Doping Agents in Sport

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The origin of doping agents

Doping is the act of taking illegal substances to improve physical performance in sports. It has been a persistent issue for decades, undermining the principles of a fair sporting environment; it is used by athletes that wish to improve their performance and gain a competitive advantage. Among the myriad of substances used as doping agents, anabolic androgenic steroids (AASs) are the most popular and common ones used today [1] and will be the focus of this review.

AASs are synthetic derivatives of the natural hormone testosterone (anabolic refers to growing metabolism and androgenic refers to the development of male characteristics). AASs are a type of exogenous steroid—hormones not naturally produced by the body—while testosterone, which naturally occurs in the body, is known as an endogenous steroid. Applications of testosterone date back thousands of years to ancient Greece, where it was first noted that male reproductive organs have healing powers.[2,3] In addition, the ancient Greeks used plant and testicular extracts that allegedly had performance-enhancing effects.[4] It was not until 1849 that research by Arnold Adolf Berthold proved that castration and reimplantation of testicular tissue had effects on the appearance and behaviour of roosters, thereby proving the existence of a ‘blood stream substance’. He found that roosters had increased combs, interest in hens, and aggressive male behaviour when testicular tissues were reimplanted after castration.[3]

As illustrated by the name, some of the physiological effects of AAS that incentive athletes to abuse them include: increased muscle strength, bone density, and red blood cell production and thus oxygen transport.[5] Typically, most abusers use steroids from 10 times to 100 times the normal therapeutic doses.[6] These steroids are taken in 4–12-week cycles with a subsequent 4–12-week abstinence period. This is to maximise the drug’s desired effects while minimising its adverse effects.[7] Many abusers will take steroids through practices known as stacking and pyramiding. Stacking describes the practice where multiple steroids are taken simultaneously, as it is thought that these steroids will have a synergistic effect, despite no scientific evidence to support such assumptions. Pyramiding is when abusers gradually increase their drug intake until they reach maximum intake during the middle of their cycle, and then gradually taper off their cycle.[6]

Nowadays, steroids are banned from sport competitions in most countries under the laws set by the World Anti-Doping Agency (WADA). These laws aim to promote a fair sporting environment for all athletes, and to protecting athletes from the many health related dangers that come with using steroids.[8] These dangers range from short-term acne, mood swings, decreased sperm count, and swelling at injection sites to more serious long-term effects on the cardiovascular, reproductive, and metabolic systems, such as fertility and heart problems, kidney failure, tumours in liver, and paranoia.[9] Bond et al. presents some of the adverse effects of steroids on the body (Figure 1).

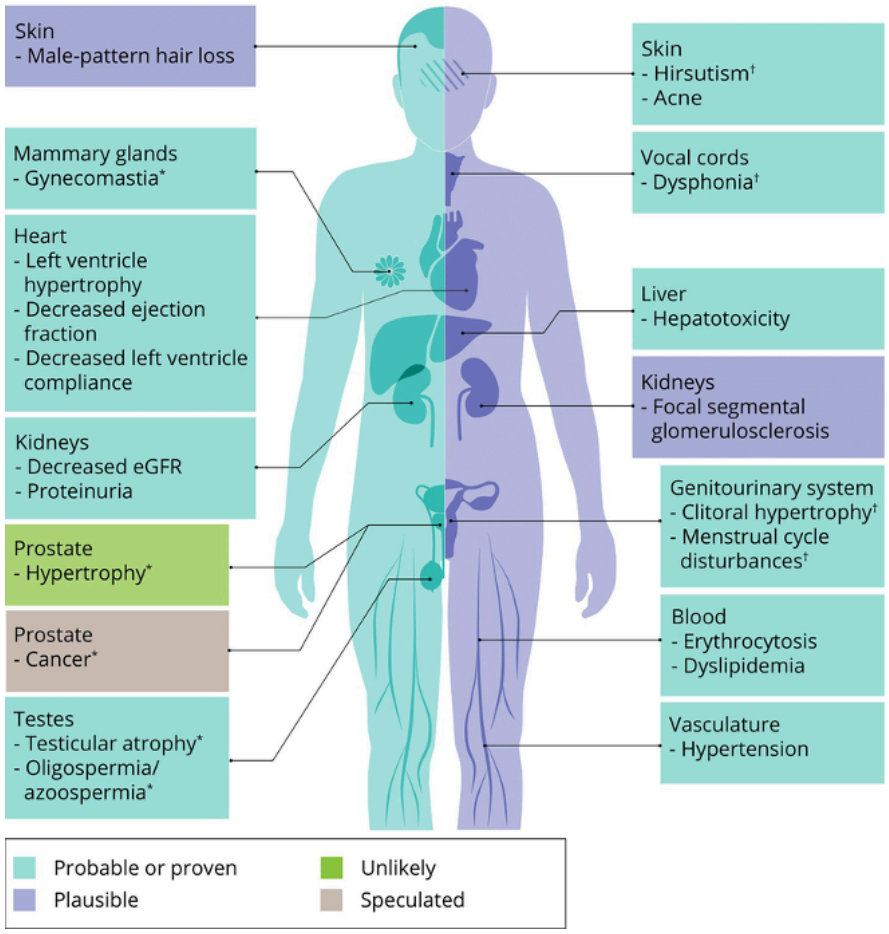

Figure 1: The various adverse effects associated with AAS use (image reproduced from Bond et al.).

