Review

Journal of INTERNAL MEDICINE

doi: 10.1111/j.1365-2796.2008.01993.x

Medicine and science in the fight against doping in sport

D. H. Catlin[1], K. D. Fitch[2] & A. Ljungqvist[3]

[1]Professor of Molecular and Medical Pharmacology, Emeritus, University of California and CEO, Anti-Doping Research Institute, Los Angeles, CA, USA; [2]School of Sports Science, Exercise and Health, Faculty of Life Sciences, University of Western Australia, Perth, Australia; and [3]Professor of Pathology, Emeritus, and Former Head of Pathology Department, Karolinska Institute, Stockholm, Sweden

Sport is motion, health, and joy, but behind the greatest triumphs, who knows whether doping took place?

Abstract. Catlin DH, Fitch KD, Ljungqvist A (University of California and Anti-Doping Research Institute, California, USA; University of Western Australia, Perth, Australia; Karolinska Institute, Stockholm, Sweden). Medicine and science in the fight against doping in sport (Review). *J Intern Med* 2008; **264**: 99–114.

The fight against doping in sports commenced as a result of the death of a Danish cyclist during the Rome Olympic Games in 1960. The International Olympic Committee (IOC) established a Medical Commission (IOC-MC) which had the task of designing a strategy to combat the misuse of drugs in Olympic Sport. Some International Sport Federations (IF)

and National Sports Federations followed suit, but progress was modest until the world's best male sprinter was found doped with anabolic steroids at the Olympic Games in Seoul in 1988. Further progress was made following the cessation of the cold war in 1989 and in 1999 public authorities around the world joined the Olympic Movement in a unique partnership by creating WADA – the 'World Anti-Doping Agency'. The troubled history of the anti-doping fight from the 1960s until today is reviewed. In particular, the development of detection methods for an ever increasing number of drugs that can be used to dope is described, as are the measures that have been taken to protect the health of the athletes, including those who may need banned substances for medical reasons.

Keywords: doping, doping in sports, mass spectrometry, olympic games, sports, substance abuse detection.

Introduction

Doping or taking substances for the purpose of enhancing sports performance has a long history. In the early era of modern sport, doping was mostly associated with professional cycling. Although some cyclists died from the intake of strong stimulants in the late nineteenth and early part of the twientieth century, sports authorities remained passive. It was not until a Danish cyclist died in 1960 during a road race at the Olympic Games in Rome that action was taken [1, 2]. The Union Cycliste Internationale (UCI) began to develop a set of rules and in 1967, the International Olympic Committee (IOC) created a 'Medical Commission' (IOC-MC) to combat the misuse of drugs in Olympic sports [3].

It became apparent that doping had been practised for many years and involved many Olympic sports. A giant gap needed to be closed. Although anti-doping efforts have intensified since 1967, and the methods for the detection of doping substances have become increasingly sophisticated, until recently it was generally accepted that the dopers and their entourage were well ahead of those who endeavoured to curtail doping. In fact, some believed that the dopers will always be ahead, and that the struggle against doping is doomed to be a losing battle and therefore futile.

Herein we describe the measures that have been taken to combat doping during the last 40 years and present evidence that the aforementioned gap has been substantially reduced. Indeed, in some respects, the anti-doping efforts are ahead in the sense that preventive actions are being taken against the use of potential future doping substances and methods. The underlying analytical methods, which are the fundamental pillar of the success of the program, are supported by peer-reviewed publications built on a growing foundation of research. A second pillar is the therapeutic use exemption (TUE), an extensive program that enables an athlete to be treated by a physician with a prohibited substance and still compete. The TUE program safeguards the health of the athlete and the integrity of the anti-doping efforts.

The use of doping substances in many sports and on all continents has become a major public health issue. The global and universal characteristics of doping led to the formation in 1999 of the World Anti-Doping Agency (WADA) [4], a unique collaboration between sports and governments. WADA is founded on equal partnership between public authorities and Olympic sport. Thus, the fight against doping has evolved from a singled-handed effort of the IOC and sport federations to one that encompasses the active support of the international political establishment.

The overall growth of testing is apparent from Fig. 1 which shows the total number of samples tested per year from 1988–2006. The first growth spurt began in 1989, possible because of a widely publicized case of stanozolol involving the first finisher of the 100-m dash final at the 1988 Seoul Olympics. From 1988 to 1991, the annual increase averaged 21%. The second spurt began in 2003. From 2003–2006 the annual increase averaged 10.8%.

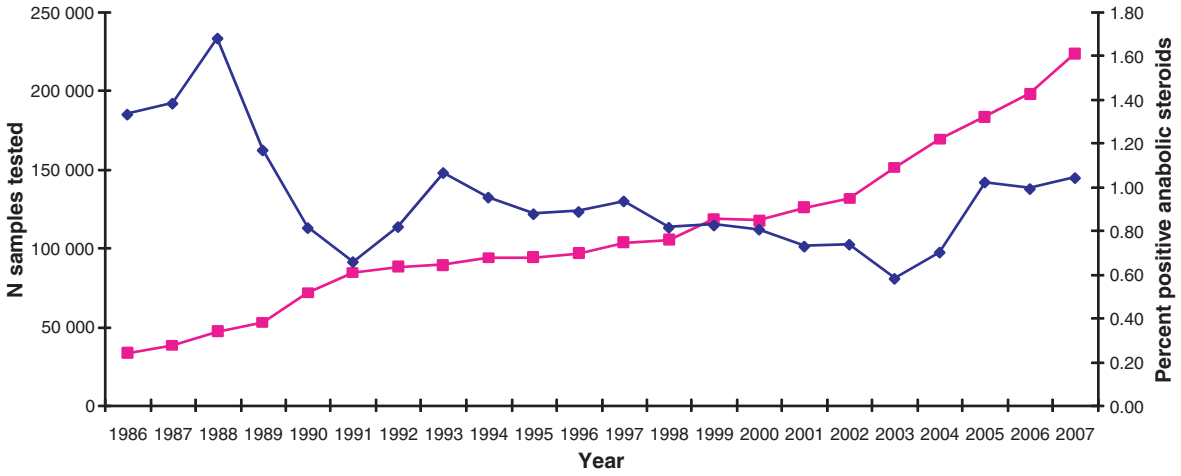

Fig. 1 The squares (left axis) indicate the total number of urine A samples tested by IOC- and WADA-accredited laboratories from 1986–2007. The diamonds (right axis) indicate the percentage of those samples that was reported as containing prohibited anabolic steroids. The percentage of the A samples reported positive will be substantially less than the percentage of the B samples reported positive. The data through 2002 were supplied by a member of the IOC Medical Commission. The post-2002 data were derived from the WADA website data (http://www.wada-ama.org). In 2004, WADA changed the T/E cut-off from 6 to 4 and this was a major factor in the increase in percentage in the figure. The percent positive figure increased at 4.6% per year for the 5 years ending in 2001, whilst for the 5 years ending in 2007, the corresponding increase was 8.2% per year.

