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Genetics, Human Physical Performance and Gene Doping: The Common Sense Versus the Scientific Reality

Original Article

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RESUMO

Atletas de elite são reconhecidos como fenômenos esportivos e o potencial para atingir níveis superiores de performance no esporte está parcialmente sob o controle de genes. A excelência atlética é essencialmente multifatorial e determinada por complexas interações entre fatores ambientais e genéticos. Existem aproximadamente 10 milhões de variantes genéticas dispersas por todo o genoma humano e uma parcela destas variantes têm demonstrado influenciar a responsividade ao treinamento físico. Os fenótipos de performance física humana parecem ser altamente poligênicos e alguns estudos têm comprovado a existência de raras combinações genotípicas em atletas. No entanto, os mecanismos pelos quais genes se interagem para amplificar a performance física são desconhecidos. O conhecimento sobre os genes que influenciam a treinabilidade somado ao potencial uso indevido dos avanços da terapia gênica, como a possível introdução de genes em células de atletas, fez surgir o termo doping genético, um novo e censurado método de amplificação da performance física, além dos limites fisiológicos. Aumentos na hipertrofia muscular esquelética e nos níveis de hematócrito estão sendo conseguidos através da manipulação da expressão de genes específicos, mas a grande parte das impressionáveis alterações foi obtida em experimentação com animais de laboratório. A compreensão dos resultados científicos envolvendo genética, performance física humana e doping genético é uma difícil tarefa. Com o propósito de evitar a contínua má interpretação e propagação de conceitos errôneos, esta revisão, intencionalmente, vem discutir as evidências científicas produzidas até o momento sobre o tema, permitindo a compreensão do atual "estado da arte".

Palavras-chave: genes, variantes genéticas, performance física, atletas de elite, doping.

ABSTRACT

Elite athletes have always been referred to as sports phenomena and their potential to reach higher performance levels in sports, far beyond normal range, is partially under the control of genes. Athletic excellence is essentially multifactorial and it is determined by a complex interaction of environmental and genetic factors. There are almost 10 million genetic variants spread throughout the entire human genome and some of them have been proven to affect physical training responsiveness. The human performance phenotypes seem to be highly polygenic and previous research has found rare genotype combinations in elite athletes. Nevertheless, the mechanisms through which genes interact with each other in order to improve physical performance are unknown. The knowledge on genes that influence trainability added to the potential misuse of advances in gene therapy, such as the possible introduction of genes into athlete cells, gave way to the terminology gene doping, a new and prohibited method of enhancing athletic performance above physiological limits. Increase in skeletal muscle hypertrophy and haematocrit levels has been achieved by the manipulation of the expression of specific genes, but great part of impressive changes in these phenotypes have been obtained using laboratory animals. The understanding on the scientific studies enclosing genetics, human physical performance and gene doping is an intricate task. This review intentionally highlights the scientific evidence that has been produced so far on this popular topic, with the purpose to avoid continuous misinterpretation and spreading of faulty concepts allowing hence the comprehension on the current "state of the art" in this field.

Keywords: Genes; genetic variants, physical performance; elite athletes; doping.

INTRODUCTION

High-performance athletes are known as "phenomena" by the common sense. Such characterization seems coherent, once becoming an extraordinary talent in sports is something rare and reached by a small amount of all who wish that. An example of it is the fact that, Olympic medalists and world Record holders are the outliers of a selective group and which stands out among athletes engaged in specific sports modalities. Although this thinking is grounded on merely observational points, the common sense view is correct, since human physical performance can be relied on scientific proof. Some people dared to say that athletes are ordinary people who are born and prepared to become athletes, raising the possibility that physical performance and sports ability are exclusively the result of hours spent in concentration and physical training [1]. These authors admit that stature and other physical structural characteristics favor success in some sports modalities, but stress the fact that attendance to physical training is an important factor and which can surpass any contribution originated from gene contribution. However, it is not very probable that this theory corresponds to the reality as human physical performance is known as a multifactorial PHENOTYPE, that is, controlled by the interaction between many environmental factors and determined by genetic factors. In practical terms, physical training (an environmental factor) undoubtedly induces to morphofunctional adaptations in the many physiological systems, but the level of adaptation depends on the interactions between the multiple genes, which on their turn are modulated by multiple genetic variants. The identification of genes and genetic variants as potential to influence physiological variables in response to physical training is the grounding for the understanding on what genetic potential of an athlete is.

In this new era of genomic medicine, the DNA mapping and sequencing enabled the human genome screening with the purpose to identify these genes and genetic variants which affect it, and consequently, to genetically characterize the "phenomena" high-performance athletes. All this laboratory technology also made the gene manipulation possible, a strategy developed for therapeutic aims, but referred in the sports environment as "genetic doping". From this context, driven by the anxiety added to the difficulty in understanding this topic, part of the sports community has been stating opinions which do not correspond to the reality of the scientific proof reached until the present time. The media, driven by the relevance of the topic "Genetics, Human Physical Performance and Genetic Doping" and based on misconceptions, has promoted a twisted reality. The final product is a increasing cycle of unreal information which feed the imagination of those Who wish for the use of high-tech illicit substances and methods to induce increase in physical performance, beyond the physiological thresholds.

Due to the simplification of the complete concepts of FUNCTIONAL GENOMICS it is possible to elaborate a comprehensive and real scenario. However, this simplification should be necessarily joined by scientific support so that statements, such as the following ones, do not hide the science behind false appearance.

"The use of some illicit ergogenic devices (e.g. anabolic androgenic steroids, GH, IGF) reverts the unfavorable genetics of an individual; athletes present genes which us, ordinary people do not; genetic mutations similarly alter all the physiological functions of the organism; looking at that athlete it is possible to identify that his genetics is favorable; an undefeatable athlete will be born if his parents have been previously submitted to genetic doping; genetic doping does not destroy the organism as drug use does; genetic doping alters the genes of the athletes. "

These statements exemplify some of the information freely spread as absolutely true concerning the high performance sports scenario. This review has the aim to censor the false statements regarding the Genetics, Human Physical Performance and Genetic Doping topic, providing understanding on current and real "state of the art". Part of the explanations was simplified to minimize difficulty in understanding genetics. Intentionally, in each explored topic there is reference on the correct way of misconceptions of the common sense in order to stimulate discussion with scientific background.

