



Effect of Exogenous Anabolic Androgenic Steroids on Testosterone/ Epitestosterone Ratio and its Application on Athlete Biological Passport in Egypt

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Abstract

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Using the Anabolic Androgenic Steroid (AAS) agents is evident not only within the competitive senior and junior athletes, but also in non-sporting contexts by individuals seeking to 'improve' their physique. No accurate data is available for the prevalence of AAS misuse among athletes. Studies suggest that it may be 1–5% of the population; with the prevalence being higher in males. Many studies documented side effects and health hazards with the misuse of anabolic steroids, where these were accused as a cause of deaths among athletes. Intake of exogenous anabolic steroids disturbed the Testosterone / Epitestosterone (T/E) ratio causing its evaluation above the normal level. This review outlines the anabolic steroids, its side effects and health impacts in both the sporting and physique development contexts. It also provides a brief review of the history of AAS as doping agents and athlete biological passport.

Conclusion: Doping among athletes is a widespread public health and social problem. Many studies have shown that both short- and long-term health complications have consequences and dependencies. There is insufficient data on the prevalence of doping in Egypt. Therefore, we recommend conducting studies on this subject.

Keywords: Doping, Athlete, Biological Passport

1. Introduction

Sport is a sublime exercise that inspires spirit and body and gives its practitioner a sense of superiority through the successes of honest competition. In addition to gaining acceptance in primitive societies, victory and excellence provide gains in terms of the economic and social status of modern societies (Unal and Ozer Unal, 2004). In today's world, however, the lucrative profit-making industry has become and is making use of

various methods, both legitimate and illegal. In sport, the use of illicit drugs comes under the category of 'doping' with the relevant sports governing bodies pursuing strategies to prevent the use of banned substances (Bird et al., 2016). The use of doping agents is evident within the competitive sport in senior and junior age groups, where they are taken by non-elite as well as elite participants. They are also taken in non-sporting contexts by individuals seeking to 'improve' their physique through an increase in muscle and/or a decrease in

fat mass (Pierre-Edouard et al., 2010). While attaining accurate data on the prevalence of their use has limitations, studies suggest the illicit use of doping agents by athletes and non-athletes may be 1-5% in the population and greater than 50% in some groups; with the prevalence being higher in males (Stephen et al., 2016). Among the many drugs or methods used to enhance physical performance, anabolic androgenic steroids are the most widely accepted substances by athletes because of its androgenic effects which are associated with masculinization and virilization, in addition to its anabolic effects that associated with protein building in the body (Kicman et al., 2008).

Anabolic-androgenic steroids (AAS) represent a class of steroidal hormones affiliated with the hormone testosterone.

Abuse of AAS is not limited to elite athletes but is also common practice among many amateurs and recreational athletes in gyms. Although the sports organizations and media pay less attention to the abuse of AAS by these athletes it is of great medical concern. In recent years many reports on the side effects of these substances in athletes have been published (Fred Hartgens and Harm Kuipers, 2004; Friedl et al., 2000).

This review will outline the anabolic, ergogenic and health impacts of AAS that may be used in both the sporting and physique development contexts. It also provides a brief tabulated overview of the history of AAS and how exogenous AAS may impact upon the analyses of clinical samples.

2. Review of the Article

2.1. AAS in sports; Brief history

For many years, androgenic-anabolic steroids (AAS) have been popular among athletes both for performance improvement and for aesthetic reasons by increasing lean body mass. The first documented incident of athletes AAS misuse dating back to the 1950s. At the world weightlifting championship in Vienna (Austria), it was rumored that the Russian team physician told the US team doctor that some Russian athletes used androgens for enhancing their performance. After that, when athletes used AAS to win competitions and championships in that period, abusing of these substances in sport

began to spread (Yesalis et al., 2000).

The International Olympic Committee (IOC) started to face doping in the 1960s. The first doping control process had applied in the Olympic Games in Mexico 1968 and in 1976 the AAS were placed on the list of banned substances. A decade later the IOC decided to introduce the so-called 'out-of-competition' doping controls.

The initial Anabolic steroids added to the prohibited list were methandienone, stanozolol, esters of nortestosterone, and related compounds.

In 1979, Testosterone and its esters were added and in 1986 the list was widened to involve any substance that leads to increasing the testosterone: epitestosterone (T: E) ratio. Initially, this ratio was set at 6:1 by the IOC, but in 2005, it was decreased to 4:1. Additional laboratory tests may be needed to decide whether the ratio is due to a physiological or pathological condition.

