

# Biochemistry and Physiology of Anabolic Androgenic Steroids Doping

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**Abstract:** Anabolic Androgenic Steroids (AASs) are chemical and pharmacological derivatives of the male hormone testosterone which are widely used for increasing burst and sprinting activities in sports. Although AASs are thought to be transversal to the plurality of sports disciplines, they are principally misused by bodybuilders, weightlifters, shot, hammer, discus or javelin throwers, rugby and American football players as well as by swimmers and runners. AAS exert a kaleidoscope of effects on human biology, principally through the 5- $\alpha$ -reductase-mediated conversion into dihydrotestosterone, the aromatase-mediated conversion into female sex hormones, a competitive antagonism to the glucocorticoid receptors, the potential stimulation of erythropoietin secretion as well as psychoactive effects on the brain. The influence of AASs on physical performance is still undefined, since the large number of studies published so far have described discordant and often contradictory outcomes. Nevertheless, animal and human investigations support the hypothesis that the administration of AASs might increase lean body mass, muscle mass, and maximal voluntary strength especially in men, so that they would represent an appealing form of doping for increasing power capacity, sustaining intensive training periods and, last but not least, as a cosmetic muscle makeover. The aim of this article is to review the biochemistry, physiology and the ergogenic effects of AASs.

**Keywords:** Doping, testosterone, sports, anabolic androgenic steroids.

## BIOCHEMISTRY OF ANABOLIC ANDROGENIC STEROIDS

The androgens naturally produced and released by glands are C19 steroids. The androgenic effect refers to a masculinising activity, which has been demonstrated by *in vivo* effects in animals and, most recently, by bioassays [1]. The biological activity of various androgens is heterogeneous and the most active molecules are indeed testosterone and 5  $\alpha$ -dihydrotestosterone (DHT), which are both characterized by a 17- $\beta$  hydroxyl and 3-oxo groups. Reduction of 3-oxo and oxidation of 17- $\beta$  hydroxyl groups are characterized by a significant reduction of the biological activity. The oxidation produces androstenedione, which has less androgenic activity, as well as dehydroepiandrosterone (DHEA) and androsterone, which contain 3- $\beta$  and 3- $\alpha$  hydroxyl groups. Epitestosterone is an epimer of testosterone characterized by a 17- $\alpha$  hydroxyl group and by a weak androgenic activity. The chemical structure of principal androgens is illustrated in Fig. (1).

The activity of androgens is mediated by a specific receptor, which belongs to the nuclear receptor superfamily. It is composed by a DNA binding domain and two transcriptional activation domains, AF-1 and AF-2. Androgen receptor transcriptional activity is mainly

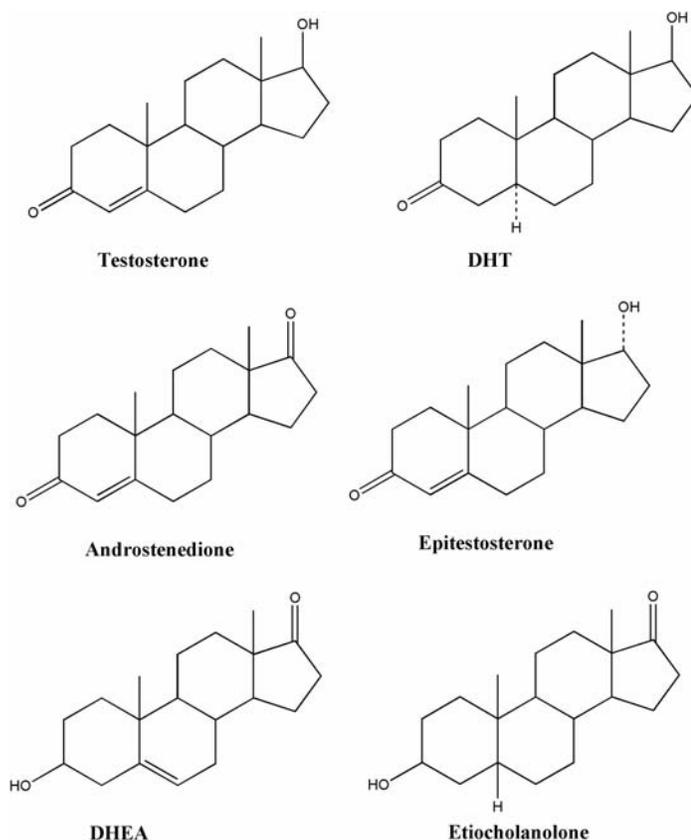
mediated by the N-terminal AF-1 domain. After reaching the target cells, the hormone binds to the receptor ligand-binding domain. Then, the receptor is dissociated from protein chaperones and becomes active, translating from the cytoplasm to the nucleus. Activated receptors interact as homodimers with the androgen response element on the chromatin, triggering the formation of a transcriptional complex. Co-activator and co-repressor complexes for nuclear-receptor-mediated transcriptional regulation are present in the cells, promoting gene activation, transcription, translation and a resultant alteration in cell function, growth or differentiation [2].

Androgens are transported in the circulation mainly bound to plasma proteins. Sex-hormone binding globulin (SHBG) is a transport protein with high affinity for testosterone ( $K_a$  about  $10^9 M^{-1}$ ) and is responsible for nearly 60% of total amount of hormones transported in the blood, whereas the remaining part is bound to albumin, which has lower affinity ( $K_a$  about  $10^4 M^{-1}$ ). Albumin is however important in steroid hormones metabolism, because it displays a high plasma concentration (3.5-5 g/dL or 0.6-0.8 mmol/L) as compared with SHBG (30-90 nmol/L in women and 10-40 nmol/L in men, respectively). Very small quantities of androgens are also transported by cortisol binding globulin, which has ten times higher affinity for corticosteroids [1, 2].

