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Doping in sport and exercise: anabolic, ergogenic, health and clinical issues

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The Association for Clinical Biochemistry & Laboratory Medicine

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Author(s)	Stephen R Bird1, Catrin Goebel4, Louise M Burke3, Ronda F Greaves1,2
Author Affiliations Including email address for corresponding author	1School of Medical Sciences, RMIT University, Victoria, Australia 2Murdoch Children's Research Institute, Melbourne, Australia. 3Australian Institute of Sport, Canberra Australia 4Australian Sports Drug Testing Laboratory Stephen.bird@rmit.edu.au
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Abstract

The use of doping agents are evident within competitive sport in senior and junior age groups, where they are taken by non-elite as well as elite participants. They are also taken in non-sporting contexts by individuals seeking to 'improve' their physique through an increase in muscle and/or decrease in fat mass. Whilst attaining accurate data on the prevalence of their use has limitations, studies suggest the illicit use of doping agents by athletes and non-athletes may be 1 – 5% in the population and greater than 50% in some groups; with the prevalence being higher in males. There is conclusive evidence that some doping agents are anabolic and ergogenic. There is also evidence that the use of doping agents such as: anabolic androgenic steroids; growth hormone and other anabolic agents; erythropoietin; and stimulants conveys considerable health risks that include, but are not limited to: cardiovascular disease, diabetes, cancer, mental health issues, virilisation in women, and the suppression of naturally produced androgens in men. This review will outline the anabolic, ergogenic and health impacts of selected doping agents and methods that may be used in both the sporting and physique development contexts. It also provides a brief tabulated overview of the history of doping and how doping agents may impact upon the analyses of clinical samples.

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Introduction

Sport and physical activity are widely encouraged by governments and health authorities for their perceived benefits to health and quality of life. Conversely, illicit drug use is deemed to have a negative impact on health and adversely affect society. However, since some drugs are known to augment physical performance and adaptations to exercise training, these two contrasting areas can intersect.

In sport the use of illicit drugs comes under the category of 'doping' with the relevant sports governing bodies pursuing strategies to prevent the use of banned substances. Doping is used in sport and exercise contexts for a number of reasons, including the desire to win and to improve aesthetic appearance. Sports are governed by rules and codes, including those relating to the prohibition of doping. Perhaps the most well-known of these is the 'World Anti-Doping Code',[1] which is implemented by the World Anti-Doping Agency (WADA) and WADA publish a list of prohibited substances and prohibited methods.[2] Doping substances and methods are included on the WADA prohibited list if they meet two of the following three criteria:[3]

(i) Evidence that it has the potential to enhance sport performance;

(ii) Use of the substance or method represents a health risk;

(iii) Use of the substance or method violates the spirit of the sport.

The WADA list clusters its doping categories into: 'Substances and Methods Prohibited at All Times (in- and out-of competition)'; 'Substances and Methods Prohibited In-Competition'; and 'Substances Prohibited in Particular Sports' (Table 1). As a broad generalisation for this distinction the '*Substances and Methods Prohibited at All Times (in- and out-of competition)*' have potential benefits in terms of enhancing adaptation to training and either directly to or as a consequence of these augmented adaptations to improve performance in competition. Whereas '*Substances and Methods Prohibited In-Competition'* are likely to enhance the immediate short term performance of the body by augmenting its ability to meet the demands of the exercise and thereby perform better. Furthermore, some substances are prohibited under the WADA code in 'Particular Sports' if they convey benefits or hazards in specific sports but not others, such as alcohol in motor

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sports. WADA publishes a list of sport organisations and countries who comply with the WADA anti-doping code and are subject to WADA testing.[4] However, it should be noted that many professional sports such as the National Football League (NFL) (American Football/Gridiron)[5] some boxing federations, and many body-building type sports do not comply fully with the WADA code and its anti-doping regulations.

In the wider context, the use of such drugs extends beyond regulated elite sport, into lower levels and younger age groups, as well as occurring outside of the sporting context; where they may be used to enhance the development of a person's physique for reasons of aesthetics and physical presence.[6] Consequently the health issues associated with the use of such drugs permeates society beyond the sporting elite. Additionally, not all ergogenics are banned from sport, examples being caffeine, creatine and bicarbonate, all of which have strong research evidence for their efficacy. [7-11] This review will briefly present some of the key events in the history of doping, consider current doping prevalence and then review the performance and health issues of the WADA doping categories.

Methods for Literature Review

The published scientific literature was searched using key words in databases, primarily Pubmed. Additionally, literature and documents were searched from key sites, such as the WADA. To ensure currency of data and information, those articles published within the last 10 years were preferentially referred to over older publications. When the identified literature referred to a key publication that had not been identified in our searches this article was located directly, likewise where the original search had been refined to reviews, key original data articles cited in the reviews were downloaded. Where applicable, confirmed examples were sought from the popular press.

For note, the nomenclature and level of detail applied to peptide hormones in the published literature varied based on the context of the information provided. Within this review when discussing the use of various hormones, such as Erythropoietin (EPO), Growth Hormone (hGH), Luteinizing Hormone (LH) and Chorionic Gonadotrophin (CG), the prefix 'h' indicates the 'human'

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form of the hormone and 'r' indicates the recombinant synthetic version, with 'rh' indicating a recombinant version of the human form. Hence the terminology may vary in places depending on the specific form of the hormone being discussed and the information provided in the source material.

The physiological basis for doping in sport and exercise

Sport and exercise initiate both short-term acute responses and longer term adaptations. The short-term acute responses include elevations in heart rate and cardiac output, redistribution of blood flow, increased pulmonary ventilation and endocrinological responses. These responses enhance the body's ability to cope with the immediate demands of the current exercise bout, for example through the facilitation of oxygen delivery and its utilisation by the muscles. The longer-term adaptations, such as structural and physiological changes to the skeletal musculature, cardiovascular system and haematology, enhance the body's ability to cope with the demands of subsequent exercise, for example through an increased capacity to deliver and utilise oxygen or an increase in contractile proteins of the muscle that thereby increase the amount of force that they can exert. Such adaptations would be seen as improvements in 'fitness' and enable the person to potentially achieve a higher level of performance in subsequent exercise bouts or events.

Doping is used to augment these responses and adaptations, thereby elevating what the person can achieve to a level above that attainable by training alone. Due to the specific demands of each sport, the prevalence of different drugs and doping procedures will differ accordingly, as they are used to target specific aspects of the fitness needed by the performer in that particular activity. Additionally, as previously indicated, some doping practises are used specifically to enhance the physique of an individual, often to gain muscle mass and/or lose body fat: and whilst for sports performers such outcomes may benefit their strength, power and endurance, for others it is the enhanced physique that is the primary objective.

A Brief History of Doping in Sport

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Historically, the second half of the 19th Century saw the start of what has become the epidemic problem of doping in sport and exercise at all levels of competition. In the first half of the 20th Century (up until the end of World War II) doping expanded with programs aimed at the individual and administration controlled by the athlete themselves or by their coach or doctor. Doping at this time was generally confined to the elite level of sport. Post World War II, doping expanded significantly with systemic team doping programs emerging. In response to the increased incidence and adverse outcomes, doping controls were first introduced in the late 1960's; partly due to the outcry resulting from the first televised doping related death.

Sport related drug use has significantly influenced competition since the mid 20th century. The 1960's and 70's saw the wide use of amfetamines in sport; the 1980's has been described as the anabolic steroid and cortisone era; the 1990's as the human Growth Hormone (hGH) and erythropoietin (EPO) era; and more recently the use of peptides has become widespread. Today we generically classify such performance enhancing drugs according to terms such as "anabolics" and "stimulants" to describe the general desired effect of their administration.

With doping control, comes the introduction of drugs to "cheat" the system. The reported instance of evasive measures became prevalent after the 1980's when the first assay to detect testosterone in urine was developed. Despite these control measures and awareness of the risks of sport related drug use, doping in sport remains endemic; transgressing all levels of activity.

At the elite level, infamous systematic doping scenarios and individual cases are cited throughout sporting history. The German Democratic Republic (GDR) government administered doping program of its athletes, particularly its female athletes, contributed to their domination of Track & Field and Swimming events for the two decades spanning the 1970s and 1980s.[12] There are also numerous highly publicised individual cases including those of Ben Johnson (Canada) who was stripped of the Gold Medal for the 100m at the 1988 Seoul Olympics when it was reported that he tested positive for stanozolol. Furthermore, it is interesting to recall that of the eight runners in this infamous race, five of the other finalists either gave positive samples or were involved in

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some way in doping scandals at some stage in their careers. Other more recent examples include: Marion Jones who won five medals at the 2000 Sydney Olympics but was stripped of all these medals, when having been implicated by the highly publicised Bay Area Laboratory Cooperative (BALCO) investigation, she admitted in 2007 to using performance-enhancing drugs; and Lance Armstrong, who won the Tour de France cycling event on seven consecutive occasions (1999 – 2005) but was stripped of these titles in 2013 having been investigated by USADA and admitting to doping during a television interview by 'Oprah'. For the interested reader a brief tacit history describing the expansion of doping in sport is provided in Table 2.

The prevalence of doping

Sport related drug use

The prevalence of doping can be indicated through surveys or by the testing of participants, but both methods have inherent problems. For example, survey data has limitations relating to differences in the definitions of doping and the taking of 'banned' substances for social rather than performance enhancing reasons, as well as a reliance upon honest self-reporting of an illicit activity.[13] Whereas the clinical testing of a blood or urine sample may fail to identify doping cheats if the timing of sample collection does not coincide with the window of time when the substance or its metabolites are present within the individual's sample.

