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Anabolic Androgenic Steroid Doping: Facts, Effects and Health Risks

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Introduction

Anabolic androgenic steroids (AAS) are a group of hormones consisting of the male sex hormone testosterone and its synthetic derivatives. AAS were initially developed in the 1930s as a drug to promote the growth of skeletal muscle and to develop male sexual characteristics, and have been used as a medication to treat conditions such as reproductive system dysfunction, breast cancer, and anaemia. In the 1950s, competitive athletes started using AAS as an ergogenic aid with the intention of promoting the growth of skeletal muscle mass. Furthermore, in the 1980s, non-athletes started using AAS for cosmetic purposes. The most common pattern followed by AAS users is "Stacking" (combining two or more oral and injectable AAS) accompanied with "Cycling" (taking multiple doses for a period of time, stopping, then restarting), which lasts for 4-12 weeks.[1,2]

World Anti-Doping Agency (WADA), which was formed as a subsidiary of the International Olympics Committee (IOC), is responsible for conducting scrutiny of doping in sports at present. Anabolic steroids were first identified as a banned class by the Medical Commission of the (IOC) in 1974. In the 1990s, the name was changed to "Anabolic Agents", so that Clenbuterol and other β_2-agonists which possess anabolic activity could also be included in the group. The anabolic agents have been reported as the most frequently detected doping substance in sports. According to WADA's Anti-Doping Administration and Management System (ADAMS) reports in 2017, 44% of the substances identified as adverse analytical findings were anabolic agents.[3,4]

Types of Anabolic Androgenic Steroids

The androgens naturally produced in the body are C19 steroids. Testosterone, the primary male hormone, is produced by Leydig cells in the testes of eugonadal men. In addition to testosterone, several other androgens are also present in the body. Testosterone and 5 α-dihydrotestosterone (DHT) are characterized by a 17-β hydroxyl and 3-oxo groups. DHT is produced by the action of the cytoplasmic action of 5-α-reductase. The oxidation of testosterone produces androstenedione, dehydroepiandrosterone (DHEA) and androsterone. Epitestosterone is an epimer of testosterone with a 17-α hydroxyl group. Testosterone and DHT are the most active forms of the androgens, and all other natural androgens show a weak androgenic activity compared to testosterone.[2,5]

The chemical structures of principal natural androgens are shown in Figure 1.

