

·Review·

## The physiological and pharmacological basis for the ergogenic effects of androgens in elite sports

Karen Choong, Kishore M. Lakshman, Shalender Bhagat

Boston University School of Medicine, Section of Endocrinology, Diabetes, and Nutrition, Boston Medical Center, Boston, MA 02118, USA

### Abstract

Androgen doping in power sports is undeniably rampant worldwide. There is strong evidence that androgen administration in men increases skeletal muscle mass, maximal voluntary strength and muscle power. However, we do not have good experimental evidence to support the presumption that androgen administration improves physical function or athletic performance. Androgens do not increase specific force or whole body endurance measures. The anabolic effects of testosterone on the skeletal muscle are mediated through androgen receptor signaling. Testosterone promotes myogenic differentiation of multipotent mesenchymal stem cells and inhibits their differentiation into the adipogenic lineage. Testosterone binding to androgen receptor induces a conformational change in androgen receptor protein, causing it to associate with beta-catenin and TCF-4 and activate downstream Wnt target genes thus promoting myogenic differentiation. The adverse effects of androgens among athletes and recreational bodybuilders are under reported and include acne, deleterious changes in the cardiovascular risk factors, including a marked decrease in plasma high-density lipoproteins (HDL) cholesterol level, suppression of spermatogenesis resulting in infertility, increase in liver enzymes, hepatic neoplasms, mood and behavioral disturbances, and long term suppression of the endogenous hypothalamic-pituitary-gonadal axis. Androgens are often used in combination with other drugs which may have serious adverse events of their own. In spite of effective methods for detecting androgen doping, the policies for screening of athletes are highly variable in different countries and organizations and even existing policies are not uniformly enforced. (*Asian J Androl 2008 May; 10: 351–363*)

**Keywords:** testosterone; dihydrotestosterone; muscle mass; mechanisms of androgen action; androgen doping; mesenchymal stem cells; detection of androgen doping

### 1 Introduction

George J. Mitchell, a former US Senator, in a recent report on the use of illegal performance-enhancing drugs in professional baseball, acknowledged pervasive use of androgenic-anabolic steroids by Major League Baseball players in the USA; the list of those linked to steroid use included many well known names in baseball. The keen observers of the professional sports scene, who have been sounding the alarm over the widespread use of ergogenic drugs worldwide—not just in

the USA, and in all professional sports—not just baseball—for the past two decades, were hardly surprised by these revelations. The use of performance enhancing agents in sports is not a new phenomenon; documentation exists of the use of a variety of potions, plants, animal extracts as far back as the original Olympiads in ancient Greece. Long before the isolation and synthesis of testosterone in the 1930s, Brown-Sequard and later Zoth and Pregl had recognized that contents of the testicular extracts could improve physical and mental energy, and muscle strength [1–4]. Shortly after successful synthesis of testosterone, Boje [5] suggested that sex hormones might enhance physical performance. The Germans are alleged to have administered anabolic-androgenic steroids to soldiers going into combat [6]. Although it has been alleged that some German athletes were given testosterone in preparation for the 1936 Berlin

Correspondence to: Prof. Shalender Bhagat, Boston University School of Medicine, Section of Endocrinology, Diabetes, and Nutrition, Boston Medical Center, Boston, MA 02118, USA.  
Tel: +1-617-414-2951 Fax: +1-617-638-8217  
E-mail: Shalender.Bhagat@bmc.org  
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Olympics [6], the most cited account of systematic use of androgens in elite sports is that of the Soviet weight lifting team in the 1952 and 1956 Olympics. In 1954, at the weight lifting championships in Vienna, Dr John Ziegler, a physician associated with the US Weight Lifting Team, learned about the use of androgens by the Russian weight lifters [6, 7]. Ziegler returned to the United States and experimented with testosterone on himself and other weight lifters in the York Babel Club [7]. When Ciba Pharmaceuticals introduced Dianabol (methandrostenolone) in 1958, he began to experiment with this new drug [6, 7]. The use of androgens that was limited initially to strength-intensive sports, spread gradually over the ensuing decades to other sports and to recreational body building [6, 7]. The media lime light surrounding the detection of androgen use by elite athletes such as Ben Johnson, Lyle Alzado, Mark Maguire, Barry Bonds, Floyd Landis, and Marion Jones has only added to the allure of performance enhancing drugs.

Admittedly, the exact prevalence of androgens use by athletes is difficult to determine because the data rely on self-reports and many users understandably do not admit to the use of these drugs. But even surveys based on self-report have found high rates of androgens use among professional athletes and Olympians [7, 8]. Yesalis estimated that approximately one million Americans had used androgens sometime in their lives [9]. Four to six percent of high school boys and one to two percent of high school girls admit to using androgens at least once [10–13]. The androgen use among girls also has increased slightly during the past decade although the overall use rates are substantially lower in women than in men [7, 10].

