# NEW TECHNOLOGY AND NO DRUGS IN SPORT: GENE DOPING REGULATION, EDUCATION AND RESEARCH

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*Review paper*

**Abstract**
*This article examines the current state of genetic doping, the use of gene therapy in sports medicine, and the ethics of genetic improvement. The purpose of gene therapy is to use the foundations of genetic engineering for therapeutic use. Gene doping is a expansion of gene therapy. Innovative research in genetics and genomics will be used not only to diagnose and treat disease, but also to increase endurance and muscle mass. The first genetic therapy tests were conducted with proteins closely related to doping (e.g. erythropoietin and growth hormone). The World Anti-Doping Agency (WADA), an international organization created in 1999 to "promote, coordinate, and monitor the fight against doping in sport in all its forms," defines gene doping as the "nontherapeutic use of cells, genes, genetic elements, or modulation of gene expression, having the capacity to enhance performance" (World Anti-Doping Agency, 2008). This method represents a new Technology, but not devoid of adverse and fatal effects; gene doping could be dangerous for the athlete. The use of the athlete's biological-molecular passport represents a possible preventive and precautionary anti-doping strategy. The best way to prevent gene doping is a combination of regulation, education, research and the known of health risks.*

**Keywords:** Doping, gene doping, sport, WADA, drugs.

## Introduction

Gene therapy aims to correct a genetic defect by introducing functioning genes or by manipulating existing genes to obtain therapeutic benefits. Gene therapy studies have managed the treatment of degenerative diseases that act at a neuromuscular level, more generally affecting the musculoskeletal system such as Duchenne muscular dystrophy (Beytia, et al., 2012; Baoutina, et al., 2008). If this therapy involves increasing the body's proteins, including those of the muscles, its possibility of being exploited and overused in sport for doping purposes has therefore also been hypothesized. (Mazzeo, 2016). This type of technology is called "gene doping" (Skipper, M, 2004; Van der Gronde, et al., 2013). The same concept of gene therapy is applied not for therapeutic use, but to improve the performance of athletes. About 250 genes are being investigated, connected somehow to the improvement of sports performance, in particular through the monument of endurance and muscle mass. Athletes used pharmaceuticals to improve their performance, a phenomenon which is commonly known as doping (Mazzeo, 2019; Montesano, 2019). Athletes use still anabolic steroids to increase physical performance, and bodybuilders use them to improve size and cosmetic appearance. Moreover, gene therapy contains potential misuse in improving athletic performance (Mazzeo, 2018a). The study on genetic doping started in 2001, when the IOC (International Olympic Committee), through its Medical Commission, discussed on the possible implications of gene therapy, i.e. the use of the development of gene therapies for the treatment of degenerative diseases, for the purpose of doping. This work was continued by the WADA (World Anti Doping Agency), which promoted several initiatives in this sense, including the preparation of a list of prohibited substances, and the financing of specific research projects to detect the presence of family members in the individual. Gene doping was identified as: "non-therapeutic use of cells, genes and genetic elements or modulation of genetic expression, with the capacity to increase athletic performance" (AMA-WADA, 2008; Mazzeo, F. (2016b). The World Anti-Doping Agency offers many different tools to assist stakeholders with their education programs, and to help them educate target groups with suitable activities (Mazzeo, F., & Raiola, G. 2018; Montesano, 2018). For athletes, inserting the modified gene into their body through a viral vector or with intramuscular injections, would be a way to make the body "naturally" produce greater quantities of erythropoietin, without resorting to the synthetic version of the hormone, which is a substance included in the WADA prohibited list (Fischetto, 2013). The hormone EPO is also used as a highly controversial performance-enhancing substance by athletes as a way to optimize oxygen delivery to muscle cells (by increasing the number of red blood cells) (Mazzeo & Volpe, 2016). A group of scientists, demonstrated that injecting mice with the gene that encodes a fat-burning protein called PPAR-δ enabled the animals to run up to twice the distance of their wild-type littermates (Wang et al., 2004). Although genetic engineering of these so-called "marathon mice" could potentially be exploited to enhance athletic performance (in long-distance runners or swimmers, for example),

Specifically, Evans and his team thus determined that increasing PPAR-δ expression effectively increased the number of type 1 muscle fibres in mice. Like IGF-1 and PPAR-δ, the EPO gene is considered by some experts to be a potential candidate for gene doping (Azzazy et al., 2005).