A small fraction of officially approved AAS are used medicinally, and are prescribed by doctors, albeit rarely. They are used to treat hormonal diseases, such as delayed puberty; to regain weight after infections, illnesses, or injuries; to treat decreased testosterone in men with medical conditions; to treat certain types of anemia; and to treat hereditary angioedema.[11,12] AASs come in the form of topical gels, creams, pills, and injectable liquids.[10]

The chemistry and biochemistry of doping agents

AAS are mimics of the hormone testosterone. The structures of six of the most commonly abused steroids are shown in Figure 2.

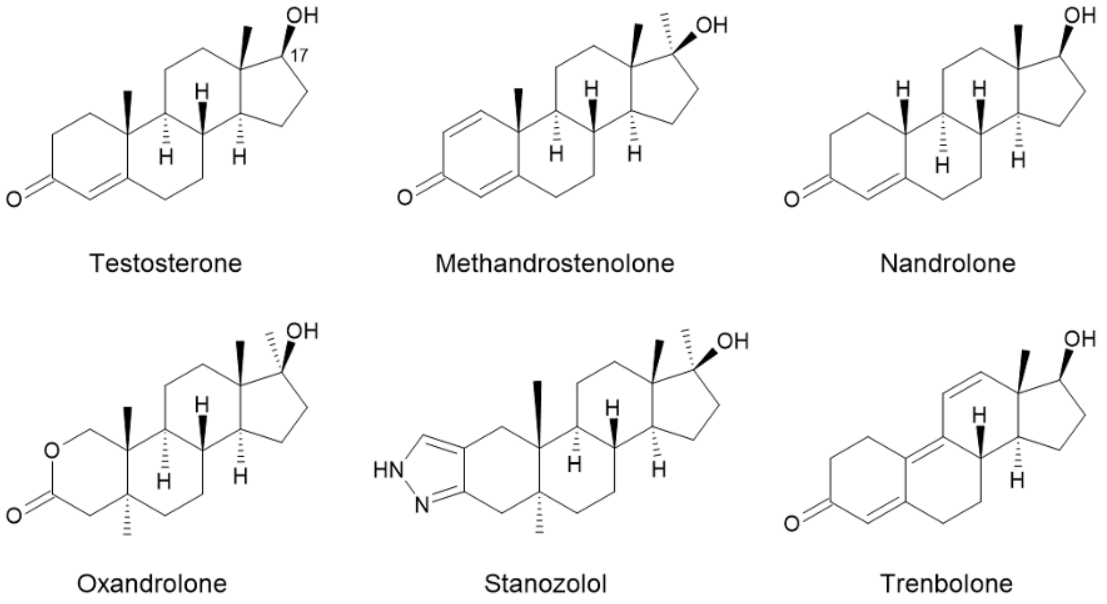

Figure 2: Six of the most commonly abused AAS (drawn with ChemSketch).

As can be seen from Figure 2, all six compounds have a steroid backbone consisting of three six-membered rings and one five-membered ring as well as a 17β-hydroxyl group (illustrated by number 17 on testosterone). The different functional groups of the compounds alter the effects of the drugs. For example, methandrostenolone (Dianabol/D-Bol), oxandrolone, and stanozolol, which are all administered orally, have a 17α-methylation. This modification assist the compounds in resisting hepatic breakdown and increases bioavailability.[10] For steroids administered in an injectable form, a potential modification is the esterification of the 17β-hydroxyl. This modification retards the rate at which the steroid is released from the injected oil-based formulation, thereby prolonging its physiological effect.[10]

The mechanism of action of AASs is analogous to that of testosterone. Testosterone (“testo” meaning testes, “ster” from sterol, and “one” from ketone) is produced naturally in the testes in men and in the ovaries and adrenal glands in women.[13] Once released into the bloodstream, the majority of circulating testosterone is bound to proteins such as albumin, corticosteroid-binding globulin (CBG) and sex hormone-binding globulin (SHBG), while the rest (typically 1% to 4%) exists in an unbound state.[14,15] After diffusion into the cell, testosterone can undergo a variety of biotransformations, either into an androgen with greater potency (such as dihydrotestosterone, which is produced by enzymes in the 5α-reductase family), into a metabolite with less or no potency (by phase I and II metabolism in the liver, kidneys and other androgen-sensitive tissues), or into an estrogen (if testosterone or its derivatives can be substrates for the aromatase enzyme).[10] Androgen receptors (ARs) are located in the cytoplasm; when an androgenic hormone, such as testosterone, binds to an AR, a conformational change occurs. Specifically, binding causes the dissociation of heat shock proteins from the AR, which previously had the effect of stabilising the receptor. This is followed by phosphorylation and dimerization of the AR, and, finally, translocation of the AR from the cytoplasm to the nucleus.

