Iranian J Publ Health, Vol. 40, No.1, 2011, pp.1-14

Review Article

Genetic Doping and Health Damages

*AA Fallahi[1], AA Ravasi[1], DD Farhud[2]

[1]Dept. of Sport Physiology, School of Physical Education and Sport Sciences, Tehran University, Tehran, Iran
[2]School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

(Received 4 Aug 2010; accepted 7 Feb 2011)

Abstract

Background: Use of genetic doping or gene transfer technology will be the newest and the lethal method of doping in future and have some unpleasant consequences for sports, athletes, and outcomes of competitions. The World Anti-Doping Agency (WADA) defines genetic doping as "the non-therapeutic use of genes, genetic elements, and/or cells that have the capacity to enhance athletic performance". The purpose of this review is to consider genetic doping, health damages and risks of new genes if delivered in athletes.

Methods: This review, which is carried out by reviewing relevant publications, is primarily based on the journals available in GOOGLE, ELSEVIER, PUBMED in fields of genetic technology, and health using a combination of keywords (e.g., genetic doping, genes, exercise, performance, athletes) until July 2010.

Conclusion: There are several genes related to sport performance and if they are used, they will have health risks and sever damages such as cancer, autoimmunization, and heart attack.

Keyword: *Genetic doping, Athletes, Gene therapy, Anti doping*

Introduction

Early information on doping has been reported from the early Olympic Games in the third century; thus doping is not a new-fangled event. In early Olympic compositions, some athletes used drugs and ergogenic substances to enhance their performances by increasing strength and overcoming fatigue. This has been continued but in new and various forms (1, 2). Current reports show the utilizing of steroids, strychnine, oxygen, and mixtures of brandy, cocaine and blood doping by cobalt (3) and considerably highly developed methods that usually involve the addition of substances naturally existing in the body such as sex hormones, erythropoietin (EPO), and so on (4). Recently, by scientific development in different branches, especially medicine, the methods, and substances of doping have been altered. Gene transfer technology or gene therapy in medicine is one of the new branches that influence the outcomes of the games and competitions (5). Genetic doping is a consequence of human genome project and gene therapy. Athletes, coaches and trainers are looking for gene therapy to enhance factors of their fitness and to overcome fatigue, but this clinical method is primarily designed to help patients with severe diseases (e.g. hemophilia, immunodeficiency) (6, 7).

There are several complexities and problems with genetic manipulation in athletes such as health risk, money, testing, detection, ethical considerations, and others (6, 8, 9). The most important reason in the prohibited list of World Anti-Doping Agency (WADA) is health of athletes. In spite of these side effects and risks, athletes use illegal substances, drugs, and methods. In a famous study on Olympic athletes, 98% of athletes reported that they would use forbidden substances if these substances were undetectable and they assured success, and over 50% reported that they would use the same undetectable substance if it did not let them win for 5 yr (10). Other reports indicate that team physicians, coaches, and trainers managed athletes to use performance-en-

***Corresponding author:** E-mail: ali.fallahi62@gmail.com

hancing substances and methods, which had some side effects and oppressed unaware athletes (2). East German physicians were instances who prescribed female athletes great dosage of anabolic steroids from 1974 to 1989. A number of women are unfruitful, have deepened voices and do not have breasts, have deformed children and, in case of one woman, she used so many steroids and developed so many male characteristics that she wanted a sex-change operation to become a man (2).

It is necessary to update a data of possible strategies on gene doping and genetic complications. In addition, there are not reviews on side effects, health risks, and damages of genetic doping in sports. In the present review, health risk and damages of various forms of genetic doping in recent years have been discussed. Therefore, the primary section discussed the potentials of gene therapy and then argued genetic doping. Then, the possibilities of using some novel genes, which are used in genetic doping in order to improve athletic performance in endurance and strength sports, are discussed including damages and risks of genes. In the final section, other complexities of gene doping including detection and ethics concepts have been discussed.

Searching Method

This review which is carried out by reviewing relevant publications is primarily based on the journals available in GOOGLE, ELSEVIER, PUBMED the in fields of genetic technology, and health using a combination of keywords (e.g. genetic doping, genes, exercise, performance, athletes). The electronic prepublications, that is, articles that are made available on the website of a journal before being published in print, are not included in the current review. One important group of updated studies is the human gene map for fitness, performance, and health-related fitness phenotypes that was reviewed in this review until July 2010.

Gene therapy is the base of genetic doping

In gene therapy, genetic materials are transferred to incorrect or damaged cells to treat human sever diseases (11). This is a new build-up method which appeared in the 20th century and involves some developed technologies such as gene division and refinement, vector choice (viral and non-viral nature), transfer technique, and so on (12). DNA, RNA or genetically altered cells can be genetic materials that are used in this approach. In this procedure, a healthy therapeutic gene that inters in a vector is delivered to a malformed cell and then therapeutic gene compensates for an absent or abnormal gene (13). This may be a new approach to treat genetic disorders such as hemophilia A and B, cystic fibrosis, infection or ischemic heart disease and others (14). Moreover, this can be used to up-regulate a healthy gene for over expression of specific genes and enhanced performance. The link between gene therapy and sports is obvious because first gene therapy trials are performed with doping-related proteins, erythropoietin, and growth hormone. Gene therapies have some undetectable side effects and in some instances cause death in persons. For instance, Jesse Gelsinger (18 yr old) who had a rare liver disease was killed by gene therapy. He participated in a gene therapy research at University of Pennsylvania (15). Hence, no gene therapy protocols have been accepted for medical practice by US Food and Drug Administration (7).

Vectors and health risks

Many vectors are used to deliver genes or genetic materials to flaw cells or tissues. These vectors are divided into two groups: viral and non-viral. The important non-viral vector is liposome. It is usually easier to produce and has relatively low toxicity and immunogenicity. The efficiency of gene delivery by non-viral vectors is low because this is hindered by a low transfection rate. A more efficient method for gene transfer is a viral vector, that is, a virus is used as a vector for gene therapy (16). In viral vector transfer system, all viral genes for pathogenic proteins are eliminated and replaced by preferred genes (s). The most common viruses have been summarized in Table 1. Infection of viral vectors with recombined wild-type virus is an important side effect of vectors that has lethal consequences (12).

