Gene therapy, genetic doping and sport: fundaments and implications for the future

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ABSTRACT

Optimal performance has been constantly sought for in high level competitive sport. To achieve this goal, many athletes use illicit drugs and methods, which could have important side effects. Gene therapy is a very recent therapeutic modality, whose results have shown to be efficient in the treatment of severe diseases so far. The basis of gene therapy is a vectorial transfer of genetic materials to target-cells in order to supply the products of an abnormal gene in the patient's genome. Recently, the potential for misuse of gene therapy among athletes has called attention of scientists and sports regulating organs. The transfer of genes that could improve athletic performance, a method prohibited by COI in 2003, was named gene doping. The most important candidate genes for gene doping are the ones which codify for the following proteins: GH, IGH-1, miostatin blockers, VEGF, endorfins and enkefalins, eritropoetin, leptin and PPAR-δ. Once inserted in the athlete genome, the gene would be expressed and produce an endogenous product capable of improving performance. Thus, current doping detection methods are not sensitive enough to detect gene doping, which in turn could stimulate its use among athletes. Moreover, gene therapy still presents known application problems, such as inflammatory response and lack of control of gene activation. It is probable that such problems would be even more important in healthy individuals, since there would be excessive product of the transferred gene. Moreover, other unknown risks specific for each gene are present. Therefore, debate on gene doping should be carried on in the academic as well as sports field, in order to study prevention, control and detection measures of gene doping, avoiding hence, future problems regarding the misuse of this promising therapy.

Keywords: Gene therapy. Genetic doping. Sport.

INTRODUCTION

Gene therapy is a fairly recent investigation field in Biomedicine which has been presenting many advances over the last years. It is believed that gene therapy represents a possibility of effective treatment for several diseases whose treatments are little effective and/or restricted to symptoms[1-7]. Since it is still in eminently experimental character stage, there are problems in the gene therapy application, being its risks control one of the most important[7-11]. However, studies in animal models[2,12-15] as well as some studies in humans[1,6-7,16-19] have presented promising results.

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Injuries derived from sports practice constitute one of the main factors of early drop out from the sportive career, extended time away from training and competitions, as well as decrease in performance, being even able to lead to functional limitations in more advanced ages[20]. In addition to that, the majority of the sportive injuries involve tissues of difficult regeneration, such as tendons, ligaments, and cartilage[17,21]. The gene therapy could, therefore, have a very important application in the sportive field, allowing among other applications the reconstitution of injured tissues. Nevertheless, this kind of treatment may carry a potential risk of misuse by athletes who search for physical performance improvement, as occurs in high level sport. The misuse of this therapy is called gene doping and has been issue of a scientific-academic debate whose importance has been growing in sports medicine and sports sciences[21-27].

In 2001 one of the first official debates on gene doping took place in a meeting of the *Gene Therapy Working Group* promoted by the International Olympic Committee (IOC)[21]. In that meeting, the Committee has declared that the gene therapy besides its importance in the treatment and prevention of diseases has a great potential for misuse in sports. Additionally, detection ways of gene doping should be developed and applied. In the beginning of 2003 the gene doping entered the list of banned methods by the IOC. In 2004, the chief editor of the journal *Molecular Therapy* published that if in the Athens Olympic Games (2004) events of gene doping could have been only scientific fiction, in Beijing (2008) they may not be[23].

Up to the present moment, there is no register of any case of athlete who had made use of genetic manipulation. On the other hand, considering that there are not gene doping control and detection devices yet, and that, theoretically, it is already possible to apply this technique in humans and other animals, one cannot state that no athlete has ever tried it.

Within this context, the aim of the present investigation was to present the gene therapy fundaments and its applications and implications for the sports field, contributing with this important debate in the national scenario. Therefore, we performed a bibliographic research in the *Medline* and *Sport Discus* databases using the keywords *gene transfer and sport, gene doping, gene therapy* and *gene therapy and sport*. The found articles were selected concerning originality and relevance to the discussion here presented, considering the strictness and suitability of the experimental outlining, the sample number and the statistic analysis. Other articles not indexed in these databases were also checked when mentioned by the obtained article in the original search and, following criteria above mentioned, they were included.