Once in the nucleus, the AR binds to specific sections of DNA called androgen response elements (AREs) and stimulates the transcription of target genes.[10,13,16]

Methods of detection

There are a variety of factors that determine which method of detection is utilized during the testing process for substance abuse. One such factor is the sensitivity and specificity of the test, which ensures that false positive and false negative results are minimized.[17] Having an accurate test decreases the possibility of false accusations, which often come with many legal implications and serious damage to the athlete's reputation. Another factor is a long detection window. All athletes know that they will be tested for steroid use before competitions, thus, it is typical for athletes on steroids to end their cycles long before competition. A long detection window ensures that steroids can be detected well after the athlete stops using them.

For this paper, only methods relating to the detection of steroids will be considered.

Types of biological samples:

Before the testing begins, a type of biological sample must be chosen to be sent to the laboratory; a variety of biological samples are available for the detection of steroids in athletes (Table 1). Some of the most common sample types, ranked in order of increasing detection window include: saliva (up to 24 hours), blood (up to 14 days), urine (up to 28 days), and hair (up to 12 months depending on hair length).[18] Saliva tests are not used as standalone methods due to both their short detection window and risk of contamination from food, drink and oral products.[19] However, saliva tests can provide rapid results and are a non-invasive approach. They work because when steroids enter the blood stream, they can diffuse into the salivary glands where they are incorporated with saliva.[20] Saliva tests are not often utilized during sporting events. Blood samples are a more invasive approach and require a longer analysis time. Although blood samples also have a relatively short detection period (up to 14 days) compared to other sample types, they are more reliable and provide information on the current circulation of steroids within the bloodstream.[18] They are typically used for the Athlete's Biological Passport (ABP), which is introduced below, or if urine tests are inconclusive.[21] Urine is the traditional type of biological sample used for detection of steroids.[22] With a relatively longer detection window (up to 28 days), urine collection is also non-invasive and is more effective and reliable; for most drugs, higher concentrations can be detected in urine compared to that of blood or other sample types.[22,23] Unlike blood, urinal samples provide an averaged presentation of the discarded metabolites from the body. However, one downside of urine testing is that without supervision, samples can easily be faked and manipulated during collection. Urine tests are typically conducted randomly before the competition or even immediately after the athlete's event; the test results are added to the ABP.[21] Hair testing has the longest detection window of up to 12 months and can provide a month-by-month report of drug usage. Although it is also non-invasive and can be obtained from any part of the body, hair is also commonly subjected to external contamination and it is not suitable in detecting recent use as it takes time for substances to be incorporated in the hair.[18] Hair testing can still be used if there are suspicions of long-term steroid use.[21]

Table 1: Summary of biological sampling types.

Type of biological sample	Detection window	Invasiveness	Downsides	How they work
Urine	Up to 28 days	Non-invasive, collected through supervised urine testing	Samples can easily be faked with a lack of supervision	Provide an averaged presentation of the discarded metabolites from the body
Blood	Up to 14 days	Invasive, obtained through blood collection with needles	Can return false negatives due to relatively short detection window.	Presents information regarding the current circulation of steroids in the bloodstream.
Hair	Up to 12 months	Non-invasive, can be collected from any part of the body	Easily subjected to external contamination	Steroids in the bloodstream can become incorporated into hair follicles
Saliva	24 hours	Non-invasive	Risks contamination from food, drinks, oral products	Steroids entering the blood stream can diffuse into the salivary glands

Athlete's Biological Passport (ABP)

The ABP is an electronic record of professional athletes' normal biological markers. From these biological markers, an acceptable range is created, and if an athlete's biological markers exceed this range, it indirectly outlines the possible use of prohibited substances.[24] ABP consists of two modules, the first including hematological markers—used to detect blood doping—and the second including urinary markers—used to detect pseudo-endogenous steroids.[24] When new samples are recorded, they are passed through The Adaptive Model in ADAMS (Anti-Doping Administration and Management System), which will produce an Atypical Passport Finding (APF) if the likelihood of each marker falling outside the predicted range is lower than 1 in 100.[25] When an APF is flagged, human experts will be informed, and expert evaluation will be conducted.

