

## Doping: towards the perfect human machine?

In a society where sports success provides fame, glory and money, managing to be the best at any price is a great temptation that can lead to employing prohibited substances and/or methods. Under this premise, international experts from anti-doping bodies, organisations that manage controls, associations of scientists interested in the issue and the athletes themselves will participate in this scientific session that will discuss the situation of doping in sport at a time of special significance, right before the Peking Olympic Games.

### **An international multidisciplinary vision**

The scientific session is organised by Dr Jordi Segura, at which international experts in the field of doping and sport will also participate.



#### **Jordi Segura, Director of the Anti-Doping Laboratory of the Municipal Institute of Medical Research (IMIM-Hospital del Mar)**

He is also the coordinator of the research group of Bio-analysis and Analytic Services of the Neuropsychopharmacology Research Programme at IMIM, member of the Medical Commission Games Group of the International Olympic Committee (IOC) and of the Doping Control Review Board of the International Swimming Federation (FINA). He is an expert in chromatography, mass spectrometry and hormone analysis. Finally, he is a tenured professor in the Department of Experimental and Health Sciences at the Pompeu Fabra University.

The Anti-Doping Laboratory of the Municipal Institute of Medical Research was created in 1985 and is accredited by standard ISO 17025 and the World Anti-Doping Agency. It was the laboratory responsible for anti-doping control during the 1992 Barcelona Olympic and Paralympics Games. Later, the laboratory has also controlled large international events such as the 1991 and 1995 Pan American Games, the 1998 Asian Games and the 2003 World Swimming Championships, among others. The laboratory receives samples and consultations from around the world each day.

Dr Jordi Segura, under the title: '*Doping and Society: towards the perfect human machine?*', which also gives its name to the entire scientific session, will give us a general introduction about the current state of doping and its control and will offer us a summary of the forecast for the near future.



#### **Alain Garnier. Medical Director of the World Anti-Doping Agency (WADA-AMA)**

He embarked on his career with WADA as a medical consultant in 2000, thanks to his prior experience as a sport specialist doctor for several French hospitals and head of the medical division of the French Ministry of Youth and Sport. In the framework of WADA, he was a member of the group that developed the World Anti-Doping Code (Code) and one of those in charge of assuring the code's acceptance by different governments. He is currently the medical director of WADA, in charge of all medical issues related to doping, especially the supervision of the

Therapeutic Use Exemptions programme and the WADA Athlete Passport project.

Under the title: '*Moving from toxicology to biology: the need for a medical approach in the fight against doping*', Dr Garnier's paper will give us a medical approach to the current situation of the fight on doping. Why must sport doctors oppose doping? Are there long-term health consequences? Can restricted substances be taken if there is no alternative pharmacological alternative? Special emphasis will be paid to the impact in the Therapeutic Use Exemption (TUE) process and the idea of the Athlete's Passport. The aim of the latter is to carry out a longitudinal monitoring of the biological parameters of athletes, an initiative that will permit the identification of abnormal profiles for the use of prohibited substances or methods.



### **Michelle Verroken, Director-founder of Sporting Integrity**

The Sporting Integrity consultancy was created in 2004 and is the first and only of its type in the United Kingdom. Its aim is to advise its clients about good sport practices and to adopt and maintain the best procedures related to sport ethics and integrity.

Mrs Verroken has two decades of experience as a world expert in the field of ethics and sport. Director of Ethics and Anti-doping at UK Sport, she was responsible for designing and implementing the internationally accepted standards for anti-doping control, results management and education, in addition to creating the UK Drug Information database and national anti-doping policies (on which the World Anti-Doping Code is based).

Under the title: '*Ethics and Doping- ethos, pathos or kudos?*' Mrs Verroken will speak about clean sport ethics and the need for all of us to be aware of the importance of this control. According to Verroken, the rules of the game should be the essence of sport. Sport's corruption through doping is destroying this sole ethical principle. Athletes often become the interpreters, who are trained using training systems that use the latest scientific innovations. Drawing the line between what is acceptable and unacceptable is becoming increasingly difficult. She will exemplify this complex issue with specific and illuminating experiences.



### **Franchek Drobnic. Director of the Physiology Department at the Olympic Training Centre (CAR) in Sant Cugat.**

He is a doctor of medicine, licensed from the Autònoma University of Barcelona, and is a specialist in Sports Medicine. He is the current director of the Physiology Department at the Barcelona Olympic Training Centre and director of Medical Services for the Spanish Federation of Taekwondo. He also collaborates directly with various sports in preparation for the Olympics.

His interest as a researcher in the sports world is broad and is oriented towards improving athletic performance through health, with a special emphasis on respiratory disorders and adaptation to physical stress, as well as on the physiology of physical performance and reparatory physiology under special conditions, such as the exercise itself, hyperbaria, hyperoxia, changes in temperature or the state of hydration and nutrition.

His presentation "*Therapeutic Use Exemptions: why and when?*" will describe the possibilities for administering prohibited products to those affected athletes who need them. In its two versions (conventional and abbreviated), "*Therapeutic Use Exemptions*" will provide the relevant medical information for the use of these medicines in pathological situations where there are no other alternatives. The presentation will highlight some of the most common therapeutic use requests, such as those related to athletes with asthma or exercise-induced asthma.



**Xavier O'Callaghan, former handball player on FC Barcelona and current manager of the handball division of FC Barcelona**

He entered the lower categories of FC Barcelona, winning three state youth and one junior championship. In the 1990-91 season, when he was 18 years old, he moved to the first team, on which he would play for 15 seasons. He is one of the athletes who has won the most state and European titles (54). He played internationally 87 times and scored 140 goals, as well as winning a bronze medal at the 2000 Sydney Olympic Games and an Olympic title at the 2005 Athens Games. At the end of the 2005 season, and after an entire career with FC Barcelona, he became the manager of the handball division of FC Barcelona.

His presentation will present his vision of doping both from a viewpoint as an athlete and as a sport manager. From his personal vision and based on his sports experience, Xavier O'Callaghan will try to answer the question of why there are athletes who use drugs and others who choose not to and what factors can influence this decision.



**Francesco Botrè, former president of the World Association of Anti-Doping Scientists (WAADS) and director of the Anti-doping Laboratory in Rome**

Dr Botrè is an associate professor at the Sapienza Faculty of Medicine at the University of Rome, a member of the WADA laboratory work group and the Medical Commission of the International Committee for the Mediterranean Games. He is also a member of several scientific societies, the author of over 200 scientific publications, conference papers and monographic works.

His presentation '*Testing: scientific aspects. Who are the laboratory experts?*' will speak of the activities performed at anti-doping laboratories that WADA-accredited; carrying out a study on the evolution they have followed in recent years to become more effective in the fight against doping. He will place special emphasis on the future evolution of doping science, taking a position that is pro fair play, health protection and society's knowledge of the work done at an anti-doping laboratory.



**Josep Guardiola, former football player and current trainer of the first football team of FC Barcelona**

He has been one of the most important midfielders in Catalan football. He played 43 times with the Spanish team and was the captain of the Catalan team for many years. In 2001, he started playing on Brescia and a mere two months later was accused of doping by the Italian National Olympic Committee. It was not until 2007, six years later, that the Brescia Court of Appeals absolved him when new scientific proof appeared that explained

the natural origin of the tests. He is now the trainer of the first team of FC Barcelona.

His participation will be done via a pre-recorded statement since at the time of the session he will be out of the country. He will give his personal viewpoint on doping. The priority issues that he will discuss are his vision of doping and its control from a sport perspective, the role that can be played by the environment closest to athletes, his own experience due to being suspected of doping, some considerations about the future of the anti-doping fight and what role science can play in the improvement of controls.

## The origins of anti-doping control

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The use of substances or other methods to improve performance is as old as competition sports. It is known that the athletes who participated at the Olympics in Ancient Greece (4<sup>th</sup>-8<sup>th</sup> century AD) employed special diets and stimulating potions to enhance their capacity. Moreover, it was not until the beginning of the 20<sup>th</sup> century that the need to control the usage of doping substances in sport was considered. Initially, there were no means for detecting the use of these substances, but scientific advances led to the instauration of progressive anti-doping controls starting in the sixties by the International Olympic Committee and the main sports federations. Unfortunate events like the death of some cyclists revealed the initial use of amphetamines and narcotics. Subsequently, the increasing use of testosterone and its derivatives (anabolic steroids) came to light as doping elements. The current list of substances includes many other pharmacological groups. The biotechnological revolution in medicine has also had a phenomenal effect on the consumption of doping substances with an identical structure to those produced by the human body. The upcoming arrival of genetic doping will add further complexity to the ethical aspects and detecting of doping in the future.

## Present-day doping

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### Present-day doping products and methods

Every year, WADA makes a list of prohibited substances. You can see the most updated list on its Web page at <http://www.wada-ama.org>. These substances are broken down into large categories such as anabolic agents, hormones and related substances, stimulants and narcotics, to name just a few examples. It also includes a list of prohibited methods, which include increased oxygen through transfusion (blood doping), chemical or physical manipulation, meaning the handling or replacement of samples or genetic doping.

Performing anti-doping controls for the components on the list is very technically complex, expensive and can only be done at 34 laboratories in the entire world, those accredited by WADA, including the Barcelona Anti-Doping Laboratory of the Municipal Medical Research Institute (IMIM-Hospital del Mar).

We include a chart hereafter with some examples of prohibited substances and their effects on sport performance and side effects with regard to health.

PROHIBITED SUBSTANCES (some examples)	EFFECTS	SIDE EFFECTS
<b>Substances that increase the quantity of oxygen in muscles</b>		
<b>Erythropoietin (EPO)</b>	Increase in red cells and increased oxygenation and resistance	Serious cardiovascular problems
<b>Darbepoetin</b>	In the EPO family, but lasts longer in the blood	Cardiovascular problems
<b>Insulin</b>	Very important in transporting nutrients to cells, permitted in diabetics	Cardiovascular problems and diabetic coma
<b>Blood transfusion</b>	Very effective in quickly improving performance	Risk of infection in the event of blood damage or poor administration
<b>Substances that increase muscle mass and strength (anabolic steroids)</b>		
<b>Testosterone</b>	Increases muscle development	Liver diseases and hair growth in women
<b>Nandrolone</b>	Increases strength, power, aggressiveness and speed	Liver problems and decreased sexual desire
<b>Estanozonol</b>	Testosterone derivative that promotes muscle development	Important sexual disorders

<b>THG</b>	Favours muscular development; synthetic drug that was designed to be undetectable	Sexual problems
<b>Clenbuterol</b>	Favours muscular and strength augmentation	Headache and tremors
<b>Stimulating substances</b>		
<b>Cocaine</b>	Lack of fatigue and increased aggressiveness	Addiction, anxiety, aggressiveness, tachycardia, tremors and cardiovascular accidents
<b>Diuretic substances</b>		
<b>Hydrochlorothiazide</b>	Masks the presence of other drugs by eliminating them through urine	Unusual fatigue, palpitations and yellowish eyes

**Number of substances identified in each group of prohibited substances** (Information provided to WADA by accredited laboratories, 2007). At this time, steroids are still the drugs that are most detected by WADA laboratories to improve athletes' performance.

<b>Substance Group</b>	<b>Number*</b>	<b>% of all Adverse Analytical Findings</b>
S1. Anabolic Agents	2,322	47.9%
S6. Stimulants	793	16.4%
S8. Cannabinoids	576	11.9%
S3. Beta-2 Agonists	399	8.2%
S5. Diuretics and Other Masking Agents	359	7.4%
S9. Glucocorticosteroids	288	5.9%
S2. Hormones and Related Substances	41	0.8%
P2. Beta-Blockers	27	0.6%
S7. Narcotics	21	0.4%
S4. Agents with Anti-Estrogenic Activity	18	0.4%
M1. Enhancement of Oxygen Transfer	3	0.1%
M2. Chemical and Physical Manipulation	3	0.1%
<b>TOTAL</b>	<b>4,850</b>	

### What path does the sample take?

Samples are collected from athletes in two recipients, A and B. Sample A is used to perform the analysis and sample B is kept in reserve in case it is necessary to perform a counter-analysis. The two samples are sent hermetically closed and sealed, only bearing a numeric code, to the laboratory doing the analysis. The sample is anonymous at all times (identified only by this numeric code) and is guarded in order to guarantee security and confidentiality. All the steps followed in the extraction and analysis of the sample are written down and all instruments and procedures employed are accredited and homologated. The accreditation process for anti-doping laboratories is a double one, both by International Standardisation Organisations (ISO) and by the WADA. The reliability of the anti-doping analysis is among the most highly-controlled activities in the scientific world. There are currently 34 anti-doping laboratories that are accredited in the world (consult the Web page: <http://www.wada-ama.org/en/dynamic.ch2?pageCategory.id=333> )

## **Which bodies can request anti-doping controls?**

There are many: the national and international federation in which the athlete competes, the National Anti-Doping Organisation (NADO) of the countries where the athlete lives or competes, the World Anti-Doping Agency, the organisers of large international competitions and the International Olympic Committee, among others.

