Enhancement Drugs and the Athlete

Francesco Botrè[a,b,*], Antonio Pavan, MD[c]

KEYWORDS

- Enhancement drugs • Athletic competition
- Doping substances • Athletic regulations

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.

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

This article originally appeared in *Neurologic Clinics*, Volume 26, Issue 1.
This work was supported in part by Grants from the Italian Department of Health ("Commissione per la Vigilanza sul Doping e la Tutela Sanitaria delle Attività Sportive").
[a] Laboratorio Antidoping, Federazione Medico Sportiva Italiana, Largo Giulio Onesti 1, 00197 Rome RM, Italy
[b] Dipartimento per le Tecnologie, le Risorse e lo Sviluppo, "Sapienza" Università di Roma, Via del Castro Laurenziano 9, 00161 Rome RM, Italy
[c] Dipartimento di Medicina Sperimentale, "Sapienza" Università di Roma and Servizio di Immunoematologia e Medicina Trasfusionale, Azienda Ospedaliera Sant' Andrea, Via di Grottarossa, 1035–1039, 00189 Rome RM, Italy
* Corresponding author. Laboratorio Antidoping, Federazione Medico Sportiva Italiana, Largo Giulio Onesti 1, 00197 Rome RM, Italy.
E-mail address: francesco.botre@uniroma1.it (F. Botrè).

Phys Med Rehabil Clin N Am 20 (2009) 133–148
doi:10.1016/j.pmr.2008.10.010 **pmr.theclinics.com**
1047-9651/08/$ – see front matter

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 multisport, 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).
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.

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, androsteonedione, 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. β_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, α-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. β-Blockers

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. Accessed October 31, 2007.

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). 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 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

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)

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.

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.	A broad variety of effects (exhaustively reviewed in Ref. [30]), including, but not limited, to the following: Cardiovascular: hypertension, elevated risk of brain hemorrhages, myocardiac damage Hepatic: abnormal liver functions, cholestasis, development of androgen-dependent adenomas, depletion of high-density lipoprotein production Skeletal: water retention Dermal: seborrhea (steroid acne), oily skin, folliculitis, furunculosis Behavioral: increase of aggressiveness (aggressive psychoses), change in the libido, mood swings (euphoria followed by depression), mental disorders, headaches, dependence, or addiction Specific effects for men: testicular atrophy, altered spermatogenesis, prostate hypertrophy, gynecomastia 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)
Other anabolic agents	Same as previously.	For the side effects of clenbuterol, see "S3. β_2-Agonists" Side effects of selective androgen receptor modulators still are under evaluation (these drugs are not yet marketed)

S2. Hormones and related substances		Risk common to all peptide hormones: immunogenicity
Human growth hormone, insulin-like growth factors	Anabolic effect	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
Recombinant erythropoietins	Increased production of red blood cells and hemoglobin, resulting in an augmented efficacy of the transport of oxygen to the muscle	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
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

(*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
S3. β_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
S4. Hormone antagonists and modulators Aromatase inhibitors (eg, anastrozole, letrozole, aminoglutethimide, exemestane, formestane, and testolactone)	To increase the production or decrease the biotransformation of endogenous AAS	At therapeutic doses: nonspecific toxic side effects, including (but not limited to) asthenia, headache, nausea, peripheral edema, fatigue, vomiting, and dyspepsia Long-term endocrinologic side effects can be severe if administered in sequence or in combination with tamoxifen or selective estrogen receptor modulators
Selective estrogen receptor modulators (eg, raloxifene, tamoxifen, and toremifene)	Same as previously	Hot flashes, flu-like syndrome, joint pain, rhinitis Blood clots, including deep vein thrombosis, and pulmonary embolus (rare)
Other antiestrogenic substances (eg, clomiphene, cyclofenil, and fulvestrant)	Same as previously	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
Agents modifying myostatin functions	To improve muscle growth by interfering with the action of myostatin.	Unknown: myostatin inhibitors never have been tested in human trials