General method of detecting steroids

The current method of detecting pseudo-endogenous steroids in athletes consists of two different steps: the first step being a longitudinal evaluation by Gas Chromatography coupled with Mass Spectroscopy (GC/MS), and a confirmation analysis by Gas Chromatography Combustion Isotope Ratio Mass Spectrometry (GC/C/IRMS).[26]

Gas chromatography coupled with mass spectroscopy (GC/MS)

Gas chromatography was first used in a professional sporting setting at the 1980 Moscow Olympic Games as suggested by Dr Manfred Donike, one of the two heads of the International Olympic Committee Medical and Scientific Department (IOC MSD).[27] At the time, the results showed that more than 20% of samples contained an unnatural ratio of testosterone.[27]

GC is a traditional technique that is used to separate organic compounds. It consists of two phases, a mobile phase (an inert carrier gas such as N_2, He or Ar) and a stationary phase (a packed column that is coated with a high boiling polymer such as silica gel) (UCLA). The analyte is introduced before the column, and is separated based on the interactions that it has with the stationary phase; the stronger the interaction is, the longer it takes to migrate through the column and therefore the longer its retention time.[28] This separation can occur by gas-liquid partition or gas-solid adsorption, based on the type of stationary phase used.[29] A gas chromatogram is drawn, where the peak position represents the retention time of the sample and the peak area is proportional to the quantity of sample.

After exiting the column, the samples are analysed using quadrupole mass spectroscopy, where ionised samples are focused and passed along the middle of the quadrupole rods. These rods have fixed direct current (DC) and alternating radiofrequency potentials (RF) potentials applied to them; by varying the DC and RF values, different ions of different mass-to-charge ratios (m/z) are filtered.[30] A mass spectrum can be obtained in three forms based on which mode the detector was set too. Full scans—where the entire mass range for the fragment ions is scanned—and selected ion monitoring (SIM)—where specific ions are chosen to be scanned.[31]

The information gathered from GC/MS can then be compared with existing data from known metabolites, thus indicating the presence of steroids within the athlete. Alternatively, the quantity of testosterone in the body can be compared to its isomer epitestosterone in the testosterone/epitestosterone ratio (T/E). This is because the isomer epitestosterone is typically found in the body in similar quantities of testosterone; when athletes dope, their testosterone levels will increase greatly while their epitestosterone quantity remains relatively stable.[32] WADA has set a 4.0 T/E ratio as an indicator for possible exogenous use. When athlete's urine surpasses this level, subsequent analysis is conducted with GC/C/IRMS.[32]

Sample preparation

Before GC/MS can be conducted, the urine sample must be prepared for GC/MS. Sample preparation is conducted with the aim of making the sample more suitable for the chromatographic environment while also maintaining the sample's integrity as much as possible.[33] Sample preparation of urine for GC/MS analysis typically entails the following steps (Figure 3).