## **Current legislation**

The creation of the World Anti-Doping Agency in 1998 (WADA), which was initiated by Josep Antoni Samaranch when he was the president of the IOC, was the first step in the fight against doping. However, the first definitive action was the political and economic involvement of the international community and the drawing up of the World Anti-Doping Code, which started to take shape at the 2<sup>nd</sup> Worldwide Anti-Doping Conference that was held in Copenhagen in 2003. The code is a universal norm in the fight against prohibited substances that was enacted with the support of 72 countries and presently has 191 member governments. More recently, the UNESCO Convention and the 3<sup>rd</sup> Worldwide Anti-Doping Conference held in November 2007 in Madrid has driven measures forward.

In Spain, the entry into force of the Organic Law against doping and in favour of health dated February 2007, much stricter than the preceding law, defines induction and collaboration in doping cases as a crime. Likewise, Royal Decree 811/2007 of 22 June establishes the new Commission on Control and Monitoring of Health and the Fight against Doping in Sport.

## **The dangers of doping**

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Some of the most widely used substances are steroids, growth hormones and EPO. All three involve significant inherent dangers in their use that we will detail hereafter:

The most dangerous side effects of steroids that have been described in medical literature include abnormalities in renal functioning and kidney tumours, endocrine and reproductive dysfunctions, testicular atrophy, cardiac effects in the lipids and psychiatric symptoms. These consequences have been exaggerated due to the common doping practice of using 10 or more times the recommended medical dose and combining them with other drugs, such as steroids and EPO or the growth hormone.

Using growth hormone can lead to important risks, especially if we take into consideration that some reports estimate that athletes who employ growth hormones to improve performance are taking 10 times more than the therapeutic dose. Some side effects of the growth hormone are abnormal bone growth, high blood pressure, cardiovascular diseases, cardiomyopathy, glucose intolerance, polyps on the colon, decreased life expectancy and cancer.

Like steroids and growth hormones, a dose that is larger than normal is also injected when doping with EPO, which can cause increased blood viscosity, coronary thrombosis, cerebral infarction and death. It is estimated that a good number of European cyclists have died due to EPO abuse, making it one of the most dangerous doping agents.

## Future challenges

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Thanks to WADA initiatives, some state anti-doping agencies (for example USADA) and national research programmes have multiple lines of research focused on detecting new doping substances.

With regard to the Anti-Doping Laboratory of the Municipal Medical Research Institute (IMIM-Hospital del Mar) in Barcelona, some of the lines of research are focused on:

- **Detection of increased oxygen availability:** Erythropoietin, gene therapy, blood transfusions

Erythropoietin: Currently, work is being done on some endogenous substances that the pharmaceutical industry produces via recombination, especially 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup>-generation erythropoietins, with the objective of characterising their differences with respect to the hormone produced endogenously.

They are also trying to develop monoclonal antibodies against N-glycolylneuraminic acid, a monosaccharide present only in recombinant material, with the objective of developing a highly-sensitive technique. Future plans are based on the development of instrumental methodologies for the detection of the presence of modifications to protein glycosylation (the addition of a carbohydrate to a molecule), which are the factors responsible for the differences between endogenous and exogenous EPOs.

Gene therapy is advancing as one of the most important therapies of the 21<sup>st</sup> century. The idea of this genetic doping technique seems relatively simple: instead of injecting a substance into the athlete's body, his muscles are enriched with the gene that produces the substance. The result is the same but detection is more complicated, since it appears as a substance generated by the body in analyses. In the world of sport, diagnostic detection of the application of gene therapy (genetic doping) could be used to prevent the bad practice of such an important medical tool. The research project is aimed at learning more about this diagnostic capability, in coordination with the main gene and image groups from the Barcelona Biomedical Research Park. There is no current proof of the utilisation of this method as a doping procedure in humans, although it is believed that it may be a reality in a very close future.

With respect to blood abuse, the objective consists of developing methods to detect abuse through blood transfusions in athletes. Two focus points will be studied: the first is based on the detection of contaminating agents present in the cavities to store blood or leukocyte concentrations; concentrations of these contaminating agents and their metabolites should be higher in the bodily fluids of individuals that undergo blood transfusions compared to subjects who do not receive transfusions. The second focus is based on the detection of aging markers in leukocytes for the storage of blood in subjects who receive transfusions.

- **Detection of growth factors:** Growth hormone, growth hormone secretagogues, chorionic gonadotropin, steroids, gene therapy and glycoconjugates.

Growth hormone (GH) is one of the most susceptible hormones due to being the object of abuse both by athletes and by the parents of children who show low idiopathic heights. Better knowledge of its detection and its structural parameters is truly useful for preventing its abuse, especially if taking into account the complexity of its structure caused by multiple isoforms (direct, joined and proteolytically derived) that are present in the human body. With respect to detection, the development of an analytical tool is very interesting to measure the ratio between the two most abundant growth hormone isoforms (20 and 22kDa). The illicit use of a drug with a single isoform would alter this ratio through a reaction mechanism and, thus, in turn, would represent an abuse indicator.

Possible abuse of GH gene therapy is also a possibility that must be prevented in the future and a project is being developed with a similar protocol to the one mentioned for erythropoietin.



Another future alternative method to produce high GH concentrations is the application of growth hormone secretagogues (analogous to ghrelin). A project is being developed to study the detection of these GH secretagogues via a single calibration protocol.

**Complementary information:**

<http://www.wada.org>

<http://www.imim.es/programesrecerca/neuropsicofarmacologia/grbsa.html>

[http://www.imim.es/ofertadeserveis/en\\_laboratoriantidopatge.htm](http://www.imim.es/ofertadeserveis/en_laboratoriantidopatge.htm)



## *Moving from toxicology to biology: the need for a medical approach in the Fight against Doping*

Dr. Alain Garnier  
Medical Director  
World Anti-Doping Agency

The intent of the presentation is to highlight the need for a medical approach in the fight against doping today. Two main reasons can be developed to support this view.

In a first hand there is an ethical reason and we will try to demonstrate why it is the duty of physicians to oppose doping by all means and not to medically assist or support doping as proposed by some authors. In the current social context of performance and drug addiction, doping is not only cheating but mainly drug misuse. The primary role of physician is to protect mental and physical integrity of athletes, far beyond supporting or enhancing their sport performance. As quoted by Prof. Frontera, President of FIMS: "all sport physicians have the duty and responsibility to oppose the use of doping practices in sports based on moral, ethical and physiological grounds." To accept medical doping results in accepting the use of medicine for other purposes than health; this is certainly not legitimate and potentially source of danger for the society. Sport reasons today could become economic reasons tomorrow in improving human performance to the detriment of health.

The second reason is more technical and based on efficiency. Effectively the classic and current approach of antidoping is mainly based on direct detection of prohibited substances in bodily specimen. Consequently it is more and more difficult to detect modern doping methods by using the traditional toxicological approach adapted to old techniques of doping.

The limits of the system have been reached if one considers the new substances used by cheats and the highly sophisticated protocols for doping abuse of EPO, blood manipulations or other growth factors use. The arrival of biotechnologies and gene therapy will certainly increase this trend. It is the responsibility of antidoping community to adapt the strategies and to

anticipate future trends if we do not want the progress of science operate only in favor of cheats. Consequently the recourse to a more biological and medical approach should be very useful. In that context the use of indirect markers of doping and its monitoring along time, as it is common in medical practice, must bring interesting results. The study of metabolic effects on the body subsequent to xenobiotic intake is known as metabonomic which is already producing encouraging results in veterinary medicine.

The presentation will develop the WADA proposed approach of the athlete passport and its principles.

# Ethics and Doping: Ethos, Pathos or Kudos?

*Michele Verroken*

*Director of Sporting Integrity, UK,  
Anti-Doping Adviser to PGA European Tour  
Secretary of the Medical Commission of the Commonwealth Games Federation  
Formerly Director of Ethics and Anti-Doping, UK Sport*

## **Abstract**

Playing by the rules should be the very essence of sport. Important lessons for life are meant to be learned through playing sport; fair play, right from wrong, what is allowed and what is not allowed, winning and losing. However sport is struggling to maintain its level playing field. Corruption of sport through doping is destroying its unique ethos. Athletes, once pure performers, are now manufactured through scientifically driven training systems, using scientifically developed equipment. Drawing the line between what is acceptable and unacceptable is becoming more difficult.

This presentation will look at the principle of playing by the new rules of sport, the anti-doping rules contained in the World Anti-Doping Code. Principally designed to reinforce the fairness of sport, anti-doping rules have become caught up in this sporting rhetoric and are now contributing to a new ethical dimension of sport. In the tragedy of sporting competition, athletes are often cast as the victims of sporting systems, vulnerable to evil scientists and malevolent coaches. Fear of doping and cheating the doping system is driving the agenda, with increasing emphasis on control systems as the evidence gathering mechanisms to verify the drug-free athlete. Yet evidence gathered from anti-doping programmes to date does not always stand up to independent examination. Analysis of thousands of urine samples still indicates a small percentage of findings and not all of these are determined to be doping offences. What is the truth behind the doping problem as defined by the Code?

Athletes are challenging our faith in sport ever being drug-free, they lie and deny. A new breed of athlete is emerging, the 'doping celebrity'. Consideration will be given to how far anti-doping systems need to go before sport has any credibility again.

# **The Anti-Doping Challenge: The Human Factor**

## **How to be (or to become) an “Anti-Doping Scientist”**

*Francesco Botrè*

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### **Abstract**

This presentation gives a general overview on the activity of the anti-doping laboratories accredited by the World Anti-Doping Agency (WADA), outlining the evolution, over the last four decades, of the strategy followed by the Anti-Doping Scientists to improve the effectiveness of the fight against doping in sport.

In particular, the focus is on the people facing their daily challenges in the laboratories to detect and deter the abuse of performance-enhancing drugs and methods by the athletes, on their scientific background, on their specific training, on their interaction with colleagues belonging to the same international scientific network (the World Association of Anti-Doping Scientists, WAADS), and, perhaps most importantly, on their constant transfer of knowledge to new generations of anti-doping scientists.

Special emphasis is given to the future evolution of the anti-doping science, as seen from the perspective of a laboratory scientist, in the wider context of fair play, health protection, and perception of the activity of the anti-doping laboratories by the general public.

# **Doping under the point of view of a former professional handball player. Incentives vs. punishments.**

Xavier O'Callaghan

*1990-2005 FC Barcelona Handball player.  
Sydney 2000 and Athens 2004 Olympic athlete.  
Since 2006 FC Barcelona Handball Team responsible.*

The intention of my presentation will try to understand the doping under the fact of incentives and punishment. All athletes have been tempted to use doping, even for a second; may be by themselves, by their coaches or by their doctors. But the question is: why there are athletes who use doping and others no? Under my point of view the relation between incentives and punishments decides this election. We have to observe incentives under a wide list of items, not just money and fame. Everybody has different incentives and takes decisions under different parameters. But at the end the election, even if is not taken rationally, is based under the balance between incentives and punishments.

Even more, this is the reason because there are differences between individual and collective sports in the doping issue. The incentive as the fastest man in the world will be more evident if you compete alone than if you are part of a team. Doping goes related to the improvement of physical conditions, which are less relevant in collective sports than in individual sports.

As a final statement I would like to express my worry as I believe incentives to commit doping grow up in the sport world as we know it today. That's the reason because all actors in this market have to act in their best way to fight against the sport's worst enemy.

## **The Anti-Doping Control Laboratory of Barcelona. Municipal Institute for Medical Research (IMIM-Hospital del Mar).**

The **Anti-Doping Control Laboratory of Barcelona** is one of the 34 laboratories accredited throughout the World by the World Anti-doping Agency (WADA).

From its accreditation in 1985, it performs anti-doping analysis in athletes in sport events and competitions, as well as during the training period, at national and international levels.

It also performs anti-doping controls in sport anti-doping controls in sport competitions involving animals.

The Laboratory is also accredited by the National Entity of Accreditation, ENAC, according to International Quality Standard ISO/IEC 17025, and the Association of Official Racing Chemist (AORC).



The main objective of the **Anti-Doping Control Laboratory of Barcelona** is to offer both high quality and reliable services. For this reason, its activity is focused on the development of research and continued education.

