Chapter 8

Anabolic agents

Neil Chester
(Orcid.org/0000-0001-8791-5040)

Abstract

Anabolic agents are a class of drugs on the World Anti-Doping Agency (WADA) Prohibited List, which comprise largely of drugs derived from the endogenous hormone, testosterone, known as anabolic androgenic steroids (AAS). These drugs are synonymous with doping and remain the most commonly detected class of drugs according to statistics produced by WADA-accredited laboratories. This chapter provides an overview of the pharmacology and side effects of AAS including evidence relating to their reputed mechanisms of action as performance and image-enhancing drugs. Landmark events involving the use of anabolic agents are highlighted, as well as new, emerging anabolic agents such as selective androgen receptor modulators (SARMs).

Chapter Contents

8.1 Introduction

Anabolic agents are a classification of substances on the WADA Prohibited List comprising of largely anabolic androgenic steroids (AAS). These substances have become synonymous with the term doping and remain one of the most prevalent groups of performance-enhancing drugs in sport. In addition, they are widely used by those engaged in sport and exercise at a recreational level due to their image-enhancing properties.

Despite the fact that these substances were first developed over 70 years ago, their efficacy is such that they remain a popular choice as a doping agent. Whilst research over recent decades has confirmed the anecdotal evidence and belief that many users held in terms of their efficacy, there remains some conjecture regarding their purported side effects. Clearly, research conducted to mirror their use by athletes and recreational users alike is difficult as a consequence of the extremely high, supra-physiological doses administered in practice. However, the availability of sub-standard preparations via the internet has led to much wider public health concerns.

Whilst the methods of detection for such substances have advanced throughout the years, athletes have attempted to circumvent such methods by turning to 'designer steroids'. In addition, the use of endogenous ASS, such as testosterone, androstenedione and dehydroepiandrosterone (DHEA) pose their own specific problems with regards to detection as a consequence of their natural occurrence in the body.

As therapeutic agents AAS use is limited but tends to centre on their anabolic properties. Indeed, potential clinical uses include combating cachexia associated with post-operative recovery and conditions such as HIV/AIDS, renal failure, chronic obstructive pulmonary disease (COPD) and burns. In addition, AAS have been used to treat osteoporosis in postmenopausal women and to treat aplastic anaemia.

Under the classification of anabolic agents on the Prohibited List, in addition to AAS, there are β_2-agonists, including clenbuterol and zilpaterol, selective androgen receptor modulators (SARMs) and tibolone and zeranol (Table 8.1). The SARMs are a class of pharmacological agents, which currently remain under development and offer the advantage over AAS in being selective to specific tissue types.

As well as the Prohibited List, WADA manage a Monitoring Program with the purpose of assessing possible misuse of various substances. Since 2020 ecdysterone has been included on the Monitoring Program and classified as an anabolic agent. Ecdysterone, is a naturally occurring steroid sourced from plants and insects and is found in various supplements used to promote muscle growth.

[Insert Table 8.1 here]

8.2 Anabolic androgenic steroids

Anabolic androgenic steroids are typically defined as compounds structurally and functionally related to the hormone testosterone. They may be categorised according to whether they exist endogenously or are synthetically derived.

Whilst testosterone was first isolated in 1935 it was soon realised that when administered orally or parentally it was broken down rapidly by the liver by first pass metabolism. This would clearly hamper its use as a therapeutic agent and consequently there was a determination to develop an exogenous AAS that could resist first pass metabolism.

Testosterone

Testosterone is the primary male androgenic hormone, responsible for the control of a wide range of processes, most notably the development and maintenance of male characteristics. These characteristics include those that develop during puberty, such as growth of the penis and testes and the development of secondary sex characteristics, such as body hair growth particularly that of facial, pubic and axillary hair and deepening of the voice, as a consequence of vocal cord thickening and enlargement of the larynx. Testosterone is produced largely in the testes by the Leydig cells (95%) but also by the adrenal glands and via conversion from weaker androgens such as androstenedione (also produced by the adrenal glands) in the periphery. It is also present in females and is produced in much smaller amounts by the ovaries, the adrenal glands and via the conversion of androstenedione produced by the adrenal glands. After puberty, plasma testosterone levels are approximately 250 to 1045 $ng.dl^{-1}$ in males and 1 to 44 $ng.dl^{-1}$ in women with levels declining steadily in males over the age of 60 years

(Salameh *et al*., 2010). Although the normal adult female and male testosterone ranges differ markedly, with a bimodal distribution, the level may differ in individuals with disorders of sex development (DSD); a factor that should be considered in discussion of female competition eligibility (Clark *et al.,* 2019; see also Chapter 27). As with males, testosterone has an important role in the development and maintenance of lean body mass (i.e. muscle and bone) in females. Testosterone also affects the central nervous system (CNS) and has a significant role to play in human behaviour, particularly sexual behaviour, namely libido.

Whilst testosterone is considered the primary androgen, it is by no means the only one, with a number of androgens serving important roles in both the development and maintenance of sex determining characteristics. Several weak androgens are synthesised by the adrenal cortex, most notably dehydroepiandrosterone (DHEA) and androstenedione (Andro). In females these act as an important circulating pool of more potent androgen precursors and their conversion is via several intracellular enzymes (Figure 8.1). After testosterone, 5α-dihydrotestosterone (DHT) is probably the most notable androgen and with a greater affinity to the androgen receptor (AR) it has a direct role is in the development of secondary sex characteristics. Indeed, testosterone typically acts as a precursor to DHT in reproductive tissue and the skin where the presence of 5α-reductase mediates its conversion, promoting prostate gland and hair growth respectively.

The presence of particular androgens at a cellular level is determined by enzyme action. In addition to the intracellular enzyme, 5α-reductase in the skin and reproductive tissue the presence of aromatase converts testosterone into oestradiol (Figure 8.1) in adipose tissue. In skeletal muscle however, 5α-reductase is undetectable (Thigpen et al., 1993) and therefore testosterone, as opposed to DHT exerts its effect directly via ARs. Thus, not only is the presence of ARs important in determining the effects of androgens, but so too is the expression of specific enzymes.