## Testosterone

Testosterone is the major human androgen. It is produced from testes in males and from ovaries and adrenal glands in

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**Fig. (1).** Chemical structures of principal natural androgens.

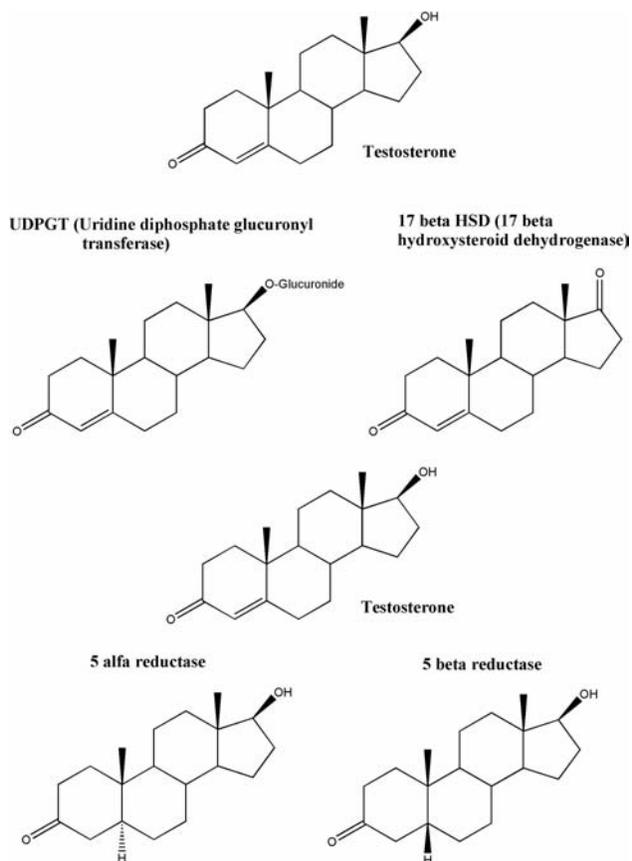
females. The rate of production is 3-7 mg/day in males and 0.1-0.4 mg/day in females, respectively [3]. The effects of testosterone are mediated by the cytoplasm receptor NR3C4, and are typically divided into anabolic and androgenic (discussed in a following section of this article). The androgenic effects are however responsible of primary and secondary male sexual characteristics (testes, distribution of body hair, voice), which are maintained during the entire lifespan by a physiological concentration of the hormone. The secondary male characteristics are maintained by the testosterone metabolite DHT, which is especially active in skin and prostate. Testosterone, as well as all the steroids characterized by hormonal functions, is derived from cholesterol (Fig. 2). The first step encompasses the cleavage of chain on position 17 in the cholesterol molecule. Then, the pathway to conversion into testosterone follows two parallel and related pathways. Pregnenolone is generated from cholesterol by a single but multifunctional enzyme called P450<sub>scc</sub>. It still retains the 5-6 double bond of cholesterol (a pathway defined as “ $\Delta 5$ ”) [3]. Progesterone is derived from pregnenolone and differs for the position of the double bond (4-5), so that it is called “ $\Delta 4$ ” pathway. The  $\Delta 5$  pathway progresses *via* DHEA and ends with androst-5-ene-3 $\beta$ ,17 $\beta$ -diol. Androst-5-ene-3 $\beta$ ,17 $\beta$ -diol is converted to testosterone by the same enzyme that converts pregnenolone to progesterone. The  $\Delta 4$  pathway features androst-4-ene-3,17-dione, the last immediate precursor of testosterone [3].

The immediate precursors (androst-5-ene-3 $\beta$ ,17 $\beta$ -diol, androst-4-ene-3,17-dione) and DHEA are often known as

prohormones. Additional prohormones of anabolic steroids are 19-norandrostenedione (estr-4-ene-3,17-dione) and the 19-norandrostenediols (estr-4-ene-3 $\beta$ ,17 $\beta$ -diol and estr-5-ene-3 $\beta$ ,17 $\beta$ -diol) as well as androst-1-ene-3,17-dione and androsta-1,4-diene-3,17 dione. The esterification of the 17-hydroxy group is important because it is used as a storage forms and induces a release of testosterone in intramuscular preparations. The esters mainly available in the market are propionate, enanthate, decanoate, undecanoate and cypionate (cyclopentylpropanoate), although acetate, laurate, undecylenate, phenylpropionate, isocaproate and phenylpropionate can also be found. An hydrolysis of these molecules is responsible for the generation of the biologically active hormones. The catabolism of testosterone encompasses the production of metabolites released through the kidney (Fig. 3). The reduction at the 4-5 double bond is responsible for the deactivation of testosterone and initiates its catabolic pathway. The hydrogen at C-5 might be added in positions  $\alpha$  (“below” the molecular plane) or  $\beta$  (“above” the molecular plane), defining two different pathways, promoted by different enzymes which respectively produce the metabolites DHT (5 $\alpha$ -isomer) and 5 $\beta$ -dihydrotestosterone [3].