The Laboratory has performed the antidoping controls in large sport events. Some examples are the Olympic and Paralympic Games in Barcelona 1992, the Panamerican Games in Havana 1991 and Buenos Aires 1995, The Asian Games in Bangkok 1999 and the FINA Swimming World Championship in Barcelona 2003.

### **Scope of activities:**

- Sport events
- Members of sports/anti-doping Comissions
- International Research and Educational Experience
- Software for manegement of anti-doping laboratories (IMLIMS)

### **Contact**

#### **Anti-Doping Control Laboratory of Barcelona**

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## Enhancement Drugs and the Athlete

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### Performance-enhancing drugs: a (brief) historical overview

The use of performance-enhancing drugs (PEDs) is perhaps as old as sport itself. The ingestion of plant and animal extracts to improve sport performance dates back to the origins of competitive sport, when Greek athletes competed in the ancient Olympics. Later, Roman gladiators had special potions prepared using a wide variety of natural products, including mushrooms, roots, and wines [1,2], to attempt to supplement performance. The use of PEDs became more systematic, no longer based on sorcery and alchemy but instead biochemistry and pharmacology, during the twentieth century, when the Olympic Games were reinvented after the recovery and promotion of the Olympic spirit heralded by Baron Pierre de Coubertin.

To compare the lifespan of the ancient Olympics with that of the modern Olympic Games, the first ancient Olympic Games took place in 776 BC and the last one was held in 393 AD, when, although the Games already had degenerated, they officially were abolished by the Roman emperor Theodosius, who, as a Christian, was against the heathen spirit of the Games [3,4]. The modern Olympic Games, the first edition of which took place in Athens in 1896, celebrates their 112th anniversary in Beijing in August 2008. It follows that the history of the ancient Olympics, spanning more than 11 centuries, is approximately 10 times longer than that of the modern Olympic Games.

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The history of PED use strictly follows the history of scientific development that took place at the time of the ancient and the modern Olympic Games; although the drugs used by athletes competing in the first ancient Olympic Games approximately were the same of those used 1 millennium later by their colleagues or by Roman gladiators, the illicit pharmacologic support to sport performance proceeded at a much faster pace in the twentieth century, with a further dramatic increase from the early 1960s to the present.

The problem of drug abuse in sport first was tackled by the international sport authorities, in the form of the International Olympic Committee (IOC), during the 1960s. An official definition of doping first was given by the IOC in 1964 and the first programs of antidoping tests were activated by the IOC and its newborn Medical Commission in 1967 [5–7]. It was in the late 1960s when, in parallel to the official sport competitions, another race began and continues to the present: the race between testers and cheaters.

### **Classification of performance-enhancing drugs: the “prohibited list”**

The first official antidoping tests performed on the occasion of a multi-sport, international event took place at the Olympic Games of Mexico City in 1968. At that time, the only prohibited substances were those capable of producing a significant effect on sport performance only if administered, in sufficient amounts, right before or during the competition. Although short (compared with its current equivalent), that first list continuously was updated to include any new form of doping substance or method of administration. The periodic upgrades of the list were performed by the IOC Medical Commission until the constitution of the World Anti-Doping Agency (WADA) in 1999. Since then, as mandated by the World Anti-Doping Code [8], the WADA has been responsible for the upgrade and publication of the list. In the framework of the World Anti-Doping Code, the list is an international standard identifying substances and methods, classified by categories, that are prohibited in competition, out of competition, and in particular sports. In the past 40 years, the “prohibited list” has expanded progressively (Box 1): it now reports hundreds of compounds, including so-called “related substances” (ie, substances with similar chemical structure or similar biologic effects to those of a banned prototype) and several prohibited methods, including blood transfusions and gene doping [9].

The chronologic evolution of the “prohibited list” over the past 4 decades leads to identifying three main steps in the parallel expansion of the abuse of drugs in sport:

1. The first period, ranging from the origin of the modern Olympic Games to the early 1970s, coincides with the use of drugs whose efficacy, as discussed previously, is maximal if the administration takes place right before or even during the competition. This is the case with stimulants, narcotics, and some drugs of abuse (eg, cocaine).

**Box 1. World Anti-Doping Code: the 2008 “prohibited list”**

*Substances and methods prohibited at all times (in and out of competition)*

Prohibited substances

- S1. Anabolic agents
  1. Anabolic androgenic steroids (AAS)
    - a. Exogenous AAS (eg, methyltestosterone, nandrolone, and stanozolol)
    - b. Endogenous AAS (eg, testosterone, androstenedione, DHT, and DHEA)
  2. Other anabolic agents (eg, clenbuterol and selective androgen receptor modulators)
- S2. Hormones and related substances (eg, EPO, human growth hormone, insulin-like growth factors, gonadotropins, insulins)
- S3.  $\beta_2$ -Agonists (eg, salbutamol, salmeterol, terbutaline, and formoterol)
- S4. Hormone antagonists and modulators (eg, antiestrogens and myostatin inhibitors)
- S5. Diuretics and other masking agents (eg, diuretics, epitestosterone, probenecid,  $\alpha$ -reductase inhibitors, and plasma expanders)

Prohibited methods

- M1. Enhancement of oxygen transfer (eg, blood transfusions and use of blood derivatives and analogs)
- M2. Chemical and physical manipulation (eg, tampering and intravenous infusions)
- M3. Gene doping

*Substances and methods prohibited in competition*

- S6. Stimulants (eg, amphetamines, cocaine, strychnine, and ecstasy-like drugs)
- S7. Narcotics (eg, morphine and opioids)
- S8. Cannabinoids (eg, hashish and marijuana)
- S9. Glucocorticosteroids

*Substances prohibited in particular sports*

- P1. Alcohol
- P2.  $\beta$ -Blockers

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*Abbreviations:* DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; EPO, erythropoietin.

*Data From* The World Anti-Doping Code. The 2008 prohibited list international standard. World Anti-Doping Agency. Montreal (Canada). 2007. Available at: [www.wada.ama.org](http://www.wada.ama.org). Accessed October 31, 2007.

2. In the second period, the PEDs also included those compounds—mainly AAS—requiring repeated administration over a prolonged period of time to be effective. It is with the use of synthetic AAS that doping substances start to be used off label (ie, with the aim of achieving one or more effects that are different from those for which a specific drug originally had been developed and authorized). This period also marks the transition from pinpoint, in-competition doping, to carefully planned, out-of-competition, systematic doping.
3. The third period follows the pharmaceutical industry development of routine techniques in protein chemistry, molecular biology, and genetic engineering, and led to the abuse of peptide hormones (including, but not limited to, erythropoietin, growth hormone, and gonadotropins). The use of PEDs belonging to the class of peptide and glycoproteic hormones led to the development of new analytic strategies for their detection, including the use of “indirect” methods based on the measurements of specific markers.

A fourth period (the recourse to gene doping) is feared by many as the next step in the illicit search for the ultimate PEDs and methods. It is expected that gene doping will develop as soon as gene therapy is available practically.

Regardless of its complexity and length, the prohibited list stands as the fundamental reference document classifying all prohibited PEDs, prohibited methods, and masking agents. The fight against doping in sport has been based—and still continues to be based—on the capability of the antidoping laboratories to develop and apply analytic procedures for the most effective detection of all substances and methods included in the prohibited list.

### **The role of the World Anti-Doping Agency–accredited antidoping laboratories**

There currently are 33 antidoping laboratories accredited by the WADA in the world (Box 2), performing more than 200,000 antidoping tests per year. A comprehensive report of the results of the analyses performed by the WADA laboratories worldwide is released yearly by WADA and made available for consultation through their website ([www.wada-ama.org](http://www.wada-ama.org)). In spite of the high number of tests, little information can be drawn simply on the basis of results of the antidoping analyses on the real toxic potential and the related mechanism of action of the many PEDs included in the WADA prohibited list. The antidoping analyses are forensic, but not diagnostic, tests. This means that the aim of the analysis is not to verify the “state of health or disease” of athletes but instead “to supply evidence”—based on the principle of strict liability—of the presence in the biologic sample of a substance (drug/metabolite/marker) included in the WADA prohibited list. It follows that the information supplied by the WADA-accredited

**Box 2. Geographical distribution of the 33 antidoping laboratories accredited by the World Anti-Doping Agency**

Africa: South Africa (Bloemfontein), Tunisia (Tunis)

Americas: Brazil (Rio de Janeiro), Canada (Montreal), Colombia (Bogota), Cuba (La Habana), United States (Los Angeles, Salt Lake City)

Asia: China (Beijing), Korea (Seoul), Japan (Tokyo), Malaysia (Penang), Thailand (Bangkok)

Europe: Austria (Seibersdorf), Belgium (Ghent), Czech Republic (Prague), Finland (Helsinki), France (Paris), Germany (Cologne, Kreischa), Greece (Athens), Italy (Rome), Norway (Oslo), Poland (Warsaw), Portugal (Lisbon), Russian Federation (Moscow), Spain (Barcelona, Madrid), Sweden (Stockholm), Switzerland (Lausanne), Turkey (Ankara), United Kingdom (London)

Oceania: Australia (Sydney)

antidoping laboratories refers to the identification of “markers of exposure,” not of “markers of effect,” of doping agents and methods.

The data supplied by the WADA-accredited antidoping laboratories also are of little epidemiologic value for the following reasons:

1. Despite the outstanding number of antidoping tests performed worldwide, the total number of positive samples is too limited to support any epidemiologic conclusions.
2. All samples analyzed by the laboratories are anonymous and, therefore, critical information necessary for the correct compilation of a reference database is not available (eg, ethnicity, age, height, weight, body mass index, genetic endowment, training level and regimen, and diet).
3. Samples are not collected as a part of a controlled study, and, therefore, it is impossible to carry out a real toxicity study correctly because of the potential influence of other confounding factors.
4. Finally, the WADA rules state clearly that the biologic samples collected in the framework of official antidoping tests cannot be used for purposes other than the antidoping test itself: this means that the activity of the laboratory has to be limited to the identification of specific compounds (drugs/metabolites/markers) whose presence (or whose concentration above a threshold value) is to be considered a proof of doping. No additional tests (including diagnostic tests) are allowed.

The same points hold true for the research activity performed within the network of the WADA-accredited laboratories via the World Association of Anti-Doping Scientists (WAADS), the international scientific society

promoting the sharing of knowledge among the accredited laboratories and the basic and applied research in development of new analytic methods. Because the result of a positive test constitutes the basis for the possible sanctioning of an athlete, all efforts are not devoted to diagnosing the health risks consequent to the use of PEDs but instead to guaranteeing the maximum of solidity of the experimental results. The International Standard Organization 17025 accreditation has been imposed since 2000 as a further prerequisite of accredited antidoping laboratories, and criteria for reporting positive samples must be in compliance with the WADA rules.

It is self-evident that there is little or no room, at present, for toxicologic evaluations. The potential toxicologic risks for abuse of performance-enhancing substances and methods cannot be evaluated fully by a single measurement of urinary/blood concentration values of drugs, metabolites, or other representative indicators of administration. Therefore, no toxicokinetic information can be estimated.

A further step forward will be represented by the final implementation of longitudinal studies, also known as the “athlete passport”: the goal is to build a database for all athletes in which the main hematologic and hormonal parameters are recorded and monitored. Although these strategies are being developed with the main purpose of detecting, via the evaluation of indirect parameters, some forms of doping otherwise problematic to identify (eg, autologous blood transfusions), they also will contribute to shedding further light on the chronic effects of the abuse of PEDs. The implementation of novel diagnostic approaches, to be performed independently of the forensic antidoping tests, for the overall assessment of the toxicity of PEDs will remain mandatory to fully accomplish the requirements of an effective antidoping strategy [10].

### **The adverse side effects of performance-enhancing drugs: what is known and unknown**

The possible health risks of doping substances and methods have been the subject of several review articles, monographs, and conference proceedings [11–16]. Mostly, these studies have been based on and supported by review of the scientific and medical literature, which have considered the results obtained in controlled, randomized clinical trials and the direct evidence obtained from clinical practice. It is impossible in this context to review, discuss, and outline the biochemical mechanisms of all the adverse effects of the PEDs described so far. To give an approximate idea of the variety of potential side effects of the different classes of substances included in the WADA-prohibited list (with the exception of alcohol, not a drug in the strict sense of the word), [Table 1](#) lists the most common potential direct and indirect effects and the corresponding side effects of PEDs. It is evident that the risks/benefits ratio is always unbalanced toward the risks. Also, it is

virtually impossible for a single drug to produce all or none of the effects listed in [Table 1](#) in one subject.