The immediate precursor of testosterone in the biosynthetic sequence is androstenedione, which is converted to testosterone via the action of 17β hydroxysteroid dehydrogenase (Figure 8.1). This enzyme also acts on other steroids with similar structures, including 19-norandrostenedione, to form 19-nor testosterone otherwise known as nandrolone (Figure 8.1).

Testosterone production is controlled by the hypothalamic-pituitary-gonadal axis. Specifically, testosterone is produced in response to luteinising hormone (LH) and follicle-stimulating hormone (FSH) secreted by the pituitary gland. Gonadotropin-releasing hormone (GnRH) produced by the hypothalamus stimulates the release of LH and FSH from the pituitary gland. This integrated system

enables regulatory feedback control, whereby increased levels of circulating testosterone inhibit the release of GnRH, LH and FSH. Androgen production from the adrenal gland is however less clear. Control, nevertheless involves adrenocorticotropin (ACTH) as well as other factors including gonadal sex steroids, insulin, growth hormone and other signalling molecules (Alesci et al., 2001).

Testosterone circulates in the bloodstream either freely (approximately 2 to 3%) or bound to proteins such as sex hormone-binding globulin (SHBG) or albumin. Testosterone bound to SHBG (approximately 44%; Dunn *et al*., 1981) is essentially inactive due to the high binding affinity of these molecules and the fact that in this state testosterone is unable to penetrate the cell walls of target cells. However, free testosterone and that which is weakly bound to albumin is deemed to be bioavailable since in its free form it may penetrate the cell wall and bind with the AR.

[Insert Figure 8.1 here]

8.3 Pharmacology of anabolic androgenic steroids

The structural modifications of testosterone to create synthetic AAS have been introduced in order to achieve one or more of the following: maximise anabolic effects; minimise androgenic effects; increase metabolic half-life; limit first pass metabolism; and the reduction of absorption rate from an intramuscular depot. Clearly, such modifications aim to improve the efficacy of testosterone as an anabolic agent and optimise its delivery according to the route of administration. Whilst the anabolic potency, compared to testosterone may have been enhanced in some AAS, the disassociation from androgenic effects has not been achieved and therefore as performance and image enhancing drugs (PIED) they all carry potential androgenic side-effects.

[Insert Figure 8.2 here]

Testosterone has a 19-carbon skeleton consisting of four fused rings, which may be commonly modified at three positions to create synthetic AAS (Figure 8.2; positions A, B and C). Such modifications include esterification of the 17-β-hydroxyl group (A) to form a group of AAS collectively known as testosterone esters. Testosterone esters have enhanced lipid solubility and when administered intramuscularly they

are released slowly into the circulation thus enabling prolonged activity and reduced hepatotoxicity. Alkylation at the 17-α position (B) produces 17α-alkylated AAS, which are orally active due to their ability to limit first-pass metabolism. Structural modifications to other positions on the testosterone skeleton (C) will tend to increase anabolic potency. Examples of AAS categorised according to their modification and route of administration are provided in Table 8.2.

Besides oral or parenteral administration via injection, AAS may also be administered topically as gels or creams. Unmodified testosterone is available as a dermal gel or patch and is administered daily due to its short half-life (Table 8.2).

In addition to testosterone, the naturally occurring18-carbon AAS, 19-nortestosterone (otherwise known as nandrolone) is considered a doping agent and so too are its modifications particularly its decanoate ester known by its brand name, Deca-durabolin (Table 8.2). As a PIED nandrolone is attractive since it has fewer androgenic effects in comparison to other AAS as it is not converted to DHT and its 5α-reductase metabolite, 19 nor-dihydrotestosterone has a low binding affinity to the AR (Toth & Zakar, 1982).

[Insert Table 8.2 here]

8.4 Clinical uses of anabolic androgenic steroids

Despite the wide-ranging effects of AAS in terms of stimulating not only muscle growth but also bone and red blood cell production their current use in clinical settings is limited. Whilst the traditional use of AAS was to promote muscle growth in those with degenerative conditions their effectiveness has been questioned. Indeed, since the late 1980s the licence of many AAS has been removed. Remaining AAS are typically used in hormone replacement therapy in males with hypogonadism. However, the use of AAS to combat muscle atrophy has been reconsidered. Both testosterone esters and 17α-alkylated steroids may now be considered useful therapeutic agents for the treatment of muscle wastage associated with HIV/AIDS, severe burns and renal failure (Basaria et al., 2001). Nevertheless, although evidence exists to support AAS use in post-operative recovery and in those with chronic conditions such as HIV/AIDS, further research is needed to establish the effects of sustained use on health and wellbeing before clear recommendations can be made (Woerdeman & de Ronde, 2011).

8.5 Anabolic androgenic steroids as performance and image enhancing agents

Despite the wealth of anecdotal evidence supporting the efficacy of AAS as performance enhancers, it has only been in recent decades that the scientific community has provided confirmation. The landmark study by Bhasin *et al*. (1996) demonstrated that individuals who were not deficient in testosterone could increase both muscle mass and function following the administration of supraphysiological doses of testosterone enanthate. Whilst exercise training was shown to have an additive effect on muscle growth and function, significant increases were still demonstrated in those who did not perform exercise training. These findings suggested that AAS were able to exert their effects by traditional genomic action via the AR without the need for anti-catabolic effects.

As regards genomic action, AAS passively diffuse into the cell of target tissue and bind with the AR and through interaction with the DNA mediate transcription thus leading to protein synthesis. DNA binding domains on the AR then bind with steroid response elements on the DNA and with the addition of co-regulators, gene activation is initiated. Gene activation then leads to gene transcription, translation and ultimately in the production of contractile protein and muscle hypertrophy.