After the generation of androstenediols, the oxo function in position 3 is reduced. The 3- $\alpha$  isoforms are predominant in humans and the 17- $\beta$  hydroxy group may still be oxidized. This reaction produces 3- $\alpha$ -hydroxy-5 $\alpha$ -androstane-17-one (androsterone) and 3- $\alpha$ -hydroxy-5 $\beta$ -androstane-17-one (etiocholanolone). These two compounds are the most



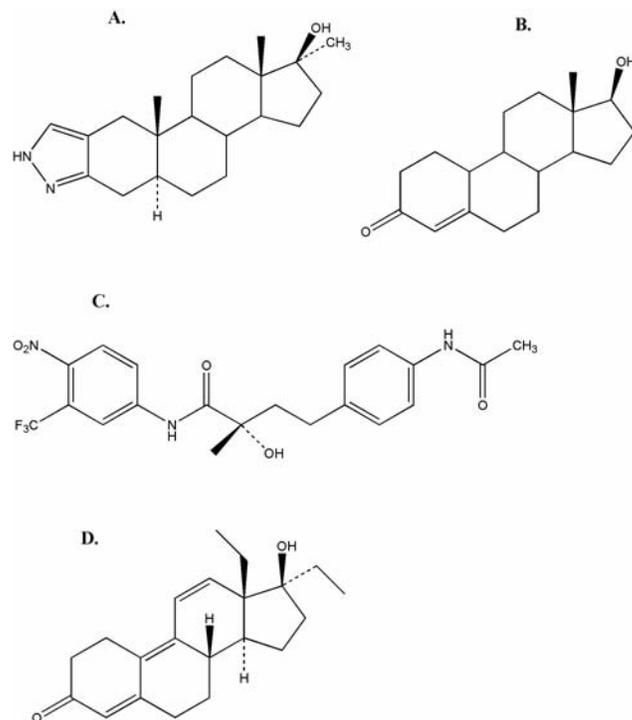


**Fig. (3).** Urinary catabolites of testosterone. The activity of UDPGT produces epitestosterone, while 17 $\beta$ HSD produces androstenedione. The activity of 5 $\alpha$ reductase produces DHT, and 5 $\beta$ reductase 5 $\beta$ dehydrotestosterone.

toxicity, and their administration has also been associated with some for of cancers (e.g., liver and pancreas). Additional structural modifications, including attachment of methyl groups at C-1, C-2, C-6, C-7, or C-11, chlorination or hydroxylation at C-4, C-6 or C-7, introduction of additional double bonds in ring A, B, and/or C, attachment of an additional ring at C-2/C-3, removal of the C-19 methyl group by minimizing aromatization, have all contributed to enhance the stability and potentiating the anabolic properties [2, 3].

The metabolism of synthetic anabolic androgenic steroids generally follows analogous pathways (e.g., 3 and 17 reduction and oxidation, hydrogenation of  $\Delta 4$  steroids, and hydroxylations) as those described for testosterone. Additional double bonds in ring A or B or additional substitutes in position C-4 or C-6 shift the reduction of the  $\Delta 4$  double bond towards 5 $\beta$  orientation [8]. Recent technological and biological advances have allowed the generation of additional molecules, such as the selective androgen receptor modulators (SARMs). These compounds effectively promote anabolic effects in muscle and bone, with very limited activation or occasionally inhibition of reproductive organs such as prostate or seminal vesicles [9]. While DHT induces proliferative extracellular signal-

regulated kinase (ERK) in prostate cells, SARMs activate the antiproliferative p38 mitogen-activated protein kinase (MAPK) [10]. In bone cells, DHT and SARMs both activate the ERK signalling. Moreover, SARMs and DHT recruit the same co-activator complexes in anabolic tissues, but different complexes in androgenic organs [11]. Specific anabolic steroids potentially used for doping purposes are illustrated in Fig. (4). The anabolic androgen steroids are listed in Table 1.



**Fig. (4).** Specific anabolic steroids. **A:** stanozolol; **B:** nandrolone; **C:** andarine; **D:** tetrahydrogestirone.

**Stanozolol**

Stanozolol (17 $\beta$ -hydroxy-17 $\alpha$ -methyl-5 $\alpha$ -androst-2-en-3-one[3,2-c]pyrazole) was originally synthesized from oxymetholone and has been commonly used for doping purposes, since it displays high anabolic properties. The metabolism of stanozolol generates eleven molecules, as revealed by gas chromatography coupled with mass spectrometry (GC-MS) after separation of the urine extract by high pressure liquid chromatography (HPLC). The main metabolites are 3'-hydroxystanozolol, 3'-hydroxy-17-epistanozolol, 4 $\beta$ -hydroxystanozolol and 16 $\beta$ -hydroxystanozolol [12].

**Nandrolone**

Nandrolone (17 $\beta$ -hydroxy-4-en-3-one; also named 19-nortestosterone) is characterized by a metabolism that resembles that of testosterone. The main metabolites are 3 $\alpha$ -hydroxy-5 $\alpha$ -estran-17-one (3-norandrosterone) and 3 $\alpha$ -hydroxy-5 $\beta$ -estran-17-one (2-noretiocholanolone). The hormone, which is naturally produced in humans in small quantities especially during pregnancy, has been synthesized nearly 60 years ago, and it is still widely used as a doping

**Table 1. List of Anabolic Androgenic Steroids. The Metabolism of these Steroids, for which Analytical Methods for Identifying and Measuring Metabolites in Urine have been Developed, is Described in the Reviews of Schänzer [1996] and Parr *et al.*, [2010]**

