

## Abstract

Together with the rapidly increasing knowledge on genetic therapies as a promising new branch of regular medicine, the issue has arisen whether these techniques might be abused in the field of sports. Previous experiences have shown that drugs that are still in the experimental phases of research may find their way into the athletic world. Both the World Anti-Doping Agency (WADA) and the International Olympic Committee (IOC) have expressed concerns about this possibility. As a result, the method of gene doping has been included in the list of prohibited classes of substances and prohibited methods. This review addresses the

possible ways in which knowledge gained in the field of genetic therapies may be misused in elite sports. Many genes are readily available which may potentially have an effect on athletic performance. The sporting world will eventually be faced with the phenomena of gene doping to improve athletic performance. A combination of developing detection methods based on gene arrays or proteomics and a clear education program on the associated risks seems to be the most promising preventive method to counteract the possible application of gene doping.

## Key words

Doping · gene therapy · Epo · IGF

## Abbreviations

CCMO	Central Committee on Research Involving Human Subjects
COGEM	Committee on Genetic Modification
Epo	erythropoietin
IGF-1	insulin-like growth factor
IOC	International Olympic Committee
NeceDo	Netherlands Centre for Doping affairs
NVGT	Dutch Foundation for Gene Therapy
VEGF	vascular endothelial growth factor
WADA	World Anti-Doping Agency

## Introduction

Gene therapy is a promising technique capable of treating seriously sick people, which in some cases results in a cure of the disease. In theory, all existing protein levels in the body may be changed by using gene therapy. As the first gene therapy trials are performed with doping related proteins, such as erythropoietin and growth hormone, the link between gene therapy and sports is evident.

The potential misuse of this kind of therapy is a new form of doping: gene doping.

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## Bibliography

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In this review, the potential of gene doping for improving athletic performance is described. First, the relevant background of genetics and gene therapy is given, followed by defining doping and genetic doping. Then the possibilities for using gene doping for improving athletic performance are discussed, including health risks, detection of gene doping and preventive measures.

The discussion on gene doping was initiated in June 2001, when the Gene Therapy Working Group, convened by the Medical Commission of the International Olympic Committee (IOC) had a meeting on the theme "Gene therapy and its future impact on sport". This committee concluded:

"We endorse the development and application of gene therapy for the prevention and treatment of human disease. However, we are aware that there is the potential for abuse of gene therapy medicines and we shall begin to establish procedures and state-of-the-art testing methods for identifying athletes who might misuse such technology. This will require investment in modern detection methods including antigen detection, gene chip and proteomic analysis which are now becoming available. We are confident that we shall be able to adequately monitor abuses and establish the procedures for doing so using ethically acceptable methods" [12].

The World Anti-Doping Agency (WADA) convened a conference on this issue in March 2002 [30] and it also was one of the main topics of the "European Working Congress on Harmonization and Future Developments in Anti-Doping Policy" in Arnhem, the Netherlands in April 2002. The results of these discussions were a call for a worldwide effort to deal with this new sort of doping and especially a joined effort of scientists, medical doctors, governments, anti-doping organizations, and the pharmaceutical industry to exchange all relevant information including educational ideas, research results and detection methods on this potentially new technique for doping.

The International Olympic Committee (IOC) has included the method of gene doping in their list of prohibited classes of substances and prohibited methods as of January 1, 2003. Starting in 2004, WADA has taken over the responsibility to publish an international doping list, which is updated every year. The method of gene doping is included and defined as: the nontherapeutic use of cells, genes, genetic elements, or of the modulation of gene expression, having the capacity to enhance athletic performance, is prohibited [39].

The WADA, in collaboration with the Karolinska Institute and the Swedish Sports Confederation, held a second workshop in Stockholm on the subject of gene doping in sport on December 4–5, 2005.

The participants discussed the current scientific, ethical and public policy issues related to the possibility of gene transfer for the purpose of enhancing athletic performance. The participants concluded that clinical results indicate that gene transfer for the purpose of therapy (gene therapy) now represents a proven, although very immature and still experimental, field of human medicine. Clinical research in human gene therapy is filled with many recognized and unrecognized pitfalls and dangers. Aware-

ness of the potential illicit use of gene transfer techniques for athletic and other enhancement purposes should be stimulated and appropriate sanction mechanisms for illegal unethical application of gene transfer in sport should be developed. New detection methods are likely to emerge and will help to prevent tainting of sport by gene doping.

## Gene Therapy

### Genes

The human genome represents the whole genetic information of each individual. This information resides on the chromosomes (23 pairs), which are present in each nucleus of all the cells that make up the organs. The chromosomes contain DNA (Deoxyribo Nucleic Acid) which is a double helix composed of four bases (A = adenine, G = guanine, T = thymine and C = cytosine). The genetic information is determined by the sequence of these bases in a chain of nucleotides. This sequence determines the order of amino acids to create a specific protein, such as enzymes or structural proteins. The sequence information that is necessary to obtain one specific protein is called a gene.