The potential anti-catabolic effect relates to the fact that the release of cortisol in response to heavy exercise has a catabolic effect as regards skeletal muscle. Cortisol is released from the adrenal cortex in response to stress to aid energy provision. This is achieved by promoting gluconeogenesis in the liver and the provision of gluconeogenic substrate in the form of protein through the breakdown of skeletal muscle. Clearly, the ability of AAS to limit the effects of cortisol might have a profound effect on skeletal muscle growth and function. It has been suggested that AAS act as antagonists to glucocorticoid receptors thus reducing the effects of cortisol. However, by and large AAS are considered to have a low binding affinity to glucocorticoid receptors (Hickson *et al*., 1990). It is now thought that AAS might affect gene expression of glucocorticoid receptors (Kicman, 2008).

Clearly, hypertrophy through genomic pathways is reliant on the apparatus within myonuclei. Muscle fibres contain hundreds of myonuclei however, there is thought to be a ceiling in terms of the level of skeletal muscle hypertrophy, supported by a finite number of myonuclei. It is therefore suggested that by

increasing myonuclei number, AAS are able to enhance hypertrophy (Kadi, 2008). Satellite cells in skeletal muscle are the source of new myonuclei and muscle fibres and their number has been shown to increase following AAS administration (Sinha-Hikim *et al*., 2003).

Research evidence supports the view held by many that the performance enhancing effects of AAS last for much longer than the period of administration. Egner *et al*. (2013) demonstrated, in an animal model, that increased myonuclei number following the administration of AAS are not lost during atrophy in response to AAS abstinence and that following subsequent training there was significant hypertrophy compared to a control group. This research suggests that the increased myonuclei are an important reserve that directly relate to hypertrophic potential, which is much greater in those that have used AAS. Further research is clearly needed to determine whether these findings might be transferable to humans.

Whilst the work by Bhasin and colleagues (1996) was able to show unequivocally that AAS were effective as a PIED, the mechanisms by which this result is achieved are complex. It is likely that positive results in terms of muscle hypertrophy and improvements in function are as a consequence of a combination of both physiological and behavioural responses. In addition to the mechanisms directly within skeletal muscle, activation of the CNS via AR and non-genomic actions to promote psychological effects may be particularly useful as an indirect mechanism by increasing exercise training intensity and volume (Kicman, 2008).

AAS, known to play an important role in sexual behaviour in both male and females, are also believed to be important in elevating mood and reducing depression. Conversely, reports of increased aggression in those misusing AAS (Yates *et al*., 1992) might be useful to promote increased exercise tolerance and competitiveness in the sporting arena.

8.6 Adverse effects following anabolic androgenic steroid use

As the mismatch between anecdotal and scientific evidence in support of AAS as effective ergogenic aids fuelled the mistrust between users and the scientific and medical community so has the limited scientific evidence with regards to potential serious adverse health effects. The general acceptance by the medical community that AAS are indeed hazardous is a clear attempt to discourage non-therapeutic

use of AAS, however scientific evidence appears to be growing in support of this position (Bird *et al*., 2016; Horwitz *et al.,* 2019). Nevertheless, difficulty remains, in terms of good scientific evidence due to the constraints relating to ethics and controlling many of the confounding variables that exist including concomitant supplement use and the practice of polypharmacy amongst users and the difficulty in establishing the authenticity of products used. The adverse effects associated with AAS use in humans have been reported in a number of ways, including: surveys, case studies and placebo controlled trials involving subjects with hypogonadism. The main reported side effects of AAS use are widespread and may be categorised accordingly:

Liver effects

The 17 α-alkylated AAS would appear to offer the largest threat to liver damage as a consequence of first pass metabolism. Hepatotoxicity attributed to AAS use may take several forms including transient elevations in liver enzyme concentrations, cholestasis, vascular injury and hepatic tumours. Numerous studies have reported increased concentrations of liver enzymes amongst AAS users (Sanchez-Osorio *et al*., 2008; Nasr & Ahmad, 2009). Whilst raised hepatic enzymes may signal toxicity they may fall over time and hide the real extent of injury during prolonged AAS exposure. Alternatively, raised hepatic enzymes may simply reflect muscle damage as a consequence of heavy training (Hoffman & Ratamess, 2006). However, in those with liver damage other symptoms may predominate such as jaundice and pruritus (itchiness) (Sanchez-Osorio *et al*., 2008; Elsharkawy *et al*., 2012). Such symptoms are typical of cholestasis, a condition where there is retention of bile in the biliary capillaries of the hepatic lobules. Whilst there are reports of vascular injury in individuals using AAS these tend to be rare. The condition of peliosis hepatis is a vascular condition, which is characterised by the development of blood-filled cysts throughout the liver and has been reported in numerous cases involving AAS use (Cabasso, 1994). Reports of carcinomas attributed to AAS use are uncommon but increasing (Tentori *et al*., 2007).

Cardiovascular effects

Numerous reports have shown that the use of AAS have a diverse effect on the cardiovascular system. The most severe include death, as a consequence of cardiovascular disease (CD) with chronic AAS use (Angell *et al*., 2012a; Montisci *et al*., 2012; Lehmann *et al*., 2019). Evidence of CD is based upon incidence of acute myocardial infarction, heart failure, coronary disease or cerebrovascular accidents (i.e. stroke) all reported via case studies.

There is a growing body of research that links AAS use with CD and the prevalence of numerous CD risk factors, however the mechanisms behind such effects are unclear (Angell *et al*., 2012b). Reports of the incidence of hypertension (Kuipers *et al*., 1991; Riebe *et al*., 1992), adverse blood lipid profiles (Lane *et al*., 2006), coronary atherosclerosis (Baggish *et al*., 2017), myocardial hypertrophy (Angell *et al*., 2012c), ECG abnormalities (Maior *et al*., 2013) and myocardial dysfunction (Baggish *et al*., 2017) amongst AAS users are numerous. However, there is a lack of more controlled prospective cohort studies which are necessary to establish possible causal mechanisms between AAS use and adverse cardiovascular health.