Whilst the highly publicised cases highlighted above create awareness through the media, quantifying the actual prevalence of sport related drug use is more problematic and incurs the aforementioned limitations. However, as an initial indication, WADA report the results of 269,878 samples analysed in 2013, of which 5,962 (2.21%) indicated either an 'adverse analytical finding' or 'atypical finding'.[14] Of these: 63.0% were due to anabolic agents; 10.1% stimulants; 7.5% diuretics and other masking agents; 6.3% glucocorticosteroids; 3.8% peptide hormones, growth factors and related substances; and 3.6% cannabinoids; with all other categories contributing <3% to the total adverse/atypical findings and their combined total being about6% of all adverse or atypical results.(Figure 1) The relatively recent introduction of testing blood samples has added to these results with a study that included the samples of 2,737 international track and field

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athletes, mainly endurance athletes, from whom blood samples were collected indicating the prevalence of blood doping (any method that increases red cell mass and enhances oxygen transport) to be 14%, but with considerable variation in prevalence between nationalities.[15]

Doping violations have also been reported in Paralympic sport, albeit with a relatively low prevalence of <1% and when such violations are detected it is most commonly for anabolic agents, with sports such as powerlifting having the highest prevalence.[16] At lower levels of sport the reported prevalence includes: 43% Anabolic Androgenic Steroids (AAS) and 12% hGH or Insulin-like Growth Factor (IGF-I) in young male weight lifters.[17] Indeed it is suggested that 4 - 6% of adolescent male athletes and 1.5 - 3.0% of adolescent female athletes will have used AAS at some time.[18]

Non-sport related drug use

In addition to sport specific doping, social drug use is also evident amongst athletes, and an Italian study indicated that 18% of all positive results were for cannabis and 7% for cocaine which whilst constituting a positive test, were likely to be cases of social drug use rather than for performance enhancing purposes.[19] In May 2013, the WADA raised the in-competition threshold for marijuana (cannabis) tenfold to 150 ng/mL to avoid such use being included as a doping offence.[20] Likewise, the results from a study that questioned members and junior members of German national teams from 43 different sports, indicated a 7% prevalence of illicit drug use, and a study of over two thousand German adolescents, in which 15.1% indicated that they had used a WADA banned substance, cannabis was the most prevalent (13.2%),[21] followed by stimulants (2.4%), cocaine/heroin (2.2%) and Anabolic Androgenic Steroids (AAS) (0.7%).[22]

What is also evident from other studies is that the use of doping agents is not confined to those involved in competitive sport, but are also used for aesthetic reasons and 'body styling' in young and adult males and females, notably for the aforementioned increase in muscle mass and/or fat reduction, with AAS being the most common drugs used.[23-27] With scenarios such as the seizure of millions of doses of steroids and hGH by the US Drug Enforcement agency further indicating a

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widespread illicit use of doping agents.[23] Indeed, a Swedish study indicated that the use of AAS was more prevalent in society than in regulated sport and the use amongst Swedish high school pupils has been reported as 2.7% in males and 0.4% in females.[28,29] This concurs with other studies in which prevalence rates for high-school-aged students range between 1% and 3% across several countries.[30] Similar prevalence rates have been reported for tertiary education students from six developed countries, with a higher usage by males compared to females.[31] Furthermore, studies indicate a 3 – 11% use in US high school students, 4 – 12 % in US male adolescents and up to 2% in young US women,[32] and use by around 3% of young males in many 'western' countries,[33] with their use being more prevalent amongst body builders and weightlifters (3 – 5%).[33] AAS use has even been reported in younger age groups such as adolescent boys (1.7%) and girls (1.4%); as well as pre-adolescents where the prevalence was 1.2 – 3.0%.[35] At the other end of the age spectrum, the therapeutic prescription of AAS and related anabolic agents have the potential to benefit older adults through their anti-ageing properties, such as the prevention of sarcopaenia and, as a consequence, they are also taken illicitly by some adults without medical supervision for their perceived physical enhancement.[30]

The illicit access to these drugs by both those involved in competitive sport and those seeking physical enhancement for aesthetic reasons appears to be through a combination of: physicians, pharmacies (both with and without the required prescription), fellow gym users, external suppliers and the internet.[24] Indeed some studies indicate that more than 50% of AAS users in some groups acquire their drugs through physicians.[24]

It is also interesting to note that a number of studies have reported an association between the use of sport performance/physique enhancing doping agents and other risk behaviours, such as increased alcohol intake and use of recreational drugs such as cannabis and cocaine.[36] Additionally, other studies have found an increased likelihood of adolescents using AAS to smoke, shared needles, possess a weapon, and exhibit suicidal behaviour, all of which result in further health risks.[37]

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Review of doping categories: their effects on physique, performance and health

S1 – Anabolic Agents

This category includes: Anabolic Androgenic Steroids (AAS) and other Anabolic Agents such as selective androgen receptor modulators (SARMs).

Anabolic Androgenic Steroids (AAS)

Anabolic and ergogenic properties

AAS are synthetic derivatives of the hormone testosterone, which is also included in this category and is the most prevalent AAS. Other commonly used AAS include: nandrolone decanoate, methandienone, stanozolol, androsterone, and androstane.[38] AAS are primarily used to increase muscle mass and as a consequence are associated with activities that require strength and high levels of peak power, such as weight lifting, throwing events and sprinting. They are also used by those seeking to increase muscle mass *per se*, which includes those seeking to attain a greater musculature and physical presence, as well as competitive body builders. Additionally. their use is known to extend to endurance athletes and cyclists who use AAS in smaller doses to increase red blood cell mass and haematocrit, which may augment oxygen delivery and utilisation, as well as aiding recovery.[39] Furthermore, the reported psychotrophic effects of AAS include the elevation of mood, determination and aggression, all of which may aid in training and competition, but may result in psychological health problems as well as inappropriate and undesirable behaviour in social as well as sporting contexts.[40]

The anabolic properties of AAS promote increases in muscle mass and aid recovery, whilst the androgenic properties promote masculinisation, which has particular health implications when taken by females due to their virilising effects. The production of endogenous testosterone is 20 – 30 times higher in males than females, which results in males having around a ten-fold greater blood testosterone level.[40] Hence it could be suggested that in terms of gains in muscle mass and strength, women have the capacity to gain a greater relative increase from AAS use than men: a feature that was evidently exploited in the GDR doping program.

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AAS may be taken orally, via intra-muscular injections or through topical gels and creams. The use of AAS as a doping agent involves doses that are considerably greater than those prescribed medically for therapeutic purposes, which has implications for research studies in which the use of therapeutic doses are unlikely to produce the performance benefits of the supraphysiological doses taken by athletes.[30] Hence many early studies failed to detect performance benefits, whereas more recent studies using supraphysiological doses provide evidence for their effects in enhancing muscle mass and strength.

As previously indicated, AAS are used by individuals seeking to become more muscular for aesthetic rather than sport performance goals and here their potential effectiveness is unequivocal.[41] However, whilst increases in muscle strength and aerobic capacity are evident, what is less certain is the extent to which these translate into improvements in performance, but the lack of unequivocal evidence may be due to the ethical and research design issues associated with undertaking double-blind clinical trials of a banned substance on competitive sportsmen and women.[40] Indeed, the prevalence of AAS use in these groups, despite their use being banned and the reported health risks, would suggest a belief in their effectiveness amongst elite sportsmen and women.

Health risks

As indicated above, doping practices use doses of AAS that are considerably greater than those prescribed therapeutically, consequently many of the elevated health issues associated with doping are due to these supraphysiological intakes. Anabolic androgenic steroids use has been associated with adverse effects upon the cardiovascular system, liver, kidney, endocrinological and reproductive systems. The cardiovascular health issues include: elevated concentrations of low density lipoprotein cholesterol (LDL-C) and lower concentrations of high density lipoprotein cholesterol (HDL-C); increased triglyceride levels,[30, 33, 42-44] elevated systolic and diastolic blood pressure,[42, 43] which is reported in some studies but not others;[45] endothelial dysfunction;[46] increased concentration of clotting factors, thrombosis,[47] hyperinsulinaemia and reduced glucose

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tolerance;[30, 43, 47] left ventricular hypertrophy, [42, 43] cardiomyopathies,[30, 33] fibrosis and myocytolysis,[48] and polycythaemia.[49] Right as well as left ventricular function may be impaired, including slower diastolic velocities,[50] and there are reported cases of acute myocardial infarction and non-fatal and fatal ventricular arrhythmias.[30, 42, 48, 49 51] These aforementioned changes, which represent an increased risk of atherosclerosis and other cardiac pathologies may be evident for some time after AAS use has been discontinued and damage to vital organs may be permanent.[23, 42, 45]

AAS use is also associated with alterations to liver function, cholestatic jaundice, peliosis hepatis, hepatocellular hyperplasia and hepatocellular adenomas, with these changes being linked to the use of orally taken 17 α-alkylated AAS.[30, 44] Additionally there are reported increased risks of liver tumours with the death from hepatic carcinomas of some athletes being linked to AAS use.[44, 52] Similarly renal dysfunction and Wilms tumours have been reported in athletes using AAS.[44, 53]

The adverse effects on the male reproductive system relate to the effects of exogenous AAS suppressing the hypothalamic-pituitary-testicular axis (HPT) and reducing the levels of the gonadotrophic hormones, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which ultimately reduces the levels of circulating endogenous testosterone.[43] There is also a reduction in sex hormone-binding globulin (SHBG).[30] These endocrine changes are associated with reductions in testicular size, sperm count, sperm motility and changes to sperm morphology.[30, 44, 54] However, in many cases these changes appear to be reversible upon cessation of AAS use.[33] In males there are also reports of prostatic hypertrophy,[55] and AAS use is also associated with gynecomastia, due to the peripheral conversion of androgens to estradiol and estrone.[44] Indeed anti-estrogenic drugs are taken by some AAS users to ameliorate this effect.[55]

In females AAS use can result in menstrual abnormalities and virilisation, such as: deepening of the voice, breast shrinkage, male-pattern baldness, increased libido, acne, body hair, and an increase in the size of the clitoris, with some of these changes not being reversible.[30, 44]

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Other effects in both men and women include the androgenic stimulation of the sebaceous glands, resulting in acne, which can be severe, affecting the face, back, shoulders and chest.[44, 56] Additionally, there have been reports of spontaneous subdural haematomas in weight lifters using AAS, which may have caused vascular changes that made the individuals vulnerable to these events when performing the valsalva manoeuvres whilst lifting.[57] Other physical consequences of AAS use may include an increased risk of tendon injuries, as some researchers suggest that the muscles may increase in strength disproportionately to the tendons, which then become vulnerable.[44] Case studies have also reported that AAS use in adolescents may cause premature epiphyseal closure of the growth plates in the long-bones thereby preventing full stature being attained.[30.44]

Furthermore AAS use has been associated with adverse mental health, including aggressive behaviour, commonly referred to as 'roid rage',[44,58] and prior use of AAS has been associated with a 2 to 4 fold increased risk of suicide in former athletes.[59] Other studies have suggested a link between the use of AAS and other health behaviours, such as high alcohol intake and the use of illicit recreational drugs.[60]

Clinical implications

With the aforementioned health effects come changes to routine diagnostic laboratory test results, i.e. medical testing conducted outside of the WADA testing laboratory. As the prevalence of AAS use is thought to be high, embracing all levels of sport, it is likely that a proportion of pathologies seen in laboratory test results could be linked to AAS use. The clinical interpretation of laboratory test results is essentially about pattern recognition and Table 3 is provided to highlight potential analytes that may be altered in association with doping, in particular AAS. In addition, Figure 2 provides the relationship of the endogenous steroid hormones and their metabolites measured in various clinical diagnostic settings.[61]

As part of the differential diagnosis, abnormal hormone patterns observed in laboratory test results should include consideration of AAS administration. Case examples highlighting the pattern of low testosterone with decreased LH levels demonstrate the difficulties faced with the

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interpretation of results, as exemplified in Table 4.[62,63] After exclusion of other causes, even when a patient emphatically denies taking AAS, consideration should be given to the patient unwittingly taking substances as part of other apparently non-hormonal nutritional supplements. This is a valid consideration as up to 15% of "non-hormonal" nutritional supplements have been found to contain varying concentrations of anabolic steroids purportedly through cross contamination during the production process.[64]

Other Anabolic Agents

Other anabolic agents include Dehydroepiandrosterone (DHEA), which is secreted by the adrenal glands and is a precursor to testosterone. Whilst it is reported to be taken by individuals seeking to enhance their muscle mass, research studies on its effectiveness in male athletes are equivocal. It is also taken for its suggested anti-aging and anti-obesity properties, but again its effectiveness remains unclear.[30] In women it appears to increase the circulating concentrations of testosterone and thereby increase the health issues associated with virilisation, including acne and hirsuitism, as well as reducing HDL-C, insulin sensitivity and glucose tolerance,[30] which have implications for increased risk of Type 2 diabetes. Through its influence on the concentration of circulating testosterone, the health risks associated with DHEA are accordingly those stated above for AAS.[55]

Selective Androgen Receptor Modulators (SARMs) are drugs that may be taken to enhance the action of the testosterone receptor,[43-45] and thereby attain a greater anabolic effect. Clinical trial reports related to non-steroidal selective androgen receptors suggest that they are likely to induce the desired anabolic effects of circulating anabolic hormones, whilst minimising the often unwanted androgenic effects that present with AAS, such as the virilisation of women and feminisation of men.[65] To date the health issues associated with their use remain unclear. However, it is suggested that if they are taken without AAS, the individual may gain some hypertrophic benefits to the muscles, without incurring the aforementioned health risks associated with AAS use. This would be a result of the SARMs acting on the receptors within muscle and

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bone, but not affecting other organs such as the prostate or virilising other tissues.[23] Clenbuterol is another anabolic agent, but is discussed under the category of beta 2 agonists.