To correctly assess the real toxicologic potential of PEDs (which easily can include additional effects not considered in [Table 1](#)) is not an easy task. Most of the side effects tend to be the same as those reported after the therapeutic use of the same drugs. It is even more difficult to evaluate the actual toxicity for athletes, because information supplied by the WADA-accredited antidoping laboratories is insufficient. Also, administration of a drug for the enhancement of sport performance clearly is different from rules regulating the administration of the same drug when used within correctly planned therapeutic schemes in patients. The range of side effects can be wider than expected and intensity more severe (discussed later).

#### *Use of off-label drugs*

With the noteworthy exception of designer steroids (discussed later), all drugs administered for nonphysiologic enhancement of sport performance are well known drugs; but when they are administered within the framework of a doping strategy, they are used off label (ie, out of the range of therapeutic application for their original intent). In most cases, athletes understand that a drug is being used beyond its indicated uses. Under these circumstances, it could be difficult to extrapolate the theoretic side effects and compare with those observed in routine medical practice to obtain a representative picture of the actual risks for athletes.

#### *Overdosing (acute or chronic)*

Doping agents generally are used at doses higher than therapeutic doses. Therefore, it is reasonable to think that adverse effects could be more severe as the administered dose increases. Although good pharmacologic practice recommends minimizing administered doses and duration of use, this situation is reversed when the desired effect instead is improvement of sport performance.

#### *Drug-drug interaction*

PEDs seldom are administered alone. Many are used in association with other drugs (banned or allowed) and with a wide variety of nutritional supplements. Drugs may be combined to reach different goals, such as maximizing overall efficacy of the doping treatment, reducing risks for undesired side effects, and complicating their detection by accredited laboratories. Because the range of desired effects is broad, it is reasonable to expect that most of the corresponding drug-drug interactions never have been considered. No therapeutic scheme has been considered for the parallel administration (again, to a healthy person) of combined “therapeutic” schemes, which may

Table 1  
Most common undesired side effects of the main classes of prohibited substances considered in the World Anti-Doping Agency list

Class of the World Anti-Doping Agency prohibited list	Potential direct/indirect effects enhancing sport performance	Side effects reported most commonly
S1. Anabolic agents AAS (endogenous and exogenous)	Generic anabolic effect, produced with the aim of enhancing muscle growth and weight and increasing strength, power, speed, endurance, and aggressiveness. Recovery times also should be improved.	<p>A broad variety of effects (exhaustively reviewed in Ref. [30]), including, but not limited, to the following:</p> <p>Cardiovascular: hypertension, elevated risk of brain hemorrhages, myocardiac damage</p> <p>Hepatic: abnormal liver functions, cholestasis, development of androgen-dependent adenomas, depletion of high-density lipoprotein production</p> <p>Skeletal: water retention</p> <p>Dermal: seborrhea (steroid acne), oily skin, folliculitis, furunculosis</p> <p>Behavioral: increase of aggressiveness (aggressive psychoses), change in the libido, mood swings (euphoria followed by depression), mental disorders, headaches, dependence, or addiction</p> <p>Specific effects for men: testicular atrophy, altered spermatogenesis, prostate hypertrophy, gynecomastia</p> <p>Specific effects for women: virilization, atrophy of the uterus, effects on the ovary (polycystic ovary syndrome, ovary inflammations), reduction of the breast gland, hirsutism, hypothyroidism, lowering of the voice, alteration of the menstrual cycle, alopecia, effects on the connective tissue (striae distensae)</p>

Other anabolic agents

Same as previously.

For the side effects of clenbuterol, see “S3.  $\beta_2$ -Agonists”

Side effects of selective androgen receptor modulators still are under evaluation (these drugs are not yet marketed)

Risk common to all peptide hormones: immunogenicity

Data on the effects of prolonged recombinant human growth hormone treatment in adults are limited

Acute overdosing could lead to hyperglycemia.

Long-term overdosing could result in signs and symptoms of gigantism or acromegaly consistent with the known effects of excess growth hormone

Other reported effects are hypertension, cardiomyopathy, respiratory disease, diabetes, abnormal lipid metabolism, and osteoarthritis

Increase risk for breast and colorectal cancer

Hypertension, thromboses (thrombophlebitis, microvascular thrombosis, and thrombosis of the retinal artery, and temporal and renal veins), pulmonary embolism, cerebral embolism, seizures

Other effects include pyrexia, headache, arthralgias, nausea, edema, fatigue, diarrhea, vomiting, chest pain, skin reaction (on the site of injection), asthenia, dizziness

*(continued on next page)*

## S2. Hormones and related substances

Human growth hormone, insulin-like growth factors

Anabolic effect

Recombinant erythropoietins

Increased production of red blood cells and hemoglobin, resulting in an augmented efficacy of the transport of oxygen to the muscle



Table 1 (continued)

Class of the World Anti-Doping Agency prohibited list	Potential direct/indirect effects enhancing sport performance	Side effects reported most commonly
Gonadotropins (human chorionic gonadotropin, luteinizing hormone, and follicle-stimulating hormone)	To stimulate the endogenous production of androgens, and to contrast the negative effects of testosterone doping	Prostate carcinoma or other androgen-dependent neoplasm Sudden ovarian enlargement resulting from ovarian hyperstimulation, ascites with or without pain, or pleural effusion, rupture of ovarian cysts with resultant hemoperitoneum Arterial thromboembolism, headache, irritability, restlessness, depression, fatigue, edema, precocious puberty, gynecomastia, pain at the site of injection
Insulin	To improve glucose transport to muscle	All adverse effects of hypoglycemia (including loss of consciousness, coma, and death) Respiratory adverse effects Chest pain, dry mouth, otitis media
S3. $\beta_2$ -Agonists	To achieve stimulants and anabolic effects after systemic administration of high doses, significantly higher than those prescribed—by inhalation—for the treatment of asthma	Cardiac arrest and even death may be associated with the abuse of any sympathomimetic medications. Other cardiovascular effect include, but are not limited to, increased pulse rate and blood pressure, ECG changes, seizures, angina, hypertension or hypotension, tachycardia with rates up to 200 beats/minute, arrhythmias. Hypokalemia also may occur Nervousness, headache, insomnia, tremor, dry mouth, palpitation, nausea, dizziness, fatigue, malaise, and sleeplessness

<p>S4. Hormone antagonists and modulators Aromatase inhibitors (eg, anastrozole, letrozole, aminoglutethimide, exemestane, formestane, and testolactone)</p>	<p>To increase the production or decrease the biotransformation of endogenous AAS</p>	<p>At therapeutic doses: nonspecific toxic side effects, including (but not limited to) asthenia, headache, nausea, peripheral edema, fatigue, vomiting, and dyspepsia</p>
<p>Selective estrogen receptor modulators (eg, raloxifene, tamoxifen, and toremifene)</p>	<p>Same as previously</p>	<p>Long-term endocrinologic side effects can be severe if administered in sequence or in combination with tamoxifen or selective estrogen receptor modulators</p> <p>Hot flashes, flu-like syndrome, joint pain, rhinitis</p> <p>Blood clots, including deep vein thrombosis, and pulmonary embolus (rare)</p>
<p>Other antiestrogenic substances (eg, clomiphene, cyclofenil, and fulvestrant)</p>	<p>Same as previously</p>	<p>At high doses, nonspecific toxic side effects, including (but not limited to) nausea, vomiting, vasomotor flushes, visual blurring, spots or flashes, scotomata, ovarian enlargement with pelvic or abdominal pain</p>
<p>Agents modifying myostatin functions</p>	<p>To improve muscle growth by interfering with the action of myostatin.</p>	<p>Unknown: myostatin inhibitors never have been tested in human trials</p>
<p>S5. Diuretics and other masking agents Diuretics</p>	<ol style="list-style-type: none"> <li>1. To obtain a rapid and reversible reduction of the total body mass, an evident potential advantage in sports where weight categories are involved</li> <li>2. To alter the normal urinary excretion of other PEDs or their metabolites (eg, by increasing the volume of urine and diluting them), making their detection by the antidoping laboratories more problematic</li> </ol>	<p>Hypotension</p> <p>Kidney dysfunction, dehydration (risk for central volume depletion), salt and water imbalance, electrolyte dispairement (eg, hyperosmolality, hyponatremia, hypokalemia, hypomagnesemia, hypocalcemia), muscle cramps</p> <p>Dizziness or lightheadedness, gastric effects, rash, impotence, secondary gout</p>

*(continued on next page)*

Table 1 (continued)

Class of the World Anti-Doping Agency prohibited list	Potential direct/indirect effects enhancing sport performance	Side effects reported most commonly
Probenecid	To interfere with the normal excretion of other PEDs, especially AAS	Metabolic effects: precipitation of acute gouty arthritis Central nervous system: headache, dizziness Gastrointestinal: hepatic necrosis, nausea, anorexia, sore gums, vomiting Genitourinary: nephritic syndrome, uric acid stones with or without hematuria, renal colic, costovertebral pain, urinary frequency Hematologic: aplastic anemia, leucopenia, hemolytic anemia Integumental: dermatitis, alopecia, flushing (Rarely) severe allergic reactions and anaphylaxis
Epitestosterone	To adjust the value of the ratio of testosterone to epitestosterone	Unknown (epitestosterone is not a registered drug), even if likely overlapping to many of the side effects of the AAS
$\alpha$ -Reductase inhibitors (eg, finasteride and dutasteride)	Alteration of the endogenous steroid profile, interfering with the quantitation of some AAS and with the correct evaluation of longitudinal data	Alteration of the sexual function (impotence, decreased libido, decreased volume of ejaculate and other ejaculation disorders, breast enlargement, breast tenderness)
Plasma volume expanders (eg, dextran, hydroxyethylstarch and other modified polysaccharides)	To mask the effects of blood doping by blood dilution	Febrile response, infection at the site of injection, venous thrombosis or phlebitis extending from the site of injection, extravasation, hypervolemia
S6. Stimulants Including, but not limited to Central nervous system stimulants	Increased alertness Improvement in coordination Increased strength and endurance, as a consequence of a decreased perception of pain and fatigue	Increased alertness Insomnia, anxiety Inhibited judgment

Respiratory stimulants  
Cardiovascular stimulants  
Appetite suppressants

Glycogen sparing effect in muscle

Increased competitiveness, aggressiveness, and hostility  
Reduced fatigue (risks for muscle and cardiac overload)  
Tremor  
Effect on the cardiovascular systems (increased heart rate and blood pressure)  
Increased risk for stroke, heart attack, or sudden death

S7. Narcotics

Increased tolerance to pain and fatigue  
Transient reduction of tremor in precision events

Effects on the skeletal muscle (rhabdomyolysis).  
Addiction (also as gateway to other drugs), tolerance, physical and psychological dependence  
Increased pain threshold  
Euphoria  
Excitement, psychological stimulation  
Incorrect perception of danger  
Loss of coordination/equilibrium  
Reduced capacity of concentration  
Nausea, vomiting, constipation  
Depression  
Reduced breath capacity  
Reduced cardiac frequency/output  
Overdosing can lead to respiratory depression and death

S8. Cannabinoids

To relieve precompetition tension  
Social drugs: motivation for their use or abuse may be different from the illicit enhancement of sport performance

Effects on the skeletal muscle (rhabdomyolysis)  
Drug dependence  
Psychomotor changes  
Antimotivational syndrome (loss of ambition)

*(continued on next page)*

Table 1 (continued)

Class of the World Anti-Doping Agency prohibited list	Potential direct/indirect effects enhancing sport performance	Side effects reported most commonly
S9. Glucocorticosteroids	<p>Effect on glucose metabolism (stimulation of de novo synthesis of glucose, conversion of amino acids into glucose, release of glucose from glycogen storage, and activation of the lipolysis in fat cells)</p> <p>Anti-inflammatory and analgesic properties, accompanied by euphoria</p> <p>Effects on the immune system</p>	<p>Acute:</p> <p>Hyperglycemia</p> <p>Fluid retention</p> <p>Mood alteration</p> <p>Chronic:</p> <p>Immunosuppression</p> <p>Suppression of the hypothalamic-pituitary-adrenal axis</p> <p>Musculoskeletal problems, also due to alteration of calcium metabolism and bone homeostasis</p> <p>Nonspecific effects (cataracts, diabetes mellitus, hypertension, peptic ulcer disease, weight gain, skin thinning, ecchymoses, striae, acne, hirsutism, fat redistribution, and various psychiatric disorders)</p>
P2. $\beta$ -Blockers	<p>To reduce tremor, which gives a competitive advantage in specific sports/disciplines (eg, shooting, archery, curling, gymnastics)</p>	<p>Cardiovascular effects: bradycardia, cold extremities, postural hypotension, leg pain</p> <p>Central nervous system/neuromuscular effects: reversible mental depression progressing to catatonia, emotional lability, dizziness, vertigo, tiredness, fatigue, lethargy, drowsiness, depression, insomnia</p> <p>Hematologic effects: agranulocytosis</p> <p>Allergic: fever, sore throat, laryngospasm, respiratory distress</p> <p>Gastrointestinal: mesenteric arterial thrombosis, ischemic colitis, diarrhea, nausea</p> <p>Respiratory effects: wheeziness, dyspnea</p> <p>Other effects: impotency, hypoglycemia</p>

include (1) erythropoietin, (2) anticoagulant agents, (3) anabolic steroids, (4) branched-chain amino acids, (5) glucocorticosteroids, and (6) diuretics [17]. It is evident that in such conditions the range of undesired side effects cannot be foreseen adequately.