In 1993, the Anabolic Agents were separated into two groups:

- i. Anabolic Androgenic Steroids; and
- ii. Another Anabolic agent (e.g. beta-2 agonists)

In 1995 Dihydrotestosterone was added and considered to be positive when its urinary concentration exceeds the normal range values.

The sample is not positive for testosterone or dihydrotestosterone, as the ratio or concentration is due to a pathological or physiological condition which is proved by the athlete. This rule was applied in 2002 for any banned substance that could be produced by the body naturally. In 2000, evidence acquired from the measurement of metabolic rate and/or metabolic isotopes could be used to derive specific results. Epitestosterone was listed in 1995 under Prohibited Techniques.

2.2. Mechanism of action

The mechanism of action of AAS is not completely understood and is still subject to research. Several general mechanisms have been demonstrated to explain action of AAS due to the presence of a variety of the steroid molecule, while in recent years several specific

mechanisms and theoretical models of action on tissue and organs have also been recognized. This may be related to the variation in affinity to androgen receptors (Fred Hartgens and Harm Kuipers, 2004). Figure 1 illustrates the general mechanism of action of AAS.

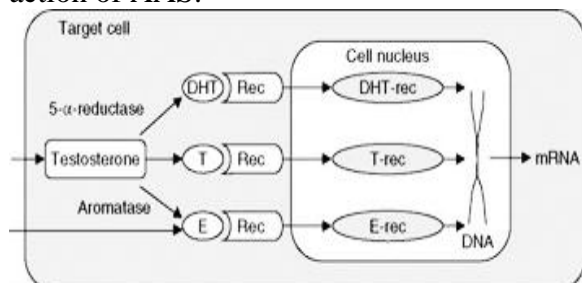


Fig. 1. Mechanism of action of testosterone. DHT = dihydrotestosterone; E = estradiol; DHT-rec = dihydrotestosterone-receptor complex; E-rec = estradiol-receptor complex; Rec = receptor; T = testosterone; T-rec = testosterone-receptor complex.

One of the most important enzymes is the 5 α -reductase, which plays a crucial role in converting testosterone to dihydrotestosterone (androstanolone). The enzyme 5- α -reductase seems to play an important role by converting AAS into dihydrotestosterone (androstanolone) that acts in the cell nucleus of target organs, such as male accessory glands, skin, and prostate. Other mechanisms comprise mediation by the enzyme aromatase that converts AAS in female sex hormones (estradiol and estrone), antagonistic action to estrogens and a competitive antagonism to the glucocorticoid receptors.

Furthermore, AAS stimulate erythropoietin synthesis and red cell production as well as bone formation but counteract bone breakdown. The effects on the cardiovascular system are proposed to be mediated by the occurrence of AAS induced atherosclerosis (due to unfavorable influence on serum lipids and lipoproteins), thrombosis, vasospasm or direct injury to vessel walls, or may be ascribed to a combination of the different mechanisms. The AAS-induced increment of muscle tissue can be attributed to hypertrophy and the formation of new muscle fibers, in which key roles are played by satellite cell number and ultrastructure, androgen receptors & myonuclei (Fred Hartgens

and Harm Kuipers, 2004). Must be taken into consideration that the exogenous administration of AAS is not the only way to produce a sustained rise in testosterone levels. This can occur by several indirect doping strategies to produce the same effect. Antiestrogens or aromatase inhibitors antagonists the estrogen receptor causing its blockade. Both estrogen blockers stimulate sustained increases in endogenous luteinizing hormone secretion, and, successively, increases in blood testosterone concentrations but with different pharmacologic action. The elevated level of testosterone caused by estrogen blockade in men is known to be sufficient to produce ergogenic and performance-enhancing effects (Pierre-Edouard et al., 2010).

2.3. Adverse Health Effects of AAS misuse

Concurrent with the increased misuse of AAS for non-medical reasons, more attention was paid to the adverse effects. The use of anabolic steroids is associated with adverse effects on both physical and psychological health, including fatalities. To date, only a few reports investigating the self-reported adverse effects in athletes using AAS have been published (Andrew et al., 2003). These reports employing questionnaires showed clearly that the majority of athletes experienced undesired health effects not only when on AAS, but also after drug withdrawal. On the other hand, case reports have the disadvantage of highlighting the most severe adverse effects and complications of AAS misuse, which may be primarily due to a temporal association rather than to a causal relationship (Fred Hartgens and Harm Kuipers, 2004).

Many adverse effects have been associated with AAS misuse, including disturbance of endocrine and immune function, alterations of sebaceous system and skin, changes of the hemostatic system and urogenital tract (Handelsman et al., 2008).