The IOC medical commission era

The IOC-MC under the Chairman, Prince de Mérode, a member of the IOC from Belgium, took the dauntless task in the seventies and eighties of creating and implementing anti-doping activities for the Olympic movement. One of the two main tasks was to create, maintain and circulate a list of prohibited substances ('the List'). Initially, the concept was that the List should only contain drugs that laboratories can test for. This requirement was dropped in the mid-eighties when some Olympic cyclists were transfused with whole blood just before the opening of the 1984 Games of Los Angeles. Their clandestine activities were uncovered and later widely reported in the media and elsewhere [5]. At the time blood doping was considered unethical but it was not formally banned for lack of a test. Thereafter the IOC-MC prohibited drugs and doping methods even though no detection was available at the time. The second main task was to accredit laboratories for doping control. After some pioneering work by the International Amateur Athletics Federation (IAAF – later renamed 'International Association of Athletic Federations'), the IOC took over the accreditation programme in 1983 and developed it further. At first, the tool for this was an IOC-MC-operated proficiency program. Urine samples from volunteers treated with doping agents were circulated and the laboratories were required to identify any substance on the prohibited list and complete their analyses within 7 days. They knew that the samples were for proficiency testing but they did not know what drugs they contained (open blind samples). Later, as the number of IOC-accredited laboratories increased, on-site visits were introduced and the rules and regulations governing the accreditation process markedly expanded. Today, the accreditation of laboratories has been taken over by WADA. There are presently 33 such laboratories around the world.

In the mid-nineties, the IOC and its doping control laboratories came under increasing pressure from the legal and worldwide laboratory community to harmonize and standardize its anti-doping methods and procedures. Following considerable discussion, the IOC-MC required all laboratories to become accred-

ited by the International Organization for Standardization (ISO) as a prerequisite to IOC accreditation. ISO is an international body that sets standards for a wide variety of manufacturing processes and commercial products [6]. Although the process was time-consuming and expensive, ISO accreditation led to considerable harmonization and it markedly enhanced the quality and stature of the laboratories.

In the early days, it was generally believed that only certain stimulants such as amphetamine, cocaine, strychnine and ephedrine were used as doping substances. Since such drugs are taken at the time of a competition to temporarily enhance performance, a strategy was developed which included not only the production of a set of rules together with information and education about doping, but also doping control tests at competitions. These became known as 'in-competition' tests.

Small-scale tests for stimulants were introduced at the 1964 Olympic Games followed by a somewhat larger doping control program at the 1968 Games in Mexico City. Then the first known positive Olympic case occurred. A Swedish modern pentathlete was found with significant levels of ethyl alcohol. Small doses of alcohol can improve shooting scores. It is therefore forbidden in shooting events. This resulted in the Swedish team losing its bronze medal. At the 1972 Munich Olympic Games, the urine samples were tested by gas chromatography (GC) for stimulants and narcotics. Seven positive cases were reported [3], including one case of ephedrine that led to a wide-ranging and long-lasting discussion of the issues involved when commonly used therapeutic agents are placed on the prohibited list.

It soon became apparent that the drug use by Olympians extended beyond stimulants, and the general premise developed that any drug that had the capacity to enhance human performance would find its way into the hands of sports persons and their entourage. Androgenic anabolic steroids (AAS) began to be used by athletes during the 1960s or earlier [7, 8], and by the early 1970s, widespread use of AAS was reported from different parts of the world [9, 10]. In 1974 the IAAF was the first sports body to ban AAS and testing was instituted at the European Athletic Championships in Rome the same year. No positive cases were identified. By the time of the Olympic Games of Montreal in 1976, the IOC had prohibited AAS and conducted testing for them for the first time. In those Games eight athletes tested positive.

The introduction of AAS posed a new problem to sports administrators because unlike stimulants which act immediately, the effects of AAS have a gradual onset. This includes their ability to allow for a more intense and efficient training. AAS users learned to 'bulk-up' during training and discontinue the drugs in advance of the competition when the tests are conducted. This led to 'out-of-competition' testing and later to totally unannounced testing. In 1991, the IAAF was the first international sports body to introduce 'out-of-competition' testing. The decision to proceed was difficult for the IAAF, but eventually they prevailed. Initially, it was argued that 'out-of-competition' testing is unethical and an unacceptable intrusion into the private life of the athlete. Today, unannounced 'out-of-competition' testing is regarded as an indispensable part of any effective doping control program. The World Anti-Doping Code [11] even includes the responsibility of the athlete to continuously provide his or her whereabouts so that he or she can be contacted for unannounced testing at any time. Failure to provide correct whereabouts information is considered as an anti-doping rule violation and renders the athlete ineligible for competition.

The WADA era

The World Anti-Doping Agency was created for four main reasons. First, there was a lack of harmonization of anti-doping rules. Different IFs and national anti-doping organizations (NADOs) had different rules which resulted in an increasing number of doping cases being contested in civil courts. Harmonization of anti-doping rules became an absolute necessity. Secondly, the use of certain doping substances, in particular AAS, expanded beyond the sports arena and tended to become a public health problem. The intake of AAS has the potential to have serious

adverse effects on the individual user and society at large [12–16]. Thirdly, there was a need to undertake research to keep abreast of developments within the pharmaceutical industry. In particular, analytical methods needed to be available as new substances and methods came on the market. Fourthly, it was essential to promote anti-doping activities both at the national and international level, and to have those activities monitored by a central body.

The IOC accepted that this could not be achieved by sport alone. The support of public authorities was critical. Governments were invited by the IOC to make joint efforts, and after considerable discussion, WADA was created in 1999. The financial underpinning of WADA is jointly shared by the IOC and the participating governments. By the time of the Olympic Games in Athens in 2004, the World Anti-Doping Code which spells out the anti-doping rules had been prepared by WADA and accepted by the majority of Olympic sports. In 2007 a UNESCO Convention was ratified which lends governmental support to the Code and WADA activities. The Code and its various subsections are constantly updated and amended.

After WADA was created, experience revealed that doping – or rather 'an anti-doping rule violation' – should mean more than just the intake of a prohibited substance or use of a prohibited method by an athlete. The Code now states that '*Doping is defined as the occurrence of one or more of the anti-doping rule violations set forth in Articles 2.1 through Article 2.8 of the Code*', meaning that not only is (i) 'the presence of a prohibited substance or its metabolites or markers' banned, but also (ii) 'use or attempted use of a prohibited substance or a prohibited method', (iii) 'refusing or failing without compelling justification to submit to sample collection', (iv) 'violation of applicable requirements regarding athlete availability for out-of-competition testing', (v) 'tampering or attempting to tamper, with any part of doping control', (vi) 'possession of prohibited substances and methods', (vii) 'trafficking in any prohibited substance or prohibited method' and (viii) 'administration or attempted administration of a prohibited substance or prohibited method to any athlete'.

