From the Anti-Doping Library

THE HISTORY OF DOPING AND ANTIDOPING

A systematic collection of published scientific literature 2000-2015

Åke Andrén-Sandberg

From the Department of Surgery, Karolinska Institutet at Karolinska University Hospital, Huddinge, S-141 86 Stockholm, Sweden
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Semantics

Throughout history, athletes have sought to improve their performance through dietary and medical help. Doping is the use of banned pharmacological substances or methods with the unique aim of improving physical performance, sometimes even to the price of serious adverse effects on health. Doping is as old as sport itself. The word itself is likely derived from the Dutch word, dop, the name of an alcoholic beverage made from grape skins and used by Zulu warriors to enhance their prowess in battle – however, first of all a drink used as a stimulant in South African ceremonial dances. The term “doping” progressed into mainstream use in the early twentieth century, originally referring to drugging of racehorses.

However, what substances and methods that should be regarded as "doping" is still a matter of opinion, and it must be understood that the definitions of doping is dependent on time and who make the proposal. Moreover, there are differences regarding definitions also depending on in which situation doping is discussed:

- The international sports federations relies on WADAs list which is updated yearly
- National laws which is different in different countries and usually only concerns hormones (amphetamines etc are dealt with by other laws) and are updated with many years delay
- The public, including the mass media, which is very moralistic and nationalistic and swings from one opinion to the other with little time delay

In this booklet: doping = substances and methods on the WADA list

In the 1980s, Jean D Wilson citing the singularity of androgen receptor, suggested that androgenic and anabolic activity of androgens could not be dissociated. Therefore, he and others have argued that the term androgenic-anabolic steroid is a misnomer and should be abandoned.

By laymen the use of androgenic anabolic steroids are usually shortened to “steroids”, which for the fundamentalistic chemist of course is very incorrect. In this booklet it is used the middle way, which means “anabolic steroids or AS (sometimes AAS)” – right or wrong, that is what is commonly use in the anti-doping vocabulary.

Ancient Greek doping

The practice of enhancing performance through foreign substances or other artificial means is as old as competitive sport itself. As early as BC 776, the Greek Olympians were reported to use substances such as dried figs, mushrooms, and strychnine to perform better.

19th and 20th centuries

The modern era of doping dates to the early 1900s, with the illegal drugging of racehorses. Alfons Bukowski (1858-1921) is commonly regarded as the pioneer of anti-doping research. In 1910, he developed a method to detect alkaloids in horse saliva. One hundred years later, this is a good moment to remember Bukowski, an outstanding Polish pharmacist, often mistakenly represented in world literature as a Russian chemist. It is also an occasion to mention that the real driving force in the history of doping were events related to horse rivalry.
In modern times, there is a correlation between the discovery of a drug and its use in sports. During the end of the 19th Century and the beginning of the 20th Century, new illicit substances were incorporated into sport. In 1896, Arthur Linton, a Welsh cyclist, died under the influence of stress and speed ball (cocaine + heroin) during the Tour de France. In 1904, and he has the dubious distinction of being the first known person to die from such drug abuse. The first documented case of drug use in the modern Olympic Games took place when Thomas Hicks, a marathoner, nearly died from a mixture of brandy and strychnine.

With the advent of modern pharmacology in the early 20th century, many athletes began to experiment with cocktails of drugs to improve strength and overcome fatigue. As this practice was not illegal, there are good records of the lengths athletes would go to in order to win. Alongside the benefits, came the dangers and following several fatalities, a need for a code to ban performance enhancing drugs was gradually understood.

In the modern era, its practice mostly continued with the use of stimulants and narcotics. Sports federations took notice and in 1928 the International Association of Athletics Federations (IAAF) became the first federation to prohibit the use of performance-enhancing drugs (PEDs), although there would be no testing in sport for another 40 years.

Anti-doping efforts started in earnest after the 1960 Olympic Games in Rome. During a biking team time trial, 23-year-old Danish cyclist Knud Enemark Jensen collapsed, fractured his skull and died. An autopsy reportedly found traces of amphetamine and a blood-vessel dilator nicotinyl tartrate in his system. Although the drugs might not have caused his death, the episode forced cycling officials to take a closer look at doping. The UCI banned some performance enhancers.

Thus, amphetamine use was involved in the deaths of cyclists Knud Jensen and Tommy Simpson in the 1960 Olympic Games and the 1967 Tour de France respectively: this spurred the development of the International Olympic Commissions (IOC) Medical Commission, which published the first IOC Prohibited List in 1967. This became the de facto Prohibited List for Olympic Sport Federations [020]. In 1960, during the Rome Olympics, for the first time, the International Olympic Committee Medical Commission instituted drug testing for athletes. However, it was only in 1968 that the IOC officially took control of testing for the use of certain doping methods and substances. When IOC began doping enforcement during the 1968 Winter Olympic Games in Grenoble, France, the limitations were immense due to the poor methods of analysis, so only a few stimulants and narcotics were detected [013]. However, already in 1966 the International Cycling Union and the Fédération Internationale de Football Association (FIFA) were among the first international sports federations to introduce doping tests in their respective world championships.

The 1972 Olympic Games in Munich, Germany, ushered in testing for stimulants, but athletes had then already started to take anabolic steroids. A test for steroids arrived at the next summer Olympics, in Montreal, Canada. But four years later, at the Moscow Olympiad, athletes had moved on to less easily detectable, naturally occurring, hormones, such as testosterone. Anti-doping authorities later on learned to measure the ratio of testosterone in the blood to a related molecule called epitestosterone. In response, some athletes have reportedly found ways of regulating epitestosterone to keep the ratio in check.

Better testing for anabolic steroids were implemented in 1976 Montreal Olympic Games through radioimmunoassay analysis: the technique, however, still only allowed for unspecific detection of a limited number of exogenous steroids. Over the years, always new doping substances are synthesized and, as a consequence, the list of prohibited compounds is continuously updated and new suitable analytical methods for their detection and determination in biological matrices are continuously required. In doping control analysis the knowledge of steroid metabolism pathway in human body is of primary importance and the
analytical methods must permit the simultaneous detection and determination not only of the forbidden precursor agents but also of their metabolites. In addition, the potential presence and amount in the biological samples of species that can interfere in the analysis should be evaluated. Also the several anabolic steroids, specifically designed to circumvent doping control, put on the market have been incorporated in the list of the prohibited substances of the World Anti-Doping Agency (WADA). In WADA list steroids figure in three main classes, namely anabolic steroids, corticosteroids and substances with anti-estrogenic properties. It must also be reminded that the WADA prohibited list establishes criteria to highlight the alteration of the natural steroid profile caused by exogenous administration.

**The World Anti-Doping Agency (WADA)**

In 1967, the International Olympic Committee (IOC) established a Medical Commission responsible for developing a list of prohibited substances and methods. Drug tests were first introduced at the Olympic winter games in Grenoble and at the summer games in Mexico City in 1968. In February 1999, the IOC convened the World Conference on Doping in Sport in Lausanne, Switzerland. The Lausanne Declaration on Doping in Sport recommended creation of an International Anti-Doping Agency. The World Anti-Doping Agency (WADA) was formed in Lausanne, Switzerland on the basis of equal representation from the Olympic movement and public authorities (international sport federations and most of the governements in the world). One of the mandates of WADA was to harmonize the Olympic antidoping code and develop a single code applicable and acceptable for all stakeholders. The world antidoping code developed by WADA included creation of several international standards (IS). The purpose of each IS was harmonization among antidoping organizations. The ISs were developed for laboratories, testing, the prohibited list, and for therapeutic use exemptions (TUE). The objective of one manuscript was to present a brief history of doping in sport and describe creation of WADA in 1999. The components of the World Anti-Doping code (in particular, the Therapeutic Use Exclusion program or TUE) is described. The WADA code defines a TUE as "permission to use, for therapeutic purposes, a drug or drugs which are otherwise prohibited in sporting competition." Experiences of the Canadian Centre for Ethics in Sport Doping Control Review Board are presented because this national TUE committee has been operational for over 12 years. The challenge of developing a rigorous global antidoping program requires acceptance of doping as a problem by sport organizations, athletes, and public authorities. Individual stakeholders must be prepared to preserve the values of sport, which means free from doping. This will require vigilance by all interested parties for the benefit of elite athletes and society overall.

WADA's success in establishing an international drug code has been underpinned by three developments. First, WADA is funded jointly by the IOC and a group of national governments. This has provided the agency with both capital and influence. Secondly, WADA has secured a series of international declarations that have commended and ratified the policy code it has developed. Thirdly, WADA policy has recently been approved by the United Nations Educational, Scientific and Cultural Organisation (UNESCO) as an international convention. The UNESCO convention is the first legally binding international framework setting out the responsibilities of national governments and is currently signed either as ratification, acceptance, approval or accession by all major countries. These achievements have consolidated WADA's position as the central international agency for regulating drug use in sport.

**Two Nobel prizes**

The Austrian physiologist Oskar Zoth (1864-1933) was the first person to propose injecting athletes with a hormonal substance, as published in his 1896 paper describing how the use of an “extract” improved muscular strength and the “neuromuscular apparatus,” thus
potentially improving athletic performance. He and his physician partner, Fritz Pregl (1869-1930), self-injected testosterone extracts from bulls and measured the strength of their middle fingers by plotting them on “fatigue curves”. They recognized that testicular extracts could improve physical and mental energy, as well as muscle strength and won the Nobel Prize in chemistry in 1923.

It was not until 1929, when a German chemist and professor, Adolf Butenandt (1903-1995), isolated the first sex hormone, that a new path of discovery was initiated. He isolated estrone from the urine of pregnant women and later isolated 15 mg of androsterone (“andro” = male, “ster” = sterol, “one” = ketone) from 15,000 L of urine from local policemen. Butenandt found evidence of a “bloodstream substance” from roosters that affected their appearance and behavior. He postulated internal secretion from his testicular transplantation experiments. His theory was correct, but it was not widely accepted by his contemporaries. He was awarded the Nobel Prize in chemistry in 1939 for his “work on sex hormones” (together with Leopold Ruzicka).

Charles-Edouard Brown-Séquard

Following clinical observations, testicular preparations were not used for therapy until popularized by self-experiments by Charles-Edouard Brown-Séquard (1817-1894) – “the father of andrology” in Paris (1889), which – as we nowadays knows – at best have had placebo effects. Thus, almost half a century before the discovery of androgens, Brown-Séquard recognized that the contents of testicular extracts could improve libido, energy, and muscle strength. Brown-Séquard was a prominent French physiologist and Harvard professor. He had a strong interest in endocrinology, and he studied adrenal glands, testes, thyroid, pancreas, liver, spleen, and kidneys. He is probably most famous for his autoexperimentation with testicular substances (extracted from guinea pigs and dogs), the results of which were published in the Lancet. He gave himself 1 mL injections of a mixture of one part testicular vein blood, one part semen and one part juice extracted from dog or guinea-pig testes daily, and after 20 days made astonishing observations on himself: “A radical change took place in me. I had regained at least all the strength I possessed a good many years ago. I was able to make experiments for several hours. After dinner I was able to write a paper on a difficult subject. My limbs, tested with a dynamometer, gained 6 to 7 kg in strength. The jet of urine and the power of defecation became stronger”.

He thus reported increased strength, mental abilities, and appetite and even claimed that the process relieved constipation and increased the arc of his urine stream. Although no one is sure why he experienced these effects, his experiment caused others to investigate the testicular substance as a possible cure for various ailments, such as diabetes, tuberculosis, epilepsy, paralysis, gangrene, anemia, influenza, arteriosclerosis, Addison’s disease, hysteria, and migraine headaches. He encouraged testing of his testosterone products by providing free samples to physicians. Unfortunately, with such widespread use, shoddy researchers subjected animals and humans alike to high risks for infection and inflammation.

Even though what was reported was placebo effects, the world had obviously waited for such quackery, because in no time the “extracts of animal organs by the Brown-Séquard method” were sold all over the (Western) world and factories sprang forth in Europe as well as in America, for example, next to Central Park in New York. There must have been a real craze for these products and physicians concerned about the image of the young field of endocrinology started worrying. The famous neurosurgeon Harvey W Cushing (1869–1939) and the president of the Association of the Study of Internal Secretions, Edward H. Rynearson even talked about “endocriminology” in the context of this organotherapy. This assessment of the medical scene at the time is also reflected in contemporary cartoons and comic songs from the early twentieth century. Eventually, this type of quackery stimulated science and decent pharmaceutical companies to search for real hormones.
Anabolic steroids

The evolution of the history of testosterone therapies is thus as interesting as the history of its development. Erectile dysfunction is one of the most researched ailments treated with testosterone, although any positive effects are questionable. In men with absent to low circulating levels of testosterone, treatment with testosterone increased libido, improved erectile function, and helped to maintain secondary sexual characteristics. In men with normal or mild hypotestosteronemia, studies have not shown consistent response to therapy. Those treated were reported to have increased sexual interest, increased arousal, increased frequency of intercourse, and nocturnal erections. In the early twentieth century, there was much interest in the hormonal influence of testosterone on sexuality and sexual preferences. It even was prescribed to “treat” homosexuals because it was theorized that male homosexuals had higher estrogen level.

Testosterone has even played an important role in various ailments affecting women, such as treatment for some metastatic breast cancers. Approximately one third of breast cancers are hormone dependent and respond to androgen therapies. Other uses for testosterone are as postmenopausal hormone replacement therapy, for sexual dysfunction (by increasing libido), and for increasing bone density. Some clinical case studies showed an increase in appetite, lean muscle mass, and strength and an improved overall sense of well-being. Before the use of erythropoietin and bone marrow transplants, testosterone was used to help treat anemia (i.e. chronic renal failure/hemodialysis). Psychiatrists prescribed anabolic steroids from the 1930s to the 1980s to treat psychoses, depression, and melancholia. Testosterone has been used as an adjunct in people with growth hormone deficiency or in boys with pubertal delay.

The first report concerning the use of anabolic steroids by an athlete who searched for increased weight and power dates 1954. A reliable test method to detect anabolic steroids was finally introduced in 1974 and the IOC added anabolic steroids to its list of prohibited substances in 1976. This resulted in a marked increase in the number of drug disqualifications in the late 1970s, notably in strength related sports such as throwing events and weightlifting.

Anabolic-androgenic steroids (AAS) are now the most common illicit drugs used to enhance performance at the modern Olympic Games along with stimulants, primarily by weight lifters and athletes in track-and-field. The AASs are a group of synthetic derivatives of testosterone with both skeletal muscle building (anabolic) and masculinizing (androgenic) effects. In 1889, physiologist Charles E. Brown Sequard reported improvement in a variety of his body functions (strength, intellect, and force of urine stream) following the injection of an extract of testicles from the dog and guinea-pig. The primary natural male hormone, testosterone was first isolated from the testis of bulls in1935. Butenandt and Hanisch and Ruzicka independently synthesized testosterone in the same year, and the chemists received the Nobel Prize in 1939 for their work. Most of the AASs were developed during the1950s when chemists attempted unsuccessfully to separate the anabolic and androgenic properties of these testosterone derivatives. Nandrolone, the19-nor analog of testosterone was the first anabolic steroid with sufficient dissociation of androgenic and anabolic properties to justify introduction into clinical practice during the 1950s. Dr John Ziegler, an American physician-weight lifter, administered AASs to three future American weight lifting champions after learning of the success of AAS-using Russian weight lifters at the 1954 World Championships. In 1958, the US Food and Drug Administration (FDA) approved the use of methandrostenolone (Dianabol) for the treatment of hypogonadism, resulting in the increased availability of this steroid. By the mid-1960s, the use of AASs to enhance performance in sports spread, particularly among weight lifters and other strength athletes. An estimate done was that a third of the US track-and-field athletes in the 1968 pre-Olympic training camp
were using AAS.

Anabolic steroids remain a mainstay in the performance enhancement drug arena given that they are really the only major class of steroids that are unequivocally anabolic with salutary effects on athletic performance. The arms race will continue as long as designer steroids are produced, tested in vitro, and then, for the more difficult parameter, that they and their metabolites will not lead to a positive test at the doping control laboratory.

**Development of new testosterone preparations**

Soon after its synthesis testosterone became clinically available, first in the form of pellets and then as injectable esters, that is, testosterone propionate with a short half-life and, from the mid-1950s on, the longer-acting testosterone enanthate appeared, which remained the major testosterone preparation for half a century. Also in 1935, 17alpha-methyl-testosterone was synthesized and its oral effectiveness was demonstrated.

Testosterone propionate (Testoviron), the prototype of the anabolic steroids was synthesized already in 1936 and appeared in sport sometime after the 1948 Olympic Games. The subsequent synthesis of methandrostenolone (Dianabol) in the United States in 1958 and oral chlordehydromethyl-testosterone (Turinabol) in East Germany after 1966 marked the beginning of the “virilization” of modern sport.

However, due to its 17alpha-structure it turned out to be liver toxic, a fact that gave testosterone in general a bad name among physicians, as this toxicity was also suspected for testosterone without reason; eventually in the 1980s this androgen became obsolete for clinical use in Europe. In the late 1970s the orally effective testosterone undecanoate, absorbed from the gut via the lymph to avoid the first-pass effect in the liver, was added to the spectrum of testosterone preparations used clinically.

However, testosterone was soon studied using different forms. Scientists quickly learned it was ineffective, and even toxic (like 17 alpha-methyl testosterone), when taken orally; instead, it was synthesized into tiny pellets that were inserted subcutaneously. Longer-acting injectable forms of testosterone were synthesized in the 1950s (i.e. testosterone enanthate). Over the following decade, the hormone was modified into derivatives that possessed more anabolic qualities. In the 1970s, oral testosterone undecanoate was synthesized; however, it did not fare well in the oral form because of hepatic clearance and hepatotoxicity. Transdermal scrotal patches were derived in the 1990s. These allowed physiologic levels of testosterone to be acquired. Nonscrotal skin patches were later developed, and testosterone gels were marketed. Today, there are short-acting buccal forms as well as the long-acting injectable testosterone undecanoate today.

In the 1950s and 1960s, the pharmaceutical industry became more interested in new androgens than in testosterone itself and concentrated its androgen research on the chemical modification of steroid molecules in order to disentangle the various effects of testosterone and produce predominantly erythropoietic or anabolic steroids. In 1956, contemporary textbooks on androgens had already described 256 androgenic steroids and by 1976 the number had increased to more than 1000.

However, it proved impossible to produce androgens with only one effect out of the spectrum of testosterone activities; at best, one of these effects could be emphasized, but the other effects remained. The steroid with pure anabolic effects on muscles or bones to treat cachexia, osteoporosis or small stature, or pure erythropoietic effect for the treatment of anemia without androgenisation could not be found. Nevertheless, anabolic and similar steroids were clinically used, but disappeared again in the wake of evidence-based medicine.
However, they continued their existence for illegal use and abuse for doping in sports and bodybuilding potentially causing considerable undesired effects. Regrettably, at that time the pharmaceutical industry neglected the chance to develop testosterone preparations better suited for the substitution of hypogonadal patients than the existing testosterone esters. It remains to be seen whether the current search for SARMs will take a more rewarding course than did anabolic steroids.

From the 1970s, the newly developed testosterone immunoassays made serial testosterone determinations in blood possible and, when applied to pharmacokinetic studies, it turned out that all available testosterone preparations resulted in unphysiologically high or low serum levels, which were undesirable in substitution therapy. Clinicians assembled at a workshop on androgen therapy sponsored by WHO, NIH and FDA in 1990 came to the conclusion: “The consensus view was that the major goal of therapy is to replace testosterone levels as close to physiologic concentrations as is possible” and demanded that new testosterone preparations better suited for clinical use be manufactured.

In the mid-1990s, transdermal testosterone patches applied to the scrotal skin became the first transdermal testosterone preparation in clinical use. They had been invented by Virgil Place at ALZA in Palo Alto, a company specializing in new forms of delivery of known drugs. However, although clinical results with this preparation were excellent and for the first time physiological serum levels could be achieved under testosterone substitution, physicians were reluctant to prescribe a medication to be applied to the scrotum and preferred a subsequently developed nonscrotal system. This, however, caused unpleasant skin reactions as it required an enhancer to drive testosterone through the skin. For this reason, the advent of the first transdermal testosterone gel was welcome. This gel became available in 2000 for the treatment of male hypogonadism, first in the US and later also in other countries. Since then, several other gels have been developed and brought to the market, differing slightly in composition and concentrations. The one with the highest testosterone concentration (2.5% Testotop®) has also been tested for scrotal application and because of the high absorptive capacity of the scrotal skin only 20 percent of the gel needed for nonscrotal application is required, making this form of application economically and ecologically more desirable.

Bob Hoffman and the Soviet connection

In 1923 Bob Hoffman formed the York Barbell Company in the United States. A dominant figure in US weightlifting, he published the “Strength and Health magazine” and sold health and food supplements in his gym. As a weightlifting coach, his success led to him being named the head coach of the US Olympic weightlifting team. In the 1950s, Russian weightlifters began to outpace American Olympians through performance-enhancing injections. At the 1954 World Championships in Vienna, he met with a Soviet colleague who told him of a synthetic form of testosterone developed by the Nazis which produced dramatic improvements in strength and power. Hoffman and his colleagues contacted Ciba Pharmaceuticals in pursuit of synthetic testosterone. Attempting to make up lost ground, the then US Olympic physician thus teamed with chemists to produce an anabolic steroid for the Americans, now known as Dianabol (methandrosteno lone). Dr. John B Zeigler began experimenting with Dianabol on weightlifters at the York Barbell Club in 1958. The weightlifters became strength and conditioning coaches in a variety of other sports in the United States and spread use of anabolic steroids to other sports, such as American football.

In the decades that followed, steroids and stimulants spread throughout sports, and in 1959, the first reported case of a high school football player’s taking steroids surfaced. Ciba had conducted a number of studies on the use of synthetic testosterone in pain patients and the physically disabled. This resulted in the development of danazol, which rapidly became a doping substance abused by weightlifters.
In the 1960s, the International Olympic Committee banned steroid use and began formal drug testing in the ensuing decade. During the 1980s, the reported positive test results ranged from 2 to 50 percent, depending on whether the tests were announced or conducted at random. Then, in 1994, an often-referenced survey was conducted by Goldman when aspiring Olympians were asked 2 simple questions. The first was, “If you were offered a banned performance-enhancing substance that guaranteed that you would win an Olympic medal and you could not be caught, would you take it?” Remarkably, 195 of 198 athletes said yes. The second was, “Would you take a banned performance-enhancing drug with a guarantee that you will not be caught, you will win every competition for the next 5 years, but will then die from adverse effects of the substance?” Still, more 50 percent of the athletes said yes. This survey made it clear that modern athletes often approach their sports with a “win at all costs” mentality.

German Democratic Republic

Anti-doping work was complicated in the 1970s and 1980s by suspicions of state-sponsored doping practised in some countries. It has later been extremely well documented the the German Democratic Republic (GDR) government administered doping program of its athletes, particularly its female athletes, contributed to their domination of track & field and swimming events for the two decades spanning the 1970s and 1980s.

From 1966 until the collapse of the German Democratic Republic in 1990, hundreds of East German physicians and scientists performed doping research and administered prescription drugs as well as unapproved experimental drug preparations to adult and adolescent athletes of both sexes. From the 1960s through the 1980s, the German Democratic Republic established a systematic doping program for thousands of their athletes that included the use of parenteral preparations of epitestosteronepropionate to avoid detection of illicit AASs.

Side effects

Athletes from former East Germany who were given performance enhancing drugs for many years and who consequently experienced longstanding health problems will receive payments of several thousand euros, the German federal parliament decided on 13 June 2002. A special law has been passed which sets up a compensation fund of about EUR 2m (GBP 1.3m; USD 1.9m). The fund is meant to be supplemented by the sports industry and by national sports associations, but neither of these groups has been keen to join the initiative. It is estimated that between 500 and 1000 men and women will apply for compensation by the end of the year and will receive about EUR 3000 each. Currently, the association representing athletes who have had health problems as a result of doping has about 150 members. Soon after the fall of the Berlin wall in 1989, it became apparent that many East German athletes had had to pay a high price for the overwhelming success of the nation in many disciplines. Continuous doping from a young age and for a very long time, mainly with anabolic drugs, ruined their health. Doping was often done without the athlete's consent or knowledge. East German trainers and doctors merely followed the socialist party's instructions. The list of health problems is long: acne, hirsutism, deep voice, muscle tension, gynaecomasty, breast cancer, bone deformation, vascular disease, and teratogenic malformations. In some cases female athletes changed their sex as a result of the continuous intake of male hormones. The association representing such athletes, as well as single athletes, is not satisfied with the new law, which will come into force in 2003.

From Ben Johnson to BALCO

The most famous doping case of the 1980s concerned Ben Johnson, the 100 meter runner
who tested positive for stanozolol (anabolic steroid) at the 1988 Olympic Games in Seoul. Johnson’s case focused the world’s attention to the doping problem to an unprecedented degree. In the same year, the distribution or possession of AASs with intent to distribute without a valid prescription became a felony, when US Federal Food, Drug, and Cosmetic Act (FFDCA) was amended as part of the Anti-Drug Abuse Act. In 1990, the Anabolic Steroids Control Act defined an AAS as any drug or hormonal substance chemically and pharmacologically related to testosterone (other than estrogens, progestins, and corticosteroids) that promotes muscle growth. These synthetic compounds became DEA schedule III drugs as defined by the US Controlled Substances Act. Later, this act was amended by the Anabolic Steroid Control Act of 2004; on January 20, 2005, the amended Controlled Substance Act added both anabolic steroids and prohormones to the list of controlled substances, making possession of the banned substances a federal crime. In response to continuing demand for illicit AASs, designer AAS appeared as a means to avoid detection of these illicit drugs. An example was the synthesis of tetrahydrogestrinone from the palladium-charcoal catalyzed hydrogenation of gestrinone by the Bay Area Laboratory Cooperative, BALCO), an American nutritional supplement company. However, analyses and legal action resulted in the banning of several athletes as a result of the use of these synthetic steroids. Subsequently, major league baseball revamped their AAS policy calling for a 50-game ban for first-time offenders (up from 10 days), a 100-game penalty for second-time offenders (up from 30 days), and a life time ban for a third positive test. Previously, a baseball player could be suspended for life only after the fifth positive test.

An ethical problem

Researchers seem to agree that doping is unwelcomed in sport. However, opinions are divided between doping being a serious deviance one must fight against and doping as undesirable but unavoidable consequence of the institutionalized sport. Notably, the reason behind banning doping initially was the growing concern about athletes’ health. Doping only became established as unethical after that point.

Not only athletes

The use of doping agents, once restricted to professional athletes, has nowadays become a problem of public health, since it also concerns young people and non-competing amateurs in different sports. The use is also diffused in social life for improving physical appearance and enhancing performance and even dietary supplements assumed to improve performance often contain anabolic steroids. While decades ago the so-called “classical doping agents” (like stimulants and narcotics) were used, to-day anabolic steroids are more widely diffused.

Testing for growth hormone

Growth hormone was added to the prohibited list in 1989. In 1998 at the Tour de France that French customs arrested Willy Voet, a physiotherapist of the Festina cycling team, for the illegal possession of needles, syringes and over 400 bottles containing erythropoietin, human growth hormones, steroids, amphetamines, narcotics and stimulants.

In the mid-1990s, the IOC and the European Commission co-funded a three-year international project called GH2000 to develop tests to detect growth hormone. The project, which concluded at the end of 1998, was led by Peter Sonksen, an endocrinologist at St Thomas’ Hospital in London. The GH2000 consortium delivered its report to the IOC in January 1999. It had developed a series of blood markers that could be used to test for
elevated hGH levels, including insulin-like growth factors and proteins that bind to them. Working independently of GH2000, researchers led by Christian Strasburger at the Ludwig-Maximilian University in Munich have developed a direct test for recombinant hGH. This relies on the fact that hGH exists in different molecular forms, the two major fractions of which have molecular masses of 22 kilodaltons and 20 kDa. Although only half of the body's own hGH is in the heavier form, for recombinant hGH the figure is 95 percent. The test uses antibodies to identify the two forms, and so allows any shift in the natural ratio to be spotted. Sonksen costed validation studies for the GH2000 and Munich tests at around US$5 million, and requested continued funding. The consortium also responded to a formal call for proposals for research issued by the IOC in August 1999, but was turned down. The German Sports Research Institute in Cologne then supported further work to validate the Munich test, drawing on some of the GH2000 samples. Strasburger hopes that the then newly created World Anti-Doping Agency (WADA), based in Lausanne, would follow through on statements that tackling hGH abuse will be its top priority, and provide the money needed to bring the test into general use.

**Blood doping**

Blood transfusions as a means for improved endurance were researched as early as 1947 by Pace and coworkers. Transfusion of 500 ml of allogeneic erythrocytes on four consecutive days reduced the pulse rate during exercise in simulated hypoxia. However, even though the methods would not meet today’s standards; this was the first of many studies to confirm the possible performance enhancing effect of blood transfusions. Accordingly blood transfusions in sports were later banned by the International Olympic Committee (IOC) in 1986.

A relatively straightforward way to increase the hemoglobin concentration (Hb), and, hence, oxygen delivery to the muscles is, thus, by blood transfusions. This was documented in the classic study by the Swedish sports physiologists Björn Ekblom and co-workers published in 1972 where a high correlation between hemoglobin and performance capacity after blood withdrawal and reinfusion was presented. An overnight increase in hemoglobin by 13 percent caused by the reinfusion of 3 units of stored autologous blood resulted in an increase in maximal oxygen uptake and physical performance capacity of 9 percent and 23 percent, respectively. The method of transfusing blood in a sport setting was hereafter dubbed “blood doping” by the media, and its potent effect on athletic performance was quickly noted in the sports community.

**Eorphropoietin**

For cycling and other endurance sports, human recombinant EPO, however, fuelled a doping revolution. EPO is a natural hormone that promotes production of oxygen-carrying red blood cells. The first synthetic, or recombinant, version was developed by the biotechnology company Amgen in Thousand Oaks, California, and in 1989 it was approved by the US Food and Drug Administration to treat anemia. It also offered cyclists an easy endurance boost that helped them to excel in grueling stage races. The drug is nearly identical to the hormone naturally churned out by the kidneys, so was impossible to detect. It is also easier to administer than blood transfusions, which had been used to the same effect. Typically, red blood cells account for 40-45 percent of the blood, but in the heyday of EPO doping, some riders were showing up at starting lines with hematocrits of more than 60 percent. The UCI instituted a “no-start” rule, disqualifying riders if their hematocrits on the morning of a race were above 50 percent for men and 47 percent for women. So cyclists began diluting their EPO-boosted blood with saline solution to keep their hematocrits below the threshold. The drug companies that produce EPO have, however, helped anti-doping laboratories to develop direct tests based on subtle biochemical differences between the recombinant molecules and the natural form. The first of these was approved for use in 2000. But athletes
increasingly obtain knock-off forms produced in China and India, and researchers have struggled to keep up.

**Amphetamines**

The amphetamines were the first “effective” performance-enhancing drugs, which were used widely by soldiers in the Second World War, crossed over into sports in the early 1950s. These drugs – nicknamed *la bomba* by Italian cyclists and *atoom* by Dutch cyclists – minimize the uncomfortable sensations of fatigue during exercise. By setting a safe upper limit to the body’s performance at peak exertion, these unpleasant sensations prevent bodily harm. The artificial manipulation of this limit by drugs places athletes at risk for uncontrolled overexertion. The first cases of fatal heatstroke in athletes using *atoom* were reported in the 1960s. In the 1967 Tour de France, elite British cyclist Tom Simpson died on the steep ascent of Mont Ventoux, allegedly because of amphetamine abuse. The precise extent to which amphetamines enhance athletic performance is unknown, since, as with all performance-enhancing drugs, there are few modern studies quantifying their effects. The convenient absence of such information represents further evidence of a hidden problem. A popular opinion is that *la bomba* can turn the usual Tour de France domestique, or support rider, into a stage winner. Since amphetamines must be present in the body to be effective, the sole method of avoiding the detection of their use during competition is to substitute a clean urine sample for the doped specimen. A multitude of innovative techniques have been developed to accomplish this swap. Cortisone, a potent but legal performance-enhancing drug used to dampen inflammation, also reduces the discomfort of heavy daily training and competition and lifts the mood. It is also widely abused by professional cyclists.

**Laboratory progress**

The 1976 radioimmunoassay analysis technique, however, only allowed for unspecific detection of a limited number of exogenous steroids. In 1983 the first endogenous steroid, testosterone, was added prohibited substances list. It was a further year before the detection method for testosterone was introduced.

The initial test for testosterone in urine was developed by Donike and coworkers, who showed that administered testosterone appeared in the urine as testosterone glucuronide. They also showed that for a population of athletes, the ratio of testosterone to epitestosterone (T/E) had a positively skewed distribution, with a modal ratio of about 1:1. Initially, an athlete sample having a T/E ratio > 6:1 was considered a doping violation. The concept of intraindividual reference ranges (as opposed to population-based reference ranges) was introduced into the T/E test in the early 1990s. Computer programs are now used to compare an athlete's current sample result to their previous sample results. Results that are inconsistent with previous results are investigated and could result in targeted testing or an antidoping rule violation. The measurement of $^{13}$C/$^{12}$C ratios in testosterone and its metabolites has allowed the differentiation of pharmaceutical testosterone from natural testosterone. Donike's group also began the concept of the urinary “steroid profile,” which used a combination of other urinary steroids to increase the sensitivity of the test. Other antidoping research has identified a del/del genotype of UGT2B17 as the cause of a subpopulation of individuals who have low (<0.5) T/E ratios in urine, the use of 11 steroids in urine to improve test sensitivity, new metabolites of testosterone (e.g. testosterone cysteinate) in the urine, and several substances that affect the metabolism and excretion of testosterone.

To measure testosterone in the urine to detect doping is not adequate because of large interindividual and intraindividual differences in urinary steroid concentration. However, the nearly constant ratio of urinary testosterone glucuronide to epitestosterone glucuronide
became the basis of a better test. Epitestosterone is the 17alpha epimer of testosterone and has no known physiological function. It is not a metabolite of testosterone [054]. An upper normal limit of six was calculated for the testosterone/epitestosterone ratio based upon population studies. In 1983 the Medical Commission of the International Olympic Committee (IOC) introduced this value as a criterion for testosterone abuse. Ratios above six should be considered suspicious, and the person concerned should be subjected to further testing. In 2004 the approved upper limit was set at four.

In 1984, testosterone was analyzed during the Olympic Games in Los Angeles, where art, technology, instrumentation, and skilled personnel were brought together to combat the use of doping substances.

**Background to T/E ratio**

It was reported that after oral, rectal, or intramuscular T administration, the excretion of TG increased more than other T metabolites. Epitestosterone (E) was found not to be a metabolite of T because deuterated T administration did not result in significant deuterated EG excretion. The origin of epitestosterone is still discussed. Although Dehennin showed that half of total E production is of testicular origin, the remaining 50 percent is still debated. Administration of adrenocorticotropic hormone (ACTH) results in an increased EG production, indicating an adrenal origin. Also, adrenal insufficiency as observed in Addison's disease correlates to significantly decreased T and E excretion rates. Also peripheral production is possible. The mean T/E ratio of urine samples of Caucasian males and females in the first population study of Donike et al was 1-2. The values showed a logarithmic normal distribution with an upper limit value lower than 6. Using these data, the Medical Commission of the International Olympic Committee (IOC) banned the use of T in 1982 and stated that a T/E ratio above 6 was sufficient proof of T abuse. When applying this criterion in research and routine analyses, cases of naturally occurring T/E ratios above 6 appeared. Dehennin et al administered testosterone enanthate in several doses intramuscularly to healthy men over a period of six months. They found via linear interpolation between doses that the T/E ratio exceeded the cutoff point of 6 when natural production (around 45 mg/week) was doubled by weekly administration of a comparable dose of exogenous T.

**A proxy for failing erythropoietin testing: Athlete biological passport (ABP)**

In the late 1990s, as a first step, “no start” rules were introduced with the official objective to protect the health of the athletes when certain blood markers exceeded definite limits (e.g. hematocrit (Hct) above 50 percent or hemoglobin (Hb) above 17 g/dL (International Cycling Union, UCI) or Hb above 17.5 g/dL in men and 16.0 g/dL in women (International Ski Federation, FIS). In this time, the widespread use of rhEPO can be assumed on the basis of indirect evidence; e.g. in elite cross-country skiers extreme Hb values up to 20 g/dL were common between 1994 to 1996 but disappeared after the “no start” rule was introduced in 1997. Yet, mean Hb values continued to rise, suggesting the further use of artificial methods with fewer extremes. It became obvious that the use of upper limits of definite blood values may result in athletes who would titrate rhEPO to approach the target Hb or Hct without exceeding it.

In an investigation of samples obtained as part of routine International Ski Federation blood-testing procedures in participants at the World Ski Championships, abnormal hematological profiles, defined as those deviating from the 1989 Nordic Ski World Championships and the IOC Erythropoietin 2000 project data set, were identified in 36 percent of the skiers tested and finishing within the top 50 places in the competitions. In addition, 50 percent of medal winners and 33 percent of those finishing from 4th to 10th place had highly abnormal hematological profiles. In contrast, only 3 percent of skiers finishing from 41st to 50th place
had highly abnormal values. Although these data cannot be immediately associated with blood doping practices, including blood transfusions, and it is very unlikely that blood doping would be less common in other endurance sports, the present situation is highly suggestive of a phenomenon that is not being controlled by the ongoing antidoping testing program. In fact, it has been hypothesized that a combination of blood transfusion and recombinant human erythropoietin administration could also be used by such athletes.

Serial analysis of biomarkers was already in practice by a number of federations with some programs predating the formation of the World Anti-Doping Agency (WADA) and the implementation of the World Anti-Doping Code. However after the 2006 Torino Winter Olympic Games, at the request of a number of International Federations (IFs), WADA formed an ad hoc Haematological Working Group to look at the issue of blood doping and to develop a harmonised longitudinal profiling programme that was both scientifically and legally robust. This resulted in the creation of the ABP Guidelines and Related Technical Documents which were first published in 2009. In 2011, WADA re-established a Haematological Expert Group to further refine and develop this module.

**Gene doping**

In 1997, Leiden et al used an adenovirus to deliver the EPO gene in mice and monkeys. This boosted the haematocrit from 49 to 81 percent in the mice and from 40 to 70 percent in the monkeys. The effects lasted for over a year in the mice and for approximately 12 weeks in the monkeys.

The International Olympic Committee (IOC) in 2002 released its new list of banned substances and methods. The list was effective from 1 January 2003 and replaces the 1 September 2001 list. Amongst the important changes, the category of genetic doping as a banned method is listed for the first time. At the 1964 Winter Olympics in Innsbruck, a Finnish competitor Eero Mäntyranta, won two gold medals in cross country skiing. Though his training programme wasn’t radically different from his rivals, Mäntyranta had a distinct advantage. He was born with a genetic mutation that increased the oxygen carrying capacity his red blood cells by 25-50 percent. Mäntyranta had a mutation in the gene coding for the erythropoeitin (EPO) receptor which prevented the normal feedback control of red blood cell mass.

Gene therapist Ted Friedmann and multiple Olympic gold medallist Johann-Olav Koss were the first to describe the possibility of misusing the techniques and experiences of gene therapy in the athletic arena. In 2006, before the Turin Winter Olympic games, the president of the World Anti-Doping Agency (WADA), Dick Pound, called gene doping “the new threat that is now a reality.” Although Pound did not expect gene doping to pose a problem in Turin, he indicated that it could be a problem at the Summer Games, 2 years hence in Beijing. In fact, the problem did not materialize in China, in 2008, nor at the London 2012 Olympics, as far as the then available detection measures could determine.

**Influence on world records in running by doping and anti-doping testing**

Improvements in track and field sports have been attributed to factors such as population increase, drugs and new technologies, but previous research has found it difficult to distinguish the contributions from specific influences. Here it is shown how this is possible by means of a performance improvement index based on useful work done combined with modelling of the annual top 25 performances. The index was set to 100 in 1948 and showed that, by 2012, it had increased in running events to between 110.5 and 146.7 (men’s 100 m and marathon). Underlying global effects accounted for the majority of all improvements (16.2 to 46.7) with smaller influences attributable to an influx of African runners (3.6 to 9.3),
and a 4-year oscillation that arose from staging of the Olympic Games (±0.2 to ±0.6). Performance decreased with the introduction of compulsory random drug testing (-0.9 to -3.9) the World Anti-Doping Agency (WADA; -0.5 to -2.5) and fully automated timing (-0.6 to -2.5). Changes in elite sporting performance since the 1890s are attributable to societal changes caused by the industrial revolution and globalisation superimposed on millennia of human evolution.
INTRODUCTION

Semantics

Throughout history, athletes have sought to improve their performance through dietary and medical help. Doping is the use of banned pharmacological substances or methods with the unique aim of improving physical performance, sometimes even to the price of serious adverse effects on health. Doping is as old as sport itself. The word itself is likely derived from the Dutch word, dop, the name of an alcoholic beverage made from grape skins and used by Zulu warriors to enhance their prowess in battle [001] – however, first of all a drink used as a stimulant in South African ceremonial dances [002]. The term “doping” progressed into mainstream use in the early twentieth century, originally referring to drugging of racehorses.

However, what substances and methods that should be regarded as "doping" is still a matter of opinion, and it must be understood that the definitions of doping is dependent on time and who make the proposal. Moreover, there are differences regarding definitions also depending on in which situation doping is discussed:

- The international sports federations relies on WADAs list which is updated yearly
- National laws which is different in different countries and usually only concerns hormones (amphetamine etc are dealt with by other laws) and are updated with many years delay
- The public, including the mass media, which is very moralistic and nationalistic and swings from one opinion to the other with little time delay

In this booklet: doping = substances and methods on the WADA list

In the 1980s, Jean D Wilson citing the singularity of androgen receptor, suggested that androgenic and anabolic activity of androgens could not be dissociated. Therefore, he and others have argued that the term androgenic-anabolic steroid is a misnomer and should be abandoned [003].

By laymen the use of androgenic anabolic steroids are usually shortened to “steroids”, which for the fundamentalistic chemist of course is very incorrect. In this booklet it is used the middle way, which means “anabolic steroids or AS (sometimes AAS)” – right or wrong, that is what is commonly use in the anti-doping vocabulary.

Overview of doping and anti-doping history

Background

Athletes have a long history of using substances in an attempt to gain an advantage in sporting competitions. The ancient Greeks and Romans used herbs, fungi, poppy seeds and stimulants such as strychnine in order to boost performance. In the modern era, this practice continued mostly with the use of stimulants and narcotics. Sports federations took notice and in 1928 the International Association of Athletics Federations (IAAF) became the first federation to prohibit the use of performance-enhancing drugs (PEDs), although there would be no testing in sport for another 40 years. Amphetamine use was involved in the deaths of cyclists Knud Jensen and Tommy Simpson in the 1960 Olympic Games and the 1967 Tour de France respectively: this spurred the development of the International Olympic Commissions (IOC) Medical Commission, which published the first IOC Prohibited List in 1967. This became the de facto Prohibited List for Olympic Sport Federations. The “Festina
affair” (1998 Tour de France), where a team trainer's car was found to contain a panoply of PEDs, was the catalyst to create a new organisation to harmonise, coordinate and promote the fight against doping in sport in all its forms. The IOC convened the first World Conference in Doping in Sport in 1999, which resulted in the formation of the World Anti-Doping Agency (WADA) [004].

**Fundamental aims of anti-doping**

Some of the leading sporting associations have stated that the fundamental aims of doping controls and anti-doping policies are to [005]:

- uphold and preserve the ethics of sport
- safeguard the physical health and mental integrity of the players
- ensure that all competitors have an equal chance.

However, the task may be thankless as the anti-doping agencies risk to thwart one cheating strategy, only for another to emerge [006].