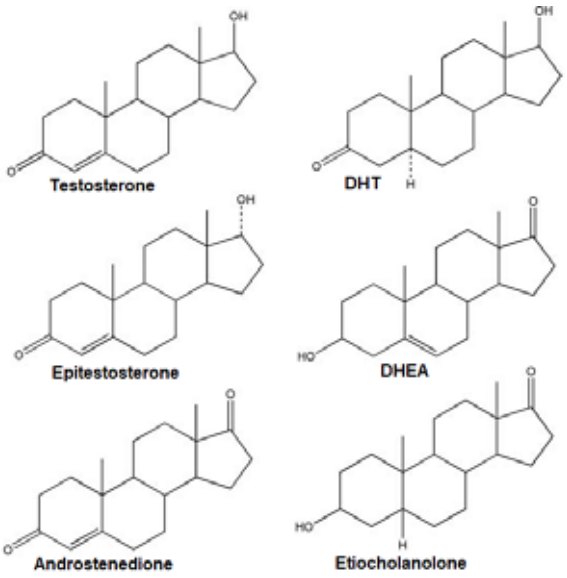

Figure 1: Principal natural androgens

Testosterone was first isolated and synthesized in 1935. Testosterone has been used as the template molecule to synthesize anabolic steroids by incorporating several structural modifications. Structural optimization of testosterone was done to minimize androgenic effects and to maximize anabolic effects while increasing its stability. Figure 2 summarizes the structural modifications to testosterone that affect its androgenic and anabolic activities. Examples of some commonly used AAS are given in Figure 3.[5,6]
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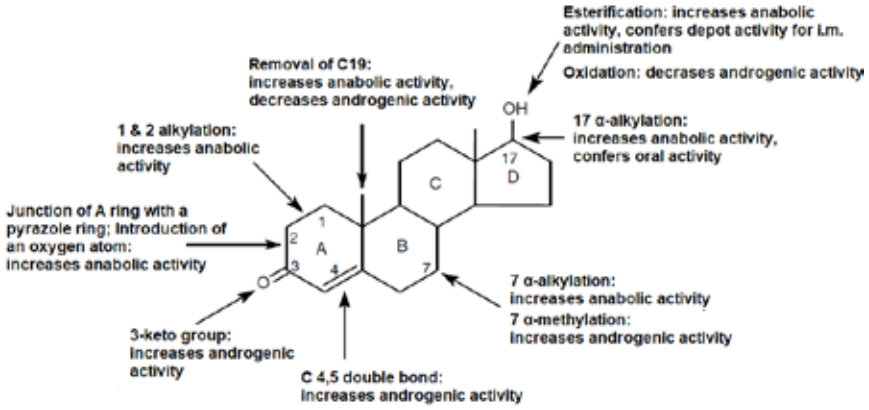


Figure 2: Structural modifications to testosterone that affect its anabolic and androgenic activities

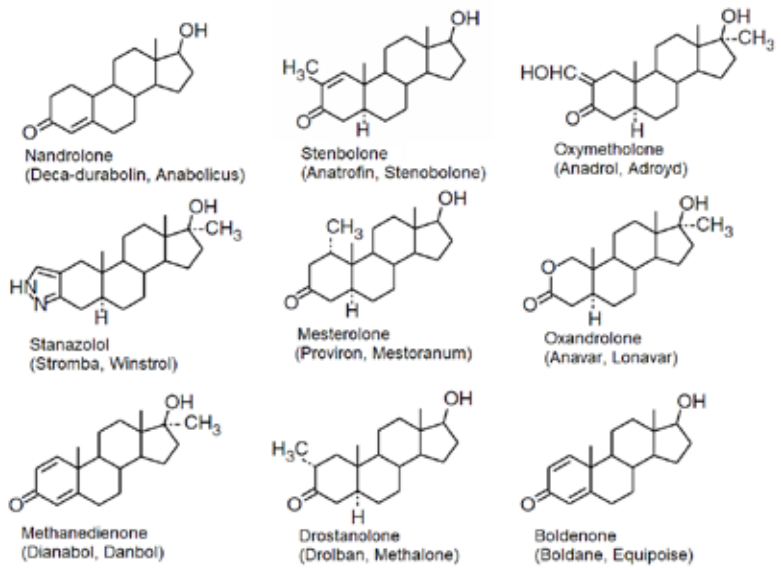


Figure 3: Chemical structures of some commonly abused anabolic androgenic steroids (example brand names are given in parenthesis)

Designer Steroids – Designer steroids are AAS synthesized from a known parent steroid and chemically modified with the intention of circumventing controlled substances laws. Similar to testosterone, designer steroids exert their anabolic activity via androgen-receptor mediated mechanisms. A vast array of designer steroids are presently available, but most of them have not undergone clinical trials. Figure 4 provides an example of structural similarities between an AAS and its designer steroid counterpart.[3,5,7]

Selective Androgen Receptor Modulators – Selective androgen receptor modulators (SARMs) were initially developed by the pharmaceutical companies as they provide a promising alternative as a group of compounds that exert anabolic effects without significant androgenic effects. An ideal SARM should possess good oral bioavailability, tissue specificity, and minimal off-target effects. With the ability to target specific tissues and organs, SARMs may play a significant role in therapeutic applications. Identifying the possibility of athletes using SARMs as a potential avenue to achieve performance enhancement, WADA have included SARMs in their List of Prohibited Substances since 2008.[5,9]