Reproductive system effects

Hypogonadotrophic hypogonadism is a side effect of AAS use as a consequence of the hypothalamic negative feedback loop responding to increased circulating androgens. Testicular atrophy and impaired spermatogenesis are symptomatic of reduced release of gonadotrophins. In females, suppressed secretion of gonadotrophins leads to menstrual irregularities and increased circulating androgens leads to clitoral hypertrophy following long-term AAS use. The inhibition of spermatogenesis may persist for many months after AAS withdrawal however, such side effects are deemed to be reversible. Similarly, in females the menstrual cycle will recommence soon after AAS use is discontinued. However, side effects such as clitoral hypertrophy appear to be less reversible (Clark, 2009).

Cosmetic effects

Cosmetic effects are most pronounced in females as AAS result in an overall masculinising effect, which may be irreversible following the discontinuation of the drugs (Clark, 2009). These effects include hirsuitism and deepening of the voice. Chronic use of AAS may produce male pattern baldness, which has been reported in both males and females. Acne is a common condition reported by many AAS users due to androgenic stimulation of the sebaceous gland.

High dose AAS regimes practiced by males may ironically result in high levels of circulating oestrogens as a consequence of aromatisation of androgens which may result in gynecomastia, which is the development of breast tissue. Consequently, users will often co-administer Tamoxifen, a selective oestrogen receptor modulator (SERM), to combat such side effects (see Chapter 11).

Psychological effects

One of the most reported side effects attributed to AAS misuse is that relating to the CNS. Increased aggression may be regarded as a positive effect in terms of facilitating increased training load. However, numerous reports have cited episodes of aggression, violence and mania as common amongst AAS users (Pope & Katz, 1988). During abstinence from AAS, users have been reported to exhibit anxiety and depression (Kanayama *et al*., 2009), deemed to be attributed to the low circulating levels of endogenous AAS as a consequence of reduced production occurring in light of prior sustained circulating exogenous AAS. Opposing views are that those exhibiting marked changes in mood and behaviour are predisposed to such psychological effects, which are only heightened with AAS use (van Amsterdam *et al*., 2010). Smit *et al*. (2020a), reported that there were no significant changes in well-being, quality of life and depression in long-term AAS users.

Additional side effects

Amongst adolescents the administration of AAS can result in the premature closure of epiphyseal growth plates and thus lead to stunted growth (Johnson, 1990). Other musculoskeletal issues relate to the potential for AAS to inflict problems associated with ligament and tendon damage (Giannotti *et al*., 2014). This is thought to be associated with the development of dysplasia of collagen fibrils thus decreasing the tensile strength of tendons (Laseter & Russell, 1991) and the disproportionate loading related with increases in muscle strength (Wood *et al*., 1988).

Inappropriate drug administration, such as needle sharing and use of non-sterile equipment is a particular issue amongst AAS users. This poses serious risks in terms of infection and the acquisition of blood borne diseases such as hepatitis and HIV/AIDS (Hope *et al*., 2013).

Possibly the greatest risk to AAS users are the health risks associated with the use of products obtained from the illicit market. The illicit market contains drugs that are no longer licenced, those that are marketed as veterinary products, new drugs that have not been fully tested and AAS that have not been authorised but developed as part of pharmaceutical research projects (Evans-Brown *et al*., 2012). In addition to a wide range of AAS that do not carry with them the required safety checks and information that exists with licenced products there are real concerns relating to the sterility and authenticity of such products (Weber *et al*., 2017). Product analysis carried out by Abbate *et al.* (2015) revealed that from 24 products marketed as bodybuilding supplements, 16 contained AAS and prohormones different to those listed as an ingredient on the label.

8.7 Use of anabolic androgenic steroids in sport

Whilst there is uncertainty as to who were first to use AAS in competition the 1950s heralded the beginnings of their use in sport (Dimeo, 2007). In 1954 it is alleged that Russian athletes under the influence of AAS won numerous gold medals in the World Weightlifting Championships in Vienna. Throughout the 1950s and 1960s AAS use escalated and whilst the IOC introduced the first list of prohibited substances in 1967 it was not until 1976 that AAS were added to the list following the development of a reliable test for their detection in urine in 1974.

Following the reunification of Germany in 1990 it was revealed that the German Democratic Republic (GDR; the former state of East Germany) had operated a systematic doping programme as part of their state-run sports programme. This programme ran from the mid-1960s, throughout the 1970s and 1980s and was instrumental in the success of the GDR in international sports competitions throughout this period. Hundreds of scientists and physicians were involved in both research and administering AAS and other doping agents to athletes with the sole purpose of improving sports performance and raising the profile of the nation on a world stage (Franke & Berondonk, 1997). Particular emphasis was placed on females and their performance where the effects of AAS are more pronounced. The real impact and legacy of the programme was the incidence of serious doping-related side effects that numerous athletes experienced of which many were irreversible.

Probably one of the most well-known cases of AAS use in sport is that of the Canadian track and field athlete, Ben Johnson at the 1988 Olympics in Seoul. Having been crowned champion of the 100 m he was subsequently stripped of his medal after failing a routine post-competition drugs test. He tested positive for the AAS, stanozolol and put the issue of drug use in sport firmly in the spotlight. As a consequence, Charles Dubin, a Canadian lawyer led a Canadian inquiry into the use of drugs in sport. The Dubin Inquiry was to last one year and included the admission of AAS use by 48 athletes (including Ben Johnson) and recommendations that would help to improve doping control globally through increased and improved drug testing and stricter penalties for those that violate the rules (Moriarty *et al*., 1992).

Designer steroids have become a particular issue in elite sport, developed specifically to circumvent routine anti-doping tests. In the early part of this century several international athletes, including sprinters Marion Jones, Dwain Chambers and Kellie White were convicted for the use of tetrahydrogestrinone (THG), a designer steroid. This became known as the BALCO affair whereby THG was manufactured and distributed

to numerous athletes from the Bay Area Laboratory Cooperative (BALCO) in California, led by its founder, Victor Conte (see section 8.9).