The abuse of performance-enhancing drugs is not limited to the USA; similar high prevalence rates of androgen use have been reported in surveys conducted in other countries [14, 15]. The most egregious example of state-sponsored anabolic steroid doping was uncovered in the former German Democratic Republic after the fall of the communist government in 1990 [16]; classified documents revealed a secret state program from 1966 to improve national athletic performance using androgens with complicity of the sports medicine physicians.

A recent study of the use of anabolic steroids among US college students found that the overall prevalence of androgen use was 1% or less [17]. The number of students reporting past-year use of androgens increased among men from 1993 (0.36%) to 2001 (0.90%) [17] and has decreased slightly since then [18]. The lifetime and past-year steroid use were associated with being male, participation in intercollegiate athletics, and risky behaviors, including cigarette smoking, illicit drug use, drinking and driving, and alcohol use disorders [17].

One of the most alarming finding of this survey was that approximately 70% of lifetime users of anabolic steroids met criteria for an alcohol use disorder [17]. Thus, college athletes who abuse androgens are at increased risk for other risky health behaviors.

## **2 Patterns of androgen abuse by athletes and recreational body builders**

Nandrolone, testosterone, stanozolol, methandienone, and methenolol are the most frequently abused androgens [19–21]. Intramuscular formulations of androgens are used far more frequently than oral formulations [19]. Combinations of androgens are used more frequently than single agents [19, 21]. Typically, athletes use two or more androgens in progressively increasing doses over a period of several weeks in a practice known as “stacking”. The doses of testosterone or other androgens used by athletes are substantially larger than those prescribed for the treatment of androgen deficiency. In one survey [19], 50% of androgen users reported using at least 500 mg of testosterone weekly or an equivalent dose of another androgen; in another survey [21], almost one fourth of androgen users used 1 000 mg testosterone weekly or an equivalent dose of other androgens. Cycling of androgens refers to the intermittent use of androgens in which weeks of androgen use are followed by periods of drug holiday; this practice is based on the unproven premise that cyclic prevents desensitization to massive doses of androgen.

In addition to the use of androgens, athletes also abuse other drugs to purportedly enhance muscle-building, muscle shaping, or athletic performance [19]. These accessory drugs include stimulants, such as amphetamine, clenbuterol, ephedrine, and thyroxine, other anabolic agents such as growth hormone, IGF-1, and insulin, and drugs perceived to reduce adverse effects such as hCG, aromatase inhibitors or estrogen antagonists [19]. The potential adverse effects of some accessory drugs may be more serious than those of androgens.

## **3 Do androgens improve athletic performance?**

Surprising as it might seem in light of the widespread abuse of androgens, the evidence demonstrating improvements in athletic performance after androgen administration is sparse and weak. There is strong evidence that androgens increase skeletal muscle mass, maximal voluntary strength, and leg power [22–27]; even this assertion was debated rancorously for almost five decades. Much of the controversy stemmed from the well recognized difficulties in conducting placebo-controlled, randomized, masked trials in athletes [27, 28]. It is not surprising that many of the earlier andro-

gen trials were neither randomized nor blinded. Some studies included competitive athletes whose adherence to rigid research protocols is always suspect [27, 28]. The protein and energy intake was not standardized; in some studies, the participants continued to ingest protein supplements *ad lib*. The exercise stimulus was not standardized and, therefore, the effects of resistance exercise could not be separated from those of androgen administration.

However, a growing body of data over the past decade has established that androgens increase muscle mass

[25, 26] and that the androgen-induced gains in skeletal muscle mass and muscle strength are correlated with the administered dose and the circulating concentrations (Figure 1) [23, 24, 29, 30]. Thus, administration of replacement doses of testosterone to healthy, hypogonadal men [31–35] and of supraphysiologic doses to eugonadal men [22, 23] increases lean body mass, muscle size and strength (Figure 2). Systematic reviews of randomized clinical trials have confirmed that testosterone therapy is associated with greater gains in lean body mass and grip strength than placebo in older men with low or low normal testosterone levels [25]. Similarly, test-