GENETICS

"Ordinary" people and elite athletes have absolutely the same genes. What the genome f athletes may present differently when compared to the genome of 'ordinary' people are the variants in the specific genetic coding involved in the modulation of physical performance phenotype.

The conclusion of the human genome mapping and sequencing had its turning point in 2004, announced by the International Human Genome Sequencing Consortium in the October issue of the Nature journal [2]. The human DNA contains approximately 3.1 billion base pairs (A – adenine; G – guanine; C – cytosine; T – thymine) divided in 20-25 thousand genes. Subsequently to transcription, the nucleotide sequence of each gene is translated in a polypeptide sequence, giving origin to a specific protein. The human genome contains almost 10 million SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs). However, not all SNPs are known as functional, that is to say, not all of them have potential to affect the gene expression or function of the codified protein by a mutant gene. Thus, among the almost 10 million existing genetic variants, only a small part of them could influence a specific genotype [3]. For example, the C34T variant of the AMP deaminase gene (AMPD1; chromosome 1p13-p21) nonsense type, transition of C nucleotide → T in the 34 position of the exon 2, results in a stop codon and, consequently, premature interruption of the protein synthesis. HOMOZYGOUS individuals to the mutant gene (TT genotype) present activity of the AMPD1 enzyme lower than 1% of what is found in wild-type individuals (CC genotype). Since this gene is involved in the maintenance of the energetic needs of the skeletal musculature during contractile activity, the variant C34T of the AMPD1 gene could influence on physical performance in specific sports modalities. The acknowledgement of how genetic variants in some specific genes may influence on physical performance of elite athletes was previously described and interested readers can refer to the review by Dias et al.[4]. Following the thought that genetic variants may affect responsiveness of physical training, approximately 200 variants in specific genes have been identified and shown influence on the phenotypes of cardiorespiratory capacity, endurance, muscle strength and power as well as intolerance to physical exercise [5].

The phenotypes of cardiorespiratory capacity, endurance, muscle strength and power and intolerance to physical exercise are multigenic, that is, controlled by many genes. The physiological adaptations in response to physical training occur as consequence of the gene expression alterations. Each gene with altered expression contributes with part of the total modulation which occurs in a phenotype.

The great majority of these 200 identified variants is originated from association studies in genetics which tested a genetic variant in an isolate gene has to affect a multigenic phenotype. As a result, genes which provide from small to moderate participation in the regulation of those phenotypes have been identified. In practical terms, it is equal

to say that the sumo f the influence of each genetic variant involved in the modulation of the cardiorespiratory capacity is which will determine the level of adaptation to physical training. It is worth mentioning that, occasionally, a single genetic variant in a specific gene may present great participation in the regulation of a multigenic phenotype.

An Olympic and Record holder athlete in a given modality may present genetic variants which amplify or inhibit specific physiological functions. This genetic background can only be known through the genotyping of the athlete. The discussions concerned with the genetic influence in the biotype determination become relevant in the context of sports talents detection based on the genetic analysis; however, few contribute to the understanding on how genetics influences on human physical performance.

Genetic and environmental factors contribute to the modulation of the body dimensions and composition [6]. Studies of family aggregation and inheritance demonstrate that morphological characteristics such as stature and bone and limb length are greatly under gene control. However, the complete scenario of the genetic variants and the gene-gene and gene-environment interactions in the different phases of development are little known. Considering that distinct sports modalities require specific biotypes, an individual may present the necessary genetic variants to determine the precise body dimensions, but not necessarily the genetic variants which affect the responsiveness to physical training. Among the genes and their respective genetic variants indentified so far, some seem to favor the development of high physical performance in modalities which require strength/power and others in modalities which require endurance. Since these phenotypes are multigenic, the existence of a genetically perfect athlete would depend on the number of favorable and unfavorable genetic variants present in his genome. The frequency of genetic variants in different genes involved in the physical performance modulation presents great variation. An example of it, are the uncoupling protein 2 genes (UCP2; chromosome 11q13) and of the alpha - 2A - adrenergic receptor (ADRA2A; chromosome 10q24-q26) in which the genotype frequency which favor physical performance may reach to 17% and 62%, respectively [7,8]. In this case, a specific individual has 62% of chances of presenting the genotype 6.7/6.7 from gene ADRA2A. Nevertheless, the probability of this same individual presenting the genotype 6.7/6.7 from gene ADRA2A plus the genotype V/V for the gene UCP2 is of 10.5%. Each preference genotype added will result in multiplicative decrease of the combined probability calculation, supposing the allele independence. Currently, genetic variants in 23 genes have shown influence on the endurance phenotype. Williams and Folland[9], while using the same flow of thinking previously mentioned, demonstrated that the probability of an individual to present the preference genotypes to the 23 genes, that is, to be the owner of the "optimum polygenic profile to endurance" is extremely low, of $8.2x10^{-14}$%. It means that the chance of the world population presenting the 23 preference allelic pairs is of one out of 1.212 trillion. In other words, the world population would need to be approximately 200 thousand times bigger so that this genetically favored individual could exist. Nonetheless, under the real circumstances it would be improbable that the "optimum polygenic profile for endurance" would exist in a single individual in the world. Gonzalez-Freire et al.[10] genotyped seven long race athletes from the cross-country modality for seven genetic variants (genes ACTN3, ACE, PPARGC1A, AMPD1, CK-MM, GDF-8 and HFE) associated with physical performance in endurance events. Curiously, only the world champion of 2007, known for his high performance during the year of 2008 and in previous editions, presented the seven preference genotypes, suggesting hence that part of his success may attributed to the rare genotype combination.