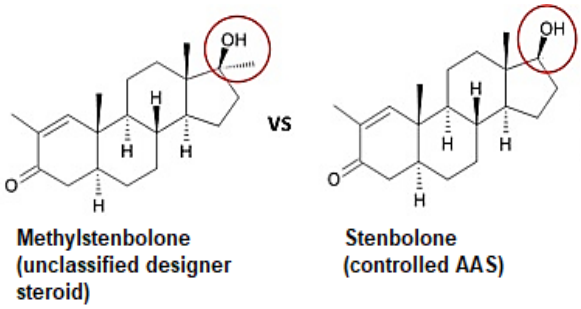


Figure 4: Structural similarities between Methylstenbolone (a designer steroid) and Stenbolone (a controlled AAS) (Adapted from Rahnema *et al.*[7])

How do Anabolic Androgenic Steroids Work in the Body?

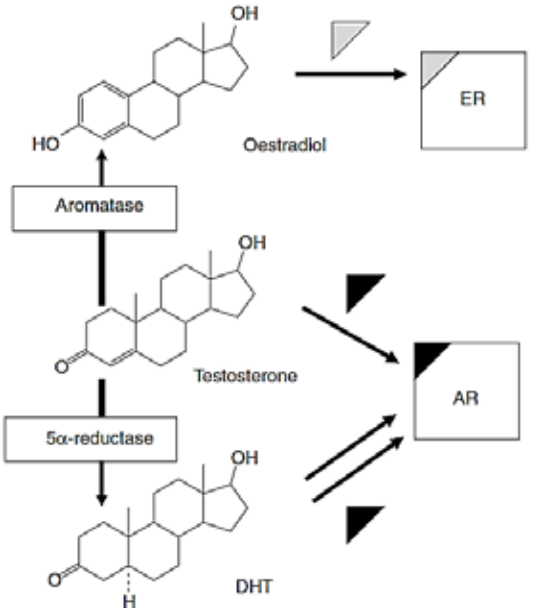
The physiological effects of androgens can be broadly categorized into two groups: anabolic (muscle-building) effects and androgenic effects (development of male sex characteristics). Various ergogenic effects associated with AAS are listed in Table 1.

Table 1: Ergogenic effects associated with anabolic androgenic steroids[8]

Increase in lean body mass and muscle cross-sectional area
Increase in muscle strength and power
Reduced muscle damage
Enhanced recovery between workouts
Enhanced recovery from injury
Increase in protein synthesis and muscle endurance
Increase in erythropoiesis, haemoglobin and hematocrit
Increase in bone mineral density
Increase in glycogen storage
Increase in lipolysis
Increase in neural transmission
Increase in pain tolerance

Several mechanisms have been suggested to explain the anabolic and androgenic activities of AAS. These mechanisms include modulating androgen receptor (AR) expression via intracellular metabolism and directly affecting the topology of the AR, interfering with glucocorticoid receptors of target tissues and acting on the CNS via non-genomic pathways.[9,10]

The mechanisms by which testosterone exerts its anabolic and androgenic effects are well-understood. Following synthesis, testosterone is transported to the target cells where it can be further converted to either DHT or 17β-estradiol by the action of the enzymes 5α-reductase and aromatase, respectively (Figure 5). These conversions play an important role in regulating the effects of testosterone within the target tissues. Once inside the target cells, either testosterone or DHT will bind with AR to form a testosterone-receptor complex. DHT has a greater affinity toward AR compared to testosterone. The hormone-receptor complex will then undergo a structural modification which allows it to be translocated into the nucleus, where it forms a homodimer that binds directly to androgen response elements on target genes, promoting gene transcription and ultimately, protein synthesis.[5-10]