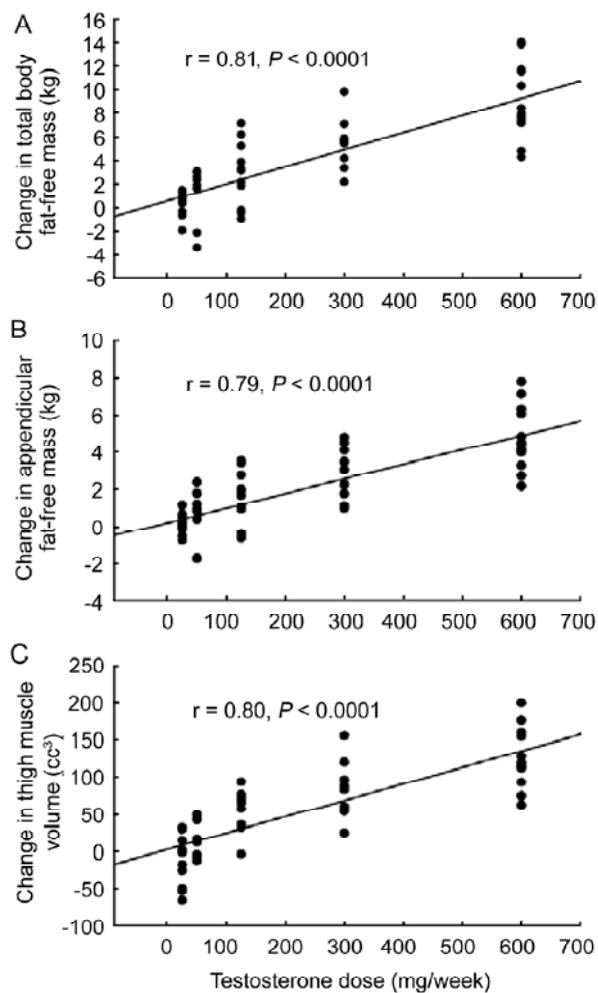


Figure 1. Linear regression of the testosterone enanthate (TE) dose and change in total body fat-free mass (A), appendicular fat-free mass (B) and thigh muscle volume (C). TE dose was strongly and significantly correlated with changes from baseline in total body fat-free mass, appendicular fat-free mass, and thigh muscle volume (Pearson product-moment correlation coefficients of  $r = 0.79$ –0.81,  $P < 0.0001$  for each model), although there was substantial heterogeneity in individual responses to testosterone administration. Reproduced with permission from Woodhouse *et al.* [30].

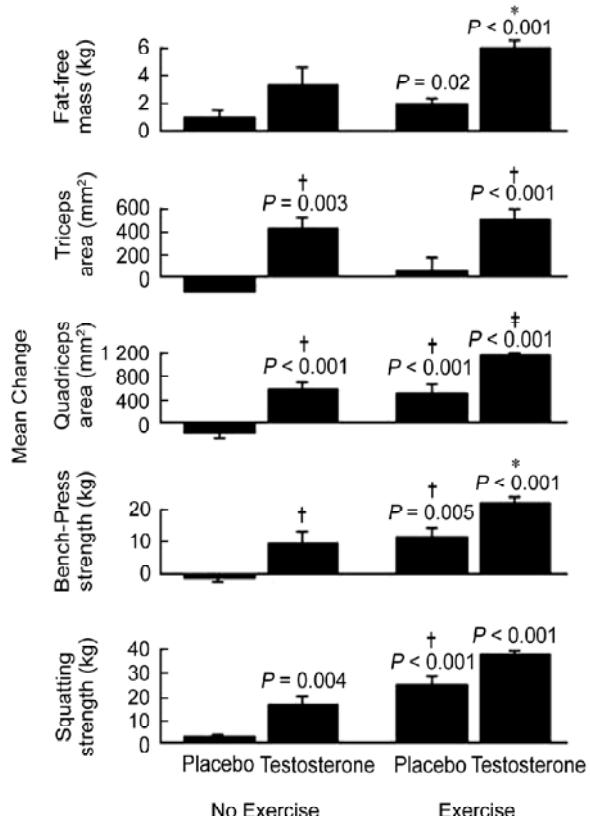


Figure 2. Changes from baseline values in fat-free mass, triceps and quadriceps cross-sectional areas, and 1-repetition strength in the bench-press and squat exercises. Healthy young men were randomly assigned to receive either placebo or 600 mg testosterone enanthate (TE) intramuscularly weekly with or without a standardized program of resistance exercise training. The  $P$  values shown are for the comparison between the change indicated and a change of zero. \* $P < 0.05$  for the comparison between the change indicated and that in either no-exercise group; † $P < 0.05$  for the comparison between the change indicated and that in the group assigned to placebo with no exercise; and ‡ $P < 0.05$  for the comparison against the changes in all three other groups. Reproduced with permission from Bhushan *et al.* [22].

sterone therapy in HIV-infected men with weight loss and in men with chronic obstructive lung disease promotes greater gains in lean body mass and muscle strength than placebo. There is considerable inter-subject variability in the anabolic response to androgen administration. A large part of the variance in anabolic response can be explained by the circulating androgen concentrations; however, polymorphisms in the polyglutamine and polyglycine tract length in androgen receptor protein, testosterone metabolism, and other unknown genetic factors may contribute to this variance [30].