**From gene therapy to genetic doping: WADA education strategy**

We could identify three levels of genetic doping as genetic manipulation can take place:
• before the competition - effect is anabolic;
• during the competition - substances that improve performance are administered;
• after the competition - through the use of defensive substances.

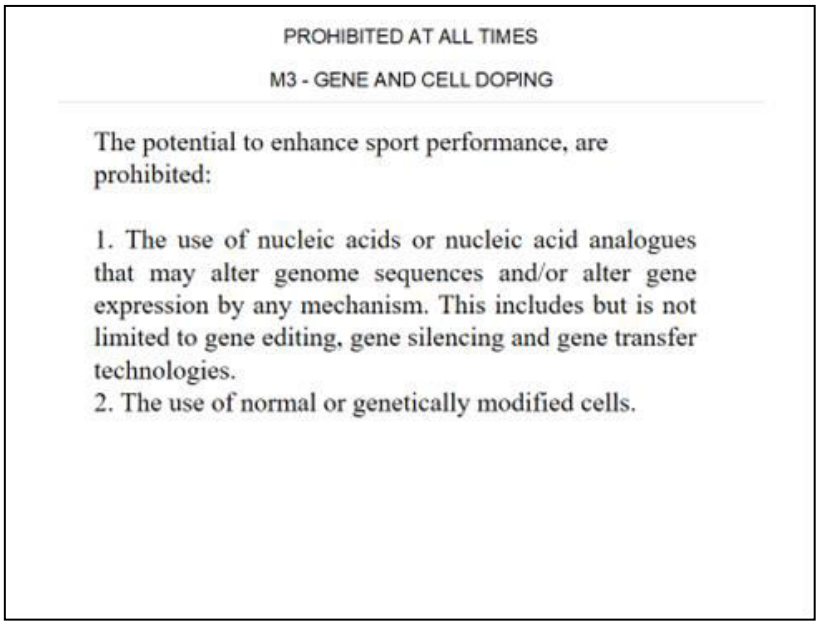
Studies carried out over the past twenty years highlight the various types of genes whose changes have led to an increase in fatigue resistance or in muscle mass (Mazzeo et al., 2016).
In summary, the main potential targets of gene therapy for doping purposes currently appear mainly as follows:

1) *search* for effects similar to those obtained by administering erythropoietin (EPO), in which the EPO increases the haematocrit value up to 80% in animals;

2) *promote* and *improve* the hemodynamic level by acting on the vascular endothelium using VEGF (vascular endothelial growth factor);
3) *search* for similar IG / IGF1 (Insulin-like Growth Factor-1) effects in order to selectively promote the growth of muscle mass.
4) *increase* muscle mass through selective blockade of myostatin, as in animals this pathway has proven effective in treating muscular dystrophy.

In Rio 2016 Olympics, the test was used for the first time to find out if the athletes in the race had tried to manipulate their genetic heritage, to strengthen the muscles and increase endurance: this was identified as genetic doping.

In 2003, the Prohibited List of Substances and Methods was revised to include gene doping as a prohibited method (Figure 1).


PROHIBITED AT ALL TIMES

M3 - GENE AND CELL DOPING

The potential to enhance sport performance, are prohibited:

1. The use of nucleic acids or nucleic acid analogues that may alter genome sequences and/or alter gene expression by any mechanism. This includes but is not limited to gene editing, gene silencing and gene transfer technologies.
2. The use of normal or genetically modified cells.

Figure 1. Wada 2020 list of prohibited methods.

The Gene and Cell Doping Expert Group gives direction to the Health, Medical and Research Committee in relation to the threat of gene doping by developing strategies to prevent and detect non-therapeutic manipulation of gene/protein in sport.

Since 2001, WADA funded projects in the following areas:

- Compounds/Methods Enhancing Growth;
- Compounds/Methods Enhancing Oxygen Delivery;
- Exogenous and Endogenous Anabolic Steroids;
- Detection of prohibited substances/methods: classic methodologies e.g. chromatographic, immunological and biochemical;
- Gene and Cellular Technologies applied to Sports;
- Identification and detection of substances with doping potential;
- Pharmacology of prohibited substances.

The Expert Group provides support in establishing policies in the area of gene transfer in sport, and in selecting research projects or programs in genomics and proteomics. The expert counsel of the Health, Medical and Research Committee enables WADA to serve as a world leader on health,

medical and research issues related to drug-free sport (WADA, 2020). Among many of the Committee's activities are the monitoring of scientific developments in sport, with the aim to protection doping-free sport practice, as well as the overseeing of the following Expert Groups: Prohibited List, Therapeutic Use Exemptions (TUE), Laboratory accreditation, and Gene Doping.