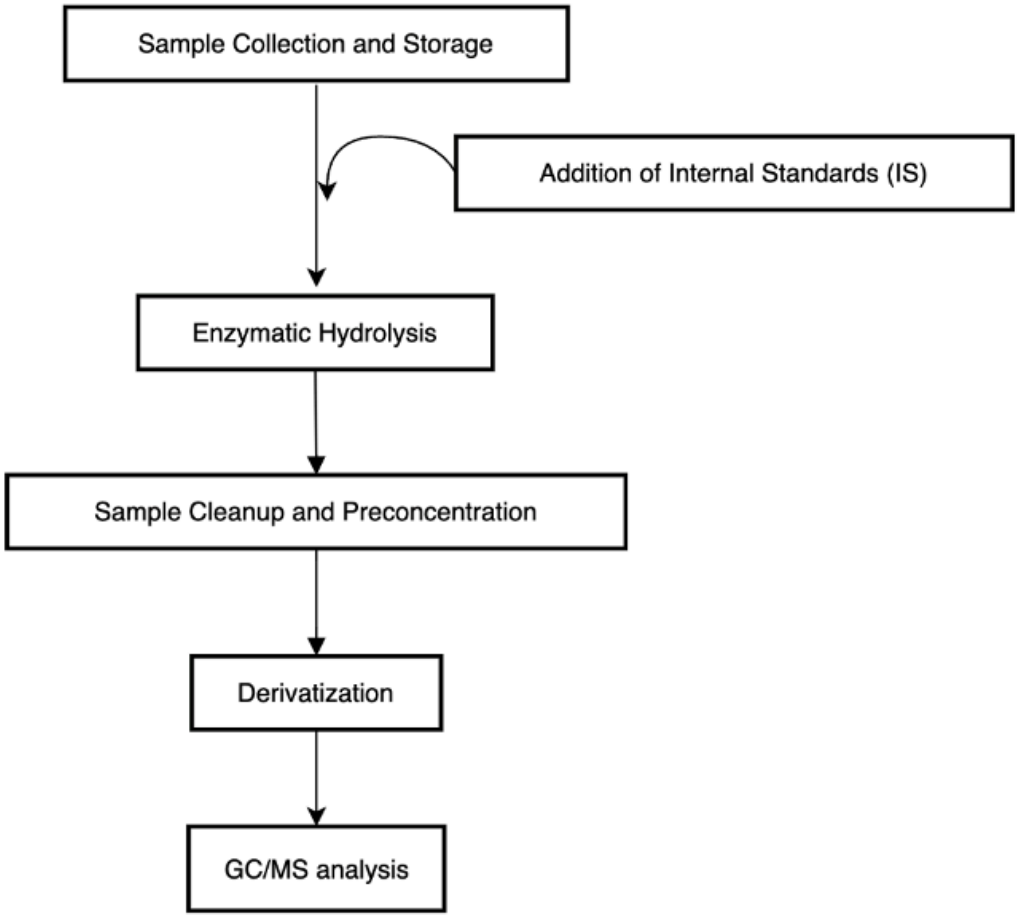

Figure 3: An illustration of sample preparation for GC/MS analysis.

During sample collection and storage, urine samples should be frozen at temperatures around -80 °C to ensure the targeted metabolites within the sample are preserved and stable; however, the temperature can be adjusted specifically for a known metabolite.[33]

Internal Standards (IS)

In the laboratory, the sample undergoes treatment in preparation for GC/MS analysis. One of the primary reasons for this is to decrease signal suppression from other matrix components and to make it easier to read the data produced by GC/MS. To start off, an IS is chosen and a known quantity is added to the sample. The sample is then calibrated, and the peak area of the drug is compared to that of the internal standard Internal samples are compounds that have almost identical chemical properties to the analyte, typically isotopically labelled analogues of the target compounds.[34] Common ISs are deuterated forms of the target compounds or ones labelled with ^{13}C; these are known as stable isotope labelled internal standards (SIL IS).[33] They are used to calibrate the data.

One of the main purposes of ISs is quantification. The area under the peak of an analyte or an IS is proportional to the concentration of that compound. By adding known quantities of IS, the concentration of the analyte can be calculated. The different detection efficiencies of the analyte versus the IS can be corrected for via the calculation of a response factor (the ratio of peaks areas of analyte/IS when equal concentrations of the two compounds are added). This allows for quantification of the drug, despite potential errors in volumetric recovery or amount of injection solvent added.[35]

Another reason for using ISs is to account for signal suppression or enhancements between the analyte and matrix components.[33] Suppression can occur due to coelution between endogenous substances in the urine and analytes. Coelution is when multiple compounds elute from the chromatography column at the same time, and compete with the analyte for the total available charge in the MS detector. This can weaken the signal and reduce the accuracy of the results by as much as 26%.[36] ISs are often coeluted with the analyte and are added first before treatment so that they are subjected to the same treatment process as the analytes. This means that these ISs will be able to provide a consistent reference to the entire analytical process. As a result, the analyte peak response is usually corrected for the ion suppression matrix effects.[36]

Enzymatic hydrolysis

After the addition of an IS, enzymatic hydrolysis in conducted. Before excretion of the target compounds from the body, the compounds are typically converted into more hydrophilic products such as conjugated metabolites.[33] These conjugated forms (commonly sulfate and glucuronide derivatives) are more difficult to detect and quantify with GC/MS and other analytical tests.[37] To counteract this, enzymatic hydrolysis reverts conjugated metabolites back into their free steroid form. Some of the common biocatalysts used in the preparation of urinary samples for steroid detection include β-glucuronidase (for hydrolysing glucuronide conjugates), arylsulfatase (for hydrolysing sulfate conjugates), or microbial enzymes, such as those derived from *Cunninghamella blakesleeana.*[38]

Sample cleanup and preconcentration

Sample cleanup and preconcentration removes interfering substances while concentrating the analytes for better sensitivity and results. The most common procedure is solid-phase extraction (SPE). One typical method for SPE uses syringe-shaped SPE tubes, containing the solid phase—such as $C_{18,}$ $C_{5,}$ or C_8 for water rich urine samples—kept in place by two filters.[39] When a urine sample is applied and washed through the solid phase with a wash solvent, the metabolites bind to the solid phase due to hydrophilic, hydrophobic, or ionic interactions.[40,41] Matrix components are washed through as waste. Then, an elution solvent strong enough to extract the analyte is applied and the analyte is collected.[40,41]

Derivatization

Derivatization is the process of converting the chemical structure of a compound to a structure that has better analytical capabilities. Types of derivatization include silylation (using trimethylchlorosilane (TMCS) to replace the active hydrogens in the functional groups of the compound with trimethylsilyl (TMS)), acylation (using acetic anhydride or trifluoroacetic anhydride (TFAA) to introduce an acyl group to compounds containing active hydrogen groups),

and alkylation (using methyl iodide or dimethyl sulfate to replace the active hydrogen groups with alkyl groups).[42–44]