GENE THERAPY

Gene therapy can be defined as a set of techniques which allow the insertion and expression of a therapeutic gene in target-cells which present some kind of disorder of genetic origin (not neces-

sarily hereditary), enabling the correction of inadequate genetic products which cause diseases. The genetic material inserted in the patient's cells may generate the functional form of a protein which due to structural alterations in this gene, is produced in small or with biological activity amounts. It is also possible to regulate the expression, activate or inactivate other genes[17,28-29].

The insertion of therapeutic gene implicates in its introduction through transfer vectors which are able to recognize the target cells. There are many insertion systems of the genetic material *in vivo*. The viral vectors are the commonest (being the retrovirus and adenovirus the most widely used); however, other types of non-viral vectors have also been used, such as liposomes and macromolecules conjugated to the DNA[6,28,30]. The injection of genetic material straight to the target tissue is also a way of performing the gene therapy without the use of virus[6,28,30].

There is also the gene therapy system *ex vivo*, in which the cells of the patient himself are removed through biopsies, modified and reimplanted in the patient, so that the therapeutic gene is inserted outside the patient's organism[28].

Before being introduced in the patient, the virus used as vectors suffer many genetic alterations in order to have the therapeutic gene inserted, while several other genes which give it virulence are removed or inactivated[6,28,30]. Thus, when joining and invading the target cells, the viral vectors inject their genetic material containing the therapeutic gene in the patient's DNA, enabling the transcription and translation of the gene for its corresponding functional protein, or they use the molecular equipment of the host cell to express its genes.

Haisma and Hon[21] affirm that around 3000 patients have received some kind of gene therapy. Several diseases have been treated, including endothelial dysfunctions, hemophilia, immune deficiency and many kinds of cancer[6,18-19,31-32]. Generally speaking, gene therapy has brought good outcomes, and its side effects seem to be reduced to a small number of patients, which is an encouraging indication of the treatment's safety. These procedures should be carefully handled, and the security certification tests of the preparations are countless and very strict, what makes the treatment extremely costly[21].

Despite the scientific and technological advances, there are still many doubts concerning the side effects of the gene therapy. The introduction of genetically modified organisms generates a great uncertainty, especially if the virus mutagenic potential is considered[22]. Moreover, the less known effects are concerned with the long run expression of the genes introduced as well as the lack of control of the expression of such genes[21]. Another very important point is the possibility (despite being small) of modification not only of the somatic cells, but also of the germinative ones[21]. Nevertheless, there is no doubt that the main problem the gene therapy faces in the current stage of development is the high immunogenic capacity of the viral vectors introduced in the patient, which can be an important complication factor derived from the treatment[7,9-11]. Although non-viral vectors are an interesting treatment alternative, they also present efficiency, toxicity and inflammatory response problems[29].

GENE DOPING

According to the 2004 definition of the *World Anti-Doping Agency* (WADA), gene doping is the non-therapeutic use of cells, genes and genic elements, or the modulation of the gene expression, which have the capacity of increasing sportive performance[24].

Despite being developed with the purpose to treat severe diseases, gene therapy, as well as several other therapeutic interventions, has great potential of abuse among healthy athletes who wish to improve performance. History has shown that athletes are able to ignore many risks in the trial to surpass their competitive limits[33]. As pharmaceuticals of unknown side effects, it is very probable that athletes submit to gene therapy in order to gain competitive performance despite the knowledge of known risks, besides the existence of unknown risks as well[21].