**Ancient Greek doping**

The practice of enhancing performance through foreign substances or other artificial means is as old as competitive sport itself. As early as BC 776, the Greek Olympians were reported to use substances such as dried figs, mushrooms, and strychnine to perform better [007].

Though we may still sing today, as did Pindar in his eighth Olympian Victory Ode, “... of no contest greater than Olympia, Mother of Games, gold-wreathed Olympia...”, we must sadly admit that today, besides overt over-commercialization, there is no more ominous threat to the Olympic games than doping. Drug-use methods are steadily becoming more sophisticated and ever harder to detect, increasingly demanding the use of complex analytical procedures and networks, biotechnology and molecular medicine progress [008].

The foundation of amateur athletics is the Olympic triad:

- creed
- oath
- motto

The creed reads: “The most important thing in the Olympic Games is not to win but to take part, just as the most important thing in life is not the triumph but the struggle. The essential thing is not to have conquered but to have fought well.” In the 2000 Olympics, the reference to non-use of drugs was added to the Olympic oath. “In the name of all competitors, I promise we shall take part in these Olympic Games, respecting and abiding by the rules which govern them, committing ourselves to a sport without doping and without drugs, in the spirit of true sportsmanship, for the glory of sport and the honor of our teams.” Still, however, the Olympic motto stands as: “citius, altius, fortius” or “swifter, higher, stronger.” What is said about the Olympic Games in this context may be said of all other parts of sports as well [009].

**20th century**

With the advent of modern pharmacology in the early 20th century, many athletes began to experiment with cocktails of drugs to improve strength and overcome fatigue. As this practice was not illegal, there are good records of the lengths athletes would go in order to win. Alongside the benefits, came the dangers and following several fatalities, a need for a code
to ban performance enhancing drugs was gradually understood [010].

By the 1920s it had become evident that restrictions regarding drug use in sports were necessary. In 1928 the International Amateur Athletic Federation became the first international sport federation to ban the use of doping (use of stimulating substances). Many other international federations followed suit, but restrictions remained ineffective as no tests were performed [011].

Biking (and football)
Anti-doping efforts started in earnest after the 1960 Olympic Games in Rome. During a biking team time trial, 23-year-old Danish cyclist Knud Enemark Jensen collapsed, fractured his skull and died. An autopsy reportedly found traces of amphetamine and a blood-vessel dilator nicotinyl tartrate in his system. Although the drugs might not have caused his death, the episode forced cycling officials to take a closer look at doping. The UCI banned some performance enhancers [012].

In 1966 the International Cycling Union and the Fédération Internationale de Football Association (FIFA) were among the first international sports federations to introduce doping tests in their respective world championships [011].

The urgency of anti-doping work was further highlighted by another tragic death, that of the doped cyclist Tom Simpson during the 1967 Tour de France [011].

Testing for doping substances in the urine

In 1960, during the Rome Olympics, for the first time, the International Olympic Committee Medical Commission instituted drug testing for athletes. However, it was only in 1968 that the IOC officially took control of testing for the use of certain doping methods and substances. When IOC began doping enforcement during the 1968 Winter Olympic Games in Grenoble, France, the limitations were immense due to the poor methods of analysis, so only a few stimulants and narcotics were detected [013].

The 1972 Olympic Games in Munich, Germany, ushered in testing for stimulants, but athletes had then already started to take anabolic steroids. A test for steroids arrived at the next summer Olympics, in Montreal, Canada. But four years later, at the Moscow Olympiad, athletes had moved on to less easily detectable, naturally occurring, hormones, such as testosterone. Anti-doping authorities later on learned to measure the ratio of testosterone in the blood to a related molecule called epitestosterone. In response, some athletes have reportedly found ways of regulating epitestosterone to keep the ratio in check [012].

Better testing for anabolic steroids were implemented in 1976 Montreal Olympic Games through radioimmunoassay analysis: the technique, however, still only allowed for unspecific detection of a limited number of exogenous steroids. Over the years, always new doping substances are synthesized and, as a consequence, the list of prohibited compounds is continuously updated and new suitable analytical methods for their detection and determination in biological matrices are continuously required. In doping control analysis the knowledge of steroid metabolism pathway in human body is of primary importance and the analytical methods must permit the simultaneous detection and determination not only of the forbidden precursor agents but also of their metabolites. In addition, the potential presence and amount in the biological samples of species that can interfere in the analysis should be evaluated. Also the several anabolic steroids, specifically designed to circumvent doping control, put on the market have been incorporated in the list of the prohibited substances of the World Anti-Doping Agency (WADA). In WADA list steroids figure in three main classes, namely anabolic steroids, corticosteroids and substances with anti-estrogenic properties. It
must also be reminded that the WADA prohibited list establishes criteria to highlight the alteration of the natural steroid profile caused by exogenous administration [014].

**International Olympic Committee (IOC)**

The practice of enhancing athletic performance through foreign substances was known from the earliest Olympic Games. In 1967, the International Olympic Committee (IOC) established a Medical Commission responsible for developing a list of prohibited substances and methods. Drug tests were first introduced at the Olympic winter games in Grenoble and at the summer games in Mexico City in 1968. In February 1999, the IOC convened the World Conference on Doping in Sport in Lausanne, Switzerland. The Lausanne Declaration on Doping in Sport recommended creation of an International Anti-Doping Agency. The World Anti-Doping Agency (WADA) was formed in Lausanne, Switzerland on the basis of equal representation from the Olympic movement and public authorities (international sport federations and most of the governments in the world). One of the mandates of WADA was to harmonize the Olympic antidoping code and develop a single code applicable and acceptable for all stakeholders. The world antidoping code developed by WADA included creation of several international standards (IS). The purpose of each IS was harmonization among antidoping organizations. The ISs were developed for laboratories, testing, the prohibited list, and for therapeutic use exemptions (TUE). The objective of one manuscript was to present a brief history of doping in sport and describe creation of WADA in 1999. The components of the World Anti-Doping code (in particular, the Therapeutic Use Exclusion program or TUE) is described. The WADA code defines a TUE as "permission to use, for therapeutic purposes, a drug or drugs which are otherwise prohibited in sporting competition." Experiences of the Canadian Centre for Ethics in Sport Doping Control Review Board are presented because this national TUE committee has been operational for over 12 years. The challenge of developing a rigorous global antidoping program requires acceptance of doping as a problem by sport organizations, athletes, and public authorities. Individual stakeholders must be prepared to preserve the values of sport, which means free from doping. This will require vigilance by all interested parties for the benefit of elite athletes and society overall [015].

**A continuing challenge: from mushrooms to testosterone**

The use of drugs to enhance physical performance has occurred since the beginning of recorded time. Ancient Greeks ate mushrooms and sesame seeds to enhance performance, and Roman gladiators used stimulants to increase endurance. In modern sports, documentation of the abuse of performance enhancing drugs appeared in the early 1900s, when athletes ingested stimulants (alcohol, cocaine, amphetamines, ephedrine, and strychnine) to alleviate fatigue and increase focus. Thomas Hicks ran to victory in the Olympic marathon of 1904 in Saint Louis with the help of raw eggs, injections of strychnine, and doses of brandy administered to him during the race [011].

**Testosterone and its analogues**

The first report concerning the use of anabolic steroids by an athlete who searched for increased weight and power dates 1954. A reliable test method to detect anabolic steroids was finally introduced in 1974 and the IOC added anabolic steroids to its list of prohibited substances in 1976. This resulted in a marked increase in the number of drug disqualifications in the late 1970s, notably in strength related sports such as throwing events and weightlifting [011].

Anabolic-androgenic steroids (AAS) are now the most common illicit drugs used to enhance performance at the modern Olympic Games along with stimulants, primarily by weight lifters and athletes in track-and-field. The AASs are a group of synthetic derivatives of testosterone
with both skeletal muscle building (anabolic) and masculinizing (androgenic) effects. In 1889, physiologist Charles E. Brown Sequard reported improvement in a variety of his body functions (strength, intellect, and force of urine stream) following the injection of an extract of testicles from the dog and guinea-pig. The primary natural male hormone, testosterone was first isolated from the testis of bulls in 1935. Butenandt and Hanisch and Ruzicka independently synthesized testosterone in the same year, and the chemists received the Nobel Prize in 1939 for their work. Most of the AASs were developed during the 1950s when chemists attempted unsuccessfully to separate the anabolic and androgenic properties of these testosterone derivatives. Nandrolone, the 19-nor analog of testosterone was the first anabolic steroid with sufficient dissociation of androgenic and anabolic properties to justify introduction into clinical practice during the 1950s. Dr. John Ziegler, an American physician-weight lifter, administered AASs to three future American weight lifting champions after learning of the success of AAS-using Russian weight lifters at the 1954 World Championships. In 1958, the US Food and Drug Administration (FDA) approved the use of methandrostenolone (Dianabol) for the treatment of hypogonadism, resulting in the increased availability of this steroid. By the mid-1960s, the use of AASs to enhance performance in sports spread, particularly among weight lifters and other strength athletes. An estimate done was that a third of the US track-and-field athletes in the 1968 pre-Olympic training camp were using AAS [016].

Anabolic/androgenic steroids remain a mainstay in the performance enhancement drug arena given that they are really the only major class of steroids that are unequivocally anabolic with salutary effects on athletic performance. The arms race will continue as long as designer steroids are produced, tested in vitro, and then, for the more difficult parameter, that they and their metabolites will not lead to a positive test at the doping control laboratory [001].

German Democratic Republic

Anti-doping work was complicated in the 1970s and 1980s by suspicions of state-sponsored doping practised in some countries. It has later been extremely well documented the the German Democratic Republic (GDR) government administered doping program of its athletes, particularly its female athletes, contributed to their domination of track & field and swimming events for the two decades spanning the 1970s and 1980s [017].

From 1966 until the collapse of the German Democratic Republic in 1990, hundreds of East German physicians and scientists performed doping research and administered prescription drugs as well as unapproved experimental drug preparations to adult and adolescent athletes of both sexes. From the 1960s through the 1980s, the German Democratic Republic established a systematic doping program for thousands of their athletes that included the use of parenteral preparations of epitestosteronepropionate to avoid detection of illicit AASs [016].

An international epidemic

In 1963, the Council of Europe defined doping in sports as a result of the death of a Danish cyclist at the 1960 Olympics, the death of a UK cyclist at the Tour de France, and the prevalence of potentially life-threatening drugs in sports. In 1964, the International Olympic Committee (IOC) unanimously voted to ban doping in sports. By 1967, the IOC established a Medical Commission with responsibilities to prohibit doping, to develop the Olympic Movement Anti-Doping Code, and to formulate a list of prohibited substances. In 1974, the IOC banned the use of AASs, and testing for AASs by immunoassay screening and gas chromatography-mass spectrometry confirmation began in 1976. In 1984, the use of testosterone was also banned [016].
The most famous doping case of the 1980s concerned Ben Johnson, the 100 meter runner who tested positive for stanozolol (anabolic steroid) at the 1988 Olympic Games in Seoul. Johnson's case focused the world's attention to the doping problem to an unprecedented degree. In the same year, the distribution or possession of AASs with intent to distribute without a valid prescription became a felony, when US Federal Food, Drug, and Cosmetic Act (FFDCA) was amended as part of the Anti-Drug Abuse Act. In 1990, the Anabolic Steroids Control Act defined an AAS as any drug or hormonal substance chemically and pharmacologically related to testosterone (other than estrogens, progestins, and corticosteroids) that promotes muscle growth. These synthetic compounds became DEA schedule III drugs as defined by the US Controlled Substances Act. Later, this act was amended by the Anabolic Steroid Control Act of 2004; on January 20, 2005, the amended Controlled Substance Act added both anabolic steroids and prohormones to the list of controlled substances, making possession of the banned substances a federal crime. In response to continuing demand for illicit AASs, designer AAS appeared as a means to avoid detection of these illicit drugs. An example was the synthesis of tetrahydrogestrinone from the palladium-charcoal catalyzed hydrogenation of gestrinone by the Bay Area Laboratory Cooperative, BALCO, an American nutritional supplement company. However, analyses and legal action resulted in the banning of several athletes as a result of the use of these synthetic steroids. Subsequently, major league baseball revamped their AAS policy calling for a 50-game ban for first-time offenders (up from 10 days), a 100-game penalty for second-time offenders (up from 30 days), and a life time ban for a third positive test. Previously, a baseball player could be suspended for life only after the fifth positive test [016].

In 1998 a large number of prohibited medical substances were found by the police in a raid during the Tour de France. The scandal led to a major reappraisal of the role of public authorities in anti-doping affairs. As early as 1963, France had been the first country to enact anti-doping legislation. Other countries followed suit, but international cooperation in anti-doping affairs was long restricted to the Council of Europe. In the 1980s there was a marked increase in cooperation between international sports authorities and various governmental agencies. Before 1998 debate was still taking place in several discrete forums (IOC, sports federations, individual governments), resulting in differing definitions, policies, and sanctions. One result of this confusion was that doping sanctions were often disputed and sometimes overruled in civil courts. The Tour de France scandal highlighted the need for an independent international agency, which would set unified standards for anti-doping work and coordinate the efforts of sports organizations and public authorities [011].

**WADA**

The IOC took the initiative and convened the World Conference on Doping in Sport in Lausanne in February 1999. Following the proposal of the Conference, the World Anti-Doping Agency (WADA) was established on 10 November 1999. On 5 March 2003, at the second World Conference on Doping in Sport, some 1200 delegates representing 80 governments, the IOC, the International Paralympic Committee, all Olympic sports, national Olympic and Paralympic committees, athletes, national anti-doping organisations, and international agencies supported the World Anti-Doping Code as the basis for the fight against doping in sport. The Code entered into force on 1 January 2004. On 19 October 2005, the World Anti-Doping Code was adopted at the 1st International Convention against Doping in Sport by the General Conference of UNESCO at its plenary session. Some 184 countries have signed the Copenhagen Declaration on Anti-Doping in Sport, the political document through which governments show their intention to implement the World Anti-Doping Code by the ratification of the UNESCO Convention [011].
WADA's success in establishing an international drug code has been underpinned by three developments. First, WADA is funded jointly by the IOC and a group of national governments. This has provided the agency with both capital and influence. Secondly, WADA has secured a series of international declarations that have commended and ratified the policy code it has developed. Thirdly, WADA policy has recently been approved by the United Nations Educational, Scientific and Cultural Organisation (UNESCO) as an international convention. The UNESCO convention is the first legally binding international framework setting out the responsibilities of national governments and is currently signed either as ratification, acceptance, approval or accession by all major countries. These achievements have consolidated WADA's position as the central international agency for regulating drug use in sport [018].

One of the first reports on doping (known as the HARDOP report) was commissioned in 1998 and published in 1999, followed by targeted research projects under the EU's Competitive and Sustainable Growth run under 5th Framework Program [019].

An ethical problem

Researchers seem to agree that doping is unwelcome in sport. However, opinions are divided between doping being a serious deviance one must fight against and doping as undesirable but unavoidable consequence of the institutionalized sport. Notably, the reason behind banning doping initially was the growing concern about athletes' health. Doping only became established as unethical after that point [019].

Not only athletes

The use of doping agents, once restricted to professional athletes, has nowadays become a problem of public health, since it also concerns young people and non-competing amateurs in different sports. The use is also diffused in social life for improving physical appearance and enhancing performance and even dietary supplements assumed to improve performance often contain anabolic steroids. While decades ago the so-called "classical doping agents" (like stimulants and narcotics) were used, to-day anabolic steroids are more widely diffused [014].

Blood doping

Blood boosting or blood doping, which involves removal and subsequent reinfusion of the athlete's blood in order to increase the level of oxygen-carrying hemoglobin, has been practised since the 1970s. The IOC banned blood doping as a method in 1986 [011].

For cycling and other endurance sports, human recombinant EPO, however, fuelled a doping revolution. EPO is a natural hormone that promotes production of oxygen-carrying red blood cells. The first synthetic, or recombinant, version was developed by the biotechnology company Amgen in Thousand Oaks, California, and in 1989 it was approved by the US Food and Drug Administration to treat anemia. It also offered cyclists an easy endurance boost that helped them to excel in grueling stage races. The drug is nearly identical to the hormone naturally churned out by the kidneys, so was impossible to detect. It is also easier to administer than blood transfusions, which had been used to the same effect. Typically, red blood cells account for 40-45 percent of the blood, but in the heyday of EPO doping, some riders were showing up at starting lines with hematocrits of more than 60 percent. The UCI instituted a "no-start" rule, disqualifying riders if their hematocrits on the morning of a race were above 50 percent for men and 47 percent for women. So cyclists began diluting their EPO-boosted blood with saline solution to keep their hematocrits below the threshold. The drug companies that produce EPO have, however, helped anti-doping laboratories to develop direct tests based on subtle biochemical differences between the recombinant molecules and the natural form. The first of these was approved for use in 2000. But athletes increasingly obtain knock-off forms produced in China and India, and researchers have
struggled to keep up [012].
SOME SPECIFIED DATES IN DOPING AND ANTI-DOPING HISTORY

The first recorded drug-related fatality occurred in 1886 when Andrew Linton died on the Bordeaux-Paris cycle race, allegedly from an overdose of strychnine, heroin, and a compound known as “trimethyl”. In 1967, the International Olympic Committee established a Medical Commission and formulated an official list of prohibited substances. The first systematic testing began at the 1972 Olympic Games in Munich with the analysis of more than 2000 urine samples by gas chromatography (GC) with nitrogen-selective detection for stimulants. Systematic urinary screening was introduced in 1983 at the Pan American Games, and blood testing was used for the first time in 1994 in the Lillehammer XVII Olympic Winter Games in an attempt to detect blood doping [020].

Some other important dates in the history of anti-doping [003, 009, 021]:

Second half of 19th Century
1865 Early report of drug use in sport by canal swimmers reported in Amsterdam
1800’s (last third) Cyclists of the day are reported to have used coffee “spiked” with caffeine at the start of races and then add increasing doses of cocaine and strychnine to the mixture during the races. Boxers reported to take strychnine tablets with a mixture of cocaine and brandy
1879 Six day bicycle race - an 144 hour continuous event. Various doping agents used: caffeine based mixtures (preferred by French racers); sugar cubes in ether (preferred by Belgian cyclists); alcohol-containing cordials; and nitro-glycerine (used specifically by sprinters).
1886 First fatality attributed to doping reported (unconfirmed). English cyclist, Arthur Linton, was reported to have overdosed on “tri-methyl” (presumably containing caffeine or ether) during a 600 km cycling event held in France (between Bordeaux and Paris). Note: Evidence to this report is however conflicting with others reporting he died 10 years later.
1887 Amphetamines first synthesised
1800’s (last third) The use of stimulants among athletes is common-place and not concealed unless the drug combination was unique and thought to provide a competitive advantage that the athlete did not want to share.

First half of 20th Century
1904 Thomas Hicks, the winner of the marathon in the 1904 St. Louis Olympic Games, takes strychnine and brandy several times during the race.
1928 Doctor Wilhelm Knoll – a Swiss physician, administers a stimulant (Coramin) to skiers at the St. Moritz Olympic Games
1928 The IAAF becomes the first federation to ban doping
1932 In the Los Angeles Olympic Games, the victories of Japanese swimmers were rumored to be the result of their being “pumped full of oxygen”
1933 The word doping is now part of the English language
1935 The first steroid (testosterone) isolated
1936 Rumors of testosterone injection at the Berlin Olympics among German athletes mid 1930’s
1937 Amphetamines identified as a central nervous system stimulant and became available via prescription
1939 Ruzicka and Butenandt synthesized testosterone
1940s Widespread use of testosterone and other AAS to treat “male climacteric” and other medical conditions
late 1940’s Amphetamines are used for the first time in professional sport
1945 First evidence of formal discussions about the viability of doping in sport through the use of stimulants in Soviet Union
1948 Soviet sport established their goal of meeting or exceeding all world records
Second half of 20th Century
1950  Nandrolone (19-nortestosterone) first synthesized by AJ Birch
1950's-1960's  First reports of female anabolic steroid use relates to Soviet female track and field athletes
1954  Soviets employ systematic use of testosterone with their weightlifters
1954  Russians use AAS for weightlifting at championship in Vienna
1956  Growth hormone (hGH) first isolated from the human pituitary gland by Li and Papkoff
1958  Ciba Pharmaceutical Company released Dianabol (methandrostenolone) and US sports doctor doctor John Ziegler begins experimenting with testosterone for the US weight lifting team. Efficacy of these drugs apparently spread by word of mouth during the early 1960s to other strength-intensive sports, from field events to football
1959  High school sport related drug use rumors to have commenced – a physician in Texas allegedly administered Dianabol to the high school football team
1960  In Rome Olympic Games, Knud Jensen, a 23-year-old Danish cyclist, collapsed during competition and dies. Autopsy results revealed the presence of amphetamines. This is the second death in an Olympic competition and the first doping related death. Note: The first Olympic death occurred in 1912 at the Stockholm Olympics when a marathon runner died of heat exhaustion during the race.
1960's  Anabolic steroid use became widespread.
1962  Mr Olympia body building contest premieres
1964  Urine samples taken from cyclists after the races during the Tokyo Games "were actually blue in color due to the use of various drugs."
1965  Tests conducted on Belgian cyclists in 1965 showed that 37 percent of professionals and 23 percent of amateurs were using amphetamines, while reports from Italy showed that 46 percent of professional cyclists tested positive for doping.
1966  First clearly documented National doping program commences – GDR doping program documented by the STASI.
1966  The IAAF, the Union Cycliste Internationale (UCI), and the Fédération Internationale de Football Association (FIFA) introduce urine drug tests in their respective championships
1967  The International Olympic Committee (IOC) institutes its Medical Commission and sets up the first list of prohibited substances
1967  First doping death televised. 29 year old English cyclist (Tom Simpson) collapses during the 13th stage of the Tour De France. The autopsy revealed high levels of methamphetamine in his system. Simpson was carrying a vial of methamphetamine on him at the time of his death.
1967  The masculine appearances of a number of female track and field athletes from the Eastern bloc countries in the mid-1960s led to speculation that they were either hermaphrodites or men disguised as women. In response, a chromosome test was initiated in 1967 at the European Cup
1967-1968  Doping controls first introduced – at the end of 1967 the International Olympic Committee (IOC) votes to adopt a drug-testing policy banning the use of specific drugs – not including anabolic steroids. This policy is released in 1968.
1968  the Swede modern pentathlete Hans-Gunnar Liljenwall was stripped of a bronze medal for dipping into the local cerveza at the Mexico City Summer Games
1968  IOC first performs drug testing at Montreal games
1968  Soccer player Jean-Louis Quadri collapses during a game in France. He is
pronounced dead on arrival to hospital. His autopsy reveals amphetamines are in his system.

1968  
A cyclist, Yves Mottin, died from “excessive amphetamine use” two days after winning a race.

1969  
First application of RIA for the measurement of steroids in biological fluids published.

late 1960's  
Blood doping by the reinfusion of an athlete's own concentrated oxygen carrying red blood cells or those of a typed-matched donor, shortly before competition is thought to have begun.

1970s  
Widespread use of AAS throughout elite sports. Marked increase in the number of doping-related disqualifications after the introduction by the IOC of anabolic steroids to its list of prohibited substances.

1972  
Unofficial poll taken by a 1972 Olympic Track and Field team member, Jay Sylvester, find that 68 percent of male track and field contestants have used anabolic steroids during their training.

1972  
Ric Demont, an asthmatic, finished first in the 400 metres swimming event, but was disqualified for using a proprietary medicine containing ephedrine.

1972  
Antoinette Bevilacqua, 4th in the high jump in Atlanta, after testing positive for ephedrine, present in ginseng, in an out-of-competition test, had her placing annulled.

1973  
Two tests developed by British scientists to detect for anabolic steroids. One test is by radioimmunoassay and the other is by gas chromatography couples with mass spectrometry. The IOC decides to adopt both tests in tandem to ensure accuracy. These assays do not detect the use of testosterone.

1974  
AAS introduced as a banned class of compounds by the IOC following the positive screening results of the 1974 Commonwealth Games.

1976  
The first female athlete tests positive for anabolic steroids at the Olympics Games and East German women emerge as a dominant force internationally.

1977  
IOC meeting in Prague discusses placing “approved” drug testing labs all around the world.

1977  
American College of Sports Medicine publishes position paper stating that AAS are ineffective for muscle gains.

1978  
In the World Cup Willie Johnstone of Scotland was positive for an ephedrine compound, which he said he took to help him sleep!

1980  
First assay to detect testosterone in urine to retrospectively detect doping in sport developed by doctor Manfred Donike. Tenty percent of all athletes tested positive, including 16 gold medallists in 1980 Olympics.

1980's  
Significant improvement in mass spectrometry particularly GC-MS.

1980s  
Introduction of out-of-competition testing.

1981  
First edition published of “Underground Steroid Handbook”.

1981  
First recombinant form of human hGH (rhGH) produced.

1982  
Caffeine and exogenous testosterone are added to the IOC doping list of prohibited substances.

1982  
“Conan the Barbarian” and “Rambo” released by Hollywood.

1983  

1983  
First use of the testosterone/epi-testosterone ratio. At the Pan American Games held in Venezuela 15 athletes (including 11 weightlifters) tested positive. In addition after these results were announced, 12 American track and field athletes withdraw and returned home before competing.

1983  
Recombinant erythropoieitin produced - Patent number US 5441868 A.

1982-1984  
Human growth hormone (hGH) recognised to be part of doping regime for bodybuilders. hGH described as the “fad anabolic drug” of the Los Angeles Olympic Games.

1984  
Beta blockers used by most pentathlon competitors to reduce tremors and anxiety at Olympic Games. Note: bet-blockers were not banned at this time.
Post the Olympic Games, 24 members of the US men's cycling team admitted to blood doping prior to competition.

1984 Media reports related to the 1984 Olympic Games suggest that some athletes were given instructions on how to evade drug tests for anabolic steroids.

1985 Beta blockers are added to the banned substance list and blood doping is prohibited. There is no test at this time however to detect blood doping.


1986 Blood transfusion banned by IOC.

1986 Diuretics are added to the IOC banned substance list.

1986 Statistical analysis of IOC approved lab positive results shows that anabolic steroid comprise two-thirds of drugs detected in this year and of these two-thirds of these positives were for nandrolone.

1987 West German Heptathlete, Birgit Dressel dies at the age of 26 years of anabolic steroid related complications.

1987 In revised position stand of American Colleges of Sports Medicine concedes that AAS is effective for muscle gains.


1988 Buckley and colleges report that 6.6 percent of 12th grade boys report use of AAS.

1988 Seoul Games, two gold medallist weightlifters tested positive for diuretics.

1988 Ben Johnson, winner of the 100-metre dash, tested positive for an anabolic steroid. The follow-up investigation identified at least half of the athletes testing positive for anabolic steroids.

1988 Linford Christie in the sprint events in Seoul had problems with pseudoephedrine that was present in ginseng. His urinary levels of pseudoephedrine were not above the cut-off level and he was allowed to keep his silver medal.

1980's Speculation that >12 deaths were the result of EPO during this decade.

1988 Peptide hormones are added to the IOC banned substance list.

1989 Monitoring of Future Study adds AAS to its annual high school questionnaire.

1989-1990 Fall of communist Europe – Berlin Wall fell in late 1989 followed by the collapse of the GDR in 1990 and therefore the end of the GDR National doping program.

1990's Some GDR coaches found employment with other teams including within China's sports programs. A number of Chinese athletes test positive during 1990's, including 29 track and field athletes and 19 swimmers.

Early 1990s The East German state-sponsored doping program revealed.

1990s DEA enforcement largely eliminates domestic illicit AAS production, but has little effect on oversees import.

1990s ePO included in the IOC’s list of prohibited substances.

1990s Introduction of blood tests.


1991 Argentinian footballer, Diego Maradona, banned for 15 months in 1991 for testing positive for cocaine.

1994 In the 1994 World Cup, Diego Maradonna tested positive, not for cocaine this time, but for ephedrine, pseudoephedrine, norpseudoephedrine, methylephedrine and the related propanolamine and was.

1994 Doping using DHT detected in 11 Chinese athletes participating in the Asian Games by the IOC accredited laboratory in Tokyo.

1995 World's youngest athlete tests positive for anabolic steroid use a 14-year old female long jumper and sprinter from South Africa.

1996 GC-HRMS testing employed for the first time at the Atlanta Olympic Games.

1996 “GI Joe Extreme” action toy, with the equivalent of a 26 inch bicap and a 55
inch chest, released

1998  Baseball player, Mark McGwire, acknowledges he uses androstenedione. Anabolic steroids were banned by the IOC and NCAA but not in professional baseball at the time of the report.

1998  Cyclist Willy Voet of the Festina team, arrested by French customs police for transporting performance-enhancing drugs. This arrest resulted in an extensive investigation which exposed the extent and 30+ year history of doping use in this sport.

1999  WADA is established

21st Century

2000  Australian Government provided a special research fund in the lead up to the Olympics to ensure that state of the art testing available for Sydney Olympics. Sydney Olympics – first to use the EPO testing by IEF. National Measurement Institute of Australia’s WADA testing facility develops bases of what is now used for the haematology module of the ABP. Population studies for IRMS which allowed the first positive finding by IRMS during the Paralympics for testosterone

2000  National Institute on Drug Abuse (NDA) announces national multimedia public education program on AAS

2000 to present  Increasingly frequent cases of elite athletes exposed for using performance-enhancing drugs

2001  ASDA (now ASADA) became the first National Anti-Doping Organisation to establish a domestic blood-testing program

1999-2005  Lance Armstrong wins the Tour de France on seven consecutive occasions

2000  Three cyclists fail the mandatory health test just prior to commencement of the 2000 Tour de France – they were not permitted to start because they had a haematocrit >50 percent

2003  First World Anti-Doping Code first adopted

2003  Gene doping was added to the list of Prohibited Methods from 1 January

2004  Caffeine, probably the most popular drug in the world, is removed from the IOC banned substance list, notably; research indicates that the ergogenic benefits from caffeine ingestion may be gained from relatively low doses, including those attained from drinking strong coffee

2004  Athens isoform assay for hGH

2004  Anabolic Steroid Control Act of 2004 signed into law, expands list of prohibited AAS and urges increased penalties

2004  The World Anti-Doping Code is adopted worldwide

2005  United Nations Educational, Scientific and Cultural Organization (UNESCO) adopts the International Convention against Doping in Sport 2005 In letter to House Committee of Government Reform, GAD, reports of hundreds of websites selling AAS

2005  Congressional hearings on the use of AAS in baseball and other aspects of AAS abuse

2005  Operation Gear Grinder: DEA targets eight Mexican manufacturers estimated to sell US 5,000,000 of AAS annually in the United States

2006  Floyd Landis was stripped of his title after testing positive for synthetic testosterone

2007  Mitchell report on AAS use in Major League Baseball generates widespread publicity

2007  Operation Raw Deal: DEA seizes 11.4 million dosage units of AAS in largest seizure ever

2007  BALCO investigation – Marion Jones stripped of the five medals she won at the Sydney Olympic games in 2000

2008  The UCI is the first federation to introduce the Athlete Biological Passport
2008 to present News stories regarding use of AAS by military and by private security contractors in Iraq and Afghanistan

2008 to present News stories regarding use of AAS by law enforcement officers in many US cities

2009 WADA Code amended

2009 Athlete Passport Haematological variables approved

2009 Brazilian/American cyclist Flávia Oliveira was suspended for 2 years after taking a supplement known as "HyperDrive 3.0+" which contained methylsynephrine, a chemical equivalent of Oxilofrine, among other substances. Her sentence was eventually reduced to 18 months after an appeal as there was enough evidence that she had unknowingly consumed said substance as the old label did not list methylsynephrine.

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2011 Norwegian terrorist Anders Behring Breivik describes use of steroids in preparation and execution of mass murder of 77 people

2012 Lance Armstrong retroactively stripped of his titles

2012 London Olympics biomarker assay for hGH introduced

2013 ABP Steroidal Module approved – monitors selected urinary steroid concentrations over time in order to detect steroid doping

2013 July 14 Jamaican runners Asafa Powell and Sherone Simpson tested positive for Oxilofrine prior to the 2013 World Athletics Championships. Powell, however, maintained that he did not take any banned supplements knowingly or willfully. Powell voluntarily withdrew as a result of the test. On 10 April 2014, both athletes received an 18-month suspension from competing. However, after appealing to the Court of Arbitration for Sport (CAS), both athletes' suspensions were lifted on 14 July 2014.

2013 Lance Armstrong admits to doping during his television interview with "Oprah" and was subsequently stripped of his seven Tour de France medals

2014-2016 The Russian Doping scandal

December 3, 2014: The German TV channel ARD accuses Russian athletes for systematic doping led by among others the chairman Valentine Balachnitjev

November 4, 2015: the former chairman of IAAF, Lamina Diack, is taken to custody of French police for being bribed for not forward information of Russian doping

November 9, 2015: WADA presents an investigation of systematic and state-supported doping

November 13, 2015: IAAF suspend all Russian athletes from internation competing

March 7, 2016: The Russian tennis player Maria Sjaparova tells the press in Los Angeles that she has been caught for using meldonium

March 8, 2016: Gold medalist Semjon Elistratov, short track, and skating star Pavel Kulizjnikov are tested positive for meldonium

March 17, 2016: Swimmer Julia Jefimov is caught formeldonium

March 21, 2016: Four track-and-field athletes caught for using meldonium

March 22, 2016: At least 10 Russian wrestler caught for meldonium

March 23, 2016: The Times acuses Russian swimming for systematic doping by the doctor Sergej Portugalov

2015 On July 16 Boston Red Sox pitching prospect Michael Kopech was suspended without pay for 50 games after testing positive for Oxilofrine, which is a banned substance under the Minor League Drug Prevention and Treatment Program. Kopech denied knowingly taking the substance

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2012-2016 40 track-and-field athletes, mostly runners, from Kenya, were caught for doping. Two of the first were two females, Joyce Zakary and Koki Manunga, at the Olympics in Beijing

To be continued!
Individual cases of certain importance for the history and future

There are also numerous highly publicised individual cases more than Ben Johnson (Canada) who was stripped of the Gold Medal for the 100m at the 1988 Seoul Olympics. Other more recent examples include Marion Jones who won five medals at the 2000 Sydney Olympics but was stripped of all these medals, when having been implicated by the highly publicised Bay Area Laboratory Cooperative (BALCO) investigation. She admitted in 2007 to using performance-enhancing drugs. Lance Armstrong, who won the Tour de France cycling event on seven consecutive occasions (1999-2005) but was stripped of these titles in 2013 having been investigated by USADA and admitting to doping during a television interview by “Oprah” [017].

Knud Jensen, 1960

In the Olympic Games in 1960, a Danish cyclist, Knut Jensen, collapsed during an Olympic race and later died in hospital where amphetamine was found in his system (his was the second Olympic death after Portuguese marathon runner Francisco Lazaro died from heatstroke in 1912) [021].

Tom Simpson 1967

July 13, 1967, on Mont Ventoux in southern France, 29 year-old British rider Tom Simpson was lying seventh overall in Tour de France when the 13th stage of the race set off from Marseilles. The temperature was well over 40°C (105°F) but Simpson was an experienced competitor, having turned professional 10 years before, and would presumably pace himself. Unexpectedly, he slowed almost to a halt, wobbled and veered to his right. Helpers, sensing his distress, rushed to help as Simpson fell from his cycle. Simpson appeared to lose consciousness as he fell; he never recovered and died. Three tubes were found in Simpson’s pocket, one full of amphetamines, and two empties. The British team’s luggage was searched and more supplies of the pills were found. At the time, the drugs element did not cause the sensation that might be expected today: the death itself was of most concern. In continental Europe, there was substantial and open advocacy of the use of stimulants to alleviate the strain of long-distance cycling. There is little doubt that many of the leading contenders in the 1967 and other tours were taking amphetamines. Simpson’s death occasioned soul-searching among Tour organizers. It was not the first time they had considered the use of stimulants. A tentative attempt in the previous year to introduce drug testing was opposed by leading cyclists, including the five-times Tour winner Jacques Anquetil, who told the publication France-Dimanche: “Yes, I dope myself. You would be a fool to imagine that a professional cyclist who rides 235 days a year in all temperatures and conditions can hold up without a stimulant.” Interestingly, Simpson was not denounced as a cheat at the time; his death opened up a rather different discourse about the perils of drug taking rather than the morality of it [021].

Ben Johnson 1988

Ben Johnson of Canada, the 100 meter dash champion who tested positive for the anabolic steroid stanozolol at the 1988 Olympic Games in Seoul, was stripped of his gold medal. Furthermore, it is interesting to recall that of the eight runners in this infamous race, five of the other finalists either gave positive samples or were involved in some way in doping scandals at some stage in their careers. While increased muscle mass was the goal of early doping with steroids, since the late 1990s steroids in Olympic sport have been primarily used to enhance recovery to allow more frequent and more intense workouts. At that time it was also understood that testosterone is also largely responsible for the larger red blood cell
(RBC) mass in men as opposed to women, so it has benefits beyond its effect on muscle. Other anabolic steroids have a similar effect on RBC production [021].

Katrin Krabbe et al

Manipulation of urine sampling in sports drug testing is considered a violation of anti-doping rules and is consequently sanctioned by regulatory authorities. In 2003, three identical urine specimens were provided by three different athletes, and the identity of all urine samples was detected and substantiated using numerous analytical strategies including gas chromatography-mass spectrometry with steroid and metabolite profiling, gas chromatography-nitrogen/phosphorus detector analysis, high-performance liquid chromatography-UV fingerprinting, and DNA-STR (short tandem repeat) analysis. None of the respective athletes was the donor of the urine provided for doping analysis, which proved to be a urine sample collected from other unidentified individual(s). Samples were considered suspicious based on identical steroid profiles, one of the most important parameters for specimen individualization in sports drug testing. A database containing 14,224 urinary steroid profiles of athletes was screened for specific values of 4 characteristic parameters (ratios of testosterone/epitestosterone, androsterone/etiocholanolone, androsterone/testosterone, and 5alpha-androstane-3alpha,17beta-diol/5beta-androstane-3alpha,17beta-diol) and only the three suspicious samples matched all criteria. Further metabolite profiling regarding indicated medications and high-performance liquid chromatography-UV fingerprinting substantiated the assumption of manipulation. DNA-STR analyses unequivocally confirmed that the 3 urine samples were from the same individual and not from the athletes who provided DNA from either buccal cell material or blood specimens. This supportive evidence led to punishment of all three athletes according to the rules of the World Anti-Doping Agency. Application of a new multidisciplinary strategy employing common and new doping control assays enables the detection of urine substitution in sports drug testing [022].

Lance Armstrong

One article examined the metabolic performance of an elite cyclist, Lance Armstrong, before and after his diagnosis with testicular cancer. Although a champion cyclist in 1-day events prior to his diagnosis of testicular cancer at age 25, he was not a contender in multi-day endurance cycle races such as the 3-week Tour de France. His genetic makeup and physiology (high VO_{2max}, long femur, strong heavy build) coupled with his ambition and motivation enabled him at an early age to become one of the best 1-day cyclists in the world. Following his cancer diagnosis, he underwent a unilateral orchiectomy, brain surgery and four cycles of chemotherapy. After recovering, he returned to cycling and surprisingly excelled in the Tour de France, winning this hardest of endurance events 7 years running. This dramatic transformation from a 1-day to a 3-week endurance champion has led many to query how this is possible, and under the current climate, has led to suggestions of doping as to the answer to this metamorphosis. Physiological tests following his recovery indicated that physiological parameters such as VO_{2max} were not affected by the unilateral orchiectomy and chemotherapy. It was proposed that his dramatic improvement in recovery between stages, the most important factor in winning multi-day stage races, is due to his unilateral orchiectomy, a procedure that results in permanent changes in serum hormones. These hormonal changes, specifically an increase in gonadotropins (and prolactin) required to maintain serum testosterone levels, alter fuel metabolism; increasing hormone sensitive lipase expression and activity, promoting increased free fatty acid (FFA) mobilization to, and utilization by, muscles, thereby decreasing the requirement to expend limiting glycogen stores before, during and after exercise. Such hormonal changes also have been associated with ketone body production, improvements in muscle repair and haematocrit levels and may facilitate the loss of body weight, thereby increasing power to weight ratio. Taken together,
these hormonal changes act to limit glycogen utilization, delay fatigue and enhance recovery thereby allowing for optimal performances on a day-to-day basis. These insights provide the foundation for future studies on the endocrinology of exercise metabolism, and suggest that Lance Armstrong's athletic advantage was not due to drug use [023].

Maria Sharapova

On 26 January 2016 at the Australian Open Maria Sharapova played against Serena Williams in the quarter-final. Following that match a urine sample was taken from Sharapova under the rules of the Tennis Anti-Doping Programme 2016 (“TADP”). It was found that she had taken meldonium, a substance which had been added to the Prohibited List with effect from 1 January 2016. She told the Anti-Doping Organization that in 2004, at the age of 17, after winning the women’s singles championship at Wimbledon was suffering from frequent cold-related illnesses, tonsil issues and upper abdomen pain. Her doctor, Anatoly Skalny of the Centre for Biotic Medicine in Moscow, concluded that his patient had a mineral metabolism disorder, insufficient supply of nutrients from food intake and other abnormalities which made it necessary to boost the immune system. He proposed a detailed medicinal and nutritional regime which at the outset comprised about 18 medications and supplements. Included in the regime were courses of meldonium, for periods of 7-14 days, in doses of 500 mg to 1 g per day. In 2006 the advices from dr Skalny was:

- meldonium 1-2 x 10, repeated in 2 weeks (before training or competition)
- one hour before competition, 2 pills of meldonium
- during games of special importance meldonium can be increased to 3-4 pills 1 hour before the match
- 30 minutes prior to a training session: meldonium 1 capsule. 30-45 minutes prior to a tournament melddonium: 2 capsules

By the end of 2012 Sharapova decided to follow a different approach to her nutritional intake. She found the taking of lots of pills overwhelming and she thought there was a better way to handle her health than by taking a large number of pills. However she took her own decision to continue to use 3 of the substances previously recommended: Magnerot, Riboxin and meldonium. Magnerot is a mineral supplement which contains magnesium. Riboxin contains inosine, a natural compound which may have some anti-ischaemic benefit. That decision to continue to use those 3 substances from Dr. Skalny’s list of 30 was taken without any medical advice. She relied on the medical practitioners provided by the WTA from whom she would seek medical advice when she suffered injury or became sick in competition. She also underwent MRI scans and ECG tests and examination by a number of specialists during this period, particularly in 2015. To none of the medical practitioners or specialists who treated her over 3 years did she disclose the fact that she was taking meldonium. Her explanation in evidence is that none of them had asked what medication she was taking. There is no document after 2010 in the player’s records which relates to her use of meldonium. Nor was the use of meldonium disclosed to the anti-doping authorities on any of the doping control forms which she signed in 2014 and 2015. On 29 September 2015 WADA published the 2016 Prohibited List. The website included a 2 page summary of major modifications document which included the statement: “Meldonium (Mildronate) was added because of evidence of its use by athletes with the intention of enhancing performance.”
ANCIENT HISTORY OF DOPING AND ANTI-DOPING

The first marathon

In 490 BC, the Persian Army landed on the plain of Marathon, 25 miles from Athens. The Athenians sent a messenger named Feidipides to Sparta to ask for help. He ran the 150 miles in two days. The Spartans were late. The Athenians attacked and, although outnumbered five to one, were victorious. Feidipides was sent to run back to Athens to report victory. On arrival, he screamed “We won” and dropped dead from exhaustion. The marathon was run in the first modern Olympics in 1896, and in many ways the athletic ideal of modern athletes is inspired by the myth of the marathon. The ideal in that myth is of superhuman performance – and at any cost [024].