The elucidation of the complete human genome with approximately 30 000 different genes will lead to new possibilities for diagnosis and prevention of a wide variety of diseases. In addition, this knowledge may be used for the design of new therapeutics, based on the DNA sequence information.

Genomics is the study of genes and their function, which includes genome mapping, gene sequencing and gene function. One way to study the genome is by DNA-chip or gene array; a microchip that holds DNA probes that form half of the DNA double helix and can recognize DNA from samples being tested. DNA chips are widely used to study the composition and activity of different genes under different circumstances, including disease and fitness. One such chip may hold up to 30 000 genes and thus covers almost the complete human genome.

Pharmacogenetics is the study of the interaction of an individual's genetic makeup and response to a drug. People differ in their response to drugs. Some people may lack a receptor that the drug is interacting with whereas other people may have an enzyme that rapidly degrades the administered drug. Pharmacogenomics will allow a better fine tuning of drug administration for patients based on their individual gene make up. This will not only diminish side effects because of optimal dosing, but will also improve the outcome of treatments because patients will get tailor-made treatments. People from different ethnicities have a different genetic make-up which results in differences in susceptibility to certain diseases. People of African descent, for example, are more prone to sickle cell anemia and among people of Jewish origin the hereditary disease Tay-Sachs (childhood dementia) is more common. Personalized medicines will also have an impact on elite athletes. Not only will they benefit from the selection of the right dose and type of drug to treat a possible disorder with fewer side effects, they may also benefit from pharmacogenomics because of a better balanced food intake to optimize their performance.

## Genes and sports

“Athletes are not born equal” is a controversial quote from Sir Roger Bannister, the first man to run one mile under 4 minutes. People from a specific ethnic origin seem to have an advantage over others. West-African runners dominate the short distance running whereas athletes from East Africa do well in the marathon. On the other hand, Caucasians dominate in swimming contests.

In this age of genetics and genomics, it will be possible to elucidate the genes that predetermine ones predisposition for a specific sport [32]. Genetic screening at an early age may indicate the greatest potential for a specific child to develop into a top athlete and a specific training program may be designed. On the other hand, genetic screening of athletes may be used to select specific training methods to enhance or improve his or her genetic predisposition [32].

Will these screening methods result in better athletes? Marion Jones and Tim Montgomery are both record holders for the 100 meter sprint. They had a baby in the summer of 2003. Also Steffi Graf and Andre Agassi (both previous number ones on the tennis world ranking list) have children, and there are more examples. These children will certainly have a genetic advantage over many other babies born, but other aspects such as psychological and environmental factors will ultimately determine whether these children will be top athletes.

## Gene Therapy

Gene therapy may be defined as the transfer of genetic material to human cells for the treatment, or prevention of a disease or disorder. Genetic materials can be DNA, RNA or genetically altered cells.

The principle of gene therapy is based on the delivery to a cell, of a therapeutic gene which may compensate an absent or abnormal gene. In general, DNA is used as the genetic material. This genetic material encodes for a therapeutic protein and needs to be delivered to the cell nucleus to be active. In order to deliver the genetic material, this material can be encapsulated into a virus such as adenovirus or retrovirus or into a lipid such as a liposome. The viruses are crippled so that they are no longer pathogenic. The encapsulated genetic material is mostly referred to as a vector and is introduced into the body by direct injection into the target organ or administered by aerosol for lung delivery. Also, it is possible to isolate cells from a patient and treat these cells with the vector in the laboratory and then reimplant these into the patient.

The DNA encodes for a protein which will give the therapeutic effect. In the DNA, sequences are present that turn on and off the protein expression, the promoters. Depending on the nature of the promoter and the vector used for delivery, the protein expression may be of short (days-weeks) or long (weeks-years) duration. The expressed protein may be confined to the cell that was treated, or in the case of a secreted protein, the protein may travel from the cell into the surrounding tissue or into the circulation.

Up to now, more than 3000 patients have received some form of gene therapy, with very little side effects. Patients suffering from different diseases, ranging from cancer to heart failure, have been treated. Recent clinical data show encouraging gene therapy results in major diseases: patients with x-linked severe combined immunodeficiency disease [17], adenosine deaminase deficiency [2], chronic granulomatous disease [5] or patients with hemophilia B [19]. In addition, angiogenic gene therapy with vectors expressing the human vascular endothelial growth factor for the treatment of coronary artery disease, showed improvement in angina complaints [27]. These studies showed that, although side effects were observed in a limited number of patients, gene therapy is generally a safe treatment, capable of curing patients with, in some cases, life threatening diseases, where no other alternative treatment is available.

Currently, there are two registered pharmaceutical products based on gene technology, several other products are experimental and are being administered in a research setting in hospitals. The first registered product, Vitravene, is based on anti-sense technology, which is a piece of DNA complimentary to the DNA of a virus which will block the replication of this virus. This is used in eye-drops for the treatment of cytomegaly virus infections [38]. A second product is from the company Gendicine in China. The Chinese government has approved a drug consisting of an adenoviral vector expressing the tumor suppressor gene p53 in cancer cells. The p53 gene is mutated in many cancers and the adenoviral p53 could compensate for this mutation resulting in a reduced tumor growth [29].