The projects WADA is funding in this field give a good indication of the type of methods researchers are examining for the detection of gene doping (Table 1).

Table 1. Summaries of research projects now being funded by WADA into gene doping and its detection Surce: Play True an official publication of WADA.

| GENE DOPING PROJECTS FUNDED BY WADA AS OF JANUARY 2005 | | | | |
|---|---|---|---|---|
| **Investigator** | **Project** | **Location** | **Started** | **Short summary** |
| Dr. Geoffrey Goldspink | Manipulation of muscle mass via the growth hormone (GH)/insulin-like growth factor (IGF-1) axis | Royal Free and University College Medical School University College London, UK | 2002 | Both GH and IGF-1 are human peptides involved in muscle growth. These factors are naturally upregulated during athletic training. Therefore, the distinction between endogenous and exogenously administered GH and IGF-1 is difficult. It has been shown that intake of GH, but not exercise, modifies the expression of a muscle- specific variant of IGF-1. This property is being used to design a test allowing the ability to distinguish between the introduced and the endogenous substances. |
| Dr. Günter Gmeiner | Application of microarray technology for the detection of changes in gene expression after doping with recombinant human growth hormone (hGH) | ARC Seibersdorf Research Seibersdorf, Austria | 2004 | Microarray technology will be used to search for changes in white blood cell gene expression following application of human growth hormone. Gene expression profiles of treated and untreated cells will be compared, with the objective of defining a set of genes modulated after hGH treatment. |
| Dr. Theodore Friedmann | Microarray detection methods for growth hormone and insulin-like IGF-1 | University of California San Diego, CA, USA | 2004 | Administration of growth hormone and IGF-1 or of the genes expressing them will be associated with reproducible and detectable secondary changes in gene expression in many affected tissues, including peripheral blood. New methods for gene expression screening, such as global microarray techniques, will be used to detect such changes in cells from peripheral blood of mice exposed to GH and IGF-1 and to gene transfer vectors expressing them. |
| Dr. Jordi Segura | IMAGENE: non-invasive molecular imaging of gene expression useful for doping control: pilot study in animals after erythropoietin gene transfer | Pharmacology Research Unit Institut Municipal d'Investigació Mèdica (IMAS-IMIM) Barcelona, Spain | 2005 | An important field of application of imaging will be the prevention of the prohibited misuse of gene therapy in athletes. For this purpose, imaging will be used to detect the RNA being formed in unusual tissues after the gene transfer process. This approach is applicable to any gene transfected to tissues not usually expressing the "doping" protein, such as muscle for EPO. Imaging of mRNA will be carried out by the use of antisense peptide nucleic acids oligonucleotide probes labeled for tomographic detection. A pilot project will be carried out to image the presence of transfected EPO genes into muscle of mice. |
| Dr. Jane Roberts | The application of cellular chemistry and proteomic approaches to the detection of gene doping | HFL Laboratory Inc. Fordham, Cambridgeshire UK | 2005 | A different and more global approach for the detection of doping is proposed. Following doping with doping substances or the use of genetic manipulation, the expression of one or more genes and/or proteins will be altered in several accessible tissues, such as blood cells or bucal mucosa cells. These changes in gene/protein expression will be detected through the application of high performance transcriptomic or proteomic techniques. Ultimately, this will lead to the identification of abnormal RNA/protein patterns, representing molecular signatures associated to the use of doping substances, such as IGF-1 or growth hormone. |

**Gene doping test**

Several methodologies have been proposed for the identification of genetically doped athletes. The fundamental principle of the Athlete biological passport (ABP) is to monitor selected variables (`biomarkers of doping´) over time that indirectly reveal the effect of doping, as opposed to the traditional direct detection of doping by analytical doping controls (Sottas P & Vernec, 2012; Mazzeo & Volpe, 20016). This has always been a highly discussed topic. Specifically, it was objected that routine tests were unable to detect the presence of modified genetic material in the athletes' bodies (Brzeziańska, 2014; Mazzeo et al., 2016). Moreover, until today, there are no specifics WADA standardized methods (Guilherme, 2007). The only probable alternative could be tissue sampling, but this type of control, as well as being highly expensive, involves ethical problems linked to the invasiveness of the control (Hermann, 2014; Breivik, 2005). Furthermore, this kind of test could be able only to identify the direct injection of genes in the desired target organ, but it is not valid in