History of sports medicine in ancient Greece

Cheating in sport is nothing new – only its form has changed. In ancient Greece, athletes attempted to lay their rivals low with curses, or simply turned to bribery. Fines levied on those caught were used to build bronze statues of Zeus, which lined the entrance of the Olympic stadium, reminding competitors of the perils of cheating [025].

Religious ceremonies and athletic competitions

“The story of organized athletics in the ancient world,” writes the historian of sport William Baker, “is primarily the story of Greece”. James Longrigg opens his account of the history of medicine in the classical world with a similar generalization: “One of the most impressive contributions of the ancient Greeks to Western culture was their invention of rational medicine.” It is not surprising, then, that the origins of Western sports medicine, and the ethical problems associated with it, are to be found in ancient Greece, the civilization that gave birth to both organized athletics and rational medicine. Initially combinations of religious ceremony and athletic competition, athletic festivals were a significant part of Greek life for more than a millennium. Hundreds of athletic festivals were held each year throughout Greece and its colonies. By the fifth century BCE, four major festivals dominated the scene. The so-called “Circuit Games” included the biennial Isthmian Games at Corinth; the biennial Games at Nemea; the quadrennial Pythian Games at Delphi; and, the oldest and most prestigious of the games, the quadrennial Olympic Games. According to tradition, the first Olympic contest was held in 776 BCE. Games-playing became a profession not later than 680 BCE, and it remained that until the end of antiquity. As professionals, athletes specialized in particular sports, and engaged in full-time, supervised training. Municipal pride led to public funding of athletes' training, and many cities provided athletes with cash awards for victories and with retirement pensions. The modern distinction between professional and amateur, which rests upon whether or not one is paid for competing, did not enter the picture. All Greek athletes expected and accepted material rewards for victory. It was not until about 50 BCE though, that athletes finally organized themselves into formal collegia – social and religious societies that also served the economic function of advocating on behalf of their members [026].

Training under supervision

Athletic training became more methodical and systematic with the introduction of the gymnasium in the early sixth century BCE, and the subsequent appearance of professional trainers. According to Harris “By the fifth century BC it seems to have become normal for every athlete of any pretensions to be trained by a professional.” Typically former athletes themselves, trainers were expected to be experts not only in the techniques of various
sports, but also in massage, diet, physiotherapy, and hygiene. Competitors in the Olympic Games and their trainers were required to be in Elis one month before the Games began, where they trained under the strict supervision of the judges. The judges prescribed a stringent regimen of exercises, and were free to disregard the training habits of individual athletes. On the opening day of the Olympics, athletes, along with their family members and trainers, swore an oath to Zeus that they would obey the rules of the Games, and that they had faithfully trained in a proper manner. According to Philostratus, the judges directed the athletes, “If you have worked in a manner worthy of coming to Olympia, and have done nothing in an offhand or base way, proceed with good courage; but as for those who have not so exercised, go away wherever you like.” The relationship between physicians and trainers was close, though often bitter. The relationship was strained by professional rivalry and, ultimately, by the fact that physicians and trainers were committed to very different fundamental values. In the ancient world, physicians and trainers were fierce rivals in the world of hygiene and therapy [026].

Critique of the diet by Hippocrates and Galen

There is a common notion of health underlying Hippocrates and Galen’s criticisms. It is the notion of health pioneered by Alcmaeon of Croton in the second-quarter of the fifth century BCE, onto which Empedocles grafted his four-element theory a generation later. In this view, health is considered a balance or equilibrium (isonomia) of the various constituents of the body, and illness is the result of imbalance. The influence of this view was enormous, as it was adopted within the Hippocratic Corpus and subsequently endorsed by Galen, and it is at the core of both the hygienic and therapeutic regimens prescribed by physicians in this tradition. Though the professional trainers held an unassailable monopoly on the training and conditioning of athletes, physicians had ample opportunity to observe, record, and analyze the effects of the various training and dietary practices of athletes. Galen, as it is well known, began his career as a doctor for gladiators. It was on the basis of their empirical findings that physicians condemned the athletic lifestyle. A case in point is the analysis of the athletes’ compulsory diets, which were typicall heavy in meat consumption. A heavy meat diet was not normal in ancient Greece. The typical diet consisted of a vegetable stew, fish, and bread; usually meat was eaten only at religious celebrations. Some athletes, though, were known to eat up to 10 pounds of lamb per day, and Hippocratic physicians cataloged a variety of intestinal disorders observed in athletes who were compelled to eat this much meat [026].

Critique of athletics by Philostratus

The best-known attempt to reform athletic training in light of the ancient physicians’ medico-ethical critique of athletics came not from a trainer, but from the sophist Philostratus. His Gymnastic is the most significant source of our knowledge of Greek athletic training, and despite the fact that this work is highly critical of contemporary training methods, Finley and Pleket argue that Philostratus should be seen as “a defender of athletics and of trainers, in particular against the doctors.” One of the primary targets of criticism is the trainers’ rigid enforcement of the system of the tetrad, which was developed at some point before the first century CE. The 4-day training cycle began with a day of preparatory exercises, followed by a day of intensive workout, a day of relaxation, and finally, a day of medium-intensity training. By alternating periods of maximum effort with periods of relative rest, the system of the tetrad bears an obvious resemblance to the system of interval training that became popular in the 1950s. Philostratus provides the cautionary tale of his contemporary, the Egyptian wrestler Gerenus. Having just won at Olympia, Gerenus celebrated with friends for 2 days. When he reported for training, hung-over and exhausted, on what was the second day of the tetrad, his furious coach insisted that Gerenus give the all-out effort that the day’s training schedule demanded. In the midst of his workout, Gerenus collapsed and died. The trainer’s mindless adherence to schedule and disregard for the athlete’s condition typified the conduct that the
physicians criticized. But again, Philostratus was not offering such tales simply to reinforce the physicians' critique of athletics; rather, it was his hope that this work would bring about the significant reform of training methods [026].

**Attempts for performance-enhancing in ancient Greece**

*Dried figs*

The laws of antidoping are new, but the use of doping substances and methods is as old as the history of sport. In this sense, it was reported that the Greeks ate mushrooms, believing the mushrooms could improve the performance of athletes in competition and that Roman gladiators used stimulants to alleviate fatigue. The ancient Greeks supported the humanistic ideals that people were born into aristocracy and that one’s position in society was established through blood lines. Participants in the Olympic Games were eligible because of their place in society. That did not stop early reports of drug misuse. Galen, in the third century BC, reported that Greek athletes used stimulants to enhance their physical performance. At the ancient Olympic Games, athletes had special diets and were reported to have taken various substances to improve their physical capabilities. The winner of the 200 m sprint at the Olympic Games of 668 BC was said to have used a special diet of dried figs. This scenario is not so far removed from the supplements that feature in today’s sports nutrition support programmes for athletes. Women were excluded from these Games, although there is a suggestion that one unofficial female participant – a Greek woman called Melpomene – “crashed” the Marathon in protest [027].

*A dangerous game*

The odes to athletes who died in the pursuit of victory and the epitaphs on their graves bear witness that the athletes' attitude of “victory or death” was popularly regarded as both reasonable and praiseworthy. A well-known case in point involves Arrichion, who died in the final round of the pankration at the Olympic Games of 564 BCE but still won, because his opponent signaled submission as Arrichion collapsed. Reflecting on a painting of Arrichion, Philostratus wrote: “Though indeed it is a great thing that he already won twice at Olympia, what has just now happened is greater: he has won at the cost of his life and goes to the land of the Blessed with the very dust of the struggle”. The epitaph of a boxer at Olympia reads: “Agathos Daimon, nicknamed the Camel, from Alexandria, a victor at Nemea. He died here, boxing in the stadium, having prayed to Zeus for victory or death. Age 35. Farewell” [026].
EARLY (MODERN) HISTORY OF DOPING AND ANTI-DOPING

The use of drugs and ergogenic substances to augment athletic performance, commonly referred to as doping, has evolved along with sporting events. Ancient Olympic athletes thus consumed mushrooms, plants, and herbs in an attempt to gain a competitive edge. The modern Olympic Games made their debut in 1896, and mixtures of cocaine, ephedrine, and strychnine were used to enhance performance [028].

First racehorses then strychnine

The modern era of doping dates to the early 1900s, with the illegal drugging of racehorses. Alfons Bukowski (1858-1921) is commonly regarded as the pioneer of anti-doping research. In 1910, he developed a method to detect alkaloids in horse saliva. One hundred years later, this is a good moment to remember Bukowski, an outstanding Polish pharmacist, often mistakenly represented in world literature as a Russian chemist. It is also an occasion to mention that the real driving force in the history of doping were events related to horse rivalry [029].

In modern times, there is a correlation between the discovery of a drug and its use in sports. During the end of the 19th Century and the beginning of the 20th Century, new illicit substances were incorporated into sport. In 1896, Arthur Linton, a Welsh cyclist, died under the influence of stress and speed ball (cocaine + heroin) during the Tour de France. In 1904, and he has the dubious distinction of being the first known person to die from such drug abuse. The first documented case of drug use in the modern Olympic Games took place when Thomas Hicks, a marathoner, nearly died from a mixture of brandy and strychnine [013].

Strychnine was used by marathon runners in the 1904 London Olympics; amphetamines came into their own at the Berlin Olympics in 1936. Up until the 1920s, mixtures of strychnine, heroin, cocaine, and caffeine were not uncommonly used by higher level athletes in different disciplines. Each coach or team developed its own unique secret formulae. This was common practice until heroin and cocaine became available only by prescription in the 1920s. During the 1930s, it was amphetamines that replaced strychnine as the stimulant of choice for athletes. In the 1950s, the Soviet Olympic team first used male hormones to increase strength and power [030].

By 1930, use of perform-enhancing drugs in the Tour de France was an accepted practice, and when the race changed to national teams that were to be paid by the organizers, the rule book distributed to riders by the organizer reminded them that drugs were not among items with which they would be provided [031].

Amphetamines

The amphetamines were the first “effective” performance-enhancing drugs, which were used widely by soldiers in the Second World War, crossed over into sports in the early 1950s. These drugs – nicknamed la bomba by Italian cyclists and atoom by Dutch cyclists – minimize the uncomfortable sensations of fatigue during exercise. By setting a safe upper limit to the body’s performance at peak exertion, these unpleasant sensations prevent bodily harm. The artificial manipulation of this limit by drugs places athletes at risk for uncontrolled overexertion. The first cases of fatal heatstroke in athletes using atoom were reported in the 1960s. In the 1967 Tour de France, elite British cyclist Tom Simpson died on the steep ascent of Mont Ventoux, allegedly because of amphetamine abuse. The precise extent to
which amphetamines enhance athletic performance is unknown, since, as with all performance-enhancing drugs, there are few modern studies quantifying their effects. The convenient absence of such information represents further evidence of a hidden problem. A popular opinion is that la bomba can turn the usual Tour de France domestique, or support rider, into a stage winner. Since amphetamines must be present in the body to be effective, the sole method of avoiding the detection of their use during competition is to substitute a clean urine sample for the doped specimen. A multitude of innovative techniques have been developed to accomplish this swap. Cortisone, a potent but legal performance-enhancing drug used to dampen inflammation, also reduces the discomfort of heavy daily training and competition and lifts the mood. It is also widely abused by professional cyclists [032].

During the 1960s at least three top athletes died from the misuse of performance-enhancing drugs [033].

Anabolic steroids

Hormones then came on the scene and although most experts believe that hormone use is relatively recent, history shows that this is not the case. For example, in 1939 the Wolverhampton football team in England was trying out testosterone. However, the main epidemic of anabolic steroids began in the 1950s among weightlifters and spread rapidly through a variety of sports in both professionals and amateurs, men and women. By the time of the 1972 Olympics in Munich, nearly 70 percent of athletes in middle or short distance running and all the US weightlifters admitted to having taken AS. The escalation of drug abuse resulted in the inclusion of the AS on the list of banned substances for all Olympic Games since 1976 [002]

Women in sports

The entry of women into the Olympics occurred as a result of the laissez-faire arrangements between the International Olympic Committee (IOC) and the host cities of Paris in 1900 and St Louis in 1904, and culminated in more formal arrangements to include women’s events in the London Olympics in 1908. The IOC’s response was to restrict the inclusion of women to a few events appropriate to an ideal of feminine activity but to locate them outside the official program. Women’s events were made official but were not given equal status with men’s competitions until 1924. So the early Olympics sidestepped one of the more modern controversial issues about hormones and sport by allowing only men to participate; there was no question of gender testing in those days as men competed naked. The very exclusion of women from the Olympic Games was itself an ethical question but undoubtedly of cultural origin. One might argue that it was concerns about the hormone profiles of some of the more masculine women athletes that led to the introduction of gender testing in 1968. Was it to identify hormone abuse by women or was it to stop men participating as women? [027].

The first doping in the modern Olympics

Olympic nationalism

In July 1912, the Boston Medical and Surgical Journal celebrated “American Supremacy,” noting that the “overwhelming success of the American athletes at the current Olympic games in Stockholm is as interesting physiologically as it is nationally gratifying. Although Sweden won the most medals, athletes from the United States won the gold medal count by the thinnest margin, 25 to 24.” The large population of the United States “is more mixed of all
races, and we should therefore be able to select the best strains and breed the best mixtures." American athletes were “better nourished and conditioned” than their competition, “which again should conduces to racial physical superiority.” To top it off, “the intensity of our national disposition leads our athletes to train much more eagerly and consistently, and with a keener professional intentness for winning.” Though eugenic undertones have faded, physicians have maintained their interest in the Olympics. The strobe of the quadrennial competitions illuminates dramatic changes in medicine and sport [034].

First doping

The use of performance enhancing drugs in the modern Olympics is on record as early as the games of the third Olympiad, when Thomas Hicks won the marathon after receiving an injection of strychnine in the middle of the race. The first official ban on “stimulating substances” by a sporting organization was introduced by the International Amateur Athletic Federation in 1928 [023].

The Olympics 1976-1992

In 1976, the East German swimming team won 11 out of 13 Olympic events, and later sued the government for giving them anabolic steroids. In 1992, Vicky Rabinowicz interviewed small groups of athletes. She found that Olympic athletes, in general, believed that most successful athletes were using banned substances [023].

The Second Olympic Games in Athens

The Olympic Games in Athens were the first to follow the introduction of a global anti-doping code. From the lead up to the games to the end of competition, 3000 drug tests were carried out: 2600 urine tests and 400 blood tests for the endurance enhancing drug EPO. From these, 23 athletes were found to have taken a banned substance – the most ever in an Olympic games. Ten of the men’s weightlifting competitors were excluded. The goal of “cleaning” up the sport is unattainable. Further down the track the spectrum of genetic enhancement looms dark and large [023].

Football

In Forward Arsenal, published in 1952, the Arsenal and England player Bernard Joy described Arsenal’s use of “pep pills” before an FA Cup match against West Ham United in the 1924-25 season. The fact that Joy was perfectly open about Arsenal’s use of stimulants, and that his matter of fact style of writing is devoid of any suggestion that Arsenal might have been cheating or doing anything improper, provide an interesting sidelight on attitudes to performance enhancing drugs in the period before their use in sport was banned [035].

Drug testing

Drug testing of athletes was first introduced at the FIFA World Cup of 1966 in England and at the Olympic Games of 1968 in Mexico City, instigated by the deaths of athletes participating in the Rome Olympic Games of 1960 and the Tour de France in 1967 linked to amphetamine and nicotinyl tartrate. This early antidoping activity culminated in the creation of the 1966 FIFA antidoping regulations that comprised a list of seven groups of prohibited substances, including narcotics and stimulants. The fundamental aims of the current antidoping policy were developed in the late 1960s by the IOC and the IFs. They are to: (1) uphold and preserve the ethics of sport, (2) safeguard the physical health and mental integrity of players and (3) ensure that all competitors have an equal chance. Since then, doping controls have
been performed at most of the major sporting competitions such as the Olympic Games, World Championships in track and field and other sports, the FIFA World Cup and major cycling competitions. In the process, the IOC in the first instance, and subsequently the WADA accredited laboratories, gained considerable expertise and experience in detecting prohibited substances [036].

Biking

The 2003 year's Tour de France ended with no big surprises: US cyclist Lance Armstrong won the race for the fifth time, again defeating his greatest competitor, German Jan Ullrich. And it was a surprisingly “clean” contest, as only one athlete was found to be using the performance-enhancing drug erythropoietin (EPO). This is clearly an improvement on the infamous 1998 race when the whole Italian Festina team were disqualified after their coach was found with more than 400 doping products, including EPO. Indeed, the Tour might have become cleaner since then: in 2001, Spanish cyclist Txema del Olmo was the only athlete who tested positive for EPO and who was subsequently banned from the tour. But the Tour de France is not the only international sports contest tainted by doping. US sprinter Ben Johnson, who rewrote the record books during the 1998 Olympic Games in Seoul, South Korea, ran to victory with the help of a cocktail of steroids and was later stripped of his medals. The greatest case of misuse, however, came to light after the reunification of Germany in 1990, when investigators found that East German athletes had been systematically doped for several years, which explained their many records and gold medals. Some critics maintain that, without drugs, today's athletes would not be any better than their predecessors in the 1960s, before doping became a widespread problem in competitive sports. Indeed, watching the Olympic Games or the Tour de France nowadays, the speed and endurance of the competitors is incredible [037].

Antidoping

IAAF banned first

Many sports organizations have come to ban the use of PEDs and have very strict rules and consequences for people who are caught using them. The International Association of Athletics Federations was the first international governing body of sport to take the situation seriously. In 1928, they banned participants from doping, but with little in the way of testing available, they had to rely on the word of athletes that they were not doping. It was not until 1966 that the Federation Internationale de Football Association and Union Cycliste Internationale joined the International Association of Athletics Federations in the fight against drugs, closely followed by the International Olympic Committee (IOC) the following year [031].

Testing

The first actual drug testing of athletes occurred at the 1966 European Championships, and 2 years later the IOC implemented their first drug tests at both the Summer and Winter Olympics. Anabolic steroids became even more prevalent during the 1970s, and after a method of detection was found, they were added to the IOC's prohibited substances list in 1976. This resulted in a marked increase in the number of doping-related disqualifications in the late 1970s, notably in strength-related sports, such as throwing events and weightlifting [031].
**Blood doping**

While the fight against stimulants and steroids was producing results, the main front in the anti-doping war was rapidly shifting to blood doping. This removal and subsequent reinfusion of an athlete’s blood in order to increase the level of oxygen-carrying hemoglobin has been practiced since the 1970s. The IOC banned blood doping in 1986. Other ways of increasing the level of hemoglobin were being tried, however. One of these was erythropoietin. Erythropoietin was included in the IOC’s list of prohibited substances in 1990, but the fight against erythropoietin was long hampered by the lack of a reliable testing method. An erythropoietin detection test was first implemented at the 2000 Olympic Games [031].
Unlike other endocrine organs, well-hidden within the body, the testes, as the source of testosterone, have an exposed position and are thus quite vulnerable and also easily accessible for external manipulation, including forceful removal. Therefore, quite early in the history of mankind, the effects of testosterone or rather their lack, became known and history is full of examples how this knowledge was applied [038].

Medical challenges

The evolution of the history of testosterone therapies is as interesting as the history of its development. Erectile dysfunction is one of the most researched ailments treated with testosterone, although any positive effects are questionable. In men with absent to low circulating levels of testosterone, treatment with testosterone increased libido, improved erectile function, and helped to maintain secondary sexual characteristics. In men with normal or mild hypotestosteronemia, studies have not shown consistent response to therapy. Those treated were reported to have increased sexual interest, increased arousal, increased frequency of intercourse, and nocturnal erections. In the early twentieth century, there was much interest in the hormonal influence of testosterone on sexuality and sexual preferences. It even was prescribed to “treat” homosexuals because it was theorized that male homosexuals had higher estrogen levels [039].

Testosterone has even played an important role in various ailments affecting women, such as treatment for some metastatic breast cancers. Approximately one third of breast cancers are hormone dependent and respond to androgen therapies. Other uses for testosterone are as postmenopausal hormone replacement therapy, for sexual dysfunction (by increasing libido), and for increasing bone density. Some clinical case studies showed an increase in appetite, lean muscle mass, and strength and an improved overall sense of well-being. Before the use of erythropoietin and bone marrow transplants, testosterone was used to help treat anemia (i.e. chronic renal failure/hemodialysis). Psychiatrists prescribed anabolic steroids from the 1930s to the 1980s to treat psychoses, depression, and melancholia. Testosterone has been used as an adjunct in people with growth hormone deficiency or in boys with pubertal delay [039].

Studies of the effects of supplemental testosterone on aging men in the 1990s suggested an increase in word memory, special cognition, increased libido, decreased bone resorption, and increased lean body mass and strength. McKinlay reported in the Journal of Urology that testosterone does not treat impotence. In theory, prostatic tissue, including cancer and benign prostatic hypertrophy, can be stimulated by testosterone, but no compelling evidence has been reported that suggests an increased risk [039].

Anti-anabolic castration

The history of anabolic steroids is a tale that has its roots in ancient “endocrinology.” More than 6000 years ago, farmers noted an enhanced ability to domesticate animals after castration. Years later, the medical theories of “humoralism” developed. This doctrine was based on a theory that attempted to explain diseases based on imbalances among the four humors: sanguine, choleric, melancholic, and phlegmatic. In addition, ancient Egyptians and Romans believed that testicles and animal penises held special healing powers. Ancient Greek athletes used a wide variety of alleged performance-enhancing drugs, such as plant extracts and testicular extracts. The biological effects of the testes and testosterone are thus
known since antiquity. Aristotle knew the effects of castration and his hypothesis on fertilization is one of the first scientific encounters in reproductive biology [039].

**Castration**

Castration has been practised for socio-cultural and political purposes since antiquity. Its major purpose was to generate obedient slaves who were loyal to their masters or rulers and, being infertile, could not create competing offspring. Set to guarding harems, they also, and in larger numbers, obtained influential administrative and political positions as in China and formed elite troops in Islamic countries. In different cultures and over centuries “wealthy women preferred intercourse (or rather other sexual pleasures) with castrated slaves for a good reason: there was no risk of pregnancy” [038].

**Egypt**

Eunuchs probably already existed in ancient Egypt. From the times of the legendary Queen Semiramis (about 800 BC) eunuchs were reported from Assyria and the system developed and continued into the Islamic world in the Middle – East and North Africa. Over centuries, slaves were deported from Sub-Saharan Africa to the Islamic cities and courts and many of the slaves who survived the exhausting march through the desert were then castrated to serve as laborers, guards, administrators and even soldiers [040].

**Greece**

In Greek mythology, castration already occurred among the first generation of gods. Gaea, mother earth, grew out of the chaos and produced Uranos by parthenogenesis with whom she then generated the titan Chronos. When Uranos prevented Gaea from creating children with their son Chronos, she induced Chronos to castrate his father. Uranos’ testes, thrown into the sea, caused the water to foam and out of these bubbles the foam-born goddess of love Aphrodite (Venus) was born. Quite extraordinary events in terms of reproductive physiology! This episode has been depicted beautifully in a fresco by Giorgio Vasari (1511–1574) in the Palazzo Vecchio in Florence [040].

**Medieval Europe**

It has been estimated that the transatlantic deportation of Africans to the Americas between 1450 and 1870 comprised about 11.5 million people while the entire Islamic deportation of slaves from Africa between 650 and 1920 amounted to 17 million people and several million of these African slaves were castrated. This constant drain of manpower effectively prevented economic and cultural development of Sub-Saharan Africa. In medieval times, slaves were also exported from Europe to the Islamic countries. These slaves were mainly from Eastern European (Slavic) and Central Asian countries. There were well-established slave routes through Europe and Verdun in France enjoys the questionable historical fame of having been the European center for castration of slaves on their way from the East to the South at those times [040].

Castration has also been practised as lawful punishment. In medieval Scandinavia, castration combined with blinding was administered for high treason, especially when the insurgent was a close relative whom one did not want to kill directly. As told in the Islendinga Saga, Sturla of the Sturlungar Clan in Iceland castrated and blinded his rebelling relative Oraekja Snorrason in 1236. When the Normans migrated south, they also introduced this penal practice in the areas they invaded. When he established his reign in Britain after 1066, William the Conqueror abolished the Anglo-Saxon death penalty and replaced it by castration and blinding: “I also forbid that anyone shall be slain or hanged for any fault, but let his eyes be put out and let him be castrated”. As a further example, when the Normans invaded Sicily, King William III was castrated and blinded in 1194 after a rebellion against Emperor Henry
VI. This episode forms the historical background for Klingsor's castration in the Parsifal epos. The Toulouse Law Codex of 1296 describes (and depicts) castration for high treason [040].

Slave trade
It has been estimated that the transatlantic deportation of Africans to the Americas between 1450 and 1870 comprised about 11.5 million people while the entire Islamic deportation of slaves from Africa between 650 and 1920 amounted to 17 million people and several million of these African slaves were castrated [038].

More recent events in Europe
Throughout the centuries castration was applied to beaten enemies by victorious soldiers for revenge and as a measure to eliminate the enemies without outright killing. This continues into recent times. When Italian troops invaded Ethiopia and lost the battle of Aduwa in 1896 supposedly 7000 Italian soldiers were castrated. As reported by Babtschenko, this still happened on both sides during the Chechen War in the Caucasus in 1996 [040].

Castration has also been reported as self-mutilation for religious reasons since ancient times in order to make a life in chastity easier. The early church father Origines (186–254) is one of the most prominent examples. In the eleventh to fourteenth centuries, the sect of the Catharers with their strongholds in Southern France promulgated self-castration as part of a "pure" life. More recently, castration was practised in Southern Russia among members of the Scoptic sect founded in the eighteenth century and the medical consequences were documented. The largest contemporary group of castrates is among the hijras in India who also comprise persons with disorders of sexual development (DSD). They function as professional well-wishers at birth rites and weddings and receive considerable financial rewards. Several thousand of them exist [040].

Castration has also been used as revenge for seduction and adultery through the centuries. For example, Paris – presumably in the twelfth century BC and preceding the Trojan War – has been reported to have castrated Peritanos after he had seduced his famous wife Helena. The case of the great medieval theologian and philosopher Peter Abaelard (1079–1142) has been celebrated in history and literature. As master of the cathedral school in Paris he seduced one of his disciples, Heloise (1100–1164), whose uncle in revenge then had Abaelard castrated by paid criminals. Despite the lack of testosterone, one of the most romantic love stories documented by literature developed. This type of revenge continues into most recent times as demonstrated by an incident in Germany in 2011 when the father of a 17-year-old girl castrated her 57-year-old lover. These people had migrated to Germany from Kazakhstan and obviously brought their rules of self-justice with them. The German court sentenced the father to 6 years imprisonment and Euros 80,000 penalty [040].

Sopranos
Castration before puberty maintains the high voice of boys so that soprano and alto voices with the acoustic volume of an adult male result. Such high-pitched voices were considered desirable among music lovers, especially at times when women were not allowed to sing in church or in operatic performances. Prepubertal castrates belonged to casts of operas in the seventeenth and eighteenth centuries; in the Vatican choirs these voices could be heard until the early twentieth century. Some of these castrates became famous soloists, such as Carlo Farinelli (1705–1782) or Domenico Annibaldi (1705–1779). The middle Italian cities of Norcia and Preci were a center for the operation on young boys. In the solitude of this hidden Apennine valley, a surgical school had already been established in the thirteenth century and the 30 family dynasties monopolizing the trade there guarantied utmost secrecy concerning this illegal operation. Strangely enough, while castration was forbidden in the Vatican state, which extended over most of middle Italy, it was not forbidden to employ castrated singers. However, most of the thousands of prepubertal castrates lost their virility in vain as they did
not achieve the promised career as a singer, developed only mediocre voices and were ridiculed by their contemporaries. An impression of the castrato voice, although of very low recording quality, is preserved from the last Vatican castrato Alessandro Moreschi (1858–1922) in one of the earliest gramophone recordings, made in 1902 (available today on CD). Today countertenors applying a trained falsetto sing the castrato roles in, for example, Händel operas, but their head voices only approximate those of seventeenth century castrati. Another impression of the castratos’ enormous artistic talents is provided by the recordings of the mezzo-soprano Cecilia Bartoli who trained her voice to sing the extremely demanding arias by Nicola Porpora (1686–1768), Georg Friedrich Händel (1685–1759) and others [040].

Prepubertal castration provides an involuntary experiment on the influence of testosterone on longevity. A retrospective comparison of the life expectancy of singers born between 1580 and 1858 and castrated before puberty, in order to preserve their high voices, to intact singers born at the same time did not reveal a significant difference between the lifespan of castrated intact singers (66 ± 14 vs 64 ± 14). This would imply that the presence or absence of normal male testosterone levels at and after the age of puberty has no influence on life expectancy [040].

China
Castration has been practised for socio-cultural and political purposes since antiquity. Its major purpose was to generate obedient slaves who were loyal to their masters or rulers and, being infertile, could not create competing offspring. Set to guarding harems, they also, and in larger numbers, obtained influential administrative and political positions as in China and formed elite troops in Islamic countries. In different cultures and over centuries “wealthy women preferred intercourse (or rather other sexual pleasures) with castrated slaves for a good reason: there was no risk of pregnancy.” The earliest documentation of creating eunuchs in China dates back to about 1300 BC. The Chinese eunuch system, with several thousand's existing at a time, continued until the end of the imperial period in 1912. The last Chinese eunuch, Sun Yaoting, died at the age of 94 in 1996. Only the fact that imperial eunuchs could obtain high-ranking positions and considerable power as well as wealth makes it plausible that adult men underwent this gruesome operation. It was performed by “licensed surgeons” just outside the imperial court in Beijing by cutting off testes and penis. About 25 percent of the volunteers did not survive this bloody operation. The severed genitals were kept in a box, as shown vividly in the film “The Last Emperor”, and were eventually buried with their owner. During the Ming Dynasty (1368-1644) eunuchs attained outstanding influence and wealth. The prime example is represented by Liu Jin (1451-1510) who is counted among the richest persons in history; he accumulated 449 750 kg of gold and 9 000 000 kg of silver, but eventually his criminal intrigues led to his execution. In the nineteenth century there were still about 2000 eunuchs at the imperial court in Beijing. The impact of peri- and postpubertal castration on the phenotype of these men was described extensively by Wagenseil who in 1922 established an Anatomical Institute at the Chinese–German Tung-Chi University in Shanghai where he examined a series of 31 Chinese eunuchs aged 45–57 years. These eunuchs had no beard growth and sparse body hair and 21 of the 31 had developed kyphosis as a clear sign of osteoporosis [040].

Organotherapy
As it was known that removal of the testes caused the clinically evidenced symptoms of hypogonadism including impotence, prescribing ingestion of testes to remedy the symptoms was a medical reflex inherent in organotherapy, practised since antiquity. Thus the Roman Gaius Plinius Secundus recommended the consumption of animal testes to treat symptoms of testosterone deficiency. Slightly more refined was the prescription of testicular extracts for the same purpose in Arabic medicine, for example, by Mensue the Elder (777–837) in Baghdad. Also in China, raw and desiccated testes were prescribed, documented at least in the twelfth century by Hsue Shu-Wei. Around the same time Albertus Magnus (1193–1280)
in Cologne, better known as a philosopher, recommended powdered hog testes, but refined his recipe by offering the powder in wine [040].

It is interesting to find that as late as 1960 Paul Niehans wrote the book "Introduction to Cellular Therapy", in which the main emphasis was on testicular secretions. He believed that testicle cell injections increased testosterone derivative excretion. Some of Paul Niehans' famous patients included Pope Pius XII, Bernard Baruch, and Aristotle Onassis [039].

Scientific exploration of the testes and their function

The declaration of the Netherlands as an independent state during the 30 Years War (1618–1648), and legalized at the Westphalian Peace Treaty in Münster in 1648, resulted in an enormous upswing in economy, culture and science in this country. The medical sciences also boomed, based on proper research, especially in anatomy, as shown in Rembrandt's contemporary painting "Anatomy of Dr Tulp" (1632). The reproductive sciences benefited from this boom as well. It was Regnier de Graaf (1641–1673), who not only described the Graafian follicle (1672), but also published a book about the anatomy of the male reproductive tract as well as the treatment of its disorders. He produced very detailed drawings and descriptions of the male genital organs and was the first to discover that the testes were composed of a "collection of minute vessels or tubules, which confect semen; if these tubules were disentangled without being broken and tied to one another, they would far exceed 20 Dutch ells in length" (about 13 m). Having first described this in the edible dormouse, he then went on to the human: a classical case of translational medicine. Unfortunately, de Graaf became involved in a quarrel with his contemporary Jan Swammerdam (1637–1680) about the question of who had first described the ovarian follicles and during that phase he died under nebulous circumstances at the young age of 32 [040].

A few years after Regnier de Graaf's early and mysterious death, his friend Antoni A. Leeuwenhoek (1632–1723), together with the student Johan Hamm, used his newly invented prototype of a microscope and described the "little animals of the sperm" in a letter to the Royal Society in London in 1677 (Collected Letters 1948). Considering the primitive appearance of his microscope, the details of his morphological descriptions of sperm are amazing and it is even more amazing that 300 years later we are still quarreling about normal and abnormal sperm morphology [040].

But it took another century until Lazzaro Spallanzani (1729–1799), a priest and scientist in Modena, artificially inseminated frogs and dogs and demonstrated the real function of sperm. By using sperm that he had preserved on ice he also became the father of cryopreservation without which modern reproductive medicine and medicine in general would be unthinkable. He was a very systemic investigator and insisted – quite in contrast to others at the time – that experiments needed to be repeated before results could be accepted, a principle that prevails until today [040].

The anatomist Franz Leydig (1821–1908) in Würzburg described the interstitial cells of the testes in 1850. Although he did not know their function, they still carry his name. Finally in Milano, in 1865 Enrico Sertoli (1842–1910) discovered the supporting cells in the seminiferous tubules, also carrying his name until today. Thus, over roughly two centuries, the basic morphological elements of the testes had been described, as well as the one major product of the testes, the sperm. Even the function of sperm and fertilization had been elucidated so that the time had come to explore the basis of testicular endocrine function [040].

Although the endocrine function of the testes was known through their physiological and clinical effects, their nature remained completely obscure. Although William Harvey (1578–
1657) had discovered the role of the heart and blood circulation in 1628, in some medical schools Galen's (129–216) concept of the four bodily humors prevailed well into the nineteenth century. Against this background it is not surprising that the idea of a hormone working as a signal transduced by circulating blood took so long to be born [040].

Transplantation of testes in 1786

John Hunter (1728-1793) was a Scottish surgeon who was later appointed as Surgeon General for the British army. He made many noteworthy contributions to science, including contributions to the understanding of digestion, fetal development, venereal diseases, dentistry, and lymphatics. As a surgeon in the Seven Years' War (1756–1763) he saw the need for transplantation of organs and limbs, and this is what stimulated his research. He never described his testis transplantations himself, but we learn about them through a scholar, Dr W Irvine, in a letter to Prof Th Hamilton in Glasgow in 1771: "...Nay more, he has many hens just now into whose abdomen when young, he has put the testes of a cock just separated from his body and his testis has got blood vessels and nerves from the part of the abdomen or viscera to which it is applied...". Far from any endocrine thought the goal was to demonstrate the survival of the transplant due to nerve growth [040].

An era of testis transplantation and organo-therapy was thus initiated after his transplantation of testes into capons in 1786. The intention of his experiments was to prove the “vital principle” as the basis for modern transplantation medicine, but Hunter did not consider endocrine aspects. He conducted the first testicular transplant in 1786 in which he removed a testicle from a rooster and implanted it into a hen [039].

Transplantation of testes in 1849

Such thoughts were precipitated by Arnold Adolph Berthold's (1803–1861) experiments, which also concerned transplanting chicken testes. Berthold was a professor at the University of Göttingen, and he performed experiments on roosters while he was a curator at a local zoo. He observed the impacts of castration and the reimplantation of testicular tissues on roosters. Once castrated, the roosters’ combs decreased in size, they lost interest in the hens, and they lost their aggressive male behaviors. Those effects were reversed after reimplanting testicular tissues or extract, despite denervation. As published in 1849, he castrated four cocks, two received an ectopic transplantation of one testis, the two others remained untreated and he observed: "They (the transplanted roosters) crowed quite considerably, often fought among themselves and with other young roosters and showed a normal inclination to hens ...Since the testes can no longer remain in connection with their original nerves after being transplanted to a strange place ... it follows that the consensus in question must be affected through the productive relationship of the testes, that is to say, through their action on the blood, and then through the suitable ensuing action of the blood on the organism as a whole." The paper describes only four animals and comprises only four pages – in contrast to the extensive style of that time, but was epochal. However, Berthold's rival at the University of Göttingen, Rudolf Wagner (1805-1864) was jealous, tried to repeat the experiments, but failed and declared them as rubbish. And as he became the full professor of physiology, his opinion prevailed. Berthold's personality did not allow him to fight for recognition of his findings, which fell into oblivion [040].

More modern transplantation of testes

Next to organotherapy there was another sad approach to treat hypogonadism and bring about rejuvenation and treatment for all sorts of disorders: the transplantation of testes. G Frank Lydston (1858-1923) in Chicago was one of the first to perform human testicular transplantation from donors after experimentation in animals [040].
The US
In 1913 in Chicago, Victor D Lespinasse (1878-1923), an urologist, claimed that he cured a patient who had sexual dysfunction by transplanting a testicle from a donor. He removed the organ, made three transverse slices, and inserted them into muscle tissue around the patient's scrotum. His most famous patient was Harry F McCormick (husband of Edith Rockefeller), the case of who was described in The New York Times. Five years later, the first journal of Endocrinology was published [039].

Iceland
These surgeons had followers in many parts of the world, for example, even in Iceland where, in 1929, the surgeon Jonas Sveinsson transplanted testis slices from a poor farmer in need of money to a rich Norwegian businessman who, he then claimed, satisfied his 23-year-old wife so that he even had three children with her. In the Soviet Union experimentation with human testicular transplantation continued at least into the 1980s. The only testicular transplantation resulting in fertility of the recipient was performed by Silber between twin brothers [040].

Isolated germ cells
The idea of transplanting tissues and cells continued in a transformed fashion as “cellular therapy” by injecting suspensions of fresh cells of sheep embryos including testis cells, also for rejuvenation and revitalization, well into the second half of the twentieth century. Meanwhile, however, science has progressed and, in the age of cell biology, testicular transplantation continues with the aim of inducing fertility, but now uses isolated germ cells, and fertility has indeed been restored by this method in gamma-irradiated cocks. Whether this may become a method to treat male infertility, for example, in Klinefelter patients remains to be seen, but at least it is pursued on a rational scientific basis [040].

Charles-Edouard Brown-Séquard
Following Berthold’s observations, testicular preparations were not used for therapy until popularized by self-experiments by Charles-Edouard Brown-Séquard (1817-1894) – “the father of andrology” in Paris (1889), which – as we nowadays knows – at best have had placebo effects. Thus, almost half a century before the discovery of androgens, Brown-Sequard recognized that the contents of testicular extracts could improve libido, energy, and muscle strength. Brown-Sequard was a prominent French physiologist and Harvard professor. He had a strong interest in endocrinology, and he studied adrenal glands, testes, thyroid, pancreas, liver, spleen, and kidneys. He is probably most famous for his auto-experimentation with testicular substances (extracted from guinea pigs and dogs), the results of which were published in in the Lancet. He gave himself 1 mL injections of a mixture of one part testicular vein blood, one part semen and one part juice extracted from dog or guinea-pig testes daily, and after 20 days made astonishing observations on himself: “A radical change took place in me. I had regained at least all the strength I possessed a good many years ago. I was able to make experiments for several hours. After dinner I was able to write a paper on a difficult subject. My limbs, tested with a dynamometer, gained 6 to 7 kg in strength. The jet of urine and the power of defecation became stronger” [040].

He thus reported increased strength, mental abilities, and appetite and even claimed that the process relieved constipation and increased the arc of his urine stream. Although no one is sure why he experienced these effects, his experiment caused others to investigate the testicular substance as a possible cure for various ailments, such as diabetes, tuberculosis, epilepsy, paralysis, gangrene, anemia, influenza, arteriosclerosis, Addison’s disease, hysteria, and migraine headaches. He encouraged testing of his testosterone products by providing free samples to physicians. Unfortunately, with such widespread use, shoddy
researchers subjected animals and humans alike to high risks for infection and inflammation [039].

In 1889 Brown-Sequard announced at a scientific meeting in Paris that he had found a substance that reversed his 72-year-old body's ailments. He reported having injected himself with the extract of dog and guinea pig testicles under the assumption that these organs had “internal secretions that acted as physiologic regulators” [06003]. An aging Brown-Séquard thus eagerly reported “a decided gain in strength” after injecting himself with the “orchitic fluid” of laboratory animals. His report created enthusiasm and controversy alike [040].

Even though what was reported was placebo effects, the world had obviously waited for such quackery, because in no time the “extracts of animal organs by the Brown-Séquard method” were sold all over the (Western) world and factories sprang forth in Europe as well as in America, for example, next to Central Park in New York. There must have been a real craze for these products and physicians concerned about the image of the young field of endocrinology started worrying. The famous neurosurgeon Harvey W Cushing (1869–1939) and the president of the Association of the Study of Internal Secretions, Edward H. Rynearson even talked about “endocriminology” in the context of this organotherapy. This assessment of the medical scene at the time is also reflected in contemporary cartoons and comic songs from the early twentieth century. Eventually, this type of quackery stimulated science and decent pharmaceutical companies to search for real hormones [040].

**Birth of endocrinology**

Substances referred to as “chemical messengers” were discovered in 1902 by English physiologists and professors, William Maddock Bayliss (1860-1924) and Ernest Henry Starling (1866-1927), at University College London. Bayliss’ research team was the focus of an animal rights controversy in 1903 – the Brown Dog Affair – in which Bayliss was alleged to have performed a live dissection of a brown dog in his laboratory. He, of course, denied the accusation and won a civil suit, donating the money to the University for further research; he even wrote articles promoting the humane treatment of animals. Other accomplishments included contributions on shock, digestive system, and endocrinology; being knighted in 1922; and authoring four editions of Principles of General Physiology. Starling officially coined the term “hormone” in 1905 when giving a Croonian Lecture (prestigious lectureships) titled “The Chemical Control of the Functions of the Body” to the Royal College of Physicians. The term “hormone” means “to urge on” or “impulse or arouse” in the sense of “to set in motion” in Greek. Years later, reports suggested that a Cambridge physiologist, William B. Hardy, actually suggested the term “hormone” to Bayliss and Starling [039].

**The Steinach operation**

In Vienna, an Austrian physician, Eugen Steinach (1861-1944), developed the “Steinach operation,” an “autoplastic” treatment for the “middle-aged and listless”. The 20-minute operation involved ligation of the vas deferens, often at the most proximal position to the testicle. This allegedly increased testosterone production. He believed that the incision produced a “back pressure” on the testicle, thus increasing testosterone production by the interstitial cells. He also implanted testicular tissue grafts between the peritoneal muscles. He reported that his patients were able to regrow hair, had better erections with less premature ejaculation, and had increased libido. Despite little clinical evidence of his claims on “rejuvenation,” the results of his operations, at best, likely were due to the power of suggestion; however, he performed this procedure on some famous patients, including Sigmund Freud and William Butler Yeats. He also discovered, by transplanting male sex glands into females and vice versa, that guinea pigs developed sexual behaviors of the
opposite sex. Later research proved that sex hormone injections have no effect on sexual orientation but that high doses of testosterone may increase sexual desire [039].

_Serge Voronoff_

One of the followers of Steinach was Serge Voronoff (1866–1951), a Russian-French physician and surgeon, who made a fortune from removing testes from animals (including the controversial monkey and chimpanzee gland transplants by way of vivisection, sparking campaigns from animal rights groups and satirical cartoons and books on the subject) and transplanting them into men. The chimpanzee tissue was not implanted inside the scrotum but instead in the tunica vaginalis. He concluded that his experiments with testicular transplants helped to relieve pain and provided a sense of well-being [039].