One of the major problems are the AAS substances are obtained from the black market with a quality is not guaranteed, that requiring an increase of doses administered as these vials and tablets obtained on the black market contain only a fraction of the declared dosage (Fred Hartgens and Harm Kuipers, 2004). The side effects reported in at least 40% of the male subjects in these studies included increased sexual drive, the occurrence of acne, increased body hair and an increase in aggressive behavior (De

Boer et al., 1996). Furthermore, many other side effects affecting several body systems were mentioned by the steroid users. These include fluid retention, elevated blood pressure (BP), sleeplessness, increased irritability, decreased libido, increased appetite, enhanced transpiration, and increased the feeling of well-being, depressive mood states, loss of head hair and the occurrence of gynecomastia (Fred Hartgens and Harm Kuipers, 2004). Data relating to female athletes are very scanty (De Boer et al., 1996; Strauss et al., 1985). Strauss et al. interviewed ten females who all reported lowering of the voice brought on by AAS use. Furthermore, nine of ten females admitted increased growth of facial hair, enlargement of the clitoris and an increase in aggressiveness and appetite (Strauss et al., 1985). Recently, in the study by De Boer et al., nine of ten interviewed female athletes had experienced side effects due to steroid use (De Boer et al., 1996). The side effects reported were acne (50%), fluid retention (40%) and alteration of libido (50%). Other side effects were only mentioned by <20% of the women. The discrepancy between the reported side effects in both studies can, at least in part, be attributed to the difference in substances and dosages used. The subjects in the study of Strauss et al. self-administered more androgenic agents and used much higher doses than the female bodybuilders of DeBoer et al.'s study which may be responsible for the masculinizing effects (De Boer et al., 1996; , Strauss et al., 1985). Of great concern is that athletes are not aware of many side effects during steroid administration, since several unwanted health effects may be detected only after a thorough medical examination, including blood analysis.

2.3.1 Effect on hormonal status

Since AAS are derived from testosterone they exert important effects on the sex hormones and the reproductive system. They will suppress the hypothalamic-pituitary-gonadal axis, which acts as a feedback system. Consequently, exogenous administration of AAS will disturb the endogenous production of testosterone and gonadotrophins (luteinizing hormone [LH] and follicle-stimulating hormone [FSH]). In males, suppression of gonadotropin production induces testicular atrophy and reduces semen production and quality. Studies have shown that the use of AAS may dramatically lower serum gonadotrophin concentrations (Fred Hartgens and

Harm Kuipers, 2004; Bhasin et al., 2001) a decline can be observed within 24 hours (Anderson et al., 1996). Serum testosterone levels will also decrease, except when exogenous testosterone is administered in amounts usually practiced by strength athletes. However, Bhasin et al proved that a close dose-response relationship exists between the administered dose of testosterone and serum levels of testosterone. Administration of weekly doses of intramuscular testosterone >300mg increase serum levels of testosterone and free testosterone, whereas weekly doses of 25 or 50mg resulted in lower serum levels of testosterone and free testosterone (Bhasin et al., 2001).

Previously, it has been demonstrated that the administration of high doses of testosterone in polydrug abusers will induce supraphysiological levels of serum total and free testosterone and estradiol (Turillazzi et al., 2011). Serum concentrations of androstenedione and dihydrotestosterone closely follow the same pattern. The high serum levels of estradiol, androstenedione, and dihydrotestosterone can be explained by peripheral conversion of AAS (Fred Hartgens and Harm Kuiper, 2004).

Long-term administration of high doses of AAS may provoke hypogonadotropic hypogonadism, characterized by testicular atrophy, oligo- or azoospermia, low serum concentrations of LH and FSH, and of endogenous testosterone and precursors (Wilson et al., 1996; Martikainen et al., 1986).

2.3.2 Testosterone/epitestosterone (T/E ratio)

Epitestosterone (17 α -hydroxy-4-androsten-3-one) is a 17-epimer of testosterone that is also secreted by the Ley- dig cells of the testes. It was first described in 1947 as an androgen metabolite; however, the first direct evidence of its urinary excretion was seen in the 1960s (Korenman et al., 1964). It is biologically inactive, and there is no inter-conversion between testosterone and epitestosterone. Although its production rate is less than 5% of testosterone, its urinary excretion is 33% that of testosterone. It is mainly excreted in the urine as a glucuronide (Kicman et al., 2008); however, a small amount is also excreted as a sulfate. Due to its rapid clearance, excretion rates of testosterone and epitestosterone are similar, making the urinary T/E ratio approximately 1. The nearly constant ratio of urinary testosterone to epitestosterone became the basis of the method of detection of exogenously administered testosterone.

Because there is no interconversion between the two compounds, the administration of exogenous testosterone results in an increase in the T/E ratio (Kicman et al., 2008).