Gene therapy for sport injuries by growth factors

Another application of gene therapy is to heal severe sport injuries. In some cases, sport injuries are so hard that they force athletes into eliminating sports. Gene therapy by the use of specific genes can help to treat some injuries of anterior cruciate ligament, meniscus, and bones (Table 2). It is important that the approach should be safe and have health and healing consequences. In healing severe disorders such as Duchene muscular dystrophy cancer, Gaucher's disease, or cystic fibrosis, gene therapy may be the final chance; therefore, side effects are undesirable in athletes. However, as there are not any reports on the risks of this process, it may have some health risks such as the risk of insertional mutagenesis (17), abnormal regulation of cell growth, toxicity from chronic over expression of the growth factor and cytokines, and malignancy.

Genetic doping

Genetic doping is used to explain the potential misuse of gene therapy as a performance-enhancing agent (18). The problem of genetic doping was discussed for the first time in 2001 Commission of International Olympic Committee (IOC) that argued the use of gene therapy in sports. In addition, WADA discussed genetic development in sports at Cold Spring Harbor in New York and included the gene doping in 2004 WADA prohibited list and World Anti-Doping Code. Both the WADA and the International Olympic Committee (IOC) have expressed some topics about the possibility of genetic doping in sports. Accordingly, the method of genetic doping has been included in the list of illegal classes of substances and prohibited methods. In the latest updated of prohibited list of WADA (2010) the transfer of cells or genetic elements (e.g. DNA, RNA, Peroxisome Proliferator Activated Receptor δ [PPARδ] agonists [e.g. GW 1516] and PPARδ-AMP-activated protein kinase [AMPK] axis agonists [e.g. AICAR]) and the use of pharmacological or biological agents are prohibited. (19).

Risks of gene delivery in animal and human researches

There are several risks associated with gene doping that are general and also specific and unique for any genes. Some general risks of genetic manipulation are summarized in Fig.1. Other risks associated with particular gene products have been demonstrated in the same animal models showing the capability of gene doping. For example, recently, Hakimi et al. reported the over-expression of PEPCK-C re-patterns of energy metabolism, which led to greater prolonged existence in PEPCK-Cmus mice (20). Decisions about the safety of gene therapy are based on interventions data in animal models as compared to similar interventions of human beings. Pathogenesis of human diseases and advances in gene therapy primarily can be studied in animal models of human diseases. It is a problem that animal models do not completely mimic human conditions. For example, the CFTR gene that related to cystic fibrosis does not have the same pulmonary effects in animals as in human beings. Health risks related to the specific proteins expressed in genetic doping in human beings are more severe than other forms of doping.

Genetic doping and human gene map for performance and health-related fitness phenotypes

It is possible to use all genes that have an effect on physiological procedure related performance, muscle activity and potentials of systems involve exercise. Some researchers around the world are trying to identify genes that have potentials to enhance athletic performance. Human gene map for performance and health-related fitness phenotypes are new researches that have been annually updated since 2000.

These updated review papers have discussed the specific genes and genetic data related to the physical performance phenotypes and cardio-respiratory endurance, elite endurance, athlete status, muscle strength, other muscle performance traits (21, 22). In early version of the gene map, 29 loci were described (23). The 2001 map included 71 loci on the autosomes and two on the X chromosome (23) and 2003 map included 109 autosomal gene entries and QTL as well as 2 X chromosome assignments. Moreover, this paper discussed some sequence variants in 15 mitochondrial genes relevant to fitness and performance phenotypes (21). In their

newest review, Bray et al. evaluated all studies related to human gene map for performance and health-related fitness phenotype published until 2006 and 2007 (24). Results of this study indicated that there were 214 autosomal gene entries and quantitative trait loci and seven others on the X chromosome that have relevance with sport performance and fitness map. Furthermore, a new result in this study was the influence of 18 mitochondrial genes on fitness and performance phenotypes. In another new paper, it was reported that more than 239 fitness genes were discovered (25). These results together with advances in human gene therapy have been shown to create an outlook for using genes, genetic elements, and cells that have the capacity to raise athletic performance (25). Some important genes related sport performance phenotypes are summarily listed in Table 3.

Genes in Endurance and Muscle-Strength Phenotypes

Aerobic or oxidative phosphorylation is the major energy system in sports events or exercises, which lasts more than 1 min. Oxygen delivery system in active muscle may be limited by metabolism capacity of skeletal muscles that relates to capillary density, mitochondrial content, and type of fiber, proportion of slow twitch (ST) to fast twitch (FT). This is a problem and limits the athlete's performance. Therefore, it may be concluded that any genes that can enhance capacity of oxygen-carrying system (e.g. EPO gene), capillary density (e.g. VEGF), mitochondrial content (e.g. PPAR δ) and slow twitch fiber (e.g.: ACTN2) may have potentials for doping of endurance athletes.

Some common genes that may be used to enhance endurance capacity include Erythropoietin (EPO), Hypoxia inducible factors (HIFs), Actinin binding protein 2 (ACTN2), Angiogenic factors (VEGF, FGF, Angiopoietins, TGF-b, PDGF-BB), Peroxisome proliferator activated receptor-delta (PPAR δ), Endorphins, Enkephalins, Growth hormone (GH)/Insulin-like growth factor-1 (IGF-1). In the other athletic performances, strength, speed, power and anaerobic capacity are the main factors that indicate elite performance. These factors relate to some genes that are expressed more in athletes with exercise training than any other persons. Several important genes related to strength performance are Insulin like growth factor-1 and 2 (IGF-1& 2), Myostatin, Growth hormone (GH), Actinin binding protein 3 (ACTN3), Angiotensin I converting enzyme (ACE). Some important genes related to elite aerobic and strength athletic performance are summarized in Table 3 and in the following section, some of them that have potentials in genetic doping will be more discussed, with a focus on health risk and side effects (Table 4).