### *Physical activity*

The overall evaluation of PED side effects has to consider that active principles are administered during intense physical exercise in competition or out of competition (ie, during the training sessions). It is not unlikely to expect the range of undesired effects are more broad than those that listed in [Table 1](#) or their intensity much more pronounced, given their use at the time of concurrent intense training.

### **The risks of the unknown: the dark side of designer steroids**

Although used off label, many PEDs officially are approved drugs and have undergone a full toxicologic premarketing evaluation. A series of antidoping investigations performed recently have revealed that that new families of drugs, previously unknown to more mainstream pharmacology methodologies, have been developed to be used by athletes seeking enhancement of sport performance. Most of these drugs have been designed to obtain completely new substances, with only some minor modifications in their molecular structure from known synthetic AAS—these are called “designer steroids.” These previously unknown compounds have been synthesized illicitly by clandestine laboratories, operating out of the channel of the pharmaceutical industry. These steroids were supposed to be undetectable, because the practice of the antidoping laboratories is based on the availability of certified reference materials for all target substances: the final proof of the presence of a target within a biologic sample requires the comparison of the analytic signal with that obtained on a certified positive reference sample. There is no reference material available for detection of many of the designer steroids. Furthermore, no pharmacokinetic data are available regarding the metabolism and the excretion profile of the designer steroids. Therefore, it has been nearly impossible for laboratory-mediated selection of suitable urinary markers to detect designer steroids. Consequently, designer steroids have been referred to as the perfect anabolic agents: effective and invisible.

The discovery of the first designer steroid was in 2002, when a previously unknown synthetic AAS, norbolethone, was identified by the WADA-accredited antidoping laboratory of Los Angeles [18]. The discovery of norbolethone was followed by detection of other designer AAS, including tetrahydrogestrinone and desoxy-methyl testosterone (or madol) [19–21]. The antidoping laboratories reacted immediately to face this new analytic

challenge by making available suitable reference materials (most of them through the WAADS network) and developing a new series of analytic procedures for the detection of designer steroids and related substances. This task has been made possible by the development of a new generation of scientific instruments that provides additional tools for the early detection of designer steroids. A particularly promising approach couples a liquid chromatographer to a time-of-flight mass spectrometer [22]. The unique feature of time-of-flight mass spectrometer is its ability to record a broad amount of information from a single assay, giving the ability to return to a previously stored electronic data file and reassess for the possible presence of substances unknown at the time of initial analysis. Other analytic strategies, based on the use of simpler instrumentation, are those based on the use of triple quadrupole liquid chromatography coupled to mass spectrometry with sequential fragmentations (LC/MS-MS) operating in precursor ion scan acquisition mode, a technique that allows identification of compounds derived from a prototype molecular structure based on class-specific fragmentation patterns. This process can be applied to the screening not only of AAS but also other classes of structurally related compounds [23,24]. Although designer steroids no longer may be invisible to antidoping laboratories, their toxicologic profiles remain unknown. Designer steroids add a further item to the list of substances sought after, but because they are not “known” drugs, there have not been any official toxicologic studies performed on them [25].

The process by which the effectiveness and toxicity of a newly developed drug are determined with human volunteers can be structured into three stages (phases) after a drug is designed, synthesized, and preliminarily tested *in vitro* and in animal models.

1. In phase I clinical trials, a new drug or treatment is tested for the first time in a small group of people (20–80) to evaluate its activity, determine a safe dosage range, and identify the most evident side effects.
2. In phase II clinical trials, a study drug or treatment is administered to a larger group of people (100–300) to verify efficacy and further evaluate safety.
3. In phase III studies, a study drug or treatment is given to large groups of people (1000–3000) to confirm evidence obtained in phases I and II, to monitor the potential side effects further, to compare features to those of reference drugs and treatments, and to collect as much clinical information as possible to a the drug or treatment to be used safely in routine medical practice.

None of these steps ever has been performed or considered for designer steroids. For this reason, designer steroids represent perhaps the most dangerous threat to the health of athletes, and the administration of these drugs or any illicitly produced drug should be discouraged.

### **The hidden risks of nutritional supplements and the parallel market**

A final aspect that has to be considered is the massive use by athletes of nonpharmaceutical products, especially nutritional supplements. These products (originally containing only amino acids, vitamins, and mineral salts) readily are available, actively marketed, and massively used by athletes. Because nutritional supplements are not drugs and generally seen as “performance-allowing” rather than “performance-enhancing” substances (and, as such, not included in the WADA-prohibited list), they are not actively included in many studies. If used correctly, nutritional supplements generally are believed safe, with the only known health risks consequent to intolerance or overdosing [26,27]. There are some cases in which the situation is not that simple: for instance, when a product contains one or more substances (or their precursors) included in the WADA list, especially when an athlete is not aware of their presence [28]. This is the case for (1) herbal products, in which the active principles may be indicated with different names (eg, ma huang instead of ephedrine); (2) prohormones, in which the active principles, correctly indicated in the label, are metabolic precursors of endogenous steroid hormones (such as androstenedione and norandrostenedione, precursors of testosterone and nandrolone, respectively); and (3) contaminated or mislabeled products, in which an athlete may be unaware of the presence of a forbidden substance. In the last case, presence of the illicit substance can be the result of accidental contamination or fraud. This problem was identified first by WADA-accredited laboratories. The Cologne Laboratory performed a thorough investigation of the products available on the international market (including those marketed via the Internet), identifying a high percentage of contaminated products [29]. Even in those products in which the concentration of nonlabeled ingredients is low (less than 0.01%), the risks for accumulation cannot be neglected, as many athletes regularly ingest considerable doses of nutritional supplements for long durations of time.

These observations also apply to the broad variety of pseudopharmaceutical products that increasingly are available via the Internet: in these cases, the lack of any pharmaceutical-grade quality control during their productive process could add further risks to those described for “pure” substances. The basic recommendation (as stated by the IOC Medical Commission in 2001) is to limit the use of nutritional supplements to certified products. Any other product should be evaluated carefully and possibly tested by specialized laboratories before being used.

### **Some conclusions and perspectives: toward a comprehensive toxicology of performance-enhancing drugs**

The study of the adverse side effects of PEDs is far from complete. Stimulation of the development of novel investigative tools could complement



(1) the toxicologic studies performed as a part of the development of any new drug; (2) the statistic data supplied by the WADA-accredited antidoping laboratories (also considering the forthcoming activation of specific protocols for the longitudinal follow-up of athletes); (3) the indirect evidence obtained by studies performed on animal models; and (4) the anecdotic information circulated within athletes' environments. A complete assessment of the overall toxicologic profile of the many different PEDs likely will result from such thorough investigations.

The authors also believe that a decisive contribution could originate from the results of ad hoc in vitro studies, which could simulate conditions in which PEDs are used. It is ethically unacceptable to design toxicity studies on humans to reproduce the effects of a real doping protocol; at the same time, the simple extrapolation of results obtained from animal models likely are overly simplistic. The toxic effects of a drug likely are different in patients or healthy volunteers versus intensively training athletes, who are exposed to acidosis, hypoxemia, and tachycardia; toxicodynamic and toxicokinetics can be altered in those conditions. A further result of such an integrated approach would be to shift the interest in use of PEDs from a forensic to a clinical context, allowing not only the identification of markers of exposure to but also of markers of effects of doping substances and methods.

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## An Open Letter to Those Promoting the Medical Supervision of Doping

By Dr. Alain Garnier, Medical Director, World Anti-Doping Agency

Following recent declarations of certain doctors who consider that doping is necessary and even healthy for athletes, it is time to reaffirm, once again and without equivocation, some very basic principles in medical practice and deontology.

If one is considering, in one's role as a sports physician, that elite sport is not healthy, then it means that this kind of practice is not well adapted to human physiology. If this is true, then it is difficult to justify the support and involvement of physicians in sports. After all, medical doctors have the obligation to protect the health of the athletes.

If a particular situation in sports is not compatible with human physiology and may be detrimental to the health of the athlete, one has in fact only two options: to change the sport or the rules that govern that sport to make it more compatible with the human

Always and without exception, a medical doctor should follow the principles of medical practice and defend the health of the athlete, independent of the level of competition or the potential economic consequences. In turn, sport organizations should always ensure this right to physicians, guaranteeing physicians independence in their medical decisions and protecting them from conflicts of interest. When faced with a situation that poses a threat to the athlete's health, a physician should neither accept the situation, nor act to render it bearable. Not following these basic principles of medical ethics leads to very serious consequences. Should a physician confronted with torture propose medical support in order to make it less detrimental to the individual? Certainly not, but those who propose medical supervision for doping are following exactly the same distorted logic.

time, perform faster, tolerate higher workloads, or better withstand pain—but these are certainly far from beneficial to health. To illustrate this point, one should consider a question frequently asked of physicians: in case of injury or fever, what should the legitimate medical attitude be? In general medical practice, the answer is always clear. Why should it be any different in sport? Can one imagine a doctor prescribing amphetamines to a truck driver because he or she is too tired to continue driving?

The use of even the most common drugs is associated with risks and potential side effects. Given this basic fact of pharmacology, any physician must understand the risk/benefit ratio before writing any prescription. Promoting doping for all athletes contradicts this basic principle of medicine. To argue that medically supervised doping is safer because a doctor is in charge misses the point

Contrary to what the physicians defending doping pretend, accepting the idea of medical supervision of doping would immediately and irremediably lead to a generalization of doping and an exclusion from sport of all clean athletes who are opposed to using unnecessary drugs and want to defend the spirit of sport.

condition, or to adapt athletes to the sport. The former is the action supported by the scientific literature in physiology, public health, and occupational medicine. The latter, regrettably chosen by certain doctors, leads one to justify doping as "indispensable."

To change sport or to change humans? That is the question. Given the imminence of gene therapy, we must not delay in addressing this question once and for all.

In addition to the ethical reasons presented above, many other medical arguments oppose the acceptance of medically supervised doping.

Regardless of whether drugs or methods used for doping purposes can effectively enhance performance, there exists no scientific evidence that such practices are healthy, particularly in the mid- and long-term. Depending on the nature of the substance used for doping, the athlete may be able to compete for a longer

entirely. There exists no credible data indicating that a drug is less dangerous when prescribed by a doctor. Everyday, in hospitals and clinics worldwide, patients experience the side effects of drugs despite strict monitoring by highly experienced doctors.

In medical practice the use of drugs is very strictly codified with indications and contra-indications. There is no evidence that competing in sports or exhausting exercise is an indication

# Use of doping agents, particularly anabolic steroids, in sports and society

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The use of doping agents, particularly anabolic androgenic steroids (AAS), has changed from being a problem restricted to sports to one of public-health concern. We review the prevalence of misuse, the evidence that some drugs improve performance in sport, their side-effects, and the long-term consequences of AAS misuse for society at large. There is substantial under-reporting of the side-effects of AAS to health authorities. We describe neuropsychiatric side-effects of AAS and their possible neurobiological correlates, with particular emphasis on violent behaviour. Analytical methods and laboratories accredited by the World Anti-Doping Agency can detect the misuse of all doping agents; although the analysis of testosterone requires special techniques, and recently discovered interethnic differences in testosterone excretion should be taken into account. The prevention of misuse of doping agents should include random doping analyses, medical follow-ups, pedagogic interventions, tougher legislation against possession of AAS, and longer disqualifications of athletes who use AAS.