He first offered his surgery in Paris, but after some scandals continued his questionable operations in Algiers, where he was obviously visited by patients all from over the world. Voronoff had followers in many countries who xenotransplanted animal testes or pieces thereof to patients in need of rejuvenation, also in the USA, where this type of treatment caused great interest among the laymen and the media. As unrest among the medical profession continued to grow, in 1927 the Royal Society of Medicine (London) sent an international committee to Voronoff in Algiers, which concluded that Voronoff's claims were all poppycock. However, the effectiveness of this kind of transplantation was later disproved [040].

_A Nobel Prize for testosterone research_

The Austrian physiologist Oskar Zoth (1864-1933) was the first person to propose injecting athletes with a hormonal substance, as published in his 1896 paper describing how the use of an “extract” improved muscular strength and the “neuromuscular apparatus,” thus potentially improving athletic performance. He and his physician partner, Fritz Pregl (1869-1930), self-injected testosterone extracts from bulls and measured the strength of their middle fingers by plotting them on “fatigue curves”. They recognized that testicular extracts could improve physical and mental energy, as well as muscle strength and won the Nobel Prize in chemistry in 1923 [039].

_Dubious experiments on prisoners_

Perhaps the most famous, and perhaps most unethical, research of “organotherapy” occurred in the 1920s and 1930s at San Quentin prison in California where Leo Stanley transplanted the testicles from executed prisoners into impotent prisoners. He reported 20 cases and the recipients reported signs of revitalization. Later on he turned to animals as sources for his testicular grafts (ram, sheep, goat, deer, and boar) and reported satisfaction on the part of the patients including 13 physicians [14016]. He treated men who suffered from senility, epilepsy, and paranoia. Over the years he performed hundreds of operations [039].

_Further research on testosterone_

In 1911, Andre Pezard first noted a direct relationship between the amount of testicular extract injected into a rooster and the size of his comb [039].

Synthetic androgens were born in the 1930s when Foss first described the medical use of orally bioavailable methyltestosterone [003].
In the late 1930s, experimentation using humans involved testosterone propionate (slow-release derivative) and methyl testosterone (oral form that was slower to metabolize). Most of the research at that time was focused on treating hypogonadism in men (inducing and maintaining secondary sexual characteristics and treating impotence). Charles D Kochakian discovered an increase in protein anabolic processes, thus opening the door for the treatment of a variety of disorders by restoring tissue and stimulating growth. In 1939, it was reported that daily topical application of testosterone by females enlarged the clitoris and increased sexual desire. The use of synthetic testosterone skyrocketed after publication of the book “The Male Hormone” by Paul de Kruif in 1945, which made claims of increasing libido and boosting athletic performance. Testosterone was a proposed treatment for menorrhagia, dysmenorrhea, estrogen-derived breast cancers, and other breast conditions. It was reported to help relieve pain, increase appetite, and promote a “sense of well-being.” Despite these claims, physicians remained reluctant to begin widespread use among women because of the virilizing side effects. Most of the profits from sale of this substance were obtained by way of the black market [039].

Chemical isolation of testosterone

Modern androgen therapy started in 1935 when Enrest Lacquer isolated testosterone from bull testes in Amsterdam. In the same year testosterone was chemically synthesized independently by Adolf Butenandt in Göttingen and Leopold Ruzicka in Basel. Testosterone was identified as 17beta-hydroxyandrost-4-en-3-one (C_{19}H_{28}O_2), a solid polycyclic alcohol with a hydroxyl group at the 17th carbon atom [041].

Since testosterone was ineffective orally it was either compressed into subcutaneous pellets or was used orally as 17alpha-methyl testosterone, now obsolete because of liver toxicity. The early phases of testosterone treatment coincide with the first description of the most prominent syndromes of hypogonadism by Klinefelter, by Kallmann, DelCastillo and Pasqualini. In the 1950s longer-acting injectable testosterone enanthate became the preferred therapeutic modality. In the 1950s and 1960s, research concentrated on the chemical modification of androgens in order to emphasize their anabolic effects. Although anabolic steroids have largely disappeared from clinical medicine, they continue to live an illegal life for doping in athletics. In the 1970s the orally effective testosterone undecanoate was added to the spectrum of preparations. Recent transdermal gels and long-acting injectable preparations provide options for physiological testosterone substitution therapy [040].

A second Nobel Prize for studies on testosterone

It was apparent to researchers that some substance circulating in the blood was responsible for their findings; however, it was not until 1929, when a German chemist and professor, Adolf Butenandt (1903-1995), isolated the first sex hormone, that a new path of discovery was initiated. He isolated estrone from the urine of pregnant women and later isolated 15 mg of androsterone (“andro” = male, “ster” = sterol, “one” = ketone) from 15,000 L of urine from local policemen. Butenandt found evidence of a “bloodstream substance” from roosters that affected their appearance and behavior. He postulated internal secretion from his testicular transplantation experiments. His theory was correct, but it was not widely accepted by his contemporaries. He was awarded the Nobel Prize in chemistry in 1939 for his “work on sex hormones” (together with Leopold Ruzicka). Butenandt initially rejected the award in accordance with government policy (he was a member of the Nazi party), but accepted it in 1949 after World War II. Butenandt spent a large part of his career studying the sex hormones and their relationship with one another but his work also laid the foundation for the production of cortisone [039].
From German soldiers to Marion Jones

Shortly after the successful synthesis of testosterone, Boje suggested that sex hormones might enhance physical performance. The Germans allegedly administered AAS to soldiers going into combat. The Germans also allegedly gave athletes testosterone in preparation for the 1936 Berlin Olympics. However, the most cited example of systematic use of AAS in elite sports is that of the Soviet weightlifting team in the 1952 and 1956 Olympics. Dr John Ziegler, a physician associated with the US weightlifting team, learned about the use of AAS by the Russian team at the weightlifting championships in Vienna in 1954, and experimented with testosterone on himself and other weightlifters in the York Barbell Club, New York. That is considered to be the beginning of AAS abuse in sports in the United States, which later spread from high-intensity strength-training games to sports such as field athletics, baseball, swimming, etc. Thus AAS use, which had been exclusive to strength-intensive sports, spread gradually to other sports and to nonathlete weightlifting over the ensuing decades. Later, the relentless glare of media lime light surrounding the detection of PED use by elite athletes, such as Lyle Alzado, Mark Maguire, Barry Bonds, Floyd Landis, Marion Jones, and Lance Armstrong, has added to the allure of PEDs and contributed to the widely-held misperception that PED use is largely limited to elite athletes and is therefore not a widespread public health problem [003].

Repeated economic success

Different potions containing substitutes for endogen testosterone continued to be prescribed and consumed up into the twentieth century. In the 1920s, Testifortan® became a financially successful drug for treatment of impotence. Its main constituent was testis extracts and yohimbin; after the war 17alpha-methyl testosterone was added without changing the name. Another famous preparation from the 1920s and marketed until today is Okasa®, which, among other components, also contains testis sicca and thereby small amounts of testosterone, as could be determined in the 1970s. However, as the testes synthesize testosterone but do not store their products in contrast to other endocrine organs such as the thyroid and the pancreas, the daily production by an adult man of about 6-8 mg is contained in roughly 1 kg of (bull) testes and even if this amount of testosterone were to be consumed, the testosterone taken orally would be inactivated by the first-pass effect in the liver. Therefore, all testicular organ therapy administered orally can only be considered as a placebo medication, which, however, may not be without its own effects. Ultimately this type of testicular organotherapy was terminated by the advent of phosphodiesterase inhibitors [040].

Interests of the pharmaceutical industry

During the 1930s, three pharmaceutical companies each hired research teams to isolate the testicular hormone. The term testosterone ("testo" = testes, "ster" = sterol, "one" = ketone) was coined in 1935 by Karoly David and his research team. Ernst Laqueur isolated testosterone from bull testes. The research team was funded by the pharmaceutical company Organon in Oss, The Netherlands. Later that same year (on a team funded by Schering Corporation in Berlin, Germany), Butenandt and Gunicr Hanisch published “A method for preparing testosterone from cholesterol” in a German journal. Only a week later, Leopold Ruzicka (who synthesized androsterone in 1934) and Albert Wettstein (working for the Ciba corporation Switzerland) published “On the artificial preparation of the testicular hormone testosterone (andro-sten-3-one-17-ol)” in Helvetica Chimica Acta and applied for a patent [039].
A black market

Most of the PEDs that athletes and nonathlete weightlifters used prior to the 1990s were pharmacological agents approved for medicinal or veterinary use. By the 1990s, various androgen precursors became available over-the-counter as unregulated “nutritional supplements” [003].

By the early 1990s, several pharmaceutical companies had stopped producing AASs. It was about at this time that the black market sales of AASs and counterfeit products increased secondary to the ease of Internet shopping and availability. Authentic steroids, as well as placebos and unpurified forms, were sold and abused [039].

Development of new testosterone preparations

Soon after its synthesis testosterone became clinically available, first in the form of pellets and then as injectable esters, that is, testosterone propionate with a short half-life and, from the mid-1950s on, the longer-acting testosterone enanthate appeared, which remained the major testosterone preparation for half a century. Also in 1935, 17alpha-methyl-testosterone was synthesized and its oral effectiveness was demonstrated [032].

Testosterone propionate (Testoviron), the prototype of the anabolic steroids was synthesized already in 1936 and appeared in sport sometime after the 1948 Olympic Games. The subsequent synthesis of methandrostenolone (Dianabol) in the United States in 1958 and oral chlordehydromethyl-testosterone (Turinabol) in East Germany after 1966 marked the beginning of the “virilization” of modern sport [032].

However, due to its 17alpha-structure it turned out to be liver toxic, a fact that gave testosterone in general a bad name among physicians, as this toxicity was also suspected for testosterone without reason; eventually in the 1980s this androgen became obsolete for clinical use in Europe. In the late 1970s the orally effective testosterone undecanoate, absorbed from the gut via the lymph to avoid the first-pass effect in the liver, was added to the spectrum of testosterone preparations used clinically [040].

However, testosterone was soon studied using different forms. Scientists quickly learned it was ineffective, and even toxic (like 17 alpha-methyl testosterone), when taken orally; instead, it was synthesized into tiny pellets that were inserted subcutaneously. Longer-acting injectable forms of testosterone were synthesized in the 1950s (i.e. testosterone enanthate). Over the following decade, the hormone was modified into derivatives that possessed more anabolic qualities. In the 1970s, oral testosterone undecanoate was synthesized; however, it did not fare well in the oral form because of hepatic clearance and hepatotoxicity. Transdermal scrotal patches were derived in the 1990s. These allowed physiologic levels of testosterone to be acquired. Nonscrotal skin patches were later developed, and testosterone gels were marketed. Today, there are short-acting buccal forms as well as the long-acting injectable testosterone undecanoate today [039].

In the 1950s and 1960s, the pharmaceutical industry became more interested in new androgens than in testosterone itself and concentrated its androgen research on the chemical modification of steroid molecules in order to disentangle the various effects of testosterone and produce predominantly erythropoietic or anabolic steroids. In 1956, contemporary textbooks on androgens had already described 256 androgenic steroids and by 1976 the number had increased to more than 1000 [040].

However, it proved impossible to produce androgens with only one effect out of the spectrum of testosterone activities; at best, one of these effects could be emphasized, but the other
effects remained. The steroid with pure anabolic effects on muscles or bones to treat cachexia, osteoporosis or small stature, or pure erythropoietic effect for the treatment of anemia without androgenisation could not be found. Nevertheless, anabolic and similar steroids were clinically used, but disappeared again in the wake of evidence-based medicine. However, they continued their existence for illegal use and abuse for doping in sports and bodybuilding potentially causing considerable undesired effects. Regrettably, at that time the pharmaceutical industry neglected the chance to develop testosterone preparations better suited for the substitution of hypogonadal patients than the existing testosterone esters. It remains to be seen whether the current search for SARMs will take a more rewarding course than did anabolic steroids [040].

From the 1970s, the newly developed testosterone immunoassays made serial testosterone determinations in blood possible and, when applied to pharmacokinetic studies, it turned out that all available testosterone preparations resulted in unphysiologically high or low serum levels, which were undesirable in substitution therapy. Clinicians assembled at a workshop on androgen therapy sponsored by WHO, NIH and FDA in 1990 came to the conclusion: “The consensus view was that the major goal of therapy is to replace testosterone levels at as close to physiologic concentrations as is possible” and demanded that new testosterone preparations better suited for clinical use be manufactured [040].

Stacking and cycling

The two common patterns of AAS abuse are “stacking” and “cycling.” Stacking involves the use of two or more androgens in progressively increasing doses over a short period of time. Cycling refers to the intermittent use of AAS where use of steroids is followed by a drug holiday. The practice of “cycling” is based on the notion that drug holidays prevent desensitization to large doses of androgen [042].

Transdermal testosterone

In the mid-1990s, transdermal testosterone patches applied to the scrotal skin became the first transdermal testosterone preparation in clinical use. They had been invented by Virgil Place at ALZA in Palo Alto, a company specializing in new forms of delivery of known drugs. However, although clinical results with this preparation were excellent and for the first time physiological serum levels could be achieved under testosterone substitution, physicians were reluctant to prescribe a medication to be applied to the scrotum and preferred a subsequently developed nonscrotal system. This, however, caused unpleasant skin reactions as it required an enhancer to drive testosterone through the skin. For this reason, the advent of the first transdermal testosterone gel was welcome. This gel became available in 2000 for the treatment of male hypogonadism, first in the US and later also in other countries. Since then, several other gels have been developed and brought to the market, differing slightly in composition and concentrations. The one with the highest testosterone concentration (2.5% Testotop®) has also been tested for scrotal application and because of the high absorptive capacity of the scrotal skin only 20 percent of the gel needed for nonscrotal application is required, making this form of application economically and ecologically more desirable [040].

Finally in 2004, the intramuscular testosterone undecaonate preparation entered the market and soon achieved great popularity as a real testosterone depot preparation. Testosterone undecanoate had originally been used in oral capsules, but had been turned into an injectible preparation by Chinese investigators using tea seed oil as a vehicle. A long half-life could be confirmed in volunteering hypogonadal men who all showed serum levels in the normal range. When finally a company could be interested in this fascinating preparation, it was
“Europeanised” by using castor oil as vehicle and was developed as Nebido® (or Reandron®) for clinical use and is licensed today in 97 countries [040].

The era of anabolic steroids in doping

Bob Hoffman and the Soviet connection

In 1923 Bob Hoffman formed the York Barbell Company in the United States. A dominant figure in US weightlifting, he published the “Strength and Health magazine” and sold health and food supplements in his gym. As a weightlifting coach, his success led to him being named the head coach of the US Olympic weightlifting team. In the 1950s, Russian weightlifters began to outpace American Olympians through performance-enhancing injections. At the 1954 World Championships in Vienna, he met with a Soviet colleague who told him of a synthetic form of testosterone developed by the Nazis which produced dramatic improvements in strength and power. Hoffman and his colleagues contacted Ciba Pharmaceuticals in pursuit of synthetic testosterone. Attempting to make up lost ground, the then US Olympic physician thus teamed with chemists to produce an anabolic steroid for the Americans, now known as Dianabol (methandrostenolone). Dr. John B Zeigler began experimenting with Dianabol on weightlifters at the York Barbell Club in 1958. The weightlifters became strength and conditioning coaches in a variety of other sports in the United States and spread use of anabolic steroids to other sports, such as American football [043].

In the decades that followed, steroids and stimulants spread throughout sports, and in 1959, the first reported case of a high school football player's taking steroids surfaced. Ciba had conducted a number of studies on the use of synthetic testosterone in pain patients and the physically disabled. This resulted in the development of danazol, which rapidly became a doping substance abused by weightlifters [044].

An epidemic

In the 1960s, the International Olympic Committee banned steroid use and began formal drug testing in the ensuing decade. During the 1980s, the reported positive test results ranged from 2 to 50 percent, depending on whether the tests were announced or conducted at random. Then, in 1994, an often-referenced survey was conducted by Goldman when aspiring Olympians were asked 2 simple questions. The first was, “If you were offered a banned performance-enhancing substance that guaranteed that you would win an Olympic medal and you could not be caught, would you take it?” Remarkably, 195 of 198 athletes said yes. The second was, “Would you take a banned performance-enhancing drug with a guarantee that you will not be caught, you will win every competition for the next 5 years, but will then die from adverse effects of the substance?” Still, more 50 percent of the athletes said yes. This survey made it clear that modern athletes often approach their sports with a “win at all costs” mentality [045].

US professionals

Despite years of aggressive anti-doping testing by international sports federations such as those for cycling, athletics and soccer, steroid abuse scandals involving high profile athletes continue to be front page news across the globe. Professional sports in the United States were not subject to extensive anti-doping programs, as players' unions and collective bargaining agreements prevented such extensive testing to be put into place. However, they did establish limited anti-doping programs, as the professional sports organizations recognized the potential of doping to harm athletes and their sport. In 1998, when Mark McGuire, an American baseball player, broke Roger Marris' home run record, it was revealed
that he had been taking a supplement containing a precursor to nandrolone, a steroid. At that
time Major League Baseball did not ban steroids and did not believe that steroids were a
problem within the league. However, subsequent government investigations and former
players revealed that steroid abuse was a problem in the League, which resulted in a limited
steroid testing program [030].

**Anabolic steroid prodrugs in the US**
The potential performance-enhancing benefits of testosterone precursors were brought to the
attention of the public and athletic community in the US in 1998 when Major League Baseball
player Mark McGwire set the home run record and openly admitted to using
androstenedione. Sales skyrocketed by 500 percent, and many supplements containing
prohormones became available in the United States market. Questions and concerns of
contamination with other supplements arose but their purity was unknown because these
supplements were not regulated by the FDA. Also, their popularity was fueled by the
misperception that nutritional supplements are natural, and, therefore, safe. In 2004, after
much controversy and debate, the US Department of Health and Human Services (HHS) and
the FDA announced a crackdown on companies that manufacture, market, and distribute
products containing androstenedione. They recognized the potential serious adverse health
risks that were similar to those associated with AASs. As part of their concern about its
safety, the FDA and HHS sent warning letters to 23 companies asking them to stop
distributing dietary supplements that contained androstenedione and warned them that
enforcement actions would be taken if they did not comply. As a result of this action, the
Anabolic Steroid Control Act of 2004 was passed. This act added the steroid precursor
androstenedione to the list of schedule III controlled substances in the United States.
Schedule III substances have limited medicinal use, require a prescription from a licensed
physician, and allegedly can threaten public health without government regulation. DHEA
was not added to the controlled substance list; industry lobbyists contended that it had
proven effective as an anti-aging supplement and that its risks were minimal [046].

**Designer drugs**
Synthetic organic chemistry can be traced back to 1865 when Friedrich August Kekule
published two theoretical papers on the structure of aromatic organic molecules. Paul Ehrlich
postulated in the early 1870s that differences in chemoreceptors between micro-organisms,
parasites, and cancer cells from those in host cells could be exploited for therapeutic
purposes. In the absence of current ligand-based and receptor-based molecular design
techniques, there were limited approaches to identify minor structural changes in biologically
active compounds that would enhance selectivity and/or potency of therapeutic molecules.
Hammett made the first significant contribution relating structure to activity of small organic
compounds with his study correlating electronic properties of organic acids and bases with
reaction rates and equilibrium constants, focusing on benzoic acid derivatives. Moving
beyond the linear free energy relationships provided by the Hammett equation, the next
major development was the introduction of quantitative structure-activity relationships
(QSAR) by Corwin Hansch et al in two seminal papers in the early 1960s, providing a new
tool to systematically relate molecular descriptors (electronic, steric, topological, and
hydrophobic indices) to biological activity. These early efforts concentrated on naturally
occurring plant hormone mimics, and relied on statistical analysis of published accounts of
the biological activity of phenoxyacetic acid derivatives and other plant growth regulators.
Electronic indices were found insufficient for QSAR of biological systems; rather, a measure
of lipophilicity (classically measured as an octanol-water partition coefficient) was essential to
predict targeting of compounds to specific tissues, cells or organelles, and subsequent
biological activity. John Topliss developed a method to automate QSAR; however, it is of
limited utility in many experimental systems and ignores possible interactions between
multiple substituents. QSAR remains a dynamic tool for drug design and optimization.
Approximately 15 years ago, pharmaceutical companies realized that existing screening
libraries were inadequate for newly developed high-throughput efforts for lead drug design. The challenge has been to balance size and structural diversity of new libraries against screening cost, while maintaining affinity and selectivity against a portfolio of targets. Two major approaches have emerged: fragment-based screening (FBS) and diversity-oriented synthesis (DOS). Despite major advances in chemical screening and synthesis, discovery of new drugs is difficult, expensive, and the efficacy of target-based drug discovery has been questioned [047].

**BALCO**

Although a general topic of interest in the 1990s, designer drugs first made international headlines in 2003 with the Bay Area Laboratory Co-operative (BALCO) scandal involving the widespread use by athletes of tetrahydrogestrinone (THG). This simple reaction created a potent agonist for androgen and progesterone receptors whose presence could not be detected by standard multiple reaction monitoring (MRM) methods used by anti-doping laboratories for steroid detection. Subsequent characterization of this steroid derivative led to US federal prosecution of many involved with BALCO, culminating in March 2011, with the most high profile case so far, involving the former San Francisco Giant (US Major League Baseball) Barry Bonds [047].

**All sports**

High levels of anabolic-androgenic steroids abuse have been attributed to professional football players, bodybuilders, weight lifters, and track and field throwers since the 1960s. The exceptional athletic performance of the East German female swimmers in the 1976 Montreal Olympics brought further public attention to AAS athletic use. It was not until the 1980s, however, that the medical community admitted that these substances were effective. Since that time, the pervasive use of AASs by professional athletes has garnered significant media attention, culminating most recently in the ongoing investigation of the use of illegal performance enhancing drugs by some of baseball's top players. “Juiced”, a book by Jose Canseco, details his steroid use and the widespread use of anabolic steroids in Major League Baseball [048].

**Science versus ethics**

Currently, androgenic-anabolic steroids are the most frequent category of prohibited substances detected in the urine of athletes both globally and at the last two Summer Olympic Games. Scientific confirmation that AAS are effective in enhancing sports performance was difficult because ethical approval was difficult for research involving male subjects taking massive doses of androgens as some athletes and bodybuilders did. Methods to detect androgenic-anabolic steroids have evolved gradually over the past three decades and currently, despite an impressive array of sophisticated analytical equipment and methods, anti-doping authorities and analytical scientists continue to face challenges as have occurred from the use by athletes of designer AAS during the past few years. The future development and use of selective androgen receptor modulators can be anticipated to pose problems in the years ahead. Endocrinologists should be aware that on occasions, replacement testosterone therapy may be authorized in sport as a therapeutic use exemption (TUE) [049].

**Soviet and East Germany**

Anabolic androgenic steroids were used already directly after World War II by Soviet athletes to increase muscle mass and power in weightlifting and bodybuilding events and when the Soviet Union began participating in international sport after World War II it soon achieved a
dominant position in the Olympic Games and other competitions. The success of Soviet athletic programs led to charges of unfair practices but, because of secrecy surrounding Soviet research in exercise biochemistry, it has been difficult to substantiate these charges [031].

In the 1950s, the Soviet Olympic team began experimenting with testosterone supplementation to increase strength and power. This was part of a government-sponsored program of performance enhancement drugs (PEDs) by national team trainers and sports medicine doctors without knowledge of the short-term or long-term negative consequences. Additionally, when the Berlin Wall fell, the East German government’s program of giving PEDs to young elite athletes was made public. Many in the sporting world had long questioned the remarkable success of the East German athletes, particularly the females, and their rapid rise to dominance in the Olympics. Young female athletes experienced more performance enhancement than did male athletes. Unfortunately, they also suffered significant and delayed side effects, including reports of early death in three athletes [031].

During the decade of the 1960s, the German Democratic Republic discovered that sporting success could both improve the self-esteem of the population and its prestige in the international competitive sports arena. They began to finance studies on the use of anabolic–androgenic steroids (AAS), invested in the discovery of young talent, and the use of prohibited drugs. During this period, professional athletes were administered Oral-Turinabol® pills and injections of testosterone esters and nandrolone—presenting them as being vitamins and prophylactic measures. In 1968, the East Germans became pioneers in the administration of androgenic hormones to female athletes [013].

When the Berlin Wall fell, the East German government's program of performance enhancement by meticulous administration of steroids and other drugs to young athletes was exposed. These well-documented and controlled hormonal doping experiments on adolescent athletes by the East German Sports Medical Service at Kreischa and Leipzig yielded a crop of gold medalists (mostly young females as they responded more dramatically to male hormones). Some of these athletes later suffered severe medical abnormalities, including premature death [050].

Physicians dope athletes for a variety of reasons that can range from unethical service to the state to the gratifying of their own immature emotional needs. The East German doctors who participated in the doping of thousands of young athletes, including the administration of anabolic steroids to pubescent girls, functioned within a state-sponsored apparatus whose political mission of sportive nationalism trumped medical ethics. State-sponsored doping in West Germany expressed similar nationalist ambitions that could not be fully realized in a democratic society. The gold medals won by East German athletes at the 1976 Montreal Olympic Games persuaded many West German sports physicians that it was time to adopt the use of androgenic drugs as a matter of national policy. At the Congress of German Sports Physicians held in Freiburg in October 1976, the most prominent West German sports physicians minimized the medical dangers of anabolic steroids and recommended that they be administered to athletes under medical supervision. Far from being a German specialty, however, this pro-steroid mindset can be found among sports doctors around the world. Some physicians have issued therapeutic use exemption (TUE) certificates to athletes that are unwarranted but allow their use of drugs that are believed to boost athletic performance [051].

The exact magnitude of benefit from the use of combined anabolic agents is difficult to calculate, as it depends not only of the chemical substance but also on personal genetic factors, training program, social structures, etc. Previously secret East German records indicate, however, that anabolic steroids alone reduce 100-m sprinting time by as much as 0.7 second and improve performance in the 400-m, 800-m, and 1500-m running events by 4
to 5, 5 to 10, and 7 to 10 seconds, respectively. Equivalent benefits have been found among
swimmers. Effects in throwing events are also substantial: a gain of 2.5 to 5 m in the shot
put, 6 to 10 m in the hammer throw, 8 to 15 m in the javelin throw, and 10 to 20 m in the
discus throw. Benefits are greatest in women, since the natural secretion of testosterone in
young women is negligible [032].

The German Democratic Republic state-supported anabolic steroid doping program, and its
health effects for the women, was the subject of an excellent review by Franke and
Berendonk and a television special by the Public Broadcasting Service [043].

Side effects

Athletes from former East Germany who were given performance enhancing drugs for many
years and who consequently experienced longstanding health problems will receive
payments of several thousand euros, the German federal parliament decided on 13 June
2002. A special law has been passed which sets up a compensation fund of about EUR 2m
(GBP 1.3m; USD 1.9m). The fund is meant to be supplemented by the sports industry and by
national sports associations, but neither of these groups has been keen to join the initiative. It
is estimated that between 500 and 1000 men and women will apply for compensation by the
end of the year and will receive about EUR 3000 each. Currently, the association
representing athletes who have had health problems as a result of doping has about 150
members. Soon after the fall of the Berlin wall in 1989, it became apparent that many East
German athletes had had to pay a high price for the overwhelming success of the nation in
many disciplines. Continuous doping from a young age and for a very long time, mainly with
anabolic drugs, ruined their health. Doping was often done without the athlete's consent or
knowledge. East German trainers and doctors merely followed the socialist party's
instructions. The list of health problems is long: acne, hirsutism, deep voice, muscle tension,
gynaecomasty, breast cancer, bone deformation, vascular disease, and teratogenic
malformations. In some cases female athletes changed their sex as a result of the
continuous intake of male hormones. The association representing such athletes, as well as
single athletes, is not satisfied with the new law, which will come into force in 2003 [052].

The struggle against the anabolic steroids in sports

The first reports of athletes using anabolic steroids searching for an increase in weight and
power appeared in 1954. After that here has been an increasing use of doping substances
by athletes. Furthermore, it was found that not just stimulants were being used but also
anabolic androgenic steroids. However, the banned list did not include those substances.
Therefore, the IAAF banned them and developed an immunological method for their
detection. It was used for the first time at the European Athletic Championships in Rome in
1974. No cases were found as the method was still immature, but the IAAF initiative paved
the way for the IOC who banned steroids in time for the 1976 Games and found eight cases
at the Montreal Games with an improved method. The IAAF experience soon showed the
need for strict procedures to be applied at every stage of a doping control, including the
laboratory analysis. Therefore, the Federation started to work out procedural guidelines for
doping controls as well as specific requirements for laboratories that were used for the
analysis of doping-control samples. Some heads of laboratories were not so happy since
they felt that their competence was questioned, but in 1979 the IAAF decided to only
recognize analytical results from laboratories that met the specific requirements. The
"Accreditation of Doping Control Laboratories" was born. Subsequently, two years later, the
IOC adopted the IAAF system, and for a couple of years laboratories were jointly accredited
by the IAAF and IOC. In 1986 the IOC took over full responsibility for the accreditation
program. Today, doping-control laboratories are accredited by WADA [053].
“It’s pretty clear that steroids are worth the price of a metre at the highest levels of sport” (Charlie Francis, Ben Johnson’s former coach, speaking at the Dubin inquiry in 1990). The early testing program were focused upon competitions and led to a situation where “only the careless or the ill advised” were to fall foul of the testing regimes of the day, even if they had been fairly applied. Reports of manipulated sample collections, of results being destroyed and of complicit activities preventing the revelation of the true extent of drug misuse make it difficult to assess the actual prevalence of hormone misuse [027].

Laboratory progress

The 1976 radioimmunoassay analysis technique, however, only allowed for unspecific detection of a limited number of exogenous steroids. In 1983 the first endogenous steroid, testosterone, was added prohibited substances list. It was a further year before the detection method for testosterone was introduced [027]. The initial test for testosterone in urine was developed by Donike and coworkers, who showed that administered testosterone appeared in the urine as testosterone glucuronide. They also showed that for a population of athletes, the ratio of testosterone to epitestosterone (T/E) had a positively skewed distribution, with a modal ratio of about 1:1. Initially, an athlete sample having a T/E ratio > 6:1 was considered a doping violation. The concept of intraindividual reference ranges (as opposed to population-based reference ranges) was introduced into the T/E test in the early 1990s. Computer programs are now used to compare an athlete’s current sample result to their previous sample results. Results that are inconsistent with previous results are investigated and could result in targeted testing or an antidoping rule violation. The measurement of $^{13}$C/$^{12}$C ratios in testosterone and its metabolites has allowed the differentiation of pharmaceutical testosterone from natural testosterone. Donike’s group also began the concept of the urinary “steroid profile,” which used a combination of other urinary steroids to increase the sensitivity of the test. Other antidoping research has identified a del/del genotype of UGT2B17 as the cause of a subpopulation of individuals who have low (<0.5) T/E ratios in urine, the use of 11 steroids in urine to improve test sensitivity, new metabolites of testosterone (e.g. testosterone cysteinate) in the urine, and several substances that affect the metabolism and excretion of testosterone [043].

To measure testosterone in the urine to detect doping is not adequate because of large interindividual and intraindividual differences in urinary steroid concentration. However, the nearly constant ratio of urinary testosterone glucuronide to epitestosterone glucuronide became the basis of a better test. Epitestosterone is the 17alpha epimer of testosterone and has no known physiological function. It is not a metabolite of testosterone [054]. An upper normal limit of six was calculated for the testosterone/epitestosterone ratio based upon population studies. In 1983 the Medical Commission of the International Olympic Committee (IOC) introduced this value as a criterion for testosterone abuse. Ratios above six should be considered suspicious, and the person concerned should be subjected to further testing. In 2004 the approved upper limit was set at four [055].

In 1984, testosterone was analyzed during the Olympic Games in Los Angeles, where art, technology, instrumentation, and skilled personnel were brought together to combat the use of doping substances [013].

Olympic Games 1980
During the Moscow Olympic Games of 1980, a high frequency of testosterone (T) abuse was suspected. By that time, analytical methods to detect the administration of synthetic anabolic steroids by gas chromatographic-mass spectrometric (GC-MS) screening procedures had
improved. Definitive proof of anabolic steroid abuse in sports was not possible prior to the introduction of combined gas chromatography/mass spectrometry (GC/MS). It was now given a report of the early history (1960-1980) of GC/MS and radioimmunoassay, and how these techniques were utilized in the first years of steroid doping control in athletics. There were several key individuals and research groups involved in the early technical developments, and their essential contributions have been acknowledged. The Oakland USA laboratory was the first IAAF (International Association of Athletic Federations) sanctioned site to do steroid GC/MS steroid analysis resulting in athletes being disqualified from competition. This gave notable successes, including the only East German female competitor ever suspended during the tenure of the DDR [056].

**Endogenous steroids**

Due to better methods to detect anabolic steroids, athletes switched to endogenous steroids like T. Quantitation of T as a way to detect T abuse was inadequate because of its high metabolic turnover rate, circadian rhythm of T excretion, and an interindividual excretion variability. In response to that, Manfred Donike (1933-1995, a German chemist with an early career as a cyclist who in 1960 and 1961 competed in Tour de France) and coworkers at the German Sport University in Cologne in 1982 reported a method for detecting testosterone abuse. They based their method on the fact that exogenously administered testosterone is predominantly excreted in the urine as the glucuronide conjugate. By determining the ratio between testosterone and epitestosterone (T/E), they eliminated the influence of urine density variations. The mode of the population distribution of T/E ratios is about 1:1 and early research suggested that ratios above 6:1 were linked to doping. WADA has later decreased the ratio consistent with doping to 4:1. In the early 1990’s, intra-individual biological variability of the T/E ratio began to be used in combination with population ranges to detect doping [003].

**Background to T/E ratio**

It was reported that after oral, rectal, or intramuscular T administration, the excretion of TG increased more than other T metabolites. Epitestosterone (E) was found not to be a metabolite of T because deuterated T administration did not result in significant deuterated EG excretion. The origin of epitestosterone is still discussed. Although Dehennin showed that half of total E production is of testicular origin, the remaining 50 percent is still debated. Administration of adrenocorticotrophic hormone (ACTH) results in an increased EG production, indicating an adrenal origin. Also, adrenal insufficiency as observed in Addison’s disease correlates to significantly decreased T and E excretion rates. Also peripheral production is possible. The mean T/E ratio of urine samples of Caucasian males and females in the first population study of Donike et al was 1-2. The values showed a logarithmic normal distribution with an upper limit value lower than 6. Using these data, the Medical Commission of the International Olympic Committee (IOC) banned the use of T in 1982 and stated that a T/E ratio above 6 was sufficient proof of T abuse. When applying this criterion in research and routine analyses, cases of naturally occurring T/E ratios above 6 appeared. Dehennin et al administered testosterone enanthate in several doses intramuscularly to healthy men over a period of six months. They found via linear interpolation between doses that the T/E ratio exceeded the cutoff point of 6 when natural production (around 45 mg/week) was doubled by weekly administration of a comparable dose of exogenous T [057].

**US legal actions**

The US Food and Drug Administration approved methandrostenolone in 1958. Although officials have banned PEDs from Olympic competition since 1967, and the International Olympic Committee has prohibited AAS use since 1975, it was not until 1991 that the US Congress designated AASs as Schedule III controlled substances. In 1994, the Drugs
Supplement Health and Education Act (DSHEA) was approved in the United States and several new steroids were commercialized as nutritional supplements. Initially these new steroids were precursors of testosterone, commonly referred to as “prohormones”. The Anabolic Steroid Act of 1994 placed anabolic steroids as well as their precursors on the controlled substance list. Possession of the drugs without a prescription was now a federal crime. Late 2004, the US Congress approved the Anabolic Steroid Control Act (ASCA), restricting the sale of anabolic steroids as nutritional supplements. This act included testosterone and all related chemical or pharmacologic substances that promoted muscle growth. Corticosteroids, progestins, and estrogens were not included in this act. However, by 2004 a range of prohormones derived from other steroids, including 19-nortestosterone, boldenone and even 17alpha-alkylated steroids were available as over-the-counter preparations. The Anabolic Steroid Control Act amended the Controlled Substances Act and expanded its definition of anabolic steroids. The new definition, which does not require proof of muscle growth, identified 59 specific substances (including their salts, esters, and ethers) as anabolic steroids and listed them as Schedule III controlled substances. Most of the PEDs that athletes and nonathlete weightlifters used before the 1990s were pharmacologic agents approved for medicinal or veterinary use [058].

By the 1990s, various androgen precursors became available over the counter as unregulated nutritional supplements. Androgen precursors are either inactive or weak androgens that the body converts into potent androgens. These include naturally occurring precursors to testosterone such as 4-androstenedi ol, 5-androstenedi ol, 4-androstenedi one, and dehydroepiandrosterone as well as precursors to synthetic AASs, including 4-norandrostenedi one, 4-norandrostenedi ol, and 5-norandrostenedi one, which the body converts to nandrolone. The widespread, unregulated sale of dietary supplements on the Internet has greatly increased the number of anabolic steroids available. Of even greater concern is the introduction of synthetic anabolic steroids such as 17-desmethylstanozolol, methylclostebol, and methyltrienolone into the market as dietary supplements. The Steroid Control Act of 2004 banned most of these substances. However, we are now seeing novel synthetic designer androgens, such as tetrahydrogestrinone and madol. Because these designer steroids have not undergone toxicologic or safety testing in humans or animals, they potentially pose an even more serious health risk than the more traditionally used AASs, which have received some level of animal or human testing [003].

France and Belgium

The decade of 1960-1970 was marked by many historical events opposed to doping, such as the creation of a council, composed of 22 nations, proposing a resolution against the use of doping agents in sports. And, in 1963, France adopted Antidoping Legislation, and two years later (1965) Belgium followed in the footsteps of France. But the event that marked the decade was the death of cyclist Tommy Simpson due to the nonmedical use of amphetamines during the Tour de France. This episode led the IOC to take forceful steps to try to prevent the use and misuse of doping substances in sports [013].

World Anti-Doping Agency (WADA)

On November 10, 1999, in Lausanne, Switzerland, the World Anti-Doping Agency (WADA) was created. “The WADA aims to promote and coordinate the international fight against doping in sport and to foster a culture of doping-free sport.” Finally, in 2003, representatives of various governments, including Brazil, gathered in the capital of Denmark, with the international Olympic movement, aimed at the unification of doping control policies at the national and international levels, where participants signed the Copenhagen Declaration, approving the World Anti-Doping Code. Since the signing of the Declaration of Copenhagen, resulting in the adoption of the World Anti-Doping Code (2004 and later revised in 2009),
Brazil has focused on creating policies of suppression against doping. However, prior to this event, laws did exist that regulated the use of substances aimed at improving athletic performance [013].

History of nandrolone

The anabolic androgenic steroid 19-nortestosterone, also called nandrolone, was first synthesised by Birch in 1950. The use of nandrolone by athletes became popular in the late 1950s. The International Olympic Committee (IOC) prohibited the use of nandrolone in sport in 1976. A study in 1982 appeared to have found NA, or a similar compound, in the urine of athletes who had not used nandrolone. In 1996, the IOC stated that a critical concentration for nandrolone metabolites in the urine had been established. A doping offence for nandrolone was defined as a concentration of NA in human urine exceeding 2 ng/mL in men and 5 ng/mL in women [059].

History of aromatase inhibitors

The use of aromatase inhibitors has been prohibited for male athletes since September 1, 2001 [060].

History of 5-alpha-reductase inhibitors enzymes (e.g. finasteride)

Scientists in the pharmaceutical industry reasoned that if 5AR could be targeted for inhibition after the external genitalia were fully formed and mature, then a safe drug to shrink the prostate, relieve lower urinary tract infections, and ameliorate baldness and acne might be developed. Eighteen years after Imperato-McGinley's first publication, the "prostate pill" arrived; the US Food and Drug Administration (FDA) approved finasteride June 19, 1992 for the treatment of men with symptomatic BPH. FDA approval for male pattern hair loss (in men only) followed, and in October 2002, the dual 5ARI dutasteride was approved. Both drugs currently claim to improve symptoms, reduce the risk of acute urinary retention, and reduce the risk of the need for BPH-related surgery. Soon after the story became known, and the implications of 5AR deficiency became clear, Merck began an ambitious development program in the research laboratories at Rahway, New Jersey. Following synthesis of many potential 4-aza steroid molecules that would inhibit 5AR, a drug known as MK-906 was selected as the best therapeutic molecule. After successful testing in experimental animals, where the drug was found to sharply reduce DHT levels and prostate volume, finasteride went into human trials in 1986. A few years later, reports of phase I testing were reported by Stoner, Gormley and colleagues, Rittmaster and colleagues, and others. As expected, men treated with finasteride developed a marked suppression of DHT, no change or slight elevation in serum testosterone, and no change in all other serum components studied. In 1992, the phase III studies, demonstrating safety and efficacy over 1 year of treatment in men with symptomatic benign prostate hyperplasia, were published in the New England Journal of Medicine, concomitant with FDA approval. As finasteride was known to be a pure Type 2 inhibitor of 5AR, efforts soon began to develop a drug that would inhibit both Type 1 and 2, theoretically a more powerful inhibition. Merck went into phase II testing of such a molecule in the early 1990s (MK-434), but trials were quickly halted because of potential toxicity problems, and the drug was never developed. A dual inhibitor from GlaxoSmithKline, originally known as GG745 (dutasteride), was developed later in the decade, and in 1998, early-phase clinical trial results were published. The dual inhibitor was found to lower DHT serum levels significantly more than finasteride (90 % with dutasteride vs 70 % with finasteride), offering the potential for greater clinical efficacy of the new drug. Although direct,
long-term comparisons of finasteride and dutasteride in a clinical trial are not available; the phase III dutasteride data published in 2002 showed that dutasteride yielded symptomatic improvement over placebo as early as 3 months and a prostate shrinkage exceeding 25 percent, both quicker and more profound than what had been seen in the finasteride trials [061].
HISTORY OF BLOOD DOPING

Semantics

The word “doping” is said to be used in the 1860s to describe a drug used for horse racing that consisted of opium and narcotics. With human athletes, “blood doping” originally referred to a process whereby athletes increased their oxygen-carrying capacity by receiving blood transfusions from previously donated blood to increase their hematocrits a few days before competition. Today, “blood doping” is used more synonymously with cheating of any kind, including any of the various blood-boosting methods or classes of ergogenic aids that are available to athletes. Advances in genetic medicine have allowed athletes to raise their level of sophistication significantly by using PESs that are virtually undetectable [062].

Physiological background for blood doping

Shortly after the discovery of blood circulation by the English physician William Harvey in 1628, the first empiric blood transfusion was attempted. There is therefore a long history of blood doping, conventionally originating with the anecdote of athletes being encouraged to drink reindeer blood or something like that to achieve extraordinary performances. By the 1930s it was clear that champion endurance athletes had remarkably high maximal O₂ uptake (VO₂MAX). In the 1950s, 1960s, and 1970s, classic studies were performed on the physiological determinants of VO₂MAX and on its key role in endurance performance. During this time there was much debate on O₂ delivery versus O₂ extraction as the “limiting factor” for VO₂MAX. Observations during this era clearly established the role of maximal cardiac output as a determinant of VO₂MAX, and very high maximal cardiac output values were seen in champion endurance athletes. In addition, the important role of blood volume and total body hemoglobin as determinants of VO₂MAX also emerged. In an effort to better understand the physiological determinants of VO₂MAX, studies were then conducted that attempted to manipulate O₂ delivery using a variety of approaches including altered concentrations of inspired O₂, drugs that speed or slow the heart, and, as will be discussed here, techniques that altered total body hemoglobin and hemoglobin concentration. In general, by the 1970s it was clear that maneuvers that increased total body hemoglobin increased VO₂MAX and maneuvers that reduced total body hemoglobin reduced VO₂MAX. These changes in VO₂MAX appeared to be somewhat independent of total blood volume because volume loading per se had little impact on VO₂MAX, and likewise maneuvers that cause hemodilution did not increase VO₂MAX. Therefore, the importance of total body hemoglobin as a primary determinant of VO₂MAX was emphasised. In parallel with these mechanistic studies on the determinants of VO₂MAX, applied observations on athletic performance and the role of VO₂MAX, lactate threshold, and running economy emerged. As VO₂MAX was seen as a key determinant of performance, the next obvious question was whether or not maneuvers that increased total body hemoglobin and VO₂MAX would also increase performance. A number of studies confirming the positive impact of increased total body hemoglobin on performance were then conducted. In addition, a variety of rumors and innuendo suggested that at least some endurance athletes were using this technique in an effort to gain a competitive advantage in international competition. Thus, the term “blood doping” was coined. Although it is clear that blood doping improves performance, it is unclear how widespread it was in the 1970s and 1980s as detection was difficult because athletes received a reinfusion of their own red blood cells [063].