Table 1: Properties of viral and nonviral vectors that have used for delivery of specific genes in to impaired cells for cured disease or sport injuries (17, 19)

Vectors	Properties
Viral	
Adenoviruses	Infects a wide range of tissues, Low toxicity/immunogenicity, Infects only mitotically, active cells, Low capacity for gene insert.
Adeno-associated virus	Serotype determine specificity, Low toxicity/immunogenicity High persistence of gene transferred, Low capacity for gene insert.
Herpesvirus or Herpes simplex	Large virus and unable to cross connective tissue barriers in muscle, Infects. mitotic/ postmitotic cells Large insert capacity Immune rejection common.
Oncoretrovirus (retrovirus)	Requires cell division for integration, immunogenicity Infects only, mitotically active cells, low capacity for gene insert.
Lentivirus	Does not require cell division
Semliki forest virus	Short-lived gene expression
Nonviral	
Liposome	Low efficiency of gene delivery
DNA gene gun	Low immunogenicity
DNA-protein complex Naked DNA	Easy to produce

Table 2: This table indicates effects of growth factors on musculoskeletal tissues (17)

Tissue injured	Growth Factor	Actions of gene	Health risks
Skeletal muscle	bFGF, NGF, IGF-1 ,TGF-beta	Treating inherited disorders such as Duchene, muscular ystrophy, improved healing of sports-related muscle injuries	Muscle tumor
Cartilage	BMP-2, bFGF, TGF-beta, EGF, IGF-1, CDMP	Regeneration of damaged articular cartilage	?
Anterior cruciate ligament	PDGF-AB, EGF, bFGF, BMP-2, IGF-1, TGF-beta	Improve healing of the ACL or "ligamentization" of the ACL graft	Muscle or tendon rupture
Meniscus	TGF -alpha, bFGF, BMP-2, EGF, PDGF-AB, IGF-1	Acceleration of the graft healing and Restructuring of meniscus	Slow immune rejection, suppression of the immunogenicity
Bone	BMP-2, IGFs ,TGF-beta, bFGF	Promote bone healing	Skeletal Tumor

Table 3: Genes and phenotypes related to endurance and muscular strength of athletes (24, 25)

Genes	Phenotypes	Genes	Phenotypes
AMPD1	RPE	DIO1	Grip strength
PPARGC1A	PAEE/VO2max,	GDF8	Hip flexion
ADRB2	VO2max	MYLK	Isometric strength
HLAA	VO2max	NR3C1	Arm and leg strength
IL-6	PWCmax	TNF	** Stair climb time
CFTR	VO2peak	CFTR	Peak anaerobic power
ADRB1	VO2peak	CNTFR	KE eccentric; slow velocity
SCGB1A1	FEV1 after exercise	IGF2	Grip strength
UCP2	Exercise efficiency	CNTF	KE concentric, fast velocity
HIF1A	VO2max (age interaction)	ACTN3	Baseline isometric strength
BDKRB2	Muscle efficiency	VDR	Grip and quadriceps strength
HP	Walking distance	IGF1	KE one repetition maximum
ACE	VO2max	COL1A1	Grip strength
CKM	VO2max	ACE	muscle strength
MTND5	VO2max		
MTTT	VO2max		
AMPD1	VO2max		
ATP1A2	VO2max		
HIF1A	VO2max (age interaction)		
ACE	VO2max, Power output		
APOE	VO2max		
CKM	VO2max		
MTND5*	VO2max		

*Mitochondrial DNA. ** training response, VO2max, maximal oxygen uptake; VE/VCO2, ratio of ventilation to carbon dioxide consumption; Wmax, maximal power output; a-vDo2, arterial–venous oxygen difference; VE, ventilation; RPE, rating of perceived exertion; PWC, physical working capacity; FEV, forced expiratory volume.

Table 4: Potential genes and its health risks

Potential genes	system/target tissue	Physiologic response	Health risks	Sport	reference
EPO	Hematologic system	Increases RBCs and oxygen delivery gene product properties are glycoprotein hormone.	High increase in blood viscosity, Obstructing regular blood flow and heart, Severe immune response	Endurance	26-32
HIFs	Hematologic and immune systems	Regulates transcription at hypoxia response elements	Enhance cancer growth and spread heart attack , increase viscosity and blood pressure	Endurance aerobic	3,29,34
ACTN 2,3	Muscular system	ACTN2 expressed in ST and ACTN3, in endurance and ACTN3	?	Sprint and endurance	36,37
VEGF	Vascular endothelium and angiogenesis	Development of new blood vessels	Cancer , tumor , immune response and specific risk factors	Endurance	13, 38, 39,40
PPAR δ	Masculae system,	Associated with the formation of ST and can induced in FT fibers, maybe role in body weight control by promotes fat metabolism	Over expression of sex hormones metabolic disorders	Speed and Endurance	41-44
Endorphins, Enkephalins	CNS, PNS	Pain modulation	Increases risk of overuse of musculoskeletal and cardiovascular system, Increase stress and Pressure on heart, sudden dead	Endurance	26
HGH/IGF-1	Endocrine and muscular system	Increases muscle size, power, and recovery	Intracranial hypertension, Visual changes, Headache, Nausea, Vomiting, Peripheral edema, Carpal tunnel syndrome, Arthralgia, myalgia, Acromegalic features such as nose and jaw, Enlargement, Cardiomegaly, Arthralgias, insulin resistance and diabetes, Cancer	Etrength	45-49
Myiostatin	Muscular system	A negative muscle mass regulator, and this lead to limited restriction of muscle growth	Damage of tendons ligament and bone	Etrength	50,51
ACE	Skeletal muscle	and ACE-I in endurance ACE-D involved in sprint & power, regulates blood pressure	Angioedema , ?	Eprint, Power, Endurance	54-57
Interleukin-15	Skeletal muscle	Myoblast proliferation and muscle-specific myosin heavy chain (MHC) expression	Cancer risk, Musculoskeletal damage	strength	58,59

Abbreviations: EPO, erythropoietin; HIF, hypoxic inducible factors; ACTN3, actinin binding protein 3; VEGF, vascular endothelial growth factor, PPAR-delta, peroxisome proliferators-activated receptor (delta); HGH, human growth factor; IGF-I, insulin-like growth factor; ACE, angiotensin-converting enzyme; FT, fast twitch; CNS, central nervous system, PNS, peripheral nervous system ST, slow twitch.