Case-control studies, which demonstrate higher frequency of variants in physical performance-associated genes in athletes, when compared to individuals from the general population, added to the findings on the rare genotype combination in athletes, support the statement that genetics is a crucial determinant in excellence of high performance sports. Interestingly, one individual with the highest number of physical performance-associated genotypes would not necessarily be representing his nation in high performance sports. The genetic background added to the opportunities as well as social and economical context evidence an athlete. Perhaps the greatest sports talent in the world ever had never been stimulated to explore his/her athletic potential.

A positive association between a variable and a gene and a physiological response indicates that such variant participates in the modulation of a given physical performance phenotype. However, this positive association does not tell the extent to which that gene participates in the phenotype modulation. Moreover, the same gene can be expressed and modulate two or more distinct phenotypes and present different participation percentages in their modulation.

Genes can present a PLEIOTROPIC effect. Na example of this is the gene of angiotensinogen (AGT; chromosome 1q42-q43) involved both in the cardiac remodeling and vascular reactivity. Basically, in the local tissues and in the blood circulation, the AGT is cleaved in angiotensin I by renin. Angiotensin I is converted in angiotensin II by the angiotensin converting enzyme (ACE). Angiotensin II activates specific receptors found on the surface of the cardiac cells and the vascular straight muscle, inducing cardiac hypertrophy and vasoconstriction, respectively. A polymorphism of single nucleotide (transition T→C), resulting from the substitution of the methionine amino acid (M) fro treonin (T) in the codon 235 (M235T), has been associated to increased levels of AGT[11]. Recently, Alves et al.[12] verified that healthy individuals with the TT genotype present higher hypertrophy of the left ventricle in response to endurance physical training, when compared to the MM/MT genotypes. Making use of the same population Dias et al.[13] identified there is not any influence of this genetic variant on the phenotype of vascular reactivity. Muscular vasodilation induced by physical exercise is similar among phenotypes MM, MT and TT. Additionally, improvement in the vasodilating response induced by physical training was not different among genotypes. These results support the statement that the same variant in a single gene has distinct participation in the modulation of two phenotypes.

According to what has been previously mentioned, the genes involved in the modulation of multigenic phenotypes, such as the human physical performance ones, have from small to moderate participation in their regulation; however, occasionally, one single genetic variant in one specific gene can present great participation in the regulation of these phenotypes. During contractile muscle activity, part of the increase in energy demand is supported by cardiovascular adjustments. Increase of cardiac debt added to muscle vasodilation guarantees greater redirection of the blood flow to the skeletal musculature. Vascular reactivity is a multigenic phenotype modulated by constricting and dilating forces. Among the dilating ones, the nitric oxide (NO) synthesized in the vessels by the endothelial isoform of the nitric oxide

Pleiotropism: term used to characterize a single gene involved in the modulation of more than one phenotype.

sintase enzyme (eNOS) is known as one of the most important (figure 1a). the variant G894T of the eNOS gene (chromosome 7q36) results in the transcription of the glutamate amino acid (Glu) by aspartate (Asp) I the 298 (Glu298Asp) of the polypeptide sequence of the enzyme. Dias et al. (2009)[14] verified that individuals with the TT genotype (Asp/Asp) present compromised muscle vasodilation. Subsequent in vivo analyses corroborated the unprecedented fact that NO is responsible for approximately 90% of the muscle vasodilation induced by exercise (figure 1b). An example of a single gene, which sinergically to other genes, presents great participation in the regulation of the vasodilation phenotype. This context Will become important in the subsequent discussions concerned with the potential genes of the candidates to genetic doping.

Innate and acquired characteristics

A sports "phenomenon" is the result of suitable exploration of genetic potential through external stimuli such as physical training and diet, added to suitable mental preparation.

The debate related to the contributions concerning innate qualities versus personal experiences (Nature versus Nurture) for the determination of the maximum physical performance, add little to the understanding on the idiosyncrasies of elite athletes. The mistrial of separating gene and environment added to the controversy between personal reports and scientific argument increase the problematics. The designing of sports "phenomena" depends on the interaction between genes and environment, as well as psychological factors. The precise comprehension on how much each factor contributes to the expression of the final product, that is, of a sports "phenomenon", is unknown. Interestingly, genetic variants can also be found in genes with potential to influence the neural connections, being able to affect characteristics as humor, perceived exertion, emotional intelligence, positivism and aggressiveness. Lippi et al.[15] call attention to the fact that success in high performance sports depends on attributes such as ability in controlling emotions, cohesion, maturity, capacity of anticipation and decision making. Joined to motivation and persistence, these features would be connected to mental performance. The influence of