Considering that the gene therapy is only in the beginning of development and that theoretically, the athletes do not make use of this kind of ergogenic strategy yet, one may only comment on the genes which are important candidates to misuse in the sports field, namely: erithropoetin, myostatin blockers (follistatin and others), *Vascular Endothelial Growth Factor* (VEFG), *Insulin-like Growth Factor* (IGF-1), *Growth Hormone* (GH), leptin, endorphins and encephalins, and *Peroxissome Proliferator Actived Receptor delta* (PPARδ)[21,34].

Erithropoetin

Erithropoetin is a protein produced in the kidneys whose main effect is the stimulus of the hematopoese[25]. Therefore, the additional copy of the gene which codifies the erithropoetin results in the increase of the production of red blood cells; consequently, the capacity of O_2 transportation to the tissues is increased. This kind of doping would be therefore especially ergogenic for endurance athletes.

Research with rats and macaques successfully transferred an additional copy of the erithropoetin gene[12,25-26], suggesting that this kind of doping is already possible. Nevertheless, it is not very probable that the super-expression of erithropoetin had important harmful effects in healthy individuals, once a remarkable increase of the monkeys' hematocrit has been observed (from 40% to approximately 80%)[12]. This fact can obviously represent a serious risk of the cardiovascular function compromising, including difficulty of the cardiac debt maintenance and tissue perfusion, due to substantial increase of blood viscosity. Besides that, severe anemia has been reported in some animals due to an autoimmune response to the extra gene transfer[35]. These reports raise serious doubts concerning the real possibility of use of the erithropoetin gene transfer in athletes.

Myostatin blockers

Myostatin is a protein expressed in the skeletal muscles both in the embrionary period and adulthood. Its action consists in regulating the proliferation of the myoblasts during the embrionary period as well as the protein synthesis in the skeletal muscles during and after the embrionary period[36-39]. In some cattle breeds it is observed the uncommon growth of muscles of some animals (a phenomenon known as *double muscling*). It was verified some years ago that these animals presented mutations in the myostatin gene, making a non-functional protein, which demonstrated that myostatin inhibited the skeletal muscles growth[40-41]. Recently, the case of a child who presented similar phenotype to double muscling has been described. It was observed that this child had also deletions in the myostatin gene[39]. Lee and McPherron[37], using genetically modified rat models, concluded that super-expression of the myostatin blockers such as follistatin, leads to the same double muscling phenotype. Myostatin inhibits both the muscular hyperplasia and hypertrophy, with the muscular mass gain derived from the myostatin blockage primarily happens due to the increase in the number of muscular fibers[37].

Therefore, it is believed that the myostatin blockage signaling is one of the candidates with most potential of abuse in sports, once muscular mass gain may be decisive in many sports modalities. However, the use of myostatin blockers as ergogenic resource is a little distant yet, once studies with myostatin blockage involved genetically modified animals, that is, which did not produce the protein from the beginning of the development. Therefore, it is not known yet which the exact effects are when the blockage occurs only in adulthood, a period in which increase in the number of muscular fibers is not observed. Another important issue is concerned with the possibility of expression of the myostatin inhibit-

ing genes in other muscular tissues; such as the straight and cardiac ones. Although this risk is not very high, once the animals from the study by Lee and McPherron[37] expressed the transgenes only in the skeletal muscles, one cannot discard this hypothesis, considering that there are no data in the literature on vectorial transfer of these genes and which involve humans.

VEGF

VEGF (or Vascular Endothelial Growth Factor) is a protein which plays an important role in the vascular endothelial growth, in angiogenesis and vasculogenesis[18-19]. The gene therapy with VEGF is one of the few already used in humans. The introduction of the gene which codifies the VEGF in patients with endothelial dysfunction responsible for coronary arterial disease and peripheral arterial disease events have introduced good results, with formation of new vascular branches[18-19].