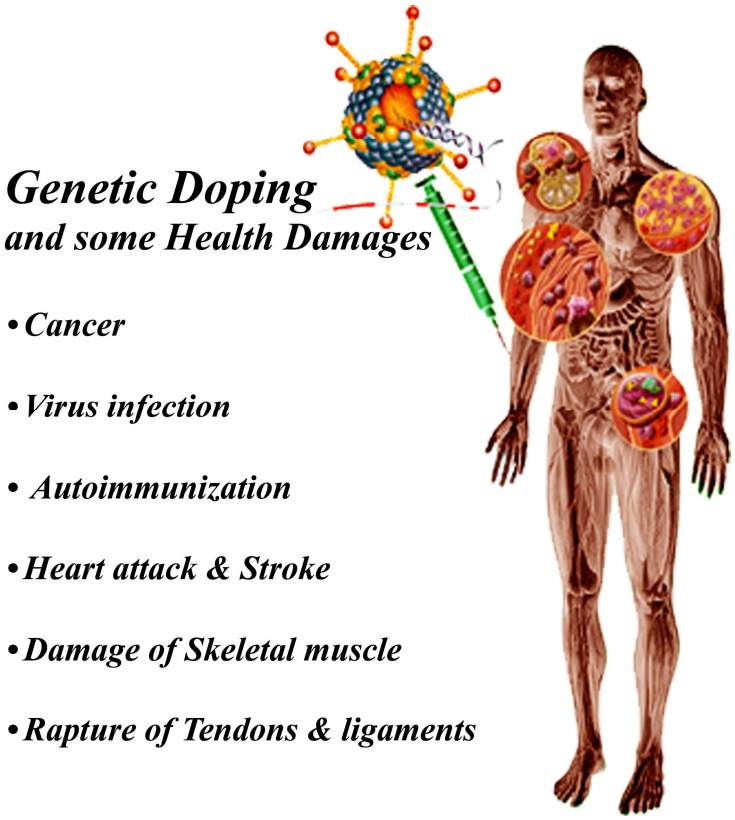


Fig. 1: Genetic doping and some health damages that may be relate to both the vector used (DNA, chemical, viral) and the encoded transgene.

Erythropoietin (EPO)

Physiological effects

In all sports particularly in endurance events, oxygen delivery system is essential to physical activity, exercise, and athletic performance. There are some illegal and banned methods to enhance capacity of oxygen delivery system including blood transfusion, altitude, hypoxic rooms, and treatment with erythropoietin (EPO). Using EPO is common in therapeutic protocols of anemia patients but is a method that athletes use, and is banned. Erythropoietin is a 165-amino-acid (34 kDa) glycoprotein hormone that by affecting kidneys increases red blood cells (RBCs). In response to hypoxia and low blood oxygen conditions, EPO is synthesized by kidneys and then EPO gene by acting on erythroid stem cells, stimulates erythropoietin and EPO hormone increases. Similar to this procedure, by injection of EPO genes into the body, EPO hormone increases in an extraordinary amount (26). However, there is a controversial natural increase in red blood cells in athletes. Eero Mäntyranta, Finnish Nordic skier won two gold medals in Winter Olympic Games in Innsbrück, Austria (1964). It was clear later that Mäntyranta had a naturally genetic mutation of EPO that enhanced capacity of oxygen delivery system by a 25%-50% increase in red blood cells (26).

Side effects and health-related risk factors

Researches on side effects and health risks of injected genes are rare and some researchers solely published a few papers on some side effects of gene delivery in human beings and animals. EPO hormone has potential health risks that may cause sudden death during and after sports. Some dangerous side effects of doping with EPO include: a) increased extraordinary hematocrit; this could enhance the probability of stroke, heart attack in monkeys and mice (13), b) increased thrombotic activity (2), c) autoimmune anemia (27, 28) and blood thromboses (29), d) increased arterial sys-

tolic BP from an average of 177 to 191 mm Hg during exercise (30), E) increased peripheral resistance during exercise (31), f) increased stress on the heart during heavy strenuous and prolonged exercises. Unexpected elevated arterial BP due to recombinant human EPO (rhEPO) injection during exercise may have been the reason for the deaths of some young athletes in the past decades (32).

Repoxygen may have been the first product to be associated with genetic doping

Repoxygen is a gene therapy drug that has recently developed and in response to low oxygen concentration, has an effect on the release of erythropoietin (EPO) in mice (33). This drug is used intramuscularly in response to hypoxia and induces syntheses of EPO transgene. Athletes especially endurance athletes could use repoxygen as a means of increasing the number of their red blood cells. Due to its supposed properties, it may be impossible to detect repoxygen currently. WADA under the 2006 Prohibited List, prohibited repoxygen both in and out of competitions.

Hypoxia inducible factors

Physiological effects

Hypoxic inducible factors (HIFs) increase EPO and oxygen transport capacity. Mechanisms of hypoxia as they occur in tissues with conditions that increase oxygen demand such as exercise are complicated, HIFs has help to known this complex mechanisms (3). In hypoxia condition, activity of genes is modulated by HIFs (29, 34). The other effects of HIFs are healing procedures for some disorders related to the alteration of oxygen metabolism such as cancer, inflammation, heart attack, and stroke.

Side effects and health-related risk factors

HIFs may have dangerous outcomes and enhance cancer by stimulating genes which encode angiogenic genes such as Vascular Endothelial Growth Factor (VEGF), Angiotensin1 (Ang1), angiotensin2 (Ang2) and Matrics Metaloprotenas (MMPs) (35) as well as other proteins related to the growth of cells (34) if used in healthy human.