In 1983, Donike and co-workers introduced a ratio of the glucuronides of T and epitestosterone (E) as a marker for exogenous testosterone administration in sports (Donike et al., 1983 & 1985) and later for the identification of the administration of related compounds. Donike suggested an upper limit of 6.0 for the testosterone over epitestosterone (T/E) ratio to detect testosterone abuse for athletes (Donike et al., 1982).

The International Olympic Committee adopted this ratio for its accredited laboratories as an arbitrary critical value as the sole test for illicit testosterone self-administration, after the studies done by Donike et al. (Schanzer et al., 1998). In 1982, the test adopted by the International Olympic Committee (IOC) for detection of T administration was based on the T/E ratio (Donike et al., 1992).

However, it was recognized that genetic differences in various populations may influence the T/E ratio. Some athletes have low endogenous epitestosterone production rate; hence, their T/E ratio always exceeds 6 (Aguilera et al., 2001; Garle et al., 1996). To the contrary, a deletion polymorphism in uridine diphosphate glucuronyl transferase 2B17, an enzyme that facilitates epitestosterone excretion, lowers T/E ratios (especially in Asian populations) (Jakobsson et al., 2006; Schulze et al., 2008).

Considering these genetic variants, the IOC recently lowered the T/E cutoff ratio to 4. Hence, any value above 4 is considered suspicious.

In 2004 this threshold value has been lowered by WADA from 6 to 4 (WADA, 2004).

Since then, other steroid ratios e.g. androsterone (Andro)/etiocholanolone (Etio), 5 α -androstane-3 α ,17 β -diol (5 α P-Adiol)/5 β -androstane-3 α ,17 β -diol (5 β P-Adiol) were also evaluated to detect changes in the steroid profile induced by administration of testosterone or prohormones (Renterghem et al., 2010). Moreover, Andostenedione administration increases epitestosterone excretion while decreasing that of

its putative precursor (Catlin et al., 2002) and also Dehydroepiandrosterone supplementation can increase the testosterone: epitestosterone ratio (Bowers et al., 1999).

Testosterone and epitestosterone levels in urine specimens commonly are measured in anti-doping laboratories by gas chromatography-mass spectrometry (GC/MS) after deconjugating the glucuronide moiety by enzymatic hydrolysis (b-glucuronidase) and derivatization (trimethylsilylation). Alternatively, testosterone and epitestosterone can be measured directly using high-performance liquid chromatography (HPLC)/tandem MS (Sottas et al., 2010; Borts et al., 2000).

Measurement of T/E ratio in a large number of athletes has shown that generally the ratio remains below 6 (Fig. 3) (Donike et al., 1984). This initially led the IOC to adopt the cutoff threshold of 6 to consider the test as positive. In agreement with Donike's publications (Donike et al., 1993; Donike et al., 1994) & Sottas et al. improved this method recently by proposing the Bayesian screening test for the detection of abnormal values in longitudinal biomarkers. This test compares sequential measurements of a biomarker against previous readings performed on the same individual (Sottas et al., 2007 & 2008).

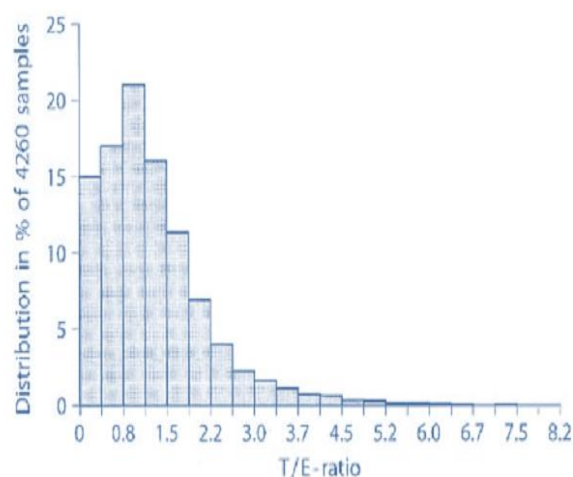


Fig. 3. Distribution of T/E ratios in 4260 samples of male athletes in an IOC accredited laboratory (adapted from Schanzer et al., 1998 with the permission of Cambridge University Press)

2.4. Athlete Biological Passport

An ABP is an individual electronic document that stores all valuable information to be an interpreter for indirect evidence of doping.

The fundamental principle of the ABP is throughout the time period, monitoring of specific biomarkers to reveal the effects of doping, as it serves as a platform for a Rule of Sport. Reviewing the ABP before the competition serve an objective proof that the athlete will participate in a healthy physiological condition that is not altered by performance-enhancing drugs (Sottas et al., 2010).