TRIVIAL NAME	CHEMICAL NAME
BOLASTERONE	7 $\alpha$ ,17 $\alpha$ -dimethyl-17 $\beta$ -hydroxyandrost-4en-3-one
BOLDENONE	1,4-androstadiene-3-one-17 $\beta$ -ol,
CALUSTERONE	7 $\beta$ ,17 $\alpha$ -dimethyl-17 $\beta$ -hydroxyandrost-4en-3-one
METYLTTESTOSTERONE	4-cloro-1,2-dehydro-17 $\alpha$ -methyltestosterone
CLOSTEBOL	4-cloro-17 $\beta$ -hydroxyandrost-4en-3-one
DROSTANOLONE	17 $\beta$ -hydroxy-2 $\alpha$ -methyl-5 $\alpha$ -androstan-3one
ETHYLESTRENOL	19-nor-17 $\alpha$ -pregn-4-en-17-ol
FLUOXYMESTERONE	9-fluoro-11 $\beta$ 17 $\beta$ -dihydroxy-17 $\alpha$ -methylandrost-4-en-3-one
FORMEBOLONE	2-formyl-11 $\alpha$ ,17 $\beta$ -dihydroxy-17 $\alpha$ -methylandrosta-1,4diene-3-one
FURAZABOL	17 $\beta$ -hydroxy-17 $\alpha$ -methyl-5 $\alpha$ -androstanol(2,3-c)-furazan
MADOL	17 $\alpha$ -methyl-5 $\alpha$ -androst-2-en-17 $\beta$ -ol
MESTANOLONE	17 $\beta$ -hydroxy-17 $\alpha$ -methyl-5 $\alpha$ -androstan-3-one
MESTEROLONE	17 $\beta$ -hydroxy-1 $\alpha$ -methyl-5 $\alpha$ -anderostan-3-one
METANDIENONE	17 $\beta$ -hydroxy-17 $\alpha$ -methylandrosta-1,4-dien-3-one
METHENOLONE	17 $\beta$ -hydroxy-1-methyl-5 $\alpha$ -androstan-3-one
METHANDRIOL	17 $\alpha$ -methylandrost-5-en-3 $\beta$ ,17 $\beta$ -diol
METHYLTESTOSTERONE	17 $\beta$ -hydroxy-17 $\alpha$ -methylandrost-44en-3-one
NANDROLONE	17 $\beta$ -hydroxyestr-4-en-3-one
NORBOLETHONE	13-ethyl-17-hydroxy-18,19-dinor-17-pregn-4-en-3-one
NORCLOSTEBOL	4-chloro-17 $\beta$ -hydroxyestr-4-en-3-one
NORETHANDROLONE	17 $\beta$ -hydroxy-17 $\alpha$ -etylestr-4-en-3-one
OXANDROLONE	17 $\beta$ -hydroxy-17 $\alpha$ -methyl-2-oxa-5 $\alpha$ -androstan-3-one
OXYMESTERONE	4,17 $\beta$ -dihydroxy-17 $\alpha$ -methyandrost-4-en-3-one
OXYMETHOLONE	17 $\beta$ -hydroxy-2-hydroxymethylene-17 $\alpha$ -methyl-5 $\alpha$ -androstan-3-one
QUINBOLONE	17 $\beta$ -(1-cyclopenten-1-yloxy)-androsta-1,4-dien-3-one
STANOZOLOL	17 $\beta$ -hydroxy-17 $\alpha$ -methyl-5 $\alpha$ -androst-2-eno[3,2-c]pyrazole
STENBOLONE	17 $\beta$ -hydroxy 2 $\alpha$ -methyl-5 $\alpha$ -androst-1-en-3-one
THG	tetrahydrogestrinone
TRENBOLONE	17 $\beta$ -hydroxyestra-4,9,11-trien-3-one

agent in some sport disciplines. The substitution of a methyl group to the carbon atom at position 19 by an hydrogen atom in the testosterone molecule changes considerably the ratio between anabolic and androgenic activities, increasing the concentration of the former compound. Some esters, such as the 17-propionate and decanoate substitutes, are characterized by a higher activity than the original molecule.

Since estrogen production increases during pregnancy and plasma nandrolone can be detected during gestation [13],

it has been assumed that this hormone is a product of the aromatization process (as might be 19-norandrostenedione, which can be then reduced to nandrolone by 17 $\beta$ HSD) [2]. The concentration of its metabolites in urine are typically comprised between 0.05 and 0.60 ng/mL (i.e., 3-norandrosterone), while 2-noretiocholanolone is generally undetectable. Interestingly, the urine concentration increases by a factor varying between 2 and 4 after physical exercise [14], due to increased of production or increased release

from fat mass where the steroid is stored after intake by diet or dietary supplements [15].

### Andarine

Andarine (also referred to as S-4, S-3-(4-acetylamino-phenoxy)-2-hydroxy-2-methyl-N-(4-nitro-3-trifluoromethyl-phenyl)-propionamide) is a typical SARM. A method based on HPLC interfaced with high-resolution/high-accuracy (tandem) MS has been used to identify phase I and II metabolites of this candidate drug, confirming the predicted target analytes for sports drug testing purposes including the glucuronic acid conjugates of the active drug, its monohydroxylated and/or deacetylated product, the hydrolysis product resulting from the removal of the compound B-ring, as well as the sulphate of the monohydroxylated and the deacetylated phase I metabolite [11].

### Tetrahydrogestrinone (THG, “The Clear”)

The THG [(13S,17S)-13,17-diethyl-17-hydroxy-1,2,6,7,8,13,14,15,16,17-decahydrocyclopenta[a]phenanthren-3-one], produced by drug designers and marketed as a nutritional supplement, has originally been referred to as “*The Clear*”, because of its virtual undetectability with common antidoping testing techniques. It has affinity for both the androgen and the progesterone receptors, but not for the estrogen receptor. The potency of THG to transactivate androgen receptor-dependent reporter gene expression is two orders of magnitude lower as compared with DHT. Moreover, THG exhibits a high binding affinity to all steroid hormone receptors and binds with the highest affinity to the glucocorticoid receptor. THG displays both anabolic and androgenic effects, but the repression of tyrosine aminotransferase in the liver, dependent from glucocorticoids, demonstrates that THG also interferes with the biology of this hormonal family [16]. THG is detected in urine samples by liquid chromatography/tandem mass spectrometry (LC/MS/MS) [17].

### EPIDEMIOLOGY OF ANABOLIC ANDROGENIC STEROIDS USE IN SPORTS

Anabolic-androgenic steroids were first used by athletes in the mid 1950s and although they were commonplace in various sporting events, they only received major attention once their misuse became apparent at the Olympic Games [18]. The most notorious era in the doping history of the Olympic Games was the use of AAS by the athletes of Russia and German Democratic Republic during the decades 1950s to 1980s [19]. The introduction of AAS among the American athletes has been attributed to Dr. John Ziegler (a physician member of the U.S. Weight-Lifting Team), who learned about the use of AAS by the Russian team in 1954 during his trip to weight-lifting championships in Vienna. Upon his return, Dr. Ziegler experimented with testosterone on U.S. weight lifters. This is considered to be the beginning of AAS abuse in sports in the US, which later spread from high-intensity strength-training games to other sports such as field athletics, baseball, swimming, *etc.* [20, 21].