The effects of testosterone on muscle performance are domain specific; testosterone administration improves muscle strength and power, but does not affect specific force or muscle fatigability. The gains in maximal voluntary strength during testosterone administration are highly correlated with increments in muscle mass; testosterone does not improve the contractile property of the skeletal muscle. In contrast, resistance exercise training increases muscle mass as well as specific force. Androgens have not been shown to improve measures of whole body endurance, such as  $\text{VO}_{2\text{max}}$  and lactate threshold.

Based on their demonstrable effects on maximal voluntary muscle strength, androgens would be expected to improve performance in events such as power lifting in which performance is dependent upon muscle strength. Therefore, not surprisingly, high rates of androgen use have been reported among power lifters. Body builders use androgens to increase skeletal muscle mass and decrease fat mass, which provides greater definition to the muscles. However, the use of androgens by athletes participating in endurance events such as long distance running and bicycling is not easily comprehensible because androgens have not been shown to improve endurance measures [36, 37]. It is possible that the androgen-induced increments in hemoglobin may improve the oxygen carrying capacity of blood [38]. The speculation that androgens might allow the athletes to train harder by improving the regenerative response of the skeletal muscle to injury has not been tested rigorously. Others have suggested that androgens might increase “motivation” and “aggression”, which may be advantageous in competitive sports.

The widespread use of androgens by baseball players and sprint runners also is not easily explained by the available data on the effects of androgens. The ability to hit a home run against a ball traveling at speeds approaching 100 mph requires extraordinary degree of hand-eye coordination—the ability to locate the ball in a specific coordinate of space and to place the bat in that precise coordinate with considerable strength and power. There is some evidence that androgens decrease

reaction time by improving neuromuscular transmission [39, 40]. Improved reaction time in conjunction with increased strength and power could potentially explain the perceived improvements in athletic performance by baseball players, although the evidence to support these hypotheses is lacking.

The use of androgens by legendary sprinters like Ben Johnson is even more difficult to explain. Among sprint runners, androgen-induced gains in body weight might potentially increase the amount of work done in carrying that body weight against gravity and resistance across the race track. Thus weight gain might be viewed as potentially deleterious to performance. The improved reaction time, the psychological edge gained because of the motivational effects of androgens, and the ability to train harder, have been cited as possible explanations without verifiable evidence.

#### 4 Mechanisms of anabolic effects of androgens

Testosterone-induced increase in skeletal muscle mass is associated with dose-dependent increase in cross-sectional area of both type I and type II muscle fibers [41]. Testosterone administration does not affect the absolute number or the relative proportion of type I and type II muscle fibers [41]. Testosterone administration increases the numbers of myonuclei and satellite cells [42], muscle progenitor cells that reside in a unique niche adjacent to the muscle fiber. Androgen receptors are expressed in the satellite cells and other stem-like cells in the interstitium of the skeletal muscle fibers and in some myonuclei of the myofibers [43]. A growing body of evidence supports the hypothesis that androgens promote the differentiation of mesenchymal multipotent stem cells into the myogenic lineage and inhibit their differentiation into the adipogenic lineage [44–46]. Thus, in cultures of mesenchymal multipotent C3H10T1/2 cells, androgens upregulate markers of myogenic differentiation, such as MyoD and myosin heavy chain II and downregulate markers of adipogenic differentiation, such as PPAR-gamma and C/EBP-alpha [46]. These effects of testosterone and DHT on myogenesis are mediated through the classical androgen receptor-mediated signaling and are blocked by bicalutamide, an androgen receptor antagonist [46].

Upon binding to its cognate ligand, androgen receptor undergoes conformational change and associates with its co-activator beta-catenin [45]. The androgen receptor-beta catenin complex moves into the nucleus, forms a complex with LEF/TCF-4, and activates a number of Wnt target genes, including follistatin [45]. The signal from androgen receptor is cross-communicated to the TGF-beta pathway through beta-catenin and TCF-4. Beta-catenin and follistatin play an

essential role in mediating the effects of testosterone on myogenic differentiation [45].

Testosterone also has been reported to promote satellite cell entry into the cell cycle [47–51]. Additionally, testosterone and DHT inhibit the differentiation of preadipocytes into adipocytes [45]. Androgens also stimulate fractional muscle protein synthesis and to increase the efficiency of reutilization of amino acids by the skeletal muscle [32, 52–54]. The effects of testosterone on muscle protein degradation need further investigation.