In athletes, the VEGF vectorial insertion could produce vasculogenesis. Thus, the blood flow for all tissues would be increased, as well as its oxygenation and nutrition. Considering that it occurs in tissues with skeletal and cardiac muscles, one may expect increase in the energetic production, decrease of metabolites production and fatigue delay. Endurance athletes would theoretically be the most interested in the gene therapy with VEGF insertion[21]. Once this kind of therapy has been already used in humans with therapeutic aims, gene doping involving VEGF could be currently illegally applied in order to improve the sportive performance.

IGF-1 and GH

In laboratory animals, the introduction of the virus through adenovirus vector which modifies the IGF-1 protein and its consequent super-expression increases the protein synthesis in the skeletal muscles. Such episode was observed both in animals which were submitted to strength training and sedentary ones. When the extra IGF-1 gene introduction was combined with strength training, hypertrophy and strength development were higher than the ones observed in only strength-trained animals (and not super-expressed IGF-1) and in the ones which only super-expressed IGF-1 (and did not train strength)[13]. Thus, it can be said that the IGF-1 super-expression may boost in great magnitude the muscular responses to physical training, especially in strength training. Due to the great success obtained in animal studies as well as the apparent safety of gene therapy with IGF-1, it is possible that within few years it is a fact in humans. Obviously, it will be able to be used by athletes who search for performance improvement; however, it will be possibly used also by people with severe muscular disease, such as Duchenne muscular dystrophy and others.

Theoretically, gene doping with GH would lead to effects very similar to the ones produced by the IGF-1, once the GH action is mediated by the IGF-1 itself. Therefore, it can be expected that the gene doping with GH produces strength gains and muscular hypertrophy. It is probable that the risks involved with the insertion of the GH and IGF-1 genes are related with the imbalance of the hypothalamus-hypophisary axis and especially with the increase of occurrence of several dysplasias. There is also the risk of GH super-expression which can lead to glomerulosclerosis, which has been already demonstrated in animal models[42].

Leptin

Leptin, a peptidic hormone especially produced in the adipose tissue whose main action is related to the control of the hunger feeling and satiety, reduction of eating intake and consequent weight loss[43], is also a candidate for abuse as gene doping[22].

In 1997 a study demonstrated that the introduction of the leptin gene through viral vector produced significant weight loss in rats[14]. On the other hand, maybe the same phenomenon is not observed in humans, since obese individuals, who present high plasma concentration of leptin, do not have reduced appetite[44]. Such resistance to leptin action may represent an important obstacle for gene therapy with this hormone. Moreover, differently from the animal models, the human eating behavior depends also on other factors (nutritional, psychological, social and cultural).

Endorphins and encephalins

Endorphins and encephalins are endogenous peptides of analgesic activity. The use of gene therapy with the endorphin and encephalin genes could, therefore, improve sportive performance through decrease of pain sensation associated with any kind of injury, fatigue or excessive training[21]. Theoretically, it would allow athletes to train more, or would avoid their temporary leave from trainings and competitions due to minor injuries. Actually, analgesic drugs are within the mostly ingested by athletes[21], which indicates the possible interest on the insertion of these genes. Animal studies have demonstrated that this kind of gene therapy was able to decrease the perception of the inflammatory pain[15]. Nevertheless, due to great lack of data in the literature, it is probable that gene doping involving endorphins and encephalins is still far from really happening[21].

PPAR-δ

The proteins from the PPARs family act as transcription factors of genes involved in the metabolism of carbohydrates and lipids. They have been firstly discovered playing a role in the peroxissomes synthesis, and for that reason, were called *peroxissome proliferator-actived receptors*[45]. There are several PPAR proteins, but the one which presents, at least from the theoretical point of view, greater potential for abuse in gene doping is the PPAR-δ[34].

PPAR-δ is a key-regulator protein of the lipids oxidation process. Acting in the liver and skeletal muscle, it stimulates the transcription of several enzymes which participate in the β-oxidation[46]. The PPAR-δ is also associated with the energy dissipation in the mitochondria which occurs through the decoupling proteins, so that its action leads to decrease of energy production. As a result, the PPAR-δ decreases the amount of adipose tissue, reduces body weight and increases the thermogenesis[46]. This is therefore, one of the justifications for the possible interest from athletes in using gene doping with PPAR-δ. Improvement in lipid oxidation, besides decrease of adiposity (an effect which would call interest from athletes of almost all sports modalities), would preserve the glycogen supplies, increasing the tolerance time to effort[47] and also probably in endurance events.