## Prevalence of doping in sport and society

The use of pharmacologically active substances to improve performance in work or sports goes back centuries but has increased in the past 40 years since the introduction of anabolic androgenic steroids (AAS; table 1).<sup>1–7</sup> In an article entitled “The toxic torch of the modern Olympic Games”, Prendergast and coauthors state that the quest for greatness has driven many athletes and coaches to push for unfair advantages by the use of performance-enhancing (ergogenic) drugs, commonly referred to as “doping”.<sup>6</sup> After the reunification of Germany, the horrifying features of the doping of Olympian competitors in the former East Germany were revealed.<sup>7</sup>

The use of doping agents is no longer restricted to competing athletes; young sportspeople in schools and non-competing amateurs also use them. Misuse of AAS is increasing among gym customers for whom bodily appearance is a priority. Estimates of misuse have to be interpreted with great caution due to the difficulties of reliable studies of illicit drug use. In the USA, between 1 million and 3 million people are thought to have misused AAS;<sup>8,9</sup> the estimate for Sweden is 50 000–100 000, among a population of 9 million. These estimates roughly equate to 1% of the respective populations.

Interviews of high-school students in several European countries and the USA reveal that 1–5% have used AAS, but this measure is of doubtful relevance for the population at risk of serious side-effects, which develop during long-term use.<sup>10</sup> An investigation of 6000 Swedish people age 16–17 years with an anonymous multiple-choice questionnaire revealed that 3.2% of males had used AAS, but none of the females had.<sup>11,12</sup> There was an association between the misuse of AAS and the use of substances such as alcohol, growth hormone, and narcotic drugs. In males, visible results of physical training were thought important for self-confidence, respect from girls, and security in nightlife and beach culture.<sup>13</sup> An informational intervention programme led to a decrease of almost 50% in misuse in males.<sup>14</sup>

Much higher estimates of misuse of AAS have been obtained in groups such as bodybuilders, weight-lifters, and prison populations.<sup>10</sup> A German study assessed the use of AAS among visitors to fitness centres by use of anonymous questionnaires. Although only 34.5% of these were returned, 13.5% in this selected group reported that they had used AAS at some point.<sup>15</sup> In this study, only 3.9% of women had used AAS, and studies in Great Britain and the USA have found similar levels of use among women.<sup>16</sup>

The best estimates of stimulant drug misuse are available for ephedrine. Ephedra alkaloids are popular components of many nutritional supplements and are also used as stimulants on their own. As many as 2.8 million US recreational athletes might have used ephedrine in 2001.<sup>16</sup>

Since 1993, the Swedish government has sponsored the antidoping hotline—a telephone service answering questions about doping from anonymous callers involved in or exposed to doping.<sup>17</sup> Between 1993 and 2006 the organisation received about 40 000 calls. Callers connected with gyms were the largest group (30%). Most calls were about AAS such as testosterone, nandrolone decanoate, methandienone, and stanozolol (table 2).

## Substances prohibited in sports

The World Anti-Doping Agency (WADA) publishes a yearly list (panel 1) of substances and practices prohibited at all time in and out of competition. When prescribing listed drugs, physicians must be prepared to verify that the drug is medically justified and can be given a therapeutic-use exemption,<sup>18</sup> a decision that requires assessment by the relevant sports organisation. As an example, a therapeutic-use exemption is needed if a  $\beta$ 2-adrenoceptor-agonist or corticosteroid inhalation is prescribed for bronchoconstriction.<sup>19</sup>

There are different rationales for including a drug on the WADA list. The original idea was to list drugs known or suspected to improve performance in sports. After

For the World Anti-Doping  
Agency see [www.wada-ama.org](http://www.wada-ama.org)

confrontation with the realities of doping, other reasons were accepted, such as the safety of the athletes, social unacceptability, and attempts to make doping analyses insensitive. Anti-oestrogens are on the list because they are sometimes used to antagonise the oestrogenic side-effects of AAS and other drugs.

There is also a list of substances prohibited in competition (panel 2). Alcohol is prohibited only in certain sports (eg, racing with automobiles and motorcycles).  $\beta$  blockers are prohibited in sports in which absence of a high pulse rate and tremor is advantageous (eg, shooting).

Prohibited practices in sports include enhancement of oxygen transfer (eg, blood doping), chemical and physical manipulation of samples collected during doping controls, and gene doping to administer erythropoietin or other genes that might affect athletic performance, which is a possible future development. Blood doping is any method or substance used for non-medical purposes that improves aerobic performance by increasing oxygen flow to peripheral tissues in athletes, and it includes blood transfusion and the use of recombinant erythropoietin.<sup>20</sup>

## Do doping agents improve sporting ability?

### Experimental studies

There is plenty of empirical evidence that doping agents improve performance in sport but very few experimental studies of the kind that are needed for a drug to be approved for marketing. An exception are Smith and Beecher's classic studies,<sup>21,22</sup> results of which showed that amphetamine in therapeutic doses (14 mg per 70 kg) improved performance in short-distance swimming and sprinting. The difference between the effects of placebo and amphetamine was small but enough to make the difference between competitive renown and obscurity.<sup>23</sup> In a double-blind, randomised, crossover study, a 180 mg dose of pseudoephedrine (three-times the therapeutic dose) decreased the time to complete a 1500 m run by 2.1% with no reported side-effects.<sup>24</sup> The use of ephedrine itself is thought unsafe because of its cardiovascular side-effects,<sup>25</sup> and its ergogenic effects are equivocal.<sup>26,27</sup> The same is true for several other central stimulants such as phenylpropanolamine, cocaine, and methylphenidate.<sup>9</sup> Despite this risk, ephedra and other central stimulants are commonly used, for example, by college athletes before a hockey game.<sup>28</sup> A combination of ephedrine and caffeine is popular for doping purposes, and evidence suggests that the combination is more effective than either drug alone.<sup>27</sup>

There is no conclusive evidence that growth hormone improves athletic performance.<sup>29,30</sup> This drug is usually taken in doses that are ten to 20 times the therapeutic level and commonly in combination with AAS in cycles of 4–6 weeks.<sup>30</sup> Up to 5% of US high-school students have tried growth hormone as an anabolic agent.<sup>30</sup>

Use of doping agents	
Centuries ago	Incas chewed coca ( <i>Erythroxylon</i> spp) leaves to sustain strenuous work; Berserkers, Norse warriors, ate mushrooms containing muscarine before battle
Ancient Olympians	Bread soaked in opium, mushrooms, strychnine
Early 1900	Canal swimmers and cyclists taking central stimulants
World War II	Amphetamine to counter fatigue among soldiers and pilots
1950	Anabolic androgenic steroids introduced in doping; dianazol synthesis inspired by people in sports
1959	Classical controlled studies show that amphetamine improves performance in short distance swimming and running
1960 Olympics	First documented doping fatality—amphetamine induced heatstroke
1964	The International Olympic Committee (IOC) bans doping for Olympic athletes
1966–72	East Germany introduces a secret national system for hormone doping of both men and women with methandrostenolone and state manufactured oral-turinabal
1967	Doping death during Tour de France, IOC adopts a drug-testing policy
1970	Diuretics used to reach the "right" weight and to dilute urine before drug testing
1973	Olympic champion Connolly testifies on the common use of anabolic steroids among athletes to US Senate committee
1974	Anabolic androgenic steroids (AAS) put on the doping list
Up to 1980	Amphetamine, cocaine, caffeine, and strychnine dominate doping incidents
1980	AAS spread to many sports
1980	$\beta$ -blockers used to improve shooting; misuse of growth hormone appears
1988	First Olympic gold medal in track and field stripped due to doping with AAS
2000	Tetrahydrogestrinone (THG or "the clear"), an AAS designed to escape detection in doping analyses, is developed
2007	Marion Jones admits having taken "the clear", a performance-enhancing drug listed to the Bay Area Laboratory

Table 1: Historical overview of doping in society and sports<sup>27</sup>

Early experimental studies of the ergogenic effects of AAS in sports were inconclusive. Many of these studies lacked adequate controls and had other weaknesses in design,<sup>31,32</sup> such as small groups of volunteers (10–24) and doses far below those used in sports.

Athletes commonly take megadoses of steroids—doses 50–100 times the amount needed to replace physiological steroid concentrations. Steroids are taken out of the competition season in cycles lasting 4–12 weeks. Many athletes take multiple steroids at once, known as stacking, and "pyramid" the dosing schedule, taking the highest total doses in the middle of the cycle. Breaks, known as drug holidays, of varying duration are common between the cycles.<sup>5</sup>

The opinions about the efficacy of AAS have gradually shifted from scepticism<sup>31,33,34</sup> to a consensus that these drugs might have some positive effects on strength when combined with muscular training, such as bench presses and lifts.<sup>35,36</sup> Athletes taking anabolic steroids can expect increases in muscular strength but not in aerobic gains.<sup>37</sup> In a randomised controlled study, healthy men received 600 mg of testosterone enanthate or placebo weekly for 10 weeks, the highest dose reported in volunteers.<sup>38</sup> When combined with strength training, testosterone increased fat-free mass, muscle size, and strength.<sup>38</sup> AAS might cause hypertrophy in human skeletal muscle even in the absence of strength training.<sup>39,40</sup>

	Substance being misused (%)		Substance being discussed (%)	
	1996–2000	2001–06	1996–2000	2001–06
AAS	(n=5505)	(n=3876)	(n=3372)	(n=1426)
Testosterone	27%	26%	26%	19%
Methandienone ("Russian")	26%	16%	19%	14%
Nandrolone	16%	24%	18%	22%
Stanozolol	9%	12%	13%	11%
Others	23%	21%	21%	34%
Other hormones and related agents	(n=421)	(n=679)	(n=509)	(n=186)
hCG/tamoxifen	58%	58%	37%	53%
GH/IGF1/insulin	42%	42%	63%	47%
Other substances	(n=1733)	(n=1594)	(n=4161)	(n=687)
Ephedrine	23%	24%	21%	27%
Clenbuterol	12%	10%	12%	10%
GHB	6%	2%	7%	2%
Narcotics unspecified	16%	17%	4%	21%
Alcohol	1%	2%	0.3%	8%
Creatine	11%	10%	8%	3%
Dietary supplement	16%	19%	14%	14%
Prescription drugs not specified here	15%	17%	33%	14%

AAS=anabolic androgenic steroids. hCG=human chorionic gonadotropin. Grt=growth hormone. IGF1=insulin-like growth factor 1. GHB=γ-hydroxybutyric acid.

**Table 2: Substances reported to the Swedish Anti-Doping Hot-Line during 1996–2000<sup>17</sup> and 2001–06 (unpublished data)**

Although there was academic controversy about published results, the secret doping programme using megadoses of AAS in East Germany confirmed the ergogenic effects of this class of drugs. Franke and Berendok<sup>7</sup> reported how hundreds of physicians and scientists became involved in unethical doping to promote the sporting success of East Germany from 1966 until the reunification of Germany in 1990.

Recent studies of muscular biopsies from athletes involved in doping<sup>41–43</sup> showed that AAS further increased the muscle-fibre hypertrophy induced by strength training. The number of nuclei per muscle fibre was higher in powerlifters using AAS than in controls. Unexpectedly, the number of myonuclei remained high in people who had stopped taking AAS several years previously.

Blood transfusion has been used for doping purposes as an effective way to increase the oxygen-carrying capacity of the blood.<sup>20</sup> Recombinant human erythropoietin, the ergogenic effects of which were documented in professional skiers in 1991,<sup>44</sup> has replaced this practice. Erythropoietin provides significant benefits due to substantial increases in haemoglobin, haematocrit, maximum oxygen uptake, and exercise endurance time.<sup>45</sup>

### Empirical evidence

Many case reports suggest that doping with AAS is effective. Notable examples include Ben Johnson's gold medal for the 100 m at the Seoul Olympics in 1988, and

hundreds of other winning elite athletes who have been caught in doping tests. Furthermore, world records in power sports seem to have reached a steady state since the introduction of sensitive doping tests.

## Adverse reactions to doping agents

### Central stimulants

Central stimulants still dominate doping in sports, but their dose-dependent adverse reactions preclude the use of megadoses. The most prominent side-effects include adrenergic effects in the CNS and the cardiovascular system. Amphetamine causes euphoria, relieves fatigue, and promotes self-confidence. Somatic effects include increased pulse-rate, hypertension, arrhythmias, and hyperthermia. High doses may produce aggressive behaviour and psychosis.<sup>21,26</sup>

Ephedrine has a particularly bad reputation for its many side-effects. In 2001, ephedra accounted for 0.8% of herbal product sales in the USA but for 64% of adverse herbal reactions.<sup>46</sup> These include cardiovascular symptoms (hypertension, arrhythmias), central nervous symptoms (anxiety, tremor, paranoid psychoses), potentially life-threatening events (myocardial infarction), and even death.<sup>47,48</sup>

Furthermore, exposure to amphetamine and cocaine in sport can lead to misuse later in life.