It wasn't however until 1974 that the AAS abuse was prohibited by the International Olympic Committee IOC

(IOC) [22]. Actually, the World Anti-Doping Agency (WADA) publishes a yearly prohibited list of drugs and methods, among which AAS are the most commonly used ergogenic substances [23].

The main reason supporting the use of AAS by the athletes is to increase the mass of the muscles and therefore to boost power performance. Thus, they are highly appealing for weightlifters who seek to lift the maximum amount of weight and field athletes who want to put the shot, or throw the hammer, discus or javelin farther than their competitors. Swimmers and runners seek to perform frequent, high intensity, long duration workouts without physical breakdown. American football players want to increase lean mass and strength, so that they can be successful at the high school, university or professional level.

The survey of the published studies suggests that the AAS use among athletes ranges between 1% and 6% [21]. Unfortunately, the abuse of AAS has penetrated from sports to mainstream population and nowadays most AAS users are not competitive athletes, but simply individuals who want to improve their body image [24-27]. Western cultural developments probably contributed to increase the prevalence AASs use, since media images focused increasingly on male muscularity [28]. A number of survey carried out in the US show that 4% to 6% of high school males use of AAS at some time in their life [29, 30]. Some of these studies also assessed the use of AASs among high school females, reporting that 1% to 2% of the responders used AASs, with a significant increase during the past decade [31]. Other reports estimate that approximately 1 million Americans have used androgens during their life [32].

The use of AAS by adolescents is not limited to the US [31]. Surveys carried out in Canada, Sweden, England, Australia and South Africa have reported overall prevalence rates for high school-aged males and females students ranging between 1% and 3% [33]. For instance, among 687 British college students, 4.4% of males and 1.0% of females currently or previously used AASs. Fifty six percent of them had first used anabolic steroids at the age of 15 or younger [34]. The use of anabolic steroids has further increased in the UK over the past decade as documented by the fact that as many as half of the members of dedicated bodybuilding gyms admitted to take anabolic agents [33]. An investigation among 6,000 Swedish adolescents revealed a prevalence of 3.2% in males, whereas steroids were not used by females. As expected, higher rates of AASs were observed in groups such as visitors of fitness centers, bodybuilders and weightlifters [35-37].

Interestingly, the profile of AASs abusers has notably changed over the past two decades. According to US studies, the typical AASs abuser in the past decade was more frequently a male with a poor self-esteem and school performance and with high levels of antisocial behaviour and self-reported violence and aggression [38,39]. Conversely, a cohort study conducted in 2007 on 1,955 North American male AASs users found that the typical user was a Caucasian 30-year old male, highly educated and gainfully employed