Another reason for the possible interest in using the PPAR-δ as gene doping is its probable role in converting type II muscular fibers into type I[48]. Therefore, athletes whose modalities do not depend on strength, but demand low weight and low fat percentage from the athlete (such as marathoners, gymnasts, skaters and so forth) would be potentially the most interested in the PPAR-δ gene transfer.

GENE THERAPY IN ATHLETES

Besides the potential interest for the use of gene therapy as a sophisticated and undetectable kind of doping, the athletes could benefit from the transfer techniques as any ordinary individual whose clinic report imposes such need. According to what has been mentioned before, the genetic reconstruction techniques of injured tissues could be widely used in the sports field for the treatment and rehabilitation of injuries[21]. The transfer of genes which code growth factors could facilitate as well as improve the treatment of osteo-musculo-articular injuries which currently require surgery and long period of rehabilitation[17].

A very important issue on the relationship between gene therapy and sport was raised in 2006 by Haisma and Hon[21]. According to the definition from the WADA, the non-therapeutic use of gene transfer techniques which are able to improve sports performance

is considered doping and, therefore, banned. Such definition, despite being clear, does not contemplate several possibilities, nor mention the consequences of the athletes' right to use gene therapy. For instance, could a subject who suffers from any muscular dystrophy or severe anemia become an athlete after the therapeutic use of gene transfer such as IGF-1, follistatin or erithropoetin? Or else, an athlete who needs gene therapy and as a consequence of this treatment acquires any competitive advantage should continue competing? According to the definition it would be possible; however, such permission clashes with some ethical and moral issues which serve as grounding for the whole doping prohibition.

Undoubtfully, the debate of these and other issues on gene therapy and sport need to be intensified with the purpose to avoid abuse without the right of therapeutic use from athletes who need gene therapy is harmed.

ASSOCIATED RISKS TO GENE DOPING

In order to evaluate the involved risks with gene doping, firstly it is necessary to know the risks of the gene therapy. It is known that one of the main concerns of the gene therapy is the use of a virus as a vector. Despite strict control in all steps of the genetically modified virus preparation, there is a risk that it would cause important inflammatory responses in the patient, despite being this topic one of the most researched for solutions[7-11,29]. The chance that the gene is erroneously introduced in germinative cells, despite being very low, should also be considered as an inherent risk to the procedure.

Another problem related to the viral vector is the capacity of mutation and replication it could have[49], especially if there are flaws in its preparation, what can be common in illegal laboratories which accept to perform gene doping[21]. Additionally, there are also the risks non-related to the vector, but to the effects of the inserted gene, such as increase of blood viscosity by super-expression of erithropoetin[12], or increase of neoplasias occurrence by the super-expression of growth factors. The lack of control over the inserted gene expression may similarly represent a risk to the therapy. The risks specific to each gene are less predictable than the general risks inherent to the genetic transfer technique, especially if it is considered that the clinic tests are performed in their majority in sick subjects, with deficiency in the tested gene. Thus, it would not be possible to previously know how healthy subjects, which is the case of athletes, would respond to the same treatment.

Nonetheless, the reported number of problems derived from the gene therapy is very low, and up to the present time, there are indications that if the procedures are all according to the security criteria, the possibility of problems occurrence is low, showing that it has been relatively safe[21].