What mechanism should decide what substances and methods should be on the 'Prohibited List?' Earlier, that decision had been the responsibility of the IOC-MC. Once WADA was created, its executive committee decided that a substance or method should meet at a minimum two out of three criteria before it could be considered for inclusion in the List, namely, (i) the substance or method can be performance-enhancing, (ii) the use of the substance or the method can endanger the athlete's health and (iii) the use of the substance or method is against the spirit of sport. Not one of the criteria is compulsory meaning that, a substance or a method can be listed without being performance-enhancing. Therefore, the general perception that doping means the intake of certain substances or use of certain methods for the sole purpose of enhancing sports performances is incorrect. The protection of the health of the athlete is of major importance, as is the public health aspect. Athletes should be healthy and clean role models for young people, not drug takers who place their health at risk. The List includes substances that are banned only 'in-competition', for example, glucocorticoids and those that are banned at all times. The latter includes, for example, erythropoietin (EPO) and AAS. The reason for the distinction is that athletes should be allowed to take medications if they need it for medical reason in their daily life when not competing without being forced to apply for a TUE.

During recent decades, more and more substances and methods have been used by athletes for the purpose of doping, and the List has been expanded accordingly. In addition to stimulants and AAS, the List today contains drugs such as EPO, human growth hormone (hGH), selective androgen receptor modulators, immunoglobulin factor-1, insulin, anti-oestrogens, beta-2 agonists and diuretics, and methods such as blood doping, chemical and physical manipulation of the sample and gene doping. In anticipation of the likelihood that new doping agents will appear, the List is non-exhaustive. This means that existing or future drugs with similar effects are likewise banned.

The cornerstones of the anti-doping strategy are education, information, doping control tests and research.

The education and information parts fall outside the scope of this paper and the doping control test systems have been outlined above. Previously, sports had lagged behind the development of doping because of lack of research funding. Not until WADA was created, was there a permanent international fund for anti-doping research. From its inception, in 1999 to the end of 2007, WADA had allocated 31.4 million USD to various research projects, and 26% of WADA's budget is presently reserved for research. Priority is given to projects aimed at improving existing detection methods and developing new methods when needed. Currently, it is feared that progress in gene therapy will be put to use by unscrupulous people for the purpose of doping. That fear is supported by some incidents in recent years [17], although there is no evidence that 'gene doping' has indeed occurred. But before that occurs, WADA aims to have detection methods in place. To that end, projects for the development of such methods have received substantial support during the last few years following the WADA gene doping symposia at Cold Spring Harbour Laboratory in New York in 2002 and at the Karolinska Institute in Stockholm in 2005. There are different avenues to pursue [18]. This is an example of attempts by WADA to stay ahead of the dopers.

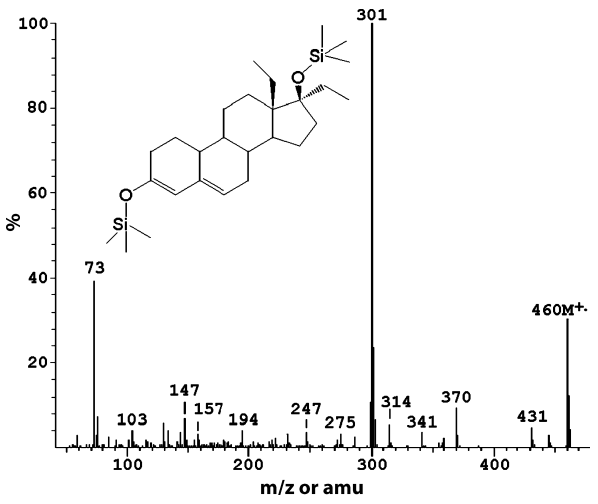

Fig. 2 Chemical structure of the di-trimethylsilyl (TMS) derivative of norbolethone shown above the mass spectrum of a norbolethone reference standard.

Detection of doping substances and methods

Stimulants detection by gas chromatography

In the era following the death of the Danish cyclist at the Rome Olympic Games in 1960, as sporting organizations began prohibiting stimulants and collecting urine from athletes for testing, GC was the only analytical method with sufficient reliability to justify using its results to penalize athletes. GC separates the compounds present in a mixture. After the mixture is injected into GC, the time it takes for a particular compound to come out alone is its retention time. Matching retention times between unknown and authentic reference standard is one element of partial drug identification. At the 1972 Games of Munich, the samples were screened by GCs equipped with nitrogen–phosphorus detectors (NPD) [19]. Most stimulants have a nitrogen atom in their chemical structure; therefore they are highly detectable by the NPD detector, especially because the urine extract contains virtually no other nitrogen-containing molecules other than the target compounds. One GC–mass spectrometry (MS) was used for confirmations and this technology is explained below.

The 1960s also witnessed the beginning of AAS abuse, but it took another 15 years for the first detection methods to appear. Testosterone (T) and methandrostenolone (Dianabol®) were the most popular AAS at the time. The former was only available for parenteral administration. Dianabol® was an instant hit with the doping community because it was potent and it was available as an oral formulation. In fact, its use became so popular that Ciba-Geigy eventually took it off the market. The key difference between methandrostenolone and T is the 17α-methyl group that renders the former active by the oral route, and also makes it distinguishable from T by immunoassay (IA).

Anabolic steroid detection by immunoassay

In the early 1970s, AAS could not be tested for by GC and MS and were not amenable to large-scale routine testings. This led to the development of IA screening tests even though the use of IA for exogenous, pharmaceutical AAS detection is a challenge because human

urine is full of endogenous AAS such as T and a cascade of its metabolites. If an IA is responsive only to synthetic AAS whose chemical structure is substantially different, such as the 17α-methyl analogues of T, it will miss the synthetic AAS that is similar, such as boldenone. If on the contrary, an IA is responsive to boldenone, it will cross-react with T and other endogenous hormones, and correctly indicate the presence of AAS in all samples, which would render the screen useless. Yet AAS screening began with IA for 17α-methyl steroids at the 1974 European Athletics Championships in Rome and later at the 1976 Summer Olympics in Montreal. IA could detect many, if not all synthetic steroids that were popular at the time [20], however, the GC–MS methods were not yet practical for immediate confirmation of IA results. Therefore, the eight IA positives in Montreal were confirmed by GC–MS a few weeks after the Games.

Gas chromatography–mass spectrometry and liquid chromatography–mass spectrometry

As an alternative to the NPD, an MS is another type of detector that can be coupled to a GC. The combined instrument is known as GC–MS. An MS comprises an ion source where the sample is bombarded with electrons to scare off one electron from the molecule, leaving behind an ionized molecular ion, which fragments into smaller ions; a way to separate ions according to their mass—often a quadrupole; and a detector to record the abundance of ions of each mass. The mass spectrum is a graph of ion abundance as a function of the mass/charge ratio or m/z (Fig. 2). A given chemical will always fragment in the same, reproducible way; therefore the mass spectrum is characteristic of the compound. Matching mass spectra between an unknown and a reference standard is key element of identification. Matching only three diagnostic ions (e.g. 301, 445, 460) and their abundance relative to the most intense of the three (e.g. 301) is broadly considered a sufficient proof of identification.

