

Blood Doping

Blood doping is the practice of illicitly boosting the number of red blood cells (RBCs) in the circulation in order to enhance athletic performance. Because they carry oxygen from the lungs to the muscles, more RBCs in the blood can improve an athlete's aerobic capacity (VO_2 max) and endurance.

Methods

The term blood doping originally meant literally doping with blood, i.e. the transfusion of RBCs. RBCs are uniquely suited to this process because they can be concentrated, frozen and later thawed with little loss of viability or activity. There are two possible types of transfusion: homologous and autologous. In a homologous transfusion, RBCs from a compatible donor are harvested, concentrated and then transfused into the athlete's circulation prior to endurance competitions. In an autologous transfusion, the athlete's own RBCs are harvested well in advance of competition and then re-introduced before a critical event. For some time after the harvesting the athlete may be anemic.

Both types of transfusion can be dangerous because of the risk of infection and the potential toxicity of improperly stored blood. Homologous transfusions present the additional risks of communication of infectious diseases and the possibility of a transfusion reaction. From a logistical standpoint, either type of transfusion requires the athlete to surreptitiously transport frozen RBCs, thaw and re-infuse them in a non-clinical setting and then dispose of the medical paraphernalia.

In the late 1980s an advance in medicine led to an entirely new form of blood doping involving the hormone erythropoietin (EPO). EPO is a naturally-occurring growth factor that stimulates the formation of RBCs. Recombinant DNA technology made it possible to produce EPO economically on a large scale and it was approved in US and Europe as a pharmaceutical product for the treatment of anemia

resulting from renal failure or cancer chemotherapy. Easily injected under the skin, pharmaceutical EPO can boost hematocrit for six weeks or longer. The use of EPO is now believed by many to be widespread in endurance sports.

EPO is also not free of health hazards: excessive use of the hormone can cause polycythemia, a condition where the level of RBCs in the blood is abnormally high. This causes the blood to be more viscous than normal, a condition that strains the heart. Some elite athletes who died of heart failure—usually during sleep, when heart rate is naturally low—were found to have unnaturally high RBC concentrations in their blood.

Testing and enforcement

General methods

A time-honored approach to the detection of doping is the random and often repeated search of athletes' homes and team facilities for evidence of a banned substance or practice. Professional cyclists customarily submit to random drug testing and searches of their homes as an obligation of team membership and participation in the UCI ProTour. In 2004, British cyclist David Millar was stripped of his world time-trial championship after pharmaceutical EPO was found in his possession. Because athletes sometimes inject or infuse non-banned substances such as vitamin B or electrolytes, the possession of syringes or other medical equipment is not necessarily evidence of doping.

It has also been possible to link athletes to blood doping entirely through documentary evidence, even if no banned substance has been found and no athlete has failed a doping test. The Operación Puerto case is a recent example.

A more modern approach, which has been applied to blood doping with mixed success, is to test the blood or urine of an athlete for evidence of a banned substance or practice, usually EPO. This

approach requires a well-documented chain of custody of the sample and a test method that can be relied upon to be accurate and reproducible. Athletes have, in many cases, claimed that the sample taken from them was misidentified, improperly stored or inadequately tested.

Yet another detection strategy has been to regard any apparently unnatural population of RBCs as evidence of blood doping. RBC population in the blood is usually reported as Hematocrit (HCT) or as the concentration of hemoglobin (Hb). HCT is the fraction of blood cells by volume that are RBCs. A normal HCT is 41-50% in adult men and 36-44% in adult women^[2]. Hemoglobin (Hb) is the iron-containing protein that binds oxygen in RBCs. Normal Hb levels are 14-17 g/dL of blood in men and 12-15 g/dL in women. For most healthy persons the two measurements are in close agreement.

There are two ways in which HCT and Hb measurements can suggest that the blood sample has been taken from a doping athlete. The first is simply an unusually high value for both. The Union Cycliste Internationale (UCI), for example, imposes a 15-day suspension from racing on any male athlete found to have a HCT above 50% and hemoglobin concentration above 17 grams per deciliter (g/dL). A few athletes naturally have high RBC concentrations (polycythemia), which they must demonstrate through a series of consistently high hematocrit and hemoglobin results over an extended period of time.