S2 – Peptide Hormones, Growth factors and Related Substances

This category includes:

(i) Erythropoiesis-stimulating agents, such EPO;

(ii) Chorionic Gonadotrophin (CG) and LH and their releasing factors in males;

(iii) Corticotrophins and their releasing factors; and

(iv) Various growth factors, including but not limited to hGH and its releasing factors and IGF-1.

Erythropoietin (EPO)

EPO is a glycoprotein hormone that is produced endogenously by the kidney and to a lesser extent (<10%) by the liver.[66] Its function is to promote the production of erythrocytes (erythropoiesis). Under normal aerobic exercise conditions, transient hypoxia is detected by cells within the kidney and liver, with a resultant increase in EPO production. This stimulates the proliferation and differentiation of erythroid precursor cells in the bone marrow and results in the post-exercise generation of additional erythrocytes.[23] In turn, this enhances the capacity to deliver oxygen to the exercising muscles in subsequent exercise sessions. With recombinant EPO (rhEPO) being developed and becoming available in recent decades this training effect can be augmented pharmacologically, resulting in even greater red blood cell production and haemoglobin mass.

The detection of rhEPO use has been problematic, with both endogenous and rhEPO having the same amino acid sequence. However in some versions of rhEPO there are small differences in the side chains with rhEPO exhibiting fewer sialic acid residues due to glycosylation, although the recent increase and widespread production of rhEPO has resulted in other variations which further complicate the detection process.[67] Recent developments in anti-doping strategies now include an athlete's 'Biological Passport'. This requires a record to be kept of the results of several blood samples and new samples to be compared against the athlete's historical record.

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Experts evaluate the records to determine whether any changes to the athlete's blood profile suggest doping. Those with suspicious profiles can then be targeted for additional testing by the anti-doping agencies.[67-69] Other doping methods that have the potential to enhance oxygen delivery to the muscles are covered in section M1.

Ergogenic properties

Clinical studies with trained athletes have shown rhEPO to increase haemoglobin mass from 12.7 ±1.2 g/Kg to 15.2 ± 1.5 g/Kg.[70] This increases the capacity to utilise oxygen (VO_2 max) by around 5 – 10% and reduce the time to run 3000 metres by approximately 6%.[70,71] Perhaps the most infamous use of rhEPO has been in the Tour de France, with several prominent cyclists later confessing to using rhEPO [72] and subsequent revelations about rhEPO use by seven times Tour de France winner Lance Armstrong are well publicised.[73] The endemic use of EPO in cycling events is supported by the experiences of elite cyclist Tyler Hamilton [74] and whilst some individual riders had been withdrawn or suspended from the race in previous years its systematic use by teams was revealed in 1998 tour when one team was ejected from the race for using EPO and six other teams quit the event.[75] Other sports in which rhEPO use have been implicated include endurance running and endurance events in the Winter Olympics.[23]

Health issues

The use of rhEPO results in an increase in red blood cell mass that is greater than an increase in the plasma volume, hence the blood becomes more viscous,[76] and studies have demonstrated haematocrit increasing from 42.7 ± 1.6% to 50.8 ± 2.0%.[71] Notably an haematocrit >50% has been used in some sports, such as cycling, as a threshold, above which the individual is not permitted to participate, the inference being that they may have doped.[67] From a health perspective the increased blood viscosity is believed to increase the risk of thrombosis, which may be further elevated if the person becomes dehydrated.[52] Indeed some authorities have implicated rhEPO abuse with the death of competitive cyclists and other endurance athletes,[55] although the association does not appear to have been proven unequivocally.[77] Other reported

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health risks include: hypertension and headaches,[22] and some authorities suggest that rhEPO use may be associated with the development of some cancers.[52]

hCG and hLH in males

In males, endogenous hCG and hLH act on the Leydig cells of the testes to stimulate testosterone production.[78] Because of this action, hCG and hLH and their recombinant versions (rhCG and rhLH), have the potential to be used as doping agents by males to promote the body's own production of testosterone and epitestosterone. The use of hCG/rhCG and hLH/rhLH in this way may thereby circumvent the risks of detection associated with using exogenous testosterone since both testosterone (T) and epi-testosterone (ET) are increased. This helps to maintain the normally occurring ratio of T/ET at around 1 - 2 and avoids the problem of a T/ET ratio exceeding six, which can occur when exogenous testosterone is used [79] and such test results initiate further investigations.[23, 80] However, whilst a high T/ET ratio is used as an indicator of a possible doping offence by anti-doping authorities, it is acknowledged that some individuals have naturally occurring low levels of epi-testosterone which result in an elevated ratio.[81] Alternative tests involve assessing the T/LH ratio on the basis that the use of AAS suppresses LH production and thereby elevates the T/LH ratio.[81,82] Recombinant hCG may also be taken by males to restore endogeonous T production by the testes, which may be suppressed through the use of exogenous AAS.[83] In males hCG/rhCG and hLH/rhLH concentrations above certain levels suggest a possible doping violation, however it is also known that hCG can become elevated in cases of testicular cancer.[39,83] Indeed there have been occasions when a doping test has resulted in tumours being identified in athletes who were unaware of their condition.[84]

In females, endogenous hCG and hLH stimulate progesterone and estradiol production by the ovaries. Endogenous hCG increases in pregnancy and the hLH surge in the menstrual cycle triggers ovulation. Consequently these hormones have different levels and functions for males and females. In females hCG/rhCG is not believed to substantially alter T levels.[23] Therefore, for this and the previously indicated confounding reasons, which include an intrusion of privacy,

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neither hCG/rhCG nor hLH/rhLH are believed to be effective as doping agents in females nor are they systematically tested for.[85]

Corticotrophins

These are hormones secreted from the anterior pituitary gland that act on the adrenal cortex to influence the secretion of various hormones. The key hormone is Adrenocorticotrophic hormone (ACTH) and its primary function is to promote the release of corticosteroids such as cortisol (see section S9). The performance effects of corticotrophins are equivocal.[86]

hGH and IGF-1

Human Growth Hormone is a polypeptide hormone that affects many metabolic activities within the body, notably the growth and division of cells. Endogenously it is secreted in a pulsatile pattern from the anterior pituitary whilst exogenous sources include synthetic recombinant Growth Hormone (rhGH), which is difficult to distinguish from endogenous sources. Endogenous production of hGH has been shown to increase in response to high intensity aerobic exercise.[87] Some of the indirect effects of hGH are mediated IGF-1, which is produced in the liver and up-regulated by hGH. Among its numerous effects, IGF-1 promotes amino acid uptake and protein synthesis in muscles, which makes it an anabolic agent increasing muscle mass and strength.[23] However in contrast to hGH, it can cause hypoglycaemia and does not appear to be lipolytic.[88] Unlike the pulsatile release of hGH levels, IGF-1 serum concentrations are less variable across a day.[23]

Anabolic and ergogenic properties

Medically, exogenous hGH (including rhGH) promotes muscle growth in people with growth hormone deficiency (GHD). This has led to its use as an anabolic doping agent by bodybuilders, weightlifters and people involved in sports requiring high levels of strength and power.[89] It is also taken for its lipolytic effects that facilitate the loss of body fat, which also makes it attractive to body builders. However the extent to which the increases in muscle mass translate into improved sports performance are unclear.[23,88,90-92] Some authorities question whether there is strong

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evidence for its effectiveness if taken as a sole doping agent by those who are not GHD, although they do suggest that it could have a synergistic effect if taken in combination with AAS.[93] As with studies on AAS, clinical studies with hGH may use lower doses than those taken by sports performers and hence may not provide a true reflection of its ergogenic potential.[91] The use of rhGH to benefit aerobic/endurance exercise lies in its potential to enhance lipolysis and thereby spare muscle glycogen, which is a limiting factor in prolonged (>2h) exercise.[94] However, despite the equivocal nature of the clinical evidence, hGH is being used by athletes across a range of sports events from the 100m sprint to the Tour de France; and the conviction of Sylvester Stallone for possession of hGH when entering Australia would suggest that its use extends beyond the sporting arena.[95]

Health issues

The use of hGH in supra-physiological doses, which in athletes may be ten times higher than the therapeutic dose,[95] is known to cause fluid retention due to its effects on increased sodium retention by the kidneys, with peripheral oedema resulting in swollen hands and feet as well as headaches and hypertension. It may also cause carpal tunnel syndrome and long term use can produce aspects of acromegaly[54] (the abnormal growth of the bones) which occurs in those with inherently elevated levels of hGH. Additionally, since acromegaly is associated with muscle weakness it has been argued that it could theoretically have an adverse effect on performance,[52] although its widespread use by power athletes and bodybuilders would suggest otherwise.[23]

Human Growth Hormone abuse has also been reported to increase the risk of cardiomyopathy, possible arrhythmias, insulin resistance that can lead to diabetes mellitus, bone abnormalities, adverse lipid profiles, acute renal failure and osteoarthritis.[23,52,55,96] As a doping agent it can be obtained as synthetically produced recombinant human growth hormone (rhGH), but has also been available from cadaveric sources, which incur the risk of contracting Creutzfeldt-Jacobs disease.[93,95]

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Some studies report the adverse effects of IGF-1 to be hypoglycaemia, myalgia and fluid retention,[97] whilst others indicate that the long term effects of its abuse are largely unknown.[98] Growth hormone and IGF-1 abuse have also been associated with colon, breast and prostate cancers.[52,77]

S3 - Beta 2 agonists

Adrenaline and noradrenaline are catecholamine hormones that are released from the adrenal medulla and also function as neurotransmitters. Their secretion increases at times of stress and facilitates a physiological response to a situation. In a sporting context this relates to increases in cardiac output, vasodilation, ventilation and circulating glucose, with the response being proportional to the intensity of the exercise. These catecholamines bring about their effects through binding to ß-adrenoceptors (ß-AR), both $ß_1$-AR and $ß_2$-AR.[99] These ergogenic properties have been exploited by the use of doping agents in the classes of 'beta 2 agonists' and 'stimulants' (see section S6).