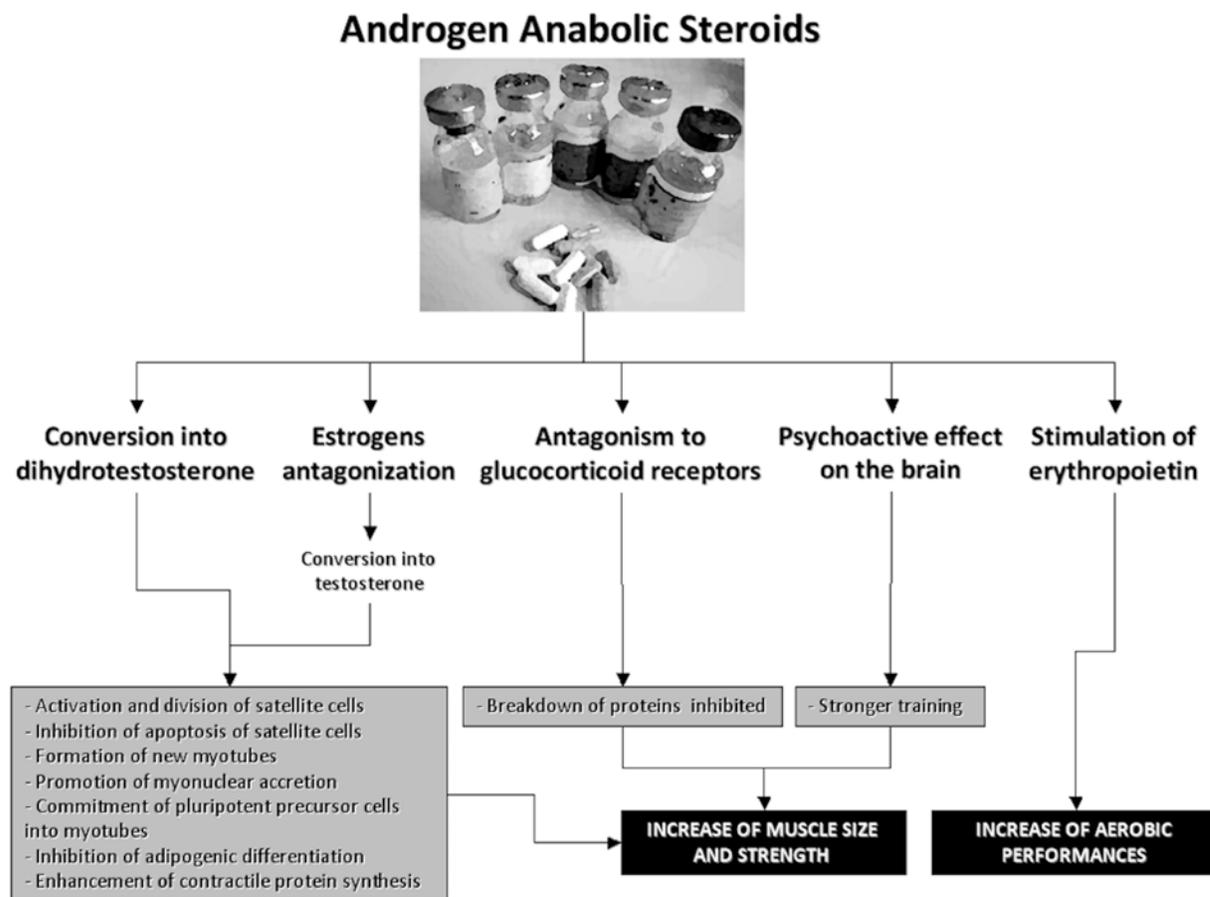


Fig. (5). Postulated mechanisms of action of AASs for increasing the sport performances.

professional who was motivated to use androgens in order to improve his attractiveness [40].

#### ILLICIT ADMINISTRATION OF ANABOLIC ANDROGENIC STEROIDS

Although there are three typical forms of AASs intake (i.e., oral pills, injection, and skin patches), oral administration is by far the most common and convenient. Oral testosterone is rapidly absorbed but it is rapidly converted into inactive metabolites, so that nearly 15% persists in active form. Testosterone derivatives are alkylated at the position 17 (e.g., methyltestosterone and fluoxymesterone) to reduce liver catabolism and ultimately enhance bioavailability. No differential effects for increasing sport performances have however been reported according to the different pattern of administration (Fig. 5).

There are two main aspects that characterize the use of AASs as ergogenic aids, that are dosage and pattern of administration. The therapeutic dosage of androgens (e.g., in subjects with hypogonadism) is typically comprised between 6 to 10 mg/day, which should allow a physiologic replacement of blood levels. Conversely, the dosage used by steroid-abusing athletes largely exceeds the therapeutic regimen, with supraphysiological doses being administered with the aim to reach 40 to 100 times the physiologic blood

levels. Paradoxically, the long-term administration of these supraphysiological doses might produce unexpected and even opposite outcomes (the endocrine system is plastic and incline to achieve a further homeostatic balance by disrupting or down-regulating various pathways, e.g., suppression of endogenous production of follicle stimulating hormone, luteinizing hormone, gonadotropin-releasing hormone), other than being associated with numerous and serious side effects (i.e., azoospermia, hypertension, cardiac arrhythmias, mood swings, cholestatic jaundice, myocardial hypertrophy, gynecomastia, masculinization, tendon weakness/rupture, hypothyroidism, hepatocellular adenoma and carcinoma) [41].

AASs are usually taken by the steroid-abusing athletes in cycles rather than continuously. "Cycling" refers to a pattern of use according to which steroids are taken for periods of weeks or months, after which their administration is suspended for a period of time and then restored. Several different types of steroids are often combined, to maximize their effectiveness, a practice which is typically called "stacking". This latter practice allow athletes to take advantage from the heterogeneous pharmacokinetic and biological properties of the different molecules. As such, the most typical cycle of administration encompasses a period of supraphysiological doses of AASs for 4 to 18 weeks, followed

by a drug-free holiday period varying from 1 up to 12 months. The main scope of the period of suspension is to minimize the side effects, promoting restoration of various endocrine homeostasis and, least but not last, preventing detection during antidoping testing. Occasionally, the AASs dose might be progressively decreased at the end the cycle before complete withdrawal, a practice known as “pyramiding” [41].

## PHYSIOLOGY OF ANABOLIC ANDROGENIC STEROIDS

AASs exert a kaleidoscope of effects on the human biology, some of which may be ultimately beneficial for the athletic performance. Several physiological pathways of action have been described so far, including the 5- $\alpha$ -reductase-mediated conversion into DHT (androstanolone) which ultimately targets a variety of organs (e.g., glands, skin and prostate), the aromatase-mediated conversion into female sex hormones (i.e., estradiol and estrone) which antagonizes the estrogens, a competitive antagonism to the glucocorticoid receptors, the potential stimulation of erythropoietin with an increase in the red blood cell mass and bone formation.

The enzyme 5- $\alpha$ -reductase has a pivotal role in AASs metabolism, in that it converts these hormones into the more active DHT. The anabolic effects of testosterone on the skeletal muscle are mediated through androgen receptor signalling [42]. AASs are mostly fat-soluble hormones, so that they can easily permeate both the plasma and nuclear membranes. Within the cell, both the native AASs and DHT bind to specific cytoplasmatic androgen receptors, producing a steroid-receptor complex which is transported into the nucleus and triggers protein synthesis by specific interaction with DNA and/or RNA. The cellular content of 5- $\alpha$ -reductase varies widely among the target tissues. As such, organs with a high content (e.g., skin, prostate, lungs, brain, fat cells and bone) are more sensitive to androgenic compounds whereas those with a lower content exert a stronger response to anabolic substances [43]. Testosterone binds to androgen receptor and thereby induces a conformational change in androgen receptor protein, causing it to associate with beta-catenin and TCF-4 and activating downstream Wnt target genes. This process promotes myogenic differentiation of multipotent mesenchymal stem cells into satellite cell and contextually inhibits their differentiation into the adipogenic lineage [42]. The outcome of this process encompasses the activation and division of satellite cells, the inhibition of apoptosis of satellite cells, the formation of new myotubes and promotion of myonuclear accretion when existing myonuclei become unable to sustain further enhancement of protein synthesis over a finite volume of cytoplasm (a concept traditionally known as “nuclear domain”). Some so-called “daughter cells” generated from satellite cell proliferation escape differentiation and return to quiescence, helping to restore the satellite cell reserve pool. Testosterone also favours the commitment of pluripotent precursor cells into myotubes and inhibit adipogenic differentiation [44]. The enhanced contractile protein synthesis is an additional mechanism by which testosterone enhances the size of muscle fibres while leaving protein breakdown unchanged. Basically,

testosterone does not modify inward aminoacid transport to muscle but markedly increased re-utilization of intracellular aminoacids.