case of human-origin gene transfer due to the equality of genes between donor and recipient. In this case, only the protein level in blood could be indicative for doping abuse, but genes may be turned on and off by taking specific drugs (Baoutina, 2010; Mazzeo et al., 2020). Over time, different alternatives have been proposed, unfortunately all characterized by invasive approaches. Recently, WADA has worked on implementing the information in the Biological Passport through the identification of the genes necessary for the improvement of sports performance (Sottas, 2012; de Boer, 2019; Wada, 2020). Indeed, the periodic detection of some proteins is able to identify any abnormal alterations in the gene expression, which could result as a suspicion of gene doping. Unfortunately, it is possible to carry out this examination only within approximately 60 days after administration of any doping substance, thus it is not possible consider it an effective and valid method (Gaffney, 2007). Despite was ascertained so far, it seems that winter 2019 brought important changes (WADA, 2019; Brown. 2019). In fact, WADA and IOC enthusiastically announced important developments in the search for doping substances and in genetic doping (WADA, 2019a; IOC, 2019). The first one regards the implementation of a particular blood test that employs the Dried Blood Spot (DBS) method, which could be considered a revolutionary weapon in the fight against doping (WADA, 2019a; IOC, 2019; Kojima, 2016, Thevis, 2020). Differently from the "traditional" blood tests, DBS involves the use of a drop of blood through a simple puncture on the finger.

This method could also be preferred by athletes as an alternative to the inconvenience of the classic collection method. Others advantages are linked to the reduction of costs associated with the transport and storage of the biological sample, and its less degree of degradation (WADA, 2019a; Thevis, 2020; Schenone et al., 2003). The last - and very important - news is that the research on genetic sequencing, financed by the IOC and conducted by Professor Yannis Pitsiladis, is leading to good results (IOC, 2019; Wang, 2017). The Pitsiladis's research, started in 2006, involves identifying the changes in the body's genetic signature after a transfusion or taking a banned product, which increases the production of red blood cells - including erythropoietin (EPO), the most widely used drug used to increase the sports performance - (Yan, 2017; Pitsiladis , 2014). The great novelty and importance of Pitsiladis' work is the discovery that there are approximately 21,000 genes in the body, and that several hundred ones are activated when an athlete has taken EPO or has undergone a blood transfusion. This change in the athlete's genetic signature will remain detectable for weeks - perhaps months - after the use of these types of doping. (Wang, 2017; Yan, 2017;). The studies, which are now in their final phase, will lead to a test for the detection of genetic doping which, if approved by WADA, can be used already in Tokyo Olympic Games 2020 (IOC, 2019).

**Risk factors of Gene Doping**

The potential health risks associated with doping concern multiple organs: heart, kidneys, and liver with consequent development of cancer. As for genetic doping and for any gene therapy intervention, the risk includes the development of a violent immune response to viral vectors, autoimmune responses towards the proteins encoded by the gene introduced, and the possibility of pathologies deriving from mutations that may arise later insertion of the gene with serious adverse effects (Gould, 2013; Körner, 2016; Mazzeo& Volpe, 2016). Patients subjected to IGF-1, after the gene transfer, increase the risk of developing cancer and of insufficient growth. In the case of muscle mass increase, due to a high expression of IGF-1 or resulting from a blockade of myostatin, muscle and/or tendon rupture can be generated due to overload. Another unexpected adverse effect has been described in some patients with severe immunodeficiency. After gene therapy, some of these patients developed severe immune responses, and others developed leukemic-type disorders (de Boer, 2019).

A further harmful effect may be that to which patients undergoing recombinant EPO are subjected. Moreover, an abuse of EPO through drugs is regularly reversible as soon as it is no longer taken, but if it occurs through the integration of additional EPO genes, the levels of erythrocytes will be excessive for life; consequently, this would increase the onset of thrombo-embolitic disorders and other permanent cardiovascular damage. There are many uncertainties about the long-term effects of gene modification; many of these effects may even never be discovered, either because they are not studied in depth (due to financial problems, since the techniques require profitable financing), or because it is difficult to define reliable samples for the study of the side effects of completely new application methods. Gene therapy could be applied to athletes solely to enhance performance dangerously, and can present a significant risk to health.

**Conclusion**

In this era of genetics and genomics, it will be possible to identify the genes determining a person's genetic predisposition for a specific sport. The study of genes in youth can be the best way to develop a great athlete starting from childhood, and to create a specific "personal training" program (Di Onofrio et al., 2019; Montesano et al., 2020). Many of the substances used for doping actually represent great steps forward in the fields of science and medicine.