Although the ABP had the initial exclusive intent of biological monitoring, today, the ABP contains more than a simple series of individual biomarker values. Heterogeneous factors, such as age, sex, and genotype; confounding factors, such as exposure to higher altitudes for the hematological module; and some information regarding the conditions of sample collection, transport, and analysis are also stored in the passport for improved decision making (Sottas et al., 2010).

The term “athlete biological passport” was first introduced in the early 2000s when monitoring and tracking of a longitudinal record of selected hematological parameters (Markers of blood doping) that identified as a means to define an individual's hematological profile and used as a method for defining the individual's hematological profile (Sottas et al., 2008).

In 2014, the initial system was established with the steroid module, which was launched to create longitudinal profiles of Athlete's steroid variables. The framework proposed in these guidelines builds on existing anti-doping infrastructure to enhance coordination in ABP programs, facilitate the exchange of information and mutual recognition of data, thereby enhancing efficiency in the operation of anti-doping activities (WADA, 2017).

Two main modules can be distinguished in the ABP: the hematological and steroidal modules.

The Hematological Module provide an information for markers referred to blood doping. This model reveals the use of prohibited substances and/or prohibited methods that enhance oxygen transport or delivery. The hematological variables used as markers in the ABP Haematological Module are: Haemoglobin,

Red blood cell (erythrocyte) count, Reticulocytes percentage, Reticulocyte count, Mean corpuscular volume, Mean corpuscular haemoglobin, Mean corpuscular haemoglobin concentration, Red cell distribution width (standard deviation) , IRF Immature reticulocyte fraction, OFF-hr Score & Abnormal Blood Profile Score (abps).

With using the Steroidal Module, many informations are revealed for steroid doping direct and indirect forms of doping with anabolic agents (Sottas et al., 2010). This Module is useful to identify the exogenous administration of EAAS, in addition, other anabolic agents, such as selective androgen receptor modulators (SARMS) mentioned in the Prohibited List under Section S1.2.

The component of the steroid profile that involved in the ABP Steroidal Module are:

- Testosterone;
- Epitestosterone;
- androsterone;
- etiocholanolone (Etio);
- 5 α -androstane-3 α ,17 β -diol (5 α Adiol);
- 5 β -androstane-3 α ,17 β -diol (5 β Adiol);

The followings are the ratios:

- testosterone to epitestosterone (T/E);
- androsterone to testosterone (A/T);
- androsterone to etiocholanolone (A/Etio);
- 5 α -androstane-3 α ,17 β -diol to 5 β -androstane-3 α ,17 β -diol (5 α Adiol/ 5 β Adiol); and
- 5 α - androstane-3 α , 17 β -diol to epitestosterone (5 α Adiol/E).

The following cut-off concentration levels of endogenous steroids equivalent to the glucuronide: testosterone >200 ng/mL, epitestosterone >200 ng/mL, androsterone >10'000 ng/mL, Etio >10'000 ng/mL and DHEA >100 ng/mL are considered as putative markers of steroid doping [Cazzola et al., 2000]. In contrast to absolute steroid concentrations, ratios such as T/E, androsterone (A)/Etio, A/T, a-diol/E, and a-diol/b-diol are robust to circadian rhythm or changes in physiologic conditions such as exercise workload for athletes [WADA, 2009]. On the other hand, these parameters may be altered

significantly according to the administered steroid and its application mode.

Generally, in athletes not using AAS, the T/E ratio remains fairly constant (Fig. 2). Hence, monitoring T/E ratio serially in the same athlete is occasionally performed to detect any change that may suggest illicit use.

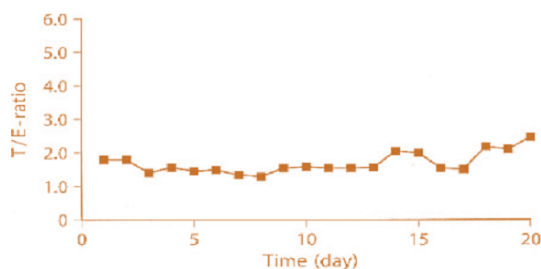


Fig. 2. Stability of T/E ratio over 3 wk of a professional athlete (adapted from Schanzer et al., 1998 with the permission of Cambridge University Press).

3. Conclusion

Doping among athletes is a widespread public health and social problem. Many studies have shown that both short- and long-term health complications have consequences and dependencies. There is insufficient data on the prevalence of doping in Egypt. Therefore, we recommend that studies be conducted on this subject to determine the extent of this phenomenon and to define the common side effects and health hazards resulted from abusing these substances through conducting a survey and case-reports studies.

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