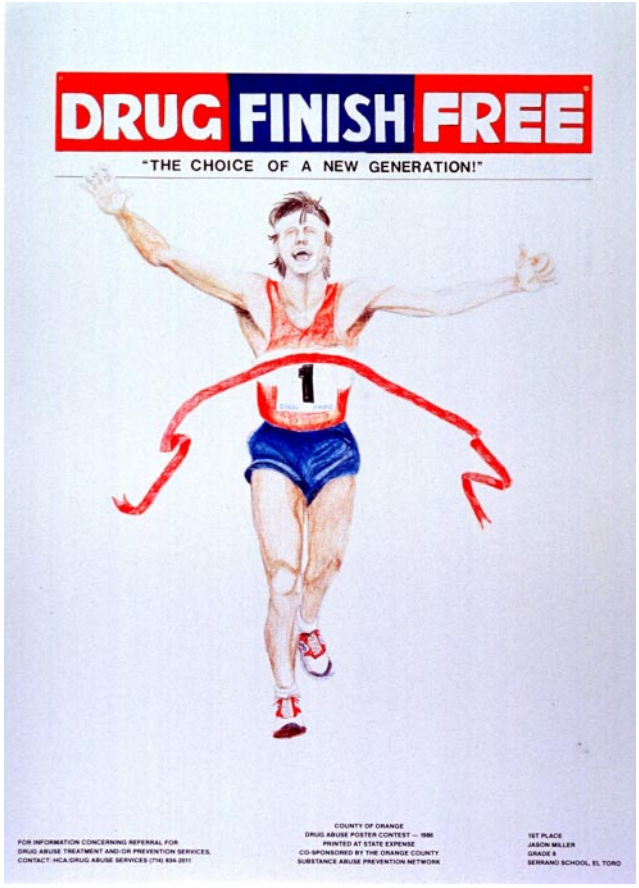
Anti-Dope Testing in Sport: The History and the Science

Larry D. Bowers[1]
U.S. Anti-Doping Agency, Colorado Springs, Colorado, USA



Finish Drug Free poster, 1986. Reprinted with permission from the Orange County Health Care Agency (Santa Ana, CA, USA). Image courtesy of National Library of Medicine.

This summer, as the XXX modern Olympic Games and the XIV Summer Paralympic Games were held in London, millions watched magnificent efforts from the best athletes in the world. The ability of the athletes to produce their best personal performances in the spotlight and the pressure of the Olympic Games was a testament to the human spirit. But, because the margin between winning and losing is so small, one imprecisely placed foot or hand, one moment of indecision, one gust of wind at the wrong moment, and the gold medal is gone.

Behind the scenes at these Olympics—properly so—was a scientific team performing under great pressure to ensure safe and fair competition by testing urine and blood for traces of performance-enhancing prohibited substances and methods. Their efforts were crucial, because, in partnership with the clean athletes, they support the integrity and the spirit of sport. More than 1000 samples were analyzed within a few days after each event for stimulants, steroids, masking agents, recombinant proteins like erythropoietin and growth hormone (GH), and other substances on the World Anti-Doping Agency (WADA) Prohibited List (1). The List of Prohibited Substances and Methods is maintained by a committee of internationally recognized scientists and sport administrators. A revised list is released annually by the WADA. In deciding whether to add a compound or method to the list, the committee considers whether there is potential to enhance performance; a potential health risk for the athlete; or if use of the substance or method violates the spirit of sport. If two of the three criteria are met, the substance is added to the Prohibited List.

The inclusion of the word "potential" recognizes that a substance may emerge for which there are no toxicological data. For example, the identification of the "designer" steroid tetrahydrogestrinone (THG) in a drop of fluid in a syringe turned in to the U.S. Anti-Doping Agency (USADA) in 2003 illustrates the problem. THG had been developed by Wyeth in the 1960s and taken to clinical trials as a potential anabolic agent, but never brought to market. It was impossible to locate any records from the clinical trials 40 years after the fact. THG had been shown to have anabolic activity (2). There were no publications about toxicological studies, but as a 17α-methyl steroid, comparison to similar structures strongly suggested the potential for some degree of hepatotoxicity. Based on this information, THG was added to the Prohibited List in 2006.

