Unhealthy competition

Advances in biomedical research provide new drugs and technologies in medicine, but also new ways for athletes to illegally enhance their performance

This 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 seems to 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. Many athletes certainly achieve this by relentless training, but there are always some who turn to biotechnology and medical research for help. The development of more sophisticated tests to weed out these cheats has often not been able to keep pace with the development of new products that could be abused in the 'quest to be the best'. While analytical laboratories still struggle

to develop new tests for the latest round of doping drugs, experts fear that sophisticated medical technology, such as gene therapy, may be the next to find its way to the darker side of competitive sports.

EPO is a classical example of how medical research is exploited in competitive sport. This naturally occurring hormone is produced by the kidneys and stimulates the production of red blood cells. It is now widely used to treat anaemia after the US company Amgen (Thousand Oaks, CA) first produced recombinant EPO, called Epogen, in the late 1980s. But as the 1998 Tour de France proved, athletes and their coaches were equally eager to abuse it, because it increases the oxygen supply to the muscles and improves performance in endurance sports, such as cycling or long-distance running. Rumours in the dressing room then contributed to its continual spread until the International Olympic Committee (IOC) became aware of EPO's misuse and developed tests within its accredited laboratories to prove it. But EPO is not the only substance abused, as

some athletes and coaches are always on the hunt for new ways to improve their performance. The most common group of compounds used for doping are anabolic steroids, which increase muscle bulk and strength. Other drugs banned by the IOC are stimulants, narcotics, diuretics, peptide hormones, mimetics and analogues, anti-oestrogen agents and masking agents (Table 1).

The urge to achieve victory drives athletes and coaches to disregard the many health problems that are associated with doping substances. One thing is certain: most of these substances are harmful to healthy people. Although the primary use of EPO is to treat patients with kidney malfunctions who produce low levels of the hormone, athletes, who use it to increase their oxygen uptake, face considerable health risks. As Peter Schjerling, Associate Professor at the Copenhagen Muscle Research Centre (CMRC) in Copenhagen, Denmark said, "Monkeys tested with EPO injections showed a 70% increase in red blood cell concentration,

Table 1 | Examples of substances that are prohibited in competitive sport

Prohibited classes of substances	Examples	Effects	Risks
Stimulants	Amphetamines, bromantan, cocaine, ephidrine, mesocarb	Reduce tiredness, increase alertness and aggression	Raise blood pressure, irregular heartbeat, addiction, heart failure
Narcotics	Buprenorphine, diamorphine (heroin), methadone, morphine	Reduce tiredness and pain	Addiction. Illegal in any case
Anabolic agents	Closterbol, nandrolone, testosterone, androstenediol, androsteredione	Increase muscle strength and size, increase aggression and competitiveness	Impotence, damage to kidneys, liver and heart. Can stunt bone growth. Disrupts menstruation
Diuretics	Acetazolamide, bumetanide, chlortalidone, etacrynic acid	Enable fast weight loss if needed	Dehydration, muscle cramps, kidney failure
Peptide hormones, mimetics and analogues	Corticotrophins, human growth hormone (GH), insulin-like growth factor 1 (IGF1), erythropoietin (EPO)	EPO increases red blood cell numbers. GH sheds fat and builds 'muscle' tissue	GH can cause heart and kidney failure and lead to diabetes. EPO thickens the blood, increasing blood pressure
Masking agents	Diuretics, probenecid, plasma expanders	Used to avoid detection of other drugs	–
Beta blockers	Acebutolol, alprenolol, atenolol, nadolol, sotalol	Induces calmness and stop trembling	Drowsiness, fatigue, nausea, possible breathing problems
Painkillers	–	Allows athlete to continue despite the pain and injury of prolonged exertion	Injuries get worse, addiction, breathing problems

Source: Olympic Movement Anti-Doping Code, January 1, 2003 (to be changed by 2004); www.wada-ama.org

and the monkeys needed to have their blood drained to keep them alive." According to Don H. Catlin, M.D., Professor at the University of California, Los Angeles, USA, and Director of the UCLA Olympic Analytical Laboratory, "EPO increases the number of red blood cells, leading to thicker blood, blood clots, thrombosis and eventually heart attack." And EPO is not an exception. Anabolic steroids taken by power athletes, such as sprinters and weightlifters, increase muscle size, leading to stronger, harder muscles and thus enhance their performance. But as they promote tissue growth, these steroids also damage the liver and heart, and increase the risk of cancer. Another recent development that concerns anti-doping agencies is the use of human growth hormone (GH), which is used to treat children suffering from stunted growth. It increases muscle size and allows tired muscles to recover more quickly, but it also triggers the growth of bones and organs, which leads to potentially life-threatening problems.