A recent, more sophisticated method of analysis, which has not yet reached the level of an official standard, is to compare the numbers of mature and immature RBCs in an athlete's circulation. If a high number of mature RBCs is not accompanied by a high number of immature RBCs--called reticulocytes--it suggests that the mature RBCs were artificially introduced by transfusion. EPO use can also lead to a similar RBC profile because a preponderance of mature RBCs tends to suppress the formation of reticulocytes. A measure known as the "stimulation index" or "off-score" has been proposed based on an equation involving hemoglobin and reticulocyte

concentrations. A normal score is 85-95 and scores over 133 are considered evidence of doping. (The stimulation index is defined as Hb (g/L) minus sixty times the square root of the percentage of RBCs identified as reticulocytes.)

These threshold levels, and their specific numeric values are sources of controversy. Establishment of incorrect threshold values is one way that false positive test results can be produced by a doping control program.

EPO

Some success has also been realized in applying a specific test to detect EPO use. An inherent problem, however, is that whereas pharmaceutical EPO may be undetectable in the circulation a few days after administration, its effects may persist for several weeks. In 2000 a test developed by scientists at the French national anti-doping laboratory (LNDD) and endorsed by the World Anti-Doping Agency (WADA) was introduced to detect pharmaceutical EPO by distinguishing it from the nearly identical natural hormone normally present in an athlete's urine. The test method relies on scientific techniques known as gel electrophoresis and isoelectric focusing. Although the test has been widely applied, especially among cyclists and triathletes, it is controversial and its accuracy has been called into question. The principal criticism has been the ability of the test to distinguish pharmaceutical EPO from other proteins that may normally be present in the urine of an athlete after strenuous exercise.

The validity of a doping conviction based on the EPO test method was first challenged successfully by Belgian triathlete Rutger Beke. Beke was suspended from competition for 18 months in March 2005 by the Flemish Disciplinary Commission after a positive urine test for EPO in September 2004. In August 2005 the Commission reversed its decision and exonerated him based on scientific and medical information presented by Beke. He asserted that his sample had become degraded as a result of bacterial contamination and that the

substance identified by the laboratory as pharmaceutical EPO was, in fact, an unrelated protein indistinguishable from pharmaceutical EPO in the test method. He claimed, therefore, that the test had produced a false positive result in his case.

In May 2007 Bjarne Riis, Rolf Aldag, Erik Zabel and Brian Holm, all former members of the Telekom cycling team, admitted to using EPO during their cycling careers in the mid 1990s. Riis also relinquished his title as champion of the 1996 Tour de France. EPO was again a factor in the various doping scandals at the 2007 Tour de France, including the suspension of Spanish cyclist, Iban Mayo.

Transfusions

In the case of detecting blood transfusions, a test for detecting homologous blood transfusions (from a donor to a doping athlete) has been in use since 2000. The test method is based on a technique known as fluorescent-activated cell sorting. By examining markers on the surface of blood cells, the method can determine whether blood from more than one person is present in an athlete's circulation.

The American cyclist Tyler Hamilton failed this test during the 2004 Olympics but was allowed to keep his gold medal because the processing of his sample precluded conducting a second, confirmatory test. He appealed a second positive test for homologous transfusion from the 2004 Vuelta a España to the International Court of Arbitration for Sport but his appeal was denied. Hamilton's lawyers proposed Hamilton may be a genetic chimera or have had a 'vanishing twin' to explain the presence of RBCs from more than one person. While theoretically possible, these explanations were ruled to be of 'negligible probability'.

At present there is no accepted method for detecting autologous transfusions (that is, using the athlete's own RBCs) but research is in progress and the World Anti-Doping Agency (WADA) has promised that a test will eventually be introduced. The test method and its introduction date are to be kept secret in order to avoid tipping off

doping athletes. The assay under development may be a measure of 2,3-bisphosphoglycerate (2,3-BPG) levels in an athlete's red blood cells. Because 2,3-BPG is degraded over time, the stored blood used in autologous transfusions will have less 2,3-BPG than fresh blood. A 2,3-BPG concentration lower than normal may therefore be an indication of autologous transfusion.

Military Use

In 1993, U.S. Special Forces commanders at Fort Bragg started experimenting with blood doping, also known as blood loading. Special forces operators would provide two units of whole blood, from which red blood cells would be extracted, concentrated and stored under cold temperatures. Twenty-four hours before a mission or battle, a small amount of red blood cells would be infused back into the soldier. Military scientists believe that the procedure increases the soldiers endurance and alertness because of the increase in the bloods capability to carry oxygen in the blood.