HISTORY OF DOPING IN SPORT

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

[1] Correspondence: U.S. Anti-Doping Agency, 5555 Tech Center Dr., Ste. 200, Colorado Springs, CO 80919, USA. E-mail: lbowers@usada.org
doi: 10.1096/fj.12-1001ufm

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.

The 1960s through 1980s were the golden age of anabolic steroid use in sports. After learning that the success of the Russian weightlifting team was in part due to their use of testosterone, Dr. John B. Zeigler began experimenting with Dianabol (methandrostenolone) 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. The German Democratic Republic operated a state-supported anabolic steroid doping program that produced many medals in the 1970s and 1980s, especially for women in swimming and track and field. The doping program, and its health effects for the women, was the subject of an excellent review by Franke and Berendonk (3) and a television special by the Public Broadcasting Service (4). Ben Johnson of Canada had anabolic steroids detected in his urine at the 1988 Olympic Games in Seoul, and he was stripped of his gold medal. 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. 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.

CONGRESS STEPS IN

Concern regarding the effects of anabolic steroids on athletes resulted in U.S. 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 (5), 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.

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 (6). The U.S. 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. 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-α), 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.

THE WORLD ANTI-DOPING AGENCY

In 1999, the IOC recognized that an effective fight against doping required cooperation between sport and government. The First World Conference on Doping in Sport resulted in the formation of the WADA in 2000, which was charged with harmonizing the international antidoping efforts. WADA has developed a World Anti-Doping Program, which has been adopted by all Olympic sports. 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 U.N. Educational, Scientific, and Cultural Organization. To date, over 160 countries, including the United States, have approved the convention.

The USADA was formed in 2000 by the U.S. 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.

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.

TESTING FOR DOPE: THE SCIENCE

While the testing in London was critical, the groundwork for establishing a level playing field has been laid over the months and years leading up to the Olympic and Paralympic Games. While a large number of samples are collected at competitions, the majority of samples for national antidoping agencies like the USADA are collected with no notice and out of competition. The perception of potentially being tested and caught is an important component of deterring performance-enhancing drug use. This applies not only to pharmaceutical substances that should not be found in human biological fluids, but also to naturally occurring substances, like testosterone, that could be abused.

The initial test for testosterone in urine was developed by Donike and coworkers (7), 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 ~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 (8) 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 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 (http://www.wada-ama.org/en/Science-Medicine/Athlete-Biological-Passport/Operating-Guidelines/ Retrieved September 4, 2012) uses the predictive model of Pottgiesser *et al.* (9) 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.

Testing results show that cheating athletes alter their drug abuse behaviors to avoid detection. For example, when a urinary test was developed for recombinant EPO, athletes changed doses and routes of administration to shorten the detection window. This is confirmed by information obtained from athlete blogs and government investigations. Thus, continued research into innovative test methods and strategies is necessary to deter drug abuse. Perhaps more importantly, the scientific contributions from antidoping research go beyond the field itself to affect other scientific disciplines, such as analytical chemistry, endocrinology, genetics, laboratory medicine, pharmacology, physiology, and sports medicine. More collaborative research with experts in these fields should enhance the rate of discovery of innovative approaches to solve doping problems. It is critical to recognize that new tests and methods must be fit for purpose. The tests and methods developed must make the transition into routine testing use. Thus, antidoping research is yet another field where translational research is a key component to applying new technology to solve problems.

With a steady stream of new therapeutic agents—from stimulants to steroids to protein hormones—with potential for abuse in sport entering the marketplace, antidoping scientists and collaborators are continually developing new approaches for detection of prohibited substances and methods. WADA has signed a declaration with the International Federation of Pharmaceuti-

cal Manufacturers and Associations, whose members will voluntarily assist in identifying products with doping potential in advance of their introduction into the marketplace.

ANTI-DOPING RESEARCH OPPORTUNITIES

The key areas of antidoping research include detection of compounds and methods for increasing the absorption, transportation, and delivery of oxygen from the lung to the muscle; detection of compounds or methods that increase the efficiency of conversion of oxygen to intracellular energy; detection of compounds and methods that enhance muscle growth and recovery; detection of genetic modifications applied to sport performance; identification of additional matrices (oral fluid, dried blood spots, *etc.*) to detect doping; and identification and detection of masking techniques.