CONTROL AND DETECTION OF GENE DOPING

Although it does not completely eliminate doping, the detection possibility and the actions derived from the confirmation of antidoping testing at least inhibit the use of illicit drugs by the athletes. The fact that gene doping at first cannot be detectable[27], may stimulate its wide use in the sports field. For this reason, it is very important that prevention measures are immediately discussed by the scientific community as well as the sports regulating organs. Educational programs which involve coaches, physical trainers, athletes and their families, which clearly demonstrate all the risks inherent to the indiscriminate use of gene therapy, are probably more efficient ways to avoid gene doping in the near future.

Concomitantly, new detection strategies should also be developed. However, it is not known yet if the gene doping will be actually detected. Would the transferred gene have its expression confined to some tissues and its products do not reach the blood stream, only through biopsies it will be possible to perform a reliable antidoping test. This fact clearly becomes a huge barrier in the control of gene doping[21,34].

Currently, there are comments on some means of detecting gene doping. It is known for instance, that it is possible to differentiate through the protein glycosylation standard, the erithropoetin produced by the native gene produced by the transferred gene[26]. Obviously, such differentiation will be only possible in cases where the gene doping involves genes whose products reach the circulation. It is still necessary to know if the difference in the glycosylation pattern occurs only for the erithropoetin or if it also occurs for other proteins.

Other possible methods of gene doping detection would be: detection of antibodies aimed at the inserted virus, whose possibility of application is very low, once the athlete could be infected with the flu virus for example, and have a false positive result in the test; and gene expression pattern through microarrays technique, in which it is established how much of a set of genes is being expressed, but which requires also tissue samples collection as well as reference values for each analyzed gene[34].

FINAL CONSIDERATIONS

After the discussion proposed in this paper, one can realize that gene therapy is a very promising therapeutic technique in medicine, whose fast advances have become every time more tangible. However, it is worth mentioning the huge potential for its misuse in the sports scenario by athletes who ignore its risks in order to gain competitive advantage. Many genes would have the capacity to promote substantial gains in athletic performance, which could be decisive in several modalities. Since the traditional methods of doping detection are not able to reveal the use of gene doping, the broadening of this debate in the scientific and sports fields is mandatory in order to study and implement control and detection strategies.

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REFERENCES

1. Schmidt K, Hoffend J, Altmann A, Kiessling F, Strauss L, Koczan D, et al. Troponin I overexpression inhibits tumor growth, perfusion, and vascularization of Morris hepatoma. J Nucl Med. 2006;47:1506-14.
2. Enquist IB, Nilsson E, Ooka A, Mansson JE, Olsson K, Ehinger M, et al. Effective cell and gene therapy in a murine model of Gaucher disease. Proc Natl Acad Sci USA. 2006;103:13819-24.
3. Kim JM, Kim SJ, Lee HC, Kim KS. Development of ligand-dependent regulatory system and its application to gene therapy of insulin-dependent diabetes mellitus. Exp Mol Med. 2006;31,38:385-92.
4. Cope DK, Lariviere WR. Gene therapy and chronic pain. Scientific World Journal. 2006;6:1066-74.
5. Kendirci M, Gur S, Sikka SC. Gene therapy for erectile dysfunction. Front Biosci. 2005;10:2758-69.
6. Wilson DR. Viral-mediated gene transfer for cancer treatment. Curr Pharm Biotechnol. 2002;3:151-64.
7. Tan PH. 9th American Society of Gene Therapy annual meeting. Expert Opin Biol Ther. 2006;6:839-42.
8. Reifenberg K, Hildt E, Lecher B, Wiese E, Nusser P, Ott S, et al. IFN gamma expression inhibits LHBs storage disease and ground glass hepatocyte appearance, but exacerbates inflammation and apoptosis in HBV surface protein-accumulating transgenic livers. Liver Int. 2006;26:986-93.
9. Ritter T, Lehmann M, Volk HD. Improvements in gene therapy: averting the immune response to adenoviral vectors. Bio Drugs. 2002;16:3-10.
10. Bessis N, Garcia Cozar FJ, Boissier MC. Immune responses to gene therapy vectors: influence on vector function and effector mechanisms. Gene Ther. 2004; 11:S10-7.
11. Bangari DS, Mittal SK. Current strategies and future directions for eluding adenoviral vector immunity. Curr Gene Ther. 2006;6:215-26.