### Anabolic androgenic steroids (AAS)

#### Endocrine effects and side-effects

All AAS bind to the one type of androgen receptor, albeit with different affinities. Since these receptors are saturated in unmedicated men, supraphysiological doses of AAS may exert secondary effects, for example, by displacing cortisol from its receptors thereby inhibiting its catabolic effects.<sup>49</sup>

The structure, distribution, and regulation of the androgen receptors are well characterised<sup>50,51</sup>—they are located not only in the male reproductive and accessory sex tissues but also in other tissues, such as skeletal muscle, skin, and parts of the brain.<sup>50</sup> The steroids bind to androgen receptors in the cytoplasm. In the nucleus, the binding of receptors to target genes triggers DNA transcription and the synthesis of specific proteins that mediate hormonal function.<sup>50,51</sup> All androgenic hormones exert both masculinising and anabolic effects. The endocrine effects are dominated by testicular atrophy, sterility, disfiguring gynaecomastia in males, and virilisation in females—including hirsutism, amenorrhoea, clitoral hypertrophy, and a hoarse voice.<sup>49,52–54</sup> Androgenisation of sportswomen in the former East Germany has had severe adverse results, such as hirsutism with gynaecological disorders, such as long-term amenorrhoea and ovarian cysts.<sup>6</sup>

#### Somatic side-effects

The unfavourable changes in blood lipid profiles caused by AAS<sup>10</sup> include an increase in the concentration of

### Panel 1: Substances and methods prohibited in sports at all times according to WADA, 2008

#### Anabolic agents

- AAS
- Exogenous AAS (eg, danazol, nandrolone, stanozolol)
- Endogenous AAS (eg, testosterone)
- Other anabolic agents (eg, desbuterol, androgen-receptor modulators)

#### Hormones and related substances\*

- Erythropoietin
- Growth hormone, insulin-like growth factors (eg, IGF1), mechano growth factors (MGFs)
- Gonadotropins (eg, LH, human chorionic gonadotropin; prohibited in males only)
- Insulins
- Corticotropins

#### β-2-agonists

- All β-2-agonists including their D and L isomers
- Inhalation of β-2-agonists requires a therapeutic-use exemption

#### Hormone antagonists and modulators

- Aromatase inhibitors (eg, anastrozole, letrozole)
- Selective oestrogen-receptor modulators (eg, tamoxifen)
- Other antioestrogenic substances (eg, clomiphene)
- Agents modifying myostatin functions (eg, myostatin inhibitors)

#### Diuretics and other masking agents

- Diuretics
- Epitestosterone
- Probenecid
- α-reductase inhibitors (eg, finasteride, plasma expanders)

\*Unless the athlete can prove that the concentration is due to a physiological or pathological disorder.

### Panel 2: Substances prohibited in competition according to WADA, 2008

- Stimulants, both optical isomers (eg, amphetamine, cocaine, ephedrine\*, methylephedrine, fenfluramine, D-methamphetamine, methylphenidate, modafinil, pemoline, selegiline, sibutramine, strychnine)
- Narcotics (eg, all opiates)
- Cannabinoids (eg, hashish, marijuana)
- Glucocorticosteroids, all are prohibited and their use requires a therapeutic-use exemption

\*Threshold value of 10 µg/mL.

changes in his blood profile, and died with extreme hypertrophy of the heart. He had hypertension, obesity, mood disorders, ruptures of various muscles, and secondary hypogonadism. A recent case report also showed the serious cardiac side-effects of AAS.<sup>61</sup> Echocardiographic examinations indicate an association between AAS abuse and left-ventricular hypertrophy.<sup>62-64</sup>

62 Finnish powerlifters who were strongly suspected of having used megadoses of AAS over several years were followed up for 12 years.<sup>65</sup> Mortality in this group was 12·9% (mean age at death was 43 years) compared with 3·1% in the control group of 1094 people participating in the WHO MONICA study (mean age at death not reported). Suicide and acute myocardial infarction accounted for six of the eight deaths.

Rare cases of hepatic complications have also been reported, such as cholestasis, peliosis, adenomas,<sup>66-68</sup> and raised concentrations of liver transaminases.<sup>66</sup> Premature closure of the epiphyseal growth plates is a concern among adolescents taking AAS.<sup>69</sup> Up to 2·7% of middle-school students (age 9–13 years) have used steroids.<sup>70</sup> Physical signs of high doses of AAS are summarised in panel 3.<sup>71</sup>

LDL, a decrease in the concentration of HDL by 30–50%, and a reduction in the concentration of apoprotein A1.<sup>55-58</sup> These metabolic changes explain the many reports of cardiovascular disease and hypertension in people who misuse AAS.

In 1993, Kennedy and Lawrence<sup>59</sup> reported the deaths of two young footballers who had misused oxymesterone,<sup>59</sup> both sustained fatal cardiac arrests during training and hypertrophic cardiomyopathy, irritability, and sudden rages had been noted soon before death. The authors also described six published cases of myocardial infarction, of which three were fatal, associated with the use of anabolic steroids.

Madea and Grellner<sup>60</sup> described several serious somatic side-effects in a 25-year-old man on multiple steroids. The patient had practised body-building with steroids from the age of 15 years and developed severe disturbances of lipid metabolism, had hormonal

#### Neuropsychiatric side-effects

In 1974, Wilson and colleagues observed that small doses of methyltestosterone added to imipramine provoke paranoid delusions in patients with depression.<sup>72</sup> And 20 years ago, Pope and Katz<sup>73</sup> reported psychosis in sportsmen misusing steroids. In a controlled but retrospective study, 20 male weight-lifters using AAS were compared with 20 male weight-lifters who had never used steroids.<sup>74</sup> The steroid users had more psychiatric side-effects than the control group; these included anxiety, depression, hostility, and paranoia. In a controlled study of 156 athletes, 88 using steroids, 20 (23%) of the users reported major mood changes, such as mania, hypomania, and major depression—symptoms that were not seen in non-users.<sup>75</sup>

The first placebo-controlled trial of an AAS (methyltestosterone in doses of 40 mg/day and 240 mg/day) in 20 healthy male volunteers was published

**Panel 3: Physical signs in patients using megadoses of AAS<sup>71</sup>****Vital signs**

Increased blood pressure (relatively uncommon)

**Skin**

Acne, male pattern baldness, striae, jaundice with liver disease, hirsutism in women

**Head and neck**

Jaundiced eyes with liver disease, deepening of the voice in women

**Chest**

Gynaecomastia with tenderness in men

**Abdominal**

Right-upper-quadrant tenderness and hepatomegaly with liver disease

**Genitourinary**

Testicular atrophy and prostatic hypertrophy in men  
Clitoral hypertrophy in women

**Musculoskeletal**

Generalised muscle hypertrophy with disproportionately large upper-body mass (especially neck, shoulders, arms and chest)

**Extremities**

Oedema due to water retention for which diuretics may be used

in 1993.<sup>76</sup> This trial had a 2-week, double-blind, fixed-order, placebo-controlled, crossover design. The healthy volunteers were medication-free, somatically and psychiatrically healthy, not involved in athletic training, and had no history of AAS use. There were small but statistically significant increases in symptom scores at the 240 mg dose both in positive (euphoria, energy, and sexual arousal) and negative mood (irritability, mood swings, violent feelings, and hostility) and in cognitive impairment (distractibility, forgetfulness, and confusion). One of the participants developed an acute manic episode and one became hypomanic, thus indicating pronounced interindividual differences in the effects on CNS functions.

Several articles have confirmed the psychological and behavioural side-effects of endogenous testosterone and AAS and documented increased aggressive behaviour in volunteers.<sup>77-79</sup> The range of psychiatric side-effects induced by AAS and their severity increase with the intensity of misuse.<sup>80</sup>

The first case report of violent criminal acts induced by an anabolic steroid (oxymethalone) was published 20 years ago.<sup>81</sup> Aggression and violence toward women who are partners of strength athletes illicitly using AAS was later described.<sup>82</sup> In our experience, many female

callers to our antidoping hotline worry about aggression and violence from partners using AAS.

In 1997, Thiblin and colleagues<sup>83</sup> pointed out that alcohol and AAS seem to be strongly synergistic in producing impulsive violent behaviour. The authors retrospectively analysed information from forensic psychiatric assessment, police reports, and court records of 14 users of AAS. This series comprised five cases of murder, five cases of assault, and four of robbery, one resulting in homicide. In eleven of these cases the perpetrators were intoxicated with alcohol while committing the crime. In agreement with anecdotal reports, there is greatly increased mortality in young people who misuse AAS.<sup>84</sup>

Early attempts to relate violent crime to misuse of AAS have been made in studies of Swedish prisoners; although no associations were found, the studies were hampered because a high percentage of the prisoners refused to give urine samples.<sup>85</sup> Petersson and co-workers recently used a new approach to the study of morbidity and mortality in users of AAS.<sup>86</sup> Patients referred from inpatient and outpatient clinics and who had tested positive for AAS (n=248) while receiving medical care were compared with patients who tested negative (n=1215). The proportion of patients who had received institutional care for substance use or psychiatric disorders was significantly higher in the AAS-positive group, as was the standardised mortality rate.<sup>86</sup>

The same research group<sup>87</sup> also reported the manner of death in 52 autopsied users of AAS (confirmed by drug analysis); the control group comprised 68 dead users of amphetamine, heroin, or both who tested negative for AAS. The AAS positive individuals died at a mean of 24.5 years, compared with 34 years for users of heroin and amphetamine and 40 years in amphetamine users; suicide or homicide were also more common among the users of AAS. The AAS users additionally used other illicit drugs to a great extent, particularly opiates. These data strongly suggest that AAS users are more likely to become involved in incidents leading to violent death than are other users of illegal drugs. In another study, weapons offence and fraud were more common in individuals testing positive for AAS than among control individuals.<sup>88</sup>

Further epidemiological studies are needed to assess whether the AAS precipitates antisocial behaviour or whether people prone to such behaviour are particularly inclined to use these drugs.

Research into mechanisms involved in the behavioural effects of AAS has focused on the serotonergic system. Chronic administration of testosterone to rats induces a decrease in brain concentrations of serotonin and its main metabolite, 5-hydroxyindolacetic acid (5-HIAA), which is associated with increased aggression.<sup>89,90</sup> AAS also decreases the number of serotonin receptors in the limbic system.<sup>91</sup> However, there are many other neurobiological changes caused by AAS in animals,<sup>92</sup>



such as increases in the concentrations of nerve growth factor in the hippocampus and decreases in the hypothalamus.<sup>93</sup> Extrapolation to human beings is difficult because of species differences in drug metabolism. Furthermore, plasma and brain concentrations of AAS have not been measured in animals to permit such a comparison.

In human volunteers who received methyltestosterone at a dose of 40 mg per day for 3 days and 240 mg per day for a further 3 days,<sup>94</sup> there were increases in 5-HIAA concentrations in cerebrospinal fluid, which were related to the hypomanic behaviour.<sup>94</sup> Mood and behavioural effects of AAS may, in part, reflect secondary hormonal changes,<sup>95</sup> but the 5-HIAA findings are particularly interesting because low cerebrospinal-fluid concentrations of this serotonin metabolite are linked to depression and suicidal behaviour.<sup>96–98</sup>

People who use AAS are more likely than non-users to misuse other drugs and alcohol.<sup>12,99</sup> Rats treated with AAS have higher voluntary alcohol consumption than control rats.<sup>100</sup> A much debated question is whether the misuse of AAS is a gateway to substance abuse in general.<sup>101</sup> In a case-control study, many users of AAS misused several other substances—either recreational or prescription drugs.<sup>102,103</sup>

According to Brower,<sup>71</sup> no misuse has occurred after therapeutic doses of AAS. By contrast up to 2002, there have been 165 reported instances of AAS dependence among body-builders and weight-lifters taking supra-physiological doses, commonly in combination with misuse of other drugs.

### Erythropoietin

The risks with erythropoietin doping are serious and include myocardial infarction, cerebrovascular disease, and serious thromboembolic events,<sup>20</sup> such as cerebral sinus thrombosis.<sup>104</sup> Predictable complications include polycythaemic disorders and hypertension.<sup>20</sup>

### Under-reporting of side-effects

As most of these substances are illegal and cannot be obtained by prescription, physicians rarely report their side-effects to national centres of pharmacovigilance. During 1996–2000, 4335 people reported about 10800 side-effects to the Swedish antidoping hotline.<sup>17</sup> In the same period, prescribers reported only 27 cases involving doping agents to the Swedish adverse-drug-reactions committee. Side-effects of doping agents, particularly AAS, are a much bigger problem in society than hitherto recognised.