In 1998, the Australian Military approved this technique for the Special Air Service Regiment. Senior nutritionist at the Australian Defence Science and Technology Organization, Mr Chris Forbes-Ewan, is quoted as saying that unlike in sport, "all's fair in love and war". "What we are trying to gain is an advantage over any potential adversary," Mr Forbes-Ewan said. "What we will have is a head-start."

In this study, over 50 performance enhancing drugs and techniques were rejected. The six approved were caffeine, ephedrine, energy drinks, modafinil, creatine and blood-loading.

Preventative measures

It was revealed in autumn 2007, following another troubled year for professional cycling, that the sport's governing body (UCI) would introduce mandatory "blood passports" for all professional riders. The scheme, thought to be the the first of its type in any sport,

involves using blood and urine samples to create a medical profile that could be compared to results of subsequent doping tests.

EPO is not the only genetically engineered compound that could help cyclists and other endurance athletes on the market. Growth hormone, which stimulates the growth of bones and muscle, became so popular that some athletes took to calling the 1996 Atlanta Olympics the "Growth Hormone Games." Like EPO, growth hormone cannot be reliably detected in abusers. Growth hormone can cause carpal tunnel syndrome and swelling in adults who are normally deficient in the hormone; the effects of the hormone on people with normal natural levels are not known.

If EPO and growth hormone are the wave of the future, anabolic steroids are the wave of the present.

New weapon in the fight against blood doping

With the test methods that are known today, it is extremely difficult to detect doping with your own blood. But with a new blood doping test developed by Australian and Danish researchers, the era of blood doping might have limited days.

Unlike blood doping with another person's blood, homologous transfusion, blood doping with an individual's own blood, autologous transfusion, has always been very difficult to detect, because the doping method isn't based on intake of any kind of synthetic drug or foreign cells. Now though, a new test method is very close to a breakthrough.

Together with Australian colleagues, Jakob Mørkeberg, a doctoral student at Bispebjerg Hospital in Copenhagen, Denmark, is in the midst of developing a precise test to detect whether athletes have illegally doped themselves with their own blood. Jakob Mørkeberg and his scientist colleagues have been developing the test during the

past year and their method uses a number of indirect measurements such as comparing different blood parameters longitudinally

Four new methods under consideration

In developing a test that can detect autologous transfusion, the researchers are working on four different test methods examining: blood parameters, total haemoglobin, gene-expression and membrane proteins.

The test material for all the analyses comes from experiments made on twenty-four young and healthy test persons, of whom sixteen each had 1.5 litres of blood withdrawn. The remaining eight worked as a control group. The blood was either stored in a freezer or refrigerator and was re-infused after a certain period of storage when the test persons had regenerated most of the withdrawn blood.

This method increases the amount of haemoglobin in the blood, thus more oxygen is carried from the lungs to the muscles, which enables the athletes to improve their performance. During the test period the test persons had blood samples taken on the average of 20 different times.

If the research results in a usable test, the blood parameter method could be compared with the indirect test method (off-score) used for tracking the use of EPO. One of the aims is to set up a blood algorithm, based on changes in various blood parameters unique for autologous transfusion.

Another test aims to measure the **total haemoglobin**. With this method, athletes inhale a small amount of carbon monoxide before and after a competition. The odourless gas is able to tie itself more than 200 times better to the red blood cells than oxygen. By measuring the percentage of carbon monoxide bound to the haemoglobin on different time points, the researchers are able to calculate the precise amount of total haemoglobin and thereby get an

indication of whether an athlete has been manipulating with his own blood.

The method requires several tests carried out well in advance before the competition. The carbon monoxide is normally out of the body after 6-12 hours.

The third test method, gene-expression, examines whether some genes are up or down regulated after autologous blood transfusions.

The last method, which is still confidential, examines membrane proteins, looking at the changes in the red blood cells when stored compared to non-stored cells.

“The test’s sensors are calibrated to identify minute changes in athletes’ blood systems that can only occur through transfusions,” Mørkeberg tells Play the Game. “We’re on to something,” he said. “But I expect it will still require some work to reach our goal.”