Beta 2 agonist drugs that have the potential to be doping agents in sport focus on the $ß_2$-AR in the brain and peripheral tissues. Stimulation of these receptors result in diverse effects such as, bronchodilation, anabolic actions and the enhancement of anti-inflammatory corticosteroids.[99] Beta 2 agonist drugs may be inhaled or taken orally and are commonly used as medications to treat and prevent asthma. WADA prohibits the use of beta 2 agonists, including bronchodilators unless the participant has a 'therapeutic exemption', which must be applied for.[100] This exception covers a limited list of drugs such as formoterol, salbutamol, salmeterol and terbutaline when taken via inhalation. Studies suggest that these drugs do not appear to have an ergogenic benefit when inhaled, but salbutamol may improve strength and endurance if taken orally.[101] One beta 2 agonist that has been used as a doping agent in sport is clenbuterol. Clenbuterol is a non-steroidal anabolic agent that increases muscle mass, which would explain its use by weightlifters and other strength athletes. It is also used in the livestock industry and some recent reports suggest that eating the meat of animals given clenbuterol could result in a positive test.[102]

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Ergogenic effects

In terms of performance benefit for the elite non-asthmatic athlete ß$_2$- agonists do not improve aerobic capacity (VO_2 max), performance in endurance events or peak cycling power.[103] Similar results were found by Elers and colleagues who also found no effect on oxygen kinetics.[104] However Pluim et al.'s analyses of studies that had used systemic ß$_2$- agonists produced equivocal results, with some studies indicating that they could benefit endurance performance and cycling sprint power, and others concluding that oral ß$_2$- agonists, combined with resistance training could improve strength.[103,105] Hence the therapeutic inhaled doses of ß$_2$- agonists do not appear to be ergogenic, but systemic and supra-physiological doses may enhance the effects of strength training and benefit endurance performance.

Health risks

Clenbuterol and salbutamol are the most commonly reported doping agents in this class, with clenbuterol in particular being taken for its anabolic properties. Since beta 2-AR agonist drugs bind to the ß$_2$-AR in the heart they thereby elicit health risks such as cardiac arrhythmias, palpitations and myocardial ischemia.[106] They are also reported to cause muscle tremor and may increase circulating glucose concentrations due to their action on the liver. Additionally clenbuterol has also been associated with reducing bone mineral content.[91]

<u>S4 – Hormone and Metabolic Modulators</u>

The prohibited list divides this category into five groups:[2]

(i) Aromatase inhibitors

(ii) Selective estrogen receptor modulators (SERMs)

(iii) Other anti-estrogenic substances

(iv) Agents modifying myostatin function(s)

(v) Metabolic modulators

Aromatase inhibitors, selective oestrogen receptor modulators (SERMs) and other anti-estrogenic substances

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These drugs are used clinically in the treatment of breast cancer or other tumours that are hormone dependent. They are also used in the treatment of osteoporosis. In males oestradiol is produced by the aromatization of testosterone and this oestradiol plays a key role in the negative feedback regulation of testosterone production via the hypothalamic-pituitary-testicular axis. Oestrogen blocker drugs include anti-oestrogen drugs that block oestrogen receptor action and aromatase inhibitors that block the synthesis of oestradiol. Hence when males take these drugs the negative feedback process is interfered with, resulting in increased secretion of gonadotrophins from the pituitary and a subsequent increase in circulating testosterone.[40] They may therefore convey an ergogenic benefit to men from the elevated levels of testosterone. Additionally, they may be taken by men trying to prevent the development of gynecomastia, which occurs with AAS abuse that results in elevated oestrogens.[23] Due to the physiological and endocrinological differences between men and women, oestrogen blocker drugs are unlikely to increase testosterone levels in women to levels that would measurably increase muscle mass or strength. When used inappropriately the health risks include cardiac arrhythmias, dizziness, osteoporosis and joint pain. Some can also result in breathlessness.

Agents modifying myostatin function(s)

Myostatin (growth differentiation factor 8 (GF-8)) is a naturally occurring hormone that regulates muscle growth. Its function is to limit growth and hence muscle mass. Naturally occurring genetic mutations occur in which the myostatin is non-functional and this results in an abnormally large muscle mass, with homozygous conditions being more extreme than the heterozygous condition. This is exploited in the livestock industry with the Belgian Blue and Piedmontese cattle breeds in which abnormalities in the myostatin genes result in cattle with greater muscle mass and hence increased meat production. Further examples occur in greyhounds and there are a few recorded examples in humans. Clinically, myostatin inhibitors endeavour to inhibit the normal action of myostatin and thereby have the potential to treat muscle wasting conditions such as sarcopaenia, muscular dystrophy and cancer cachexias.[23] As doping agents this inhibitory effect may enable muscle hypertrophy to exceed that attainable under non-doping conditions.

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Metabolic modulators

Metabolic modulators include insulins, and Peroxisome Proliferator Activated Receptor δ (PPAR δ) agonists. Insulin is a key regulator of glucose levels within the blood, and is secreted by the β cells of the pancreas primarily in response to high blood glucose concentrations. Clinically, it is used by insulin dependent diabetics whose endogenous production of insulin is insufficient or non-existent. The use of exogenous insulin by non-diabetics can lower blood glucose to hypoglycemic levels, resulting in the risk of dizziness and coma in more extreme cases. As a doping agent, insulins may increase the rate of glucose uptake into the muscles and thereby aid recovery by facilitating muscle glycogen replenishment. In addition to their hypoglycemic properties, insulins have an anabolic action, which makes them potential doping agents for increasing muscle mass.[23,107] However, whilst there are some reports of insulin abuse it does not appear to be as widespread as other anabolic agents.

Peroxisome Proliferator Activated Receptor δ (PPAR δ) agonists specifically mentioned in the WADA prohibited list are 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR; peroxisome proliferator–activated receptor-δ [PPAR-δ]-AMPK agonist) and GW1516 (PPARδ-agonist).[2] Their inclusion on the prohibited list relates to their effect in augmenting the adaptations to endurance training, such as the enhancement of mitochondrial biogenesis, angiogenesis and insulin sensitivity[108] , with the overall outcome being muscle fibres with a greater aerobic capacity and greater fatigue resistance. Mechanistically PPARδ are believed to be involved with AMPK and PGC-1α responses, which have well established roles in the promotion of biogenesis post-exercise.

Health risks

As mentioned previously, one of the consequences of AAS abuse is their conversion to oestrogens, with a resultant risk of gynaecomastia. To combat this, oestrogen anatagonists such as SERMS may be used, as are aromatase inhibitors that inhibit the synthesis of oestrogen. The health risks associated with the specific use of these drugs as doping agents by 'healthy' individuals is currently unclear, since they are taken in combination with other doping agents.

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However it is suggested that oestrogen blocking agents and aromatase inhibitors do not have direct androgenic effects, and therefore do not convey the health risks associated with the use of androgens such as AAS.[40] The health risks associated with the use of myostatin inhibitors are likewise unclear at this stage, but their potential for development as therapeutic agents means that their use may become more widespread in the future.

S5 - Masking Agents

Masking agents are not considered performance-enhancing, but they are taken to conceal the use of other doping agents and abuses. They include diuretics, epi-testosterone, probenecid, 5α-reductase inhibitors and plasma expanders. The mechanism of action varies, with the intention being to reduce the concentration of the doping agent or its metabolites in the sample through increasing the volume of urine or reducing the rate of excretion of the doping agent/metabolites into the urine; or alternatively by interfering with the parameters used by anti-doping labs to identify doping offences.[109]

Diuretics

Therapeutically, diuretics are used to increase urine production and sodium excretion. They are prescribed for a variety of conditions, such as hypertension, heart failure, liver, kidney and lung diseases.[110] Diuretics promote urine production. As doping agents they are used in a number of sporting context for a variety of reasons, with the 2008 WADA laboratory statistics indicating that nearly 8% of positive samples involved diuretics.[111] The diuretic induced increase in urine production has two effects that may be exploited by the doping athlete. Firstly it can produce a rapid and temporary weight reduction when endeavouring to make a weight category in sports such as boxing. It may also be used to counteract fluid retention, which can occur when using other drugs, notably in body building where the fluid excess would conceal the definition of the musculature. Secondly the increased urine volume will lower the concentration of doping agents and/or their metabolites in a urine sample, with the intent of reducing the concentration to below detectable levels. Thirdly, some diuretics can alter urinary pH and inhibit the excretion of some drugs into the urine, thereby lowering their concentration in urine samples.[110]

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Health risks

The use of diuretics either to reduce weight or mask the use of other doping agents through the production of copious amounts of dilute urine, entails the risk of dehydration and excessive loss of minerals such as potassium and calcium.

Probenecid

Therapeutically, probenicid is used to treat chromic gout and may also be prescribed alongside antibiotics for the treatment of some bacterial infections. Probenecid acts on the renal tubules of the kidneys to increase the excretion of uric acid and reduce the excretion of some acidic compounds.. In a doping context this would include a reduced excretion of testosterone, epi-testosterone and AAS, which are excreted mainly as their glucuronic acid compounds. In such contexts the purpose of using probenecid is to lower the concentrations of exogenous doping agents and their metabolites to below the detectable levels.[109] It may also interfere with the levels of endogenous T and E in urine samples.

Plasma expanders

Clinically, plasma expanders, in the form of colloid solutions containing dextrans, are used in situations of blood or fluid loss. As doping agents they may be used to mask the use of EPO and blood infusions. The rationale for their use is that EPO and blood infusions will increase the haematocrit and Hb levels, which are advantageous in endurance sports. Sports authorities set thresholds for haematocritand the concentration of haemoglobin, above which a possible EPO and/or blood transfusion doping offence is indicated. Plasma expanders will temporarily dilute the blood, lowering the haematocrit and Hb concentration, thereby bringing them below the levels that are used as indicators of doping.