The enzyme aromatase is also involved in AAS metabolism, although its function seems less important. Basically, aromatase is a member of the cytochrome P450 superfamily, whose function is to aromatize androgens for producing estrogens (estradiol and estrone) by excising the C19 methyl group to convert the C19 androgen structure to a C18 structure with an aromatic A ring. The enzyme is highly expressed in reproductive tissues (e.g., placenta, ovary, testis, breast, uterus, prostate), in non-classical oestrogen target tissues such as the brain, bone and fat, whereas its expression is low in skeletal muscle [45]. As such, the biological basis of blocking the oestrogenic negative feedback supports the hypothesis that drugs acting as oestrogen receptor antagonists (antioestrogen) or aromatase inhibitors - when present in supraphysiological concentration - might efficiently counteract the activity of female sex hormones. The induction of this oestrogen blockage replicates the effects of castration and unleashes a reflex increase in episodic hypothalamic GnRH secretion into the pituitary portal system, a pathway that finally promotes LH release, which in turn stimulates luteinizing hormone (LH)-dependent Leydig cell testosterone biosynthesis and secretion (i.e., the AAS-induced secretion of LH in physiological pulsatile patterns would be sufficient to maintain a modest increase in blood testosterone concentrations). Nevertheless, no reliable evidence has been provided so far that oestrogen blockers such as AAS might cause any significant (indirect) effect on increasing blood testosterone concentration, so that the supposed performance enhancement might be modest (at best) through this pathway [45].

Some evidence also suggests the existence of androgen-receptor-independent pathways, the most important of which is thought to be the competitive antagonism with the glucocorticoid receptors. Glucocorticoids are typically released after various forms of stress, including physical exercise, especially when strenuous and prolonged. Since cortisol possesses a strong catabolic activity on human proteins, the inhibitory properties of AASs against glucocorticoids might therefore limit breakdown of proteins and thereby promote muscle hypertrophy [43].

AASs might exert additional anabolic (indirect) actions, which include a psychoactive effect on the brain (e.g., long-term administration might influence training intensity, predisposing the athletes to exercise more and thereby facilitating the increase in muscle size and strength), glucocorticoid antagonism, and stimulation of the growth hormone (GH) insulin-like growth factor-1 (IGF-1) axis [46].

## OVERVIEW OF THE EFFECTS OF ANABOLIC ANDROGENIC STEROIDS ON SPORTS PERFORMANCES

The effects of anabolic steroids on physical performance are rather unclear since well-controlled and double blind studies have reported discordant, often contradictory

outcomes. Although the earlier package insert of the AAS Dianabol reported unequivocally that "*anabolic steroids do not enhance athletic ability*" [20], the broad knowledge gained so far on this topic attests that these substances might instead exert a significant ergogenic effect. Anabolic steroids are particularly attractive for bodybuilders and weightlifters, but they still can be used by athletes of other sports disciplines where an increase in size and strength of the muscles might be required to increase or optimize power sport performances. As such, the American College of Sports Medicine and the American Academy of Orthopaedic Surgeons (AAOS) have already acknowledged that AASs, especially associated with adequate diet and high-intensity exercise, can contribute to increase muscular size, strength of athletes as well as aggression and motivation, and thereby improve sport performances [47].

Although the mechanism of action of AASs may differ widely among the various molecules due to the different pharmacokinetics and affinity with the androgen receptors (i.e., high-affinity binding such as 19-nortestosterone and metenolone; low-affinity binding such as stanozolol and fluoxymesterone; no binding such as oxymetholone), there is an overall agreement that the administration of these drugs might produce two major effects, that are increase of both strength and bodyweight [43]. Overall, the described effects of AASs on lean body mass (i.e., muscle hypertrophy and development of new muscle fibers) are time- and dose-dependent, whereas lean mass hydration is mostly unaffected. Accordingly, muscle mass and bodyweight typically increases by nearly 5-20% and 2-5 kg from the baseline, respectively. The upper bodily district (i.e., thorax, neck, shoulders and upper arm) seems to be more responsive to AAS due to a larger presence of androgen receptors. The type I muscle fibres of the vastus lateralis and the trapezius muscles are those more increased as a result of long-term AASs administration. Globally the effects of AASs on muscle may persist for up to 6-12 weeks after cessation [43]. Based on the historical researches of Hervey *et al.*, published nearly 30 years ago [48-49], it has been assumed for long that the effects of AAS were limited to experienced strength athletes. Nevertheless, in 1996 Bhasin *et al.*, demonstrated that supraphysiological doses of testosterone (600 mg of testosterone enanthate for 10 weeks) may increase fat-free mass, muscle size and strength also in normal men, especially when combined with strength training [50]. In a following investigation on eugonadal men aged 18-35 years, randomized to receive monthly injections of a long-acting gonadotropin-releasing hormone (GnRH) agonist and weekly injections of 25, 50, 125, 300, or 600 mg of testosterone enanthate for 20 weeks, it was demonstrated that changes in circulating testosterone concentrations were associated with testosterone dose- and concentration-dependent changes in fat-free mass, muscle size, strength and power, according with a single linear dose-response relationship [51].

The same team of authors further demonstrated that the increase in muscle volume is mainly due to muscle fiber hypertrophy, since it is associated with concentration-dependent increases in cross-sectional areas of both type I and type II muscle fibers and myonuclear number [52].