In the GC–MS, compounds separated by the GC go into the MS and the MS continuously records mass spectrum scan after scan. Substances are identified by matching chromatographic retention time and mass spectra between unknown and authentic reference standard. The standard may be the pure chemical or a urine sample from a research subject known to have taken the drug. GC–MS makes it possible to identify chemicals unambiguously. GC–MS is the gold standard in small molecule identification, therefore by 1976 the use of GC–MS for confirmation was required by the IOC.

As an alternative to GC, liquid chromatography (LC) achieves compound separation in the liquid phase and there is no heat involved. Therefore, thermolabile compounds can survive LC, whereas they cannot be analysed by GC. The combined instrument, LC–MS, has many advantages over GC–MS and is becoming the analytical method of choice even though it is not well-suited for detecting certain AAS. Except for glycopeptide and peptide hormones such as EPO and hGH, most of the substances prohibited in sports are now identified by GC–MS or LC–MS.

Detection of testosterone and other endogenous steroids

No positive doping cases were reported during the 1980 Summer Olympic Games in Moscow; however, rumours of AAS use were circulating. As doping control tests improved at identifying synthetic steroids, users moved on to T and other endogenous steroids. Unfortunately, neither GC–MS nor LC–MS can distinguish endogenous T made by the human body from exogenous T from the pharmacy or the black market. Between the Games of Moscow and Los Angeles, extensive studies of the urine steroid profile were conducted. It was found that human urine contains a T isomer with no known biological function, epitestosterone (E). The median ratio of urinary T to E (T/E ratio) is approximately 1 : 1 and increases upon T administration [21, 22]. Donike proposed that the T/E ratio be used to detect T administration [21]. At first the IOC-MC set the cut-off at 6 : 1. As the T/E ratio could only be determined by GC–MS, in 1984, for the first time all Olympic samples (n = 1510) were screened for AAS by GC–MS [23]. Sixteen were reported positive for AAS (nine nandrolone metabolites, five T/E, two methenolone) but only 11 of these

were documented and acted on during the Games. The other positive steroid cases were lost because of 'accidental' shredding of the codes. Later, the IOC-MC increased the T/E cut-off to 10 : 1, and then back to 6 : 1. Now the WADA defines an adverse analytical finding as having a T/E greater than four.

One problem with T/E is that drug-free individuals can have a naturally elevated T/E. Another problem is that T/E never exceeds four in some users, either because their T/E does not change much upon administration for genetic reasons [24] or because they use microdoses to titrate themselves. One approach to resolve such ambiguous cases is longitudinal profiling, which consists in plotting T/E and other urinary androgen concentrations and ratios over time. Drug-free individuals are expected to have stable values whereas users are expected to show abrupt variations correlating with drug use [22]. In the late 1990s, a new approach was introduced: isotope ratio MS (IRMS) [25].

Isotope ratio mass spectrometry

Most of the carbon in the natural world is carbon-12, with six neutrons and six protons in the nucleus of the atom. Approximately 1% of all carbon, however, is carbon-13, with seven neutrons instead. Fortunately for anti-doping scientists, exogenous, pharmaceutical T contains slightly less carbon-13 than endogenous, natural T. Pharmaceutical T is manufactured by semi-synthesis from plant starting materials; natural T arises from biosynthesis from cholesterol, which comes from endogenous or dietary sources. The carbon in all of these compounds and all living things originates from atmospheric CO_2, fixed by plants during photosynthesis. Because the pathways between atmospheric CO_2 and each T of interest differ, the resulting carbon-13 content differs between endogenous and exogenous T, and this difference is measurable with a GC-combustion-IRMS. In practical terms, it means that the carbon isotope ratio value for a urinary steroid determines if the steroid is natural or synthetic. The WADA Technical Document on reporting and evaluation guidance for testosterone, epitestosterone, T/E ratio and other endogenous steroids, recommends that a urine sample in which any one of certain criteria such as T/E greater than four, is met during the screening procedure, it will be routinely submitted to the IRMS analysis [26]. IRMS analysis in sports doping control is commonly referred to as a carbon isotope ratio. In addition, the sports authority may request IRMS analysis on samples of its choice selected by a targeting approach. The IRMS test is not required to identify the actual substance that was taken. In many cases, that substance is simply not known.

In addition to moving on to endogenous steroids, users also moved on to smaller doses of exogenous steroids and cocktails, because it was increasingly difficult for the laboratories to detect the smaller amounts of AAS in the urine and most laboratories did not have research grade specialized MS. However, costly high resolution MS (HRMS) or trap MS, had the increased sensitivity that made it possible to find long-lasting metabolites in lower amounts [27]. At the Games of Atlanta in 1996, HRMS was used for the first time at an Olympics to screen all samples. Several samples were found to contain long-lasting metabolites and low concentration of AAS, however, these cases were administratively dropped when disagreements arose regarding some of the findings.

Erythropoietins

Meanwhile in 1989, recombinant human EPO (rHuEPO) became available for patients and was soon misused by sportspersons. In the late 1980s, there were a number of unexplained deaths amongst young, healthy, elite male European cyclists. The prevailing hypothesis is that they had overdosed on rHuEPO [28]. Sport first attempted to cope with EPO by instituting indirect tests on blood. The simplest indirect test consists of measuring the haematocrit or haemoglobin. Some sports organizations conduct 'health tests' shortly before the start of races, for example, the UCI rules define a maximum haematocrit of 50% for men and 47% for women, above which an athlete is not allowed to compete because exercise-induced dehydration and the resulting increase in haematocrit results in a greater risk of thromboembolic event [29].

Such a finding would not be an adverse analytical finding (or 'positive case' in common language), nor would it lead to any sanction or disqualification. Instead it is an 'Atypical Value' which leads to declaring the athlete 'unfit' to compete. The difference in language is designed to lower the risk of legal action.

Combining blood parameters in a mathematical formula also makes it possible to calculate an on-score or off-score which indicates whether the individual is on a recombinant EPO or recently stopped using one. The off-score has a retrospectivity of about 2 weeks [30]. These blood tests are indirect tests because they do not detect the presence of recombinant EPO, only the footprints of use.

In contrast the direct test detects rHuEPO itself. Endogenous human EPO is a peptide hormone consisting of a family of isoforms, which differ from each other not by the peptide sequence, but by its degree of glycosylation. This family of isoforms is different for recombinant EPO and natural EPO, in other words, the two types of EPO carry different charges and can be separated by electrophoresis. Although electrophoresis had practical limitations, it demonstrated conclusively that the isoform pattern of urinary endogenous EPO differs from the pattern of urinary rHuEPO, and it was the first successful attempt to develop a direct test for urinary rHuEPO [31].