<u>M1 – Manipulation of Blood and Blood Components</u>

Autologous and allogenic Blood Doping

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As previously stated, increasing RBC elevates the capacity of the body to deliver oxygen to the exercising muscles and increases aerobic performance.[112,113] Key to this improvement is the increase in the total Hb mass rather than [Hb] or Hct, although these tend to increase alongside the increase in Hb mass.[114,115] Consequently drugs such as rhEPO are banned (see section S2). Prior to the availability of rhEPO in the 1980s, endurance athletes and cyclists are known to have achieved an increase in RBC via the transfusion of matched blood from another person (homologous or allogenic blood doping) or through the reinfusion of their own blood, which had been removed around 4 weeks previously and then reinfused 1 – 7 days before the event (autologous blood doping). Since the development of tests to detect rhEPO and allogenic blood doping in the early 2000s,[113] autologous blood transfusion has made a resurgence amongst those wishing to avoid detection.[116] Tests to detect autologous doping assess inconsistencies in the blood profile of athletes in accordance with the Athlete's Biological Passport, in which a record of their blood profile from previous tests has been recorded. Longitudinal comparisons of the athlete's blood involve assessing a combination of hematologic markers.[117] These tests work on the basis of detecting altered erythropoiesis and include measures of total mass of haemoglobin and the ratio between the amount of Hb in the mature erythrocyte population and the reticulocytes (RBCHb:RetHb ratio).[116,118,119] Other suggested markers relate to the body's response to the withdrawal of blood (anaemic phase) in which [Hb] is reduced, whilst EPO, % reticulocytes and sTfR increase for a number of days until the blood loss is replenished.[120] Another approach for the detection of autologous blood doping is to test for markers that indicate that blood has been in storage. These include decreases in 2, 3-bisphosphoglycerate, altered cell membrane structure,[112] changes to gene expression related to T lymphocytes that occurs due to a transfusion induced immune response, and the presence in the urine of metabolites of plasticisers such as di-ethylexylphthalate (DEHP) that have leaked from the bags in which the blood was stored.[121,122]

Ergogenic properties

A review of early studies conclude that blood doping using 1 – 3 units of blood increased Hct by up to 13%, improved aerobic capacity (VO_2 max) by 0 – 10% and endurance by 3 – 37%.[123]

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These figures were supported by later studies. In the 1984 Los Angeles Olympics, some members of US cycling team utilised blood transfusions and achieved exceptional performances including nine medals; the US having not won a cycling medal in the previous 72 years of the games.[124] The more recent revelations around the Tour de France further support the efficacy of this practice in enhancing aerobic performance in cycling.[74] These doping practises extend into other sporting arenas, such as distance running and endurance skiing events.[121]

Health risks

Health issues relate to the risks of infection and in cases of allogenic blood doping, the risk of transferred infection and mismatching of blood.

Artificial enhancement of oxygen uptake, transport and delivery

Other potential agents that enhance oxygen delivery to the muscles include synthetic O_2 carriers such as haemoglobin-based oxygen carriers (HBOCs) and perflurocarbons (PFCs).[72,122] However, unlike endogenous Hb, which releases more of its oxygen to the tissues in hypoxic conditions, the aforementioned exogenous artificial agents do not, which may limit their effectiveness. They also have adverse side-effects and may result in tissue damage, which would not make them conducive as doping agents.[72] Likewise, whilst endogenous 2,3-diphosphoglycerate (2, 3 – DPG), which promotes oxygen release to the tissues is clearly beneficial to the endurance athlete, artificial 2, 3 DPG mimetics do not as yet appear to be effective in a sporting context due to easy detection and a short half-life.[72] However others suggest that efaproxiral, which alters the haemoglobin-oxygen saturation curve, may be of benefit.[122]

<u>M2 – Chemical and Physical Manipulation</u>

This refers to tampering with a sample taken as part of the anti-doping testing procedures, in order to alter its integrity and validity. It also includes the adulteration of samples by the addition of proteases and other chemicals that alters parameters of the steroid profile, a characteristic that appears to be relatively consistent within individuals, but is altered by the use of doping agents.

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For the interested reader, aspects of this were investigated and are described in detail by Kuzhiumparambil and Fu.[125] "Intravenous infusions and/or injections of more than 50 mL per 6 hour period except for those legitimately received in the course of hospital admissions or clinical investigations" are also prohibited.[2]

M3 - Gene Doping

The capacity to attain an elite level of performance in sport has a genetic component, and it is said that to become an elite athlete you need to choose your parents very carefully. The current human gene map for performance and health-related fitness phenotypes identifies over 200 genes that appear to be associated with athletic performance:[126] some phenotypes favouring endurance, whilst others favour strength, anaerobic power and sprinting.

Gene therapy is a concept that has the potential to treat genetically based diseases such as Cystic Fibrosis,[127] Duchenne Muscular Dystrophy and many others. It works primarily on the principle of adding a functional gene and/or genetic material into the cells of the recipient and thereby enabling the expression of a gene. This may increase the number of copies of the gene within these cells, or provide a functioning form of the gene to cells when the recipient's own genotype includes a less effective or non-functioning mutated version of that gene. The means by which this new genetic material gets into the cells is usually via a virus (viral vector) with the virus being introduced into the body via injection or nasal spray. Once in the target cells the introduced genetic material will be activated to produce the mRNA and protein that the cell/body currently lacks. To date there are relatively few examples of effective gene therapy, but as a concept it has great potential.

In a sporting context, the principles of gene therapy have been foreseen as having the potential for abuse as a doping procedure. For example, in theory it could be abused to enhance an athlete's production of proteins such as EPO,[128] hGH and IGF-1,[129] vascular endothelial growth factor (VEGF) and myostatin antagonists that will promote muscle hypertrophy.[130] So, whilst there are no recorded cases of gene doping or even its efficacy in enhancing performance, the

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procedure has been added to the WADA banned list. Furthermore, the process of gene doping, which has the potential to temporarily enhance or suppress the production of key proteins could be further developed into genetic enhancement, whereby the introduced genetic material becomes fully integrated into that of the recipient and is consequently replicated with the host's own genetic material and is thereby be perpetuated with each cell division. If such procedures become a reality they may again convey great benefits in a therapeutic setting, but would also be open to abuse in sport. For example, as the details of the human genome are further elucidated, the influence of specific genetic polymorphisms upon the individual's aerobic capacity and endurance or ability to produce high anaerobic power and sprint become evident,[126] manipulating the genetic make-up of the individual has the potential to augment their performance.

In the context of health risks, the science of gene therapy is in its infancy with relatively few successful clinical trials, but even these have exposed risks with the procedures, and some of these have fatal consequences, even in carefully regulated clinical trials.

S6 – Stimulants

Stimulants are a diverse group of banned drugs that are 'banned in competition'. They have the confounding aspect of including drugs that whilst having possible ergogenic properties may also be found in over the counter medicines and/or be taken as 'recreational drugs'. The former would include ephedrine and pseudoephedrine, whilst the later would include cocaine and ecstasy. Studies indicate that stimulants account for around 6 – 18% of the positive samples detected in the sporting context, which makes them the 3rd most prevalent category, behind AAS and cannabinoids.[131] The stimulant caffeine, which is included in a number of sports drinks and gels and generally consumed broadly around the world, was removed from the WADA banned substance list in 2004 but is briefly considered in this review.

The potential ergogenic properties of stimulants primarily relate to their effects on the central nervous system and their capacity to: reduce the perception of fatigue; increase alertness, promote self-efficacy and confidence; and in some cases stimulate cardiac output and blood flow

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to the exercising muscles. Typically, they work via their pharmacological effects on increasing the release of neurotransmitters, blocking the re-uptake of neurotransmitters, and the activation of receptors. Some mimic the responses of the sympathetic neuroendocrine system, notably adrenaline and noradrenaline, whilst others affect the dopamine and serotonin systems.[132] WADA list over 60 specific drugs within this category and include a general statement aimed at covering similar drugs with similar properties.

Evidence for the ergogenic efficacy of many stimulants on sport performance is often equivocal and the use of such drugs can entail serious health risksincluding hyperthermia, stroke, respiratory and cardiac arrest.[99] A difficulty with clinical research on the ergogenicity of stimulants is that the laboratory/experimental setting can rarely, if ever, fully mimic the competition environment. As a consequence an enhanced performance in experimental trials, when arousal may not be maximal, may not translate into the competition environment when the athlete's arousal is likely to be higher. Within this review, a few examples will be mentioned briefly to illustrate the characteristics of different stimulants. For a more extensive coverage of the pharmacological aspects, see the review by Docherty.[132]

Amphetamines

Amphetamines may be used in sport to reduce the perception of fatigue and promote concentration, alertness and self-confidence. They enhance the brain activity of noradrenaline and dopamine.[133] They also increase blood pressure and peripheral vasoconstriction.

Health risks

Their use has been associated with ventricular dysrhythmias, increased blood pressure and peripheral vasoconstriction.[76] As a consequence they increase the risk of tachycardia and cardiovascular failure, as well as reducing the body's capacity to lose the heat generated during strenuous exercise, which may result in hyperthermia.[134] Some fatalities in endurance cycling events have been attributed to this, including the televised death of Tommy Simpson in the 1967 Tour de France. Other health risks include anxiety, hallucinations and insomnia. [32,134] Their

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regular use can result in tolerance and dependence that may cause depression following withdrawal.[134]

Sympathomimetics (ephedra alkaloids including ephedrine and pseudoephedrine).

This group includes the drugs ephedrine and pseudoephedrine, which are agonists at both α- and β-adrenergic receptors, increasing heart rate and blood pressure.[135,136] Ephedrine and pseudoephedrine are sympathomimetics commonly included in decongestant drugs. WADA monitor these substances on the basis of a urine concentration threshold due to their presence in many over the counter medicines, including those for nasal congestion.[2] The concentration for ephedrine is set at >10 micrograms per millilitre and pseudoephedrine is set at >150 micrograms per millilitre. Ephedrine acts on the CNS promoting the release of noradrenaline,[134] with resultant increases in heart rate and blood pressure. Other ephedra alkaloids have similar properties and may be found in traditional Chinese medicines, examples being the herb *ma huang* or *guarana*.[137] They may be abused by athletes because of a perceived benefit to strength, aerobic performance and even fat loss, however studies on performance are equivocal, although there is some evidence that they may convey performance benefits if taken with caffeine.[136]

Health issues

Reviews of sympathomimetic drugs indicate that their use has been linked to a number of adverse cardiovascular events and fatalities, including stroke, arrhythmias and myocardial infarction, as well as hypertension.[134,135]

Cocaine

Cocaine is reported to reduce the perception of fatigue, and promote confidence and euphoria, which may aid performance. It exerts its effects via the CNS, increasing the release of catecholamines, dopamine and inhibiting the reuptake of noradrenaline.

Health risks

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The health risks of cocaine are reported in the wider context of its use as a recreational drug and include: elevated heart rate and blood pressure, myocardial ischemia, myocardial infarction, arrhythmias and cardiac arrest even in the absence of atherosclerosis.[134,135] It's considered highly cardiotoxic when taken with alcohol.[134] It can also adversely affect the vascular, pulmonary, gastrointestinal, musculoskeletal and genitourinary systems, as well as causing cerebrovascular events.[134,138] Additionally, its vasoconstrictive action may adversely affect thermoregulation.[76]

Caffeine

Caffeine is a stimulant that is not currently banned by WADA, despite its proven ergogenicity. In the past it was included on the banned list at urine concentrations above (12 µg/mL), on the basis that concentrations below this level may be attained from the consumption of coffee, coca cola and similar sources, whereas above this concentration indicated a deliberate consumption, probably via tablets, with the intent of performance enhancement. It was removed from the 'banned list' in 2004 but is still subject to monitoring, although it should be noted that the ergogenic benefits for a range of sports appear to be attained at modest doses (3 mg/kg) doses that are easily achieved via intake of everyday dietary sources such as coffee, cola drinks and energy drinks. Indeed, the ergogenic properties of caffeine are broad and evident at relatively low doses of 3 $mg \cdot kg^{-1}$ body mass. The performance benefits include: reductions in the perception of fatigue and direct effects on muscle contractility, although previously claimed mechanisms such as the promotion of fat as a fuel sources during endurance events is no longer considered important.[139]

S7- Narcotics

The category of narcotics includes substances such as morphine and related compounds. Morphine is known for its analgesic effects in increasing tolerance to pain, which may thereby improve performance. Another notable drug in this category is heroin, the health risks of which are reviewed elsewhere in the context of its wider use in society.