There is anecdotal evidence of widespread use of AAS at a recreational level including those engaged in gym exercise and bodybuilding. With increased media focus on image and the ideal body, coupled with increased accessibility to illicit products, via the internet, it would seem that the market amongst recreational users is burgeoning (Evans-Brown *et al*., 2012; see Chapter 25).

Sources, supply and control of Anabolic Androgenic Steroids

The use of AASs is clearly associated with their availability and legality. In the UK AAS are controlled as class C drugs under the Misuse of Drugs Act 1971. Whilst it is illegal to possess these drugs, individuals who possess AAS for personal use are unlikely to face prosecution. The control measures for AAS varies between different countries with some legislating specifically against the use of doping agents in sport. Nevertheless, the supply of such drugs has never been greater, with the internet opening up a global market in the trade of illicit products, many of which may be manufactured in clandestine laboratories outside of any regulatory system (Evans-Brown *et al*., 2009).

The trade in substandard and counterfeit products is a significant health concern since their quality and thus safety cannot be guaranteed. In addition to sub-standard manufacturing, there is an issue concerning counterfeiting, that is deliberately mislabelling products. Consequently, there are no assurances that the alleged ingredients are present and in the correct quantity (Graham *et al*., 2009; Evans-Brown *et al*., 2009; Abbate *et al*., 2015).

Patterns of administration

Anabolic steroids are broadly available as three types of preparation, oral, oil-based or water-based injections and topical gels or patches. Oral preparations have a structure resistant to breakdown by stomach acid, can be absorbed by the gastrointestinal tract and tend to withstand total breakdown by liver enzymes, however they have a short half-life and require frequent dosing. Injectable oil-based preparations have a longer half-life but produce a degree of pain at the injection site. They have a slow absorption rate into the blood stream, so that lower concentrations pass through the liver, thereby reducing liver toxicity. Injectable water-based steroids have a long half-life, though normally less than oil-based preparations, produce less discomfort at the site of injection and can be mixed with other water-based steroids or other drugs (George & Mottram, 2011). Topical gels (or creams) or patches result in low dose administration of un-modified testosterone which has a short half-life requiring daily application.

There are a number of administration regimes in use, known as ‘cycling’, ‘pyramiding’ and ‘stacking’. Experienced users will typically follow a combination of these regimes concurrently. Each regime is reputed to offer a particular advantage in terms of heightening the effect of a particular drug (or drugs) or limiting the potential side effects experienced. However, there is no scientific evidence to support such regimes.

Cycling is the administration of a particular drug over a period of time followed by a period of abstinence before the administration is recommenced. Cycling patterns are typically short (i.e. 6 to 8 weeks of administration followed by 6 to 8 weeks of abstinence) or long (i.e. 6 to 18 weeks of administration followed by up to 12 months of abstinence). The rationale behind cycling is that the periods of abstinence may reduce the incidence of side effects.

Pyramiding is a variation of cycling whereby the dose is gradually increased during the cycle to a peak and then gradually reduced towards the end of the cycle. This regime allegedly results in fewer behavioural side effects caused by rapid withdrawal of the drug, such as lowered mood.

Stacking is a word used to describe the use of more than one AAS at a time. In its simplest form, this regime might involve the simultaneous use of both an orally administered steroid and an injectable one. More sophisticated regimes involve intricate schedules of administration using many different AAS each with supposedly different pharmacological profiles. The aim of this technique is to avoid the development of tolerance to a particular drug (George & Mottram, 2011). In addition to AAS, common ancillary drugs include, growth hormone, clenbuterol, insulin, insulin-like growth factor-1 (IGF-1), human chorionic gonadotrophin (HCG), ephedrine and tamoxifen of which some will be taken for their alleged synergistic action whilst others because of their ability to combat unwanted side effects.

8.8 Prevalence of anabolic androgenic steroid use in sport

As with all illicit drug use, it is difficult to establish an accurate indication of how prevalent AAS use is within sport. Most evidence in elite sport comes from data obtained from WADA-accredited laboratories, which highlights, on a yearly basis the number of positive drug tests (Table 8.3). Whilst such data cannot provide an accurate estimate of absolute numbers administering AAS, they do reveal that AAS

comprise half of all adverse analytical findings and atypical findings reported by WADA-accredited laboratories (WADA, 2020a).

[Insert table 8.3 here]

The sports within which AAFs for anabolic agents were most frequently recorded in 2019 are shown in Figure 8.3. These data reflect the sports within which anabolic affects are a pre-requisite for optimal performance and are in line with previous findings (Aguilar-Navarro *et al*., 2020). These figures do not reflect the true extent of AAS use in sport particularly at a sub-elite and recreational level. Use of AAS appears to be a significant problem in collegiate sport in the United States (McCabe *et al*., 2007). Also, anecdotal evidence would suggest that AAS use is widespread amongst those engaged in recreational sport and numerous reports suggest that it is a developing public health issue (Evans-Brown *et al*., 2012).

Over recent years there appears to have been an increase in the 'drive for muscularity' amongst adolescents (McCreary & Sasse, 2000) but also the general population. This would seem to be as a consequence of the way in which the media portray health and attractiveness particularly from the perspective of the male body form. Indeed, there is evidence of increased prevalence of body dissatisfaction and low self-esteem amongst males with respect to the level of musculature which has been termed muscle dysmorphia (Pope *et al*., 1997). The focus on enhanced musculature is coupled with an increase in the use of image-enhancing drugs such as AAS (Kanayama *et al*., 2006) particularly amongst gym users (Smit *et al*., 2020b). Unfortunately, as with elite sport, it is difficult to establish reliable figures, in terms of prevalence of AAS use. Data from harm reduction programmes, such as needle exchange clinics offer some indication to the extent of use (McVeigh *et al*., 2003). Such increases cannot necessarily be attributed to increased AAS use but clearly illustrates the success of harm reduction programmes in attracting different types of recreational drug users.