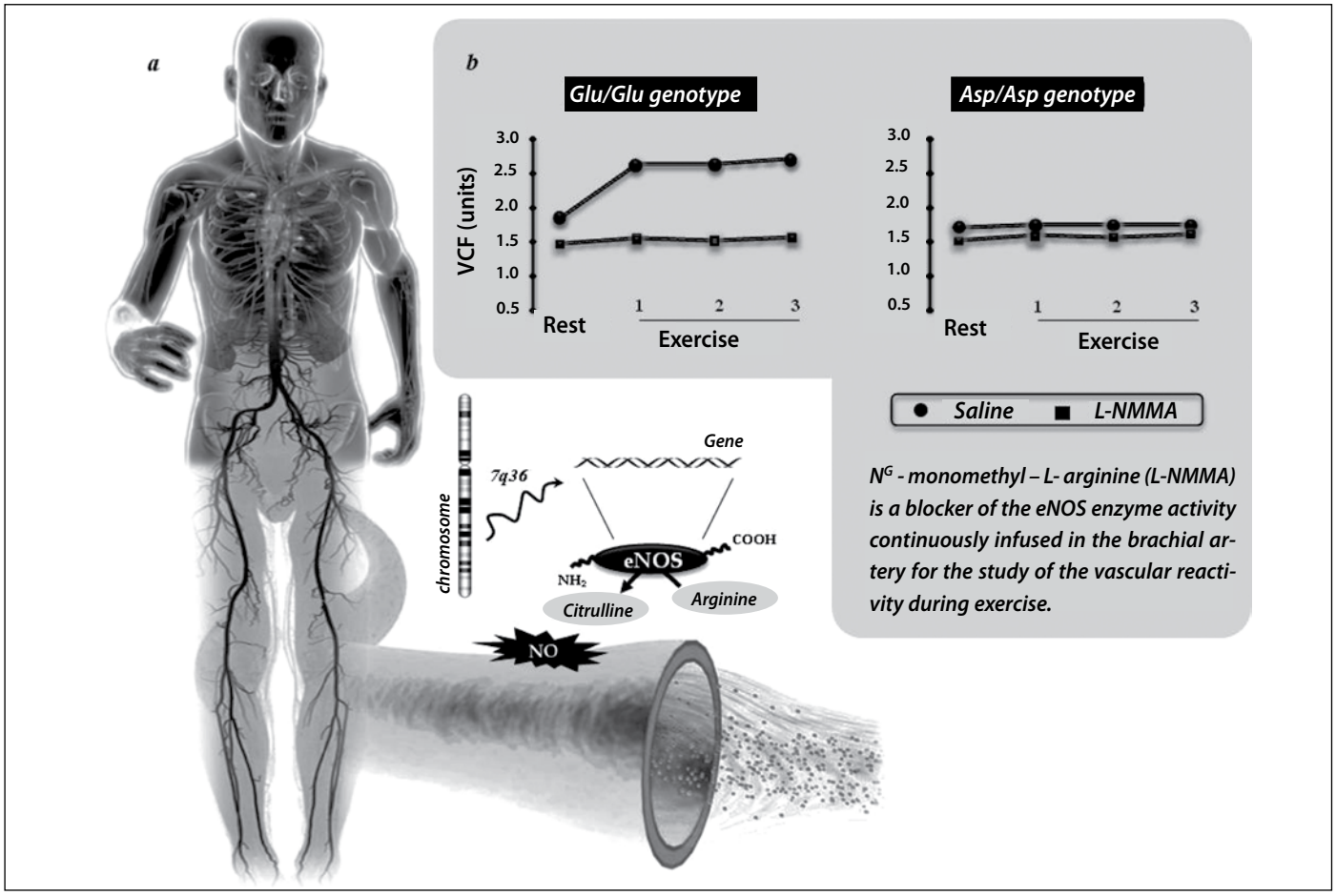

Figure 2. Physiological systems modulated by genetic variants which can affect the expression of a specific gene or the function of the protein codified by the mutant gene, influencing the human physical performance phenotypes. The skeletal muscle is the main target to genetic doping, being able to play direct or indirect effect on the physical performance amplification. a) The muscle strength and power phenotypes are partially modulated by skeletal muscle hypertrophy. Some of the genes involved with increase in the muscle protein synthesis are: ACE, IGF1A, GDF8, FST and GH1. Among these, the main candidates to genetic doping are genes IGF1A, GDF8 and FST. b) The endurance phenotypes as well as muscle strength and power are partially modulated by the typing of skeletal muscle fibers. Some of the genes involved with the determination of the percentages of the different types of fiber are: ACE, PPAR-β/δ, PPARGC1A and PPARGC1B. Among these, the main candidates to genetic doping are genes PPAR-β/δ, PPARGC1A and PPARGC1B. c) Endurance phenotype is partially modulated by the oxygen transportation to the skeletal muscle. One of the genes involved with increase of red blood cells is EPO gene, being it the main candidate to genetic doping. d, e) The endurance phenotype is partially modulated by the oxygen transportation and macronutrients to the skeletal muscle. Some of the genes involved with the increase of tissue perfusion through the muscle vasodilation or angiogenesis are: eNOS, VEGF, FGF, HGF and HIF-1α. Among these, the main candidates to genetic doping are genes VEGF, FGF, HGF and HIF-1α. IGF1A – growth factor similar to insulin 1; GDF8 – myostatin; FST – follistatin; GH1 – growth hormone 1; ACE – angiotensin converting enzyme; PPAR-β/δ – peroxisome proliferator–activated receptor beta/delta; PPARGC1A – transcriptional coactivators PGC-1α; PPARGC1B – transcriptional coactivators PGC-1β; EPO – erythropoietin; eNOS – endothelial nitric oxide synthase; VEGF – vascular endothelial growth factor; FGF – fibroblast growth factor; HGF – hepatocyte growth factor; HIF-1α – hypoxia induced factor 1α.

variants in genes associated with psychological phenotypes has been investigated. Details related to this topic are found in Bryan et al.[16] and Maliuchenko et al.[17].

Maximal performance of elite athletes is determined by the maximum exploration of his genetic potential through external stimuli added to maximum expression of mental performance. However, the extent to which each factor will contribute to the designing of a sports "phenomenon" is partly dependent on the sports modality.

Distinct sports modalities differently demand from the genetic, environmental and psychological components. Perhaps a cyclic modality (e.g. 100m tracking) may depend more on physical performance and less on cohesion and decision making when compared to an acyclic modality (e.g. soccer). In the latter, greater mental performance could result in success even in the absence of excellent physical performance. Regardless of this detail, in the high performance sports world there is predominance of the idea that success depends on surpassing the physiological thresholds, even if it is necessary to make use of unconventional substances and methods for improvement of human physical performance.