Actinin binding proteins (ACTN2, ACTN3)

Physiological effects

Two important members of actinin-binding proteins that relate to exercise and athletic performance are a-actinin alleles ACTN2 and ACTN3. Alpha-actinins maintain the structure of the myofibrillar array and regulate myofiber contraction (36). It is reported that sprint athletes in comparison with control groups have more A-Actinin-3. With correlation of A-Actinin-3 frequencies with fast twitch myofibers, it could be concluded that this allele has a positive effect on force and velocity of muscle action (37). Other reports indicate that ACTN2 genes code has effects on muscular endurance (7, 36).

Side effects and health-related risk factors

No investigation surveyed the side effects and health risks of ACTN alleles.

Angiogenic factors (VEGF, FGF, Angiopoietins, TGF-b, and PDGF-BB)

Physiological effects

Angiogenic factors are mediators of angiogenesis process in response to hypoxia and low oxygen condition and metabolic changes after training in skeletal muscle. The important angiogenic factors are vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), Angiopoietins, transforming growth factor beta (TGF-b), Platelet-derived growth factor (PDGF-BB). Angiogenesis process is controlled by stimulators and inhabitations. Exercise is a strong stimulator by induced a low oxygen condition and increases metabolic factors that active formation new blood vessels to meet needs of skeletal with exercise training. Angiogenic gene therapy is primarily a potential technique to treat pathological states related to reduce blood flow to tissues such as coronary heart disease and peripheral vascular obstruction, which is the death of tissues in the body's extremities because of inadequate oxygen supply (38-40). Use of angiogenic factors by athletes increases the potentials of circulation system by increasing blood vessels, oxygen, and nutrients for all organs.

Side effects and health-related risk factors

Some health risks of gene delivery of angiogenesis factors in patients and in athletes are cancer, tumor, immune response and specific risk factors that can relate to each factor (13).

Peroxisome proliferator activated receptor-delta (PPAR δ)

Physiological effects

One of the newest groups of genes that may be used in gene therapy protocols is the peroxisome proliferator-activated receptors (PPARs). These modulated nuclear receptors transform the expression of specific genes. There are three distinct subtypes of PPARs including PPAR alpha (α), delta (δ), and gamma (γ). Each subtype is expressed in a particular tissue and generates heterodimers with the retinoid X receptor to affect transcription of a specific gene (41). The peroxisome proliferator-activated receptor delta (PPAR-delta) gene may be used for endurance athletes with increasing biogenesis of mitochondria and oxidative phosphorylation capacity, and changing the FT to ST fibers (42). These results have been confirmed by reports of some investigators on increased PPAR-delta gene in elite endurance athletes (43, 44).

Side effects and health-related risk factors

There are not any studies about health risks and side effects of PPARs genes. Regarding the physiological effects, it can be mentioned that some side effects may exist by PPARs over expression of sex hormones and metabolic disorders.

Endorphines, Enkephalins

Physiological effects

Pain is a warning signal and should not be connived. In addition, athletic performance may be restricted by pain in injuries as well as in competitions. Athletes who use a plenty of anti-inflammatory drugs and pain-relieving remedies try to maintain numerous painful injuries and to enhance performance. Nowadays, pain relievers are used as an over-the-counter by the majority of athletes. Endorphins and enkephalins are pain-relieving peptides that help individuals to work for a longer period without pain. The genes that increase these peptides could be used in the relief of severe pain in diseases and injuries of athletes (26). These genes also increase the capacity of pain tolerance and time of performance in response to increase painful metabolic factors such as lactate acid, acute and chronic injury in athletes.

Side effects and health related risk factors

Endorphins and enkephalines by blocking pain receptors enhance pain threshold, while these receptors act as a defensive mechanism against injury. By blocking pain receptors, risks of overuse of musculoskeletal system and cardiovascular system and also stress and pressure on heart and injury of this systems and sudden death will increase.

Growth hormone (GH)/Insulin-like growth factor-1 (IGF-1)

Physiological effects

GH is an anabolic hormone that increases growth of all tissues specifically skeletal muscle by induced insulin like growth factor (Igf-1). IGF-1 has anabolic effects and is made in the liver as well as muscles and other locations like brain. IGF-1 genes give rise to an increase in muscle bulk in mice and regulate skeletal muscle growth and regeneration (45). Over expression of exogenous IGF-I in mice results in increased muscle fiber size and number without any exercise program (46). Combining IGF-1 with other growth factors or with strength training programs may lead to even greater responses in muscle growth (46). It combines with a phenotypic shift in more fatigue-resistant and oxidative slow fibers. People with degenerative muscle conditions such as muscular dystrophy may benefit from gene therapy with anabolic hormones like growth hormone or insulin-like growth factor-1 (IGF-1).

Side effects and health-related risk factors

There are some severe side effects in GH treated patients or athletes such as intracranial hypertension, visual changes, headache, nausea, vomiting, peripheral edema, carpal tunnel syndrome, arthralgia, myalgia, acromegalic features such as nose and jaw enlargement, hypertension, cardiomegaly, increased cardiovascular risk arthralgias, insulin re-

sistance and diabetes (45). In addition, IGF-I-treated individuals may be stricken with tumor, because some studies reported that IGF-1 was expressed in 17 different tumors (47- 49). Therefore, use of these genes may have a potential risk of several tumors and cancers.