## Regulations

The clinical application of gene therapy is strictly regulated. In Europe, three directives were adopted by the Council: 1. Containment of genetically modified organisms (GMOs) to protect workers and the environment during the production process (Council of the European Communities [CEC] 1990a, revised 1998; 1991b); 2. The potential adverse effects of the deliberate release of GMOs (CEC200); and 3. Directive on the protection of workers from the risks related to exposure to biological agents at work (CEC 1990c). In addition, strict adherence to Good Laboratory Practice (GLP) and Good Manufacturing Practice (GMP) is mandatory. Early phases are reviewed at the national level, whereas marketing authorization is covered by the European Medicines Evaluation Agency (EMA) [11]. In the USA, specific guidelines are available from the FDA and National Institutes of Health. The Center for Biologics Evaluation and Research (CBER) regulates human gene therapy products – products that introduce genetic material into the body to replace faulty or missing genetic material, thus treating or curing a disease or abnormal medical condition. CBER uses both the Public Health Service Act and the Federal Food Drug and Cosmetic Act as enabling statutes for oversight.

## Vector production

As described in the previous section, gene therapy is delivered by vectors, which may be of biological origin in the case of viruses or chemical compounds such as liposomes. The preparation of DNA itself is relatively simple. The DNA containing the gene encoding the desired protein, such as a growth factor, can be obtained from commercial sources covering almost the entire hu-

man genome or may be isolated with routine molecular biology techniques. The DNA can be produced in large quantities in bacteria, in the form of a plasmid, which is a piece of circular DNA. Bacteria are easy to grow at low costs. Then the DNA can be purified with commercially available kits. The whole production process for DNA takes only 1–2 days and results in a pure preparation which may be used in patients after safety testing.

The preparation of liposomes and other chemical vectors starts with the production of DNA, which is then followed by the addition of the chemical compounds, which serve to improve the cellular uptake of the DNA. Depending on the methods used, several purification steps are needed to obtain an injectable product. Because all ingredients are relatively pure, this procedure is easily scaled up to pharmaceutical grade and scale.

In contrast to plain DNA or chemical vectors, the viral vectors are much more efficient in gene delivery, but impose a more difficult production method. The viruses are produced in cell lines of murine or human origin. These cell lines need to be cultured under specific conditions to allow the viruses to be produced. The virus is then harvested and purified from the cell debris and culture supplements. In some cases, the crippled virus containing the desired gene may be contaminated with recombinant wild-type virus, which is of potential danger for patients and needs to be avoided. Therefore, care is taken into analysis of the virus produced, to define the possible contamination.

The costs for production of gene therapy vectors highly depend on the vector used. The simplest systems, starting with plain DNA, are obviously the cheapest to produce, whereas the more complicated vector systems such as the viral systems are the most expensive. For a large part, the costs are due to the many safety precautions and testing that is performed on clinical batches.

### Doping

Athletic performance may be increased in many ways, some of which are permitted and some of which are prohibited by the governing sports bodies. An example of the latter is the use of performance-enhancing drugs, or doping. Doping can be both of chemical and protein nature or may involve prohibited methods, such as illegal blood transfusions. The majority of athletes take drugs [13]. A survey from the Centre for Drugs Research [1] concluded that less than 1% of the Dutch population has ever used doping products, a total of approximately 100 000 people. Forty percent of these people use doping on a yearly basis, the majority of which is active in strength training or body building.

The prevalence of doping use in elite sports (athletes performing at the level of international championships) is likely to be higher than the mentioned 1% in the general population, but an exact percentage cannot be given. The percentage of athletes that test positive in doping tests has oscillated between 1.3% and 2.0% over the last few years [14].

The past has shown that some athletes (and their entourage) go to great lengths to gain a competitive edge. Pharmaceutical products have been found that had not yet been sufficiently tested for side effects (as was the case with efaproxiral or RSR-13 in

2001) or that have been produced specifically for the use by athletes, without even knowing possible side effects (which was proven by the discovery of tetrahydrogestrinone or THG in 2003). This shows that even when it can be expected that the use of certain products will have serious health consequences, some athletes are willing to serve as subjects in an uncontrolled trial.

### Gene doping

According to the WADA, gene or cell doping is defined as “the nontherapeutic use of cells, genes, genetic elements that have the capacity to enhance athletic performance” [39]. This definition leaves room for questions. What exactly is nontherapeutic? In the future it may be possible to treat patients with muscle disorders by genetic medicines that will improve their muscle strength. Will these patients be allowed to perform? The same holds true for patients who were treated for cancer by chemotherapy and now receive a gene encoding Epo to boost recovery of the bone marrow but may also increase their hematocrit levels. Studies have also been conducted to speed up wound healing and to ameliorate muscular soreness after exercise, a practice that might not be considered as “therapeutic” by everybody and their performance enhancement properties might be questionable. Once genetic therapies have become commonplace, it will not be fair to deny these therapies to all athletes. From a clinician's point of view, it would be better to specify the definition of gene doping so it will solely address the unapproved use of genetic transfer technologies.