DOPING

It has been tried hard to create an organization which could promote, coordinate and monitor the movement against doping in sports. The World Anti-Doping Code was designed and implemented by the WADA – World Anti-Doping Agency) in a trial to harmonize the political issues and anti-doping guidelines to all sports modalities in all countries. Additionally, the WADA is responsible for issuing every year an updated list of the composts and procedures which characterize doping. Doping is defined as illicit use of substances and methods with the goal to artificially improve physical and/or mental performance. The intention of the anti-doping control is to watch for the athletes' health, besides promoting equality in the competition for the single goal of winning. Recently, the term genetic doping was introduced on the WADA's list (Prohibited List – International Standard) as being a new method possible of usage for modulation of physical performance and that hence, would be banned. Generally speaking, genetic doping uses advanced strategies in gene transfer technology, developed to prevent and treat diseases through the manipulation of expression of specific genes. The WADA defines genetic doping as being the non-therapeutic use of cells, genes, genetic material or modulation of gene expression with potential to increase athletic performance. The idiosyncrasies pertaining the use of genetic therapy techniques for doping purposes will be revised in order to explain the real high performance sports scenario in a moment when the possibility of designing a genetically modified athlete is already real.

Gene therapy

Gene therapy is characterized by the introduction of genetic material in cells with the purpose to measure the functionality of a gene or substitute a non-functional gene. This strategy was developed and has been improved with the aim to prevent, treat or alleviate the symptoms of hereditary diseases or acquired disorders. Basically, to identify the signaling way in which a gene is involved, to identify a possible mutation in this gene and to confirm the disorder caused by the mutant gene are the initial steps which justify the use of this technique. Gene therapy can be performed in lineages of GERMINATIVE OR SOMATIC CELLS. The introduction (knock in) or deletion (knock out) of an exogenous gene in germinative cells will result in the propagation of this modification to the new originary cells, while alterations through the introduction of an exogenous gene in somatic cells of an organ would be restricted to the TRANSFECTED CELLS. In the first case, subsequent generations would inherit the genetic alterations, while in the case of transfection, these alterations would be restricted to the transfected individual. For technical and ethical reasons, the application of gene therapy in human germinative-cell lineages is not allowed. On the other hand, gene therapy in somatic cells represents a promising technology to the therapeutics, but with few positive results in CLINICAL RESULTS yet. Some deficiencies concerned with the method have not been solved yet, which can result in risk of death or oncogenic complications, as in cases already reported in the literature [18,19]. Although the discussion on the the creation of perfect athletes by manipulation of genetic material of germinative cells is already present in the high performance sports environment, genetic doping represents the possibilities of manipulation of genes in lineages of somatic genes. Additionally, among the possible candidates to genetic doping, not all genes would be modulated by the classic gen therapy, which consists of the introduction of an exogenous gene in specific cells in order to obtain its suitable expression. An example of this exception is myostatin (GDF-8, growth differentiation factor 8; chromosome 2q32.2) which can benefit from the non-classic form in which theoretically, inhibition of the GDF-8 gene through silencing of protein expression should be conducted to produce the expected hypertrophic effect in the skeletal musculature.

The technology for protein production through the manipulation of genes is already a reality. The fact that the potential therapeutic effect of these molecules is still being tested in pre-clinical and clinical studies does not exclude the possibility that athletes are still making use of them with aim to amplify physical performance.

Molecular biology laboratories already use gene therapy for animal experimentation and clinical studies. Minimization of the risks related to the method requires suitable environment with appropriate technology to the preparation of the TRANSDUCTION VECTORS and safety and toxicity control through laboratory tests. Permission for use of gene therapy in humans requires extreme control and approval from the regulation organs. This severity aims to reduce the death risks and development of diseases associated with the viral vector and with the exogenous gene, besides avoiding possible replications and recombinations of competent virus [20]. One thousand five hundred and thirty-seven clinical investigations with gene therapy for many different disorders are being carried out all around the world [21]. The lack of total efficiency of the method, due to the sumo f small deficiencies such as short life of transfected cells, toxicity and activation of immune and inflammatory response to the viral vector, partly explain the reason why the FDA (Food and Drug Administration) did not approve for com-

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GERMINAL CELLS AND SOMATIC CELLS – germinal or reproductive cells have (n= haploids; 23 chromosomes) and are represented by oocytes and sperm, in humans and animals. Somatic cells (2n=diploids; 2x23 chromosomes) are all the cells, with exclusion of the ones destined to formation of gametes (n).

TRANSFECTION – transfer of an exogenous gene to somatic cells.

PRE-CLINICAL AND CLINICAL – The investigation on gene therapy is divided in: pre-clinical, a phase in which the tests are performed with the use of laboratory animals; and clinical, phase in which the tests are conducted with humans.

VECTOR TRANDUCTION: the vector which will conduct the exogenous gene to the target tissue is usually a virus. The use of virus for transduction is one of the used methods for gene transfer.

POSTMITOTIC – Somatic cells not in cell division any longer

mercialization, until the present moment, any product derived from genetic manipulation. Although the therapeutic purpose of the gene therapy techniques seems to please those dedicated to the advances in the regeneration processes of injured tissues, the main application to high performance sports is really supported in genetic doping. The difference between the use of gene manipulation with therapeutic purposes or doping seems to rely on the fact that the latter, by nature, does not require permission, and safety is not a real concern. Developed for therapeutic investigations, Repoxygen is a vector loaded with the erythropoietin gene (EPO) and controlled by an element responsive to hipoxia (HRE – hipoxia-responsive element). Rumors indicate that Repoxygen is already available in the "black market" and has been used for artificial amplification of human physical performance [22]. Studies by Lasne et al.[23] indicate the possibility of detecting doping with the EPO gene. However, until the present moment, no anti-doping test has been implemented by the WADA, which results in lack of evidence, in case this genetic doping is actually being used.