Ideally, if the analyte can be tested in its original form, it should be. Derivatization of an analyte into another form adds costs, uncertainties and impurities. However, there are reasons why derivatization might be used for an analyte. Lin et al. suggests three reasons including: to make the analytes more chemically compatible with the chromatographic environment, to achieve required separation or improve separation efficiency, and to improve detection effectiveness. An example where derivatization is used to improve analyte compatibility with the chromatographic environment is when analytes have carboxyl or amine functional groups, which can form hydrogen-bonds with the chromatography system. The formation of hydrogen bonds with the chromatography system results in peak loss by irreversible adsorption and peak tailing by reversible adsorption. Derivatization can change these species into their inactive form prior to GC analysis and prevent hydrogen bonding.

An example where derivatization is used to achieve the required separation for MS analysis is for enantiomers; typical GC cannot separate enantiomers due to their identical physical properties. Derivatization with chiral reagents can be used to separate enantiomers by conversion to diastereomers. Diastereomers have different physical properties and can be separated by GC. Derivatization is often preferred over the use of chiral GC columns—which also separate enantiomers—due to its cost-effective nature.[45]

Gas chromatography paired with tandem mass spectroscopy GC/MS/MS

There are many types of GC/MS/MS, including GC-TQMS (triple quadrupole), GC-ITMS (ion trap MS) and GC-QTOF-MS (quadrupole time of flight). The most commonly used in the detection of steroids from urinary samples is GC-TQMS.[31] The main difference between GC/MS and GC-TQMS is the presence of three quadrupole mass filters on GC-TQMS compared to one on GC/MS. This allows for fragmentation, which enhances the effectiveness of the method by reducing noise and increasing specificity and sensitivity.[46]

The first of these mass filters operates identically to that in GC/MS, it filters ions that will be scanned. The second quadruple acts as a medium for collision induced fragmentation, creating new fragments that are passed to the third quadruple, while filters and analyses them.[31] GC-TQMS is powerful in this way, larger fragments can be broken down to confirm the correct structure of the molecule. Another reason why GC-TQMS is known as a “multidimensional” technique is that all of the quadrupoles can be set for different functions. For instance, all three quadrupoles can be set to the function of the first quadrupole, which achieves a traditional GC/MS analysis. Alternatively, the first quadrupole can be set to SIM for a single ion and third quadrupoles can be set to SIM for a single fragment from that ion. This allows for ultimate sensitivity; it is very unlikely for an ion (or even isomer) to have the same transition after being fragmented and thus the same retention time. One of the disadvantages of using GC-TQMS is the cost of the machine and the special training required to use the machine. Additionally, errors in sample preparation and contamination can be amplified when using GC-TQMS.[31]

Gas chromatography/combustion/paired with isotopic ratio mass spectroscopy

GC/MS is used in the initial stages of detection due to its fast and cost-effective nature. On the other hand, GC/MS cannot differentiate between endogenous steroids and synthetic analogs.[47]

However, GC/C/IRMS can, and is used as a conformation procedure after anomalies are detected using GC/MS. GC/C/IRMS works on the principle that the relative ratio of light stable isotopes in natural compounds are different to those in synthetic compounds.[48] For example, De La Torre et al. found that the ^{13}C /^{12}C isotopic ratio for natural human testosterone deviated from the ^{13}C /^{12}C ratio of a defined standard by -21.3‰ to -24.4‰ while synthetic testosterone had deviations ranging from -26.18‰ to -30.04‰. GC/C/IRMS rules out false positives that could potentially be simply variations in the athlete's endogenous steroid profile. Some of the isotopes that GC/C/IRMS looks at includes carbon isotopes ($^{13}C/^{12}C$), hydrogen isotopes ($^{2}H/^{1}H$), nitrogen isotopes ($^{15}N/^{14}N$), and oxygen isotopes ($^{18}O/^{16}O$). However, GC/C/IRMS faces difficulties when the steroid is purposefully created with a similar carbon isotopic composition value to those reported for endogenous urinary steroids.