During the Olympic Games held at moderate altitude (2250 m at sea level) in Mexico City in 1968, altitude-induced blood adaptations such as an increase in hemoglobin concentration were considered primarily responsible for athletes living at altitude winning most of the
endurance races and this phenomenon instigated a new research focus on altitude training in the field of sports physiology [064].

In 2000, Birkeland et al demonstrated that rHumanEPO administration for 4 weeks in 20 male athletes increased hematocrit from 43 to 51 percent, VO\textsubscript{2}max by 7 percent as well as time to exhaustion by 9 percent. The practice of red cell transfusions has made a strong comeback in recent years in response to the development of antidoping tests. Based on testimonies from athletes, it is now known that athletes are using rHumanEPO in combination with blood transfusions [064].

**Blood transfusions**

Blood transfusions as a means for improved endurance were researched as early as 1947 by Pace and coworkers. Transfusion of 500 ml of allogeneic erythrocytes on four consecutive days reduced the pulse rate during exercise in simulated hypoxia. However, even though the methods would not meet today's standards; this was the first of many studies to confirm the possible performance enhancing effect of blood transfusions. Accordingly blood transfusions in sports were later banned by the International Olympic Committee (IOC) in 1986 [065].

**Research**

Although the earliest proof of improved sport performances after blood transfusions was provided already in 1947, the first evidence of blood doping came later, in 1972. Despite that blood transfusions, as a method to enhance endurance performance, gained attention already after the Olympic Games (OGs) in Mexico City in 1968. Before these OGs, evidence was presented that a lowered atmospheric pressure would decrease performance in all athletic disciplines dependent on a high level of sustained oxygen uptake. This was indeed confirmed in Mexico City, where all winning times in running races above 800 m were significantly worse than the world records at that time. This highlighted the impact of the oxygen delivery to the working muscles as a limiting factor during whole body endurance exercise. It also became evident that runners hailing from higher altitudes tended to be superior to competitors from lowlands because they had “thick blood” with high hemoglobin content. A relatively straightforward way to increase the hemoglobin concentration (Hb), and, hence, oxygen delivery to the muscles is, thus, by blood transfusions. This was documented in the classic study by the Swedish sports physiologists Björn Ekblom and co-workers published in 1972 where a high correlation between hemoglobin and performance capacity after blood withdrawal and reinfusion was presented. An overnight increase in hemoglobin by 13 percent caused by the reinfusion of 3 units of stored autologous blood resulted in an increase in maximal oxygen uptake and physical performance capacity of 9 percent and 23 percent, respectively. The method of transfusing blood in a sport setting was hereafter dubbed “blood doping” by the media, and its potent effect on athletic performance was quickly noted in the sports community [066].

**Practical doping**

The first alleged use of blood boosting in sport was in the 1960s, when a French four times winner of the Tour de France (1961-1964) was named as one of the first cyclists to use the technique. Blood doping was certainly used already at the OG in 1972 by a Finnish steeplechaser, and during subsequent OGs, several athletes admitted having used blood doping. The technique became more popular during the 1980s and was used by distance runners (5000 m, 10000 m, marathon runners), cyclists, and skiers. Specific accusations were made against the Russians, Italians, Finns, Americans, and East Germans, particularly during the 1980 and 1984 Olympics. Athletes who admitted using the technique included the
Italian cyclist who beat the one hour world record in 1984 and a Russian distance runner who specifically admitted to autologous transfusion with two units by team doctors in 1980 [067].

Not until after the OG in Los Angeles in 1984, where the US cycling team used blood doping and won 9 medals after not having won a medal in cycling for 72 years, the method (both homologous and autologous transfusions) was prohibited by the International Olympic Committee, although no method was available to detect its use. Since then, there are consistent records of athletes experimenting with blood transfusions who achieved incredible success in competitions. Besides the first anecdotal reports, this technique became fairly popular during the 1980s and was widely used by distance runners, cyclists, and skiers, particularly during the 1980 and 1984 Olympics. Although no reliable test had been devised for unequivocal detection, the International Olympic Committee (IOC) officially banned blood doping after the 1984 Olympics. In the same year, the USA Olympic Committee declared that seven cyclists, including four medallists, out of 24 athletes of the national team who participated in the Olympic Games, used transfusions [068].

Lasse Viren, a Finnish long distance runner who won gold medals at the 1972 and 1976 Olympic Games in the 5,000 m and 10,000 m, is believed to be among the first athletes to have used blood transfusions to improve performance. It should be noted that this technique was not banned at the time and although the ethical debate on the topic was in full swing, it was only in 1986 that the International Olympic Committee banned blood transfusions. Other than the anecdotal evidence from the Nordic distance runners, there are other reports on more systematic use of transfusions in the context of major sporting events in the 1980s. Notably, it is well established that a large part of the US cycling team was involved in a systematic blood doping program that earned them unprecedented success at the 1984 Olympic Games in Los Angeles. There is also some evidence that blood transfusions were an integral part of the doping regime used for the enhancement of performance for athletes from the Eastern bloc (Soviet Union, East Germany) at that time. Nevertheless, it can be assumed that because of the logistic requirements of blood withdrawal and reinfusion, the technique was not widespread, as the technical necessities were only available to a small number of athletes, but nevertheless available to certain elite athletes [069].

Years later, following the implementation of reliable strategies for detecting doping with recombinant erythropoietin and analogues, blood transfusions, which had fallen out of favor, made a strong comeback. In March 2002 at the Salt Lake City Olympics, the IOC investigated the discovery of discarded blood transfusion equipment at the quarters of the Austrian cross-country skiers. Following DNA testing, two Nordic skiers (who had been placed in the 40s, and not the Austrian team’s three medallists) were disqualified and had their results cancelled. For the same reason, some professional cyclists, one of whom nearly died after being injected with poorly stored blood, were found guilty and suspended in 2004 [068].

Although testing for homologous blood transfusions had been performed at the Lillehammer OG in 1994 by use of antigen testing cards, it was not until 2004 at the OG in Athens that a test had been validated and implemented. At this event, the gold medal winner of the men's time trial in cycling was first tested positive, but because the backup sample (B-sample) was frozen and, thereby, the red blood cells (RBCs) destroyed, no doping offense could be proven. After he failed further doping tests at the 2004 Vuelta a España, the rider was suspended for 2 years [066].

The suspension of several professional road cyclists from the 2006 Tour de France could represent the tip of the iceberg, with more than 200 athletes in different sports disciplines implicated in an international doping probe including blood transfusions and exogenous hormone administration. In an apartment building in Madrid occupied by a doctor, Spanish police discovered clandestine equipment for international performance enhancement, seizing
more than 200 450 mL blood bags, along with records and several other doping substances, which allowed investigators to finally match code names of athletes with their highly detailed doping records. This sophisticated pan-European doping ring either treated athletes locally or arranged the transport of stored blood through a system of couriers to athletes at race sites. Hence, based on the riders named in this one investigation, the problem is endemic [068].

In 2004, UCI introduced a blood test for the detection of homologous blood transfusion to enhance the analysis of indirect hematological parameters indicating blood manipulation. The two observations from the UCI and FIFA clearly indicated that the risks of blood doping abuse are different in individual endurance sports compared with Olympic team sports, for example. Consequently, appropriate risk management must be based on data from adverse or atypical analytical findings as well as after considering the estimated risk of doping in different sports. It was acknowledged that the latter may be difficult to determine accurately [036].

**Autologous blood transfusion (ABT)**
The obvious alternative to homologous blood transfusions is autologous blood transfusions (ABTs). In 2006, just before the Tour de France, a large Spanish doping scandal evolved, known as Operación Puerto. A doping ring involving several physicians was uncovered by the Spanish police, and more than 200 autologous blood units belonging to professional athletes were found in freezers and refrigerators for subsequent reinfusion. Detailed doping calendars from individual athletes were published, and the modus operandi of involved athletes and their physicians was uncovered. From these calendars, it became evident that besides the massive abuse of a wide range of different performance-enhancing drugs and masking agents, ABTs were used during important competitions. The procedure of blood withdrawal and reinfusion was performed numerous times for each individual athlete during the year by using specialized equipment for phlebotomy and storage. Typically, blood was withdrawn after competitions and reinfused few days before 1-day races or before and during multiple-day competitions. Since then, athletes testing positive for other substances have confirmed the ongoing abuse of ABT today [066].

**Erythropoietin**

*Physiological background*

Increasing delivery of oxygen to the active muscles and making energy efficiently from the oxygen is the most effective way to increase performance. Increasing the number of RBCs is the most effective way to increase aerobic performance. Erythropoietin, Epo, represents a prototypical success of molecular biology. The presence of Epo was first suggested in the nineteenth century based on the high blood viscosity of people living in or returning from high altitude areas. The production of red blood cells was linked to blood oxygen pressure more than a century ago. In 1906 Carnot and DeFlandre described a substance capable of stimulating the production of red blood cells, later known as erythropoietin (Epo). Experimentally, the presence of Epo as an erythropoietic humoral factor was discovered due to the erythropoietic property of serum from phlebotomized rabbits. Most notably, in 1977, it was purified Epo from 2550 liters of urine from patients of aplastic anemia and determined its amino acid sequence. Based on this finding, the human Epo genes were cloned in 1985. Erythropoietin has been commercially available since 1989 principally for disease states such as for treating the anaemia seen in chronic renal failure. Then, recombinant human Epo (rHuEpo) was successfully used to treat anemic patients with end-stage renal diseases (ESRD). Furthermore, studies on Epo gene regulation led to the identification of hypoxia-inducible transcription factors (HIFs) and hypoxia response elements (HREs) as the HIF-binding consensus sequence on genome, and to the current understanding of the molecular mechanisms of cellular adaptation to hypoxia [070].
Doping with erythropoietin

The blood doping situation changed dramatically with the commercial introduction of recombinant human erythropoietin (rhEPO), and the first recombinant erythropoietin (rhEPO) was approved by the FDA for treatment of anemia in renal failure in 1989. The first rHuEPO product marketed in the United States was isolated and purified from Chinese hamster ovaries and reproduced using DNA recombinant techniques. One year later the IOC banned the use of erythropoietin (EPO) [069].

Soon after the approval for medical use in 1990, the American Medical Association and the International Olympic Committee banned the abuse of rHuEpo in athletes. However, athletes were quick to exploit the drug, especially in professional cycling, where the scandal at the 1998 Tour de France highlighted this issue when a team employee was caught with a carload of performance enhancing agents, including EPO [071].

Studies investigating the effect of rhEPO on performance were soon published and demonstrated positive effects on maximal oxygen uptake of 6-12 percent. Although the authorities rapidly banned rhEPO, the easy access to the substance and the huge impact on performance resulted in widespread abuse of rhEPO during the 1990s/2000s. It is believed that this substance had a considerable impact on the development of peak performances in all endurance sports during these years and there are even scientific attempts to prove this for several sports on the basis of performance analysis. The abuse was facilitated by the fact that no detection method was readily available at that time. From a practical point of view, the impact of rhEPO on performance in endurance sports is best illustrated by a quote from Greg Lemond, an American cyclist who won the Tour de France in 1986 and 1989, i.e., before rhEPO became available, recalling the 1991 race: “I was the fittest I had ever been, my split times in spring training rides were the fastest of my career, and I had assembled a great team around me. But something was different in the 1991 Tour. There were riders from the previous years who couldn’t stay on my wheel who were now dropping me even on modest climbs.” These words accurately describe how rhEPO changed the entire world of endurance sport in the following decades and divided the athletes’ performance primarily between rhEPO users and non-users [069].

Because of its logistic advantages compared with blood transfusions, human erythropoietin (rhEPO) became the preferred blood boosting method by athletes after it had been available. At the OG in Sydney in 2000, two tests for rhEPO were introduced: a “direct test” that was able to distinguish rhEPO from endogenous molecules by isoelectric focusing and an “indirect test” based on changes in blood parameters caused by rhEPO administration. Because of the introduction of these tests, old-fashioned blood doping reentered the scene [066].

Logically, following widespread abuse from the 1990s onwards, doping scandals involving rhEPO or blood transfusions have shaken the world of sport on a regular basis, culminating with the investigation of Lance Armstrong, who subsequently – after many years of denying and without one single positive doping test – admitted the use of both rhEPO and blood transfusions throughout his career. Although, it is therefore common belief that many recent doping cases were not unveiled by conventional anti-doping testing, but rather by police investigations or admissions from athletes or staff, thus non-analytical approaches, anti-doping laboratories were able to detect about 400 cases testing positive for rhEPO between 2003 and 2011 (World Anti-Doping Agency (WADA) statistics). Analytics have therefore come a long way in the detection of blood manipulation in sports and still outperform police investigations by 10 to 1 [069].

The popularity and effectiveness of rHuEPO in elite endurance athletes is demonstrated by a long list of anecdotes associated with its misuse during international competition. When the
average speed of the cyclists racing in the Tour de France began to increase suddenly during the 1990s, rumors of rHuEPO use began to circulate. rHuEPO was available in Europe by 1987. Finally, suspicions of rHuEPO use in professional cyclists competing in Europe were confirmed during the 1998 Tour de France; boxes of ampules containing rHuEPO were found in team vehicles and the personal rooms of riders from many of the biggest and most successful teams. It became embarrassingly clear that rHuEPO use in elite professional cyclists was organized, widespread, and sophisticated [062].

A proxy for failing erythropoietin testing

In the late 1990s, as a first step, “no start” rules were introduced with the official objective to protect the health of the athletes when certain blood markers exceeded definite limits (e.g. hematocrit (Hct) above 50 percent or hemoglobin (Hb) above 17 g/dL (International Cycling Union, UCI) or Hb above 17.5 g/dL in men and 16.0 g/dL in women (International Ski Federation, FIS). In this time, the widespread use of rhEPO can be assumed on the basis of indirect evidence; e.g. in elite cross-country skiers extreme Hb values up to 20 g/dL were common between 1994 to 1996 but disappeared after the “no start” rule was introduced in 1997. Yet, mean Hb values continued to rise, suggesting the further use of artificial methods with fewer extremes. It became obvious that the use of upper limits of definite blood values may result in athletes who would titrate rhEPO to approach the target Hb or Hct without exceeding it [069].

Plasma volume fluctuations resulting from changes in posture, exercise, and training, altitude exposure, season as well as storage conditions influence concentration-based blood values such as Hct and Hb and thus represent a major limitation of their use with absolute limits. Additionally, cheating athletes may manipulate abnormally elevated Hb and Hct values by intravenous infusions of normal saline leading to hemodilution. On the other hand, even clean athletes may be declared unfit as Hb and Hct in a normal distribution may exceed the given limits. The panel of indirect markers was extended and more evidence was gathered on the effect on blood values of rhEPO administration in training athletes. It was suggested the use of a combination of indirect markers of altered erythropoiesis (reticulocyte Hct, serum EPO, soluble transferring receptor, Hct, %macrocytes) in a multivariate statistical model for detection of rhEPO during a possible administration phase (ON models) and after recent cessation of rhEPO use (OFF models). The sensitivity of these models was improved with larger numbers of subjects and resulted in the introduction of the so-called second-generation blood tests of which the OFF-hr model, a score combining Hb and %retics, is part of the current ABP according to the WADA ABP operating guidelines. Although OFF-hr was originally described for the detection of rhEPO use, it is also sensitive to other forms of blood doping such as blood transfusion [069].

The application of these models by sports authorities and anti-doping organizations was problematic despite their scientific impact. The OFF-hr model was used by certain sports federations as another ‘no start’ criterion. Yet, infringements of the “no start” rule were equal to failing a “health test” but not considered a violation against WADA’s anti-doping code and therefore only yielded short mandatory interruptions of competition, e.g. 2 weeks. As even these improved biomarkers were only compared with a population-based reference range in a cross-sectional setting (e.g. universal limit of Hct above 50 %, OFF score greater than 122), it already seemed likely in 2000 that a longitudinal, individual hematologic profile, the so-called hematologic passport, could be advantageous to prevent and perhaps detect blood doping. Various attempts were made to define the natural within-subject and between-subject as well as analytical variability to use longitudinal measurements as an instrument against blood doping [069].

Originally, the only means to test for doping by blood transfusion was the adoption of arbitrary thresholds for hematocrit and/or hemoglobin. Blood doping practices were
suspected when blood tests showed hemoglobin values exceeding 175 g/L for men and 155 g/L for women (International Ski Federation), and hematocrit values above 0.50 for men and 0.47 for women (according to the International Cycling Union). Athletes with random values exceeding such limits were prevented from racing in official competitions. Nevertheless, such a questionable strategy involved several drawbacks, including the difficult interpretation of several hematological parameters because of wide inter-individual variability, the possible occurrence of false positive results that would have penalized clean athletes with naturally increased values, and the possibility to arbitrarily expand or titrate the RBC mass up to the allowable threshold [068].

The International Olympic Committee (IOC) added rHuEPO to its list of banned substances in 1990, even though all forms of blood doping had been officially prohibited since 1984. Despite justified suspicions of rHuEPO use in cycling and the inability of current methods to detect its use, in 1997, the governing body of the International Cycling Union (UCI) enacted hematocrit cutoffs for male (50 %) and female (47 %) cyclists while more reliable methods of detection could be developed. The hematocrit cutoffs were based on existing normative data on elite athletes, taking into consideration the expected effect of dehydration, in an attempt not to exclude athletes with normal variations but to protect athletes from danger. Anyone over that limit would be considered “unfit to race” and could not compete for 2 weeks, although they were not subjected to official sanctions. To circumvent this, an athlete could inject rHuEPO every 2 to 3 days over 3 to 4 weeks, along with some form of iron supplementation, to get a desired effect and then reduce the dose to match the basal rate of endogenous EPO production to maintain one's hematocrit just below the “legal limit” [072]

**Doping problems in cycling**

The introduction of recombinant human erythropoietin (rHuEPO) in the early 1990s sparked a new capacity in performance enhancement primarily for endurance sports such as cycling and track and field – detection during this time was unlikely. The US pursuit cycling team unexpectedly won gold at the XXIII Olympic Games in Los Angeles. It was later revealed that they transfused blood and that some of the cyclists had suffered severe transfusion reactions [12006]. In 1990, erythropoietin was included on the list of prohibited substances by the International Olympic Committee because misuse by athletes was suspected, although no approved test existed. There were rumors during this time that athletes used large doses of rHuEPO to induce very high-blood parameter values. For example, Bjarne Riis, the 1996 Tour de France winner (who admitted doping in 2007), acquired the nickname of “Mr 60 percent” which referred to his hematocrit level. To help athletes avoid detection, individual physicians in countries such as Italy and Spain “masterminded” drug prescription. This resulted in high profile cases in the Tour de France (especially the Festina affair in 1998) and Giro d'Italia such as Marco Pantani, Tyler Hamilton and, most recently, Lance Armstrong. Arguably, these cases represent only the “tip of the iceberg” and exposed the insufficiencies of the current antidoping system. During the 1990s, “blood doping” in the form of rHuEPO use and/or blood transfusions became widespread. In response, the IFs such as the International Cycling Union (UCI), FIFA and the IOC introduced the direct urine test and the sampling of blood to detect blood manipulation as evidenced by direct and indirect parameters. The analysis of indirect blood parameters conducted during the Tour de France in 1997 revealed significantly higher levels of hemoglobin, hematocrit and reticulocytes compared to the normal population, while a similar analysis of blood samples from players participating in the 2002 FIFA World Cup in Korea/Japan was consistent with normative data. During the 2000 Sydney Olympics, the IOC approved the use of a test developed by the Australian Institute of Sport to detect rHuEPO users [062].

Despite the new hematocrit rule, the Festina scandal at the Tour de France in 1998 provided the proof of organised and widespread doping in professional cycling and highlighted the need for the creation of an independent international agency, which would set unified
standards for antidoping work and coordinate the efforts of sports organisations and public authorities [064].

Evidence from many believable anecdotes, from sworn testimony to the United States Anti-Doping Agency (USADA), and from police raids at the 1998 Tour de France and before the 2006 Tour de France shows that cyclists continue to abuse rEPO. Indeed, top South African cyclist David George was caught on rEPO (and admitted it) in August 2012. The culture of competitive cycling dies hard; despite the deaths and the black box warning, rEPO abuse in cycling has endured for 25 years. In his affidavit to USADA, Stephen Swart, a teammate of Lance Armstrong in 1994 to 1995, said that their Motorola Team used rEPO for the 1995 Tour de France, and that most riders, including Lance Armstrong, had a hematocrit over 50 percent. It is widely reported that Marco Pantani abused rEPO and had a hematocrit of 60 percent in a 1995 race. As noted in Tyler Hamilton’s recent tell-all book, Bjarne Riis won the 1996 Tour de France on rEPO, and his peak hematocrit was an astonishing 64 percent. For cyclists who abuse rEPO, there may be a thin line between winning and dying. Alas, the deaths continue. From early 2003 to early 2004, eight more European cyclists died, and up to five fit the profile of a likely rEPO death. Notable were French cyclist Fabrice Salanson, 23, found dead in his hotel room just hours before he was to start the Tour of Germany, and Belgian cyclist Johan Sermon, 21, who went to bed early to rest up for a planned 8-h training ride the next day, but was found by his mother dead in bed at dawn. In early 2009, the promising Belgian cyclist Frederick Nolf, 21, died at night in his Ritz-Carlton hotel room, after the fourth stage of the Tour of Qatar. He went to bed laughing and happy and never woke up. No autopsy was done. How the autopsy results on Salanson were described to the press may come closest to the truth in this long, sad saga. Dr Jan Dressler of the University of Dresden Medical Institute said the death was probably caused by the heart enlarging and the coronary vessels failing to pump enough blood [073].

**Early testing strategies for blood doping**

The IOC’s decision to adopt the two EPO tests for the Sydney games was a genuine milestone in antidoping science. Recombinant EPO, a peptide of 165 amino acids produced by genetic engineering, is among the world’s top selling pharmaceuticals. In approving one of the EPO tests, the IOC is for the first time requiring athletes to give blood samples for doping control. Developed by Michael Ashenden and his colleagues at the Australian Institute of Sport near Canberra, the test measures the EPO concentration in blood as well as four other factors affected by raised EPO levels. Precursors of red blood cells, known as macrocytes and reticulocytes, are overproduced in bone marrow when EPO levels are raised, and they leak out into the circulation. So Ashenden’s test measures the levels of red blood cells and these two precursors. It also measures the serum concentration of a protein called soluble transferrin receptor, which is involved in iron metabolism – and as such influences the production of the oxygen-carrying hemoglobin complexes found in red blood cells. The other test, described earlier in 2000 by Françoise Lasne and Jacques de Ceaurriz of the French National Anti-Doping Laboratory in Châténay-Malabry, near Paris, detects directly the presence of recombinant EPO in urine. The test is based on a subtle difference between human EPO and that produced in vitro for pharmaceuticals. The recombinant EPO has the same amino-acid sequence as the natural hormone but, because it is produced from non-human cells, it has a different number of sugar residues attached to it. As a result, the electrical charges on the two forms of EPO are different and they can be separated using an electrophoretic technique called isoelectric focusing. To avoid the possibility of false positive results in Sydney, sanctions will only be taken against athletes who fail both tests. The blood changes tested for by Ashenden’s method linger for two to three weeks after an athlete stops taking EPO, but the recombinant EPO itself is flushed out of the body within a few days. After watching the performance of the EPO tests in Sydney, IOC-accredited labs will decide
whether to include them in their battery of standard tests. In 2000, these cost around 150 euros (USD 131) for competition samples and 100 euros for out-of-competition testing. The intensity of the war on doping will always depend on money, both for carrying out the tests and for developing new ones. In 1998, the IOC decided to fast-track the development of the EPO tests by investing USD1 million. This figure was matched by the Australian government [025].

The advent of recombinant protein therapeutics in the late 1980s ushered in a new era for dopers. The lay press speculated that the deaths of 18 European cyclists were related to the availability of recombinant human erythropoietin (rhEPO). rhEPO stimulates the production of red blood cells in the bone marrow, resulting in increased red blood cell mass. The development of a test for rhEPO caused the athletes to change the route of administration from subcutaneous to intravenous, decrease the dosage, and increase the frequency of administration in order to avoid detection. At the Salt Lake City Games in 2002, three winter endurance athletes had Aranesp (darbepoetin-alpha), a novel erythropoietin stimulating protein, detected in their urine samples – 7 months after approval in the European Union and 5 years before the FDA approved its medical use in the United States. Information gathered from investigations confirms that with the advent of tests for prohibited peptides and proteins like EPO, some cheating athletes changed to autologous blood transfusions to increase RBC mass [043].

**History of detection of recombinant erythropoietin and derivatives**

Direct detection of a forbidden substance in a biological matrix such as urine or blood obtained from an athlete is the classical forensic approach to prove doping. This approach has long been the sole strategy, only fine-tuned by improving the sensitivity of the analytical detection methods and by optimizing the timing of testing. The basic principle of the direct detection of forbidden substances relies on the fact that these substances are different from the normal constituents of the human organism. With the introduction of recombinant drugs such as rhEPO, this principle was not valid anymore, as the recombinant constituent was virtually identical to the endogenous version of the substance. Thus, at first, doping tests could not differentiate between the natural, endogenous and the artificial, exogenous recombinant version of the drug. This has, for a long time, been a major difficulty for the testing laboratories. In 1995, it was described a method to separate the natural from exogenous EPO through electrophoresis, but other laboratories could never replicate their results and the described method never reached the stage of validation. Only in 2000, thus more than 10 years after the estimated beginning of rhEPO abuse in sports, the first practicable and validated test to directly detect rhEPO in urine was published. This test relied on a difference in glycosylation between the endogenous and the exogenous EPO molecules, which resulted in different migration characteristics during isoelectric focusing (IEF). The recombinant EPO was industrially harvested from transfected hamster kidney or ovarian cells and, owing to the difference in cell organelles, a minor posttranslational difference in glycosylation between the rhEPO (made by the hamster cells) and the endogenous EPO (made by the human kidney cells) occurred, although the amino acid sequence is identical. The rhEPO molecules are less negative and will thus move differently from endogenous EPO in an electric field, which can be demonstrated using the IEF. Further developing the approach of Wide, IEF is then followed by double blotting, which addresses the problem of non-specific binding of the EPO molecules. Although relatively cumbersome, the method soon identified the first athletes testing positive, namely Roland Meier and Bo Hamburger, both cyclists. Ironically, Hamburger was later acquitted by the court of arbitration of sports on formal grounds (i.e. a lack of harmonization of the positivity criteria for the EPO tests between laboratories was identified). This issue has since been addressed and strict positivity criteria apply, based on acceptance, identification, and stability principles. rhEPO positive samples, for example, have to show at least three acceptable, consecutive bands in the basic area and the two most intense bands measured by densitometry must be in the
basic area. When the analysis is performed in blood (serum/plasma) the intensity of those bands must be approximately twice or more than any band in the endogenous area. Many laboratories nowadays use computer-based classification algorithms to guarantee objectivity in this context [069].

At the 2000 Olympic Games in Sydney, the Australian WADA-certified laboratory first launched a sophisticated anti-doping test for erythropoietin that required both urine and a blood sample. Over 300 tests were performed for erythropoietin for the first time in Olympic history but no positives were reported. This could be due to the fact that the technology for the test was new and questions still existed about the assay [030].

In addition to EPO, there are several EPO analogues that are also effective – for example, darbypoietin. Fortunately these substances, which have a longer half-life, are more easily detectable, and several athletes were suspended for darbypoietin use at the 2002 Winter Olympics [063].

**Hypotheses on deadly erythropoietin doping practice**

It has been noted that recombinant erythropoietin (rEPO) appeared in Europe in 1987, and the unusual deaths began soon thereafter. Between 1987 and 1991, more than 20 Dutch and Belgian cyclists died at rest – some of them while sleeping – as a result of unexplainable cardiac arrest. Between 1997 and 2000, 18 more cyclists died from pulmonary embolisms, stroke, and myocardial infraction. Autopsy results were elusive, but cycling authorities said the deaths were from “heart attack,” “cardiac arrest,” or “cardiac failure.” However, about 15 of the deaths seemed to fit a profile that suggested another explanation: They were young and improving fast, “rising stars” who died not during a race but at rest before or after a race. The rationale for abusing rEPO in competitive cycling is that by raising the hematocrit without unduly raising blood viscosity, one can enhance aerobic performance by enhancing oxygen delivery to muscles. By 1990, the first experiment on the effects of rEPO on athletes had been done, and the lead researcher was quoted to the effect that rEPO might enable an elite athlete to shave 30 s off a 20-min racing time. The problem is that the higher the hematocrit, the greater the risk of clots. Blood clots are the proximate cause not only of pulmonary emboli but also of many strokes and heart attacks. Coagulability hinges partly on blood viscosity, which is set by the plasma fibrinogen level, the deformability of red cells, and the hematocrit. Hematocrit also influences platelet adhesion, the first step in arterial thrombosis. Hematocrit, then, modulates the flow, fluidity, and coagulability of blood. As hematocrit increases toward 60 percent as in mountaineering, for example, blood clots can become a menace [073].

In 1991, a warning appeared in a prestigious medical journal on how fast hematocrit can rise with large doses of rEPO and how rEPO abuse by athletes could drive hematocrit to “dangerously high levels.” By 2007, after esteemed studies in patients with renal disease, cancer, and other major illnesses tied higher dosing of rEPO to greater risk of death from thrombotic events (heart attack, stroke, or venous thromboembolism) or heart failure, the Food and Drug Administration issued a black box warning on these risks from rEPO. Even this did not end the abuse of rEPO by athletes or the deaths. Cyclists continued to dope, dupe, and die [073].

**Sudden deaths in Swedish orienteers**

A spate of deaths in orienteers paralleled the cycling deaths. Suddenly, among young, elite orienteers (but in no other Swedish sports), the death rate spiked to 1 percent a year for 3 years in a row. From 1989 to 1992, seven elite-level orienteers, all from the same small area
of central Sweden, died during or after competitions or training. They knew one another and occasionally trained together. All performed very well shortly before they died; some placed near the top in national competitions. The last, Melker Karlsson, just 24 years old, was a rising star who died after a training run and sauna. His death was the final straw that led to a meeting of Swedish health experts to probe potential causes and solutions. As in the cyclists, the deaths were considered “cardiac,” and a popular hypothesis was a transmissible myocarditis, ascribed first to Chlamydia and then to Bartonella. Their supporting evidence, however, is not compelling and does not dissuade skeptics, from speculating that a culprit in this spate of sudden deaths in top Swedish orienteers was abuse of rEPO [073].

Background to the athlete biological passport

In an investigation of samples obtained as part of routine International Ski Federation blood-testing procedures in participants at the World Ski Championships, abnormal hematological profiles, defined as those deviating from the 1989 Nordic Ski World Championships and the IOC Erythropoietin 2000 project data set, were identified in 36 percent of the skiers tested and finishing within the top 50 places in the competitions. In addition, 50 percent of medal winners and 33 percent of those finishing from 4th to 10th place had highly abnormal hematological profiles. In contrast, only 3 percent of skiers finishing from 41st to 50th place had highly abnormal values. Although these data cannot be immediately associated with blood doping practices, including blood transfusions, and it is very unlikely that blood doping would be less common in other endurance sports, the present situation is highly suggestive of a phenomenon that is not being controlled by the ongoing antidoping testing program. In fact, it has been hypothesized that a combination of blood transfusion and recombinant human erythropoietin administration could also be used by such athletes [068].
HISTORY OF DOPING WITH GROWTH HORMONE

Today, many medical interventions that begin as treatments for disease often expand into therapies that reduce disability, lessen disadvantage, or even confer advantage. Forces that propel profitable drugs, devices, and procedures dominate over considerations of efficient and equitable distribution of resources. This dominance is fueled by industry-physician collaborations often biased by prior assumptions, reliant on surrogate outcomes, and advantageous to marketing. Interventions are justified by “medicalization” of physiologic variations (e.g. short stature) as defects or disease, and nudged into “standard practice” by key opinion leaders. The story of recombinant human growth hormone (hGH) treatment of short stature is one vivid example, but others (e.g. expansion of drug treatment to “optimize” cholesterol profiles, bone health, psychological well-being) can be found throughout medicine. In the new obesity era, lessons learned from the hGH era will be needed to keep the field of pediatric endocrinology empowered to make the key clinical decisions, and free of unintended consequences for patients and runaway health care inflation for society [074].

Doping with GH is a well-known problem among elite athletes and among people training at gyms, but is forbidden for both medical and ethical reasons. The increased availability of growth hormone (GH) in the mid-1980s, as a result of advances in recombinant DNA techniques, has allowed research into the use of this hormone at physiological dosage, as replacement therapy for adults with GH deficiency (GHD) and at pharmacological dosages as a possible therapeutic agent, for a number of disease states [075].

Physiology of growth hormone in regard to doping

Human growth hormone (hGH) is a naturally occurring hormone produced by the anterior pituitary gland and is one of the major hormones influencing growth and development. Growth hormone (GH) is an important and powerful metabolic hormone that is secreted in a pulsatile pattern from cells in the anterior pituitary, influenced by several normal and pathophysiological conditions. Human GH was first isolated in the 1950s and human derived cadaveric GH was initially used to treat patients with GH deficiency. However, synthetic recombinant GH has been widely available since the mid-1980s and the advent of this recombinant GH boosted the abuse of GH as a doping agent [076].

It is mainly the anabolic and, to some extent, the lipolytic effects of GH that is valued by its users. Even though GH's rumour as an effective ergogenic drug among athletes, the effectiveness of GH as a single doping agent has been questioned during the last few years. There is a lack of scientific evidence that GH in supraphysiological doses has additional effects on muscle exercise performance other than those obtained from optimised training and diet itself. However, there might be synergistic effects if GH is combined with, for example, anabolic steroids, and GH seems to have positive effect on collagen synthesis. Regardless of whether or not GH doping is effective, there is a need for a reliable test method to detect GH doping. Several issues have made the development of a method for detecting GH doping complicated but a method has been presented and used in the Olympics in Athens and Turin. A problem with the method used, is the short time span (24-36 hours) from the last GH administration during which the test effectively can reveal doping. Therefore, out-of-competition testing will be crucial [076].

By the 1980s, the anabolic actions of growth hormone had been well described, and GH was established as a drug of abuse. The performance-enhancing potential of GH for use in sports was first advocated in the Underground Steroid Handbook in 1983, where it was described as “the most expensive, most fashionable and least understood of the new athletic drugs.” After Ben Johnson was stripped his 100-m gold medal from the Seoul Olympic Games, he
admitted to having taken a cocktail of drugs including GH. A Chinese swimmer, Yuan Yuan, was forced to withdraw from the 1999 world championship after 13 vials of human GH were discovered in her suitcase. More recently, during a grand jury testimony, Tim Montgomery (former 100-m world record holder) admitted receiving an 8-week supply of GH and a steroid compound known as “the clear” [020].

Growth hormone was added to the prohibited list in 1989 [027].

In 1998 at the Tour de France that French customs arrested Willy Voet, a physiotherapist of the Festina cycling team, for the illegal possession of needles, syringes and over 400 bottles containing erythropoietin, human growth hormones, steroids, amphetamines, narcotics and stimulants [030].

Six months prior to the 2000 Olympic Games, a pharmacy in Sydney was broken into and 1,575 multiple dose vials of growth hormone were taken while nothing else was touched. Also, on their way to Australia, the Chinese swimming team was detained, as needles, syringes, and vials of human growth hormone were found by customs officials in their baggage [030].

**Risk of Creutzfeldt-Jakob disease**

Harvey Cushing discovered the hormone in 1912 and it was isolated from human and monkey cadaver brains in 1956. Two years later it was used to treat dwarfism in children by injection. The unfortunate development of Creutzfeldt-Jakob disease, a degenerative brain disorder, in boys who were treated with cadaver growth hormone led to the discontinuation of all products derived from the human pituitary gland. Because of this ban, the abuse of hGH was rare in sport until the middle to the end of the 1980s. In 1985 Genentech received approval from the US FDA to market Protropin® for children with growth hormone deficiency. This was the first recombinant DNA form of growth hormone (rhGH) that was safer than cadaver extracts used in the past. Recombinant DNA technology made the production of pharmaceutical grade growth hormone easier and cheaper. Most human growth hormone used in medicine and diverted to sports doping is now obtained by recombinant technology, and is simply referred to as hGH (but it may also appear as rhGH or HGH) [030].

**Doped**

After testing over 1,000 samples, the first adverse analytical finding of growth hormone came in February 2010 when the British Rugby League player, Terry Newton, tested positive. Since then, several other positive tests have been reported including the announcement in September 2010 from the Canadian Center for Ethics in Sport that Matt Socholotiuk, a University of Waterloo football player, had tested positive for GH use on 31 March 2010. The following year, Colorado Sky Sox first baseman Mike Jacobs became the first baseball player to test positive for GH and was subsequently suspended for 50 games by Minor League Baseball. In 2011, Andrus Veerpalu, an Estonian Olympic gold medal winning skier, tested positive for GH. However, he pleaded his innocence and challenged the laboratory finding in the Court of Arbitration for Sport who subsequently acquitted Veerpalu on 25 March 2013 as the court was not convinced that the threshold for considering an adverse analytical finding was sufficiently reliable to uphold the doping conviction; nevertheless, the court stated “that there are many factors in this case which tend to indicate that the Athlete did in fact himself administer exogenous hGH” [077].
Testing for growth hormone

In the mid-1990s, the IOC and the European Commission co-funded a three-year international project called GH2000 to develop tests to detect this substance. The project, which concluded at the end of 1998, was led by Peter Sonksen, an endocrinologist at St Thomas’ Hospital in London. The GH2000 consortium delivered its report to the IOC in January 1999. It had developed a series of blood markers that could be used to test for elevated hGH levels, including insulin-like growth factors and proteins that bind to them. Working independently of GH2000, researchers led by Christian Strasburger at the Ludwig-Maximilian University in Munich have developed a direct test for recombinant hGH. This relies on the fact that hGH exists in different molecular forms, the two major fractions of which have molecular masses of 22 kilodaltons and 20 kDa. Although only half of the body's own hGH is in the heavier form, for recombinant hGH the figure is 95 percent. The test uses antibodies to identify the two forms, and so allows any shift in the natural ratio to be spotted. Sonksen costed validation studies for the GH2000 and Munich tests at around US$5 million, and requested continued funding. The consortium also responded to a formal call for proposals for research issued by the IOC in August 1999, but was turned down. The German Sports Research Institute in Cologne then supported further work to validate the Munich test, drawing on some of the GH2000 samples. Strasburger hopes that the then newly created World Anti-Doping Agency (WADA), based in Lausanne, would follow through on statements that tackling hGH abuse will be its top priority, and provide the money needed to bring the test into general use.
HISTORY OF DOPING WITH OTHER SPECIFIED SUBSTANCES AND METHODS

History of doping with human chorion gonadotropin

hCG was first prohibited in sport during the 1980s. The WADA statistics 2012 reports 93 adverse analytical findings for hCG, however it is not known how many of those are due to doping or as a result of disease [078].

History of doping with insulin

It was at the Winter Olympic Games in Nagano in 1998 when a Russian medical officer enquired as to whether the use of insulin was restricted to insulin-dependent diabetes. This drew attention to its role as a potential performance-enhancing drug and the IOC were swift to act and immediately placed it on its list of banned substances [027].

Insulin physiology

Sir Edward Schafer was Professor of Physiology in Edinburgh when he published in 1916 a wonderful book called The Endocrine Organs. The book is based on a series of lectures he delivered at Stanford University in California in 1913. As well as containing a wealth of interesting insights into the early days of endocrinology, this book is most notable for the fact that it was the first time that the then hypothetic hormone insulin was named (8 years before it was discovered). What is even more remarkable, he predicted the formation of insulin from “pro-insulin” 54 years before it was actually discovered! Schafer was a contemporary of Baylis and Starling – two eminent academic rivals from University College in London. Shortly before Schafer delivered his lectures to his American audiences, Baylis and Starling had isolated, characterised and published about Secretin, the first “hormone” (a term coined by them to describe a substance produced in one part of the body, carried by the blood stream and acting elsewhere in the body) to be isolated. Schafer questioned the use of the word “hormone” and proposed two alternative names:

- Autacoids – excitatory substances
- Chalones – inhibitory substances

He went on to describe how his new hypothetical hormone “insulin” exhibited properties that resembled both autacoids and chalones and that the chalonic or “inhibitory” actions were physiologically the most important. It was, he proposed, lack of this chalonic (inhibitory) action of insulin that led to a failure to store glucose in the liver with the net result that the liver overproduced glucose and glucose accumulated in the circulation, and this led to the hyperglycaemia that is characteristic of diabetes. This was indeed advanced thinking. The “black ages” of endocrinology followed early in vitro experiments in the 1950s that showed insulin to be capable of stimulating glucose uptake into bits of rat muscle and fat. Before long the biochemists had extrapolated from these experiments to conclude (wrongly) that the hyperglycaemia of diabetes was due to a “damming back” of glucose in the blood stream as a result of a failure of glucose to enter cells as a direct consequence of insulin deficiency [079].

History of doping with caffeine

Caffeine is a stimulant that is not currently banned by WADA, despite its proven ergogenicity. In the past it was included on the banned list at urine concentrations above (12 microg/mL),
on the basis that concentrations below this level may be attained from the consumption of coffee, coca cola and similar sources, whereas above this concentration indicated a deliberate consumption, probably via tablets, with the intent of performance enhancement. It was removed from the banned list in 2004 but is still subject to monitoring, although it should be noted that the ergogenic benefits for a range of sports appear to be attained at modest doses (3 mg/kg) doses that are easily achieved via intake of everyday dietary sources such as coffee, cola drinks and energy drinks [017].

From 1962 to 1972 and again from 1984 to 2003 caffeine was on the WADA banned list, with a concentration >12 microg/ml in the urine considered as doping. Caffeine has been demonstrated to be ergogenic at doses lower than those doses that result in a urine concentration of 12 microg/ml, and higher doses appear to exhibit no additional performance-enhancing effect. During the second banned period, many athletes tested positive for caffeine. The sanctions ranged from warnings up to 2 year suspensions (maximum penalty, usually only 2-6 months). Since 2004, caffeine has been removed from the prohibited list, however, it is still part of WADAs monitoring program (stimulants but in competition only) in order to monitor the possible potential of misuse in sport. According to WADA, one of the reasons caffeine was removed from the Prohibited List was that many experts believe it to be ubiquitous in beverages and food and that having a threshold might lead to athletes being sanctioned for social or dietary consumption of caffeine. Furthermore, caffeine is metabolized at very different rates in individuals and hence urinary concentrations can vary considerably and do not always correlate to the dose ingested. In addition, caffeine is added to a wide range of popular food products such as coffee, tea, energy drinks and bars, and chocolate [080].

Caffeine was removed from the World Anti-Doping Agency list of restricted or banned substances in 2004 [081].