Physiological systems and genes candidates to *doping*

Skeletal musculature seems to be the main target for gene therapy and, consequently, for genetic doping. Besides the post-miotic status of the cells, which guarantees higher expression period of the exogenous gene [24], the muscle tissue is of easy accessibility and very vascularized [20]. A skeletal muscle transfected with a specific gene can result in direct or indirect effect on human physical performance. This is equal to say that if the gene of interest results in hypertrophy or modulation of the fiber typing, the effect is direct. On the other hand, the skeletal muscle can be tranfected with an EPO gene, playing indirect effect on physical performance. In this case, the machinery of the muscle cells is only used for gene transcription and EPO protein translation, a hormone with main endocrine function of inducing erythropoiesis in the bone marrow.

Generally speaking, genetic doping would allow the athlete design the physiological systems making use of the direct and indirect methods for modulation of the musculoskeletal, cardiovascular, respiratory and blood phenotypes.

The sports modality specificity in which the athlete is inserted guides the interest for strength and/or power or endurance amplification. Subsequently, the gene with potential to trigger such response would be determined.

According to what previously mentioned, approximately 200 variants in specific genes have been identified until the present moment and showed influence on human physical performance and health-related fitness[5]. These genes are an indication of which could be transfected or blocked in the human genome, aiming the amplification of physical performance (figure 2). Since physical performance is controlled by a set of genes, those with higher participation percentage in the modulation of a given phenotype would be the candidate targets to doping. Basically, amplification of the strength/power or endurance physical capacities, besides the physiological thersholds,

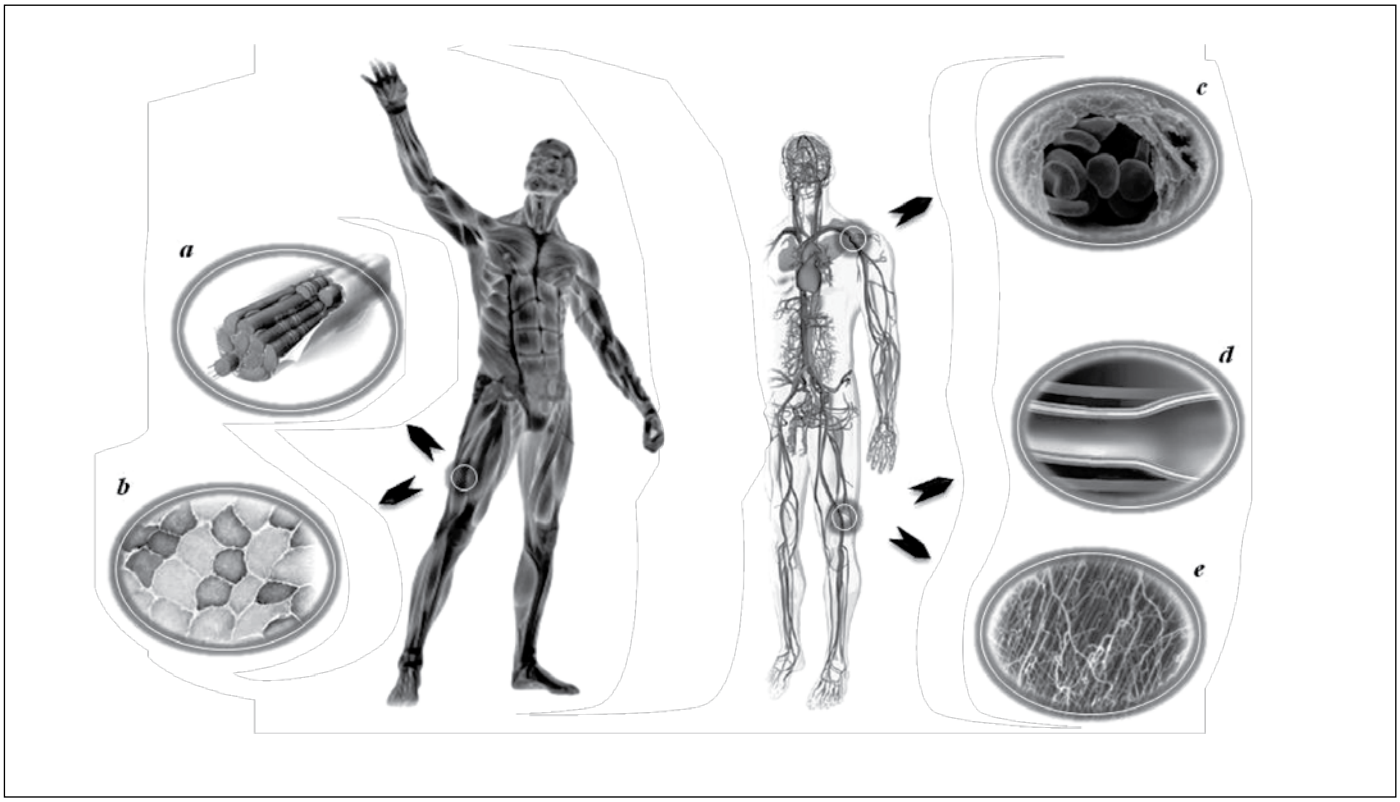

Figure 1. Nitric oxide (NO) and its participation in the regulation of the muscle blood flow during physical exercise. a) The eNOS gene is located in the chromosome 7q36 and after having been transcribed, it is translated in the eNOS enzyme. Basically, the eNOS, located in the vascular endothelial cells, is mainly activated by shear stress, converting L-arginine in L-citrulline and NO. During physical exercise, eNOS is more activated and the increase in the NO synthesis seems to be the main responsible for the muscle vasodilation. b) Vascular reactivity measured in the forearm during handgrip isometric exercise. The xharts represent the blood flow at rest and the muscle vasodilation in a single wild-type homozygous individual (Glu/Glu genotype) and in a single homozygous individual for the mutant gene (Asp/Asp genotype). Observe that the natural response (saline) of muscle vasodilation verified for the Glu/Glu individual is harmed in the Asp/Asp individual. During the intra-arterial infusion of de L-NMMA, the muscle vasodilation is virtually abolished (~ 90%) in the Glu/Glu individual, remaining almost unchanged in the Asp/Asp individual. These results confirm that the enzyme translated from the T allele does not increase biosynthesis of NO in response to the physical exercise stimulus and that NO per se, presents great participation in the modulation of the phenotype of vascular reactivity. VCF – vascular conductance in the forearm.