S5. Diuretics and other masking agents Diuretics	1. To obtain a rapid and reversible reduction of the total body mass, an evident potential advantage in sports where weight categories are involved 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	Hypotension Kidney dysfunction, dehydration (risk for central volume depletion), salt and water imbalance, electrolyte dispairement (eg, hyperosmolality, hyponatremia, hypokalemia, hypomagnesemia, hypocalcemia), muscle cramps Dizziness or lightheadedness, gastric effects, rash, impotence, secondary gout
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
α-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	Increased alertness	Increased alertness
Including, but not limited to	Improvement in coordination	Insomnia, anxiety

(*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
Central nervous system stimulants	Increased strength and endurance, as a consequence of a decreased perception of pain and fatigue	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 Effects on the skeletal muscle (rhabdomyolysis).
S7. Narcotics	Increased tolerance to pain and fatigue Transient reduction of tremor in precision events	Addiction (also as gateway to other drugs), tolerance, physical and psychologic dependence Increased pain threshold Euphoria Excitement, psychologic 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 Effects on the skeletal muscle (rhabdomyolysis)
S8. Cannabinoids	To relieve precompetition tension Social drugs: motivation for their use or abuse may be different from the illicit enhancement of sport performance	Drug dependence Psychomotor changes Antimotivational syndrome (loss of ambition)

S9. Glucocorticosteroids	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) Anti-inflammatory and analgesic properties, accompanied by euphoria Effects on the immune system	Acute: Hyperglycemia Fluid retention Mood alteration Chronic: Immunosuppression Suppression of the hypothalamic-pituitary-adrenal axis Musculoskeletal problems, also due to alteration of calcium metabolism and bone homeostasis Nonspecific effects (cataracts, diabetes mellitus, hypertension, peptic ulcer disease, weight gain, skin thinning, ecchymoses, striae, acne, hirsutism, fat redistribution, and various psychiatric disorders)
P2. β-Blockers	To reduce tremor, which gives a competitive advantage in specific sports/disciplines (eg, shooting, archery, curling, gymnastics)	Cardiovascular effects: bradycardia, cold extremities, postural hypotension, leg pain Central nervous system/neuromuscular effects: reversible mental depression progressing to catatonia, emotional lability, dizziness, vertigo, tiredness, fatigue, lethargy, drowsiness, depression, insomnia Hematologic effects: agranulocytosis Allergic: fever, sore throat, laryngospasm, respiratory distress Gastrointestinal: mesenteric arterial thrombosis, ischemic colitis, diarrhea, nausea Respiratory effects: wheeziness, dyspnea Other effects: impotency, hypoglycemia

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 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.