8.9 Designer steroids

The term 'designer steroids' first became widely recognised in response to the BALCO (Bay Area Laboratories Cooperative) affair during the early part of this century. The term refers to the use and development of AAS, specifically to evade detection by anti-doping authorities. Tetrahydrogestrinone

(THG), otherwise known as ‘the clear’, was supplied as a sublingual AAS preparation by BALCO, a US-based company, to athletes for the purpose of enhancing performance. Since THG was never marketed its existence from an anti-doping perspective was unknown and therefore was undetectable during routine doping control analysis. Indeed, its existence as a performance enhancing agent was only possible as a consequence of a ‘whistle-blower’ who alerted the anti-doping authorities, who were then able to determine its molecular structure and subsequently establish a method for its detection (Catlin *et al*., 2004). Several high-profile athletes tested positive for THG (including the British sprinter Dwain Chambers) and many others were implicated in the affair (including US sprinters, Marion Jones and Kelli White) and consequently received sanctions including suspension from competition. In addition to track and field athletes, American football players tested positive for THG and baseball players were implicated in the affair. In addition, several athletes were convicted of perjury under state law, in the US. Victor Conte and several coaches were convicted for their part in the distribution of AAS to athletes and received sanctions under state law, including imprisonment.

In addition to THG, other designer steroids were identified at around the same time including, norbolethone (Catlin *et al.,* 2002) and desoxymethyltestosterone (DMT) (Sekera *et al*., 2005). Desoxymethyltestosterone was also known as Madol and was patented in 1961 but never approved for clinical use in human patients. Probably the first designer steroid however was dehydrochloromethyltestosterone (otherwise known as Turinabol) which was used by former-GDR athletes as part of their state-run doping programme (Parr & Schanzer, 2010).

The recent cases involving designer steroids have led to heightened vigilance by anti-doping organisations and WADA-accredited laboratories clearly improving the prospect of early detection. Nevertheless, the most important issue relates to the fact that as un-marketed steroids there is limited, if any, toxicology data and thus safety information. There use by athletes therefore poses a significant, yet unknown threat to health.

8.10 Prohormones

Prohormones refer to a group of substances that are precursors to steroid hormones and are now accepted as hormones and indeed AAS in their own right. The prohormones, androstenedione (Andro)

and dehydroepiandrostenedione (DHEA) are endogenous hormones produced by the gonads and adrenal gland and form an important circulating pool of steroid hormone precursors.

Administration of exogenous prohormones is thought to enhance the circulating pool of steroid precursors and thus increase the subsequent biotransformation into testosterone. Increased circulating testosterone is then thought to impact positively on skeletal muscle hypertrophy and function. However, the scientific literature does not confirm these hypotheses. Whilst there is evidence to show that the ingestion of prohormones can increase the circulating levels of DHEA and Andro, resultant significant elevations in the circulating pool of testosterone has only been demonstrated in females (Morales *et al*., 1998). This could be explained by the fact that in females a significant proportion (up to 100%) of circulating testosterone is as a consequence of peripheral conversion of weaker androgens, namely DHEA (Labrie *et al*., 2005). However, in males, circulating testosterone is almost entirely based upon its production in the gonads (and to a lesser extent by the adrenal cortex) (Kicman, 2008).

Despite the positive outcomes, in terms of increased circulating testosterone in females, there has been no indication that this might manifest itself in gains in muscle size and strength (King *et al*., 1999). The efficacy of prohormones as PIEDs is further weakened by the evidence that they may induce a number of negative side effects. King *et al*. (1999) revealed an adverse effect on blood lipid profiles and increased levels of circulating oestrogens following the administration of 300 $mg.d^{-1}$ administration of Andro over an eight-week period.

Prohormones are commonly included as ingredients in, and marketed as, nutritional sports supplements in order to avoid the necessary controls required in the manufacture and sale of pharmaceutical products. As sports supplements they are available from sports supplements shops and via the internet and used amongst the sports and fitness community. Clearly, such widespread availability poses problems in terms of both the intentional and unintentional use of AAS in elite athletes and potential failed drug tests as a consequence.

8.11 Detection of anabolic androgenic steroids

Detection of exogenous AAS is generally based upon direct quantification of a particular AAS and its metabolites in urine. However, in the case of endogenous AAS it is reliant on the investigation of various

steroid profiles in order to establish possible androgen misuse. The concentration of testosterone and its stereoisomer, epitestosterone is determined together with additional endogenous androgens including androsterone, etiocholanolone, 5α-androstane-3α,17β-diol (5αAdiol) and 5β-androstane-3α,17β-diol (5βAdiol). In addition, ratios of several endogenous androgens are also determined including the ratio of testosterone to epitestosterone (T:E), androsterone to testosterone (A:T), 5αAdiol to 5βdiol and 5αAdiol to epitestosterone. An individual steroid profile is established as part of the steroidal module of the Athlete Biological Passport (see Chapter 5). Alteration of the endogenous androgens or ratios may constitute doping and further confirmatory analysis maybe warranted (WADA, 2020b).

The endogenous AAS, 19-norandrosterone also poses its own unique problem in that it may also be present in urine as a metabolite of the exogenous AAS 19-nortestosterone. A urinary threshold for 19-norandrosterone is therefore set a 2 $ng.ml^{-1}$.

Micro-dosing using topical application of testosterone via dermal patches and gels is a particular concern to anti-doping personnel not least because of the short half-life of the drug and thus the potential to evade a positive drugs test.

Clearly the introduction of out-of-competition drug testing was in some way effective in catching those administering exogenous AAS (or in acting as a deterrent to potential users) since the use of AAS as PIED is most effective during training and in the lead up to competition. Before the advent of out-of-competition testing many athletes would therefore simply abstain from use leading up to a competition in order to avoid detection during an in-competition test. However, the arrival of designer steroids would prove particularly difficult since their existence as a PIED was unknown and therefore would not appear on a urine drug screen. A proactive approach involving research and intelligence will no doubt help to limit the effects of such events in the future.