**History of doping with ephedrine**

Ephedra is a Chinese shrub which has been used in China for medicinal purposes for several thousand years. The pure alkaloid ephedrine was first isolated and characterized by Nagai in 1885. It was then forgotten until it was rediscovered by Chen and Schmidt in the early 1920s. Its actions on the adrenoceptors could be classified into separate alpha and beta effects – a defining moment in the history of autonomic pharmacology. Ephedrine became a highly popular and effective treatment for asthma, particularly because, unlike adrenaline (until then the standard therapy), it can be given by mouth. Ephedrine as a treatment for asthma reached its zenith in the late 1950s, since when there has been a gradual and inevitable decline in its therapeutic use. From mainstream medicine, ephedrine moved into the twilight zone of street drugs and nutritional supplements. Ephedra and ephedrine products are now banned in many countries, as they are a major source for the production of the addictive compound methamphetamine (crystal meth) [082].

Ephedrine is not only efficacious in the treatment of numerous ailments, but also has a long history of misuse. Research was needed to examine ephedrine policy over time in order to determine potential regulatory flaws that allowed misuse to continue. One review is based on primary literature derived from systematic searches of historical and scientific archives, as well as grey literature. Ephedrine managed to pass through numerous regulatory loopholes within seventy years. Despite warnings of misuse over the latter half of the century, ephedrine, and its herbal source, ephedra, were regulated in a piecemeal fashion and remained easily available to the public. Health authorities have struggled to control ephedrine, as an amphetamine "look-alike," as a methamphetamine precursor, as a dietary supplement, and as a medication. Despite being a potentially dangerous stimulant, under-
regulation was perhaps more problematic than the substance itself. Tighter control of all ephedrine products, drugs and dietary supplements alike, might have prevented adverse outcomes and allowed this substance to remain available in a safer manner. Stringent regulation of all ephedrine products is necessary to prevent misuse and to protect the public's health [083].

Ma huang or ephedra, which companies promoted as a legal alternative to ecstasy, although a natural product, contains the chemical ephedrine, which stimulates the nervous system and constricts blood vessels. FDA banned ephedra in 2004, after a 23-year-old Major League Baseball pitcher collapsed and died during practice and was found to be taking the herb. By early 1996, it had been linked to at least 15 deaths. Meanwhile, FDA was regularly issuing warnings about liver, kidney, and other health risks tied to supplements [017].

**History of doping with beta2-agonists**

Due to fear of possible doping effects of beta2-agonists, including both improved performance and possible anabolic effect upon muscle, the Medical Commission of the International Olympic Committee already in 1993 put certain restrictions upon the use of inhaled beta2-agonists, allowing only the two short-acting inhaled beta2-agonists salbutamol and terbutaline for use in sports. However, inhaled salmeterol was allowed by IOC to treat and prevent exercise induced asthma in relationship to sports from 1 February 1996 [084].

**History of doping with morphine**

A quantitative analysis of morphine and codeine in human urine was performed after oral intake of cakes containing commercially available poppy seeds in order to estimate the possibility of positive doping results. Therefore, eight products from different manufacturers (poppy seeds or baking mixtures) and origin were obtained and analyzed by gas chromatography-mass spectrometry for the presence of the alkaloids. One selected batch of poppy seeds was used as an ingredient in a typical cake and was the object of an excretion study with nine volunteers. After application, several urine specimens contained morphine with concentrations higher than 1 microg/mL, and peak values of approximately 10.0 microg/mL were detected. Because the International Olympic Committee set a cutoff limit for morphine at 1 microg/mL, high-performance athletes could possibly test positive in doping control after consumption of products containing poppy seeds [085].

**History of doping with cannabis**

The medical properties of cannabis have been known for many centuries. The first documented use of cannabinoids for medical purposes dates back to 2800 BC in the Chinese herbarium Pen-ts’ai, a herbal pharmacopoeia describing many drugs among which cannabis, which was referred to as "ma", meaning "chaotic". Pen-ts’ai described the pain-relieving, stupefying and hallucinogenic properties of cannabis and recommended cannabis for constipation, malaria, gout, rheumatism, and menstrual anomalies. Cannabis therapeutic use was introduced in Western medicine during the first half of the nineteenth century by the Irish physician William Brooke O'Shaughnessy (1809–1889), who studied forensic toxicology and chemistry at the University of Edinburgh in Scotland. He conducted a number of experiments in animals and proved that cannabis was safe even at high doses; thus, he extended the use of cannabis to patients suffering from rheumatism, seizures, and tetanus [043].
The International Olympic Committee included cannabis in the banned substance list beginning in 1989 and since 2004 the World Anti-Doping Agency has prohibited its use for all sports competition. Cannabinoids are substances prohibited in-competition only [086].

The class of cannabinoids has been subject of much debate concerning its relevancy for sports drug testing, fuelled by the increase of the urinary threshold for the main cannabis metabolite 11-nor-delta9- tetrahydrocannabinol-carboxylic acid (THCCOOH) from 15 ng/mL to 150 ng/mL (being effective since 11 May 2013) while the MRPL for cannabimimetics remained at 1 ng/mL as well as prevalence studies demonstrating the widespread availability and misuse of cannabis and its synthetic analogs. Since the raise of the urinary threshold for THCCOOH came unexpected, studies from early 2013 concerning improved/accelerated quantification approaches have become obsolete, even though the principle is certainly still valid [086].

History of doping with xenon

On September 1st 2014, a modified Prohibited List as established by the World Anti-Doping Agency (WADA) became effective featuring xenon as a banned substance categorized as hypoxia-inducible factor (HIF) activator [087].

History of doping with alcohol

Alcohol is prohibited in-competition only and it is prohibited in the following sports: aeronautic, archery, automobile, karate, motorcycling and powerboating. Until 2010, modern pentathlon was also included in this list. The limit (blood tests) eligible for a doping violation is 0.10 g/L [080].

History of doping with gamma-Hydroxybutyric acid (GHB)

GHB was first synthesized in 1960 as an alternative anesthetic to aid in surgery because of its ability to induce sleep and reversible coma. However, it had little analgesic effect, and onset of coma was often associated with seizure activity including tonic-clonic jerking movements of the limbs or face. In the late 1980s, GHB was marketed and sold in the health food industry as a “growth hormone stimulator” to help bodybuilders promote muscle mass and maintain weight, and as an over-the-counter sedative agent. In 1991, the drug was banned by the FDA after several reports of adverse reactions in individuals using nutritional and weight loss supplements containing GHB. Despite the FDA ban, GHB continues to be manufactured and sold clandestinely [088].

Occurring naturally in many parts of our body from the brain to heart, to most muscles, kidneys and brown fat, gamma-hydroxybutyric acid or GHB for short was first synthesized in 1960s by Laborit in an attempt to study the effects of GHB and GABA, producing a compound that would interfere with beta oxidation and cross blood–brain barrier. It was later discovered that GHB was an endogenous compound and an endogenous metabolite of GABA. GHB was thus discovered in search for therapeutic GABA analogs. Since its discovery, GHB has played many roles in the laboratory. It was used to create an absence seizure model. GHB was also shown to have tissue-protective effects in the setting of myocardial infarction, stroke, sepsis, small bowel ischemia, hypovolemic shock, ionizing radiation and oxygen free radicals. Despite promising beneficial effects, GHB has not found widespread clinical use. In the 1960, GHB was used as a general anesthetic agent but fell out of favor due to an association with abnormal electroencephalographic (EEG) patterns in
animals. In the year 1980, GHB could be bought in health food stores and the use began to rise amongst body builders as it was believed that taking this drug could improve muscle mass or improve exercise performance. While GHB has been present in laboratories and therapeutic trials for years, it has recently become a public health issue as a drug of abuse. Hence in the year 1990, FDA imposed ban over the counter sale of the drug throughout the United States. Simultaneously from 1997 to 1999, several states and countries passed laws to control the sale and consumption of GHB and finally designated it as a Schedule 1 substance in the United States in the year 2000 [089].

History of doping with ecstasy

The methylenedioxy-derivatives of amphetamine and methamphetamine represent the largest group of designer drugs. The most frequently used compounds are 3,4-methylenedioxy-methamphetamine (MDMA, ecstasy) and 3,4-methylenedioxy-amphetamine (MDA), first synthesised in 1910 (MDA) and 1914 (MDMA), respectively, to be used as an appetite suppressant. At the end of the 1960s, non-medical (recreational) use appeared in the USA, and in the middle of the 1980s in Europe. In Norway, MDMA and related compounds have been detected in forensic samples since the early 1990s. In order to bypass the legal regulations and to produce more potent substances, a number of related compounds have been synthesised, including derivatives with one or more substituents (methoxy, methyl, halogen or sulphur), attached at different positions to the phenylring of amphetamine or methamphetamine. A report from 1998 shows that 0.5-3 percent of the adult European population, mainly young people, has used ecstasy [090].

History of attempts to dope with nutritional supplements

The modern supplement era began in 1994, when Congress passed the Dietary Supplement and Health Education Act, or DSHEA (pronounced duh-shay-uh). In the decades before, the supplements industry was overwhelmingly focused on vitamins and minerals. Much of the regulation centered on recommended daily allowances of products like vitamin C, iron, or calcium. DSHEA established the first broad framework for regulating supplements. It also gave supplements a legal definition: as substances intended to “supplement the diet,” containing “dietary ingredients” such as herbs, botanicals, or vitamins. At the same time, the law sharply curtailed FDA’s power. Companies were not required to notify FDA provided the dietary ingredient had a history of use before the law was passed. For the first time, DSHEA allowed them to make claims on the label suggesting supplements affected the structure or function of the body – for example, by boosting the immune system or protecting prostate health. And DSHEA codified a loose arrangement: Under the law, as FDA notes on its website, “unlike drug products that must be proven safe and effective for their intended use before marketing, there are no provisions in the law for FDA to "approve" dietary supplements … before they reach the consumer.” The agency can act only after a supplement is on the market and evidence shows it’s unsafe. Since 1994, the number of dietary supplements marketed in the United States has swelled from about 4000 to more than 75,000. About USD 36 billion worth were sold last year [091].

In the mid and late 1990s, nutritional supplements gained immense popularity among professional as well as also recreational athletes, and an alarming number of positive doping cases for drugs such as nandrolone were reported in different sports including football. Athletes attributed their positive steroid cases to the intake of nutritional supplements contaminated with nandrolone or other anabolic steroids. A careful examination of more than 600 nutritional supplements by the WADA accredited laboratory in Cologne supported this claim as 15 percent of the samples analysed contained anabolic androgenic steroids not
reported on the label. Of great concern was the finding that the majority of contaminated nutritional supplements were freely available from fitness clubs, health-food stores and the Internet. Following the publication of these results, the IFs, led by the IOC and FIFA, launched an educational campaign warning athletes to avoid nutritional supplements that were not approved by relevant national regulatory bodies. This message was reinforced by a consensus statement that reaffirmed the view that there was no evidence of ergogenic effects of dietary supplements (i.e. a positive effect on health or performance) and strongly discouraged the indiscriminate use of any nutritional supplements. It was recommended that nutritional supplements should only be taken if advised by qualified sports nutrition professionals [064].

**History of caffeine-containing nutritional supplements**

Tolerability concerns related to caffeine-containing dietary supplements can be traced to caffeine's ability to augment the various pharmacologic effects of sympathomimetic amines. This effect first caught the medical community's attention in the late 1970s, when synthetic combinations of caffeine, phenylpropanolamine, and ephedrine – known as amphetamine look-alikes – gained notoriety as over-the-counter appetite suppressants and legal speed. Shortly after their appearance, amphetamine look-alikes were linked to an upsurge in serious adverse drug events, namely, myocardial infarctions, strokes, seizures, and psychoses, most of which occurred in young adults. In 1982, the FDA deemed look-alikes as unapproved new drugs that presented a potential health hazard. By 1988, the FDA disallowed the marketing of look-alikes as nonprescription medications. Outside the United States, however, some countries allowed combinations of purified caffeine and ephedrine to be sold as prescription weight loss aids as late as 2002 [092].

Beginning with Red Bull® in the early 1990s, energy drinks and shots have become the fastest growing dietary supplement category on the market in terms of number, variety of products, and sales revenue. The boom in energy drink use, especially among adolescents and young adults, has also sparked an upsurge in caffeine-related adverse events among this population. Energy drinks and energy shots differ from conventional caffeinated soft drinks in that they are not as highly carbonated (making them easier to consume quickly), have higher caffeine content, and often contain vitamins, amino acids, l-carnitine, taurine, glucuronolactone, and botanical extracts, such as guarana, ginseng, Ginkgo biloba, and milk thistle, to name a few. Like Ephedra-free dietary supplements, ambiguity of energy drink label claims for caffeine content may contribute to the purported health risks linked to these beverages. For a while certain energy drinks were formulated with alcohol, but tolerability concerns regarding caffeine and alcohol combinations prompted the FDA to preclude sale of these products in 2011. Nevertheless, the combination of energy drinks and alcoholic beverages remains a cause for alarm for both health care professionals and law enforcement officials [092].

**History of doping with creatine**

A French scientist named Chevreul is credited with first discovering creatine (Cr) in 1832, however, it was not until 1926 that the scientists Chanutin quantified Cr storage and retention in the body. The first reports that phosphocreatine content in human muscle can increase up to 50 percent following daily creatine supplement (5 g Cr monohydrate 4-6 × day for ≥ 2 days) was written by Harris et al in 1992 [093].

One article presented previously restricted information regarding the development and use of creatine supplements and blood doping in the USSR. Early work by Olexander Palladin established the role of creatine in muscle function. In the 1970s, Soviet scientists showed
that oral creatine supplements improved athletic performance in short, intense activities such as sprints. Subsequent studies in the West substantiated these investigations and have led to the widespread acceptance and use of creatine supplements to enhance muscle function and athletic performance. In addition, however, the Soviet government supported the development of blood doping, which is banned by the International Olympic Committee. Blood doping was pervasive in the USSR in the 1970s and 1980s, and was used by many Soviet athletes in the 1976 and 1980 Olympic Games. Open publication and discussion may help to prevent the abuses that can come from secret scientific research [094].

**History of doping with carnosine**

In describing carnosine among the constituents of muscle tissue in 1900, V. Gulevitsch opened the question of its real biological role. Investigation of carnosine-related phenomena occurred simultaneously with the study of its metabolic transformation within the cell. It has now been demonstrated that carnosine has the ability to protect cells against oxidative stress as well as to increase their resistance toward functional exhaustion and accumulation of senile features [095].

**History of gene doping**

In 1997, Leiden et al used an adenovirus to deliver the EPO gene in mice and monkeys. This boosted the haematocrit from 49 to 81 percent in the mice and from 40 to 70 percent in the monkeys. The effects lasted for over a year in the mice and for approximately 12 weeks in the monkeys [096].

The International Olympic Committee (IOC) in 2002 released its new list of banned substances and methods. The list was effective from 1 January 2003 and replaces the 1 September 2001 list. Amongst the important changes, the category of genetic doping as a banned method is listed for the first time. At the 1964 Winter Olympics in Innsbruck, a Finnish competitor Eero Mäntyranta, won two gold medals in cross country skiing. Though his training programme wasn’t radically different from his rivals, Mäntyranta had a distinct advantage. He was born with a genetic mutation that increased the oxygen carrying capacity his red blood cells by 25-50 percent. Mäntyranta had a mutation in the gene coding for the erythropoietin (EPO) receptor which prevented the normal feedback control of red blood cell mass [096].

Gene therapist Ted Friedmann and multiple Olympic gold medallist Johann-Olav Koss were the first to describe the possibility of misusing the techniques and experiences of gene therapy in the athletic arena. In 2006, before the Turin Winter Olympic games, the president of the World Anti-Doping Agency (WADA), Dick Pound, called gene doping “the new threat that is now a reality.” Although Pound did not expect gene doping to pose a problem in Turin, he indicated that it could be a problem at the Summer Games, 2 years hence in Beijing. In fact, the problem did not materialize in China, in 2008, nor at the London 2012 Olympics, as far as the then available detection measures could determine [096].

**Medical history of placebo**

Over the last 200 years, the placebo effect has cast a large and persuasive shadow over the medical field. In that time it has been by turn; harmless charade, charlatan’s ruse, therapeutic device, methodological tool, ethical dilemma, research theme and source of controversy. Despite popular recognition, pervasive problems underlie conceptualisation of placebos and placebo effects. With medicine now firmly entrenched in the age of evidence based practice,
there is a question as to whether it is time to leave the old placebo behind us. The idea of a magical black box from which unexplained therapeutic effects spring up is archaic and also unhelpful from a scientific point of view. If there really is an effect, surely we are best served by directly investigating what is responsible for it. This knowledge can be used in the clinic to improve treatment effectiveness and in research to inform study design. The view and role of placebos have developed over time. Modern medical understanding of placebos dates back to the late 1700s, and since then, placebo interventions appear to have been used fairly commonly in medical practice alongside other treatments. Up until midway through the 20th century, the prevailing opinion appears to have been that placebo interventions had no effect on pathophysiology. Placebos were used only to bring comfort to the patient, “a camouflage behind which to watch nature takes its course.” From the 1950s onwards, there was a shift towards regarding placebos as having genuine effects of their own. This period also saw the start of rapid growth in the use of placebos as a control intervention for testing the efficacy of other treatments. Thus, as research generally, and randomised controlled trials (RCTs) in particular, grew in stature and volume, placebo interventions also gained a more prominent face in the medical literature [097].
In 1994, it was shown that between-subject variation can be removed in doping tests by using a series of measurements obtained from the same individual. Six years later the concept of the Athlete Biological Passport (ABP) was proposed. The ABP uses a longitudinal approach where an individual's previous results are logged and compared to the new results. In 2006, Sottas et al proposed to use and combine all data contained in a single blood profile of an athlete. The hematological module of the ABP, used to detect blood doping, has successfully been in use since 2009, while the module for steroid doping is currently being finalized for implementation [098].

To detect cellular components of blood and those pharmacological agents that are too large to be excreted in urine, blood collection and analysis was begun in 2008. The Hematological Module of the WADA Athlete Biological Passport uses the predictive model of Pottgiesser et al to monitor hematological marker changes within an individual. The fact that intraindividual variations in a number of blood (and urine) parameters are lower than interindividual variations has been used in the clinical chemistry laboratory since the 1970s. Blood is also analyzed for recombinant proteins, such as GH variants and biomarkers [043].

Serial analysis of biomarkers was already in practice by a number of federations with some programs predating the formation of the World Anti-Doping Agency (WADA) and the implementation of the World Anti-Doping Code. However after the 2006 Torino Winter Olympic Games, at the request of a number of International Federations (IFs), WADA formed an ad hoc Haematological Working Group to look at the issue of blood doping and to develop a harmonised longitudinal profiling programme that was both scientifically and legally robust. This resulted in the creation of the ABP Guidelines and Related Technical Documents which were first published in 2009. In 2011, WADA re-established a Haematological Expert Group to further refine and develop this module [099].

The athlete biological passport (ABP) has been implemented in anti-doping work and is based on the individual and longitudinal monitoring of haematological or urine markers. These may be influenced by illicit procedures performed by some athletes with the intent to improve exercise performance. Hence the ABP is a valuable tool in the fight against doping. Actually, the passport has been defined as an individual and longitudinal observation of markers. These markers need to belong to the biological cascade influenced by the application of forbidden hormones or more generally, affected by biological manipulations which can improve the performance of the athlete. So far, the hematological and steroid profile modules of the ABP have been implemented in major sport organisations, and a further module is under development. The individual and longitudinal monitoring of some blood and urine markers are of interest, because the intraindividual variability is lower than the corresponding interindividual variability. Among the key prerequisites for the implementation of the ABP is its prospect to resist to the legal and scientific challenges. The ABP should be implemented in the most transparent way and with the necessary independence between planning, interpretation and result management of the passport. To ensure this, the Athlete Passport Management Unit (APMU) was developed and the WADA implemented different technical documents associated to the passport. This was carried out to ensure the correct implementation of a profile which can also stand the challenge of any scientific or legal criticism. This goal can be reached only by following strictly important steps in the chain of production of the results and in the management of the interpretation of the passport. Various technical documents have been then associated to the guidelines which correspond to the requirements for passport operation. The ABP has been completed very recently by the steroid profile module. As for the hematological module, individual and longitudinal monitoring have been applied and the interpretation cascade is also managed by a specific APMU in a similar way as applied in the hematological module. Thus, after
exclusion of any possible pathology, specific variation from the individual norms will be then considered as a potential misuse of hormones or other modulators to enhance performance [100].

Semantics

Large database of blood values was then accessible to the main endurance federations and the concept of individual follow-up slowly entered into the culture. The term of passport was first introduced by Cazzola and later Malcovati who studied the feasibility of the hematological passport for athletes competing in endurance sports. This group, as other authors previously, made at that time a clear statement that the definition of arbitrary limit in critical hematological markers to evaluate the eligibility to compete was neither a very specific nor a very sensitive strategy. In fact, the adoption of this kind of limit was risky by creating false positive cases (e.g. naturally elevated HGB) and many potential false negative cases with athletes using plasma volume expanders [100].

Manfred Donike

For many years, the concept of individual monitoring of biological markers had been studied to detect the potential abuse of doping substances. Manfred Donike, head of the Cologne anti-doping laboratory, worked with his group since the beginning of the 80s on the effect of steroid abuse. In 1989, the Cologne group described the long-term influence of anabolic misuse on the steroid profile. The idea of individual and longitudinal follow-up in the field of the fight against doping was then born naturally. The same group made this statement even clearer by observing that for an individual, the homeostasis of biosynthesis and metabolism of endogenous steroids was not disturbed by physical workload but of course was influenced by the use of testosterone or other similar substances [100].

A database since 1989

Regarding blood doping also, the idea of individual and longitudinal monitoring was established quite soon at the time when recombinant human erythropoietin (rh-EPO) was introduced in the market. In 1989, blood tests were performed at the World Cross Country ski championships in Lahti, Finland, in order to constitute a database and to show possible abnormal individual variation in blood markers due to rh-EPO doping or to blood transfusion. As the abuse of rh-EPO by endurance athletes increased dramatically in the 90s, several proposals of indirect detection by markers were suggested. The percentage of circulating MacroHypo red blood cells (RBCs) as well as the transferrin receptors was proposed. Then, the percentage of reticulocytes (RETs) was shown to be drastically increased a few days after the beginning of a treatment showing the potential of the red cell line to be used as a diagnostic tool to detect blood doping manipulation in the athlete population. All these studies were carried out at the time when blood doping (including rh-EPO and transfusion) was certainly the most severe in endurance sports and disciplines. In 1996 and 1997, two major international sports federations decided to limit blood doping in their population of interest. At that time International Skiing Federation (FIS) and International Cycling Union (UCI) decided to introduce a competition rule based on a population-based upper limit of 18 g/dL haemoglobin (HGB) for FIS and 50 percent haematocrit (HCT) for UCI. This “no-start” rule was introduced with the double aim to preserve the health of the athlete and also to protect fairness in the competition, but it became rapidly obvious that a population-based cut-off was not appropriate to maintain the competition fair due to the large interindividual variability distribution in a population of several blood markers [100].
Longitudinal monitoring

Longitudinal monitoring of athlete’s hematologic parameters holds considerable promise as a strategy to detect and thereby deter illicit blood doping. Two cornerstones of this approach will be Hb concentration and reticulocyte counts, which can both be measured on portable analyzers relocated to the event venue. Thus samples collected at the time of competition for routine blood screens could also provide a cost-effective and convenient source of Passport data. A crucial element of the Passport approach is to define in advance normal fluctuations in blood parameters, to enable authorities to discriminate between expected and suspect changes. It is also necessary to recognize whether biological variability is sport-specific or whether such changes are universal across different sports. One study documented temporal changes of hemoglobin concentration (Hb) and reticulocyte counts in elite rowers. Blood samples were obtained from members of the French National Rowing squad (n=83 males, n=31 females). Between two and eight (average 5 males and 4 females) EDTA blood samples were collected from each rower, and were measured on either an ADVIA 120 Hematology Analyzer or a Sysmex Roche XE2100 (instruments were calibrated according to manufacturers’ specifications). The results were contrasted with previously reported data comprising longitudinal evaluations from a subset of n=288 male professional football players measured on average 3 times. Analysis of variance was used to partition the total variation. These quantitative data illustrate that for Hb the major component of variance is attributable to between subject variations, which supports the intuitive belief that comparing an athlete’s current values with their own longitudinal data rather than population-derived thresholds will provide greater resolution when searching for signs of blood doping. The similarity of within subject variation apparent across different sports augurs well for the universality of this approach. Although of secondary importance to the Passport concept (which is contingent upon consistent within subject variance), there was no apparent explanation for why between subject variation in the cohort of female rowers was markedly lower than for other groups, and this deserves closer scrutiny. The results also emphasize the need to quantify, and adjust for, instrument bias for reticulocyte assays. Instrument bias can be quantified by using the mean value from a cohort of athletes as a de facto calibration agent, and bias negated by using a paper adjustment to standardize values. The “within subject” variation in rowers was comparable to that of athletes from other sports. Reticulocyte results were dependent on the type of instrument used [101].
DOPING IN THE OLYMPICS

The resolut fight during the Olympic games against doping in sports commenced as a result of the death of a Danish cyclist during the Rome Olympic Games in 1960 - directly seen by millions of people viewing TV. The International Olympic Committee (IOC) established a Medical Commission (IOC-MC) which had the task of designing a strategy to combat the misuse of drugs in Olympic Sport. It's today a far cry from the horror that ensued when drug testing was first introduced for the 1968 Winter and Summer Olympic Games and an athlete was busted, for of all things, drinking beer: the Swede modern pentathlete Hans-Gunnar Liljenwall was stripped of a bronze medal for dipping into the local cerveza at the Mexico City Summer Games [102].

The ancient Olympic games

The first instance of an athlete doping in competition is unclear, although there are examples of sportmen from the Greek era using natural substances to gain an advantage. The Olympic games, founded in 776 B.C. (date of the earliest recorded Olympic competition) in Olympia as a tribute to the gods but also to celebrate the virtues of athletic competition, peaceful coexistence and the magnificence of athletics, constitute Olympia's perennial contribution to the world, this symbolized by the eternally burning Olympic flame. We may still sing today, as did Pindar in his eighth Olympian Victory Ode, “… of no contest greater than Olympia, Mother of Games, gold-wreathed Olympia…” [103].

The ancient Olympic games were (almost) men only affairs. Successful athletes were highly honoured, and, perhaps for this reason, skulduggery was not unknown. For example, it is red in Wikipedia that “Sotades at the ninety-ninth Festival was victorious in the long race and proclaimed a Cretan, as in fact he was. But at the next Festival he made himself an Ephesian, being bribed to do so by the Ephesian people. For this act he was banished by the Cretans” [104].

In fact, doping is thus older than organized sports. Ancient Greek Olympic athletes dating back to the third century BC is documented to various brandy and wine concoctions and ate hallucinogenic mushrooms and sesame seeds to enhance performance. Various plants were used to improve speed and endurance, while others were taken to mask pain, allowing injured athletes to continue competing. Yet, even in ancient times, doping was considered unethical. In ancient Greece, for example, identified cheaters were sold into slavery [031].

The first tests in modern Olympics

The first Olympic drug testing took place at the 1968 Games in Grenoble and Mexico City, but it was the Munich Games in 1972 that marked the introduction of a comprehensive testing program. Approximately 7000 athletes participated and just over 2000 samples were collected and analyzed for various types of stimulants. Since then the testing program has expanded for each (summer) Games, both in number and percentage-wise, and at the 2008 Beijing Games where 10,500 athletes took part, 4770 samples were collected. By then, the number of banned substances to be analytically identified had increased significantly. The IOC, however, had limited possibilities to conduct an efficient anti-doping activity as they governed only two big competitions every 4 years (since 1992, one every 2 years, winter and summer alternatively). Then, as it is today, the responsibility for the year-round sport activities rested with the international federations and the national associations, but they remained remarkably inactive on the doping issue for a long time. The first international sport organization to pick up the matter in a serious way was the International Association of
Athletics Federations (IAAF), and during the time between the Munich Games and 1999 when the World Anti-Doping Agency (WADA) was created, the IAAF was the leading international organization in the fight against doping, as will be evident from the following article. A series of challenges met the anti-doping campaigners when they started in the early 1970s [053].

Doping during the modern Olympics

One of the most enduring symbols of the Olympics is the torch or flame, an icon of peace and sportsmanship that has its roots in Ancient Greece. According to the Creed of the Olympics: "The important thing in the Games is not winning, but taking part. The essential thing is not conquering, but fighting well." The modern Olympic Games (1896-2000) have been heavy laden with controversy, as athletes have abused performance enhancing drugs to thrust themselves into the limelight in search of gold. It was not until 1967 that the International Olympic Medical Commission began banning drugs. Full-scale drug testing was instituted in 1972. Retrospective review of modern summer and winter Olympics Games sources (1896-2002) was done for documentation of drug abuse, drug-related overdoses, and positive drug screens. Data were collected for the type of drug documented, the athlete's name, their country of origin, and Olympic event. Seventy cases were identified. The most common class of agents were steroids (29), followed by stimulants (22), diuretics (7), beta-2 agonists (2), and beta blockers (1). Alcohol and marijuana, while not historically prohibited, have been outlawed by several individual sport federations. Toxicities of these 2 agents were most likely under-reported. Countries of origin of individual athletes included Bulgaria (7), USA (7), Sweden (4), Spain (4), Japan (2), Poland (2), Greece (2), Canada (2), Hungary (2), Russia (2), Austria (2), and Great Britain, Norway, Romania, Armenian, and Latvian, each with 1. The most common Olympic events in which drug abuse was documented were weightlifting (25), track and field (12), skiing (5), wrestling (5), volleyball (3), modern pentathlon (3), cycling (2), swimming (2), gymnastics (1), and rowing (1). As athletic pressures and financial gains of the Olympic Games heighten, more toxicities are likely to occur despite attempts at restricting performance-enhancing drugs [105].

Athletes have always sought to outperform their competitors and regrettably some have resorted to misuse of drugs or doping to achieve this. Stimulants were taken by the first Olympic athletes to be disqualified in 1972. Although undetectable until 1975, from the 1950s androgenic anabolic steroids were administered for increased strength and power followed in the 1990s by erythropoietin for enhanced endurance. Both are highly effective doping agents. As analytical science validated improved techniques to identify these drugs, Olympic athletes, including many medallists were caught and disqualified. When the International Olympic Committee (IOC) prohibited beta blockers (beneficial in shooting), diuretics (assist weight classified athletes) and glucocorticosteroids, some athletes with genuine medical conditions were denied legitimate medical therapy. To overcome this, in 1992 the IOC introduced a system known now as Therapeutic Use Exemption (TUE). One paper discussed Olympic athletes who have been known to dope at past Games and some medical indications and pitfalls in the TUE process [106].

At the 2000 Olympics, 10 athletes were caught doping, including 6 medalists, while a record 27 athletes were caught doping at the 2004 Olympics. In total, 84 athletes, including 28 medal winners, have been caught doping at Summer Olympics, 37 of whom were weightlifters, the most notorious of sports amongst dopers. The Winter Olympics have generally witnessed fewer tests and fewer doping busts. Since 1968, only 13 athletes, including 6 medal winners, have been caught doping. Seven of them were cross-country skiers and 4 were hockey players. According to the International Olympic Committee there have been 84 infractions for doping since 1984, including such bizarre incidents as one in
2004 in which an Irish equestrian administered an antipsychotic drug to his horse. By Olympic Games, the failed, missed, refused or falsified test (and medals forfeited) have been up to 2008 [08012]:

<table>
<thead>
<tr>
<th>Year</th>
<th>City</th>
<th>Failures</th>
<th>Medals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1968</td>
<td>Mexico City</td>
<td>1 (1)</td>
<td></td>
</tr>
<tr>
<td>1972</td>
<td>Munich</td>
<td>7 (4)</td>
<td></td>
</tr>
<tr>
<td>1976</td>
<td>Montreal</td>
<td>10 (3)</td>
<td></td>
</tr>
<tr>
<td>1980</td>
<td>Moscow</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>1984</td>
<td>Los Angeles</td>
<td>12 (2)</td>
<td></td>
</tr>
<tr>
<td>1988</td>
<td>Seoul</td>
<td>10 (4)</td>
<td></td>
</tr>
<tr>
<td>1992</td>
<td>Barcelona</td>
<td>5 (0)</td>
<td></td>
</tr>
<tr>
<td>1996</td>
<td>Atlanta</td>
<td>2 (0)</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>Sydney</td>
<td>10 (6)</td>
<td></td>
</tr>
<tr>
<td>2004</td>
<td>Athens</td>
<td>27 (8)</td>
<td></td>
</tr>
</tbody>
</table>

The Winter Olympics have generally witnessed fewer tests and fewer doping busts. Since 1968, only 13 athletes, including 6 medal winners, have been caught doping. Seven of them were cross-country skiers and 4 were ice-hockey players.

In total, 84 athletes, including 28 medal winners, have been caught doping at Summer Olympics, 37 of whom were weightlifters, the most notorious of sports amongst dopers. By country, the number of medals forfeited as a consequence of drug testing up to 2004:

- Bulgaria: 7
- USA: 6 (3 gold and 2 bronze from Marion Jones)
- Hungary: 3
- Germany: 2
- Sweden: 2
- Canada, Russia, Poland, Ireland, Romania, Finland, Mongolia, Greece, Spain, Armenia, the Netherlands & Ukraine: 1

By sport 12 medals have been forfeited in weightlifting, 5 in athletics, 2 each in equestrian, wrestling and cycling, and 1 each in judo, modern pentathlon and rowing [102].

The increased number of positive tests is in part a function of the increased number of tests administered at each game. At the 2000 Olympics, about 2000 doping tests were administered. That number grew to 3700 by the 2004 Olympics. The increasing number is largely a result of the expansion of the rules governing who gets tested. In the past, the top 4 finalists in an event and 1 other athlete chosen randomly were subjected to tests. However, in Beijing, the top 5 athletes were tested in addition to 2 chosen at random in each final. As well, random tests will be conducted throughout earlier stages of competition. Beginning in 2000, Olympic athletes were also subject to pre-Olympic, out-of-competition testing to detect substances consumed prior to competition that wouldn't later appear on a test. Blood testing was introduced on a limited basis at the 1994 Winter Olympics and at the 2000 Summer Olympics. It is the International Olympic Committee itself that administered and monitored athlete testing in Beijing, not the World Anti-Doping Agency. The latter focuses on policies, regulations and monitoring the 33 facilities worldwide that have been approved for testing athletes' samples [102].

Some International Sport Federations (IF) and National Sports Federations followed suit when the anti-doping process started, but progress was modest until the world's best male sprinter (Ben Johnson, Canada) was found doped with anabolic steroids at the Olympic Games in Seoul in 1988. Further progress was made following the cessation of the cold war in 1989 and in 1999 public authorities around the world joined the Olympic Movement in a unique partnership by creating WADA, the World Anti-Doping Agency, which has doubtless
been the start of a new anti-doping era [107].

The Olympics in medical journals

Even before Pierre de Coubertin revived the Olympics in 1896, lore from the ancient games circulated in the medical literature. An 1851 essay in the Boston Medical and Surgical Journal about the power of mind over body described “the old Greek who died on the spot from excess of joy on seeing his three sons crowned with laurel at the Olympic games” (1851). Oliver Wendell Holmes invoked this same episode in his valedictory to Harvard medical graduates (1858). Other authors drew competing lessons from the Olympic legacy. One warned that excessive athletic training diverted energy from mental development, leaving adolescents “listless and stupid”: “It was especially remarked by the Greeks that no one who in boyhood won the prize at the Olympic games ever distinguished himself afterwards” (1867). An 1891 review, in contrast, expressed the hope that educators would learn from the ancient techniques and improve athletic training in US schools [034].

When the modern games began in Athens in 1896, physicians only slowly became interested – and mostly in marathons. Heat and humidity tormented marathoners in St. Louis in 1904: only 14 of 27 finished. The winner Thomas Hicks, who sustained himself during the race with strychnine sulfate, five eggs, and brandy, required the care of four physicians in the aftermath. Heat caused problems again in London and Stockholm. When the games resumed in Antwerp in 1920, athletes were subjected to physical examinations. The United States sent its first team physician – one who had fenced in Stockholm – to the Paris games in 1924. Medical scrutiny has continued ever since [034].

Physicians have been interested in the Olympics for many reasons. In the 1920s, they probed the limits of human physiology. One group studied the Yale heavyweight rowers who won gold in Paris. An ingenious contraption revealed that at their racing speed – 12 mph – the eight men produced four horsepower, a 20-fold increase over resting metabolism (1925). A 1937 study published in the Journal showed that athletes at the 1936 Berlin games consumed 7300 calories each day. Sometimes the venue itself became the issue. The United States threatened to boycott the Berlin games until Hitler relented and allowed black and Jewish athletes to compete. Ignoring these tensions, the Journal, which had published a favorable review of Nazi health insurance in 1935, advertised the exhibits and lectures on “Medical Theory and Practice in the New Germany” that had been organized for physicians who visited the Olympics (1936). Boycott politics surfaced again when the Journal's editor, Arnold Relman, visited the Soviet Union in 1980. Relations had been strained by tensions over the Soviet invasion of Afghanistan and the United States’ threat to boycott the Moscow games. Other venues created medical concerns. Roger Bannister, who eventually became a neurologist after being the first person to run a mile in under 4 minutes, “thoroughly disapproved” of holding the 1968 games at high altitude in Mexico City. And indeed, several hundred athletes collapsed at those Olympics, from migraine, shock, syncope, or emotional excitement. Fears of local pathogens emerged before the Olympics in Seoul (Japanese encephalitis) and Barcelona (multidrug-resistant strep). Each warning met with vehement rebuttal. Olympic events now attract millions of visitors and require careful medical and public health planning [034].

The safety of Olympic sports has remained an enduring concern. The French Academy of Medicine appointed a committee before the 1924 Paris games “to study the effects of modern athletics on the human system.” The resulting tests “revealed an alarming number of cases of athletic heart.” Subsequent studies from the 1920s (on athletes from the 1928 Amsterdam games) through the 1990s (on 310 Italian Olympians) have produced conflicting evidence on the question of whether intense physical training can cause cardiac hypertrophy.
Some sports received special scrutiny. On the eve of the Atlanta games in 1996, a scathing review likened women's gymnastics to child abuse, arguing that although "elite gymnastics can provide a profoundly meaningful experience for the athletes," it could also "result in serious, life-endangering physical and psychological disabilities." Citing injuries, eating disorders, and social problems, the authors warned that "talented youngsters at every competitive level should be supported rather than crippled by their sport as they enter adulthood" [034].

A different kind of medical scrutiny emerged in the 1950s. Commenting on a symposium about "Pheidippidian physiology," a 1957 editorial highlighted the recent dramatic improvements in performance at track and field events. What explained "these epidemics of broken records"? The editorial considered possible contributions from training, diet, antibiotics, and motivation but focused on "a speculative explanation": "that amphetamine is being used by some athletes to help them break otherwise unassailable records." Such practices, if they were in fact occurring, were both dangerous and "ethically undesirable." Each passing decade brought new scandals about performance-enhancing drugs. After the U.S. Olympic Committee admitted that seven cyclists (including four medalists) had received blood transfusions at the Los Angeles games, a Sounding Board article in the Journal in 1985 condemned this practice. Not only was the practice dangerous, especially in light of the emerging AIDS epidemic, but also it "represents an attempt to use a medical therapy to provide athletes with an unfair competitive advantage." When Ben Johnson was stripped of his gold medal at the Seoul Olympics, the Journal reviewed the medical risks and legal consequences of anabolic steroids in 1989. Erythropoietin came next. A Dutch physiologist wrote in the Journal that "the next Olympic Games have already been nicknamed the 'Hematocrit Olympics,'" and physicians' obligation seemed clear: "the medical profession has a responsibility to consider carefully these untoward consequences of scientific progress." [034].

Performance-enhancing drugs have cast a long shadow on the modern Olympics. Whether the agents are the strychnine, heroin, cocaine, and morphine that athletes used in Athens in 1896 or the amphetamines, steroids, and erythropoietin that some use today, the dilemma remains the same. As a sports medicine specialist noted in 2004, the "attraction of performance-enhancing drugs is simply that they permit the fulfillment of the mythical promise of boundless athletic performance – the hubristic "faster, higher, stronger" motto of the Olympic Games." The ensuing systems of medical surveillance have led, inevitably, to "a new type of competition," in which some athletes try to stay one step ahead of the authorities [034].

There are several historical journal article in the Boston Medical and Surgical Journal (1851-1925) and in the follower New England Journal of Medicine after that [034]:

Boston Medical and Surgical Journal
1858. Holmes OW. Valedictory address. 58:149-59.  
1867. The abuse of physical exercise. 77:425.  
1891. Hartwell EM. The principal types of physical training compared. 125:641-4.  
1924. French Academy of Medicine names committee to study effects on the human system of modern athletics. 190:397.  
New England Journal of Medicine
1935. Davis MM, Kroeger G. Recent changes in German health insurance under the Hitler government. 212:1037-42.
1936. Information for doctors during the Olympiad in Berlin. 215:211.

Canada

It is now a long time since the Ben Johnson scandal at the Olympics in Seoul, Korea, drew attention to the issue of doping. In the scandal's aftermath, an independent organization now known as the Canadian Centre for Ethics in Sport was established to develop a national antidoping program. It has served as a model for other national antidoping organizations. Still, 55 Canadian athletes violated antidoping rules in the past 3 years: 27 used “recreational” drugs such as marijuana, and 28 took performance-enhancing drugs, most often anabolic steroids or stimulants [108].
HISTORY OF WADA

WADA is the international independent agency that publishes the World Anti-Doping Code, which is the document harmonizing anti-doping policies in all sports and all countries. The Code was first adopted in 2003 and became effective in 2004. The Code sets forth specific anti-doping rules and principles that are to be followed by the anti-doping organizations responsible for adopting, implementing, or enforcing anti-doping rules within their authority, including the IOC, International Paralympic Committee, international sport federations (for example, the International Cycling Union), major event organizations, and national anti-doping organizations (for example, the US Anti-Doping Agency). WADA revises and publishes its list of banned substances approximately annually. It specifies those banned substances and methods that are prohibited at all times (both in-competition and out-of-competition) because of their potential to enhance performance in future competitions or their masking potential, and those substances and methods that are prohibited in-competition only. The list may be expanded by WADA for a particular sport [031].

World Anti-Doping Agency (WADA)

The World Anti-Doping Agency (WADA) was established in 1999 as an independent, international agency with the aim of creating an environment in world sport that is free of doping. WADA and associated anti-doping organisations such as the Australian Sports Anti-Doping Authority (ASADA) strive to ensure that there is a “level playing field” in high-performance sport and to optimise the safety and welfare of athletes. The World Anti-Doping Code (the Code) is the document that provides consistency of anti-doping policies across sports and across international boundaries. It is based on five international standards aimed at bringing consistency among anti-doping organisations. It covers:

- testing and investigations
- laboratories
- therapeutic use exemptions
- the list of prohibited substances and methods
- protection of privacy and personal information

The world of sports doping is constantly changing. One of the key functions of WADA is to support high-quality research in order to stay abreast and ahead of individuals and organisations who seek to illegally enhance sporting performance. The Code also requires frequent updating to adapt to changing knowledge and the changing doping environment. A new Code was introduced in 2015 with ramifications for athletes, sporting organisations and medical practitioners who deal with high-level athletes. Athletes bear strict liability for any substances found within their bodies. As some commonly prescribed drugs are prohibited in sport, it is crucial that medical practitioners and others advising athletes have access to up-to-date anti-doping information. Exemptions may need to be obtained if the athlete requires the therapeutic use of a drug [109].