## 5 Potential adverse effects of androgen use

Because of the variability in the dose, frequency, duration, and the type of drugs used, systematic investigations of the adverse effects of androgens in athletes and recreational body builders have been difficult to conduct. These analyses are further complicated by the concurrent use of accessory drugs. The low frequency of serious adverse effects reported with androgen use is surprising; it is likely that the adverse effects are under-reported. Furthermore, the accuracy of self-reported drug use is difficult to verify.

Adverse events associated with androgen use include deleterious changes in the cardiovascular risk factors, including a marked decrease in plasma high-density lipoproteins (HDL) cholesterol level [55] and changes in clotting factors [56], suppression of spermatogenesis resulting in infertility, increase in liver enzymes, hepatic neoplasms, and mood and behavioral disturbances [57–63]. Elevations of liver enzymes, hepatic neoplasms, and peliosis hepatic and even hepatic rupture have been reported with the use of oral, 17-alpha alkylated androgens [57, 58, 64], but not with parenterally administered testosterone or its esters [65]. Acne and premature hair loss can occur with androgen use. Women using large dose of androgens are at risk for menstrual irregularities, infertility, and virilizing side effects, including hirsutism, deepening of voice, changes in body habitus, and clitoral enlargement; some of these virilizing adverse effects may be irreversible.

A number of deaths due to unexpected coronary and cerebrovascular thrombotic events among androgen users have been reported [66–68], but these reports are largely anecdotal and do not establish a cause and effect relationship. In a stunning report that has received surprisingly little attention, Finnish world class power lifters suspected of AAS intake during their sports career experienced five times higher mortality than age-matched controls [69]. The findings of this small study need further confirmation. The changes in plasma lipids vary depending on the dose, the route of administration (oral or parenteral), and whether the androgen is

aromatizable or not. Thus, orally administered, 17-alpha-alkylated, nonaromatizable androgens produce greater reductions in plasma HDL cholesterol levels than parenterally administered testosterone. Orally-administered, 17-alpha alkylated androgens also have been associated with insulin resistance and glucose intolerance [70]. Androgen use has been associated with increases in hematocrit, homocysteine levels, blood pressure and peripheral arterial resistance, and left ventricular hypertrophy and diastolic dysfunction [71–81]. However, it is not clear whether myocardial hypertrophy reported in power lifters is the result of resistance exercise or androgen use. In a cross-sectional investigation [82], power athletes who had used androgens showed subclinical impairment of both systolic and diastolic myocardial function that was correlated with the dosage and duration of androgen use. Also, one controlled trial in healthy volunteers [83] and other uncontrolled, open-label studies in weight lifters, have not found significant changes in left ventricular mass or function with androgen use [84]. The long term effects of androgen abuse on the risk of prostate and cardiovascular disease are unknown.

The anecdotal reports of “roid rage” among androgen users have received much attention in lay press. However, in placebo-controlled trials, testosterone administration has not been associated with a statistically significant increase in anger scores or measures of aggressive behaviors [63, 85–90]. It is possible that the self-reporting questionnaires lacked the sensitivity to detect small but significant changes in aggression. In controlled trials, a small number of subjects have demonstrated marked increases in aggression measures with the use of supraphysiologic doses of testosterone, while a majority of participants show little or no change, leading to speculation that high doses of androgens might provoke rage reactions in a subset of individuals with pre-existing psychopathology. Kouri *et al.* [88] reported that administration of supraphysiologic doses (600 mg weekly) of testosterone enanthate to healthy, young men was associated with a significant increase in aggressive responses to provocation than placebo administration. Testosterone doses that approximated the replacement doses or were slightly above the replacement dose did not produce significant changes in aggressive response in this experimental setting [88].

A wide range of psychiatric side effects, including increased aggression and hostility, and mood disturbances (e.g. depression, hypomania, and psychosis) have been reported among androgen users [91]. Dependence and withdrawal effects (such as depression) occur in a small number of steroid users. Dissatisfaction with the body and low self-esteem is common among androgen users and may predispose these individuals to

the abuse of muscle building drugs [91]. Both increased and decreased sexual desire and function have been reported [61].

Breast tenderness and breast enlargement (“bitch tits” in street parlance) are frequently associated with the use of aromatizable androgens. It is not uncommon for athletes to use an aromatase inhibitor or an estrogen antagonist in combination with androgens to prevent breast enlargement.