A significant improvement occurred in 2000 when Lasne and de Ceaurriz [32] described a method based on isoelectric focusing (IEF) with immunoblotting plus one novel and critical step: a second blot ('double-blotting') [33]. After the isoforms of rHuEPO are separated by IEF, the first blot transfers the proteins to a first membrane, which is incubated with anti-EPO antibody. The second blot transfers only the anti-EPO antibodies to a second membrane, which is incubated with a second antibody directed against the first antibody. This step markedly reduces nonspecific binding and yields clear isoform patterns. Finally, chemiluminescence produces an image of the gel.

The electropherogram contains one lane per sample, standard, or quality control sample. In each lane, the isoform pattern consists of bands. The pattern (number of bands, positions, relative intensities) allows identification. At the 2002 Olympic Games of Salt Lake City, the IEF test with double blotting was successfully used to detect darbepoetin alpha, a long-acting form of rHuEPO, which had been released in US just 4 months before the Games [34]. Darbepoetin alpha has additional negative charges which cause the bands to migrate to the more negative portion of the IEF gel, therefore darbepoetin alpha can readily be distinguished from rHuEPO based on different isoform band patterns. The IEF detected darbepoetin alpha in the urine of three athletes who had won eight medals during the Games.

Flow cytometry to detect blood transfusion

The past few Olympic Games have seen the introduction of blood testing. At the 2004 Athens Olympics, flow cytometry was used to detect blood transfusions [35]. No adverse analytical findings were reported during the Games, because of a sample handling mishap, however, 2 weeks after the Games another sample from the same athlete had evidence for two populations of red cells in his circulation [36].

Human growth hormone

Anti-doping scientists have been working on a method to detect the use of recombinant hGH (r-hGH) for over 10 years. At this time the two leading contenders are an indirect test based on a medley of markers [37, 38], and an antibody test based on the ratio of 22 k hGH immunoactivity to that of the total immuno-hGH activity in serum [39]. Following the administration of 22 k hGH, immunoactivity increases whilst the denominator decreases. The ratio test was deployed at the Games of Athens and Torino. There were no reports of adverse findings. The retrospectivity of the ratio test is not clear but should be in the order of one to several days. The marker test is an indirect test that measures a number of variables that change when hGH is administered [37, 38]. It has not yet been validated for the purpose of doping control analysis.

Profiling of blood or urine: longitudinal testing, passports and volunteer programs

Monitoring clinical parameters is common in medicine and in everyday activities of the practicing physician. Changes in parameters may be sensitive indicators of disease or doping. The first data point is compared with population norms and subsequent points to the patient's baseline. This is exactly how the urinary T/E is first compared indirectly with population statistics by way of the cut-off and then to the individual's other values. A spike indicates T use [22]. On the other hand, a marked reduction in the concentrations of endogenous urinary steroids reflects the shut down of endogenous androgen production because of use of potent AAS. Concentrations and ratios of urinary androgens are powerful indicators of doping with any androgen. Monitoring has been expanded from urine to blood.

For example, the UCI biological passport concept consists of maintaining athletes' individual records of urine and blood test results and profiles. Doping would be detected indirectly without identifying the substance taken, by assessing significant variations, which would suffice to open disciplinary proceedings.

A revolutionary concept is the Volunteer Program, scientifically similar, but with a goal exactly opposite. Far from punishing drug users, this program would merely deny them the privilege of its benefits, and instead focus on rewarding drug-free athletes. The situation with doping in sports needs to be turned around, so why not do it literally? [40].

Dietary supplements

Later phases in the steroid abuse saga include dietary supplements, specifically those containing anabolic steroid prohormones, designed to be converted in the body to hormones such as T [41]. In 1998, a reporter spotted a bottle of 'Andro' (androstenedione, the immediate precursor of T in the body) in the locker of an American baseball star. Worldwide sales sky-rocketed overnight. At the time, Andro was not prohibited by any sports organization. At first, it was not clear if Andro had significant anabolic properties; nevertheless, in 1998 the IOC-MC placed it and a number of other endogenous steroids on the Prohibited List. This necessitated dividing the List of prohibited steroids into two categories: endogenous and exogenous. Gradually, evidence had been accumulating that Andro is anabolic. Although unequivocal and convincing evidence of increased strength has not yet been demonstrated, evidence is accumulating. For example, in young men, it was found that 300 mg per day for 7 days increases serum T [42].

Many prohormones, natural hormones and related steroids can be ordered directly from internet sources. Federal authorities in the US try to control this activity, however, sites are continually popping up on the internet. Often the products masquerade as dietary supplements, although they are typically clearly advertised as anabolic, but legal. Some US agencies have made efforts to get some of these products off the market but they can still be purchased without much difficulty.

For athletes, beyond the risk of adverse effects, there is a risk of an adverse analytical finding for an anabolic agent, because either the main ingredient is a supplement (assuming it is even present in the bottle) or more likely, an impurity from sloppy synthesis or an unclean mixing vat. In several infamous cases, athletes have been punished or kept from going to the Olympics allegedly because of a supplement contaminant. The analysis of numerous dietary supplements by a number of WADA-accredited laboratories worldwide has shown that the bottle may contain what it says on the label in the amount stated on the label, or more, or less, or none, or a different steroid altogether, including one that is a controlled substance in US [41, 43].

Designer anabolic androgenic steroids

In the latest phase in AAS abuse, designer steroids surfaced. For decades, anti-doping scientists had suspected the existence of a clandestine industry, designing doping agents and doping regimens and discovering ways to beat the official tests and make

a fortune. The first proof of concept came under the form of norbolethone, a pharmaceutical drug long abandoned by the industry, yet found in the urine of one athlete in 2002, only because diligent drug testers noticed a suppressed endogenous steroid profile, a tell-tale sign of exogenous steroid use. It took far more diligence to identify the steroid in use because it was not routinely monitored by anti-doping laboratories. Eventually, the norbolethone in the athlete's urine sample could be matched against a reference sample of norbolethone from the history vaults of Wyeth [44]. A year later, an anonymous coach mailed to US Anti-Doping Agency, a spent syringe allegedly containing a secret, an undetectable steroid. Two months later, Compound X had been identified as tetrahydrogestrinone (THG) by eight researchers [45]. THG is the most notorious designer steroid—designed, synthesized, and distributed only to beat the test—but it was not the first or the last.

Tetrahydrogestrinone marked the beginning of the BALCO affair, a wide-ranging sport drug scandal that continues today. BALCO was an American company that masqueraded as a food supplement provider whilst it distributed performance-enhancing drugs to athletes in several Olympic and professional sports. The scandal involved many high profile athletes, a chemist who synthesized hard-to-detect drugs, a drug distribution system and laboratory testing. Until that time the dominant testing method was GC/MS, however, THG disintegrated in the GC. Because LC–MS is the best way to screen for THG, all WADA-accredited laboratories now screen urine samples by LC–MS, but THG has never been found again. The LC–MS screen, however, is more sensitive than GC–MS for several other AAS, and as a result epidemics of positive cases for some of those steroids have been recorded.