### Ethical aspects

The general ethical justification for the prohibition of gene doping by WADA is given in section M3 of the World Anti-Doping Code (version January 1, 2006), in which the criteria are given for including substances and methods on the so-called prohibited list. Besides the criterion that “there is scientific evidence, proven pharmacological effect or experience that substances or methods included have the potential to enhance or enhances sport performance”, two main ethical arguments are given for including substances or methods on the doping list.

First, the use of the substance or method is causing an actual or potential health risk to the athlete. The underlying ethical principle is to protect individuals against harm or risk to health. The second argument is that the use of doping violates the spirit of sport. This spirit of sport is described in the introduction to the Code with reference to a rather miscellaneous set of values such as ethics, fair play, honesty, health, fun and joy and respect for rules. Many of these values are not specific for the practice of sports and the application of these values to the issue of doping is often ambiguous.

In reference to evidence described elsewhere in this review, it is clear that gene doping can be dangerous and detrimental to health. The case of gene doping is special in the sense that there are many uncertainties as to the long-term effects of gene modifications. Many of these effects may go unnoticed because they might never be studied in a scientifically reliable way for financial reasons, or for the reason that it is difficult to define reliable paradigms for the study of side-effects of completely new methods or new applications. This argument applies to the adminis-

tration of genetically modified substances, as is now already practicable. But it applies especially to the application of gene “therapy” for purposes of improving “bodies” to compete in sports. In contrast to somatic cell therapy, germ-line alterations are permanent and are transmitted to future generations. In addition to the possibly grave risk for the health of athletes, the uncertainties with respect to effects create moral problems concerning responsibilities with regard to third parties such as offspring, parents and partners and with regard to informing the athlete in a way that makes risk-acceptance by the athlete him- or herself a possible way of justifying the use of these substances and methods.

The aspect of fair play might be compromised by gene doping in an especially deep and potentially disastrous way for the practice of sports. In the area of pharmacogenetics, which is being developed rapidly by the combined efforts of science and the pharmaceutical industry, the objective is to develop “tailor-made” medicines for individuals. As is well-known, many drugs have quite a different effect on individual people because they are developed and defined in a general way and not in view of the differential genetic dispositions of individuals to respond to substances. Therapeutically, pharmacogenetics is a very promising area. However, if the knowledge that becomes available is used in sports, the very idea of a competition between athletes who are recognizably equal and prepare themselves in more or less comparable ways, might become obsolete. The arguments against doping in the “old” (chemical) sense might all come in here with extra force. These arguments are that doping makes competition dubious and unreliable, because the test of relative inequalities based on one’s own individual bodily efforts, talents and character is perverted. “Tailor-made” substances and methods might help the individual athletes to make the best of their abilities, but it will make sport as an essentially social and collective practice uninteresting and even no longer human-like, in the sense that people might no longer be able to identify with the human characteristics and actions of athletes and their performances, but will come to see them as manufactured “products of science”.

### Gene therapy for sports doping in an athletic setting

Gene therapy, at this time, is an experimental therapy for serious human diseases. Clinical data showed encouraging gene therapy results in patients with x-linked severe combined immunodeficiency disease [17] and patients with hemophilia B [19]. In addition, angiogenic gene therapy with vectors expressing the human vascular endothelial growth factor for the treatment of coronary artery disease, showed improvement in angina [27]. Despite these early positive results, it may be years before gene therapy is a standard treatment for one of these diseases.

### Sports injuries

Gene therapy may not only be applied for the treatment of these serious diseases, but also for less life threatening situations or injuries. Sports injuries usually involve tissues that display a limited capacity for healing. Treatment of various sports-related injuries, including muscle injuries, ligament and tendon ruptures, central meniscal tears, cartilage lesions, and delayed bone fracture healing is labor intensive and time consuming. Gene therapy using the transfer of defined genes encoding suitable growth

factors into the injured tissue may potentially result in improved regeneration of tissue defects following trauma [18]. These approaches are currently being evaluated in animal models. It may be expected that clinical studies will follow in the coming years.

### Gene therapy for sports doping

Athletes may be able to use gene therapy to re-engineer their bodies for better performance. Many genes with potential to enhance athletic performance are available (Table 1). The most relevant are discussed in more detail. These genes not only have potential to improve athletic performance of human athletes. Also in animal sports, such as horse racing, gene doping may be applied. In this report, however, the discussion will be limited to human athletes.

Controlled risk is in the case of pharmaceutical grade gene transfer vector use, with the appropriate safety testing. Uncontrolled risk is when gene therapy vectors are produced in uncontrolled laboratories, with no assurance of quality control or safety testing.