The long-term suppression of the hypothalamic-pituitary-testicular axis with its attendant risk of dependence and continued use of androgens is a serious complication of androgen use that has not been widely appreciated. Androgen administration suppresses endogenous testosterone and sperm production by suppressing the hypothalamic-pituitary-testicular axis [92, 93]. Men using androgens may experience subfertility or infertility [94]. The recovery of the hypothalamic-pituitary axis after discontinuation of the exogenous androgen, may take weeks to months, depending on the dose and duration of prior androgen use [95–98]. During the period immediately after discontinuation of androgen use when circulating testosterone levels are low, the users experience symptoms of androgen deficiency, including loss of sexual desire and function, lack of energy, depressed mood, and hot flushes. Some patients may find these withdrawal symptoms difficult to tolerate and may revert back to using androgens, thus perpetuating the vicious cycle of abuse, withdrawal symptoms, and dependence [96–98]. Others may resort to off-label use of aromatase inhibitors or hCG obtained illicitly based on the presumption that these agents accelerate the recovery of the hypothalamic-pituitary-testicular axis, although there is no evidence to support this premise and it is possible that the use of hCG may delay the ultimate recovery of the hypothalamic-pituitary-gonadal axis.

Self administration of intramuscular injections increases the risk of infection, muscle abscess, and even sepsis [20]. Transmission of HIV infection has been reported among anabolic steroid users presumably because of needle sharing or the use of improperly sterilized needles and syringes.

Excessive muscle hypertrophy without commensurate adaptations in the associated tendons and connective tissues may predispose athletes using androgens to the risk of tendon injury and rupture and unusual stress on joints [99].

A vast majority of androgen users also abuse additional drugs [19]. Some of these additional drugs of abuse, such as cocaine, amphetamine, and ephedra may be associated with potentially serious complications.

## **6 Detection of illicit androgen use**

Thirty four laboratories around the world have been accredited by the International Olympic Committee to perform doping tests. Traditional radioimmunoassay techniques were used initially to detect androgens in the urine specimens. However, since 1981, the accredited laboratories have used either gas chromatography-mass spectrometry (GC-MS) or in some instances liquid chromatography mass spectrometry (LC-MS) to detect androgen or their metabolites that show poor gas chromatographic properties or are temperature labile [100]. Also, during the past ten years, the introduction of the high resolution mass spectrometry (HRMS) and tandem mass spectrometry (MS/MS) has further improved the sensitivity of androgen steroid detection techniques. Derivatization of samples is often used to improve the sensitivity of the gas chromatography [101]. Thus, silylation reaction converts the polar groups such as hydroxyl and keto groups to less polar trimethylsilyl ethers and improves the signal to noise ratio [101].

For detection of testosterone abuse, the analysis of testosterone to epitestosterone ratio in conjunction with isotope ratio combustion mass spectrometry is used [102–109]. Urinary testosterone to epitestosterone ratio typically is less than 6 and is constant in any individual. There are genetic differences in testosterone to epitestosterone ratio. Administration of exogenous testosterone increases the urinary excretion of testosterone glucoronide and increases the testosterone to epitestosterone ratio. Testosterone to epitestosterone ratio greater than 4 is viewed suspiciously. Ratios greater than 4 need evaluation of previous urine samples or additional urine samples obtained after a time interval. If the high ratio is due to genetic variation, then all samples obtained from the subject would show the high ratio. A high testosterone to epitestosterone ratio that is higher than that observed in previous samples is viewed as a positive test.

If the results of the testosterone to epitestosterone ratio test are abnormal and suggest exogenous testosterone use, then additional confirmation by using gas chromatography combustion isotope ratio mass spectrometry is required [101, 104]. This method is based on the measurement of  $^{13}\text{C}/^{12}\text{C}$  isotope ratio in testosterone. In nature, 1.1% of carbon exists as  $^{13}\text{C}$ . Synthetic androgens are synthesized from plant sterols diosgenin and stigmasterol that have less  $^{13}\text{C}$  than their endogenous homologs. Therefore, synthetic testosterone, in a manner similar to other synthetic organic compounds, has lower  $^{13}\text{C}$  to  $^{12}\text{C}$  ratio than a reference gas standard. During the course of the GC combustion isotope ratio mass spectrometry, the steroids are separated by gas chromatography and oxidized to carbon dioxide in a combustion chamber. The ratio of

$^{13}\text{CO}_2$  (m/e 45) and  $^{12}\text{CO}_2$  (m/e 44) is monitored in an isotope ratio mass spectrometer, and the  $\delta$  value is calculated ( $\delta$  value refers to the decrease in  $^{13}\text{C}$  relative to the reference gas with a standardized  $^{13}\text{C}$  to  $^{12}\text{C}$  ratio) [110]. A negative  $\delta$  value along with a high testosterone to epitestosterone ratio suggests exogenous testosterone administration.

The procedures for the collection and transportation of samples for doping tests follow strict rules that have been established by the individual sports organizations [101]. Typically, each urine sample, collected under direct visual oversight of an accredited supervisor, is divided into two parts (A and B samples) and transported to the testing laboratory using strict “chain of custody” procedures. If A sample is deemed positive, then B sample is analyzed in the presence of the athlete or an authorized representative of the athlete. If B sample is also positive, then doping with an androgen is confirmed, and the sports organization can impose punitive sanctions [101].