### Detection of misuse

#### Analytical methods accredited by WADA

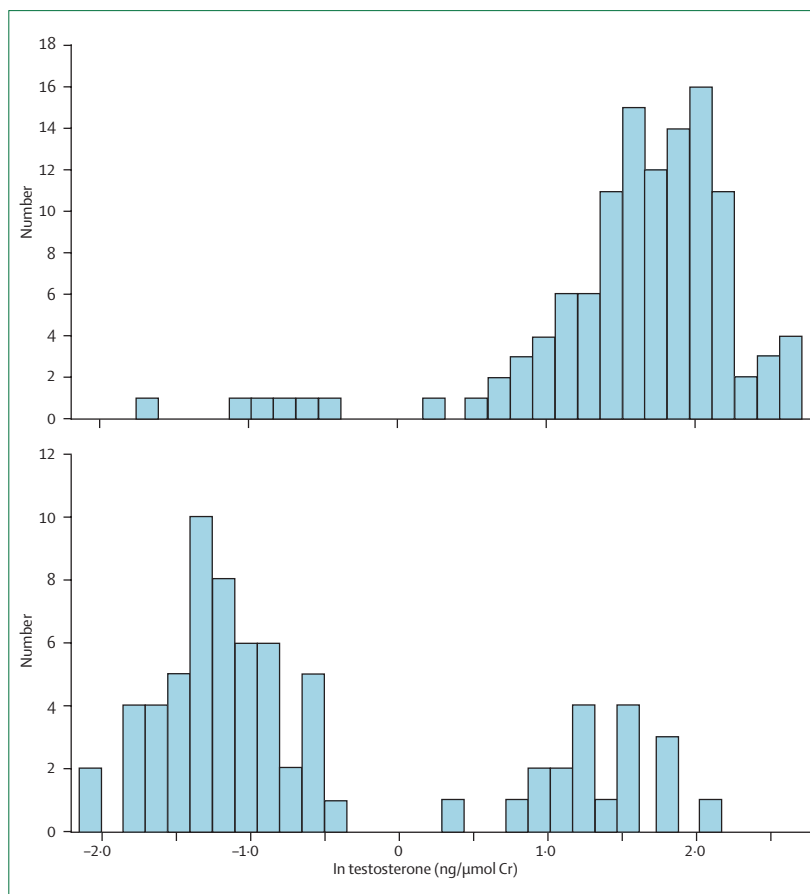
Athletics drug testing has been described in several reviews.<sup>4,105</sup> Not until the 1976 Olympic Games did suitable tests for AAS become available to enable an enforceable ban.<sup>105,106</sup> Abuse of doping agents in sports

can be verified by the 30 laboratories accredited by WADA for doping control in national and international events, including the Olympic Games. Some laboratories are also involved in random unannounced doping controls between games. The analytical methods have a much better precision and sensitivity than the usual routine methods used in clinical chemistry. Urine or blood samples are first screened and suspicious results ultimately confirmed with advanced methods based on mass spectrometry.

Attempts to mask the presence of doping agents in urine (eg, by use of diuretics and probenecid) have generally failed. New anabolic steroids have been designed to avoid detection in doping controls, but laboratories have developed tests to detect these substances as well.

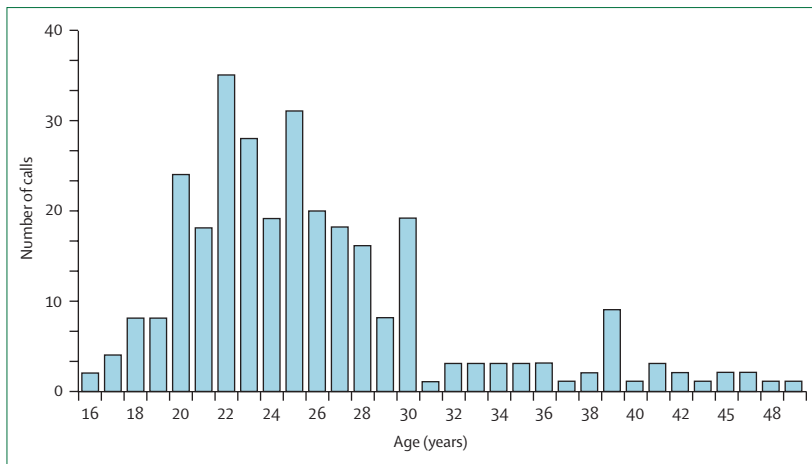
### Special problems with analysis of testosterone—pharmacogenetic aspects

The large differences in urinary kinetics of various AAS are a poorly explored problem and present a challenge



**Figure 1: Urinary excretion of testosterone**

Frequency distribution of natural logarithms of urinary unconjugated + glucuronide conjugated testosterone (ng/μmol Cr) in Swedish (n=122; top) and Korean (n=74; bottom) populations of healthy men. Both groups show a bimodal distribution of testosterone excretion rate. Fast excretors dominate in the Swedish sample but are rare in the Korean sample.<sup>123</sup>



**Figure 2: Age profile of people who contacted the Swedish antidoping hotline in 2006**  
People contacted the hotline in person or via a relative for information. Data were similar for years before 2006.

for future research. Although water soluble compounds (such as oxandrolone) yield positive doping tests for only a few days after oral administration, an intramuscular injection of nandrolone decanoate results in metabolites that can be detected several months later.<sup>4</sup>

The challenge has been to differentiate between exogenous and endogenous testosterone.<sup>106</sup> Manfred Donike suggested, in 1983, that the urinary ratio of testosterone and the naturally occurring isomer epitestosterone (T/E ratio) might indicate the use of exogenous testosterone<sup>107</sup> as the concentration of epitestosterone is not affected by intake of testosterone. Statistical reasons suggested that a T/E ratio greater than 6 was highly suggestive of testosterone doping. Subsequent observations from limited studies indicated, however, that urinary T/E ratios vary a lot between individuals,<sup>108–111</sup> suggesting an influence of genetic factors.

The adrenal glands are a major source of precursors of sex steroids. Prohormones such as dihydroepiandrosterone and androstenedione, also used in doping, are secreted into the blood and then bioactivated in the gonads and the prostate into testosterone and dihydrotestosterone. The gonadal function is partly governed by pituitary LH and FSH, which are kept in balance with the circulating hormones by virtue of an endocrine feedback system. Although most circulating testosterone is generated in the testes, the prostate also contributes substantially through bioactivation of dihydroepiandrosterone and androstenedione,<sup>112</sup> which are sometimes used in doping to increase testosterone production.<sup>112</sup>

The major drug metabolising enzymes in the cytochrome P450 (CYP) family are inherited in a polymorphic way which may confer 100–1000-fold differences in metabolic capacity among individuals.<sup>113</sup> Because the same or related enzymes, such as CYP3A:s

and CYP17, metabolise many androgens and drugs,<sup>114–117</sup> there is probably genetic variability in the metabolism of AAS.<sup>118,119</sup> This variation may affect not only renal excretion patterns but also intracrine concentrations of androgens and, hence, their organ effects.

Testosterone is excreted mainly as conjugates after glucuronidation by uridine diphospho-glucuronosyl transferases (UGT). These enzymes have a key role in the homeostasis of several endogenous molecules, including steroid hormones, facilitating their excretion in urine.<sup>120,121</sup> There are seven members of the UGT2B subfamily,<sup>122–124</sup> of which UGT2B17 and UGT2B7 are particularly active in the glucuronidation of testosterone and epitestosterone.

We compared the excretion of testosterone, epitestosterone, and many other androgens in a large sample of Swedish and Korean people. Swedish people had 16-times higher excretion of unconjugated and glucuronidated testosterone (hereafter called testosterone) than did Koreans.<sup>123</sup> These findings predict different effects of testosterone intake on the T/E ratio in the two ethnic groups. The difference is a confounder in programmes of antidoping testing. There is a bimodal distribution of the natural logarithm of urinary testosterone concentrations in both Europeans and Asians, suggesting monogenic inheritance.<sup>123</sup> The recent report of a deletion polymorphism in the UGT2B17 gene<sup>124</sup> inspired investigation of this polymorphism and the testosterone excretion pattern. All individuals lacking UGT2B17 had no or negligible testosterone excretion (figure 1). Interestingly, and consistent with the pattern of testosterone excretion, the deletion genotype was seven times more common in Koreans (67·0%) than in Swedish people (9·3%).<sup>123</sup>

Although provisional results indicate genetic variation, we found no relation between the excretion of epitestosterone in urine and the UGT2B17 genotype.<sup>123</sup> Our findings indicate that consideration of genetic variation in androgen metabolising enzymes will help refine the detection of testosterone doping.

### Erythropoietin

Methods for diagnosing the abuse of erythropoietin have been difficult to develop. A combination of indirect and direct methods seems most suitable, but there is still room for improvements, particularly of sensitivity. The indirect methods are based on multiple markers of increased erythropoiesis, while the direct method for urine analysis of erythropoietin is based on isoelectric focusing, which differentiates between the recombinant and endogenous types.<sup>20,125</sup>

### Clinical diagnosis

The diagnosis of misuse is most difficult for anabolic steroids. The mostly likely users are young men who do weight training or sports that require strength and power.<sup>71</sup> The investigation should include a compre-

hensive drug history as well as physical and mental examinations (panel 3). Clinical assessment should be complemented with laboratory tests, such as LH and FSH, and urinary screening for AAS.

## Prevention

### Doping analyses

The introduction of doping analyses has held back doping in elite sports. In Sweden, the proportion of positive doping tests among athletes has declined from 2% to below 0.5% during the past 5 years. Between 1981 and 2005, hormones (62%) were the most commonly detected, stimulants accounted for 7% and narcotics for 5%. 23% of athletes refused to participate and were disqualified. The proportion of positive doping tests is much higher among risk groups in society than among athletes (unpublished).

### Education

Information and education are the most important tasks for our antidoping hotline established in 1993 ([www.dopingjouren.nu](http://www.dopingjouren.nu)). Professional advice is given by trained nurses with physicians trained in clinical pharmacology as back-up. As most misusers are age 17–27 years (figure 2), it is particularly important to improve information about AAS and other doping agents in high schools. Similar antidoping hotlines are now available in Norway, Denmark, and Holland.

Pedagogic intervention programmes to prevent the use of AAS should be encouraged.<sup>13</sup> Physicians and other health personnel must be better educated in AAS misuse, which is an underappreciated part of prevention of narcotic misuse. In a recent Danish study, a third of 571 practising physicians had encountered patients with side-effects of AAS, usually males age less than 40 years.<sup>126</sup> Unfortunately, users of AAS commonly mistrust physicians and prefer to consult friends, internet sites, or even the people who sold them the steroids.<sup>127</sup> This credibility gap is believed to be related to the fact that members of the medical community have claimed for a long time that AAS are ineffective for gaining muscular strength.<sup>127</sup>

The educational programmes against doping should also emphasise the ethical and moral issues involved. The scientist and powerlifter Anders Eriksson elegantly expressed it as follows in his thesis: “Three times in my career I have received medals several months after the competition because lifters finishing ahead of me were caught in a drug test. This means that three of the greatest moments in my sport career were reduced to a letter with a medal.”<sup>43</sup>

### Legislation

Many countries are on the way to strengthening the laws against possession and use of AAS and now consider these drugs as equivalent to narcotics. In a recent news story in the *BMJ*,<sup>128</sup> MPs in the UK were reported to be

calling for tougher methods to tackle doping in sports. An important concern is the ease with which banned substances can be obtained by athletes and the public.

The results of studies by Eriksson<sup>41–43</sup> show the effect of AAS on muscle fibres lasts much longer than believed, which suggests that athletes using anabolic agents should be disqualified for longer than 2 years. His histological observations may be the cellular correlate to an old observation in East Germany that “androgenic initiation” has long-lasting effects, particularly in women.

## Conclusion

Our review summarises the increasing medical concerns about the widespread misuse of doping agents, particularly anabolic androgenic steroids (AAS), that started in athletes and now affects the general population. Compared with the attempts to prevent the misuse of narcotic drugs, the illegal use of AAS has not elicited sufficient interest from health authorities to hold back the problem, and the many side-effects of AAS remain largely unrecognised. As with narcotics, AAS have neuropsychiatric side-effects, including aggressive and even violent behaviour. Preventive measures include increased awareness among physicians, proper doping analyses, pedagogic interventions, and updated legislation. Doping in sport must be combated with much longer disqualifications of athletes using AAS, a proposal that has scientific support.

### Conflict of interest statement

We declare that we have no conflict of interest.

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