to estrogen. This pathway involves the aromatase (CYP19A1) enzyme found in many tissues (bone, brain, testis, adipose), which derives from a large gene with multiple promoters that allows tissue-specific regulation of its expression (Simpson et al., 1994). These pathways are illustrated in Fig. 1.

The human genome contains 57 cytochrome P450 (CYP) genes, which encode for enzymes that catalyze a wide range of oxidation reactions using hydrophobic substrates. Most of these enzymes are expressed in the liver and possess broad though low-affinity substrate specificity for drugs and other xenobiotics, yet steroids are sufficiently hydrophobic that most steroids are P450 substrates. Indeed, 6 of these P450s are biosynthetic enzymes in steroidogenesis, which perform the essential oxidation reactions to convert cholesterol into progesterone, mineralocorticoids, glucocorticoids, androgens, and estrogens. Other P450s are essential for the synthesis of sterols and/or bile acids. Cortisol biosynthesis, for example, requires 4 P450 enzymes to convert cholesterol to cortisol; however, cortisol itself is also a substrate for the hepatic enzyme CYP3A4, which also metabolizes about half of all drugs used today. CYP3A4 metabolism of cortisol generates primarily 6β-hydroxycortisol, which is biologically inert and thus a catabolic or degradative pathway. Hepatic P450 metabolism of steroids is thus equivalent to “phase 1” of drug metabolism, increasing the hydrophilicity of the compound and adding hydroxyl groups for subsequent conjugation reactions.

The liver also contains the enzyme 5β-reductase (AKR1D1), an essential enzyme for bile acid biosynthesis. The 5β-reduction chemistry creates a 90-degree bend in the A-ring, and in combination with 3α-reduction plus 7α- and 11α-hydroxylation, gives bile acids their amphipathic properties of one polar face and one hydrophobic face. Steroids and bile acid precursors are structurally similar and share the 3-keto-Δ^4-functionality, meaning a 3-ketone in conjugation with a double bond between carbon atoms 4 and 5, the site of 5β- (or 5α-) reduction. Not only is T 5β-reduced, but essentially all steroids with the 3-keto-Δ^4-functionality are subjected to both 5β- and 5α-reduction in the liver as a catabolic or degradative pathway. Subsequent to the reduction of the Δ^4-double bond, the 3-keto-5β- (or 5α-) metabolites are further reduced to mainly 3α-hydroxysteroids and some 3β-hydroxysteroids, and these tetrahydro-derivatives are the products of two sequential reduction reactions. Exogenous steroids containing the 3-keto-Δ^4-functionality typically undergo similar catabolic transformations in the liver.

The tetrahydro-steroids are now substrates for glucuronyl transferase (UGT) enzymes, a classic “phase 2” reaction of drug metabolism. Just as the human genome contains many genes encoding multiple P450 enzymes, many UGT isoenzymes exist with their own spectrum of substrates, regiochemistry, and catalytic efficiencies (Turgeon et al., 2001). For example, UGT1A1 conjugates bilirubin, and incomplete UGT1A1 deficiency causes Gilbert syndrome, a generally benign condition of elevated total and indirect (unconjugated) bilirubin. Patients with Gilbert syndrome, however, are predisposed to irinotecan toxicity, because UGT1A1 is important for phase 2 metabolism of this drug (Marsth and McLeod, 2004). The UGT2B7, UGT2B15, and UGT2B17 isoenzymes are the major enzymes for conjugation of androgens and their metabolites. About 1% of T is excreted in the urine as its 17-glucuronide. The 2 dominant metabolites of T are androsterone (An)—the 17β-oxidized, 5α-,3α-reduced metabolite—and etiocholanolone (Et), which differs from androsterone only by 5β-reduction (Krone et al., 2010). The urine An/Et ratio varies somewhat but is generally in the range of 0.5–2:1. Administration of a 5α-reductase inhibitor such as finasteride or dutasteride will decrease the An/Et ratio significantly by shifting more T metabolism to the 5β-pathway. Administration of DHT, conversely, will reduce T synthesis via negative feedback, and since DHT is already 5α-reduced, the An/Et ratio will rise.

2.2. Anabolic steroids testing

2.2.1. Testosterone/epitestosterone ratio

Testing for synthetic AAS involves collection of a urine sample, which contains conjugated steroids and steroid metabolites. The steroids are deconjugated, derivatized, and analyzed using gas chromatography/mass spectrometry. The analysis has 2 components, analysis of synthetic AAS not normal found in human urine and analysis of endogenous androgens and their metabolites. Testing for AAS is relatively straightforward, with the caveats that often one or more metabolites rather than the parent compound are targeted in the method and that an upper limit of normal must be established, to account for endogenous substances that are identical to or very similar to the analyte in question. Testing for endogenous androgens is considerably more complex, because these compounds are normally present in human urine. For T, the principle of testing employs the simultaneous measurement of epitestosterone (E), which is the 17α-epimer of tesotsterone with no known biological activity. The biosynthetic pathway to E in human beings is not known, but E is produced in a fixed proportion to T during normal testicular androgen synthesis. The T/E ratio in urine varies from about 0.4 to 2 with a bimodal distribution, but for a given individual, this ratio remains remarkably constant. Adminstration of exogenous T reduces synthesis of both T and E by negative feedback and results in an increase in the T/E ratio (Basaria, 2010). A T/E ratio of >4:1 is conservatively set as evidence of doping by WADA.

2.2.2. Individual athlete's steroid profile

Taking the analysis of endogenous AAS one step further, the steroid profile refers to longitudinal follow up of concentrations and ratios of various endogenously produced steroids, their precursors and metabolites. The urinary steroid profile measures the following substances: T, E, DHT, An, Et, dehydroepiandrosterone (DHEA), 5α-androstane-3α,17β-diol (Adiol), and 5β-androstane-3α,17β-diol (Bdiol). Because the flux along these metabolic pathways is fairly stable, these values are compared to subject-based reference ranges, which have been found to be much more reliable than population-based ranges (Sottas et al., 2010). The serial monitoring of individual athletes’ steroid profiles is the concept of a “biological passport,” which provides a sensitive approach to detect doping with endogenous AAS. Any alteration in the ratios of these substances or marked changes in concentrations raises suspicion of doping (Mareck et al., 2008).

3. Strategies to avoid detection

Athletes use several methods to avoid detection of AAS, from urine replacement or adulteration to administration of masking agents (Table 1). In general, a doping violation can be subverted in the following scenarios (Botrè, 2008):

1. The substance is either unknown or its metabolism is unknown, and thus there is no specific analyte targeted for detection as evidence of doping. Subtle modifications of known AAS often

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