REFERENCES

1. Zerbini M. Alle Fonti del Doping. Fortune e prospettive di un tema storico-religioso. Roma (Italy): "L'Erma" di Bretschneider; 2001 [in Italian].
2. Christopoulos GA, editor. The Olympic games in ancient Greece. Athens (Greece): Ekdotike Hellados S.A.; 2003.
3. Yalouris N. Origin and history of the games. In: Christopoulos GA, editor. The Olympic games in ancient Greece. Athens (Greece): Ekdotike Hellados S.A.; 2003. p. 88–93.
4. Kyrkos B. The development of sport in Hellenistic and Roman Periods. In: Christopoulos GA, editor. The Olympic games in Ancient Greece. Athens (Greece): Ekdotike Hellados S.A.; 2003. p. 289–300.
5. Wagner JC. Enhancement of athletic performance with drugs. An overview. Sports Med 1991;12:250–65.
6. Le Mondenard JP. Dopage aux jeux olympiques. Condé-Sur-Noireau (France): Editions Amphora SA; 1996 [in French].
7. Guezennec CY. Doping: effectiveness, consequences, prevention. Ann Endocrinol (Paris) 2001;62:33–41.
8. The World Anti-Doping Code. World anti-doping agency. Montreal (Canada). 2003. Available at: www.wada-ama.org. Accessed October 31, 2007.
9. The World Anti-Doping Code. The 2008 prohibited list international standard. World Anti-doping Agency. Montreal (Canada); 2007. Available at: www.wada-ama.org. Accessed October 31, 2007.
10. Botrè F. Drugs of abuse and abuse of drugs in sportsmen: the role of in vitro methods to study effect and mechanism. Toxicol In Vitro 2003;17:509–13.
11. Donohoe T, Johnson N. Foul play? Drug abuse in sport. Oxford (United Kingdom): Blackwell; 1986.
12. Wadler GI, Hainline B. Drugs and the athlete. Philadelphia: FA Davis Company; 1989.
13. Cowan DA, Kicman AT. Doping in sport: misuse, analytical tests, and legal aspects. Clin Chem 1997;43:1110–3.
14. Segura J. Sports. In: Karch SB, editor. Drug abuse handbook. Boca Raton (USA): CRC Press; 1998. p. 641–726.
15. Peters C, Schulz T, Michna H, editors. Biochemical side effects of doping. Köln (Germany): Sport & Büch Strauss; 2001.
16. Botrè F. Classi di sostanze e metodi doping. Schede riassuntive. In: Il CONI contro il doping. 2nd edition. Rome (Italy): National Italian Olympic Committee; 2001. p. 19–37 [in Italian].
17. Menthéour E, Blanchard C. Secret Défonce. Ma vérité sur le dopage. Paris: J-C Lattès; 1999 [in French].

18. Catlin DH, Ahrens BD, Kucherova Y. Detection of norbolethone, an anabolic steroid never marketed, in athletes' urine. Rapid Commun Mass Spectrom 2002;16:1273–5.
19. Catlin DH, Sekera MH, Ahrens BD, et al. Tetrahydrogestrinone: discovery, synthesis, and detection in urine. Rapid Commun Mass Spectrom 2004;18:1245–9.
20. Sekera MH, Ahrens BD, Chang YC, et al. Another designer steroid: discovery, synthesis, and detection of 'madol' in urine. Rapid Commun Mass Spectrom 2005;19:781–4.
21. Malvey TC, Armsey TD II. Tetrahydrogestrinone: the discovery of a designer steroid. Curr Sports Med Rep 2005;4:227–30.
22. Georgakopoulos C, Vonaparti A, Stamou M, et al. Preventive doping control analysis: liquid and gas chromatography time-of-flight mass spectrometry for detection of designer steroids. Rapid Commun Mass Spectrom 2007;21: 2439–46.
23. Thevis M, Geyer H, Mareck U, et al. Screening for unknown synthetic steroids in human urine by liquid chromatography-tandem mass spectrometry. J Mass Spectrom 2005;40:955–62.
24. Mazzarino M, Turi S, Botrè F. A screening method for the detection of synthetic glucocorticoids in human urine by liquid chromatography—mass spectrometry based on class characteristic fragmentation pathways. Anal Bioanal Chem, in press.
25. Botrè F. I controlli antidoping nel terzo millennio: mai più sostanze 'invisibili'? Med Sport 2007;60:119–31 [in Italian].
26. Botrè F, Tranquilli C. Uso e diffusione degli integratori in Italia: opinioni a confronto. Med Sport 2001;54:263–74 [in Italian].
27. Caprino L, Braganò MC, Botrè F. Gli integratori fitoterapici nello sport: uso ed abuso. Ann Ist Super Sanita 2005;41:35–8 [in Italian].
28. Pipe A, Ayotte C. Nutritional supplements and doping. Clin J Sport Med 2002;12: 245–9.
29. Geyer H, Parr MK, Mareck U, et al. Analysis of non-hormonal nutritional supplements for anabolic-androgenic steroids—results of an international study. Int J Sports Med 2004;25:124–9.
30. Spitzer G. Doping with children. In: Peters C, Schulz T, Michna H, editors. Biochemical side effects of doping. Köln (Germany): Sport & Büch Strauss; 2001. p. 127–39.