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S8 – Cannabinoids

Natural and synthetic cannabinoids are banned 'in competition' and there have been a number of high-profile cases in which athletes have produced positive samples following competition and been disqualified as a consequence. Due to their 'relaxing' properties, such drugs are likely to impair performance (ergolytic) rather than being ergogenic.[140] but a substance does not have to have proven ergogenic properties to be included on the WADA banned list, and the cannabinoids would tend to fall into this realm. Indeed, the reported reasons for taking them include relaxation and the promotion of sleep prior to competition. However, due to their effects on alertness, concentration and reaction time, they may present a safety problem for others as well as the user in some sporting contexts,[141] which is why they are banned. For the wider context, readers are directed to other reviews on the mental health consequences of taking cannabinoids.[142,143]

S9 – Glucocorticosteroids

Physiological glucocorticosteroids include cortisol and cortisone, which have an anti-inflammatory role. They are used to treat musculoskeletal and tendon injuries: a common example being hydrocortisone. In sport they are 'banned in competition' [2] due to their effects on pain relief and perhaps fatigue, which may aid performance, but may also exacerbate injury to the damaged tissues as the person is able to continuing loading the tissue in the absence of pain that would normally cause cessation of the activity.[144] In the past they may also have been taken under the belief that their effects on increasing gluconeogenesis and the mobilisation of amino acids and fatty acids would aid performance, although the evidence for this is equivocal.[145]

Health issues

Glucocorticosteroid injections are sometimes used within the treatment program for Achilles, quadriceps and hamstring tendons. However, whilst their use may accelerate the athlete's return to training and competition, there is some debate as to whether their use is also associated with an increased risk of subsequent rupture of the tendon due to degenerative processes and incomplete repair in the tendon.[96,144,146] This has raised concerns about the chronic use of non-steroidal anti-inflammatory drugs, which whilst producing short-term relief may retard complete

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healing and recovery of the tissue. In addition, the chronic use of glucocorticosteroids may increase the risk of diabetes mellitus, osteoporosis, necrosis of the femoral head and growth retardation in children.[76,96,147]

P1 – Alcohol

Within the WADA prohibited substances list alcohol (ethanol) is prohibited in competition in specific sports above a blood concentration of 0.10 g/L (i.e. 0.01%).[2] It is banned for reasons of safety to the participants and others, in sports including: aeronautics, archery, motorsport, karate and power-boating. The acute effects of alcohol consumption are generally ergolytic, but may be used by the participants in sports requiring 'boldness', such as downhill skiing and mountain biking to overcome inhibitions and thereby improve performance or perception of performance.[148] Its health issues will not be discussed here as they are extensively debated elsewhere and in the wider context of society.

P2 – Beta-Blockers

Beta-Blockers also come under a category of being prohibited in specific sports during competition. Since their effects are antagonistic at the β-adrenergic receptors they have the effect of slowing the heart rate and reducing blood flow to the muscles, which would be ergolytic rather than ergogenic.[99] However, their performance benefits relate to a calming effect in slowing the heart rate and reducing tremor, which has been demonstrated to improve pistol shooting and may benefit similar target sports.[149] Hence they are not permitted in archery, motorsport, billiards, darts, golf, shooting and skiing/snowboarding. The health risks of their abuse include bradycardia, orthostatic hypotension and arrhythmias, as well as reducing the capacity to thermoregulate.[55]

Concluding remarks

In summary, this review has provided an overview of the WADA doping categories, why substances or methods within these categories are used, the performance benefits, health risks and implications for clinical samples. Whilst laboratories accredited by WADA carry out testing for

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these doping agents, in reality novel mechanisms of doping are being utilised by sports people at all levels to achieve that unfair advantage.

In the context of health risks, the use of various doping agents, including the most prevalent, such as AAS, stimulants and EPO, increase the risk of cardiovascular disease, thrombosis, stroke and cancers in men and women. Additionally, the use of AAS adversely affect the hormonal axes resulting in the suppression of endogenous hormone production in males, whilst exerting a virilising affect in women. Furthermore the regular use of some anabolic agents and stimulants can have serious mental health/behavioural consequences. Hence the physical and mental health risks associated with the use of such agents are likely to exceed the health benefits associated with participation in physical activity. The prevalence of doping agents extends beyond the realms of elite sport, into lower levels of competition and into non-competitive exercise training for the development of an enhanced physique. So whilst the reliability of prevalence data and health impacts have limitations, the indications are that doping and related drug abuse represent a significant health issue, and those involved in the clinical testing of samples should be aware of the potential for some patients sample results to be affected by doping practises.

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Figures and Tables

Table 1: A summary of the world anti-doping agency Anti-Doping Code and Doping categories.

Substances and Methods Prohibited at All Times
Prohibited substances:
S1 – Anabolic Agents
Anabolic Androgenic Steroids
Other Anabolic Agents
S2 – Peptide Hormones, Growth factors and Related Substances
S3 – Beta-2 Agonists
S4 – Hormone and Metabolic Modulators
S5 - Diuretics and Other Masking Agents
Prohibited Methods
M1 – Manipulation of Blood and Blood Components
M2 – Chemical and Physical Manipulation
M3 – Gene Doping
Substances and Methods Prohibited In-Competition

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Prohibited substances:
S6 – Stimulants
S7- Narcotics
S8 – Cannabinoids
S9 – Glucocorticosteroids
Substances prohibited in Particular Sports
P1 – Alcohol
P2 – Beta-Blockers

1 S = Substance; M = Methods; P = Particular

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Table 2: A brief summary of the history of Doping in sport

Year	Event
Second half of 19th Century	
1865	Early report of drug use in sport - Doping by canal swimmers reported in Amsterdam
1800's (last third)	Cyclists of the day are reported to have used coffee 'spiked' with caffeine at the start of races and then add increasing doses of cocaine and strychnine to the mixture during the races. Boxers reported to take strychnine tablets with a mixture of cocaine and brandy.
1879	Six day bicycle race - an 144 hour continuous event. Various doping agents used: - caffeine based mixtures (preferred by French racers); - sugar cubes dipped in ether (preferred by Belgian cyclists); - alcohol-containing cordials; and - nitro-glycerine (used specifically by sprinters).
1886	DEATH - First fatality attributed to doping reported (unconfirmed). English cyclist, Arthur Linton, was reported to have overdosed on 'tri-methyl' (presumably containing caffeine or ether) during a 600km cycling event held in France (between Bordeaux and Paris). Note: Evidence to this report is however conflicting with others reporting he died 10 years later.
1887	Amphetamines first synthesised
1800's (last third)	Overview of period - The use of stimulants among athletes is common-place and not concealed unless the drug combination was unique and thought to provide a competitive advantage that the athlete did not want to share.
First half of 20th Century	
1904	Thomas Hicks, the winner of the marathon in the 1904 St. Louis Olympic Games, takes strychnine and brandy several times during the race.
1928	Doctor, Wilhelm Knoll - a Swiss physician, administers a stimulant (Coramin) to skiers at the St. Moritz Olympic Games in 1928
1932	In the 1932 Los Angeles Olympic Games, the victories of Japanese swimmers were rumoured to be the result of their being 'pumped full of oxygen'
1933	The word doping is now part of the English language
1935	The first steroid (testosterone) isolated
1936	Rumours of testosterone injection at the Berlin Olympics among German athletes
mid 1930's	Amphetamines identified as a central nervous system stimulant and became available via prescription in 1937
late 1940's	Amphetamines are used for the first time in professional sport
1945	First evidence of formal discussions about the viability of doping in sport through the use of stimulants - Soviet Union

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1948	Soviet sport established their goal of meeting or exceeding all world records
Second half of 20th Century	
1950	Nandrolone (19-nortestosterone) first synthesized by AJ Birch
1950's - 1960's	First reports of female anabolic steroid use relates to Soviet female track and field athletes
1954	Soviets employ systematic use of testosterone with their weightlifters
1956	Growth hormone (hGH) first isolated from the human pituitary gland by Li and Papkoff
1958	Ciba Pharmaceutical Company released Dianabol (methandrostenolone) and US sports doctor Dr. John Ziegler begins experimenting with testosterone for the US weight lifting team. Efficacy of these drugs apparently spread by word of mouth during the early 1960s to other strength-intensive sports, from field events to football.
1959	High school sport related drug use rumours to have commenced - a physician in Texas allegedly administered Dianabol to the high school football team
1960	DEATH: 1960 Rome Olympic Games, Knud Jensen, a 23-year-old Danish cyclist, collapsed during competition and dies. Autopsy results revealed the presence of amphetamines. This is the second death in an Olympic competition and the first doping related death. Note: The first Olympic death occurred in 1912 at the Stockholm Olympics when a marathon runner died of heat exhaustion during the race.
1961	IOC form a medical committee - in response to the death at the Rome Olympics in 1960.
1960's	Anabolic steroid use became widespread.
1964	Urine samples taken from cyclists after the races during the Tokyo Games 'were actually blue in colour due to the use of various drugs'.
1965	Tests conducted on Belgian cyclists in 1965 showed that 37 per cent of professionals and 23 per cent of amateurs were using amfetamines, while reports from Italy showed that 46 per cent of professional cyclists tested positive for doping.
1966	First clearly documented National doping program commences - GDR doping program documented by the STASI.
1967	DEATH: First doping death televised. "29 year old English cyclist (Tom Simpson) collapses during the 13th stage of the Tour De France. The autopsy revealed high levels of methamphetamine in his system. Simpson was carrying a vial of methamphetamine on him at the time of his death.
1967 / 1968	Doping controls first introduced - at the end of 1967 the International Olympic Committee (IOC) votes to adopt a drug-testing policy banning the use of specific drugs - not including anabolic steroids. This policy is released in 1968.
1967	The masculine appearances of a number of female track and field athletes from the Eastern bloc countries in the mid-1960s led to speculation that they were either hermaphrodites or men disguised as women. In response, a chromosome test was initiated in 1967 at the European Cup
1968	DEATH: Soccer player Jean-Louis Quadri collapses during a game in France. He is pronounced dead on arrival to hospital. His autopsy reveals amfetamines are in his system.