12. Zhou S, Murphy JE, Escobedo JA, Dwarki VJ. Adeno-associated virus-mediated delivery of erythropoietin leads to sustained elevation of hematocrit in nonhuman primates. Gene Ther. 1998;5:665-70.
13. Lee S, Barton ER, Lee S, Farrar RP. Viral expression of insulin-like growth factor-I enhances muscle hypertrophy in resistance-trained rats. J Appl Physiol. 2004; 96:1097-104.
14. Murphy JE, Zhou S, Giese K, Williams LT, Escobedo JA, Dwarki VJ. Long-term correction of obesity and diabetes in genetically obese mice by a single intramuscular injection of recombinant adeno-associated virus encoding mouse leptin. Proc Natl Acad Sci USA. 1997;94:13921-6.
15. Lin CR, Yang LC, Lee TH, Lee CT, Huang HT, Sun WZ, et al. Electroporation-mediated pain-killer gene therapy for mononeuropathic rats. Gene Ther. 2002;9: 1247-53.
16. Carretero M, Escamez MJ, Prada F, Mirones I, Garcia M, Holguin A, et al. Skin gene therapy for acquired and inherited disorders. Histol Histopathol. 2006;21: 1233-47.
17. Huard J, Li Y, Peng H, Fu F. Gene therapy and tissue engineering for sports medicine. J Gene Med. 2003;5:93-108.
18. Rajagopalan S, Mohler ER, Lederman RJ, Mendelson FO, Saucedo JF, Goldman CK, et al. Regional angiogenesis with vascular endothelial growth factor in peripheral artery disease: a phase II randomized, double-blind, controlled study of adenoviral delivery of vascular endothelial growth factor 121 in patients with disabling intermittent claudication. Circulation. 2003;108:1933-8.
19. Losordo DW, Vale PR, Hendel RC, Milliken CE, Fortuin FD, Cummings N, et al. Phase 1/2 placebo-controlled, double-blind, dose-escalating trial of myocardial vascular endothelial growth factor 2 gene transfer by catheter delivery in patients with chronic myocardial ischemia. Circulation. 2002;105:2012-8.
20. Patel DR, Baker RJ. Musculoskeletal injuries in sports. Prim Care. 2006;33:545-79.
21. Haisma HJ, de Hon O. Gene doping. Int J Sports Med. 2006;27:257-66.
22. Unal M, Unal DO. Gene doping in sports. Sports Med. 2004;34:357-62.
23. Verma IM. Doping, gene transfer and sport. Mol Ther. 2004;10:405.
24. World Anti Doping Agency. The world anti-doping code. The 2006 prohibited list. International Standard. Keynote address WADA health medical and research committee, 1-1-2005. Montreal: WADA; 2005.
25. Diamanti-Kandaraskis E, Konstantinopoulus P, Papailiou J, Kandaraskis AS, Andreopoulus A, Sykiotis G. Erytropoietin abuse and erytropoietin gene doping. Sports Med. 2005;35:831-40.
26. Lasne F, Martin L, de Ceaurriz J, Larcher T, Moullier P, Chenuaud P. "Genetic doping" with erythropoietin and cDNA in primate muscle is detectable. Mol Ther. 2004;10:409-10.
27. Andersen JL, Schjerling P, Saltin B. Muscle, genes and athletic performance. Sci Am. 2000;283:48-55.
28. Karthikeyan BV, Pradeep AR. Gene therapy in periodontics: a review and future implications. J Contemp Dent Pract. 2006;7:83-91.
29. Li S-D, Huang L. Gene therapy progress and prospects: non-viral gene therapy by systematic delivery. Gene Ther. 2006;13:1313-9.