Rapidly deployed proactive methods

A cardinal feature of doping is that some athletes will experiment with any new substance that might improve performance. They do not wait for regulatory approvals. If they can obtain a supply they will try it. Therefore the WADA laboratories strive to anticipate and develop tests even before the misuse of a substance as a doping agent is reported.

Three examples of rapid development and implementation of a test are the tests for RSR13 (efaproxiral), hydroxyethyl starch (HES) and the haemoglobin-based oxygen carriers (HBOCs). In the case of RSR13 (efaproxiral), an assay was developed whilst it was still in clinical trials. The pharmaceutical company that was developing RSR13 recognized that as it was an allosteric modifier of haemoglobin and had been shown to increase maximum oxygen uptake (VO_{2max}) in animal models, it was a potential doping agent. In addition, there were unconfirmed reports that RSR13 was confiscated at a sporting event. This led to proactive collaboration and a test was developed and implemented even before the RSR13 trials were complete [46].

In January 2000, HBOCs such as Hemopure and plasma volume expanders such as HES were placed on the Prohibited List. A few months later, a test for HES was described [47] and shortly thereafter, it was used to detect doping in athletes competing in the 2001 World Nordic Ski Championships in Lahti, Finland. Similarly in anticipation of the misuse of HBOCs, a method for detection was developed and deployed [48].

Doping controls at olympic games

At the Olympic Games, urine and blood samples are collected by staff members of the Local Organizing Committee and the laboratory analyses are performed at an accredited laboratory which is normally located in the host city. The laboratory is staffed by experts from the host nation plus senior and highly experienced experts from other WADA-accredited laboratories. The members of the IOC-MC supervise the work both at the venues where the sampling takes place and in the laboratory. The laboratory is staffed and equipped to provide the results of the screening analyses within 24 h of receiving the samples. The result management is the responsibility of the IOC-MC. Any decision related to positive samples or any other alleged anti-doping rule violation is taken by the IOC Executive Board.

In the eighties and nineties, the Olympic samples were collected at the completion of the competition and tested only for the 'in-competition' menu, however, for the past few Olympic Games, the 'Olympic period' or the period of time that an athlete may be tested, has greatly expanded as has the number and types of testing performed. The 'Olympic period' now extends from the opening of the Olympic Village 2 weeks before the start of the Games to the close of the Games about 16 days later. During this period of about 1 month, every athlete is available for blood and/or urine sampling whether residing in or outside the Olympic Village. In addition, NADOs usually increase the intensity of testing in the weeks immediately before the Games. In-competition tests are conducted after each final and typically include samples from the gold, silver and bronze medalists and a random selection of the remainder. In addition, samples are collected at random also after preliminary rounds.

The quality and quantity of the Games-time control program has steadily increased since the Olympic Games of Munich in 1972 when 7121 athletes participated and 2079 urine samples were analysed by GC for stimulants and narcotics [3]. At the 2004 Games of Athens, 10563 athletes participated and 2863 urine samples were analysed by the sophisticated methods described above. In addition, 416 blood samples were analysed for hGH and 263 blood samples were analysed for evidence of blood transfusion and HBOCs. In Beijing 2008, the number of participating athletes will be about the same as in Athens, but the doping control program will expand to include about 4500 urine samples. The number of blood samples collected will be about the same as in Athens but the number of tests per sample will increase.

Therapeutic use of prohibited substances

Initially, this was termed permitted use of prohibited substances and abbreviated to 'permitted use'. In 1999–2001, the term 'therapeutic use' or 'TUE' was introduced gradually and the process is known as 'therapeutic use exemption' or 'TUE'. However, as expected, global recognition and acceptance of this procedure was slow.

The List was developed first to prevent drug-induced enhancement of sports performance and secondly to protect the health of athletes. It was not intended to prevent doctors from prescribing the correct medical treatment to their athlete spatients. In 1985, the inclusion on the List of three new classes, diuretics, beta-blockers and systemic glucocorticosteroids, put some athletes and their doctors at a disadvantage. Their inability to administer any drugs in these three categories had the potential to result in either suboptimal treatment or aggravation of an athlete's medical condition if the drug had to be discontinued. In 1988, in Calgary, the IOC-MC permitted an athlete to continue to take oral corticosteroids for biopsy-proven inflammatory bowel disease and compete at the Games, whilst later that year in Seoul, an athlete with biopsy-proven nephrotic syndrome was permitted to take oral furosemide. About the same time, exemptions were being granted in a several countries (including Sweden and Australia) to a few national level athletes who had presented an incontestable need to administer a prohibited substance to treat a genuine medical illness or condition. Such approvals were valid only in that country.

In 1989, a 19-year-old Australian athlete with neonatal torsion of both testes, who had been prescribed cyclic replacement T injections since puberty, sought national approval and indicated that he hoped to compete internationally in the near future. The IOC-MC undertook studies on urine samples of the athlete's urine after T administration. There were discussions of recommending replacement T therapy, however, the proposal received little support and was dropped. In 1991, the IOC-MC established a small committee of the three members, A Ljungqvist, D Catlin and K Fitch (secretary) termed the Medication Advisory Committee (MAC) to examine the problem of TUE.

The MAC established criteria that had to be met before an athlete could be granted permission to administer a prohibited substance and compete and guidelines as to how this approval process should be managed. Criteria were:

1 the athlete would experience significant impairment of health if the prohibited medication was withheld;

2 no enhancement of performance could result from the administration of the prohibited substance as medically prescribed;

3 the athlete would not be denied the drug if he/she was not a competing athlete;

4 no available or practical alternative can be substituted; and

5 retrospective permission would not be granted.

Guidelines included the need to submit full medical details including laboratory and imaging investigations, confirmation of the necessity to administer the prohibited drug by a consultant or specialist in the appropriate medical discipline and information on the sports discipline and specific role of the athlete.

Although that committee's report was presented and approved in October 1991, the MAC was not permitted to commence its role for the 1992 Winter Olympics. At those Games, an ice hockey player, whose dermatitis was a direct consequence of wearing a mandatory protective device, was permitted by the IOC-MC to take a small daily dose of oral corticosteroid because every time he discontinued such treatment, his condition relapsed. The MAC, which operated in strict medical confidence, commenced to function prior to the 1992 Barcelona Olympic Games and provided permission for a number of athletes to take prohibited substances and compete at those and subsequent Olympic Games. It has also rejected a number of applications because they failed to fulfil the established criteria.

However, the concept was not formally approved by the IOC Executive Board nor permitted to be publicized because the Chair of the IOC-MC was concerned that a plethora of applications for TUEs may follow. During the next 8 years, the MAC provided advice to 11 National Olympic Committees (NOCs) and 15 IFs who sought assistance for athletes who may have warranted a TUE. The MAC's advice was always accepted.