Naturally, preventive action must also be taken to avoid the practice of doping, be it pharmacological or genetic. There is a very good chance that scientists will discover techniques for detecting gene doping (Mazzeo et al., 2015; Spera et al., 2019).

The first step to be taken towards the path of prevention is certainly the diffusion of knowledge about the phenomenon, with a focus on its negative effects on health, as it usually happens when describing the side effects of pharmacologically active substances (Mazzeo et al., 2019;). It needs that the medical and sports communities, as well as all those who work in the sector, use their economic resources to encourage the search for new doping methods to increase doping tests, in addition to adopting common strategies to prevent their use, both at genetic and other level. They should also promote the values of sport as a tool for healthy and loyal growth of the individual in every possible way, in particular through appropriate information and awareness campaigns starting especially from the youngest age groups, and using all mass communication tools, such as social networks, for the protection of health in sports activities (Mazzeo et al., 2016). Will the Olympic Games soon be dominated by genetically-transformed athletes? WADA is using all the resources at its disposal to battle gene doping, and that includes bringing together some of the top scientists in the field for advice.

**Disclosure statement**

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**References**


Azzazy, H. M. E., *et al.* (2005). Doping in the recombinant era: Strategies and counterstrategies. *Clinical Biochemistry,* 38, 959–965.

Baoutina A., Coldham T., Bains G.S., Emslie K.R. (2010). Gene doping detection: Evaluation of approach for direct detection of gene transfer using erythropoietin as a model system. *Gene Therapy,* 17(8), 1022-1032.

Baoutina, A., Alexander, I. E., Rasko, J. E., & Emslie, K. R (2008). Developing strategies for detection of gene doping. *The journal of gene medicine* 10(1): 3-20

Beytia, M. D. L. A., Vry, J., & Kirschner, J (2012). Drug treatment of Duchenne muscular dystrophy: available evidence and perspectives. *Acta Myologica* 31(1):4.

Breivik G. (2005). Sport, ene doping and ethics. In Genetic Technology and Sport. Tamburrini C., Tännsjö T., (eds.). London/New York, Routledge, 165-78.

Brown J. (2019). Genetic doping: WADA we do about the future of 'cheating' in sport? International Sports Law Journal, 19, 3-4, 258-280.

Brzeziańska E., Domańska D., Jegier A. (2014). Gene Doping in sport – perspectives and risks. *Biol Sport,* 31(4), 251–259.

de Boer E.N., van der Wouden P.E., Johansson L.F., van Diemen C.C., Haisma H.J. (2019). A next-generation sequencing method for gene doping detection that distinguishes low levels of plasmid DNA against a background of genomic DNA. *Gene Therapy*, 26, 7-8, 338-346.

Di Onofrio, V., Montesano, P., & Mazzeo, F. (2019). Physical-technical conditions, coaching and nutrition: An integrated approach to promote cohesion in sports team. *Journal of Human Sport and Exercise, 14*(Proc4), 981-990.

Fischetto, G., & Bermon, S. (2013). From gene engineering to gene modulation and manipulation: can we prevent or detect gene doping in sports? *Sport Med* 43(10): 965-977.

Gould, D. (2013). Gene doping: gene delivery for Olympic victory *British journal of clinical pharmacology* 76(2): 292-298.

Guilherme Giannini A., Dominguez Crespo H. R., Herbert L.J.A. (2007). Gene therapy, genetic doping and sport: fundaments and implications for the future. Revista Brasileira de Medicina do Esporte, 13(5), 317-321.

Hermann A., Henneberg M. (2014). Anti-doping systems in sports are doomed to fail: a probability and cost analysis. *J Sports Med Doping Stud*, 4(5), 1–12.

International Olympic Committee (IOC) (2019). 5th World Conference on Doping in Sport.

Kojima A., Nishitani Y., Sato M., Kageyama S., Dohi M., Okano M. (2016). Comparison of urine analysis and dried blood spot analysis for the detection of ephedrine and methylephedrine in doping control. *Drug Test. Anal*., 8, 2, 189-198.

Körner, S., & Symanzik, T. (2016). Gene doping–challenge for sport and society. *Pharmakon* 4(1):36-42.

Mazzeo, F. (2016b). Drug abuse in elite athletes: Doping in sports. *Sport Science, 9*(2), 34-41.