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1968	DEATH: Cyclist, Yves Mottin, died from 'excessive amphetamine use' two days after winning a race
late 1960's	Blood doping by the reinfusion of an athlete's own concentrated oxygen-carrying red blood cells or those of a typed-matched donor, shortly before competition is thought to have begun.
1969	First application of RIA for the measurement of steroids in biological fluids published
1972	Unofficial poll taken by a 1972 Olympic Track and Field team member, Jay Sylvester, find that 68% of males Track and Field contestants have used anabolic steroids during their training.
1973	Two tests developed by British scientists to detect for anabolic steroids. One test is by radioimmunoassay and the other is by gas chromatography couples with mass spectrometry. The IOC decides to adopt both tests in tandem to ensure accuracy. These assays do not detect the use of testosterone.
1974	AAS introduced as a banned class of compounds by the IOC following the positive screening results of the 1974 Commonwealth Games
1976	The first female athlete tests positive for anabolic steroids at the Olympics Games and East German women emerge as a dominant force internationally.
1977	IOC meeting in Prague discusses placing "approved" drug testing labs all around the world.
1980	First assay to detect testosterone in urine to retrospectively detect doping in sport developed by Dr Manfred Donike. 20% of all athletes tested positive, including 16 gold medallists. - 1980 Olympics.
1980's	Significant improvement in mass spectrometry particularly GC-MS
1981	First recombinant form of human hGH (rhGH) produced
1982	Caffeine and exogenous testosterone are added to the IOC doping list of prohibited substances
1983	First use of the testosterone / epi-testosterone ratio. At the Pan American games held in Venezuela 15 athletes (including 11 weightlifters) tested positive. In addition after these results were announced, 12 American track and field athletes withdraw and returned home before competing.
1983	Recombinant erythropoietin produced - Patent number US 5441868 A
1982 / 1984	Human growth hormone (hGH) recognised to be part of doping regime for body builders. hGH described as the 'fad anabolic drug' of the Los Angeles Olympic Games.
1984	Beta blockers used by most pentathlon competitors to reduce tremors and anxiety at Olympic Games. Note: bet-blockers were not banned at this time. Post the Olympic Games, 24 members of the US men's cycling team admitted to blood doping prior to competition.
1984	Media reports related to the 1984 Olympic games suggest that some athletes were given instructions on how to evade drug tests for anabolic steroids
1985	Beta blockers are added to the banned substance list and blood doping is prohibited. There is no test at this time however to detect blood doping.
1985	Bio-synthetic human growth hormone manufacturing commences at Genetech (FDA approved).
1986	Diuretics are added to the IOC banned substance list
1986	Statistical analysis of IOC approved lab positive results shows that anabolic steroid comprise two -thirds of drugs detected in this year and of these two-

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	thirds of these positives were for nandrolone.
1987	DEATH: West German Heptathlete, Birgit Dressel dies at the age of 26 years of anabolic steroid related complications.
1988	1988 Seoul Games, two gold medallist weightlifters tested positive for diuretics.
1988	Ben Johnson, winner of the 100-metre dash, tested positive for an anabolic steroid. The follow-up investigation identified at least half of the athletes testing positive for anabolic steroids.
1980's	DEATH: Speculation that >12 deaths were the result of EPO during this decade.
1988	Peptide hormones are added to the IOC banned substance list
1989 - 1990	Fall of communist Europe - Berlin Wall fell in late 1989 followed by the collapse of the GDR in 1990 and therefore the end of the GDR National doping program.
1990's	Some GDR coaches found employment with other teams including within China's sports programs. A number of Chinese athletes test positive during 1990's- including 29 track and field athletes and 19 swimmers
1991 / 1994	Argentinian footballer, Diego Maradona, banned for 15 months in 1991 for testing positive for cocaine and ejected from the 1994 world cup after returning a positive test for ephedrine
1994	Doping using DHT detected in 11 Chinese athletes participating in the Asian Games by the IOC accredited laboratory in Tokyo.
1995	World's youngest athlete tests positive for anabolic steroid use - a 14-year old female long jumper and sprinter from South Africa
1996	GC- HRMS testing employed for the first time at the Atlanta Olympic Games.
1998	Baseball player, Mark McGwire, acknowledges he uses androstenedione. Anabolic steroids were banned by the IOC and NCAA but not in professional baseball at the time of the report.
1998	Cyclist, Willy Voet of the Festina team, arrested by French customs police for transporting performance-enhancing drugs. This arrest resulted in an extensive investigation which exposed the extent and 30+ year history of doping use in this sport.
21st Century	
2000	Australian Government provided a special research fund in the lead up to the Olympics to ensure that state of the art testing available for Sydney Olympics. Sydney Olympics - first to use the EPO testing by IEF. National Measurement Institute of Australia's WADA testing facility develops bases of what is now used for the haematology module of the ABP. Population studies for IRMS which allowed the first positive finding by IRMS during the Paralympics for testosterone
2001	ASDA (now ASADA) became the first National Anti-Doping Organisation to establish a domestic blood-testing program
1999-2005	Lance Armstrong wins the Tour de France on seven consecutive occasions
2000	Three cyclists fail the mandatory health test just prior to commencement of the 2000 Tour de France - they were not permitted to start because they had a haematocrit >50%
2004	Caffeine, probably the most popular drug in the world, is removed from the IOC banned substance list, notably; research indicates that the ergogenic benefits from caffeine ingestion may be gained from relatively low doses, including those attained from drinking strong coffee.

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2004	Athens isoform assay for hGH
2007	BALCO investigation - Marion Jones stripped of the five medals she won at the Sydney Olympic games in 2000
2009	Athlete Passport Haematological variables approved
2012	London Olympics biomarker assay for hGH introduced
2013	ABP Steroidal Module approved - monitors selected urinary steroid concentrations over time in order to detect steroid doping
2013	Lance Armstrong admits to doping during his television interview with "Oprah" and was subsequently stripped of his seven Tour de France medals.

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Table 3: Potential effect on test results in clinical diagnostic laboratories

Measure	Example common clinical lab method of detection	Considerations for the clinical diagnostic laboratory	WADA Compound group	
CORE LABORATORY TESTS				
Analyte/s	Routine method of measurement	Clinical and analytical considerations	Category	Drug group
Clotting factors	turbidometric	Clinical - AAS is associated with ↑ clotting factors; rhEPO is associated with increased HCT which leads to increased viscosity and is believed to be associated with increased risk of thrombosis.	S1	Anabolic Steroids
Full blood count		Clinical - AAS is associated with polycythaemia; - rhEPO associated with increased RBC mass greater than the plasma volume, leading to an increase in haematocrit with some examples demonstrating HCT's >50% and increased reticulocytes.	S1 / S4	Anabolic Steroids / Hormone and Metabolic Modulators

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Glucose	Spectrophotometric / Electrodes / POCT	Clinical - AAS is associated with reduced glucose tolerance; B2-AR agonist drugs associated with an increase in circulating glucose; hGH is associated with bouts of hypoglycaemia due to the adverse effect of IGF-I.	S1 / S2 / S3	Anabolic Steroids / Peptides / Beta-2-Agonists
		Analytical - increased Hct associated with inaccurate results in some POCT glucose meters	S4	Hormone and Metabolic Modulators
GTT	Spectrophotometric / Electrodes / POCT	Clinical - AAS can reduce glucose tolerance	S1	Anabolic Steroids
Hct		Clinical - EPO causes increased RBC production and increases haematocrit. Analytical - increased Hct associated with inaccurate results in some POCT glucose meters	S4	Hormone and Metabolic Modulators
HDL Cholesterol	Spectrophotometric	Clinical - AAS is associated with ↓HDL	S1	Anabolic Steroids
LDL Cholesterol	Calculated from Freidwald formula	Clinical - AAS is associated with ↑ LDL	S1	Anabolic Steroids

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LFT's	Spectrophotometric	Clinical - AAS is associated with altered liver function and cholestatic jaundice e.g. increased conjugated bilirubin. However, exercise alone can also increase liver function enzyme tests results - AST, ALT and LDH.	S1	Anabolic Steroids
Osmolality / Osmol gap	Osmometer / Calculated	Clinical - no significant adverse effect from ethanol ingestion as levels are usually to enhance performance Analytical – no significant effect on measured and calculated osmol or osmol gap as administered levels are relatively low compared to recreational intake.	P1	Alcohol
Trigylcerides	Spectrophotometric	Clinical - AAS is associated with ↑ Triglycerides	S1	Anabolic Steroids
Urine Creatinine	Spectrophotometric (Jaffe or enzymatic)	Clinical - increased urine volume, Analytical - urine concentration reduced - diluting everything down to potentially undetectable levels	S5	Diuretics
ENDOCRINE TESTING				

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ACTH	Plasma immunoassay	Clinical - may suppress ACTH levels due to negative feedback of H-P-A axis	S9	Glucocorticoids
Androstenedione	Serum Immunoassay (a few LC-MS/MS)	Clinical - DHEA administration may result in increased blood levels. Analytical - androgen administration may possibly cross reaction in other C19 steroid immunoassays	S1	Anabolic Steroids
Cortisol	Serum Immunoassay (a few LC-MS/MS)	Clinical - chronic glucocorticoid use may suppress endogenous cortisol production. Analytical - may cross react in immunoassays measuring cortisol, resulting in higher levels	S9	Glucocorticoids
Epi-testosterone	serum LC-MMS	Analytical - immunoassay . The C3 epimer of testosterone will cross react in testosterone immunoassays and it is structurally nearly identical possessing the same number of carbon, oxygen and hydrogen atoms. Analytical - Mass spectrometry. Need to ensure chromatographic separation as this compound is the C3 epimer of testosterone and shares the same molecular weight and ion transitions.	S1	Anabolic Steroids
FSH	Serum/plasma immunoassay, can also be measured in urine but infrequently performed	In males suppress the hypothalamic-pituitary-testicular axis (HPT) reducing the levels of the gonadotrophic hormones, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which reduces the levels of circulating endogenous testosterone	S1	Anabolic Steroids

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Growth Hormone	Serum Immunoassay	Clinical - usually as recombinant growth hormone; effect on growth and metabolism Analytical - effect will depend on if immunoassay detects rhGH	S2	Peptide hormones, growth factors and related substances
Insulin	Immunoassay	Clinical - AAS is associated with ↑ Insulin	S1	Anabolic Steroids
LH	Serum/plasma immunoassay, can also be measured in urine but infrequently performed	In males suppress the hypothalamic-pituitary-testicular axis (HPT) reducing the levels of the gonadotrophic hormones, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which reduces the levels of circulating endogenous testosterone	S1	Anabolic Steroids
Oestrogen	Serum/ plasma immunoassay	Clinical - serum oestrogen concentrations can increase with androstenedione administration. This effect is significant for males and may be associated with gynaecomastia.	S1	Anabolic Steroids
SHBG	Serum/plasma immunoassay	Exogenous AAS can result in a decrease in the main binding protein for testosterone i.e. SHBG	S1	Anabolic Steroids
Testosterone	Serum Immunoassay (a few LC-MS/MS)	hCG can increase testosterone production in males. In males and females the circulating concentration of testosterone may increase following testosterone administration. Androstenedione administration can lead to altered testosterone to epi-testosterone ratio.	S1	Anabolic Steroids

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	Urine steroid metabolomic profile	Urine timed or random by GC-MS	Clinical - urine concentration of androsterone and eticholanolone and associated 6 alpha and beta metabolites are increased with androstenedione administration ; Analytical - detection of "unknown" steroid peaks in GCMS scans associated with androgen and glucocorticoid administration	S1 / S9	Anabolic Steroids / Glucocorticoids

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Table 4. Case examples highlighting the dilemma occasionally faced by clinical diagnostic laboratories.