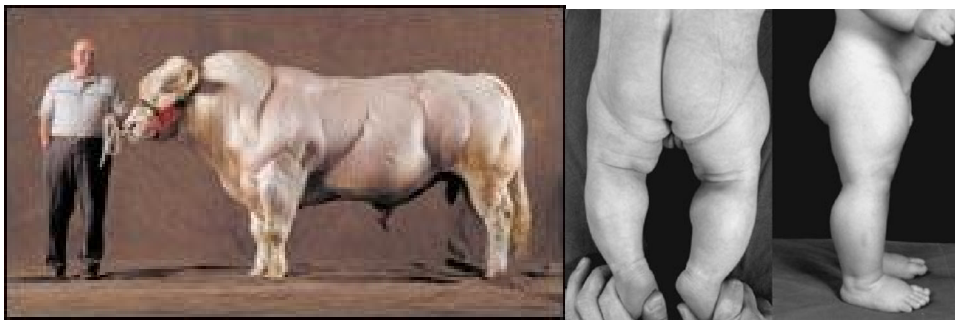
Myostatin blocker

Physiological effects

Myostatin has a negative effect on skeletal muscle growth and is a member of transforming growth factor β (TGFβ) family (50). Myostatin blockers are those groups of peptides that block the myostatin and increase muscle mass by hypertrophy and hyperplasia in null mice. Example of myostatin blocker can be seen in a certain breed of cattle through natural mutation (Fig. 2). Blocking of this negative regulatory protein has noticeable advantages for users such as muscles hypertrophy, less fat of body. A new example of mutation can be seen in a child who was born in Berlin with a stupendous mutation, which turns off the myostatin gene (51). This boy (4.5 yr old) is similar to a bodybuilder's physique (Fig. 2). The effect of blocking the antigrowth factor of myostatin in human beings and animals has been showed in Fig. 2.

Side effects and health-related risk factors

One of the side effects in gene therapy and doping with myostatin blockers is over expression of these genes and increased muscles over their natural size, which as a result increases overload on tendons and bones, or damages differential stresses on them. Increasing muscle mass within a short time without heart adaptation may promote some pathological conditions of heart such as hypertonic cardiomyopathy and increased heart attack.



a) b) c)

Fig. 2: The effect of blocking the antigrowth factor of myostatin in human beings and animals. a) A natural genetic mutation in this breed produces a truncated, ineffective form of myostatin, which allows muscle growth to go unchecked, b) an uncommon mutation in myostatin blocker genes in a German 4.5-year-old boy who is similar to a bodybuilder's physique (6, 51, 52).

Angiotensin-converting enzyme (ACE)

Physiological effects

It is reported that continually suppression of Angiotensin-converting enzyme develops the aerobic performance (53, 54). Some studies on elite high-altitude mountaineers (54), endurance rowers (55), elite short-distance and swimmers (56) suggest that there are two alleles at the ACE I/D polymorphism which have differing effects on athletic performance, with the I allele favoring endurance ability and the D allele improving performance in sprint or power events. ACE affects not only the skeletal muscle function but also exercise-induced left ventricular hypertrophy (LVH) (57).

Side effects and health-related risk factors

Angioedema is a severe side effect of ACE gene therapy with an incident 0.1%-0.5% in patients (56, 57). There are not any reports on the use of ACE genes and their side effects in healthy persons or athletes.

Interleukin-15

Physiological effects

InterIeukin-15 (IL-U) is a growth factor, which is vastly expressed, in skeletal muscle. This growth factor by increasing muscle mass may be a candidate for genetic doping (58). Some findings indicate that IL-15 affects parameters associated

with stimulated myocytes and muscle fibers to increase the amounts of contractile proteins and hypertrophy of skeletal muscle fiber (59).

Side effects and health-related risk factors

IL-15 genes may be similar to IGF-1 which increased some risks such as cancer, musculoskeletal damages.

Phosphoenolpyruvate carboxykinase (PEPCK)

Physiological effects

Phosphoenolpyruvate carboxykinase (PEPCK) has some effects on the process of metabolism in several tissues such as liver and kidney cortex. This mitochondrial enzyme catalyzes the conversion of oxaloacetate to phosphoenolpyruvate (19, 42). In liver and adipose tissues, PEPCK regulates gluconeogenesis and glyceroneogenesis. Hakimi et al. (2010) reported over expressed PEPCK in skeletal muscle, increased endurance, physical activity, and life span and decreased body fat of mice (60). These results may increase the probability of using gene encoding of PEPCK in genetic doping.

Side effects and health-related risk factors

There are no investigations into the side effects or risk of using PEPCK in sport.

Genetic doping and other complications

Ethical consideration about genetic manipulation (genetically modified infants, genetically modified athletes) in sport is one of the important issues about genetic doping. This consequence of gene doping changes the future of elite sport. Below, this issue may give rise to some ethical questions in future that are challenging the world of sport, and related ethical committees must deal with them and such committees must find answers for these questions (9).

- Is it necessary to absolutely ban gene manipulation in athletes?
- Is it necessary to allow athletes to use gene technology only to improve injuries or specific diseases?
- Despite difficulties in detection of gene doping in future, do we witness doubts about the nature of sport?
- Should we deprive genetically modified athletes from sport competitions or prepare specific competitions for them?

Detection of genetic doping is another complication (61). It is approved that genetic doping detection is a complicated process and requires experience, specific knowledge about any genes; it is very costly and needs interdisciplinary scientific procedures (62).

Conclusion

There are several complications about genetic doping and genetic manipulation in sport. It seems that damage of athletes, competitions and sports is very important. Gene therapy is the base of genetic doping and still has potential risks, is performed in laboratory settings in a protective procedure and is used to treat severe diseases. Genetic doping has several health risks and damages and is likely to be done in secret without protective actions; as a result, further unpredictable health damages are expected. Human gene map for performance now has over 220 gene entries and mitochondrial genes, these genes have potential for genetic doping. In this novel updated review, we are trying to create a new base to survey damages and health risks of genetic doping. There are some investigations into health risks of famous genes mentioned in this paper. It is expected that genetic doping will go into the athletic sports. Therefore, national and international scientific sport communities and anti-doping agencies all over the world should manage protective actions such as teaching athletes, manage practicable detection strategies, and focus more on this issue so that they can overcome it.

Ethical Considerations

Ethical issues including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc. have been completely observed by the authors.

Acknowledgements

The authors declare that there is no conflict of interests.