The challenge of developing and validating methods for the long list of prohibited substances and methods is daunting, requiring analytical skills, a thorough understanding of drug metabolism and pharmacokinetics, and an appreciation of human physiology and endocrinology. Antidoping science would benefit from the expertise of scientists working on proteomics collaborating with scientists interested in changes in the red blood cell during storage—hopefully resulting in a test for autologous blood transfusions. Other key areas of research in the future will involve recombinant protein and glycoprotein characterization and quantification using mass spectrometry and other techniques. The potential for genetic modification for performance enhancement is of great concern, and methods will need to be developed to detect gene doping. New methods of drug administration may impact detection methods. Alternative testing matrices, such as oral fluid, dried blood spots, and hair, may become more important.

According to the WADA website, they have distributed over $50 million in research funding since 2001 (10). Between 2002 and 2010, USADA distributed an additional $9 million in research funding. In 2008, the USADA, the U.S. Olympic Committee, the National Football League, and Major League Baseball joined forces to create the Partnership for Clean Competition (PCC) to support research in antidoping science. PCC has now funded 28 proposals totaling $6 million In addition to research proposals, PCC will continue to establish collaborative working groups to focus the efforts of the best researchers on the science of antidoping. While significant advances have been made in improving detection strategies and methods, increased collaboration with the basic research community should enhance the progress of drug abuse deterrence. Translation of those findings to routine testing will also require funding and effort.

There are numerous opportunities for members of the scientific community to assist in the fight against the use of performance-enhancing drugs and cheating. If you are interested in sharing your research skills, visit the PCC website (http://www.cleancompetition.org). FJ

REFERENCES

1. World Anti-Doping Agency (2011) Prohibited list. Retrieved September 1, 2012, from http://wada-ama.org/en/World-Anti-Doping-Program/Sports-and-Anti-Doping-Organizations/International-Standards/Prohibited-List/
2. Vida, J. A. (1969) *Androgens and Anabolic Agents,* Academic Press, New York
3. Franke, W. W., and Berendonk, B. (1997) Hormonal doping and androgenization of athletes: a secret program of the German Democratic Republic government. *Clin. Chem.* **43,** 1262–1279
4. Public Broadcasting Service (2011) *Doping for Gold: The State-Sponsored Doping Program [Video].* Educational Broadcasting Corp., New York
5. Dubin, C. L. (1990) *Commission of inquiry into the use of drugs and banned practices intended to increase athletic performance.* Canadian Government Publishing Centre, Ottawa, ON, Canada
6. Sawka, M. N., Joyner, M. J., Miles, D. S., Robertson, R. J., Spriet, L. L., and Young, A. J. (1996) American College of Sports Medicine position stand. The use of blood doping as an ergogenic aid. *Med. Sci. Sports Exerc.* **28,** i–viii
7. Donike, M., Bärwald, K.-R., Klostermann, K., Schänzer, W., and Zimmermann, J. (1983) Nachweiss von exogenem testosterone. In *Sport: Leistung und Gesundheit/Kongreßbd. Dtsch. Sportärztekongreß 1982 Köln* (Heck, H., Hollman, H., Liesen, H. and Rost, R., eds), Deutscher Ärzte Verlag, Cologne, Germany, 293–298
8. Jakobsson, J., Ekström, L., Inotsume, N., Garle, M., Lorentzon, M., Ohlsson, C., Roh, H. K., Carlström, K., Rane, A. (2006) Large differences in testosterone excretion in Korean and Swedish men are strongly associated with a UDP-glucuronosyl transferase 2B17 polymorphism. *J. Clin. Endocrinol. Metab.* **91,** 687–693
9. Pottgiesser, T., Sottas, P. E., Echteler, T., Robinson, N., Umhau, M., and Schumacher, Y. O. (2011) Detection of autologous blood doping with adaptively evaluated biomarkers of doping: a longitudinal blinded study. *Transfusion* **51,** 1707–1715
10. World Anti-Doping Agency (2009) Research. Retrieved September 1, 2012, from http://www.wada-ama.org/en/Science-Medicine/Research/