### Erythropoietin (Epo)

In 1964, Finnish Nordic skier Eero Mäntyranta blew away the competition and won two gold medals at the Olympic Games in Innsbruck, Austria. It was later shown that Mäntyranta had a naturally occurring genetic mutation that gave him higher amounts of red blood cells than the average person. Having more red blood cells means more cells to carry oxygen from the lungs to tissues, thus increasing his endurance. Mäntyranta had what every endurance athlete wants and what Epo can provide. Athletes of the future may be able to alter their genes in a way that mimics the natural mutation that Mäntyranta had.

This may be accomplished by inserting an additional copy of a gene into a person to boost production of the hormone erythropoietin (Epo). This hormone instructs the body to manufacture new red blood cells, which, in turn, increases aerobic capacity. Patients who suffer from severe anemia, such as people with AIDS, cancer patients after chemotherapy or patients with kid-

Table 1 Potential is defined as the relative easy to use the gene in an athletic setting in relation to the expected effect

Genes	Potential	Risks controlled	Risks uncontrolled
Erythropoietin (Epo)	++++	±	++++
Insulin-like growth factor (IGF-1)	++	–	++++
Vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF)	+	±	++++
Growth hormone (GH)	+	–	++++
Myostatin/follistatin	++++	?	++++
Endorphins, enkephalins	+	?	++++

Range: +++++: easy to use, great potential, difficult to use, little effect expected. Risk is the health risks for the user when such a gene is introduced. Range: +++++: potentially dangerous for health

ney failure, may benefit tremendously from this form of therapy because their bodies produce inadequate amounts of red blood cells. For athletes, increased Epo production would enhance oxygenation of tissues, in turn increasing endurance.

Epo may be delivered as a protein by injection, or by introduction of the gene encoding Epo into the body's cells. Researchers successfully delivered Epo genes into the cells of mice and monkeys. The hematocrits (the proportion of blood volume made up of red blood cells) of the animals were boosted by as much as 80 percent [42]. When adeno-associated virus was used for Epo gene delivery into monkeys, the animals developed supraphysiologic levels of Epo and polycythemia. However, a severe anemia ensued in some animals due to an autoimmune response to endogenous and transgene derived Epo. This inadvertent autoimmunity has not been observed in other studies, but may pose a serious problem if it would develop likewise in humans [15,10].

Given these unexpected side-effects, it will be several years before Epo gene therapy will be evaluated in clinical studies.

#### **Insulin-like growth factor-1 (IGF-1)**

Like gene therapy for Epo production, techniques to strengthen muscles are being developed to help people with illnesses: in this case, people with degenerative muscle conditions such as muscular dystrophy. Whereas the Epo therapy would be pervasive throughout the body, this approach would target specific muscles. Insulin-like growth factor-1, IGF-1, is made in the liver as well as muscle and has anabolic effects. Its concentration is related to the concentration of Growth Hormone (GH). IGF-1 gives rise to an increase in muscle bulk in mice injected with the gene [6]. This was in the absence of any special exercise program. In a subsequent study, Lee et al. showed that the combination of resistance training and overexpression of IGF-1 induced greater hypertrophy than either treatment alone [24]. Extending this treatment to athletes could mean strengthening a tennis player's shoulder muscles, a sprinter's calves or a boxer's biceps. Such gene therapy is likely to be relatively safe given that the effects seem to be localized to the targeted muscle and is likely that human trials will start in the coming years. Combining IGF-1 with other growth factors or with strength based training programs may lead to even greater responses in muscle growth. However, before clinical studies can be started, further studies in primates need to be performed to further evaluate the efficacy and toxicity of IGF-1 for gene therapy.

#### **Vascular endothelial growth factor (VEGF)**

Genes may also be used to help grow new blood vessels. This therapy is being developed to produce a coronary bypass in patients with ischemic heart disease and may help elderly people with peripheral arterial disease because of inadequate oxygen supply. The gene encoding vascular endothelial growth factor (VEGF) or other factors may turn on the formation of new vessels. Clinical studies are being performed all over the world, including Groningen, NL. These clinical trials, in many instances, show efficacy in patients with angina [6,27,37] or peripheral arterial disease [7,31]. If athletes used these treatments for improving blood vessel production, the result could be a hyper supply of oxygen and other nutrients to the tissues. With better vascularization, muscles, the heart and other parts of the body, ex-

haustion would be delayed. As VEGF is already used in several clinical studies, VEGF gene doping would be possible at this time with the gene therapy vectors used in those studies.

#### **Myostatin**

Myostatin is a negative regulator of muscle formation. Synthesized by muscle cells it acts either auto- or paracrine in heart and skeletal muscle. Its physiological role is still not yet clear. Administration of myostatin blockers such as follistatin, mutant activin type 2 receptors and myostatin propeptide, will result in a dramatic and widespread increase in skeletal muscle mass due to an increase in number of muscle fibers (hyperplasia) and thickness of fibers (hypertrophy) and less fat and connective tissue in muscle [25]. These myostatin antagonists may improve muscle regeneration in patients suffering from Duchenne and Becker muscular dystrophy [8]. At this time, myostatin inhibitors are advertised to boost muscle size without exercising. Athletes could use these agonists as gene doping in the near future [28].