Storer *et al.*, also showed that 600 mg testosterone enanthate administration for 20 weeks in 61 healthy eugonadal young men produced a dose-dependent increase in leg press strength and leg power, although muscle fatigability did not change significantly during the treatment. The observed variation in leg power and press strength were also significantly correlated with total and free testosterone, although specific tension and muscle fatigability remained unmodified. It was hence concluded that the different components of muscle performance might be modulated by different mechanisms in subjects assuming testosterone. It was also observed that the increased muscle strength was not associated with changes in specific tension, or in the amount of force generated per unit volume of muscle [53]. Similar results were obtained by van Marken Lichtenbelt *et al.*, who reported that administration of 200 mg per week intramuscularly injected nandrolone decanoate for 8 wk significantly increased body mass and fat-free mass in experienced male bodybuilders, whereas fat mass, bone mineral content, bone mineral density, and the hydration of the fat-free mass remained unaffected [54].

The effects of AASs on the improvement of field performances have also been intensively investigated for decades, although remarkable differences in study design and outcome measures have contributed to generate rather different, often contradictory outcomes. In 1969 Johnson and O'Shea reported that the strength of subjects treated with 5 milligrams of methandrostenolone (Dianabol) twice daily increased significantly at the end of the study, as did oxygen uptake ability and nitrogen retention by the blood [55]. To assess the effects of AASs on human muscle strength, Elashoff *et al.*, performed a MEDLINE search for the period from January 1966 to April 1990, supplemented by manual searches of previous reviews. From the studies included in the detailed data summary, it was concluded that previously trained athletes showed slightly greater improvements in strength in the AAS-treated group than in the placebo (median difference: 5%; range: 1.2-8.7%). A further meta-analysis of three studies with enough information to compute effect size also showed a mean difference of 1.0% [56]. In a further critical revision of the literature, Kuhn hypothesized that changes in power performance (e.g., weightlifting) in males are typically limited and comprised between 1 and 5%, but they might still be significant, representing the margin of victory for elite athletes. Despite the dramatic evidence of AASs effects on female athletes as a result of the East German sports program in the 1970s and 1980s, it was however concluded that the effects of these substances are still uncertain on women, because no controlled studies could be performed for rather obvious ethical and clinical reasons [57]. In 2007, Rogerson *et al.*, subjected healthy young men to 3.5 mg/kg testosterone enanthate once per week and observed increased muscular strength and cycle sprint performance after 3 to 6 weeks of therapy [58]. Overall, it could be concluded that the maximum benefits on burst and sprinting activities can be achieved when AASs are administered to individuals who have been training and continue to train, who consume a high protein diet throughout the training program, while such benefits are overall modest (<5%) and seem to be limited during

administration and to the narrow period immediately afterwards [59].

As regards endurance performance, although androgen receptors are present in the kidneys and seem responsible for increasing the red blood cell mass by various mechanisms (i.e., stimulation of erythropoietin release, increase of bone marrow activity and iron incorporation into the erythrocytes) [60], definitive information is lacking. Van Zyl *et al.*, originally demonstrated that Long-Evans rats receiving 0.5-mg nandrolone phenylpropionate for 8 weeks had a substantial improvement of running capacity (i.e., they were able to ran 41% longer during the test of submaximal running endurance), as compared with trained rats receiving saline over the same period. Nevertheless, submaximal running endurance was not increased in sedentary rats receiving the anabolic-androgenic steroid and no further increase in maximal sprinting speed was observed after additional 4 weeks of training and treatment with anabolic-androgenic steroid [61]. Nevertheless, it is to mention here that these results could not be further reproduced in both the animal and human model. Liang *et al.*, investigated the effects of nandrolone decanoate and exhaustive endurance exercise (as well as the combination of both treatments) on *in vitro* cardiac contractile function in male Sprague-Dawley rats, concluding that no significant improvement of LV function could be detected at the end of the experiment [62]. To investigate whether concomitant AASs treatment combined with training might enhance training effects, Gayan-Ramirez *et al.*, submitted rats to inspiratory muscle training (IMT) for 8 weeks. During the last 5 weeks of training, trained rats were divided to receive weekly either low-dose (1.5 mg/kg) or high-dose (7.5 mg/kg) nandrolone decanoate or saline. At the end of the experiment, diaphragm muscle mass and contractile properties were unchanged with either treatment [63]. Georgieva and Boyadjiev randomly allocated Wistar rats into a sedentary group and an exercising group training on treadmill for 8 weeks. Half of the trained and half of the sedentary rats received weekly either nandrolone decanoate (10 mg/Kg) or placebo for the last 6 weeks of experiment. The trained rats significantly increased their submaximal running endurance (SRE) compared with the sedentary ones, and those receiving nandrolone decanoate ran 46% longer than trained rats receiving placebo. However, no significant differences could be recorded in maximum oxygen consumption (VO<sub>2</sub>max), running economy (VO<sub>2</sub>submax) and red blood cells parameters between the trained rats receiving nandrolone decanoate and those receiving placebo [64]. Likewise, Tamaki *et al.*, assessed the influence of nandrolone decanoate or placebo in rat hindlimb during 14 days after a single exhaustive bout of weight lifting. The only significant difference was a higher fatigue resistance of a primary plantarflexor muscle in the steroid compared with the control group [65]. Baume *et al.*, evaluated the effect of multiple doses of AASs on different physiological parameters that could indirectly relate the physical state of athletes during a hard endurance training program. In synthesis, three groups of healthy male volunteers from Caucasian origin, aged between 20 and 30 who practiced sports on a regular base with weekly training times ranging within 4 and 12 h, were orally administered placebo, testosterone undecanoate or 19-

norandrostenedione in a double blind settings for 12 times during 1 month. Interestingly, data from exercise testing on submaximal and maximal level did not reveal any significant difference in performance between the three groups, nor on fatigue recovery [66]. Taken together, these data contribute to raise serious doubts that AASs may have a significant influence on endurance capacity.

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