Gene doping. Are we willing to risk it?

Dopaje genético. ¿Estamos dispuestos a arriesgar?

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Gene doping is defined as the non-therapeutic use of genes, genetic elements and/or cells that have the capacity to improve sporting performance. This can be achieved by introducing and subsequently expressing a transgene, or by modulating the activity of an existing gene to achieve an additional physiological advantage[1].

Since 2001, when the improvement of the abilities of athletes that used the principles of gene therapy was discussed for the first time in the prestigious Nature[2] magazine, gene doping has been the focus of many debates, some controversial in terms of its banning.

The decoding of the human genome has opened the door to a wide set of possibilities for genetic treatments, as well as for the technologies that will develop them. This has not just been in the field of therapy, but also in improvements in sporting performance. Considering that gene therapy is a new form of medicine, and until recently it had only been tested on patients with terminal illnesses, its long-term consequences are as of yet unknown. Therefore, the key questions continue to be unanswered regarding the possible use of transgenes in the sporting sphere.

Perhaps the fundamental question refers to the theoretical possibility that the transgenics used in gene doping could inadvertently affect germinal cells, and produce permanent alterations that could be transferred to future generations. There are currently no solid answers to this question.

The basic principles of gene doping: Much simpler than it seems

The non-therapeutic use of genes can modify genetic expression in that proteins are produced in the organism that give muscles more growth, faster recovery and greater strength. The proteins produced this way will be the same as those generated normally by the body.

The idea is simple: alter our genetic composition, the building bricks of which we are made, to make us stronger or faster. However, the practical aspects are highly complex[3-5].

Genetic therapists add a synthetic gene to the patient's genome, and reintroduce it into the body via a de-activated virus. The new gene is extracted by the patient's stem cells, and acts as a treatment, becoming permanently incorporated into the body.

It is still a rare and highly specialised treatment, but the principle is used for the research of any variety of illnesses, including those in which there is muscle deterioration, a point that makes it easier to imagine how athletes could benefit from this mechanism.

Types of genetic interventions: Are they all equally dangerous?

Generally speaking there are two types of genetic interventions:

Somatic

SThis involves the intervention into cells to modify the genome of already existing beings in order to make them more resistant to certain illnesses or to improve their physical capacities. In theory they are not variations that can be transmitted genetically from people to their descendants. In principle these would be those that are (currently) applied in gene doping.

On this level there are currently two possibilities of using somatic gene therapy to improve sporting performance:

Ex vivo: For this, a cell line must be extracted from the athlete (using a biopsy). The gene is transferred to these cells, which are then re-introduced into the body. This is an invasive method (as it requires a biopsy) but it has the great advantage that it allows for the exclusive treatment of specific cell lines (usually muscle lines).

In vivo: The gene is transferred directly to the patient using a drug. The gene would be transported in a vector such as a virus or plasmid, and the modified DNA would be injected into the athlete's cells.

Genetic modifications to the germ line

– *In vitro fertilization:* development of the embryo before its implantation and the genetic modification of the embryonic stem cells of the

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foetus, by which the genetic data of the future individual is changed.
- *Cloning:* an adult somatic cell is genetically modified. Next, the nucleus of this modified cell is introduced into an egg that has no nucleus, and fertilization is simulated, with which an embryo is created that contains the same genetic data as the first adult, plus the added genetic modification. This has not yet been applied to human beings… Are we considering applying it to be stronger, faster and more resistant?

Which genes are candidates for use in gene doping?

There are many, but perhaps the most well known are:

Erythropoietin: Achieving an increase in energy production by the aerobic metabolism. The high-profile Repoxygen

Repoxygen leapt into the media during the trial that took place in 2006: A genetic therapy developed by the British laboratories Oxford Biomedica, in 2002 found it to be a very effective treatment for severe anaemia in neoplastic processes and in kidney failure.

Treatment with Repoxygen is based on the direct intramuscular administration of an inactivated virus that carries the erythropoietin gene. The drug attaches to a specialised gene in the DNA of its host, in this case, the gene that is responsible for the synthesis of EPO. In the right conditions, the gene directs the cells to start producing extra erythropoietin (EPO).

The majority of Repoxygen studies carried out reached the conclusion that the EPO gene injected produces higher levels of circulating EPO and has a much more pronounced biological effect that the endogenous gene in all the species studied, thus revealing its high potential in gene therapy strategies for EPO[6-7].

Vascular endothelial growth factor (VEGF): Increase of the oxygen supply

Oxygen is vital for the synthesis of ATP for aerobic breathing. Oxygen, being a small molecule, is able to spread through the plasmatic membrane of endothelial cells. Therefore, an increase in vascular branching promotes quicker and more effective oxygen diffusion to tissues, and a greater availability of it to produce energy. VEGF promotes the branching of the pre-existing vessels, thus increasing capillarisation. In gene doping, various copies of the gene that codifies the VEGF are inserted into the muscle, probably using viral vectors. Therefore, if it is successful in athletes, muscle microcirculation will be stimulated and the oxygen supply to muscles will be increased[8].

Type 1 insulin-like growth factor IGF1: Increase in the growth and differentiation of the muscle

In 2007, whilst Lee Sweeney - a professor at the Pennsylvania University – researched the possible ways of restoring muscle growth in patients with muscular dystrophy, mice were created that continued to have enormous muscles and that preserved a significant percentage of their strength into old age.