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Detecting the use of these drugs is an increasingly difficult challenge for laboratories. Whereas most of the anabolic steroids can now be detected by standard methods, such as gas chromatography, mass spectroscopy or enzyme immunoassays, recombinant EPO initially posed a challenge for the analytical laboratories because it is similar in structure to the naturally occurring hormone. Olivier Rabin, Science Director of the World Anti-Doping Agency (WADA) described the two current methods of testing for EPO. "The first is the blood method where changes in blood parameters are tested, such as increase in haematocrit—the proportion of red blood cells—or in haemoglobin or reticulocytes. The second method is urine based, in which the samples are run on an electrophoretic gel. The gel shows the difference in the bands, between naturally produced EPO and recombinant EPO [rEPO]. EPO is released progressively, and the bands will show even if diuretics [used to speed up the elimination of rEPO from the urine] are taken." Before sporting events, athletes must provide a urine sample that is divided into an A and a B probe. If any illegal substances are detected in A, the B probe is used for further analysis to verify the finding. Since the 2000 Olympic games in Sydney, Australia, analytical laboratories also take blood samples to test for markers of EPO use. Now that this hormone is detectable, athletes are already looking to

alternative substances: contenders are Darbepoietin- α , which, like rEPO, increases red blood cell count, and recombinant haemoglobin or oxyglobin to improve oxygen transport. The greatest challenge now for laboratories is to detect the abuse of GH, which cannot be proven by the tests available at present. As GH doping is outlawed by the IOC, GH testing may be included in the new tests that WADA plan to introduce before the 2004 Olympic Games in Athens, Greece.

Advances in medical research are already providing the tools for the next phase in the arms race between athletes and analytical labs: gene doping. However far-fetched and futuristic this may sound, various gene-delivery methods are now being investigated to treat cancer or to replace single defective genes in inherited diseases. If this technology falls into the wrong hands, it could be abused in competitive sport. By permanently altering their genetic make-up, athletes could improve their strength and endurance, and this method could have one potentially big advantage: it would be undetectable by any existing test. The introduction of extra copies of the *EPO* gene is one possibility; however, strong transcriptional control of the gene is needed to harness its true power, Bengt Saltin, Associate Senior Professor of the CMRC, pointed out. "It increases haemoglobin by two times and

needs to be controlled because of the possibility of thrombosis and haemorrhage. It increases the red blood cell count and increases performance by 10–20%," he said. Adenovirus has already been used to transfer the *EPO* gene to mice and monkeys at the University of Chicago (IL, USA) in 1997, which increased the animals' haematocrit by approximately 30%. The biotech company Chiron (Emeryville, CA, USA) reported similar results in the following year, using adeno-associated viruses (AAV) to deliver the *EPO* gene to baboons. Avigen (Alameda, CA, USA) has also patented the use of AAV to deliver the *EPO* gene.

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Schjerling pointed out that of the growth hormones, insulin-like growth factor 1 (IGF1), would be another good candidate for gene doping as it increases the size of specific muscles. This gene is upregulated in response to mechanical signals, such as over-exercise or stretching—basically any exercise that leaves athletes feeling sore the next day—and helps in the repair of micro-tears by bulking up the muscle fibres. An *Igf1* isoform has been delivered to mice leg muscles using an adenovirus, and the muscle mass increased by 15%. If this technology were developed further, it could prove very useful against muscle-wasting diseases such as muscular dystrophy. But it could also be used, for example, to beef up the thigh muscles of sprinters or the biceps of weightlifters to improve their physical performance.

Nevertheless, gene therapy comes with its own risks. Researchers in Paris were the first to cure an inherited severe immunodeficiency disease by replacing the mutated gene, but this also increased the risk of leukaemia for the treated children, as the transferred gene had a propensity to insert near or into certain oncogenes. Furthermore, the death of Jesse Gelsinger in 1999 during a gene therapy trial at the University of Pennsylvania (Philadelphia, PA, USA), also showed that the procedure is far from safe. And if the gene is not properly controlled it can cause more direct effects on the body. "With athletes the change is permanent, they risk high blood pressure, resulting in clotting, increasing the risk of a heart attack or a blood clot in the brain," Saltin said about the dangers of transferring additional copies of the *EPO* gene.

Saltin described another method that could be abused for doping: "Transcription factors are important for several genes to be expressed, and not the least for genes encoding for proteins which enhance skeletal muscle usage of lipids and performance in prolonged efforts." Indeed, endurance athletes fear the moment when their blood sugar is used up and their body starts burning fatty acids as a new energy source. This switch takes some time, during which the muscles are not properly supplied; and this time period can only be reduced with years of training. "Thus, to identify compounds that possibly affect such transcription and introduce them into the muscles could be equivalent to years of extensive endurance training," Saltin said. But he pointed out that the possibility of gene transfer in sports is 5–10 years away, and that the necessary tests for gene doping have been or are in the process of being developed. "For the first time, we will be ahead of the athletes," he said.

However, the definition of what constitutes doping is not always clear. "Tour de

France cyclists take glucose infusions which are legal. This increases their caloric intake because the cyclists physically cannot eat enough food to raise the sort of calorie level required. This is technically not doping," Schjerling said. He went on to say that "50% of top cyclists are registered as asthmatics. Many probably don't have asthma, but they are given cortisol, and this increases muscle size, but only slightly." However, this still gives an added advantage and provides for an improvement in performance. The Tour de France is 3,427.5 km long and lasts for 20 days with only one rest day in the middle, with a daily average of 171 km being covered per day.

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So then, why not make doping legal and allow athletes to use any drug that increases performance at their own risk? Schjerling claimed that there were talks about this possibility, with children being banned from drugs but adults being allowed. Catlin also conceded that one could "create a situation where doping is legal, all performances are enhanced and the records too, but society won't like it and neither will the athletes." As all of them would have to dope to compete, it undermines the basic fundamentals of sport. "Most athletes want to succeed with their natural abilities," Catlin said.

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