To improve the fight against this new potential kind of abuse, the International Olympic Committee (IOC) and national sports federations collaborated in 1998 to establish the World Anti-Doping Agency (WADA), an agency jointly funded by the IOC and cooperating nations and committed to develop programs for detection and control of athletic doping. It carries out its tasks by compiling and constantly updating a list of substances and methods that are inconsistent with the ideals of sports and that should be banned from athletic competition. It is also responsible for developing and validating new, scientifically sound detection assays and implementing effective international programs for in-competition and out-of-competition screening of athletes. The WADA has implemented its program on drug control in sports by
issuing and continually updating the world Anti-Doping Code, including a list of banned substances and methods, the latest of which is presented as an appendix to this volume [110].

One article provided a review of the leading role of the World Anti-Doping Agency (WADA) in the context of the global fight against doping in sport and the harmonization of anti-doping rules worldwide through the implementation of the World Anti-Doping Program. Particular emphasis is given to the WADA-laboratory accreditation program, which is coordinated by the Science Department of WADA in conjunction with the Laboratory Expert Group, and the cooperation with the international accreditation community through International Laboratory Accreditation Cooperation and other organizations, all of which contribute to constant improvement of laboratory performance in the global fight against doping in sport. A perspective is provided of the means to refine the existing anti-doping rules and programs to ensure continuous improvement in order to face growing sophisticated challenges. A viewpoint on WADA's desire to embrace cooperation with other international organizations whose knowledge can contribute to the fight against doping in sport is acknowledged [111].

From the homepage of WADA

The World Anti-Doping Agency (WADA) was established in 1999 as an international independent agency composed and funded equally by the sport movement and governments of the world. Its key activities include scientific research, education, development of anti-doping capacities, and monitoring of the World Anti-Doping Code (Code) – the document harmonizing anti-doping policies in all sports and all countries.

The vision

A world where all athletes can compete in a doping-free sporting environment.

The core values

Integrity
- We are the guardian of the values and spirit inherent in the Code.
- We are impartial, objective, balanced and transparent.
- We observe the highest ethical standards and avoid improper influences or conflicts of interests that would undermine our independent and unbiased judgment.
- We develop policies, procedures and practices that reflect justice, equity and integrity.

Accountability
- We govern and manage in accordance with the values and spirit of the Code.
- We are accountable to our funding bodies, while maintaining appropriate independence from undue influence.
- We respect the rights and integrity of clean athletes.

Excellence
- We conduct business professionally.
- We develop innovative and practical solutions to assist with stakeholder Code implementation and compliance.
- We benchmark off and apply best practice standards to all our activities.
**Logo story**

The square shape of the logo background represents the customs and the rules that define sport. The color black evokes neutrality and is the traditional color of the referee. The "equal sign" expresses equity and fairness. The sign is depicted with a human touch to reflect the individuality of every athlete. The color green evokes health and nature and the field of play. The "play true" tag line encapsulates WADA's core values and is intended as a guiding principle for all athletes at every level of competition.

**The agency’s history**

After the events that shook the world of cycling in the summer of 1998, the International Olympic Committee (IOC) decided to convene a World Conference on Doping, bringing together all parties involved in the fight against doping. The First World Conference on Doping in Sport held, in Lausanne, Switzerland, on February 2-4, 1999, produced the Lausanne Declaration on Doping in Sport. This document provided for the creation of an independent international anti-doping agency to be operational for the Games of the XXVII Olympiad in Sydney in 2000. Pursuant to the terms of the Lausanne Declaration, the World Anti-Doping Agency (WADA) was established on November 10, 1999, in Lausanne to promote and coordinate the fight against doping in sport internationally. WADA was set up as a foundation under the initiative of the IOC with the support and participation of intergovernmental organizations, governments, public authorities, and other public and private bodies fighting doping in sport. The Agency consists of equal representatives from the Olympic Movement and public authorities.

**Pre-WADA history**

In the modern era, doping practice continued mostly with the use of stimulants and narcotics. Sports federations took notice and in 1928 the International Association of Athletics Federations (IAAF) became the first federation to prohibit the use of performance-enhancing drugs (PEDs), although there would be no testing in sport for another 40 years. Amphetamine use was involved in the deaths of cyclists Knud Jensen and Tommy Simpson in the 1960 Olympic Games and the 1967 Tour de France respectively: this spurred the development of the International Olympic Commissions (IOC) Medical Commission, which published the first IOC Prohibited List in 1967. This became the de facto Prohibited List for Olympic Sport Federations. The “Festina affair” (1998 Tour de France), where a team trainer’s car was found to contain a panoply of PEDs, was the catalyst to create a new organisation to harmonise, coordinate and promote the fight against doping in sport in all its forms.3 The IOC convened the first World Conference in Doping in Sport in 1999, which resulted in the formation of the World Anti-Doping Agency (WADA) [004].

One study investigated the anti-doping policy promoted by the IOC historical sociologically focusing on the period from 1968 to 1999. Public opinion surrounding doping control has emerged as a large amount of drug possession by athletes who had participated in the 1952 Olympics was caught, as well as following the accident where an athlete had died during the competition as a result of doping. From 1960, as many doping cases in sports games were exposed, several international organizations proclaimed fight against doping in order to seek a preventive measure. In 1961, the IOC newly established a medical commission within the organization. It was decided to implement doping control and female sex testing at the same time for all athletes who participated in the 1967 Olympics, and they were implemented from 1968 winter and summer Olympic Games. In 1971, the provisions for the tests were prescribed as mandatory on the IOC charter. From 1989, the OCT system was introduced as a measure to overcome limitations of the detection during competition period. As political
problems and limitations emerged, WADA (World Anti-Doping Agency) was established in 1999 to professionally manage and push for doping control. Female sex testing policy contributed to preventing males from participating in female competition by deceiving their gender to some extent. However, it was abolished due to strong public condemnation such as women's rights issues, social stigma and pain, and gender discrimination debate. In 1984, a doping control center was established in Korea, which enabled drug use or doping in the sports world to emerge to the surface in our society. Korea Sports Council and KOC articles of association that supervise doping related matters of Korean athletes were revised in 1990. The action of inserting doping related issue in the articles of association was taken 20 years after the start of IOC doping policy. Beginning with two international competitions in the 1980s, Korean athletes experienced doping test directly, yet education about doping was limited. However, some national team level athletes tested positive on the doping test and underwent disciplinary action. In addition, athletic federation or leaders acquiesced athletes doping made secretly; this indicated that South Korea was also not free from doping. It was found that Korea world of sports showed very passive countermeasures and development process [112].

**Formation of the IOC Medical Commission**

In 1961 the International Olympic Committee (IOC) created a Medical Commission (IOC–MC) at its 59th Session in Athens, Greece. The decision was triggered by the death of the Danish cyclist Knud Enemark Jensen during the road race for teams at the Rome Olympic Games the year before. He was said to have taken some stimulating drug, but was also reported to have suffered from heat exhaustion and dehydration. Probably a combination of all this caused his death, but this has never been officially confirmed. At any rate, the IOC could no longer ignore the use of stimulants that had obviously been in place in certain sports for quite some time. The creation of the IOC-MC marked the start of the modern era of the anti-doping campaign. The Commission was requested to propose a strategy for combating the use of performance-enhancing drugs in Olympic sports. It took quite a while to analyze the situation and recruit the necessary competence. Not until the 1967 IOC session were some concrete proposals presented such as a list of forbidden drugs (stimulants) in Olympic sport and drug testing at the coming Games. Therefore, 1967 is often referred to as the start of the IOC-MC [053].

**Formation of the World Anti-Doping Agency**

When antidoping activities accelerated following the Ben Johnson case at the Seoul games in 1988 and following worldwide political changes that took place soon afterwards, it became apparent that the situation and antidoping rules around the world were in chaos. Athletes received different penalties for the same offence depending on sport and their nationality. The antidoping campaign could not advance without a harmonised and universally accepted set of rules. But that could not be carried out by sport alone. Governmental support was needed. That was one of the main reasons for the creation of WADA in 1999. Remarkably, WADA had a draft set of rules – a draft Code – ready by late 2003. It received wide support mostly from the governments whereas some federations expressed reservations. The IOC declared, however, that those Olympic federations that did not adopt the Code before the Athens Olympic Games (2004) would not have their sport included in the games. Thus, when the Athens Games opened, the world also saw the birth of the “World Anti-Doping Code”. The governments took their share of the commitment by creating a Convention under UNESCO in 2005; this to encourage all governments around the world to support the fight against doping on the basis of the WADA Code [113].
The practice of enhancing athletic performance through foreign substances was known from the earliest Olympic Games. In 1967, the International Olympic Committee (IOC) established a Medical Commission responsible for developing a list of prohibited substances and methods. Drug tests were first introduced at the Olympic winter games in Grenoble and at the summer games in Mexico City in 1968. In February 1999, the IOC convened the World Conference on Doping in Sport in Lausanne, Switzerland. The Lausanne Declaration on Doping in Sport recommended creation of an International Anti-Doping Agency. The World Anti-Doping Agency (WADA) was formed in Lausanne, Switzerland on the basis of equal representation from the Olympic movement and public authorities. One of the mandates of WADA was to harmonize the Olympic antidoping code and develop a single code applicable and acceptable for all stakeholders. The world antidoping code developed by WADA included creation of several international standards (IS). The purpose of each IS was harmonization among antidoping organizations. The ISs were developed for laboratories, testing, the prohibited list, and for therapeutic use exemptions (TUE). The objective of this manuscript is to present a brief history of doping in sport and describe creation of WADA in 1999. The components of the World Anti-Doping code (in particular, the Therapeutic Use Exclusion program or TUE) is described. The WADA code defines a TUE as "permission to use, for therapeutic purposes, a drug or drugs which are otherwise prohibited in sporting competition." Experiences of the Canadian Centre for Ethics in Sport Doping Control Review Board are presented because this national TUE committee has been operational for over 12 years. The challenge of developing a rigorous global antidoping program requires acceptance of doping as a problem by sport organizations, athletes, and public authorities. Individual stakeholders must be prepared to preserve the values of sport, which means free from doping. This will require vigilance by all interested parties for the benefit of elite athletes and society overall [015].

Probably the most serious challenge for the anti-doping fight during the 1970s and 1980s was the unwillingness of most countries and international federations to join the fight. There were several reasons, namely: the costs; the lack of competence; the negative image should a top athlete in their own country or sport test positive; and, the Cold War. The East Germans used success in sport as a political weapon and other countries had followed suit, although in a less sophisticated way. Officially, sports leaders were against doping, but far too many only paid lip service and some were even sabotaging the fight. When the Cold War faded following the political events in 1989 and the years that followed, the anti-doping fight gained increased support. In 1999 the public authorities actually joined the fight by accepting the invitation of the IOC to form WADA together [053].

The World Anti-Doping Code was created on February 20, 2003 and entered into force for the first time on January 1, 2004 [013].

In order for governments to ratify an equivalent to the World Anti-Doping Program, it was necessary to develop the International Convention Against Doping in Sport through the UN Educational, Scientific, and Cultural Organization. Up to 2012, over 160 countries, including the United States, have approved the convention [043].

The later development of the World Anti-Doping Agency (WADA) was an endeavor of the international community to consolidate and coordinate efforts to minimize doping practice. In this current anti-doping environment and in view of the Athens 2004 Olympic Games, the organizing committee "Athens 2004" in collaboration with the International Olympic Committee (IOC), the Medical Committee (MC) and WADA, will develop new anti-doping policies and will deliver programs and guidelines for doping control [002].
The code

WADA is a unique, independent body representing equally sport and the governments of the world. The World Anti-Doping Code is the core document on which anti-doping programmes are modelled. The first version of the Code came into effect in January 2004. There are presently over 600 signatories, including almost all the world's sport federations. The Code applies to Athletes, as defined by their national anti-doping organisations (NADOs) or international federations. Who is considered an athlete for anti-doping purposes may vary widely and a NADO may still test recreational athletes but not apply all elements of the Code, for example, the requirement for whereabouts or advanced therapeutic use exemptions. Athletes may be subjected to sanctions based on possession or trafficking of prohibited substances and not simply due to a positive doping test. However, it is important to be aware that criminal legislation exists in certain countries (e.g. for narcotics) which may be in addition to, or completely separate from, anti-doping sanctions [004].

Doping in sport presents an ongoing challenge to fair competition and the health of athletes. The paucity of drug testing in a number of countries, varying sanctions for the same offence and the way in which some athletes have been able to avoid out-of-competition testing are causes of concern. World Anti-Doping Code (WADC) globally addresses doping in sport and represents a worldwide code for adoption by all nations and all sports. Its purpose is to protect the right of the athlete to participate in doping-free sport, thereby promoting health, fairness and equality for athletes world wide, and to ensure there are harmonised, coordinated and effective anti-doping programs. The code provides international standards for laboratories engaged in drug testing, the prohibited list of drugs and the granting of therapeutic use exemption. Education of athletes and ongoing research are aspects encouraged in the code. At the Second World Conference on Doping in Sport held in Copenhagen in March 2003, 80 countries were represented [114].

Definitions

It has never been possible to have a simple one sentence definition of doping. In the code, the definition of doping is the occurrence of any one or more of the following eight anti-doping rule violations [114]:

1. The presence of a Prohibited Substance or its Metabolites or markers in an athlete's bodily specimen
2. Use or attempted use of a prohibited substance or a prohibited method
3. Refusing or failing to submit to sample collection or otherwise evading sample collection
4. Violation of requirements regarding athlete availability for out of competition testing including failure to provide required whereabouts information and missing tests
5. Transferring or attempting to tamper with any part of doping control
6. Possession of prohibited substances and methods
7. Trafficking in any prohibited substance or prohibited method
8. Administration or attempted administration of a prohibited substance, or prohibited method to an athlete assisting, covering up or any complicity involving an anti-doping rule violation or attempted violation

National antidoping organizations (NADOs)

Doping control for national- and international-level athletes has undergone major changes in the past few years, and will continue to change at an accelerated rate. National antidoping
organizations (NADOs) such as the United States Anti-Doping Agency (USADA) are being established by major nations to work with national governing bodies of sport. The World Anti-Doping Agency has been established to coordinate worldwide antidoping efforts with the NADOs and international federations of sport, and to implement a recently drafted World Anti-Doping code, which clarifies the definition of doping and establishes procedures to harmonize international efforts in sample collection process, testing laboratory accreditation, result reporting, and result adjudication. A number of substances and methods currently used in doping present serious challenges to the scientific community, and are described briefly. In addition, brief descriptions of other issues of significance to doping control, including the role of physicians in doping and the operation of the USADA, are presented [115].

Formation of Court of Arbitration (CAS)

During the late 1970s and early 1980s, one athlete after another who tested positive challenged the IAAF before national courts and the Federation spent a lot of money on court trials. In an attempt to take care of the problem of escalating legal costs the IAAF included in its constitution an “Arbitration panel” as the highest authority to settle disputes within athletics. This could not prevent athletes from bringing their cases before national courts, but the Arbitration panel served as deterrent. This happened in 1982 and a year later the IOC created the Court of Arbitration for Sport (CAS), which today is included in the WADA Code as the final appellate body on doping matters. There are examples of enormous legal costs following an athlete’s positive sample. The IAAF was ordered by a district court in USA to pay USD 27.4 million in damage compensation to the 400 m world-record holder Harry “Butch” Reynolds after their panel had disqualified him for steroid doping in 1990. The decision was overruled by the Circuit court, and the Supreme Court refused to hear the case. Thus, after several years of legal battle Reynolds lost the case (and money) but the IAAF also lost a lot of money. The winners were the lawyers on each side. In recent years, WADA successfully defended the testosterone analysis before CAS after it had been challenged by the Tour de France winner Floyd Landis following his positive test. The defense cost WADA about USD 2 million. The ever-increasing legal costs are a major challenge for sports organizations and a true threat to the anti-doping fight [053].

Out-of-competition testing

A second challenge was to convince the world of sport that doping controls at competition only is not sufficient. Since AAS are taken during training periods in order to promote the building of muscles and strength, doping controls will also have to be conducted during training – so-called out-of-competition controls. Such controls had been in place in Norway and Sweden since the early 1980s but when the idea was brought to a broader international sports community it was met with great resistance. A majority saw it as an unacceptable intrusion into the athlete’s private life. However, the Ben Johnson scandal at the Seoul Games in 1988 made the skepticism fade and in 1990 the IAAF, as the first international federation, started testing out-of-competition after the necessary rules had been passed by the 1989 IAAF Congress. Today, out-of-competition testing is compulsory in the WADA Code, and any national anti-doping organization or international federation that does not conduct such testing will be declared noncompliant with the Code and possibly face serious consequences. The IAAF ambition to clean up its sport resulted in the identification of doped athletes, some very famous [053].
History of therapeutic use exemptions (TUE)

The introduction of what today is known as therapeutic use exemption (TUE) was far from smooth. It started in the mid-1980s when a Swedish athlete asked the nation’s anti-doping organization for permission to use testosterone as replacement therapy following the removal of both his testes (unilateral cryptorchism as a newborn followed by cancer in his remaining testis as a teenager). It was granted with the remark that it was only valid within Sweden. Soon thereafter a similar case (bilateral testicular torsion) occurred in Australia. At the 1988 Olympic Games two athletes were allowed by the IOC-MC to use banned substances (corticosteroids for inflammatory bowel disease and diuretics for nephrotic syndrome). The permissions were granted although no clear rules existed. Some Commission members, therefore, suggested that such rules be worked out. Other members were against, arguing that athletes should not be allowed to use prohibited substances. If they were in such need they should quit sport. In 1992, however, a proposal was presented to the IOC-MC with strict criteria for “permitted use of prohibited substances” and the formation of a group that should evaluate applications for such use of IOC-MC. The proposal was accepted, and a “Medications Advisory Committee (MAC)” started its operation at the Barcelona Games in 1992. Although MAC’s authority was limited to the Olympic Games it became an advisory body for international federations and national anti-doping organizations. MAC worked under strict anonymity and, actually, in secret. The fear was that if MAC became generally known it would result in a flood of requests and abuse of the system. When the IOC Juridical Commission reviewed the 1999 edition of the IOC Medical Code they first refused to include the concept of “permitted use”. Only after pressure from the IOC-MC was it added as a brief ‘addendum’ When WADA was about to be formed, however, the IOC-MC requested that the concept of permitted use be accepted and clearly explained in the coming rules. Today TUE is an important part of the WADA Code [053].

When the IOC undertook the first extensive doping controls (>2000) at the 1972 Munich Olympics, the only drugs that were prohibited were stimulants and narcotics. Androgenic-anabolic steroids (AAS) were prohibited prior to the 1976 Olympics, although endogenous steroids such as testosterone could not be identified. During this period, there was no discussion about athletes needing a Therapeutic use exemptions (TUE). However, after diuretics, beta-blockers and systemic glucocorticosteroids (GCS) were added to the IOC’s Prohibited List in the 1980s, significant interference occurred in the availability of essential medications to manage medical conditions in elite athletes. Around 1987-1988, two male athletes without testes, one in Sweden and the other in Australia, sought to continue their testosterone replacement therapy and participate in their sport. Both were granted national approval. At the 1988 Calgary Games, an ice hockey player was approved to take oral GCS for inflammatory bowel disease (IBD) and later that year in Seoul, a rower with nephrotic syndrome was approved to continue her diuretic therapy and participate. Both were ad hoc decisions taken by all members of the IOC-MC, some of whom were not medically qualified.

In February 1991, it was presented a paper “Permission for athletes to use drugs contained in the IOC List of Banned Classes” to the IOC-MC. This detailed the following criteria that should be met to approve any such application by an Olympic athlete [116]:

- The athlete would experience significant impairment of health if the prohibited medication was withheld
- No enhancement of performance could result from the administration of the prohibited substance as medically prescribed
- The person would not be denied the prohibited substance if he/she was not a competing athlete
- No available permitted or practical alternative can be substituted for the prohibited substance
- Retrospective approval would not be granted
Independently, and at the same IOC-MC meeting, professor Manfred Donike presented a paper on “Replacement therapy” which focused on testosterone in anorchia. An interim Medications Advisory Committee (MAC) was appointed (D Catlin, A Ljungqvist and K Fitch, Convenor) and requested to develop guidelines to implement the proposal. This was undertaken and included [116]:

- The complete medical details including the history, clinical findings and investigation must be submitted
- The necessity to administer the prohibited medication including the dosage, route and frequency of administration must be certified by a suitably qualified medical specialist
- The medical necessity to administer the prohibited substance cannot be the result, wholly or partially, of prior use of a drug from the banned classes or banned methods
- Additional investigations requested by the MAC will be undertaken at the athlete’s or his/her National Olympic Committee’s (NOC) expense
- Any doctor who provides the MAC with false information will be ineligible to be accredited as an Olympic team doctor or official
- Under no circumstances will permission be given to use any synthetic anabolic steroid

However, the concept had its doubters and it would be another year before the IOC-MC agreed to allow the MAC to start operating prior to the Barcelona Games. However, the IOC-MC Chairman, supported by some Commission members, would not permit any publicity for “fear of abuse of the system”. Hence, only two applications were received for Barcelona 1992. Both were for oral GCS for well-documented IBD and were approved. Each athlete won a medal, although neither was in an individual event [116].

Anorchia

During the early years, MAC members were conscious that they were embarking on a new and potentially controversial undertaking and that their decisions must be able to withstand the closest scrutiny. In the mid-1990s, the MAC compiled the first document that established some circumstances in which certain prohibited substances and methods might be approved to manage specific medical conditions encountered in athletes. A number of conditions and circumstances for which permission would not be granted were also documented. The classes of prohibited drugs under consideration were diuretics, corticosteroids, beta-blockers and AAS (only testosterone). No exemptions were contemplated for stimulants or narcotics.

In 1994, two world-class sailors applied to continue to administer testosterone at the 1996 Olympic Games if selected. Both had anorchia, one a congenital condition and the other surgical due to bilateral malignancies. Having considered these applications, the MAC sought the advice of independent expert endocrinologists who were “blinded” as to the identity of each athlete, his NOC and his medical advisors. Both were approved and although neither athlete qualified at the 1996 Olympic yachting trials, one did compete in Athens 2004 [116].

Aplastic anemia

One unusual request was for erythropoietin in a Winter Olympic athlete prior to the 1994 Lillehammer Games because she had recently been a bone marrow donor for her brother with aplastic anaemia. It was the usual policy of the oncology centre concerned to withdraw and store a unit of blood prior to harvesting bone marrow and reinfusing this postharvest. (This would have been a Prohibited Method.) Owing to not wanting to interrupt her Olympic training programme and the urgency of her brother’s need for a bone marrow donation, this was not undertaken and her haemoglobin fell by 2 g/dl. However, despite the compelling circumstances, the application was rejected [116].
Congenital adrenal hyperplasia (CAH) and hypogonadism

Some early requests included an archer with 21-hydroxylase deficiency (salt losing) congenital adrenal hyperplasia (CAH) seeking oral GCS, which was approved. However, shortly after this, a shooter with 17-hydroxylase deficiency CAH who had been granted national approval was denied permission by her International Federation (IF) to administer GCS and compete internationally. Fortunately, intervention by the MAC reversed this decision and later, she competed successfully at the 2000 Olympic Games. Prior to Atlanta 1996, a female soccer footballer with C1-esterase deficiency hereditary angioneurotic oedema was approved to use danazol daily and participated. In contrast, an aging canoeist who had been prescribed testosterone for alleged hypogonadism and had the backing of his national antidoping agency sought permission to continue this therapy at Atlanta. The MAC advised that this athlete did not have a valid justification and rejected the application [116].

Stimulation medication

A well-publicised case that regrettably reflected that the MAC was compelled to remain "clandestine" was that of an elite 22-year-old 3 m diver whose severe narcolepsy was diagnosed in 1996. Immediately, she sought permission from her NOC and then from FINA to take stimulant medication but was rejected by both bodies. She continued to compete nationally and internationally and won national titles but seemingly was not required to take a doping control until a Grand Prix event in 1999 when she admitted the use of dexamphetamine on the doping control form and tested positive. Despite being advised that her narcolepsy was so severe that she was at major risk of falling asleep on the 3 m diving platform and thus of potential injury, FINA’s Doping Control Panel imposed a 12 month suspension. Intervention by the FINA Medical Commission resulted in the sanction being reduced to 6 months, but on appeal to CAS, this was further reduced to 2 months. Ironically, less than a month after her positive test, FINA modified their doping regulations to permit the use of a prohibited medication in special circumstances. The opinion of the MAC was not sought until shortly prior to her initial FINA hearing and then at the request of her NOC. The MAC had no hesitation in advising that she was entitled to a TUE. This may or may not have influenced both the FINA Medical Commission and the CAS. Had the IOC informed the stakeholders of the MAC’s existence, this diver would have applied and been approved to take her medication in 1996. Soon after this decision by the MAC, an application was received from a track and field athlete from the same NOC whose narcolepsy had been proven conclusively by sleep studies. On this occasion, the opinion of an independent expert in sleep medicine was sought. This request was granted by the MAC for the recently released but prohibited modafinil – a wakefulness drug [116].

Danazol

Between 1992 and 2003, the IOC’s MAC (later TUEC) received numerous applications and its recommendations were believed to have been accepted on all occasions. In addition to functioning at the Olympic Games without publicity, the MAC provided advice to as many as 15 IFs and 11 NOCs who sought assistance. Few applications were received that could be deemed to be “opportunistic”, but one from a 33-year-old elite female weightlifter appeared to be. On the advice of a “Longevity Institute” doctor, she sought permission to administer transdermal testosterone following her hysterectomy and bilateral oophorectomies performed for endometriosis. This was in addition to her replacement permitted hormone therapy. The application was rejected because no woman should ever be granted approval to take any AAS except danazol for very strict criteria. From its early days, the MAC decided that no AAS, except testosterone, could be approved for any male athlete and only then with a conclusive diagnosis and if the necessity was confirmed by an independent expert. This policy remains today [116].
**Glucocorticoids and diuretics**

In late 1998, approval for oral GCS treatment was granted to a young athlete in the sport of curling who had a successful renal transplant. Earlier that year, he had competed at the 1998 Nagano Olympics with an MAC-approved TUE for furosemide to assist in managing his chronic renal failure. At that time, a condition of approval was that the specific gravity of urine collected in doping control must be 1005 or greater by refractometer. However, improved analytical hardware has made this requirement no longer necessary [116].

**Beta-blocker**

A 56-year-old elite international shooter with a history of coronary artery bypass surgery was denied permission to take a cardioselective beta-blocker and compete. Although his medical indication was not questioned, beta-blockers had been demonstrated to produce a 13 percent improvement in shooting performance and this was deemed to contravene the criterion of ‘not enhancing sports performance’. Currently, the status of beta-blockers in shooting remains unchanged, that is, TUEs should not be approved. Prior to Salt Lake City 2002, a bobsledder with low-serum testosterone after a unilateral radical orchiectomy for seminoma was denied permission to administer depotestosterone because of the presence of one intact testicle [116].

**Recognising the concept of TUEs**

Many NOCs and IFs did not accept the policy of therapeutic use, first because it was not in the Medical Code of the Olympic Movement, and second, because of ignorance on the part of many sports authorities as to the concepts of therapeutic use. Thus, recognition of the concept of TUE continued to be difficult. In 1998, the IOC’s Juridical (Legal) Committee was incredulous at being advised by the author that TUEs had been approved for 6 years and criticised the IOC-MC Chairman for denying the MAC and its operation any publicity. But it would be another 2 years before the Juridical Committee would finally permit a one sentence mention of the concept as an addendum to the 2000 edition of the Medical Code of the Olympic Movement. This permitted a small breakthrough when the Anti-Doping policy for the Sydney 2000 Olympics made mention of the principle. Australia is believed to be the first country to have a committee with legislative authority to approve TUEs from August 1999. During the Sydney 2000 Olympic Games, Australia’s TUEC organised a meeting of NOCs, IFs and interested persons which recommended that templates of established and proposed criteria for specific TUEs be prepared. This task was undertaken by Australia’s TUEC and the templates circulated to attendees for feedback in 2001 [116].

It was the World Anti-Doping Agency (WADA), not the IOC that provided global recognition and acceptance of TUE. Strenuous attempts to sell the idea of TUE occurred at the 1999 World Conference on Doping in Sport in Lausanne from which WADA had its origins. Fortunately, two members of the MAC had significant WADA roles from its start and were able to ensure that the principle of TUE was accepted in the World Anti-Doping Code. An International Standard of TUE (ISTUE) was prepared between 2001 and 2003 and became operative when WADA finally assumed global responsibility for doping in January 2004. Significantly, the TUE criteria and guidelines developed by the MAC in 1991-1992 were incorporated virtually unaltered in the initial ISTUE. WADA established a TUE Expert Group in 2004 and members reviewed the Australian TUE templates that had been updated at least annually. These were offered to WADA in 2005 but the offer was rejected. During the last 6 years, WADA has developed advice termed “Medical Information for TUE Committees”, which had a rocky start, but by involving experts in each field, it has become a valuable reference document for TUECs [116].
More recent Olympic Games

It is difficult to compare TUEs at Olympic Games as changes to the Prohibited List have necessitated TUEs for different substances and methods. Insulin was prohibited prior to Sydney 2000 and 5/8 TUEs approved were for insulin-dependent diabetes mellitus (IDDM). But the number of applications increased significantly after WADA assumed global responsibility for doping from the IOC. At the Games in Athens 2004, 24 TUEs for athletes from 19 NOCs and 15 sports were approved with 9/24 (38 %) being for insulin. An appeal against a rejected application for systemic GCS was heard by WADA and the verdict confirmed that the IOC's MAC, now termed TUEC, had acted correctly. Intravenous infusions were prohibited prior to Torino 2006, although no athlete sought a TUE and 2/4 approved TUEs were for insulin for IDDM. Two applications were considered not to meet the criteria for approval and were discussed with each athlete's Chief Medical Officer (CMO) who accepted the TUEC's rationale and both were withdrawn. In Beijing, the TUEC recognised or approved 39 TUEs, of which nine were for IDDM. These 39 athletes were from 19 NOCs and 19 sports. For the first time, five intravenous infusions were approved. Interestingly, there were two Olympic athletes with well-documented Addison's disease approved for oral GCS. One application for stimulant medication for adult onset attention deficit hyperactive disorder was approved and a second withdrawn when a second opinion was sought, which was IOC's policy and later WADA's [116].

Between 2002 and 2008, inhaled beta₂-agonists (IBA) were managed by an IOC Independent Asthma Panel but not considered to be a TUE. However, in 2009, when WADA included IBAs in the prohibited list, the requirements to approve IBA use were identical to those of the IOC's Asthma Panel. This decision was partially rescinded just prior to Vancouver 2010 but two IBAs remained prohibited, formoterol and terbutaline. These accounted for 94 of the large number of 107 TUEs approved or recognised by the IOC's TUEC at Vancouver. Of the remaining 13, there were three approvals for insulin for IDDM, two for ADHD and one for an IF infusion. One IF-approved TUE for a female athlete to take dehydroepiandrosterone was a fundamental error since no female athlete should ever be approved to take any AAS except danazol. An appeal to WADA was successful, but WADA rules permitted the athlete 14 days to cease taking this prohibited medication, which allowed her to continue until after the Games. This is unsatisfactory and should be changed [116].

A number of difficulties were encountered at the recently successful London Olympic Games. From 2004, the IOC's TUEC had agreed to recognise TUEs for participating Olympic athletes approved by both IF and NADO TUECs after being subjected to close scrutiny. For Vancouver, the IOC was required to use WADA's Anti-Doping Administration and Management System (ADAMS). This posed a number of problems which were augmented in London with almost four times the number of participating athletes and IFs. First, the IOC TUEC's access to ADAMS was restricted since members were denied the opportunity to open uploaded medical files to allow them to confirm that an approved TUE had been granted according to established criteria. Second, more than half of the participating 205 NOCs do not upload TUEs onto ADAMS. Third, few NOCs heeded the request to provide the IOC's TUEC with a list of valid TUEs for their athletes. Finally, much time was spent in fruitlessly examining ADAMS to identify athletes believed to be accredited for London 2012 who may have had current a TUE. Almost all TUEs identified on ADAMS for athletes presumed to be competing in London were either for substances that were currently not prohibited (mainly IBA) or short-term TUEs that were no longer necessary or valid. Clearly, the IOC needs WADA to provide superior access and a more efficient way of ascertaining valid TUEs if ADAMS is to be used at future Olympic Games [116].

Prior to the opening of the London Olympic Village, CMOs were requested to provide the IOC's TUEC with details of all valid TUEs for their NOC athletes who would participate at the Games. Only two complied totally and 21 of the 31 preapproved TUEs that were recognised...
were from these two NOCs. During the Games, two athletes tested positive for prohibited substances and when advised, the NOCs of both athletes confirmed that each had a valid TUE that had not been disclosed. Hence, one must question how many other athletes had TUEs and did not advise the IOC. Although at each Games between 2002 and 2010, between 1/1000 and 1/1500 athletes were known to have IDDM, in London, only three athletes were reported to have a TUE for insulin (1/3500) which begs the question: were there more insulin-dependent diabetic athletes about whom the IOC was never advised? In London, the TUEC approved another 26 TUEs, with as customary most (15) being for systemic GCS and there were six intravenous infusions. One application was considered inappropriate and the CMO was contacted and the reasons provided. The NOC agreed to withdraw the application, institute an alternative permitted treatment and this athlete won a gold medal [116].

Legislation

In the early 1900s, endurance events lasted for days without rest. Open-water swimming, cycling, and long-distance running and walking athletes used stimulants such as strychnine, heroin, and amphetamine to alter the perception of fatigue. Only later did governments and sport recognize the serious health risks associated with the use of stimulants. The International Amateur Athletics Federation (IAAF; now the International Association of Athletics Federations) banned the use of stimulants in 1928. The amphetamine-related deaths of Danish cyclist Knud Enemark Jensen during competition at the 1960 Olympic Games and British cyclist Tommy Simpson during the 1967 Tour de France illustrated the seriousness of the problem. In 1966, the cycling, soccer, and track and field international federations began testing for stimulants. The International Olympic Committee (IOC) formed its Medical Commission, which included a Subcommission on Biochemistry and Doping in Sport, in 1967 and tested for stimulants at the 1968 Olympic Games in Mexico City. France adopted antidoping legislation in 1963; the Council of Europe adopted the first international Anti-Doping Convention in 1968 [043].

In 1998, police found a large number of prohibited substances, including ampoules of erythropoietin, in a raid during the Tour de France. The scandal led to a major reappraisal of the role of public authorities in anti-doping affairs. As early as 1963, France had been the first country to enact anti-doping legislation. Other countries followed suit, but international cooperation in anti-doping affairs was long restricted to the Council of Europe. In the 1980s, there was a marked increase in cooperation between international sports authorities and various governmental agencies. Before 1998, debate was still taking place in several discrete forums (IOC, sports federations, individual governments), resulting in differing definitions, policies, and sanctions. Athletes who had received doping sanctions were sometimes taking these sanctions, with their lawyers, to civil courts and sometimes were successful in having the sanctions overturned. The Tour de France scandal highlighted the need for an independent, nonjudicial international agency that would set unified standards for anti-doping work and coordinate the efforts of sports organizations and public authorities. The IOC took the initiative and convened the First World Conference on Doping in Sport in Lausanne in February 1999. Following the proposal of the Conference, the World Anti-Doping Agency (WADA) was established later in 1999. In the 1990s, there was a noticeable correlation between more effective test methods and a drop in top results in some sports [031].

Concern regarding the effects of anabolic steroids on athletes resulted in US Congressional hearings in 1988. Anabolic steroids were scheduled under Class III of the Controlled Substance Act in 1990. That same year, the Dubin Commission report, commissioned by the Canadian government because of concerns regarding the use of public money in sport, documented widespread abuse of performance-enhancing drugs and poor testing by
Canadian sporting authorities. In 1990, two governmental agencies, the Canadian Sport Anti-Doping Agency and the Australian Sport Drug Agency, were formed to deal with drugs in sports. The second Council of Europe Anti-Doping Convention was signed. A multilateral intergovernmental agreement, the International Anti-Doping Arrangement (IADA) was formed to promote more effective antidoping practices. The IADA group developed an International Organization for Standardization (ISO) Publicly Available Specification (ISO/PAS 188730) for collection of urine samples. This document eventually became the basis for WADA's International Standard for Testing [043].

**Courtroom, economics and anti-doping**

Before any new drugs test is introduced, sports governing bodies must be convinced that it will stand up to legal challenges. If the doping control authorities lose a courtroom battle, the consequences can be disastrous. In 1997, the British Athletics Federation went bankrupt, partly as a result of court costs incurred after the middle-distance runner Diane Modahl challenged its decision to ban her following a positive test for testosterone. Modahl convinced the court that bacterial growth caused by a failure to refrigerate her urine sample properly could have led to a false positive result [00001].

**Expelled team doctor in 2000**

The International Olympic Committee (IOC) has expelled a Romanian team doctor from the Sydney games and barred him from the next two Olympic Games because he gave champion gymnast, Andreea Raducan, a cold remedy that contained a banned substance while she was competing at games. Raducan tested positive for pseudoephedrine. The IOC also stripped the 16-year old athlete of her all round gold medal but allowed her to keep a gold medal from the team competition and a silver from the vault competition. Prince Alexandre de Merode, IOC drug chief, said she had taken two tablets of an over-the-counter cold medication. “The medication was prescribed by the team doctor”, said de Merode. “She is not directly responsible. The fault falls with the medical doctor. But we have rules and we have to apply the rules.” The Romanian team doctor, named in wire service reports as Ioachim Oana, was expelled immediately from the Sydney games and is suspended for the winter games in Salt Lake City, Utah, USA, in 2002 and the summer games in Athens, Greece, in 2004 [025].

**USA**

Although officials have banned PEDs from Olympic competition since 1967, and the International Olympic Committee has prohibited AAS use since 1975, it was not until 1991 that the US Congress designated AAS as Schedule III controlled substances. In 2004 the Anabolic Steroid Control Act amended the Controlled Substances Act and expanded its definition of anabolic steroids. The new definition, which does not require proof of muscle growth, identified 59 specific substances (including their salts, esters, and ethers) as anabolic steroids and listed them as Schedule III controlled substances [003].

The USADA was formed in 2000 by the US Olympic Committee in part to avoid the perception of the “fox guarding the hen house.” In 2001, Congress designated USADA “the official antidoping agency for Olympic, Pan American and Paralympic sport in the United States.” USADA was given authority to develop a comprehensive national antidoping program including testing, adjudication, education, and research. USADA and WADA have jointly worked to advance the science (analytical chemistry, biochemistry, endocrinology, hematology, laboratory medicine, pharmacology, physiology, sports medicine, and toxicology) of detection of doping [043].
The Bay Area Laboratory Cooperative (BALCO) scandal was one of the early examples of information sharing between law-enforcement and antidoping agencies. BALCO was providing synthetic anabolic steroids not approved by the Food and Drug Administration and designed to avoid detection to a number of athletes including Kelli White, Marion Jones, and allegedly Barry Bonds. Sharing of information between the Internal Revenue Service Criminal Investigations, local law enforcement, and USADA enabled effective prosecution of the cases in criminal and sport venues, as appropriate. Prior to 2004, detection of a prohibited substance or its metabolites or markers was required to be prosecuted for a violation of the antidoping rules. The 2004 edition of the Code recognized other means for proving a case of doping, including any reliable information. USADA's prosecution of the first "nonanalytical positive" case that same year resulted in suspension of the athlete [043].

**Industrialized doping**

In 2003, another significant event in the understanding of the institutional nature of doping occurred. A syringe was anonymously sent to a WADA-accredited laboratory in Los Angeles that contained tetrahydrogestrinone (THG), a "designer" steroid that was not known and not on the current WADA prohibited list, made specifically to avoid detection by modern anti-doping technologies. This led to a series of investigations resulting in the indictment and subsequent conviction of individuals running a performance-enhancing program for professional athletes at the BALCO pharmacy in San Francisco [030].

In May 2006, Spanish police arrested five people and seized a variety of banned performance-enhancing drugs and blood-doping supplies at a Madrid doping clinic. Here, professional athletes would receive medically-supervised injections of hormones and other performance-enhancing drug regimes. The 40-page police report included a clear paper trail of doping procedures on at least 50 professional cyclists. The report was given to the International Cycling Union, which led to the disqualification of 23 professional cyclists, virtually all the top contenders from the 2006 Tour de France. The final of the 2006 Tour was also tarnished, as the champion, Floyd Landis, was found to have a positive anti-doping test for steroids. Landis was stripped of the championship and discharged from his team. At this writing the result is being challenged by Landis and his legal and medical experts, claiming that the test was invalid since several errors were made in the collection, analysis and reporting of the results [030].

In a separate investigation in Paris in 2006, 23 individuals were sentenced to 4 years in jail for trafficking a cocktail of amphetamines and other performance-enhancing drugs known as "Belgium Pot" to professional cyclists. In October of that same year, the cricket world was shocked to learn that two Pakistani fast bowlers, Shoaib Akhtar and Mohammad Asif, tested positive for the steroid nandrolone [030].

**Laboratory testing**

Mass spectrometry has played a decisive role in doping analysis and doping control in human sport for almost 40 years. The standard of qualitative and quantitative determinations in body fluids has always attracted maximum attention from scientists. With its unique sensitivity and selectivity properties, mass spectrometry provides state-of-the-art technology in analytical chemistry. Both anti-doping organizations and the athletes concerned expect the utmost endeavours to prevent false-positive and false-negative results of the analytical evidence. The Olympic Games play an important role in international sport today and are milestones for technical development in doping analysis. Mass spectrometry has played an important role in doping control from Munich 1972 to Beijing 2008 Olympics [117].
One brief note gave also a general overview on the activity of the antidoping laboratories accredited by the World Anti-Doping Agency outlining the evolution, over the last four decades, of the analytical methods and techniques in the detection of prohibited substances and methods. Special emphasis was given to the future trends of the fight against doping in sports, 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 antidoping laboratories by the general public [118].

There were 35 WADA accredited laboratories in 2009. Approximately 3,300 additional samples were analyzed in 2009 compared to 2008, with a slight increase in Adverse Analytical Findings and Atypical Finding, from 1.84 percent (2008) to 2.02 percent (2009) [118].

**Adverse findings 2005-2011 from the Doping Control Laboratory of Athens**

One article concerns the analysis of the adverse analytical findings (AAFs) and the appropriate alterations made during the period 2005-2011, so that the Doping Control Laboratory of Athens (DCLA) obeys the updated World Anti-Doping Agency (WADA) List of Prohibited Substances. The % AAFs of the DCLA was compared with those of WADA-Accredited Laboratories. In 2008, the term Atypical Finding was introduced by the WADA representing a reported but inconclusive result. A characteristic example is when a testosterone-to-epitestosterone ratio is >4 followed by a negative gas chromatography/combustion/isotope ratio mass spectrometry result. In a total of about 30,000 athlete samples, 136 athletes were found with an increased testosterone/epitestosterone ratio and 43 with tetrahydrocannabinol metabolite (THCCOOH) of 427 reported AAFs. Twenty-one athletes in total were found positive with methylhexaneamine, the 11 found after a batch of 1000 samples was reprocessed. Besides, there were AAFs below their Minimum Required Performance Level (MRPL). The increasing need for higher detectability imposed new apparatus, e.g., liquid chromatography/quadrupole/time-of-flight mass spectrometry, whereas that for lowering the capital costs and reporting times led to the unification of the screening method which includes stimulants, diuretics, anabolics and other substances [119].