can be reached with the modulation of the genes: erythropoietin (EPO; chromosome 7q22), angiotensin converting enzyme (ACE; chromosome 17q23.3), peroxisome proliferator-activated receptor-beta/delta (PPAR-β/δ; chromosome 6p21.2-21.1), transcriptional coactivators PGC-1α (PPARGC1A, chromosome 4p15.1) and -1β (PPARGC1B, chromosome 5q33.1), α-actinin 3 (ACTN3; chromosome 11q13.1), vascular endothelial growth factor (VEGF, chromosome 6p12), fibroblast growth factor (FGF, chromosome 11q13.3), hepatocyte growth factor (HGF; chromosome 7q21.1), hypoxia-induced factor 1α (HIF-1α; chromosome 14q21-q24), growth factor similar to insulin 1 (IGF1A; chromosome 12q22-q23), interleukin 3 (IL3; chromosome 5q31.1), myostatin (GDF8; chromosome 2q32.2), folistatin (FST; chromosome 5q11.2) growth hormone 1 (GH1; chromosome 17q24.2) and phosphoenolpyruvate carboxykinase (PEPCK-C; chromosome 20q13.31). Genes with potential to reduce pain and inflammatory processes caused by injuries and recurrent trauma are also candidate targets to doping [20].

Animals versus genetically-modified athletes

As previously mentioned here, the gene therapy techniques still face problems which hamper their liberation. The majority of the promising therapeutic results with potential of resulting in artificial amplification of human physical performance originated from pre-clinical studies. Additionally, the expressive results are greatly from studies with GENETICALLY MODIFIED animal models for human diseases. It cannot be expected that alterations in germinative cells produce results equivalent to the ones verified when the alterations are performed in somatic cells. It is improbable that the transfection of an in vivo gene reaches all the somatic cells of a target tissue. Transfection of the IGF gene by a viral vector in skeletal muscle of rat [25] may not trigger the same level of hypertrophy when compared to a genetically modified animals for the IGF gene[26]. Moreover, in transfection by viral vector, the hypertrophic response would occur only in the site of application and in the transfected cells. These results with animals suggest the use of the IGF gene as a possible therapeutic strategy in diseases related to muscle disorders. It is still unknown if the possible benefits to patients with muscular diseases would be reproducible in healthy individuals and athletes. No clinical study with gene therapy with the IGF gene is being performed at this moment [21]. However, the IGFgene potential in causing hypertrophy may result in extra strength/power gain for athletes.

Introduction of a DNA segment containing gene which is, who knows, able to duplicate the production of a protein of interest or genetic material which is able to silence the production of another protein in an athlete characterizes genetic doping. In addition to the intrinsic risks of the gene therapy procedure for doping purposes, there is not any proof that it is able to produce the expected physiological effect.

The EPO, mainly excreted by the liver, stimulates the erythropoiesis supporting the maintenance of the physiological values of hemoglobin and hematocrit. Transfection of the EPO gene to the skeletal musculature of monkeys increased in 75% the hematocrit[27]. Although the study has proved the efficiency of the transfection of the EPO gene in medium-sized animals, the authors stress the fact that these results only facilitate the beginning of the investigations in studies with humans. Since acquisition of maximum physical performance is depends on the oxygen supply to the skeletal musculature through the transporting capacity in the blood, high hematocrit and hemoglobin would be able to amplify performance, especially in endurance events. However, this increase, added to dehydration associated with physical exercising, increases blood viscosity. Besides causing cardiovascular work overload, this increased viscosity may result in blocking of the microcirculation followed by death.

Peroxisome proliferator-activated receptors (PPAR) are nuclear receptors involved in the plasticity control of the skeletal musculature. The PPAR-β/δ isoform is involved in the modulation of the muscle fibers typing as well as in the stimulus of the mitochondrial biogenesis. Additionally, the PPAR β/δ modulates the expression of genes involved in the synthesis of enzymes which regulate the fatty acids uptake and oxidation as well as of the genes involved in the synthesis of the sarcomeric protein isoforms specific to slow-contraction fibers. Genetically modified animals for the PPAR-δ gene present amplification of the endurance capacity, with increase of 67% and 92% in exercise time and completed distance, respectively[28]. Lunde et al.[29] confirmed the fact that the typing of muscle fibers is modulated in the Ib→IIa→I direction, even with the transfection of the PPAR-δ gene in somatic cells. These results, originated from animal models, demonstrate that fatigue and endurance can be modulated by gentic modulation in adult muscle fibers and at postmitotic status, suggesting that the use of the PPAR gene by athletes may amplify physical performance in endurance events, since it increases the proportion of type I muscle fibers.

Factor 8 of growth and differentiation (myostatin), differently from IGF and GH, limits growth of skeletal musculature and seems to play two distinct functions: 1) to control the number of myofibers of the developing muscle in the prenatal phase; and 2) to regulate the hypertrophic process in postmitotic cells[30]. Animals knockout for GDF-8 gene, present muscle mass volume approximately two times bigger, in comparison to the control animals [31]. This increase seems to result from the combination between muscle cells hypertrophy and hyperplasia. In another study, animals knockout for the GDF-8 gene and knockin for the folistatin gene presented muscle mass volume approximately four times bigger than the control animals [32]. Folistatin is an antagonist of myostatin and in this study it proved to modulate the muscle mass volume also by ways different from the ones from the myostatin inhibition. According to what has been mentioned before, a non-classic form of gene therapy for the GDF-8 would be with the use of the interference RNA (RNAi), a mechanism which inhibits the gene expression in the stage in which the RNAm translation would occur in the polypeptide sequence. Until the present moment, results similar to the ones verified in animal models have not been reproduced in investigations involving humans. However, the studies presented suggest that the myostatin inhibition and/or transfection with the folistatin gene can result in physical performance increase for athletes engaged in modalities which require muscle strength/power.