References

1. Knopp W, Wang T W, Bach BR (1997). Ergogenic drugs in sports. *Clin Sports Med*, 16(3): 375-92.
2. Bahrke MS, Yesalis C (2002). Performance-enhancing substance in sport and exercise. 1st ed, Human kinetics.
3. Lippi G, Franchini M, Guidi GC (2006). Blood doping by cobalt. Should we measure cobalt in athletes*? J Occup Med Tox,* 1: 1-3.
4. De Merode PA, Schmamasch P (1998). Harmonization of methods and measurements in the fight against doping. Technical Report Project SMT4-1998-6536.
5. Hilvoorde IV, Vos R, Wert GD (2007). Flopping, Klapping and Genetic doping: Dichotomies Between 'Natural' and 'Artificial' in Elite Sport. *Soc Stud Sci*, 37(2): 173-200.
6. Sweeney HL (2004). Genetic doping. *Sci Am*, 291: 62-9.
7. Gaffney GR, Parisotto R (2007). Genetic doping: A Review of Performance-Enhancing Genetics Pediatric. *Clin N Am*, 54: 807-22.
8. Trent RJ, Alexander IE (2005). Gene therapy in sport. *Br J Sports Med*, 40: 4–5.
9. Fallahi AA, Piry H, Farhud DD (2007). Genetic doping and ethical considerations. *Iranian J Ethic Science Tech*, 2(3, 4): 45-56.
10. Congeni J, Miller S (2002). Supplements and drugs used to enhance athletic performance. *Pediatr Clin North Am*, 49: 435-61.
11. Jin X, Yang YD, Li YM (2008). Gene therapy: Regulations, ethics and its practicalities in liver disease. *World J Gastroenterol*, 21 (15): 2303-307.
12. Lundstrom K, Boulikas T (2003). Viral and non-viral vectors in gene therapy: technology development and clinical trials. *Technol Cancer Res Treat*, 2: 471-86.
13. Haisma HJ, Hon OD (2006). Genetic doping. *Int J Sports Med*, 27: 257-66.
14. Wells DJ (2008). Genetic doping: the hype and the reality. *Br J Pharmacol*, 154: 623–31.
15. Beher M (2004).Will Genetics Destroy Sports? *Discover, Biology & Medicine,* 25(7).
16. Martinek V, Freddie H, Huard J (2003). Gene Therapy and Tissue Engineering in Sports Medicine. *J Gene Med*, 5: 93-108.
17. Crystal R (1995). Transfer of genes to humans: early lessons and obstacles to success. *Science*, 270: 404–10.
18. McKanna TA, Toriello HV (2010). Gene Doping: The Hype and the Harm. *Pediatr Clin North Am*, 57(3): 719-27.
19. WADA (2010). The World Anti-Doping Code: The 2010 Prohibited List international Standard.
20. Hakimi P, Yang J, Casadesus G, Massillon D, Tolentino-Silva F, Nye CK, et al. (2007). Overexpression of the cytosolic form of phosphoenolpyruvate carboxykinase (GTP) in skeletal muscle repatterns energy metabolism in the mouse. *J Biol Chem,* 282: 32844-55.
21. Rankinen T, Pérusse L, Rauramaa R, Rivera MA, Wolfarth B, Bouchard C (2004). The Human Gene Map for Performance and Health-Related Fitness Phenotypes: The 2003 Update. *Med Sci Sports Exerc*, 36(9):1451-69.
22. Bray MS, Hagberg JM, Pérusse L, Rankinen T, Roth SM, Wolfarth B, Bouchard C (2009). The human gene map for performance and health-related fitness phenotypes: the 2006-2007 update. *Med Sci Sports Exerc*, 41(1): 35-73.
23. Pérusse L, Rankinen T, Rauramaa R, Rivera MA, Wolfarth B, Bouchard C (2003). The human gene map for performance and health-related fitness phenotypes: the 2002 update. *Med Sci Sports Exerc*, 35(8):1248-64.
24. Rankinen T, Bray Ms, Hagberg Jm, Pe´Russe L, Roth Sm, Wolfarth B, Bouchard C (2006). The Human Gene Map for Performance and Health-Related Fitness Phenotypes: The 2005 Update. *Med Sci Sports Exerc*, 38(11): 1863-88.