Some controversy has erupted recently over the large number of positive tests for nandrolone. Small quantities of nandrolone, 17beta-hydroxy-19-nor-4-androsten-3-one, and its metabolite 19-norandrosterone, are excreted in the urine naturally in men. The International Olympic Committee has established a threshold level of 2 ng/mL for 19-norandrosterone. Levels higher than this threshold have been reported in some individuals eating a high meat diet in conjunction with intense resistance exercise [111] and in individuals ingesting dietary supplements such as delta4-androstenedione [112].

## 7 The abuse of androgen precursors and designer androgens

### 7.1 $\delta$ -4-androstenedione

$\delta$ -4-Androstenedione is a precursor of testosterone that is converted by the enzyme 17beta hydroxy-steroid dehydrogenase to testosterone. Androstenedione witnessed a brief period of rapid growth in sales following Mark McGuire's admission of its use during an extraordinary season replete with 68 home runs. Under the Dietary Supplement Health and Education Act passed by the US Congress, for many years, androstenedione was sold over the counter as a dietary supplement [113–114]. Unlike other androgens, whose sales were regulated within the dictates of Anabolic Steroid Control Act, androstenedione's sales had not been subject to regulatory oversight of Food and Drug Administration and Drug Enforcement Agency. However, the US Congress recently added androstenedione to the list of banned anabolic steroids and it is no longer sold over the counter.

Administration of 100 mg androstenedione orally daily is associated with little or no change in circulating testosterone concentrations, while administration of 300-mg dose produces only modest increments in testosterone area-under-the-curve. However, Jasuja *et al.* [115] demonstrated that 500-mg androstenedione administered thrice daily for 12-weeks to hypogonadal men increased serum testosterone and free testosterone concentrations into the eugonal range, and increased fat-free mass and muscle strength. Similarly, in women, administration of 100-mg androstenedione significantly increased serum testosterone concentrations above the physiologic range for women [116]. In female hyenas and several other mammalian species, circulating concentrations of androstenedione are higher than those in male members of these species and are associated with virilization of external genitalia and increased aggression [117]. Jasuja *et al.* [115] demonstrated that androstenedione binds androgen receptor albeit with a substantially lower binding affinity than testosterone, and that it promotes myogenic differentiation in a mesenchymal, multipotent cell line. Thus, androstenedione meets all the criteria for an anabolic steroid: it has structural resemblance to testosterone, it binds androgen receptor, and it promotes myogenic differentiation in vitro and when administered in sufficiently high doses, it increases muscle mass [115]. Based on these data, the US Congress recently classified androstenedione as an anabolic steroid and banned its over the counter sales.

Androstenedione administration produces substantial increments in serum estradiol and estrone concentrations [116, 118–121]. Most of the orally administered androstenedione is inactivated during its presystemic metabolism as indicated by a marked increase in its urinary metabolites, including testosterone glucuronide with only a small increase in serum testosterone [122].

The over the counter preparations of androstenedione have not been subject to the rigorous quality control required of the FDA-approved pharmaceuticals [123, 124]. Substantial variability has been observed in androstenedione content of different preparations and among different batches from the same manufacturer [112, 125]. Some batches of over the counter androstenedione have been found to contain one or more banned androgens such as nandrolone; thus, ingestion of androstenedione may result in the doping tests becoming positive [112].

### 7.2 Potential adverse effects of androstenedione

The long-term side effects of androstenedione use are unknown. Short term administration of androstenedione is associated with a significant increase in

estradiol levels. The long-term consequences of the marked increase in estrogen levels in men taking androstenedione are unknown. In men, the increases in serum estrogen concentrations may potentially affect semen quality, increase inflammatory markers, cause gynecomastia, and induce epigenetic and cytogenetic effects on sperm.

The supplementation of androstenedione decreases HDL levels and increases low-density lipoprotein (LDL)/HDL ratio. Other adverse effects of androstenedione stem from the potential increase in testosterone levels. These may include adverse effects on plasma lipids, erythrocytosis, acne, sleep apnea, and increased risk of detecting prostate events.

Given the lack of efficacy data and total absence of long term safety data, the use of androstenedione is not clinically recommended for any indication, including the treatment of androgen deficiency in men or women.

### *7.3 Dehydroepiandrosterone (DHEA)*

DHEA is a weak androgen by itself, but it is converted in peripheral tissues to testosterone and estradiol. In addition to being a weak androgen and an androgen precursor, DHEA has been shown to function as a neurosteroid [126].