Genetics, human physical performance and genetic *doping* idiosyncrasies

The complexity of the cellular mechanisms and molecular interactions does not allow that the thinking on genetics is "linear". Suppose Nature would go against statistics and brought to the world the only individual who, already in adulthood, finds out to possess the preference genotypes for the 23 genes, that is to say, to be the owner of the "optimum polygenic profile for endurance". Theoretically, the exposure

GMO – genetically modified animals which transmit the genome alterations to subsequent generations.

of this individual to the routine of marathon runners would, in a short time, result in the development of a "phenomenon" of the endurance events. Surprisingly, this thinking may not correspond to the reality. The complex interaction between gene and environment, added to observational details concerning the elite athletes life history, supports the hypothesis that the maximum contribution of extremely favorable genetics would be depend on the time of exposure of these genes to the training stimulus. Summing it up, this is equal to say that the potential of response of the gens in the adult phase partly depends on the level of "aggressiveness" with which these genes were stimulated from childhood.

Doping with transfection of the EPO gene is thought to increase the red blood cells and consequently, the capacity of oxygen transportation in the blood. Similar to a scenario of POLYCYTHEMIA, this conduct causes work overload to the cardiovascular system, besides increasing death risk. Additionally, decrease of plasma volume, as consequence of hydric loss during physical exercise, increases even more blood viscosity. Early central fatigue could appear as a result of cardiac work overload. If the benefit resulting from the increase in oxygen uptake by the peripheral tissues surpassed the cardiovascular wear caused by the increased blood density, would be reasonable to believe that the EPO gene could result in increase of physical performance. However, until the present moment this fact has not been corroborated.

Alternatively, higher oxygen supply to the exercising muscle could be reached through increase in the local blood flow. Since NO is responsible for approximately 90% of the muscle vasodilation capacity in response to exercise, the eNOS gene would be a candidate to genetic doping. Transfection with the eNOS gene to the skeletal musculature of lower limbs of endurance athletes could increase even more the NO synthesis during the event, resulting in, who knows, duplication of vasodilation. However, decrease in tissue perfusion pressure as well as in blood pressure would be the possible collateral effects caused by excess in vasodilating response.

In the case of use of gene therapy for therapeutic purposes, the concern with the physiological effect caused by the transfection does not seem to be relevant from the moment at which the exogenous gene would have the function to normalize the concentration of a protein, enzyme or hormone This is different from genetic doping, where the intention is to increase the protein, enzyme or hormone concentration to values above the physiological standards. In this case, the possible effects reached in the amplification of physical performance will be always followed by imminent risks to physiological integrity of the athlete.

CONCLUSION

The current developments in functional genomics prove what used to be simply suspicions. Excellence in high performance sports, which partly depends on maximum physical performance, is under control of genes. Although the screening of the modulating genes of the complexes phenotypes of physical performance is under construction, it is already possible to comprehend how variants in specific genes modulate the adaptations to physical training, supporting the hypotheses why certain more responsive individuals become the sports "phenomena". The justification for the isolate discussion on the genetic component of the high performance athlete falls on the difficulty, since it concomitantly deals with all the topics which modulate these complex phenotypes. It seems to be clear along this review that this excellence is consequence of the sum of the maximum physical performance with the maximum mental performance.

Athletes are born as ordinary people and, if stimulated, are naturally selected to express their maximum physical performance in specific modalities. Generally speaking, those Who possess genetic variants with potential influence on the capacity of strength/power, little or no chance would have to stand out in modalities which demand from the endurance capacity. Although science has corroborated the fact that elite athletes are the result of rare genotype combinations, the sports world still shows the illicit use of substances and methods with potential to artificially amplify physical performance, beyond the thresholds imposed by genetics. Concerning genetic doping, the raw thinking that two genes produce double result would justify the desperate search for the method.

The technology for the manipulation of genes is available and the use of genetic doping with the aim to create genetically modified athletes is already reality. Molecular biology laboratories, legal or illegal, which are in accordance with the genetic doping, may be using it even without any safety and positive results guarantee to the amplification of human physical performance.

The absence of proved cases of genetically modified athletes does not exclude the possibility that these athletes are already being "produced" in laboratory, since the WADA has not implemented tests for genetic anti-doping until the present time. Additionally, these genetically modified athletes would not necessarily be expressing physical performance higher than the threshold, naturally determined by his genotype combination. All expected effects of physical performance amplification in humans with the use of gene manipulation are based on results originated from studies with animal models or clinical investigations. Whether or not these same results are replicable in healthy individuals and athletes as well is unknown. Observe that in the previous topic "Animals versus genetically modified athletes" the evidence comes from studies with animal models, which allows us only to "suggest" that such effects could be reached in athletes. As far as we understand, the techniques are available and genetically modified athletes may be freely moving around in the competitions arenas. However, it is not known if these athletes would be benefiting from genetic doping. There is not proof that the genes candidates to doping result in real amplification of physical performance in elite athletes.

The complex scientific evidence added to countless generated hypotheses is not easily interpreted. The promising results of physical performance in animal models have called the attention of those involved and interested in high performance sports. Besides mistaken concepts, hypotheses and theories are being spread as absolute truths. In an excessive move, the same beliefs spread for conventional doping are being produced with genetic doping. The trial to investigate the use of gene therapy in athletes, with the aim to prove those generated hypotheses, maculates the ethical principles. Despite the consideration that there are few "certainties" related to the genetics, human physical performance and genetic doping context, common sense concepts should not surpass real scientific evidence.

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POLYCYTHEMIA – Hematocrit increase. Patients with Chuvash Polycythemia present mutation in the VHL gene, involved in the regulation of the EPQ gene transcription.

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