Attempts to recognize the concept of TUE continued and during 1998, a member of the MAC addressed the IOC Juridical Commission, the members expressing surprise that the principle of TUE had been not been generally disclosed and later, another MAC member explained in writing to the Juridical Commission the concept and the need for its recognition. In February 1999, at the World Conference on Doping in Sport in Lausanne, the MAC pleaded that the concept be supported. However, in August 1999, when the Medical Code of the Olympic Movement was released by the IOC Juridical Commission, there was no mention of TUE. In December 1999, the MAC again appeared before the Juridical Commission and requested that they include TUE in the IOC Medical Code and in an addendum that was issued in January 2000, a single sentence mentioning that the concept was included. By that time, a number of NOCs including Sweden and Australia had TUE Committees operating and providing national exemptions for many years.

During the Sydney Olympic Games, Australia's TUE Committee organized a conference on TUE. This was well attended by both NOCs and IFs. The meeting resolved that protocols of TUEs i.e. detailed requirements necessary to confirm the athlete's medical condition, treatment with a prohibited drug and any conditions attached to an approval be compiled and circulated. This task was undertaken and completed in January 2001.

As WADA commenced in 2000 to assume responsibility for doping matters, TUE was discussed at a WADA meeting in Stockholm in May 2001 attended by two members of the MAC and a representative from the Paralympic Body. These protocols were discussed and modified. When the World Anti-Doping Code was approved in March 2003, the concept of TUE was included. Following this, a WADA Committee chaired by a member of the MAC developed an International Standard for TUE which was approved by WADA's Executive Committee in September 2003.

Currently, the WADA website [49] contains the International Standard for TUE, guidelines for a TUE and an application form to formally apply for a TUEs. The WADA criteria that must be met to grant a TUE are:

1 the athlete would experience significant health problems without taking the prohibited substance or method;

2 the therapeutic use of the substance would not produce significant enhancement of performance, and

3 there is no reasonable therapeutic alternative to the use of the otherwise prohibited substance or method.

In December 2006, the German NADO and WADA conducted a symposium on TUE attended by representatives of 23 NADOs and 15 IFs which resulted in WADA preparing and in 2007, inserting on their website '*Medical Information to Support Decisions of TUE Committees* [50] which has superceded the 2001 protocols.

Some examples of TUEs that may be granted include:

1 oral corticosteroids for inflammatory bowel disease and severe asthma;

2 diuretics for cardiovascular and renal conditions;

3 stimulant medication for attention deficit hyperactive disorder in children and adolescents and for narcolepsy; and

4 insulin for type 1 diabetes.

Because of the high likelihood of improving performance, T for documented primary or secondary hypogonadism remains the most contentious and difficult TUE, necessitating an independent referee's opinion and strict monitoring. The athlete's sport and his role in that sport are sometimes relevant. No synthetic AAS can be prescribed except danazol for C-1 esterase deficiency for hereditary angioneurotic oedema. AAS can never be approved for the diagnosis of age-related hypogonadism ('andropause') and is never permitted to be administered to female athletes.

Currently, the concept of TUE has wide acceptance by NOCs, IFs and Organizing Committees of major Games. Mutual recognition is required by the WADA Code but there are occasional pitfalls because some TUE Committees of small NOCs and IFs have little or no experience and err at times, mostly in the athlete's favour, which does have the potential to disadvantage their opponents.

Conclusions

During the last 40 years, remarkable advances in analytical chemistry have allowed initially the IOC and IFs and recently WADA the capacity to identify and sanction athletes who have misused a wide range of pharmaceutical products, the vast majority of which were developed to treat diseases. Recently, drugs designed especially for athletes to enhance performance have been produced, used, and athletes punished. The administration of prohibited substances to athletes with an indisputable clinical need is now possible under strict regulations. While great progress has been achieved to catch up with the dopers and their rogue scientific advisors, many challenges remain in the future, not the least of which will be the necessity to prevent gene doping from damaging sport.

Conflict of interest statement

No conflict of interest was declared.

Acknowledgements

We thank Caroline K. Hatton, PhD, for her vivid recall of events, discussions and scientific editing. Patrick Schamasch, MD, IOC Medical Director, provided invaluable assistance by locating critical documents.

References

1 Dirix A, Sturbois X. *The First Thirty Years of the International Olympic Committee Medical Commission*. Lausanne: The International Olympic Committee, 1998.
2 Beckett AH, Cowan DA. Misuse of drugs in sport. *Br J Sports Med* 1979; 12: 185–94.
3 Dirix A. *Medical Guide of the IOC Medical Commission*. Lausanne: International Olympic Committee 1992; 53–90.
4 World Anti-Doping Agency. http://www.wada-ama.org/
5 Klein HG. Blood transfusion and athletes. *N Engl J Med* 1985; 312: 854–6.