**The 2005 WADA list**

The purpose of the World Anti-Doping Code 2003 and the 2004 Prohibited List is to create a universal international standard to fight doping in competitive sports. The result of this is a whole series of changes for doctors with regard to their work with competitive athletes. The revised definition of doping now includes physicians in the group of persons who can fulfil the elements of a doping offence. Moreover, the mere possession of substances appearing on the Prohibited List represents a violation of anti-doping regulations. The 2004 Prohibited List includes several changes to the Olympic Movement List from 2003. Caffeine, for example, was removed from the list. Cannabinoids, on the other hand, are now prohibited in competition for all sports. The same is true for all forms of glucocorticosteroids. Therapeutic use exemptions in an abbreviated process are possible for the administration of glucocorticosteroids by non-systemic routes, as well as inhalative therapy with the beta-2-agonists formoterol, salbutamol, salmeterol, and terbutaline. In other cases, a therapeutic use exemption is possible using a standard application process. Further changes will become effective in the 2005 Prohibited List. In 2005, it is essential that beta-2-agonists are prohibited in and out of competition. HCG and LH are prohibited for all athletes. Dermatological preparations of glucocorticosteroids are no longer prohibited, and intravenous infusions will be a prohibited method in 2005, except as a legitimate acute medical treatment. In cases of violations of anti-doping regulations where it is permissible for the affected person to furnish proof of exoneration, the burden of proof is not higher than that
required to prove the violation. The sanctions provided for in the World Anti-Doping Code follow a principle of rules and exceptions which at first glance seems difficult to understand. In the case of doping violations by physicians, the anti-doping code provides--as a general rule--for exclusion from sports associations for at least four years. Since several of the changes are questionable under constitutional aspects, it remains to be seen whether the World Anti-Doping Code 2003 will allow the achievement of a universal standard to combat doping [120].

The 2011 WADA list

International anti-doping efforts are harmonized and regulated under the umbrella of the World Anti-Doping Code and the corresponding Prohibited List, issued annually by the World Anti-Doping Agency (WADA). The necessity for a frequent and timely update of the Prohibited List (as the result of a comprehensive consultation process and subsequent consensual agreement by expert panels regarding substances and methods of performance manipulation in sports) is due to the constantly growing market of emerging therapeutics and thus new options for cheating athletes to illicitly enhance performance. In addition, “tailor-made” substances arguably designed to undermine sports drug testing procedures are considered and the potential of established drugs to represent a doping substance is revisited in light of recently generated information. The list that was published and has been authoritative from 1 January 2011 comprising a total of 10 different classes of banned substances (S0–S9), three different groups of prohibited methods (M1–M3), and two classes of drugs (P1 and P2) being banned from selected sports only. In comparison to the 2010 edition of the Prohibited List, few but significant modifications were made. A major novelty has been the installation of the S0 section, which interdicts the use of any pharmacological substance that has not (yet) received approval by governmental health authorities (or where development has discontinued) as a human therapeutic agent. This addendum is particularly important in the light of new drug entities that are not covered by any of the established classes of banned substances, either by their chemical nature or their biological effects. Ryanodine receptor-calstabin complex stabilizers, which have been proven to enhance performance in the laboratory setting are currently undergoing advanced clinical trials but do not represent compounds of S1–S9. These might exemplify such a new category of substances. The section S2 (peptide hormones, growth factors, and related substances) was modified concerning the examples of erythropoiesis-stimulating agents (ESAs) by explicitly listing hypoxia-inducible factor (HIF) stabilizers, which also represent a considerably heterogeneous emerging class of substances targeted for clinical approval. In contrast to these additions to S2, the use of platelet-derived preparations has been legitimized and the paragraph removed in the 2011 Prohibited List accordingly. The category M2 (chemical and physical manipulation) was extended by a new paragraph (M2.3) that particularly emphasizes the illicit nature of the “sequential withdrawal, manipulation and reinfusion of whole blood into the circulatory system”, a strategy that includes, for example, the so-called UV-activated autohemotherapy (commonly regarded as alternative medicine). M3 (gene doping) was split into three sub-groups that define (1) the transfer of nucleic acids or sequences of these; (2) the use of normal or genetically altered cells; (3) the use of drugs manipulating gene expression with impact on athletic performance as prohibited methods [121].

The 2012 WADA list

As of 1 January, the 2012 Prohibited List International Standard has come into effect, exhibiting minor but relevant alterations from to the previous 2011 version. In agreement with its predecessor, the List comprises a total of 10 different classes of banned substances (S0–
S9), three different groups of prohibited methods (M1–M3), and two classes of drugs (P1 and P2). The latter are banned from selected sports only. The major modifications can be observed in the sections: S3 (beta2-agonists), S4 (hormone and metabolic modulators), and M3 (gene doping). In the S3 group, quantitative consideration of formoterol has been considered with the allowance of a maximum daily therapeutic dose of 36 microg of inhaled formoterol and a urinary threshold of 30 ng/mL. If the determined quantity in urine exceeds this level, an adverse analytical finding is reported followed by penalty, unless the athlete can prove (e.g. by means of a pharmacokinetic study) that the concentrations were reached by the admissible route and daily dosage. The category S4 has been complemented by a new subsection named “metabolic modulators”. These host peroxisome proliferator activated receptor (PPAR)delta agonists such as GW1516 and PPARdelta-AMP-activated protein kinase (AMPK) axis agonists, such as 5-amino-4-imidazolecarboxamide ribonucleoside (AICAR). These were formerly listed among gene doping (M3.3) in the previous list. Following a re-evaluation of the impact of the use of alcohol (P1) and beta-receptor blocking agents (beta-blockers, P2) on the athletes’ performance in selected sport disciplines, the interdiction of alcohol was lifted for Ninepin and Tenpin Bowling (in agreement/on request of the Federation Internationale des Quilleurs) and so was the ban of beta-blockers for bobsleigh, skeleton, curling, modern pentathlon, motorcycling, sailing, and wrestling. In order to probe for potential patterns of abuse concerning selected substances that are currently not (or not at all times or at any concentration) prohibited, the established WADA monitoring programme has been expanded. Besides the stimulants bupropion, caffeine, phenylephrine, phenylpropanolamine, pipradrol, pseudoephedrine (< 150 microg/ml), and synephrine and the ratio of morphine over codeine, the prevalence of nicotine, hydrocodone, and tramadol was to be monitored in-competition. Moreover, the (ab)use of corticosteroids in out-of-competition periods is acquiring concern and appears as a new item on the 2012 monitoring programme. Concerning nicotine and its metabolites, a comprehensive compilation of monitoring data was published outlining an alarmingly high prevalence of nicotine use in selected sports disciplines. Further to these explicitly stated drugs, alternative medicine has necessitated greater attention in order to protect both the spirit of sport and the athletes themselves from inadvertent anti-doping rule violations. In continuation of the endeavor to keep pace with the changing trends of doping, manipulation, and innovations and improvements in analytical chemistry, anti-doping laboratories are urged to enhance their procedures in terms of comprehensiveness, speed, and/or sensitivity. This, in combination with the fact that the International Standard for Laboratories allows for the long-term storage and re-analysis of doping control samples, is considered one of the main aspects causing deterrence to cheating athletes [122].

The 2013 WADA list

Compared to its predecessor, the 2013 Prohibited List exhibited only few major modifications such as the re-categorization of insulins from S2 (peptide hormones, growth factors and related substances) to S4.5 (hormone and metabolic modulators) and the inclusion of M2.3 (chemical and physical manipulation) into M1.1 (manipulation of blood and blood components) by respective re-wording of the paragraph. In addition, the maximum daily therapeutic dose of 36 microg of inhaled formoterol (S3) was increased to 54 microg, resulting in a permissible urinary concentration of 40 ng/mL (formerly 30 ng/mL). In agreement with prior protocols, an adverse analytical finding is reported (followed by penalty) if the determined quantity in urine, including the measurement uncertainty, exceeds the threshold limit. The finding will be processed as an anti-doping rule violation unless the athlete can prove (e.g. by means of a pharmacokinetic study) that the concentrations were reached by the admissible route and daily dosage. In agreement with or on request of international federations, the interdiction of beta-receptor blocking agents (beta-blockers, P2) was lifted in selected sport disciplines including ninepin and tenpin bowling, aeronautic,


boules, bridge, and powerboating. This is a continuation of the process initiated in 2012, where another 7 international federations removed the ban of beta-blockers from their sport [086].

In continuation of the 2012 Prohibited List, the 2013 version also contained 12 classes of prohibited substances (S0–S9 plus P1 and P2) and three categories of prohibited methods (M1–M3). Compared to its predecessor, the 2013 Prohibited List exhibited only few major modifications such as the re-categorization of insulins from S2 (peptide hormones, growth factors and related substances) to S4.5 (hormone and metabolic modulators) and the inclusion of M2.3 (chemical and physical manipulation) into M1.1 (manipulation of blood and blood components) by respective re-wording of the paragraph. In addition, the maximum daily therapeutic dose of 36 microg of inhaled formoterol (S3) was increased to 54 microg, resulting in a permissible urinary concentration of 40 ng/mL (formerly 30 ng/mL). In agreement with prior protocols, an adverse analytical finding is reported (followed by penalty) if the determined quantity in urine, including the measurement uncertainty, exceeds the threshold limit. The finding will be processed as an anti-doping rule violation unless the athlete can prove (e.g. by means of a pharmacokinetic study) that the concentrations were reached by the admissible route and daily dosage. In agreement with or on request of international federations, the interdiction of beta-receptor blocking agents (beta-blockers, P2) was lifted in selected sport disciplines including ninepin and tenpin bowling, aeronautic, boules, bridge, and powerboating. This is a continuation of the process initiated in 2012, where another 7 international federations removed the ban of beta-blockers from their sport. In addition to the Prohibited List, WADA has established a monitoring program in order to probe for potential patterns of abuse concerning selected substances that are currently not (or not at all times or at any concentration) interdicted. The “in-competition” monitoring program, which included the stimulants bupropion, caffeine, phenylephrine, phenylpropanolamine, pipradrol, pseudoephedrine (< 150 microg/mL), synephrine, and nicotine as well as the ratio of morphine over codeine, hydrocodone, and tramadol in 2012, was complemented by the analgesic tapentadol in 2013. Further, as in 2012, the (mis)use of corticosteroids in out-of-competition periods has been investigated [086].

**Category 0**

The category S0 of the Prohibited List does not explicitly mention any specific substance; here, any pharmacological compound not covered by the other classes of prohibited substances and methods and without “current approval by a governmental regulatory health authority for human therapeutic use” is considered illicit. Potential candidates for this category are sirtuin-1 (SIRT1) activating drugs such as SRT1720 the characterization, metabolism. In case of the proposed routine doping control application, the mass spectrometer was a QqQ instrument with ESI source operated in positive mode and MRM, while compound characterization was conducted on a quadrupole-time-of-flight (Q-TOF) system [086].

**Monitoring program**

In addition to the Prohibited List, WADA has established a monitoring program in order to probe for potential patterns of abuse concerning selected substances that are currently not (or not at all times or at any concentration) interdicted. The “in-competition” monitoring program, which included the stimulants bupropion, caffeine, phenylephrine, phenylpropanolamine, pipradrol, pseudoephedrine (< 150 microg/mL), synephrine, and nicotine as well as the ratio of morphine over codeine, hydrocodone, and tramadol in 2012, was complemented by the analgesic tapentadol in 2013. Further, as in 2012, the (mis)use of corticosteroids in out-of-competition periods has been investigated [086].
The 2014 WADA list

Identical to the 2013 Prohibited List, the 2014 issue also contains 12 classes of prohibited substances (S0–S9 plus P1 and P2) and three categories of prohibited methods (M1–M3). Major modifications compared to the preceding 2013 version include the addition of vasopressin V2 antagonists (commonly referred to as vaptans) to the subclass of diuretics and the addition of cathinone and its analogues as well as trimetazidine to Section S6 (stimulants). Moreover, as of 1 September 2014, substances acting as hypoxia-inducible factor (HIF) activators, such as xenon and argon, have been listed as explicitly prohibited, necessitated by recently surfaced documents on an arguably licit and extensive use of xenon/oxygen mixtures among selected athletes. WADA further continued the monitoring programme in order to generate information on potential patterns of abuse concerning defined substances that are currently not (or not at all times or at any concentration) prohibited. The 2014 in-competition monitoring programme was complemented by the narcotic agent mitragynine, covering now collectively the ratio of morphine over codeine, hydrocodone, tramadol, tapentadol, and mitragynine as well as the stimulants bupropion, caffeine, phenylephrine, phenylpropanolamine, pipradrol, pseudoephedrine (<150 microg/mL), synephrine, and nicotine. Further, as in 2013, the potential (mis)use of corticosteroids in out-of-competition periods has been monitored [123].

Erythropoiesis-stimulating agents

Besides EPO and its derivatives, hypoxia-inducible factor (HIF) stabilizers/activators are considered as relevant to doping controls. This class of compounds is particularly heterogeneous and includes low molecular mass organic compounds such as FG-4592 as well as the recently added noble gas xenon and, as of January 2015 explicitly named, the inorganic substance cobalt. Ionic cobalt, more specifically Co^{2+} administered as CoCl2, was the method of choice for the treatment of renal and non-renal anemia in the pre-EPO era. Due to serious adverse effects and an undesirable cost-benefit ratio, CoCl2 was removed from the therapeutic arsenal [123].

Amendment to the 2014 Prohibited List

Having been alerted to the substance of Xenon and its potential performance enhancing characteristics in February, the WADA List Committee discussed the matter during its April meeting. Following its consideration, the Executive Committee approved the option to modify Section S.2.1 of the 2014 Prohibited List, which will be effective following the required three-month notice period:

S2. Peptide hormones, growth factors and related substances
The following substances, and other substances with similar chemical structure or similar biological effect(s), are prohibited:

Erythropoiesis-Stimulating Agents [e.g. erythropoietin (EPO), darbepoetin (dEPO), hypoxia-inducible factor (HIF) stabilizers and activators (e.g. xenon, argon), methoxy polyethylene glycol-epoetin beta (CERA), peginesatide (Hematide)]. The process means that the amendment to the 2014 Prohibited List will not come into effect until three months after UNESCO has appropriately communicated the amendment to all States Parties after the Consensus meeting on antidoping in sport, Zürich, Switzerland, November 29-30 2013

As of 1 January 2015, the revised 2015 World Anti-Doping Code will be operational in the fight against doping. This will be binding for all stakeholders who unanimously approved the
revised code at the “World Conference on Doping in Sport” in Johannesburg, South Africa on 15 November 2013 [124].


A medical and scientific multidisciplinary consensus meeting was held from 29 to 30 November 2013 on Anti-Doping in Sport at the Home of FIFA in Zurich, Switzerland, to create a roadmap for the implementation of the 2015 World Anti-Doping Code. The consensus statement and accompanying papers set out the priorities for the antidoping community in research, science and medicine. The participants achieved consensus on a strategy for the implementation of the 2015 World Anti-Doping Code. Key components of this strategy include:

- sport-specific risk assessment
- prevalence measurement
- sport-specific test distribution plans
- storage and reanalysis
- analytical challenges
- forensic intelligence
- psychological approach to optimise the most deterrent effect
- the Athlete Biological Passport (ABP) and confounding factors
- data management system (Anti-Doping Administration & Management System (ADAMS))
- education
- research needs and necessary advances
- inadvertent doping and
- management and ethics: biological data

True implementation of the 2015 World Anti-Doping Code will depend largely on the ability to align thinking around these core concepts and strategies. FIFA, jointly with all other engaged International Federations of sports (IFs), the International Olympic Committee (IOC) and World Anti-Doping Agency (WADA), are ideally placed to lead transformational change with the unwavering support of the wider antidoping community. The outcome of the consensus meeting was the creation of the ad hoc Working Group charged with the responsibility of moving this agenda forward [125].

Non-approved substances

The category S0 of the Prohibited List does not explicitly mention any specific substance; here, any pharmacological compound not covered by the other classes of prohibited substances and methods and without “current approval by a governmental regulatory health authority for human therapeutic use” is considered illicit. Potential candidates for this category are sirtuin-1 (SIRT1) activating drugs such as SRT1720 the characterization, metabolism, and analysis of which was recently presented. Using a set of five model SIRT1 drug candidates with thiazole-imidazole pharmacophore (as in SRT1720), the mass spectrometric behaviour under ESI-CID conditions was studied and the detection of the active substances in human plasma was demonstrated. The analytical system consisted of an LC equipped with a reversed-phase C-18 column (2 x 50 mm, particle size 3 microm) and aqueous acetic acid (0.1 %, containing 5 mM ammonium acetate) and acetonitrile were used as solvents A and B, respectively. At a flow rate of 350 microL/min, the analytes were separated by gradient elution. In case of the proposed routine doping control application, the mass spectrometer was a QqQ instrument with ESI source operated in positive mode and MRM, while compound characterization was conducted on a quadrupole – time-of-flight (Q-
TOF) system. Plasma samples (100 microL) were enriched with eightfold deuterated SRT1720 as ISTD and 100 microL of water prior to protein precipitation by the addition of acetonitrile (400 microL). The supernatant was concentrated, reconstituted, and analyzed by LC-MS/MS. The approach allowed for limits of detection (LODs) between 0.1 and 1 ng/mL at recoveries of 90-98 percent, demonstrating the fitness-for-purpose of the method [086].

In order to expand the analytical possibilities for SIRT1 activating drugs to urine samples, in vitro metabolism studies were conducted to provide insights into metabolic pathways and potential target analytes in human urine. Mainly hydroxylation and N-oxidation were observed, and sites of modifications were localized by chromatographic-mass spectrometric and chemical methodologies. Eventually, an existing routine doping control assay consisting of enzymatic hydrolysis and LLE with subsequent LC-MS/MS analysis was expanded to include the new target analytes and LODs of 0.5 ng/mL were accomplished. In the absence of authentic administration study urine samples, the screening for in vitro/in silico generated metabolites has proven to be a viable means to identify atypical components in doping control samples [086].

**Important persons in anti-doping**

*Richard W Pound*

When talking about performance-enhancing drugs in sport, Richard Pound does not mince words: “I think it's my job to be everyone's face”, says the 63-year-old Montreal lawyer and chairman of the World Anti-Doping Agency (WADA), “This is not a diplomatic problem; this is cheating.” Pound, who swam for Canada in the 1960 Summer Olympics and the 1962 Commonwealth Games, joined the Canadian Olympic Committee when he retired from competition, eventually becoming its president. He was elected to the Committee in 1978 and put in charge of negotiating television and sponsorship contracts. The lucrative deals he struck have been credited with freeing the organisation from its dependence on government largesse. “I was happy as a clam doing that”, Pound recalls. Then, in 1998, in the wake of a major doping scandal, the then president of the International Olympic Committee Juan Antonio Samaranch was quoted in the press as saying he thought performance-enhancing drugs should be allowed as long as they were not harmful, and that the Committee's list of banned substances was too long. A firestorm of criticism erupted. “It got worse and worse”, Pound says, “and finally Samaranch had to call an emergency meeting of the executive board and asks, ‘How are we going to get out of this?’ And we all said, ‘We?’” Pound argued for setting up an anti-doping agency independent of the International Olympic Committee and other sports organisations, “because nobody believes the IOC anymore, nobody believes the international sports federations, like the UCI (Union Cycliste Internationale), and nobody trusts the national Olympic committees, like the Canadian or US Olympic committees, to blow the whistle on our own people”. Samaranch eventually asked Pound to lead the effort. “I said I'd do it for a couple of years to get it up and running and after that I'm out of here. That was 6 years ago.” When he started the unpaid job, Pound knew nothing about doping: “The first thing that became clear to me when we started out was that when all is said and done; far more is said than done. There was an awful lot of lip service being paid and not very much actual work.” Many coaches and officials had got in to the habit of looking the other way, Pound says, “We knew our athletes were facing athletes who were doped – East Germans, athletes from the Soviet Union – and so if our kids leveled the playing field in their own way, well nobody looked too hard at it. What rules there were, Pound noted, also varied between organisations and countries. “The rules were all over the ballpark: one sports organisation had a life ban for the first positive test and another had a 2-week ban that you could serve between Christmas and New Year's.” So, in February, 1999, the International Olympic Committee convened a meeting in Lausanne, Switzerland, to draw up the Lausanne
Declaration in Sport, which led to the formation of WADA later that year. The first order of business was to draft and implement a uniform set of anti-doping rules. These were adopted, after long negotiations, in 2003 at the second World Conference on Doping in Sport, and came into effect on Jan 1, 2004. The Code has now been adopted by most major international and national Olympic organisations and, this October, by UNESCO in the International Convention Against Doping in Sport. "So now everybody will be playing off the same sheet; same rules for all athletes, all sports, all countries, and no differences between domestic laws and sports rules", Pound says. In addition to drawing up and refining the Code, WADA maintains and updates a list of banned substances, helps their members to adopt best practices, and funds scientific research, in particular into the development of drug-detection technology. But the key to solving the problem of doping in sport will be the education of athletes and the public, Pound says: “Part of my job is to make it as visceral as I can: I ask ‘How would you like it if your kid, who had trained for 10 years to go to the Olympics, lost by a tenth of a second to somebody who was all doped up?’” Now that the Code has been adopted, Pound notes that the next challenge for WADA is to make sure the Code is applied. This challenge is a difficult one, but he is hopeful. "If you and I had had this conversation in 1999 when WADA was being founded, and I was to say to you within 5 years we’re going to have an international code that will apply to all countries, all sports, in place, adopted by 202 national Olympic committees, 75 international sports federations, the IOC, and we'd have an international convention under the umbrella of UNESCO unanimously approved, you'd look at me and say, ‘You're out of your mind’ [126].

Arne Ljungqvist

Professor emeritus Arne Ljungqvist from Karolinska Institutet, Stockholm, who has served in various high positions in the IOC, International Association of Athletics Federations (IAAF), Swedish Sports Confederation, to mention some of them, has dedicated a great deal of his life to service in sports and sports medicine. Arne Ljungqvist has especially dedicated his career to the fight against doping and to the protection of the health of the athletes. It all started over 60 years ago when Arne became a Swedish senior champion in high jump in 1951, jumping as high as 201 cm. Arne was multi-talented and won also the Swedish junior championships in pole vault and javelin. He was one of the favourites for the Gold medal in the high jump competition in the Olympic Games in Helsinki 1952, but unfortunately could not compete because of an injury that he sustained during a medical student carnival in the autumn of 1951 when a group of us were jumping for the general public in the streets with numerous hard landings on asphalt. Arne had to end his jumping career as his injury could not be cured, and it was not until the 1960s that the diagnosis of patellar tendinopathy could be made. Arne, however, continued his medical studies and soon became very successful. Arne was appointed professor at the Karolinska Institutet in 1972 because of his excellent medical research in the fields of renal and cardiovascular diseases and, later, oncology. He held several high professional positions such as Vice Dean of Medical Faculty, Karolinska Institutet, 1972–1977; Pro-Rector, Karolinska Institutet, 1977–1983; Chairperson, Department of Pathology and Cytology, Karolinska Hospital, 1983–1992; President, Swedish Council of Sports Research, 1980–1992; Dean Swedish School of Sport and Physical Education, 1992–1996; and President of the Swedish Cancer Society, 1992–2001. Interestingly enough, he took time to serve Chamberlain to His Majesty the King of Sweden, 1977-1986 and has since then been Lord in Waiting to His Majesty the King of Sweden. In 1971, Arne returned to sport as he was elected to the Board of the Swedish Athletics Association, where in 1973 he became chairperson. In this time period, athletes used all kinds of medicines to enhance their bodies to achieve success. An anonymous survey among Sweden’s best athletes indicated that nearly half of them were using anabolic steroids, which indeed was legal until 1975, when reliable tests had finally been developed to identify users. Arne realised that something had to be done to create a healthy and ethical environment in sports. In 1975, he became a member of the Swedish Sports Confederation and was part of the initiation of the Swedish Commission against doping in 1977 with its own
doping rules by 1979. During 1989–2001, Arne became the President of the Swedish Sports Confederation and in 1989 a member of the Swedish Olympic Committee. Arne has had an outstanding international career, which started when, in 1976 he was elected to become a Council Member in IAAF and elected Vice President in IAAF in 1981. He served in this position until 1999 and became thereafter the Senior Vice President of IAAF until 2007 and Chairperson of the Medical Committee and Anti-Doping Commission. Since 1987 he has been a Member of the IOC Medical Commission and in 2003 he was appointed Chairperson of the Medical Commission of IOC. In 1994, Arne was elected Member of the IOC. When WADA was founded in 1999, Arne became the Member of WADA’s Foundation Board and Chairperson of WADA’s Health, Medical and Research Committee. Since 2003, Arne has been a Member of WADA’s Executive Committee and since 2008 has been WADA’s Vice President. Arne has been the front-line fighter against doping and his name is today one of the world’s most respected within international sports. Arne’s contributions in the fight against doping are second to none. He has been an athlete himself, which makes him understand the special language that is present in the athletic situation and also in the locker rooms. In his autobiography “Doping's Nemesis”, Arne gives an unrivalled insider’s view of the biggest dope scandals over the years, including the Ben Johnson and Balco affairs and the history of the Greek sprinters at the Athens Olympics in 2004. Arne’s actions together with the Italian police during the Torino Olympics 2006 against the Austrian team are classic. Doping seems to be steadily on the decline, and there is no doubt that Arne has played a key role in this successful work. Arne’s legacy in the fight against doping is lasting [127].
EFFECT OF INTERNATIONAL RESULTS DURING THE ANTI-DOPING ERA

Influence on world records in running by doping and anti-doping testing

Improvements in track and field sports have been attributed to factors such as population increase, drugs and new technologies, but previous research has found it difficult to distinguish the contributions from specific influences. Here it is shown how this is possible by means of a performance improvement index based on useful work done combined with modelling of the annual top 25 performances. The index was set to 100 in 1948 and showed that, by 2012, it had increased in running events to between 110.5 and 146.7 (men's 100 m and marathon). Underlying global effects accounted for the majority of all improvements (16.2 to 46.7) with smaller influences attributable to an influx of African runners (3.6 to 9.3), and a 4-year oscillation that arose from staging of the Olympic Games (±0.2 to ±0.6). Performance decreased with the introduction of compulsory random drug testing (-0.9 to -3.9) the World Anti-Doping Agency (WADA; -0.5 to -2.5) and fully automated timing (-0.6 to -2.5). Changes in elite sporting performance since the 1890s are attributable to societal changes caused by the industrial revolution and globalisation superimposed on millennia of human evolution [128].

World records in running as indication for doping in the elite

If recent improvements in athletic performance have been driven by doping, then improved doping control might be reflected by a leveling off or declining performances in sports where doping is thought to be ubiquitous. In recent analyses of major cycling races including the Tour de France, Giro d'Italia, Vuelta A España, the average speed has been leveling off or declining, since the introduction of improved techniques to detect use of exogenous EPO in 2005. However, the analysis of cycling is confounded by varying race distances, yearly changes in course, and weather. Endurance running eliminates many of these confounding factors. The tracks and courses are identical from year to year. The major cycling "Grand Tours" have shown an attenuation of performance over the last decade. This has been interpreted as circumstantial evidence that newer anti-doping strategies have reduced the use of performance-enhancing drugs. To examine this idea under more controlled conditions, speed trends for world class 5000 m, 10000 m, and marathon performances by men from 1980 to 2013 were analyzed. We obtained comprehensive records from the International Association of Athletics Federations, Association of Road Racing Statisticians, and the Track and Field All-time Performances database webpages. The top 40 performances for each event and year were selected for regression analysis. For the three distances, we noted cumulative performance improvements in the 1990s thru the mid-2000s. After the peak speed years of the mid 2000s, there has been limited improvement in the 5000 m and 10,000 m and world records set during that time remain in place today, marking the longest period of time between new records since the early 1940s. The world record for the 5000 m was set in 2004 while the 10000 m world record was set in 2005; these records stand today, which is the longest gap between world records since the 1940s. The number of performances below the 2012 Olympic A qualifying standard for the 5000 m and 10,000 m also appears to have leveled off since the middle 2000 s. Similarly the number of athletes breaking 2:10:00 for the marathon has also leveled off. 2:10:00 was chosen as a comparable standard for the marathon because this time is considered generally similar to or slightly slower than the A standards for shorter distances based on various empirical point tables, scoring systems and time conversion programs. Furthermore, year alone explained a large percentage of the variation in the speed trends. Consistent with these overall model estimates, the 5000 m and 10000 m had significant increases in speed during the 1990s whereas the marathon showed an increase over the entire three plus decades. In particular, the 5000 m the speed trend levels off starting around 2000. The marathon and 10000 m times do not show this as a
pronounced tendency. With the 5000 m and 10000 m, there is a pronounced “outlying” effect of the top performance from 1995 to late 2010. The marathon, however, displays no attenuation of the increased speed over the epoch sampled and the relative speed of the fastest annual time does not appear to be an outlier. The degree to which the fastest times were relatively fast (compared to other years) was observed during the 2000s in the 5000 m and 10000 m distances. The regression spline analyses supported these findings that the fastest relative times for the 5000 m and 10000 m varied over the epoch. While the speed trends for 5000 m and 10000 m track results parallel those seen in elite cycling, the marathon trends do not [129].

The speed and performance trends for top 5000 m and 10000 m distance running performers on the track show a period of increased speed among the fastest runners to the mid-2000s with an attenuation of speed in either all (5000 m) or the fastest performances (10000 m) after this period of time. For the marathon, all indices of speed show a nearly linear increase in speed with an increased number of elite performances over the three plus decades was sampled. It was believe there are a number of possible explanations for these findings. First, the findings for the 5000 m can be interpreted as consistent with the hypothesis that improved drug testing has limited the ability of elite athletes to manipulate their oxygen transport systems with EPO (or other techniques to improve oxygen transport during exercise) since the middle 2000s. These observations are also broadly consistent with recent speed trends in elite cycling races. This interpretation can also be applied to the 10000 m results, but only when considering the fastest times. By contrast, the data for the marathon shows continued improvements in running speed during the same time period along with more total elite performances and world records. These observations challenge the idea that the speed leveling seen in the 5000 m on the track and in the so-called “Grand Tours” of cycling is due primarily to better drug testing and the reduced use of performance enhancing drugs. A second possible interpretation is that world class performances are leveling off and reaching a physiological upper limit as has been postulated for equine and canine athletes. In the case of the marathon a number of empirical estimates and physiological modeling suggest the record is relatively slow in comparison to the 5,000 m and 10,000 m times and is merely catching up by comparison. In this context, it is interesting to note that top speeds have not fallen for the shorter races but only leveled off [129].

The third element of any interpretation focuses on the changing financial incentives in professional distance running. Prize money for top marathon performances has increased. Specifically, in 1980 the highest total payout for any marathon was USD 50,000; just over two decades later the first million dollar race was run. These incentives could be attracting a stronger pool of competitors to “move up” and focus on the marathon and forgo record setting attempts at shorter distances. This could lead to more competitive races among top runners at the major marathons. Second the highest profile marathon races are now being staged in a way designed for world record attempts that include the use of pacers. Along these lines, the use of pacers has been wide spread for races on the track for many years, and many top athletes have bonus plans and other financial incentives from sponsors that reward fast times at the shorter distances. There are a number strengths and limitations to this study. A major strength of our data set and analysis is that it includes standardized distances and courses with numerous competitive opportunities at the shorter two distances when environmental conditions are likely to be optimal. By contrast, a limitation to our analysis is that we have no idea if improved approaches to training or equipment (shoes and tracks) might have contributed to the trends we report. However, we favor the interpretation that the entire epoch we have analyzed has been relatively stable from a technical perspective. This includes widespread use of high volume and high intensity training, widespread availability of synthetic tracks, and adequate footwear. Additionally, while ideas about training have been refined it is not known if how uniformly these have been adopted by elite athletes, especially the East Africans. This perspective contrasts to the major
improvements in equipment for cycling that includes use of advanced materials and improved aerodynamic designs to construct faster bikes [129].

A final concern whenever the topic of doping is raised is discussed relates to what might be called the continuous “cat and mouse” game between those trying to enforce the rules with improved testing and those trying to circumvent them. This has engendered speculation that micro-doses of EPO can be titrated by athletes in a way to achieve high levels of performance and yet avoid a positive drug test. There is also widespread speculation about the use less or undetectable compounds and so-called designer performance enhancing drugs. Advocates of these points of view have argued that while doping is considered widespread the number of positive tests in major competitions is quite low. The counter argument is that the low number of positive results demonstrates that the testing is working and deterring doping. The lack of hard data on the true incidence of doping and how it has or has not been influenced by improved testing is unknown and a major limitation to any discussion on this topic. However, it is clear that anonymous questionnaire based surveys suggest the true incidence of doping it is much higher (14-39 %) than about 2 percent rate of positive tests suggests. This is clearly an area of sports sociology that requires increased attention. It should also be noted that the sociology surrounding the doping phenomenon along with the ongoing incentives to dope are complex. In this context, strategies beyond testing alone will be required to improve the efficacy of doping control [129].

Prediction of further improvements (year 2002)

The limiting factors of top athletic performance and the psycho-physiological mechanisms involved remain controversial. The aim of one study was to attempt a prediction of world records (WR) for the next ten years in five athletic track and field events. Our prediction has been produced by means of computer-aided mathematical models. In short, polynomials that could best approximate the WR of the last decades have been calculated and projected over the period 2000-2010. The predicted values for the year 2010 point to an improvement rate of the WR considered varying between 0.2 and 10.3 percent, depending on event and gender. Those values could be influenced by the use of better sports equipment, better nutrition and training and especially by the impact of doping and of anti-doping measures [130].

100 meter and 5000 meter

Human upper performance limits in the 100-m sprint remain the subject of much debate. The aim of this commentary is to highlight the vulnerabilities of prognoses from historical trends by shedding light on the mechanical and physiological limitations associated with human sprint performance. Several conditions work against the athlete with increasing sprint velocity; air resistance and braking impulse in each stride increase while ground-contact time typically decreases with increasing running velocity. Moreover, muscle-force production declines with increasing speed of contraction. Individual stature (leg length) strongly limits stride length such that conditioning of senior sprinters with optimized technique mainly must be targeted to enhance stride frequency. More muscle mass means more power and thereby greater ground-reaction forces in sprinting. However, as the athlete gets heavier, the energy cost of accelerating that mass also increases. This probably explains why body-mass index among world-class sprinters shows low variability and averages 23.7 ± 1.5 and 20.4 ± 1.4 for male and female sprinters, respectively. Performance development of world-class athletes indicates that about 8 percent improvement from the age of 18 represents the current maximum trainability of sprint performance. However, drug abuse is a huge confounding
factor associated with such analyses, and available evidence suggests that we are already very close to “the citius end” of 100-m sprint performance [131].

100 meter in Olympic Games

The introduction of doping substances and methods in sports triggers noticeable effects on physical performance in metric sports. Here, we use time series analysis to investigate the recent development in male and female elite sprinting performance. Time series displaying the average of the world's top 20 athletes were analyzed employing polynomial spline functions and moving averages. Outstanding changes in performance over time were statistically analyzed by Welch's t-test and by Cohen's measurements of effect. For validation we exemplarily show that our analysis is capable of indicating the effect of the introduction of in- and out-of-competition doping testing on women's shot put as well as the effects of the market introduction of erythropoietin (EPO) and the introduction of EPO and continuous erythropoiesis receptor activator (CERA) testing on 5000 m top 20 male performances. Time series analysis for 100 m men reveals a highly significant drop by more than 0.1 s from 2006 to 2011 with a large effect size of 0.952. This is roughly half of the effect size that can be found for the development of the 5000 m performance during the introduction of EPO between 1991 and 1996. While the men's 200 m sprinting performance shows a similar development, the women's 100 m and 200 m sprinting performances only show some minor abnormalities. It was discussed why the striking sex-specific improvement in sprinting performance is indicative for a novel, very effective doping procedure with insulin-like growth factor-1 (IGF-1) being the primary candidate explaining the observed effects. It is known that human growth hormone (hGH) has been abused in professional sports since the 1980s, disregarding its appearance on the World Anti-Doping Agency (WADA) list for banned substances. For modern elite athletes, especially for cheating sportsmen and women, growth-promoting effects play an important role and can be induced by various substances. One study demonstrates that hGH administration in recreationally trained athletes results in statistically significant improvements in sprint capacity. IGF-1 is known for evoking growth-promoting effects and for its direct anabolic impact on skeletal muscle. At least some of the hGH deployed anabolic actions are exerted through triggering the generation of IGF-1. However, so far no clinical trials have demonstrated a beneficial effect of IGF-1 administration on athletic performance. IGF-1 acts in endocrine, autocrine, and paracrine modes on skeletal muscle. Exogenous administration of IGF-1 causes powerful anabolic actions similar to the effects of hGH. Due to limited experience with long-term administration of exogenous IGF-1, only side-effects from short-term usage have been documented in clinical trials. Next to edema, arthralgia, headache, and jaw pain especially hypoglycemia should be mentioned. Furthermore, it is appealing to use IGF-1 in combination with other drugs like hGH, insulin, and anabolic steroids, as the combined application most likely provides an enormous potential to improve performance. Consequently, IGF-1 is included in the WADA list of banned substances, but its abuse by elite athletes is assumed to be lower than for hGH, because of its lower availability due to the lack of a natural resource from which IGF-1 could be harvested. Nevertheless, IGF-1 will be more attractive to use than hGH, since testing for hGH is improving. Preparations containing IGF-1 were first approved for the American market in August 2005 by the US Food and Drug Administration (FDA) for the treatment of growth failure in children. Therewith the possibilities to acquire and abuse IGF-1, despite the lack of a natural source, have been increasing even more. The results from the time series analysis disclose a time decreasing trend in male short-distance running that started around 2005/2006. Over 100 m, elite athletes underwent a large effect (0.952) in the last five years that is clearly displayed by both approaches: spline and moving average. The last six years over 200 m entailed an effect size of 0.481. In women's track and field sprinting, an effect is also detectable over 100 m. However, the moving average is at the same level in the beginning and the end of the regarded interval and over 200 m there is no effect noticeable after 2004. These explicit developments can be discussed to be associated
with doping, but it is important to mention the existence of other possible or additional explanations. Simultaneously with the time decreasing trend over 100 m for men, we noted that more athletes from the West Indies entered the annual top 20 lists. Thus changing conditions concerning training for these populations of athletes could contribute to the stated developments. When taking doping into consideration as a plausible explanation, several different substances have a strong enough physiological effect and therefore could be related to this development. HGH may still be used as presently no robust long-term testing method for the detection of hGH administration exists. Unfortunately it is also possible that new synthetic ‘designer steroids’ have been developed that cannot be detected by the tests currently available. Additionally the list of other substances that could possibly improve sprinting performance is long. Next to hGH, IGF-1 and ‘designer steroids’ another example would be oestrogen receptor antagonists. However, it was argued in the following that among the substances that were recently introduced to the market, IGF-1 is a very important candidate that could explain the observed development in sprinting performance. The mechanism of IGF-1 with its beneficial actions concerning physical performance presupposes its abuse, alone and in combination with other agents, in track and field. 2008 is the year in which we first noted a significant change over 100 m, but IGF-1 was approved in 2005. Interestingly, we detected the first sign of an EPO effect in 1992, although it entered the American market in 1989. In one opinion, athletes first need to get acquainted with drugs they want to abuse. Its handling is usually only known for medical purposes. So the doping-related application of the drug and its incorporation in the training process take some time to be optimized. Additionally, examination of the number of athletes that ran the 20 fastest times of each year over 100 m shows a fall from ten in 2005 to four in 2006 and eleven athletes ran the fastest times in 2011. It was believed that this progression illustrates a slow integration: IGF-1 abuse started with a small amount of athletes and now is integrated in the world elite of track and field sprinters. Induced by the effect detected over the women’s 100 m, it was suspected female athletes to be using IGF-1, too. Even so, it seems to be less beneficial for women, because there is no parallel effect over 200 m. Furthermore, athletes usually try to deliver top performances in Olympic Games years. Over the women’s 100 m and 200 m, 2008 – the year of the Olympic Games in Beijing – is the minimum. In contrast, the men have their minima in 2011 and there is a local maximum in 2008 over 200 m. A study showed that women responded with a smaller increase in IGF-1 levels, although they received a larger dose of hGH compared to men. So far it is not known whether treatment with IGF-1 leads to sex differences in the related physiological response. However, sex differences can be found for serum levels of IGF-1. IGF-1 would not be the only doping substance with different effects depending on sex. For example, our analysis of women’s and men’s shot put shows greater effects for female shot putters. Considering the whole period from the introduction of anabolic steroids to the beginning of out-of-competition controls, it was computed an effect size of 3.482 for men from the earlier effect onset in 1961 to 1988 and 5.154 for women from 1966 to 1988. Franke and Berendonk mentioned that enormous effects, especially in female athletes, were noticed in the 1970s during the systematically organized doping efforts of the German Democratic Republic. Anabolic steroids and EPO are the best-known doping substances and the portrayed examples account for enormous effect sizes. Anabolic steroids had an effect of 3.177 over ten years in the women’s shot putt and EPO 1.925 over a five-year period in the men’s 5000 m. Both were abused in a variety of sports disciplines. Nowadays blood doping, including potential abuse of EPO, is widespread among athletes regardless of the progress in testing. This is well in line with our notion that the introduction of EPO testing had no profound effect. In comparison, out-of-competition testing for anabolic steroids led to a clear decrease in performances displayed through the arithmetical mean of the 20 best athletes of each year. The effect sizes that was computed for short-distance running from 2001 to 2011 are not as large, but still enormous enough to animate other athletes to misuse IGF-1. In our opinion, IGF-1 could definitely also be effective concerning track and field in the jumping disciplines, although analysis of long-jump and high-jump did not display any recent significant developments, but future examination...
might. Thus, the official introduction of the recently developed test procedure for IGF-1 could prevent the expansion of the abuse of IGF-1 in professional sports [132].

Contrary to that statement, when analyzing men’s 100 m performance at the Olympic level, the winners (average 86.36 kg) have outweighed the rest of the finalists (77.72 kg) dating back thirty years. This phenomenon is also consistent when comparing the medal winners (80.45 kg) versus non-medal winners. Contrary to the claims made by the author’s there have been statistically significant improvements over the last quarter century in elite performance of the 100 m dash, both in the men's and women's division. A separate one-way ANOVAs compared the 100 m finals times for men and women from the Olympic Games over the past 20 years with Bonferroni post hoc analysis when appropriate. There were significant differences observed for men and women. Post hoc analysis revealed men's times from 2012 were significant lower than 1992 and 2000; with 2008 also being lower than 1992. Women’s times from 2012 were lower compared to 2000. This holds true for not only the medal winners of the Olympic Games, but also of the participants in the finals as a whole [133].

It is believed that the remarkable improvements observed in the 100 and 200 m are mostly due to the performance of one single athlete, Usain Bolt, who has repeatedly broken the world record of these sprint disciplines in 2008 and 2009, by lowering the limits by notable coefficients of 0.984 and 0.993, respectively. Since the dramatic drop of the polynomial lines is almost entirely due to the performance of one athlete, it cannot be attributed to an entire group of athletes. This phenomenon has been recently defined as the Usain Bolt effect, and has been attributed to stature and reduction in stiffness as a consequence of the increased contact time and lower step frequency, which both result in an advantage in relative power development and mechanical efficiency. This would not support the theory of improvement by doping but – rather – the well known possibility of “extreme outliers” that seldom occur in a normal distribution of athletes, and may remarkably account for an improvement in records. A second important aspect is that Usain Bolt, the athlete who has dramatically improved both 100 and 200 m world records, has never been found positive during anti-doping controls, either in- or out-of-competition, by whatever anti-doping authorities. Until opposite evidence can be provided, this is the only reliable proof that we have that the world records were broken by a fair athlete. Then, it is also questionable to assert that the use of hGH and/or IGF-1 explains the effect on 100 m performance between 2006 to 2011, since it has been clearly acknowledged that athletes have been abusing hGH for its anabolic effects since the early 1980s, whereas the first test was not introduced until 2004. Accordingly, it is much more likely that the abuse of hGH had been commonplace before 2006, and not afterwards. An identical consideration can be made on the potential abuse of IGF-1, since this substance appeared much earlier than 2006 on the black market [13071].
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