Liquid chromatography tandem mass spectrometry

GC/MS has a multitude of limitations including being unable to detect thermally unstable compounds, non-volatile compounds, and polar compounds. This is when an alternative technique—liquid chromatography coupled with mass spectrometry or tandem mass spectrometry—can be used. Liquid chromatography (LC) is a similar chromatographic technique to GC; however, a liquid mobile phase is used instead of a gaseous mobile phase. Unlike GC, where separation is mainly determined by the boiling points of the solute molecules, LC separation is determined by the interaction of the solute with the chromatography medium.[50] Although slower, LC is used when the solute is thermally unstable and could be structurally altered when placed in the high temperature GC column. The technique also requires less sample preparation and virtually no derivatisation, while being a more sensitive chromatographic technique in comparison to GC/MC.[51]

Preventing detection

Masking agents are a group of compounds that athletes use to prevent the detection of steroids. There are many types of masking agents including diuretics, which change the composition of body fluids by increasing the rate of urine flow;[52] probenecid, which reduces the secretion of steroids in the urine;[53] and liposomes, which work by encapsulating the drugs and modifying its physiochemical and pharmacokinetic properties.[54] Both diuretics and probenecid can be detected by GC/MS or LC/MS;[52] however, liposomes cannot. Liposomes are aqueous core filled spheres surrounded by a phospholipid bilayer; they can be mixed with steroids during injection, injected just before an expected doping test, or added into the urine sample during collection.[54] There are two ways which liposomes mask the presence of steroids: "body orientated" masking, meaning that they slow down the release of the drug altering its pharmokinetics, and "lab-orientated" meaning that they interfere with analytical methods by creating a lipid drug complex with free steroids/metabolites, decreasing the effectiveness of standardised tests such as GC and LC.[55]

Designer Drugs

Another way in which athletes avoid detection of doping is through designer drugs. Designer drugs are drugs that have been structurally manipulated to avoid detection in WADA accredited laboratories. Because of a lack of research surrounding these drugs, designer drugs are

consequently far more dangerous than typical drugs.[56] The development of detection for designer drugs is an evolving area of research. This is discussed in more detail below.

Microdosing

Microdosing is the technique of taking small doses of doping agents in just the right amount. It takes advantage of WADA's T/E ratio, which allows for a testosterone to epitestosterone ratio of 4.0. By taking micro doses, athletes can stay within the allowed range of T/E ratio fluctuation, while still achieving noticeable benefits that improve performance. The idea behind microdosing is that the athlete has the benefits of the doping agents during the event, yet evidence of its presence dissipates before the athlete is tested afterwards.[57]

New detection techniques

Currently, new techniques are being created in an attempt to improve detection of doping agents and decrease the possibilities of false positives. Two methods that are able to detect the use of liposomes as masking agents are currently under investigation: one being flow cytofluorimetry, used to detect liposomes in the blood, and the other being detection—by LC/MS—of DSPE-PEG, a product of liposome breakdown present in urine.[55]

Another emerging technique that allows for the detection of steroids is the bioassay. This is the process of measuring the potency of a drug by the effect it has on living organisms or tissues. Bioassays act as a non-targeted approach in detecting doping substances, including designer drugs.[58] Bioassays work on the principle that all steroids have the same mechanism of action in the process of asserting their effects. One common form of bioassay uses cultured cells that contain an AR in their cytoplasm. When an androgen binds to the receptor, it releases the protein complex HSP90 which then dimerises a second ligand-bound AR. The second ligand-bound AR translocates to the nucleus, augmenting DNA transcription by binding with androgen receptor elements (ARE). Bioassays take cells from different sources (e.g. HuH7 (human liver cancer cells) or MDA-kb2 (human breast cancer cells)) and genetically modify them to be able to express reporter proteins under the regulation of AREs.[59]

One specific bioassay proven to be very effective in the detection of doping substances in urine is the Chemically Activated Luciferase eXpression (CALUX) bioassay, or AR-CALUX, when applied to androgens.[60] AR-CALUX works by incorporating androgen-controlled luciferase reporter gene constructs into human U2-OS cells. When androgens enter the cells, transcription occurs and the firefly gene is produced, ultimately emitting light; luciferase activity can then be measured using a luminometer. This process is shown in more detail in Figure 4.