CASE ONE

Clinical Notes and History: 28 y.o. male elite athlete has a routine check-up post a major event two days earlier. The patient was well and had a previous routine check-up with a serum testosterone level of 9.9 nmol/L. For the current check-up the patient returned the following results

Analyte (Serum)	Result	Reference Interval
Testosterone	3.3 nmol/L	9.9 – 27.8
SHBG	30 nmol/L	15 – 45
Calculated Free Testosterone	64 pmol/L	260 – 740
Creatinine Kinase	1518 U/L	<195
FSH	3 U/L	1 – 15
LH	<1 U/L	1 – 9

Differential Diagnoses Considered:

1) Timing of testosterone sampling. The sample was collected in the morning excluding diurnal variation as the cause of the low testosterone result.
2) Male hypogonadism. This could be due to either hypothalamic/pituitary or testicular failure. In this athlete the LH was low indicating the cause was either hypothalamic or pituitary origin. i.e. hypogonadotrophic hypogonadism. This resulted in consideration of:
    a) Pituitary function including TSH, prolactin and hGH and their relevant peripheral hormones. Consideration during these investigations were given to the possibility of a pituitary tumour. No abnormality was detected.
    b) Hypothalamic dysfunction. This commonly eventuates in relation to

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some form of trauma or in specific diseases. All likely scenarios were ruled out in this patient.

c) Drug or exercise induced hypothalamic suppression. In such cases the decrease in testosterone levels is transient and will return to normal levels over time. This was considered likely in this patient. A repeat (follow up) testosterone level returned a result of 11.1 nmol/L.

Summary Comments: The testosterone level was likely to be decreased due to the recent exercise. This athlete's earlier testosterone was also borderline low. The patient had a negative drug history and normal SHBG and FSH levels. The author of this case thought that the transient nature of this patient's low testosterone levels made drug abuse less likely, but not completely ruled out.

Reference: Sacks S. Australasian Association of Clinical Biochemists - Omniscience case series. Clinical Biochemist Newsletter. December 2009, p 51-52. [62]

CASE TWO

Clinical Notes and History: 16 y.o. male presented to a paediatrician with a 6/12 history of fatigue, disturbed sleep and anxiety. He had commenced duloxetine hydrocholride 4 months ago. He entered puberty at 13 years and was considered to have normal virilisation with Tanner Stage V noted. He had lost weight associated with a decreased caloric intake over the six month period with his BMI decreasing from 23.8 to 18.1 Kg/m^2

"The patient stated that his main interests were body building and healthy eating". During the consultation he requested for his testosterone level to be tested.

Analyte (Serum)	Result	Reference Interval
Testosterone	0.9 nmol/L	8.0 – 26.0
SHBG	62 nmol/L	13 – 71
FSH	4.3 U/L	1.4 – 18.0
LH	0.9 U/L	1.4 – 7.0

Additional Testing:

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This low testosterone level was not consistent with the patient's Tanner stage of V. A repeat test for testosterone and SHBG was performed three weeks later and the results were now within the reference intervals.

Differential Diagnoses Considered:

1) Secondary Hypogonadism due to a hypothalamic-pituitary-gonadal tumour. This patient did not exhibit any symptoms such as increased intracranial pressure and the MRI of the brain did not find any abnormality. Hyperprolactinaemia due to e.g. a prolactin producing tumour. Prolactin is known to suppress GnRH. This patient had prolactin measured on subsequent bloods which returned slightly elevated (but insignificant) levels compared to the reference intervals.
2) Patient's current medication. Whilst this may contribute it was felt that duloxetine hydrochloride was unlikely to suppress the testosterone to the level observed. The patient remained on this medication throughout these investigations, during which time the patient's testosterone returned to "normal" male levels i.e. 16.7 – 27.8 nmol/L.
3) Eating disorder – males with anorexia nervosa exhibit significantly lower testosterone levels. This was a likely cause in this patient based on his recent weight loss and "healthy" eating. In males muscle dysmorphia is a recently recognised condition precipitating anorexia nervosa.
4) Excessive exercise – overtraining syndrome, strenuous exercise etc are known to result in transient low levels of testosterone as discussed in patient one above. This was also likely in this patient. This also results in a decrease in LH (and FSH) and increase in SHBG (but usually not above the reference interval).
5) Anabolic steroid induced hypogonadism. Based on the patient's history this was also thought to be likely. AAS suppresses natural androgen production by providing feedback to the hypothalamus / pituitary resulting in a decreased LH etc. The patient however denied on direct questioning the use of any AAS.

Summary Comments: As with patient one, both the testosterone and LH were low with a SHBG within the reference interval. The authors of this case thought that the transient low testosterone in this patient was likely to be due to the eating disorder, potentially compounded by AAS (even though use denied).

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Reference: Woodhill I, Cooper C, Zacharin M, Cukier K, Vuillermin P. Low testosterone in a male adolescent bodybuilder: Which diagnosis holds more weight? J Paed Child Health. 2014;50:739-741.[63]

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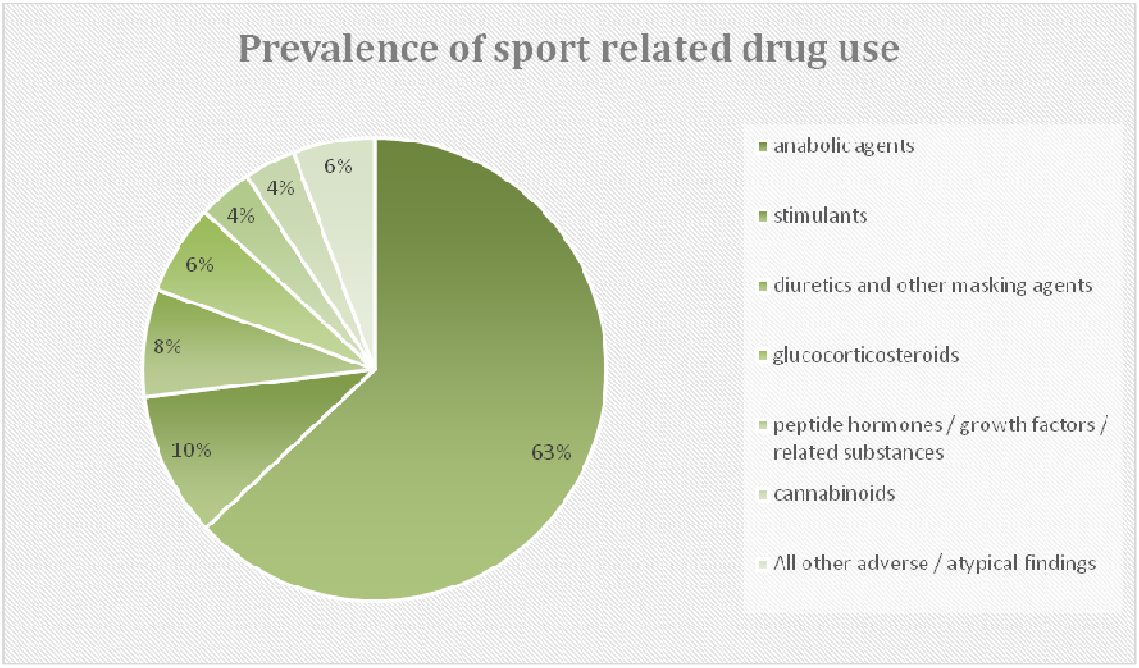

Figure 1: Prevalence of sport related drug use. Data taken from the 2013 WADA report which included the results of 269,878 samples analysed during 2013 of which 5,962 (2.21%) indicated an adverse or atypical finding.[14] The pie chart in this figure demonstrates the breakdown of this 2.21% of findings.

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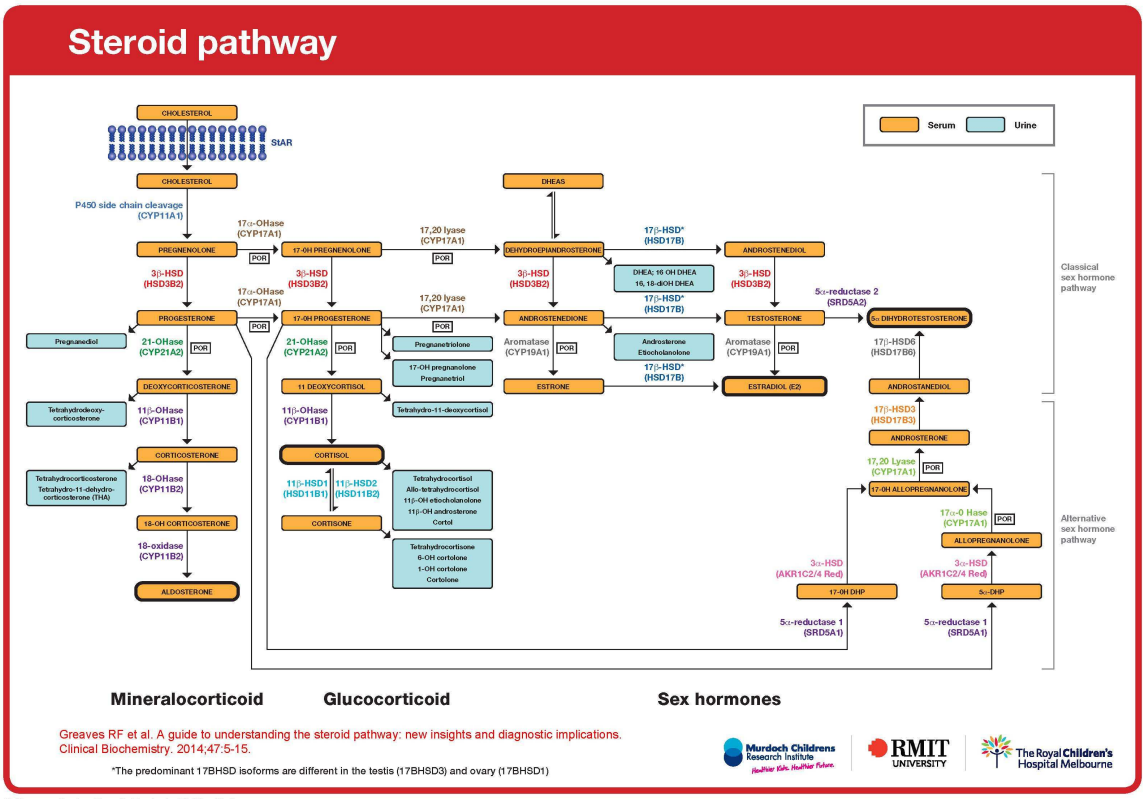
Figure 2: Endogenous steroid pathway providing a succinct overview of the relationship of steroid hormones to each other and the likely matrix for measurement (blood or urine) in the clinical diagnostic laboratory.[61]



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