25. Sharp NC (2010). The Human Genome and Sport, Including Epigenetics, Gene Doping, and Athleticogenomics. *Endocrinol Metab Clin North Am*, 39(1): 201-15.
26. Haisma HL, De Hon O, Sollie P, Vorstenbosch J (2004). *Genetic doping*. Netherlands Centre for Doping Affairs.
27. Goa G, Lebherz C, Weiner DJ, Grant R, Calcedo R, McCullough B, Bagg A, Zhang Y, Wilson J M (2004). Erythropoietin gene therapy leads to autoimmune anemia in macaques. *Blood*, 103(9): 3300–2.
28. Lasne F, Martin L, de Ceaurriz J, Larcher T, Moullier P, Chenuaud P (2004). ''Genetic doping'' with erythropoietin cDNA in primate muscle is detectable. *Mol Ther*, 10(3): 409-10.
29. Döring F, Onur S, Fischer A, et al. (2010). A common haplotype and the Pro582Ser polymorphism of the hypoxia-inducible factor-1 (HIF1A) gene in elite endurance athletes. *J Appl Physiol,* 108: 1497-1500.
30. Berglund B, Ekblom B (1992). Effect of recombinant human erythropoietin treatment on blood pressure and some hematological parameters in healthy males. *J Int Med Sci sports*, 2: 21-25.
31. Bergestrom J (1993). New aspects of erythropoietin treatment. *J Int Med*, 233:1-18.
32. Azzazy HME, Mansour MMH, Christenson RH (2005). Doping in the recombinant era: strategies and counterstrategies. *Clin Biochem*, 38(11): 959–65.
33. Diamanti-Kandarakis E, Konstantinopoulos PA, Papailiou J, Kandarakis SA, Andreopoulos A, Sykiotis GP (2005). Erythropoietin Abuse and Erythropoietin Gene Doping: Detection Strategies in the Genomic Era. *Sports Med*, 35(10): 831-84.
34. Marx J (2004). How cells endure low oxygen. *Science*, 303:1454–56.
35. Neil S, Don M (2006). Genetics and molecular biology of muscle adaptation. *Churchill Livingstone*. Italy 1st ed, pp: 185-89.
36.Lippi G, Giuseppe LongoU, Maffulli N (2010). Genetics and sports. *Br Med Bull*, 93: 27–47.
37. Scott RA, Irving R, Irwin L, et al. (2010) *ACTN3 and ACE genotypes in elite Jamaican and US sprinters*. *Med Sci Sports Exerc*, 42(1):107-112.
38. Lee JS, Feldman AM (1998). Gene therapy for therapeutic myocardial angiogenesis. A promising synthesis of two emerging technologies. *Nature Med* 4, 739-742.
39. Isner JM, Asahara T (1999). Angiogenesis and vasculogenesis as therapeutic strategies for postnatal neovascularization. *J Clin Invest*, 9: 1231-36.
40. Paavonen EBK, Ristimaki A, Kumar V, Gunji Y, Klefstrom J, Kivinen L, et al. (1997). Comparison of VEGF, VEGF-B, VEGF-C and Ang-1 mRNA regulation by serum, growth factor, oncoprotein, and hypoxia. *Oncogene*, 14: 2475-83.
41. Bocher V, Pienda-Torra I, Fruchart J-C, Staels B (2002). PPARs: transcription factors controlling lipid and lipoprotein metabolism. *Ann NY Acad Sci*, 967: 7-18.
42. Maciejewska A, Sawczuk M, Cieszczyk P (2011). Variation in the PPARα gene in Polish rowers. *J Sci Med Sport*, 14(1): 58-64.
43. Kramer DK, Ahlsen M, Norrbom J, Jansson E, Hjeltnes N, Gustafsson T, Krook A (2007). Human skeletal muscle fibre type variations correlate with PPAR a, PPAR d and PGC-1 a mRNA. *Acta Physiol* (Oxf), 189(1): 207–16.
44. Grimaldi PA (2005). Regulatory role of peroxisome proliferators-activated receptor delta (PPARd) in muscle metabolism: a new target for metabolic syndrome treatment? *Biochimie,* 87(1): 5–8.
45. Stephen DR Harridge, Cristiana PVelloso (2009). IGF-I and GH: Potential use in gene doping. *Growth Horm IGF Res*, 19: 378-82.
46. Heydemann A, Doherty KR, McNally EM (2007). Genetic modifiers of muscular dystrophy: Implications for therapy. *Biochim Biophys Acta*, 1772: 216–-28.
47. Trojan J, Johnson TR, Rudin SD, Ilan Ju, Tykocinski ML, Ilan J (1993). Treatment and prevention of rat glioblastoma by immuno-

genic C6 cells expressing antisense insulin-like growth factor I RNA. *Science*, 259: 94–97.

48. Zumkeller W (2002). IGFs and IGF-binding proteins as diagnostic markers and biological modulators in brain tumors. *Expert Rev Mol Diagn*, 2: 473–77.
49. Yu H, Robin T. (2000) Role of the insulin–like growth factor family in cancer development and progression. J *Natl Cancer Inst*, 92: 1472-89.
50. McPherron AC, Lawler AM, Lee SJ (1997). Regulation of skeletal muscle mass in mice by a new TGF-beta super family member, *Nature*, 387:83-90.
51. Schuelke M, Wagner KR, Stolz LE, Hubner C, Riebel T, Komen W, Braun T, Tobin JF, Lee S-J (2004). Myostatin mutation associated with gross muscle hypertrophy in a child. *N Engl J Med*, 350: 2682–88.
52. Bogdanovich S, Krag TO, Barton ER, Morris LD, Whittemore LA, Ahima RS, Khurana TS (2002). Functional improvement of dystrophic muscle by myostatin blockade. *Nature*, 420 (6914): 418–21.
53. Paynea J, Montgomery H (2004). Angiotensin-converting enzyme and human physical performance. *Equine and Comp Exerc Physiol,* 1: 255-60.
54. Thompson J, Raitt J, Hutchings L, Drenos F, Bjargo E, Loset A, Grocott M, Montgomery H (2007). Angiotensin-Converting Enzyme Genotype and Successful Ascent to Extreme High Altitude. *High Alt Med Biol*, 8(4): 278-85.
55. Gayagay G, Yu B, Hambly B, Boston T, Hahn A, Celermajer DS, Trent RJ (1998). Elite endurance athletes and the ACE I allele-the role of genes in athletic performance. *Hum Genet*, 103: 48–50.
56. Woods D, Hickman M, Jamshidi Y, Brull D, Vassiliou V, Jones A, Humphries S, Montgomery H (2001). Elite swimmers and the Dallele of the ACE I/D polymorphism. *Hum Genet*, 108: 230–32.
57. Hernandez D, de la Rosa A, Barragan A, Barrios Y, Salido E, Torres A, Martin B, Laynez I, Duque A, De Vera A, Lorenzo V, Gonzalez A (2003). The ACE/DD genotype is associated with the extent of exercise-induced left ventricular growth in endurance athletes. *J Am Coll Cardiol*, 42, 527-32.
58. Busquets S, Figueras M, Almendro V, López-Sorianoa FJ, J Argilés M (2006). Interleukin-15 increases glucose uptake in skeletal muscle. An antidiabetogenic effect of the cytokine. *Biochim Biophys Acta*, 1760(11): 1613-17.
59. Quinn LS, Haugk KL, Grabstein KH (1995). Interleukin-15: A Novel Anabolic Cytokine for Skeletal Muscle. *Endocrinology*, 136(8): 3669-72
60. Hakimi P, Yang J, Casadesus G, Massillon D, Tolentino-Silva F, Nye CK, et al. (2007). Overexpression of the cytosolic form of phosphoenolpyruvate carboxykinase (GTP) in skeletal muscle repatterns energy metabolism in the mouse. *J Biol Chem,* 282: 32844–55.
61. Minunni M, Scarano S, Mascini M (2008). Affinity-based biosensors as promising tools for gene doping detection. *Trends Biotechnol*, 26(5): 236-43.
62. Baoutina A, Coldham T, Bains GS, Emslie KR (2010). Gene doping detection: evaluation of approach for direct detection of gene transfer using erythropoietin as a model system. *Gene Ther*, 17(8): 1022-32.