The introduction of the ABP Steroidal Module represented a decisive improvement for the detection of endogenous AAS doping. However, there remains some concerns regarding the detection of endogenous AAS that require further attention, most notably: the short detection window following oral administration, the low sensitivity of detection of biomarkers following transdermal administration and difficulties concerning the differences in the metabolism of testosterone by the liver linked to particular genotypes (Ponzetto *et al*.,2019). Also, re-testing anti-doping samples, particularly involving testing for long-term metabolites for AAS has proved to be successful in detecting AAS misuse and is likely to be an effective deterrent (Kuuranne & Saugy, 2016; Kolliari-Turner *et al*., 2021).

8.12 Selective Androgen Receptor Modulators (SARMs)

Selective androgen receptor modulators may offer a possible advantage over AAS both clinically (Christiansen *et al*., 2020) and as PIED due to their potential for tissue selectivity and in promoting anabolic rather than androgenic effects. They are also designed to be administered orally with reduced hepatotoxicity. However, SARMs remain under development and do not have full clinical approval (Narayanan *et al*., 2018). Nevertheless, as with many doping agents their lack of availability as therapeutic agents are no barrier to their potential use as performance enhancers. Consequently, their chemical characterisation and methods for their detection have been developed (Thevis *et al*., 2013a; Thevis & Schanzer, 2018; Thevis *et al.,* 2019; Zierau *et al*., 2018). Indeed, recent anti-doping testing figures confirm that SARMs have been detected in doping control samples thus providing clear evidence of their misuse in sport (Table 8.3; WADA, 2020a).

Inevitably, SARMs are marketed in dietary supplements and available via the internet (Leaney *et al*., 2021). In a study by van Wagoner *et al*. (2017), it was shown that many contained unapproved substances and were inaccurately labelled.

8.13 Beta-2 agonists

Several drugs classified as β_2-agonists are also included on the WADA Prohibited List as anabolic agents, namely clenbuterol,and zilpaterol (Table 8.1; WADA, 2021). Beta-2 agonists are typically used to combat respiratory conditions due to the stimulation of the β_2-adrenergic receptors of the bronchioles and their bronchodilatory effects (see chapter 10). However, when administered orally, clenbuterol has been shown to possess anabolic effects in animals (Choo *et al*., 1992). Indeed, both clenbuterol and zilpaterol are used as growth promoters in livestock (Davis *et al*., 2008). Despite the evidence to support their use as anabolic agents in animals there is limited evidence for this use in humans. Nevertheless, within the bodybuilding fraternity their use is relatively widespread as both an anabolic and repartitioning agent (Jessen *et al*., 2020).

The consumption of meat infected with prohibited growth promoters and the impact on doping control

Given the inclusion of a number of livestock growth promoters on the WADA Prohibited List, what is the likelihood for the consumption of meat containing such agents culminating in a positive drugs test? This is an obvious concern to athletes subject to doping control tests. Indeed, Parr *et al*. (2009) demonstrated that by consuming meat from cattle having been given clenbuterol, there is a real possibility that this may lead to a positive drugs test. In light of this, WADA introduced a urinary threshold of 5 $ng.ml^{-1}$ for clenbuterol (WADA, 2019) in an attempt to limit the possibility of an inadvertent doping offence. In addition, detection methods have been developed to differentiate between clenbuterol from direct pharmaceutical origin and that from ingested food (Dolores *et al*., 2019).

It is ironic that there is now concern regarding the possibility of false positives from a doping perspective as a direct consequence of improved analytical methods. The potential to detect minute traces of prohibited substances brings in to question the anti-doping movement's guiding principle of strict liability, particularly as the possibility of prohibited substances entering the body unintentionally, in such small quantities is considered significant.

During the 2011 FIFA U17 World Cup clenbuterol was detected in over half of the doping control samples. This was attributed to the ingestion of food contaminated with the drug as indicated by food analysis (Thevis *et al*., 2013b). Risk of inadvertent clenbuterol doping is deemed to be a particular risk in countries where the use of the drug in livestock is not regulated. The European Union prohibit the use of hormonal active growth promoters in livestock (Stephany, 2010). However, in China where clenbuterol use in livestock is permitted it remains a real risk to athletes (Guddat *et al*., 2012).

Zeranol is a synthetic compound used in some countries as a growth promoter in livestock. Whilst there have been cases of its presence in doping control samples there is suggestion that this might be as a consequence of food contamination with a mycotoxin, zearalenone. Consumption of cereals contaminated with the mycotoxin can result in the conversion to zeranol (Thevis *et al*., 2011; Walpurgis *et al*. 2020).

8.14 Tibolone

Tibolone is a synthetic steroid hormone with an affinity for oestrogen receptors and is used in the symptomatic relief of the menopause and in the prevention of osteoporosis in women. However, whilst tibolone is indicated for its oestrogenic properties it also has weak androgenic properties hence its inclusion in class S1 of the WADA Prohibited List (Table 8.1; WADA, 2021) and detection by WADA-accredited laboratories according to anti-doping testing figures (Table 8.3; WADA, 2020a).

8.15 Ecdysterone

Whilst, not prohibited, the use of ecdysterone in sport is monitored both in and out of competition since its inclusion to the WADA Monitoring Program in 2020 (WADA, 2020c). Ecdysterone is a steroid naturally occurring in insects and plants and is an ingredient in sports supplements marketed for muscle growth and strength-enhancing properties. Despite its availability as a constituent of sports supplements and the growing evidence to support its efficacy there has been limited research carried out in humans. Nevertheless, a study by Isenmann and colleagues (2019) examined the effects of ecdysterone supplementation in healthy male volunteers during a 10-week strength training programme and reported significant improvements in both muscle mass and strength compared to in those supplemented with a placebo. Research involving an in vitro model suggests that the effects of ecdysterone may even surpass those of AAS (Parr *et al*., 2015).

Although the mechanisms supporting ecdysterone as an anabolic agent have not been fully elucidated there is evidence from research involving *in vitro* cell culture suggesting that rather than the AR it is mediated by the oestrogen receptor β subtype (ERβ; Parr *et al*., 2014). Further research is clearly required in this area, nevertheless there is growing support for the consideration of ecdysterone as a prohibited substance and addition to the WADA Prohibited List.