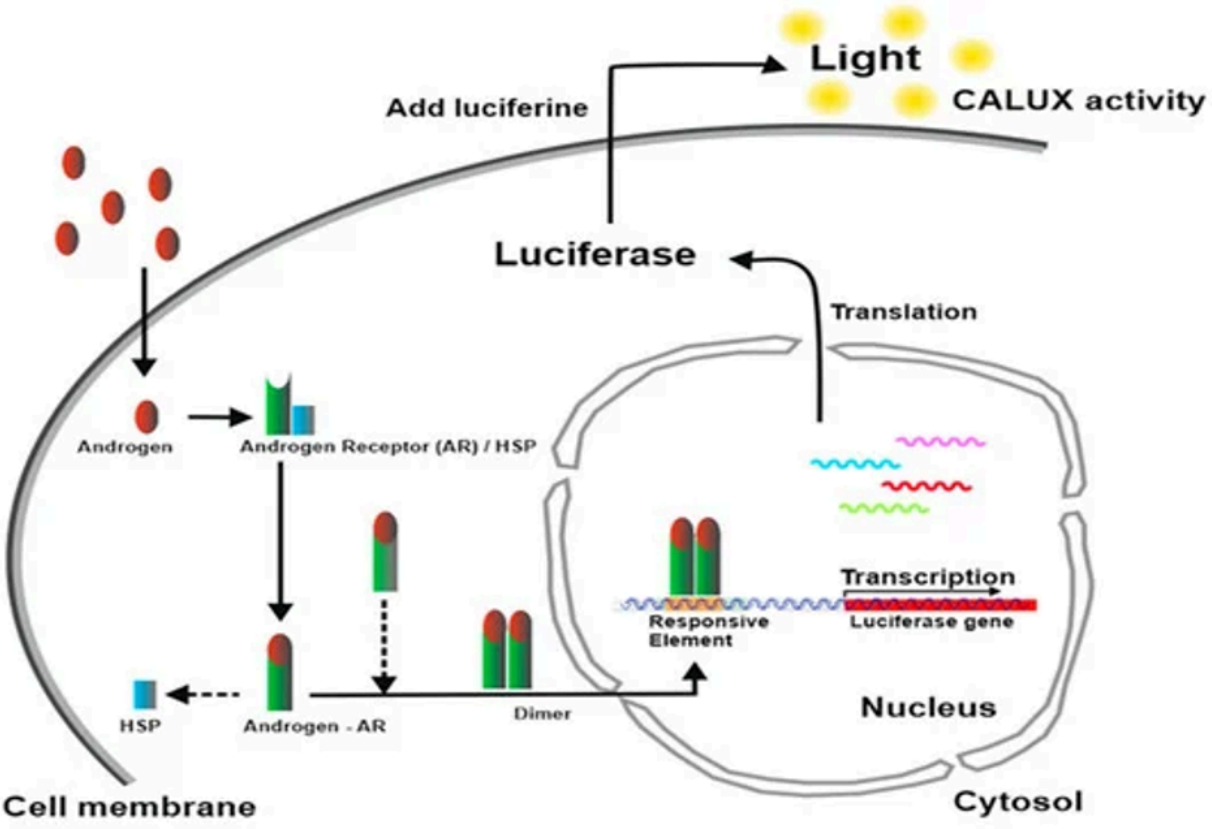

Figure 4:The process of AR-CALUX bioassays (image reproduced from (Martín-Escudero et al., 2021)).

One of the reasons why some cell lines are preferred over others is a lack of other receptors. The absence of certain receptors means that there is less chance of crosstalk between receptors, a phenomenon where similar steroid/androgen receptors recognise the same hormone response element, leading to the presence of false positives.[59] Different cell types also have different biological mechanisms. Yeast cells do not detect designer androgens in the form of prohormones due to their lack of steroid-metabolizing enzymes, but are better at measuring intrinsic androgenic potential due to this fact.[59] Mammalian cells also have steroid metabolizing enzymes; however, each type of mammalian cell has different expression patterns.

Another recent technique for steroid detection is nano liquid chromatography mass spectroscopy (nLC/MS). Reducing the column internal diameter (to < 0.5 mm) in nLC can reduce sample and solvent demands while also enhancing sensitivity with MS.[61] Lower detection limits achieved through nLC/MS will help identify use of doping substances easier. It was also found that this process could be automated.[62]

The future of doping

With the evolution of detection methods for steroids, scientists have also begun to explore and prepare for alternative methods that could be used by athletes in the future; one of which is gene doping. Gene doping is the process of employing gene editing techniques—including modifying the human genome or to introduce transgenes—to improve the sporting

performances of athletes.[63] Although no athletes have been caught doing gene doping, scientists are fully aware of its possibility and thus are still improving detection methods for it.[64]

Currently, some genes that could potentially improve athlete performances have been identified. This includes the PPARGC1A gene, which can significantly improve endurance by enhancing mitochondrial function;[65,66] the T allele on the AMPD1 gene, which improves skeletal muscle energy metabolism;[66] and the COL5A1 gene, which is correlated with ligament injuries and thus, potentially recovery times for athletes.[67,68] Because gene doping is a relatively novel technique, its long-term effects on athlete health have not yet been established.[63] However, there are also an increasing number of methods that can be used in the detection of gene doping of which include polymerase chain reaction (PCR) techniques, MS techniques, and CRISPR-based techniques.

Conclusion

The everlasting race between athletes that abuse doping agents and antidoping agencies is often compared to that of a cat and mouse chase. The development of new drugs is always met with the development of new detection methods. As long as there are incentives for performance enhancement, athletes will continue to seek ways to abuse certain substances. GC/MS, GC/MS/MS, LC/MS, LC/MS/MS, nLC/MS and bioassays are all integral techniques in the detection of steroids; their refinement disincentives athletes from pursuing unethical doping practices. Furthermore, new methods to counter gene doping are in development, with hopes of detecting gene doping even before its abuse. Understanding the chemistry and biology of steroid detection is necessary in the process of upholding a fair sporting ground for all.

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