Mazzeo, F. (2019). Attitude and practice of substance misuse and dietary supplements to improve performance in sport. *Journal of Substance use, 24*(6), 581-586.

Mazzeo, F. (2018a). Anabolic steroid use in sports and in physical activity: Overview and analysis. *Sport Mont, 16*(3), 113-118.

Mazzeo, F., & Raiola, G. (2018). An investigation of drugs abuse in sport performance. *Journal of Human Sport and Exercise, 13*, 309-319.

Mazzeo, F., & Volpe, R. A. (2016). From gene doping to athlete biological passport. *Sport Science, 9*(2), 97-103

Mazzeo, F., Monda, M., Messina, G., Santamaria, S., Messina, A., Montesano, M., . . . Tafuri, D. (2016). Doping in italy: An analysis of its spread in ten years. *Biology and Medicine, 8*(1).

Mazzeo, F., Santamaria, S., & Iavarone, A. (2015). "Boosting" in paralympic athletes with spinal cord injury: Doping without drugs. *Functional Neurology, 30*(2), 91-98.
Mazzeo, F., Santamaria, S., & Montesano, P. (2019). Gender difference, nutritional supplements and drug use in sport to enhancing performance: An Italian revision over the last decade. *Sport Mont, 17*(1), 69-73.
Mazzeo, F., Rinaldi, M., & Tafuri, D. (2020). Sports and supplements: Educating to well-being through effective nutrition. *Sport Science, 13*, 70-75.
Mazzeo, F., Monda, M., Messina, G., Santamaria, S., Messina, A., Montesano, M., . . . Tafuri, D. (2016). Doping in italy: An analysis of its spread in ten years. *Biology and Medicine, 8*(1)
Montesano, P., Masala, D., Di Silvestro, M., Cipriani, G., Tafuri, D., & Mazzeo, F. (2020). Effects of combined training program, controlled diet and drugs on middle-distance amateur runners: A pilot study. *Sport Science, 13*(1), 17-22.
Montesano, P., & Mazzeo, F. (2019). Sports activities in obese teenagers improve social inclusion and health. *Sport Mont, 17*(1), 55-60.
Montesano, P., & Mazzeo, F. (2018). Pilates improvement the individual basics of service and smash in volleyball. *Sport Mont, 16*(3), 25-30.
Pitsiladis, Y.P., Durussel J., Rabin O. (2014). An integrative 'Omics' solution to the detection of recombinant human erythropoietin and blood doping. *British Journal of Sports Medicine*, 48, 856-861.
Schenone, S., Bruno, O., Ranise, A., Brullo, C., Bondavalli, F., Filippelli, W., . . . Falcone, G. (2003). 2-aryl-3-phenylamino-4,5-dihydro-2h-benz[g]indazoles with analgesic activity. *Farmaco, 58*(9), 845-849.
Skipper, M. Gene doping: A new threat for the Olympics? *Nature Reviews Genetics,* 5, 720 (2004)
Sottas, P., Vernec, A. (2012). Current implementation and future of the Athlete Biological Passport - *Bioanalysis*. 4(13):1645-52.
Spera, R., Belviso, I., Sirico, F., Palermi, S., Massa, B., Mazzeo, F., & Montesano, P. (2019). Jump and balance test in judo athletes with or without visual impairments. *Journal of Human Sport and Exercise, 14*(Proc4), 937-947.
Thevis M., Kuuranne T., Geyer H. (2020). Annual banned-substance review – Analytical approaches in human sports drug testing. *Drug Testing and Analysis*, 12, 1, 7-26.
Van der Gronde, T., de Hon, O., Haisma, H. J., & Pieters, T (2013) Gene doping: an overview and current implications for athletes. *British journal of sports medicine* 47(11): 670-678
Wang G., Karanikolou A., Verdouka I., Friedmann T., Pitsiladis Y., 2017, Next Generation "Omics" Approaches in the "Fight" against Blood Doping. *Med Sport Sci*, 62, 119-128.
Wang, Y.X., *et al.* Regulation of muscle fiber type and running endurance by PPAR-δ. *PLoS Biology,* 2, 294.
World Anti-Doping Agency Home Page. (2008).
Yan X., Eynon N., Papadimitriou ID., Kuang J., Munson F., Tirosh O., O'Keefe L., Griffiths LR., Ashton KJ., Byrne N., Pitsiladis Y.P., & Bishop DJ. (2017). The gene SMART study: method, study design, and preliminary findings. *BMC Genomics*, 18, 8:821, 15-31.

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