6 ISO. *International Standards for Business Government and Society.*: ISO, 2008.
7 Berendonk B. *Doping Dokumente*. Berlin: Springer-Verlag, 1991.
8 Franke WW, Berendonk B. Hormonal doping and androgenization of athletes: a secret program of the German Democratic Republic government. *Clin Chem* 1997; 43: 1262–79.
9 Yesalis CE, Courson SP, Wright J. History of anabolic steroid use. In: Yesalis CE, ed. *Anbolic Steroids in Sport and Exercise*. Champaign, IL: Human Kinetics, 1993; 36–40.
10 Ljungqvist A. The use of anabolic steroids in top Swedish athletes. *Br J Sports Med* 1975; 9: 82.
11 World Anti-Doping Agency. WADA code. Montreal: WADA. http://www.wada-ama.org, accessed 12 January 2008.
12 Thiblin I, Parlklo T. Anabolic androgenic steroids and violence. *Acta Psychiatr Scand Suppl* 2002; 412: 125–8.
13 Pope HG Jr, Katz DL. Homicide and near-homicide by anabolic steroid users. *J Clin Psychiatry* 1990; 51: 28–31.
14 Pope HG Jr, Katz DL. Psychiatric and medical effects of anabolic-androgenic steroid use. A controlled study of 160 athletes. *Arch Gen Psychiatry* 1994; 51: 375–82.
15 Sullivan ML, Martinez CM, Gennis P, Gallhager EJ. The cardiac toxicity of anabolic steroids. *Prog Cardiovasc Dis* 1998; 41: 1–15.
16 Bagheri SA, Boyer JL. Peliosis hepatis associated with androgenic-anabolic steroid therapy. A severe form of hepatic injury. *Ann Intern Med* 1974; 81: 610–8.
17 Baoutina A, Alexander IE, Rasko JE, Emslie KR. Developing strategies for detection of gene doping. *J Gene Med* 2008; 10: 3–20.
18 Schneider AJ, Friedmann T. Gene doping in sports: the science and ethics of genetically modified athletes. *Adv Genet* 2006; 51: 1–110.
19 Donike M, Jaenicke L, Stratmann D, Hollmann W. Gaschromatographischer Nachweis von stickstoffhaltigen Pharmaka in wassrigen Losungen mit dem Stickstoffdetektor. *J Chromatography* 1970; 52: 237–50.
20 Dugal R, Dupuis C, Bertrand MJ. Radioimmunoassay of anabolic steroids: an evaluation of three antisera for the detection of anabolic steroids in biological fluids. *Br J Sports Med* 1977; 11: 162–9.
21 Donike M, Barwald KR, Klostermann K, Schanzer W, Zimmerman J. Nachweis von exogenem Testosteron (The detection of exogenous testosterone). In: Heck H, Holmann W, Liesen H, Rost R, eds. *Sport: Leistung und Gesundheit*. Koln: Deutscher Arzte-Verlag, 1983; 293–8.
22 Catlin DH, Hatton CK, Starcevic SH. Issues in detecting abuse of xenobiotic anabolic steroids and testosterone by analysis of athletes' urine. *Clin Chem* 1997; 43: 1280–8.
23 Catlin DH, Kammerer RC, Hatton CK, Sekera MH, Merdink JL. Analytical chemistry at the Games of the XXIII Olympiad in Los Angeles, 1984. *Clin Chem* 1987; 33: 319–27.
24 Schulze JJ, Lundmark J, Garle M, Skilving I, Ekström L, Rane A. Doping test results dependent on genotype of UGT2B17, the major enzyme for testosterone glucuronidation. *J Clin Endocrinol Metab* 2008 Mar 11. [Epub ahead of print] PMID 18334693.
25 Aguilera R, Chapman TE, Catlin DH. Performance characteristics of a carbon isotope ratio method for detecting doping with testosterone based on urine diols: controls and athletes with elevated testosterone/epitestosterone ratios. *Clin Chem* 2001; 47: 292–300.
26 World Anti-Doping Agency. *TD2004EAAS, WADA Technical Document on Reporting and Evaluation Guidance for Testosterone, Epitestosterone, T/E Ratio and Other Endogenous Steroids*. Montreal: WADA, 2008. http://www.wada-ama.org, accessed 1 June 2008.
27 Schanzer W, Geyer H, Fussholler G *et al.* Mass spectrometric identification and characterization of a new long-term metabolite of metandienone in human urine. *Rapid Commun Mass Spectrom* 2006; 20: 2252–8.
28 Catlin DH, Hatton CK, Lasne F. Abuse of recombinant erthropoietins by athletes. In: Molineux G, Foote MA, Elliott SG, eds. *Erythropoietins and Erythropoiesis*. Basel, Boston, Berlin: Birkhauser Verlag, 2006; 205–27.
29 Union Cycliste Internationale. *Cycling Regulations Part 13. Sporting Safety and Conditions*, Aigle:UCI pp. 8, paragraph 13. 1. 063.
30 Parisotto R, Wu M, Ashenden MJ *et al.* Detection of recombinant human erythropoietin abuse in athletes utilizing markers of altered erythropoiesis. *Haematologica* 2001; 86: 128–37.
31 Wide L, Bengtsson C, Berglund B, Ekblom B. Detection in blood and urine of recombinant erythropoietin administered to healthy men. *Med Sci Sports Exerc* 1995; 27: 1569–76.
32 Lasne F, de Ceaurriz J. Recombinant erythropoietin in urine. *Nature* 2000; 405: 635.
33 Lasne F. Double-blotting: a solution to the problem of non-specific binding of secondary antibodies in immunoblotting procedures. *J Immunol Methods* 2001; 253: 125–31.
34 Catlin DH, Breidbach A, Elliott S, Glaspy J. Comparison of the isoelectric focusing patterns of darbepoetin alfa, recombinant human erythropoietin, and endogenous erythropoietin from human urine. *Clin Chem* 2002; 48: 2057–9.
35 Nelson M, Popp H, Sharpe K, Ashenden M. Proof of homologous blood transfusion through quantification of blood group antigens. *Haematologica* 2003; 88: 1284–95.
36 American Arbitration Association. *United States Anti-Doping Agency v Tyler Hamilton. 30-190-111130-05*. New York: American Arbitration Association, 2005.
37 Powrie JK, Bassett EE, Rosen T *et al.* Detection of growth hormone abuse in sport. *Growth Horm IGF Res* 2007; 17: 220–6.
38 Wallace JD, Cuneo RC, Lundberg PA *et al.* Responses of markers of bone and collagen turnover to exercise, growth hormone (GH) administration, and GH withdrawal in trained adult males. *J Clin Endocrinol Metab* 2000; 85: 124–33.
39 Wu Z, Bidlingmaier M, Dall R, Strasburger CJ. Detection of doping with human growth hormone. *Lancet* 1999; 353: 895.
40 Alexander B. The awful truth. *Outside* July 2005; 100–8.
41 Geyer H, Parr MK, Mareck U, Reinhart U, Schrader Y, Schanzer W. Analysis of non-hormonal nutritional supplements for anabolic-androgenic steroids – results of an international study. *Int J Sports Med* 2004; 25: 124–9.

42 Leder BZ, Longcope C, Catlin DH, Ahrens B, Schoenfeld DA, Finkelstein JS. Oral androstenedione administration and serum testosterone concentrations in young men. *JAMA* 2000; 283: 779–82.
43 Catlin DH, Leder BZ, Ahrens B *et al.* Trace contamination of over-the-counter androstenedione and positive urine test results for a nandrolone metabolite. *JAMA* 2000; 284: 2618–21.
44 Catlin DH, Ahrens BD, Kucherova Y. Detection of norboletheone, an anabolic steroid never marketed, in athletes' urine. *Rapid Commun Mass Spectrom* 2002; 16: 1273–5.
45 Catlin DH, Sekera MH, Ahrens BD, Starcevic B, Chang YC, Hatton CK. Tetrahydrogestrinone: discovery, synthesis, and detection in urine. *Rapid Commun Mass Spectrom* 2004; 18: 1245–9.
46 Breidbach A, Catlin DH. RSR13, a potential athletic performance enhancement agent: detection in urine by gas chromatography/mass spectrometry. *Rapid Commun Mass Spectrom* 2001; 15: 2379–82.
47 Thevis M, Opfermann G, Schanzer W. Detection of the plasma volume expander hydroxyethyl starch in human urine. *J Chromatogr B Biomed Sci Appl* 2000; 744: 345–50.
48 Thevis M, Ogorzalek Loo RR, Loo JA, Schanzer W. Doping control analysis of bovine hemoglobin-based oxygen therapeutics in human plasma by LC-electrospray ionization-MS/MS. *Anal Chem* 2003; 75: 3287–93.
49 World Anti-Doping Agency. *International Standard for Therapeutic Use Exemptions*. Montreal: WADA, 2007. Accessed 29 January 2008.
50 World Anti-Doping Agency. *Medical Information to Support the Decisions of Therapuetic Use Exemption Committees*. Montreal: WADA, 2007. http://www.wada-ama.org, accessed 26 January 2008.

Correspondence: Don H. Catlin, MD, Anti-Doping Research Institute, 3873 Grand View Blvd, Los Angeles, CA 90066, USA.
(fax: 310 482 6929; e-mail: Dcatlin@ucla.edu).