8.16 Key Points

- Whilst the WADA classification of Anabolic Agents consists of some new and emerging drugs of abuse, AAS remain the major class of drugs misused by many who seek improvements in terms of muscle size and strength.

- Despite improvements in terms of drug detection and the unfolding evidence in relation to health concerns surrounding the use of AAS there are no signs to suggest that their use is abating.
- The use of AAS amongst those engaged in recreational sport or exercise, particularly gym goers, for purposes largely related to image enhancement appears to be burgeoning.
- The scientific literature clearly supports the use of AAS as PIEDs however, there remains some way to go before there is clear evidence to categorically link the use of AAS with serious, long-term and even life-threatening health effects.
- Potentially the most disturbing issues in relation to the non-therapeutic use of anabolic agents is the availability of 'black market' products where there is no evidence of their legitimacy and thus their safety. Indeed, the use of new and emerging drugs not yet trialled, such as SARMs offer no assurances in relation to safety.
- Research into ecdysterone, a naturally occurring steroid in plants and insects demonstrates its potential as an anabolic agent. There is growing evidence in support of the reclassification of ecdysterone as a prohibited substance.

8.17 References

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Table 8.1: Class S1 of the 2021 WADA List of prohibited substances and methods (WADA, 2021)

S1. ANABOLIC AGENTS

PROHIBITED AT ALL TIMES (In- and Out-of-competition)

Allsubstances in this class are non-Specific Substances

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1. Anabolic Androgenic Steroids (AAS)

    a. Exogenous AAS

    b. Endogenous AAS when administered exogenously

2. Other Anabolic Agents, including but not limited to:

    Clenbuterol, Selective Androgen Receptor Modulators (SARMs e.g. andarine, LGD-4033(ligandrol), enobosarm (ostarine) and RAD140), Tibolone, Zeranol, Zilpaterol.

Figure 8.1: The formation of testosterone and its derivatives (George & Mottram, 2011; p 52)

Figure 8.2: The molecular structure of testosterone illustrating the major sites of modification in the in the formation of synthetic anabolic androgenic steroids (adapted from Wilson, 1988)

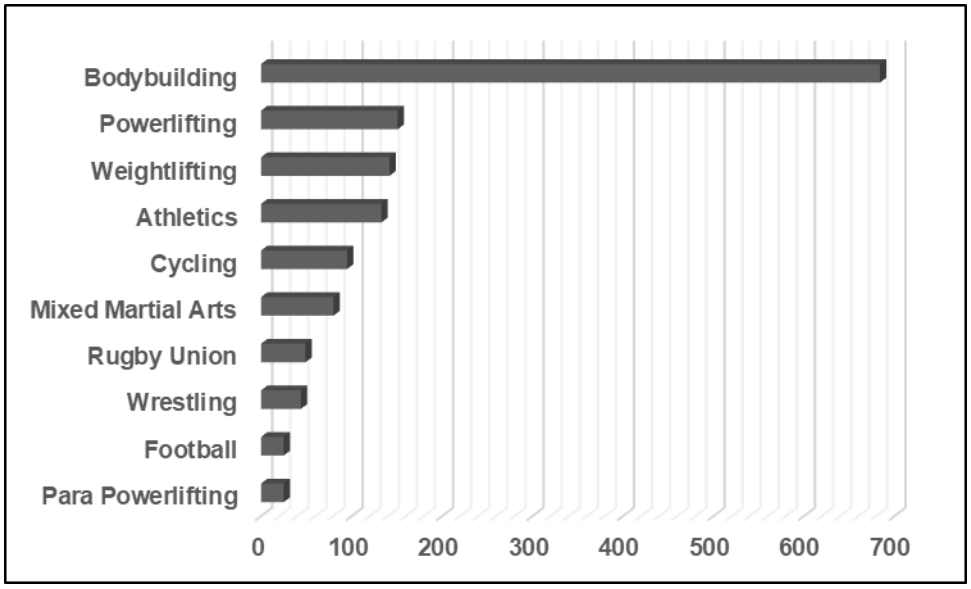

Figure 8.3 The sports within which adverse analytical findings for anabolic agents were identified through WADA-accredited laboratory statistics for 2019 (WADA, 2020a)

Table 8.2: Categorisation of AAS according to their route of administration and their structural modification from testosterone (adapted from Clark, 2009).

AAS	Trade name	Route of administration
Unmodified testosterone		
Testosterone	Androgel, Testim	Topical gel
Testosterone	Androderm, Testoderm	Dermal patch
Testosterone esters		
Testosterone enanthate	Delatetryl	Intramuscular injection
Testosterone cypionate	Depo-Testosterone	Intramuscular injection
Testosterone propionate	-	Intramuscular injection
17α-alkylated AAS		
Methyltestosterone	Android, Virilon	Oral
Fluoxymesterone	Halostestin	Oral
Stanozolol	Winstrol	Oral
Methandrostenelone	Dianabol	Oral
19-Nor testosterone esters		
Nandrolone deconate	Deca-durabolin	Intramuscular injection
Nandrolone phenylproprionate	Durabolin	Intramuscular injection

Table 8.3: Prohibited anabolic agents identified by WADA-accredited laboratories in 2019 (WADA, 2020a)

Anabolic agent	Occurrences
Anabolic Androgenic Steroids (AAS)	
Stanozalol	267
Drostanolone	163
19-norandrosterone (26 cases consistent with an exogenous origin)	162
The GC/C/IRMS result is consistent with an exogenous origin	147
Boldenone (29 cases the result is consistent with an exogenous origin)	120
Metandienone	113
Oxandrolone	92
Dehydrochloromethyl-testosterone	90
Metenolone	80
Trenbolone	69
Mesterolone	38
Methasterone	31
Clostebol	28
Others	69
Total	1469
Other Anabolic Agents	
Clenbuterol	199
Enobosarm (Ostarine)	74
LGD-4033	62
Tibolone	12
RAD140	4
Zilpaterol	2
Zeranol	1
SARM S-23	1
Andarine	1
Total	356