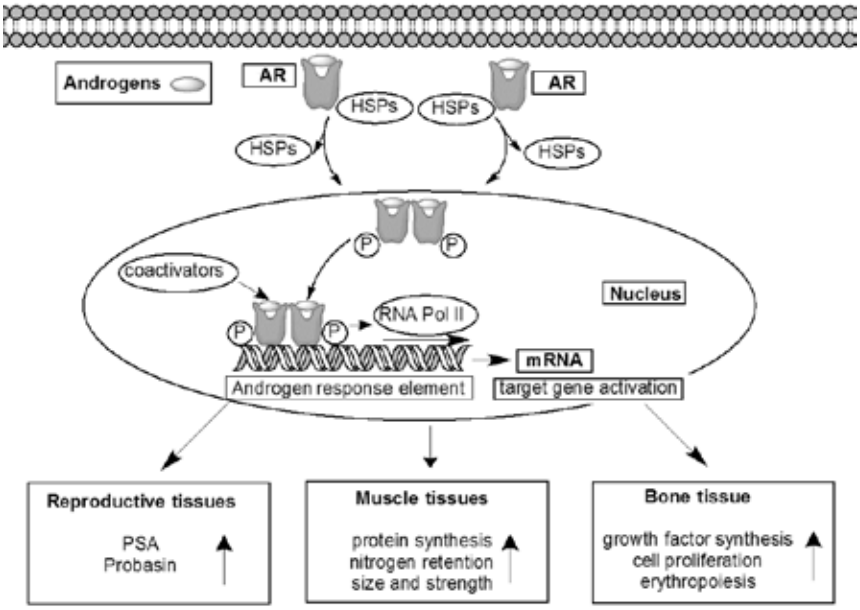


AR: Androgen receptor; ER: Estrogen receptor

Figure 5: Metabolic fates of testosterone within the target tissues[5]

Similar to endogenous testosterone, AAS exert their anabolic effects primarily through AR-mediated mechanisms. Within the target cells, AAS bind with specific AR to form a steroid-receptor complex that will translocate into nucleus and trigger protein synthesis (Figure 6). AAS have been shown to increase lean body mass, muscle size, strength, protein metabolism, bone mineral density and collagen synthesis. Skeletal muscle is considered as a primary target tissue for the anabolic effect of AAS. Testosterone has been shown to have dose-dependent hypertrophy on both Type I and Type II muscle fibres. It has been also demonstrated that AR can be up-regulated by exposure to AAS. Furthermore, AR expression can be increased by strength training, which could be a possible reason for the anabolic effects observed when supra-physiologic doses of AAS are combined with exercise.[9-12]

The anti-catabolic activity of AAS has been linked to glucocorticoid antagonism. Glucocorticoids are a group of stress hormones that exert a strong catabolic effect on human proteins via amino acid degradation, which results in a decrease in muscle mass and an increase in nitrogen excretion under stress conditions. Some evidence suggests that AAS competitively bind to glucocorticoid receptors, thereby restricting the catabolic activity of



AR: Androgen receptor; HSP: Heat shock proteins

Figure 6: Genomic action of AAS mediated by androgen receptor binding (Adapted from Parr and Müller-Schöll[11])

glucocorticoids. However, the specific impact of the anti-catabolic activity of AAS associated with glucocorticoid antagonism has not been demonstrated unequivocally because of testosterone's ability to increase net protein synthesis without decreasing protein degradation.[9-12]

Several other genomic and non-genomic mechanisms have been proposed to explain some indirect ergogenic effects of AAS. It has been suggested that non-genomic actions of AAS involve the activation of second messenger signal transduction cascades including increases in cytosolic calcium and activation of protein kinase A, protein kinase C, and MAPK (mitogen-activated protein kinase). [2,9-12]

Smooth muscle relaxation, neuromuscular and junctional signal transmission and neuronal plasticity are among the cellular effects that result from rapid non-genomic actions of AAS. Additionally, AAS have been shown to stimulate the growth hormone (GH) - insulin-like growth factor-1 (IGF-1) axis, thereby indirectly stimulating muscle hypertrophy. AAS are thought to have a psychotic effect on brain. The long-term administration of AAS may cause behavioural changes in athletes that will allow them to follow intense exercise regimes, which result in an increase in muscle size and strength.[2,9-12]

Adverse Effects of Anabolic Androgenic Steroids

It is well-documented that AAS administration could result in numerous adverse health effects that are dependent on the dose, frequency and the pattern of use. The liver and cardiovascular, reproductive, musculoskeletal, endocrine, renal, immunologic and haematological systems are among the targets for adverse health consequences of AAS use.[13]

Adverse health effects caused by AAS administration are summarized in Table 2.