These super-mice were created by injecting normal mice with a virus that held the gene for type 1 insulin-like growth factor, which has receivers in the surface of muscle cells and stimulates their growth. These mice were nicknamed "Schwarzenegger mice" after the North American body-builder.

In gene doping, multiple copies of the gene that codifies for IGF-1 could be inserted into the skeletal muscle and would produce an increase in muscle mass due to the hypertrophy of the muscle cells. This somatic gene insertion, according to experts, could be achieved by using two alternative vectors: plasmid or virus, and would always be performed using the ex vivo technique, i.e. via muscle biopsies, strengthening the muscle groups required.

It is essential to recall at this point that IGF-1 also has activities that act beyond muscular effects, including the capacity to boost tumour development and progression, that is, it is potentially pre-neoplastic[9].

The antagonists of myostatin, the gene PPAR-Delta and its agonist the gene GW501516: The increase of hypertrophy and hyperplasia of the muscle and also the perpetual movement machine

Myostatin, a member of the growth factor family, could be useful in gene doping with the aim of improving muscle percentage and sporting performance. It is closely linked to the gene PPAR-Delta. In 2008, Evans developed a strain of "marathon mice" by stimulating the gene called PPAR-Delta. The genetically modified mice were able to run twice as far as normal mice, and were capable of possessing high muscle definition, even when they were fed with a high-fat diet10. It is key to remember and reiterate that the activity of these genes goes further than the target organ, and they therefore have the capacity to proliferate tumours, as in they are potentially pre-neoplastic.

The list would be endless, as there are currently at least 181 active clinical trial treatments in the USA alone, and over 2000 internationally. The majority of them focus on treatment for severe anaemia and muscle weakness, which could undoubtedly be used in athletes. Mention should be made of growth hormone modulating genes, the hypoxia induced factor (HIF), peroxisome proliferator activated receptors (PPARα), etc.

Where does the problem lie?

Permanently changing the genome could be complex, using a de-activated virus to take medicine genetically to cells. Nevertheless, there is a shortcut that offers temporary results: injecting the purified gene directly into the muscle. In fact, this is simple, and now, thanks to the Internet, it is possible to access Repoxygen.

We could suggest that a tempting aspect for those aspiring to use doping is that this temporary improvement, after a couple of days, could be difficult to detect by the authorities.

If this is the case, what is the World Anti-Doping Agency (WADA) doing about this?

WADA: Detection or prevention strategies?

Unfortunately, science is a step behind those that turn to cheating, and when a new substance is detected, there is already another one in the market.

In 2003 the WADA banned gene doping, as from the agency's scientific management perspective, not only would the carrying out of this practice be unfair, it could also be lethal[11].

It is highly unlikely that anyone is benefitting from gene doping, and undoubtedly it is much more effective to focus attention on more standardised doping systems such as anabolic steroids and different blood doping methods. However, the WADA upholds that it is investing significant amounts of money and research resources into finding an effective diagnostic method to detect the intervention of genetic material in athletes. In fact, the latest challenge in the fight against doping is to be able to detect gene doping, for which molecular biology techniques have been used. Work is currently being undertaken from a new perspective: instead of tracking substances, as in a standard examination, changes are looked for in genetic expression and in the production of protein. Another very curious idea that is being contemplated is the shaping of images, using a similar process to magnetic resonance, to explore the body in the search for less common gene expression locations. In light of the studies that are being carried out, experts from WADA feel that it is only a matter of time before a detection test is created.

In all honesty, to date, no one can be sure of whether or not genetically modified Olympic athletes are already in the pools or on the tracks, as the temptation of winning the gold medal can lead athletes to take the hugely dangerous genetic step towards the unknown.

Some say that it may not be long before we see the first genetically modified athlete. Others, including myself, consider that the use of gene therapy to improve sporting performance is already a reality. However, given that the diagnostic methodology still lacks the sophistication needed to contest "gene doping", its status continues to be unclear.

A certainty is that the use of genetic modification to improve sporting performance is technically feasible, at least in animals, and that some athletes are prepared to risk their lives in the quest for guaranteed gold medals[12].

Yet there is an even greater question mark. Even if there were already an effective test to detect gene doping, what would happen if gene therapy became a widespread, or even routine practice? What would happen if we could all purchase genetic medicine to reduce muscle deterioration? Should we – or could we – prevent athletes from using this medicine to prolong their careers or to speed up their recovery after an injury?[13-15].

Where are we headed?

This is where I leave my own question: If gene therapy used to prevent muscle deterioration were safe, it would become an exclusively ethical issue, given that we could maintain normal quality in the field of sporting medicine for longer, and optimise ageing, which is where the issue returns to sport.

We know that the earlier the intervention, the better the ageing process will be. Could it be unethical to stop treating people with something that could truly enable their muscles to be much healthier now and in the future?

In any case, we are decades from having the need to cover this issue, given the slow rate at which gene therapy is advancing. When the moment arises, the Agency will have to mark the limits that it does with all drugs: Do they unfairly improve the athlete's performance?

Though with that in mind, it seems that the nature of gene doping will make it technically difficult and ethically cumbersome to mark these limits. Authorities, athletes and the sporting sphere agree upon a whole new definition of what they want sport to mean.

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