That myostatin antagonists may be effective in humans has recently become clear. A German boy had well developed muscles at birth. The muscle growth continued and at age 4 he could lift weights of 3 kg. The child is the son of a previously professional athlete. The grandfather and great-grandfather were known as very strong men. Genetic analysis of the mother and son revealed a mutation in the myostatin gene resulting in the lack of myostatin production [34]. Inhibition of the human myostatin gene is expected to result in the increase of muscle mass and muscle strength and may be applicable for the treatment of muscle disease and may then find its way to doping applications.

#### **Endorphins**

Pain is a warning sign and should not be ignored. Muscular exhaustion leads to a hyperacidity, because it uses up so much energy and prevents the detoxication of the lactic acid, the waste products of matrix, and causes pain. Pain relief could potentially help athletes to perform better or for a longer period of time. Most athletes will use an over-the-counter pain reliever at some time. These drugs, in fact, are some of the most widely used drugs. An alternative to these chemical drugs could be analgesic peptides, such as endorphins and enkephalins. The genes encoding for these peptides could be administered and may be used for pain relief. At this time, preclinical animal studies have shown that genes encoding for such peptides have an effect on inflammatory pain perception [26,35]. Although promising, pain relieve gene therapy is still in its infancy and far from clinical application.

#### **Gene therapy as medicine for athletes**

All forms of gene therapy which would be potentially beneficial for athletes are still experimental and not widely used at this time. In the future, this may change and it is foreseeable that the current definition of gene doping needs revision. When gene therapy becomes a regular treatment, each type of gene therapy needs to be judged in light of the doping criteria, as described in previous paragraphs, as is currently done for prescription medicines.

## Risks of Gene Doping

Gene therapy is currently an experimental therapy delivered to patients in a well controlled setting. The gene transfer vectors used have been produced in certified laboratories and have been extensively tested for toxicity and safety. If gene therapy would be used to improve athletics performance, it is very likely that such a setting will be absent. The risks involved with gene therapy would then increase tremendously.

The use of medicines by healthy people, gene therapeutics or others, always involves a certain risk. The drugs are developed to treat ill people, improving the performance of healthy people is a different indication which may result in specific side effects.

### General health risks

The risks involved in gene doping are several, and are related both to the vector used (DNA, chemical, viral) and to the encoded transgene. So far, clinical gene therapy studies have been relatively safe [20]. Over 3000 patients have been treated and only one patient died due to a chronic liver disease and an overdosing of vector [33]. In three other patients, who were cured for their life-threatening immune deficiency disease, leukemia-like symptoms developed [17]. One of these patients died from this malignancy. Since then, other groups have treated similar patients with comparable therapeutic results, without any side effects [9]. These studies aimed at a life-long cure of the patients with integrating vectors that will not be used for performance enhancement. Therefore, these side effects will not occur in athletes taking gene doping.

Recently, an autoimmune response to endogenous and transgene derived protein was reported in monkeys. Adeno-associated virus was used for Epo gene delivery and some animals developed a severe anemia due to an autoimmune response [15]. So far, this inadvertent autoimmunity has not been observed in other studies, but may pose a serious problem if it would develop likewise in humans.

Other side effects from gene therapy that have been reported are mostly flu-like symptoms. There have been no reports on transfer of gene therapy vectors from treated patients to next of kin or to germ cells [4, 16, 36].

However, if gene transfer vectors would be produced in noncontrolled laboratories, the preparations may be contaminated with chemicals and other impurities from the production and purification process, including pyrogens and virulent viruses. The potential for generating new viruses, known as replication – competent viruses (RCV) is a major safety concern. There is no way to predict the virulence or disease potential of recombinant viral vectors. These impose great safety risks for the people who would receive these agents.

### Specific health risks

Health risks related to the specific proteins expressed in gene doping are similar to those of other doping forms. Healthy people who unnaturally boost their Epo levels increase their chances of stroke and heart attack because adding red blood cells makes the blood thicker. As it gets thicker, it becomes more difficult for the

body to pump it successfully to all tissues of the body, causing clots wherever vessels cannot compensate for this increased density. Whereas the athletes using synthetic Epo today face similar risks [22], after a few weeks, the risk subsides as Epo is cleared from the body and red blood cell production returns to normal levels. But if Epo would be delivered by gene therapy, the level and duration of Epo production is less controllable. The hematocrit would be less manageable and could continue almost indefinitely giving rise to pathological Epo levels.

Other genes may give different health risks if the expression is not controlled. It may be envisioned that genetic growth hormone treatment with IGF-1 or VEGF may give rise to tumor development. Therefore, it is crucial that selected pharmaceutical grade gene delivery vectors are used, which will have a known and controlled gene expression pattern.