Detection of AAS Doping

According to the Prohibited List annually published by WADA, anabolic agents fall into 3 broad categories: exogenous AAS, endogenous AAS and other anabolic agents such as Clenbuterol and SARMs. The detection of exogenous AAS is based on the presence of their phase-I and phase-II metabolites in urine. The initial testing procedure involves the use of gas chromatography-(tandem) mass spectrometry [GC-MS(/MS)] to estimate the markers (androsterone, etiocholanolone, 5α-Androstane-3α,17β-diol, 5β-Androstane-3α,17β-diol, testosterone and Epitestosterone) in the urinary steroid profile of the subjects. The confirmation procedure shall be carried out based on the results of steroid profile analysis, which includes GC-MS or GC-MS/MS quantification and GC/C/IRMS analysis of the marker(s) of the steroid profile. Most of the available SARMs can also be detected using LC-MS/MS and LC-HRMS. [15-18]

Table 2: Adverse health effects associated with AAS use [13,14]

Organ/apparatus	Adverse effects
Reproductive system	Female: Menstrual irregularities, clitoral hypertrophy, uterine atrophy, breast atrophy, teratogenicity Male: decreased reproductive hormones, testicular atrophy, oligospermia, impotence, prostatic hypertrophy, prostatic carcinoma, gynecomastia
Liver	Hepatocellular damage, cholestatis, fatal liver cysts, hepatoadenoma, hepatocarcinoma
Cardiovascular and hematologic effects	Increased LDL-cholesterol, decreased HDL cholesterol, increased platelet aggregation, hypertension, thrombosis, left ventricular hypertrophy
Musculoskeletal system	Increased rate of muscle strains/ruptures, increased risk of musculotendinous
Urinary	Elevated blood urea nitrogen, acute renal failure, focal segmental glomerulosclerosis, nephritis, Wilm's tumour
Integument	Acne, alopecia, hirsutism, male pattern baldness, edema
Psychological effects	Mood swings, aggressive behaviour, depression, psychosis, addiction withdrawal and dependency disorders
Other	Deepening of the voice, decreased glucose tolerance, decreased IgA levels, hepatitis B or C, HIV infection

Several novel strategies have been developed to address the challenges associated with AAS doping detection while ensuring the accuracy of testing. Prolongation of the detection windows for exogenous AAS is one such strategy. Because AAS exert long-term effects on performance, the screening procedures now focus on the detection of long-term metabolites with the aid of more sophisticated mass spectroscopic methods, thereby allowing the prolongation of the detection window for AAS. Furthermore, non-targeted and indirect analytical approaches have been introduced to detect designer steroids. The non-targeted approach involves the detection of structure-specific product ions that are derivatives of designer steroids, by employing LC-MS/MS in precursor ion scan mode. The indirect approach, on the other hand, involves the detection of alterations in the steroid profile of the subjects, which result from AAS misuse.[15]

Concluding Remarks

AAS are well known for their ability to induce muscle hypertrophy. Despite mounting evidence supporting the fact that AAS abuse can have adverse acute and chronic health effects, athletes tend to misuse them in an attempt to build muscles and to enhance performance. As a result, AAS has become the most frequently detected doping agent in elite sports. In parallel to the development of new androgens, the AAS detection methods have also been advanced. The general public, especially the athletes, must be made aware of the importance of abstaining from AAS doping to prevent adverse health effects and to ensure fair play in sports.

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