30. Rubanyi GM. The future of human gene therapy. Mol Aspects Med. 2001;22: 113-42.
31. Hacein-Bey-Abina S, Le DF, Carlier F, Bouneaud C, Hue C, De Villarttay JP, Thrasher AJ, et al. Sustained correction of X-linked severe combined immunodeficiency by ex vivo gene therapy. N Engl J Med. 2002;346:1185-93.
32. Kay MA, Manno CS, Ragni MV, Larson PJ, Couto LB, McClelland A, et al. Evidence for gene transfer and expression of factor IX in haemophilia B patients treated with an AAV vector. Nat Genet. 2000;24:257-61.
33. De Francesco L. The faking of champions. Nat Biotechnol. 2004;22:1069-71.
34. Azzazy HME, Mansour MMH, Christenson RH. Doping in the recombinant era: strategies and counterstrategies. Clin Biochem. 2005;38:959-65.
35. Chenuaud P, Larcher T, Rabinowitz JE, Cherel Y, Casadevall N, Samulski RJ, et al. Autoimmune anemia in macaques following erythropoietin gene therapy. Blood. 2004;103:3303-4.
36. Matsakas A, Diel P. The growth factor miostatin, a key regulator in skeletal muscle growth and homeostasis. Int J Sports Med. 2005;26:83-9.
37. Lee SJ, McPherron AC. Regulation of myostatin activity and muscle growth. Proc Natl Acad Sci USA. 2001;98:9306-11.
38. Gonzalez-Cadavid NF, Bhasin S. Role of myostatin n metabolism. Curr Opin Nutr Metab Care. 2004;7:451-7.
39. Schuelke M, Wagner KR, Stolz LE, Hubner C, Riebel T, Komen W, et al. Myostatin mutation associated with gross muscle hypertrophy in a child. N Engl J Med. 2004;350:2682-8.
40. McPherron AC, Lee SJ. Double muscling in cattle due to mutations in the myostatin gene. Proc Natl Acad Sci USA. 1997;94:12457-61.
41. Kambadur R, Sharma M, Smith TP, Bass JJ. Mutations in *myostatin* (GDF8) in double-muscled Belgian blue cattle and piedmontese cattle. Genome Res. 1997; 7:910-5.
42. Machado MO, Hirata RD, Sellitti DF, Iotti R, Iotti A, Cusumano AM, et al. Growth hormone promotes glomerular lipid accumulation in bGH mice. Kidney Int. 2005; 68:2019-28.
43. Konturek PC, Konturek JW, Czesnikiewicz-Guzik M, Brzozowski T, Sito E, Konturek PC. Neuro-hormonal control of food intake: basic mechanisms and clinical implications. J Physiol Pharmacol. 2005;56:S5-25.
44. Sahu A. Leptin signaling in the hypothalamus: emphasis on energy homeostasis and leptin resistance. Front Neuroendocrinol. 2003;24:225-53.
45. Nelson DL, Cox MM. Hormonal regulation and integration of mammalian metabolism. In: Nelson DL, Cox MM, editors. Lehninger Principles of Biochemistry. 4th ed. Freeman; 2005.
46. Wang YX, Lee CH, Tiep S, Yu RT, Ham J, Kang H, et al. Peroxisome-proliferator receptor activates fat metabolism to prevent obesity. Cell. 2003;113:159-70.
47. Baar K. Involvement of PPAR gamma co-activator-1, nuclear respiratory factors 1 and 2, and PPAR alpha in the adaptative response to endurance exercise. Proc Nutr Soc. 2004;63:269-73.
48. Wang XY, Zhang CL, Yu RT, Cho HK, Nelson MC, Bayunga-Ocampo CR, et al. Regulation of muscle fiber type and running endurance by PPARδ. PLoS Biol. 2004;2:1532-9.
49. Nienhuis AW, Dunbar CE, Sorrentino BP. Genotoxicity of retroviral integration in hematopoietic cells. Mol Ther. 2006;13:1031-49.