### Environment risks

Athletes that would have received gene therapy may have genetically modified cells or excreta that contain the gene transfer vector. This may potentially pose a risk for people in close contact with the athlete, because they may be exposed to the gene. In current gene therapy trials, patients treated with viral gene therapy vectors are closely monitored for shedding of the gene therapy vectors and in most instances should have no detectable gene therapy vector in blood, stool, urine, semen, or saliva before they are allowed to leave the hospital. Although there have been no reports of unwanted gene transfer from shed gene therapy vectors in clinical studies, this can not be excluded when athletes are treated with these vectors in a less controlled environment.

### Detection of Gene Doping

A concern in the athletic community, especially among doping control agencies, is that no one knows how easily gene doping can be detected, if at all.

The DNA which is used for gene transfer of the gene is of human origin, and therefore not different from that of the person applying gene doping. Labelling of gene transfer products with genetic “bar codes” as has been suggested with Genetically Modified agricultural produce may be an option, however, this would require the complete cooperation of scientists, ethicists, athletes, sports authorities, medical practitioners, professional societies, pharmaceutical, and biotech industries, and public to avert misuse.

Gene doping will be delivered by a vector containing DNA, with or without chemicals to enhance gene transfer or by a viral vector. Muscle based therapies will be confined to the injection site or tissue in the direct vicinity. Therefore, many of the muscle based gene technologies are unlikely to be detected by urine or blood testing as is currently done in elite athletes. The detection of associated chemicals or viral particles may be of use, but this would involve tissue sampling. It will be unlikely that athletes can be forced to give consent to this procedure given the invasive nature of the biopsies.

Many forms of genetic doping do not require the direct injection of genes in the desired target organ, i.e., the Epo gene may be injected into almost any site of the body to locally produce the Epo protein which will then enter the blood stream and stimulate the bone marrow. Finding the site of injection will be like looking for a needle in a hay stack. With injectable Epo use, close medical monitoring ensures that red blood cell parameters can be contained within set levels making it difficult to even be suspicious that illicit gene doping may have occurred.

According to Larry Bowers, the leading toxicology and testing expert with the U.S. Anti-Doping Agency, there would be no way to test for gene doping with current technologies. The authors of a *Scientific American* article [3] conclude their assessment by saying, "For all intents and purposes, gene doping will be undetectable." It may be impossible to detect the agents used in gene doping, but the effects can be measured.

The gene doping will, in most instances, result in the production of a human protein, which by itself is identical to the persons own proteins. A recent publication indicates that even in these cases a difference between the native protein and the gene therapy product may be detected, on the basis of different glycosylation patterns in different cell types. It remains to be seen if this will be the case for all types of gene doping [23].

Only the (blood) level of the protein may be indicative for doping abuse. In the case of Epo-type treatment, this might be detectable, because of the resulting increase in hemoglobin and hematocrit. However, genes may be turned on and off by taking specific medicines. Studies in monkeys have shown that Epo levels can be controlled in this way, resulting in desirable hematocrit levels [41].

A possible solution is the use of RNA or protein markers, as indicators for disruption of normal physiology. In a recent study, microarrays were used to identify the effects of tetrahydrogestri- none (THG) on the expression of the genes of the mouse genome in muscle [21]. An anabolic steroid-specific signature was identified [40], describing a proteomics technique for the detection of hGH in blood. Plasma proteins were reduced, alkylated, and digested with trypsin, and the resulting peptides were separated on a capillary C-18 column and then detected by ion-trap mass spectrometry (1D LC/MS). This study provided a global view of the serum proteome with over 200 plasma proteins being preliminarily identified. In the MS/MS analysis, hGH was detected by characterization of the first tryptic peptide. The detectable amount of growth hormone was 10-fold above normal *in vivo* levels, representing concentrations that may be present in doping measurements. These studies have demonstrated that shotgun sequencing approaches (LC/MS/MS) not only can profile high-abundance proteins in complex biological fluids but also have the potential to identify and quantitate low-level proteins.

These techniques may be used to identify gene doping in the future. It will require the sampling and analysis of sets of RNA or proteins at the level of the individual athletes physiology over time. It is conceivable that testing will be limited to urine and blood samples as athletes are unlikely to accept tissue sampling. These techniques will require storage of samples at low temper-

atures and thus demand either on site testing or new handling regimens. With progress in RNA array and proteomic techniques, which allow the simultaneous screening of the expression of hundreds of proteins, this technique may become valuable for anti-doping testing.

In conclusion, it will be very hard to detect gene doping in the near future. Therefore, it is important to fight this new phenomenon with a combination of different preventive measures.

## Preventive Measures

### Regulations

Public authorities and sports organizations, including the International Olympic Committee, have condemned doping since the 1960s. The concerned substances were, for the most part, approved medicines from pharmaceutical companies. The recent advances in biological pharmaceuticals will have a great impact on the nature of medicines prescribed to patients but will also change the choice of performance enhancing medicines for athletes.

Clinical use of gene therapy is still experimental. One usually needs permission from offices which evaluate ethical issues and safety issues. This guarantees that pharmaceutical grade vectors are used with known safety profiles. However, there are no restrictions on the availability of genetic materials. The only European law limiting exchange of genetic materials is the EC decree Nr. 3381/94 on the export control of goods, limiting import and or export of strategic goods, including genetic materials.