DHEA binds androgen receptor with a binding affinity that is substantially lower than that of dihydrotestosterone (DHT). A separate G-protein coupled membrane receptor for DHEA has been proposed [127]; however, the existence of such a DHEA-specific membrane receptor has not been confirmed. DHEA also has been shown to modulate the activities of N-methyl-D-aspartate (NMDA) and  $\gamma$ -amino-butyric acid (GABA) receptors [128].

The literature on DHEA is difficult to interpret. DHEA studies in rodents have limited applicability to humans, because rodents have very little endogenous circulating DHEA. Many DHEA studies reporting beneficial neurotropic and anti-cancer effects, and immune enhancement were conducted in rodents.

The human trials of DHEA have been characterized by heterogeneity of doses, formulations, and study populations. DHEA studies have been conducted in patients with adrenal insufficiency [129–133], older men and women [134–139], peri- and post-menopausal women [140, 141], and in patients with autoimmune disease [142–144]. These trials used doses as high as 1500 mg daily and as low as 25 mg daily. Most human trials used 50 mg DHEA daily for three to six months, included small samples, and were of relatively short durations.

A Cochrane review of DHEA trials concluded that there was insufficient evidence of beneficial effect of DHEA on cognition in older men and women [138, 139].

One randomized trial has reported greater improvements in bone mineral density in older men and women receiving 50 mg DHEA daily than with placebo [145].

DHEA trials in women with adrenal insufficiency have yielded inconsistent results. Arlt *et al.* [129] used a double-blind, placebo-controlled, crossover study design in women with primary or secondary adrenal insufficiency who received either placebo or 50 mg DHEA daily for 16 weeks each. DHEA administration was associated with improvements in scores for depression and anxiety, sexual function, and circulating osteocalcin levels, but no significant changes in body composition [122]. Other trials of DHEA supplementation in women with adrenal insufficiency failed to confirm the beneficial effects of DHEA on mood, well being or sexual function that were observed in the Arlt study [130–132]. DHEA has not been shown to consistently improve body composition, physical function, or insulin sensitivity. The effects of DHEA administration on cardiovascular event rates or cancer incidence rates are unknown.

In a placebo-controlled trial that used pharmacological doses of DHEA (200 mg daily), modest improvements in lupus outcomes and a greater reduction in disease flares and disease activity were reported in patients receiving DHEA than in those receiving placebo [142–144]. The effects of DHEA on bone mineral density in patients with SLE have been inconsistent.

Thus, the efficacy of DHEA has not been demonstrated in any disease state and DHEA use cannot be recommended for any clinical indication at present.

### *7.4 Other androgen precursors and designer steroids*

Precursors of testosterone (4-androstenediol and 5-androstenediol in addition to 4-androstenedione and DHEA discussed above), dihydrotestosterone (5-alpha-androstan-3beta-17 beta-diol, 5-alpha-androstan-3 alpha, 17 beta diol, 5-alpha-androstan-3, 17 dione, 5-alpha-androst-1-ene-3, 17 dione, 17 beta-hydroxy-5-alpha-androst-1-en-3-one, 5-alpha-androst, 1-ene, 17 beta-diol) or nortestosterone (4-norandrostenedione, 4-norandrostenediol, and 5-norandrostenediol) [101] that are weakly androgenic by themselves, but that are converted in the body to potent androgens, have become available on the internet. Even a precursor (androsta-1,4-diene-3, 17-dione) of boldenone (17-beta-hydroxyandrosta-1, 4-dien-3-one) has been introduced [101].

The androgens abused by athletes had been synthesized initially for medicinal or veterinary indications. However, recent years have witnessed the appearance of designer steroids, such as tetrahydrogestrinone (THG) [146, 147] and madol [148] that were developed solely for abuse [149]. The detection of these novel androgens has proven challenging to the testing laboratories because detection methods have not been stan-

dardized for these new designer androgens. These designer compounds have not undergone any formal toxicological or safety testing in animals or humans; consequently, their growing use by athletes poses significant health concerns. The government agencies have found themselves stymied in their efforts to regulate this underground marketplace of designer steroids because there are no published data with the use of these novel designer steroids, and generating new data of their androgenic and anabolic efficacy that would withstand scientific and legal scrutiny is a time consuming and laborious task.

## 8 Conclusion

The abuse of androgens by athletes and recreational body builders is wide spread and worldwide. Androgens increase skeletal muscle mass through their effects on mesenchymal stem cell differentiation through an androgen receptor-mediated mechanism. In spite of significant improvement in detection methods, the problem of doping in sports is unlikely to disappear anytime soon because of societal values and economic incentives that emphasize winning at all costs and because of the lack of will on the part of governments throughout the world to enforce stricter screening and penalties.

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