Gene therapy is only permitted in nonreproductive cells ensuring that gene therapy will not affect future generations.

The International Olympic Committee (IOC) has included the method of gene doping in their list of prohibited classes of substances and prohibited methods as of January 1, 2003.

### Detection

The prohibition of gene doping by the World Anti-Doping Agency (WADA) and international sports federations provides a strong basis for the elimination of gene doping in sports, but will depend on methods to determine the compliance of athletes with these regulations. As described above, detection of gene doping is very difficult. Gene therapy vectors may be measurable only shortly after administration and, in many cases, would require tissue sampling. The resulting protein is not different from the endogenously produced protein. It may only be possible to detect gene doping by repeated physiological protein profiling of athletes, allowing changes in protein levels to be perceived. These measurements need to be developed and evaluated in order to be introduced as routine testing methods. In addition, other preventive methods to minimize the use of gene doping in sports should be stimulated.

### Education

Most athletes will not have enough background knowledge to fully understand the potential health hazards imposed by gene doping. Therefore, it is of utmost importance that athletes and

their supporting staff will be educated on this subject in order to prevent the use of gene doping. Athletes will receive information on the potential benefits of gene doping from the mass media such as newspapers, magazines and television. They will be tempted to try such a new therapy, especially when they are informed that such a form of doping may be almost impossible to detect. Athletes should be made aware of the risks involved in gene doping when used in an uncontrolled athletic setting without compromising the great possibilities that gene therapy offers for the treatment of certain illnesses. Risks that may involve themselves, their relatives and even the environment.

### Coordination

In order to develop an effective strategy for the prevention of gene doping, national as well as international coordination is required. The inclusion of gene doping as a prohibited method on the doping list is only a first step. In this regard, WADA should play a leading role. Coordination is necessary to set-up an educational program for athletes and their supporting staff, as well as the general public. Also, research needs to be coordinated in order to investigate the development of methods to detect gene doping. The pharmaceutical industry should preferably subscribe to a code in which they state that they will not produce or sell genetic products for other than therapeutic use, banning gene doping.

### Survey on Gene Doping

A limited number of people from different disciplines in science and sports were interviewed in order to obtain an idea of the notion and possible impact of gene doping. People included three sports physicians, a pharmacist, four elite athletes and five scientists from academia, as well as from the pharmaceutical industry. Questions included: Are you familiar with the term gene doping? What do you think it means, do you think gene doping will improve athletic performance? Will it be different from current doping strategies? What, in your opinion, are the health risks of gene doping? Do you think gene doping is used at present or will be used in the near future? (If yes, could you give an estimated time frame?) Do you think gene doping will be easily detectible?

From the answers, it is clear that people outside of the scientific community or pharmaceutical industry involved in gene therapy have little knowledge about the use of gene therapy. A general fear is that it may affect offspring and could cause cancer. They feel that detection of gene doping will be complicated and preventive measures will be difficult. On the other hand, they insist that gene doping will be used by athletes, as soon as it is available, which according to them will be in the coming years.

The professionals surrounding elite athletes are quite aware of the possibilities for gene doping. They think it may be used within a few years and that detection of gene doping will be very hard. They recommend the education of athletes, as well as their medical staff. In addition, they advocate the development of assays for gene doping measurement, such as physiological protein profiling in blood.

The pharmaceutical industry is well aware of the possibilities and risks of gene doping. Although gene therapy is an experimental treatment that needs further development, they foresee that gene doping will be used by (elite) athletes in the next years. They will not allow the use of their products outside prescribed use, including the use to enhance athletic performance. They see the detection of gene doping as a major problem and are willing to collaborate on the development of assays to detect the gene products from their medicines.

### Conclusions

The sporting world will sooner or later be faced with the phenomena of gene doping to improve athletic performance. The exact number of years that it will take for this issue to enter the athletic arena is difficult to estimate, but it is most likely that this will happen within five years.

Many genes are readily available which may potentially have an effect on athletic performance. These genes are evaluated in clinical trials for the treatment of illnesses. The gene therapy vectors used in these studies may find their way to athletes and their supporting staff. Alternatively, illegal laboratories may be set-up to produce the gene transfer vectors. In both instances, it seems unlikely that fail-safe detection methods will be developed. The uncontrolled use of nontherapeutic gene therapy by athletes imposes potential risks for the user and the environment. At this moment, a combination of developing detection methods based on gene arrays or proteomics and a clear education program on the associated risks seems to be the most promising preventive method to counteract the possible application of gene doping.

### Internet Sites

Ccmo: [www.ccmo.nl](http://www.ccmo.nl)

Cogem: [www.cogem.nl](http://www.cogem.nl)

IOC: [www.olympic.org](http://www.olympic.org)

World Anti-Doping Agency (WADA) websites:

[www.wada-ama.org](http://www.wada-ama.org) and [stage.wada.netcomsus.com](http://stage.wada.netcomsus.com)

Necedo: [www.necedo.nl](http://www.necedo.nl)

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