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The potential implications of exercise-induced epigenetic modifications

Potencijalne implikacije epigenetskih modifikacija uzrokovanih vežbanjem

Biljana Vitošević*, Miroslav Smajić†, Goran Dimitrić†, Dino Buković‡, Ivana Bojić§‖, Božana Radanović§‖, Branka Protić-Gava†, Milica Filipović*

University of Kosovska Mitrovica, *Faculty for Sport and Physical Education, Leposavić, Serbia; University of Novi Sad, †Faculty of Sport and Physical Education, §Faculty of Medicine, Novi Sad, Serbia; University of Zagreb, School of Dental Medicine, ‡Department of Prosthodontics, Zagreb, Croatia; University of Niš, ‖Faculty of Sport and Physical Education, Niš, Serbia

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Introduction

Genetics provides a versatile approach and highlights the mechanisms responsible for the successful sports phenotype. Despite the stability of the genome, the environment has a potential to act as a trigger for chemical changes that activate, or silence genes and so affect the phenotype [1]. These changes could be reflected in the health beneficial epigenetic modifications that may leave a significant and permanent mark on the epigenetic profile of the individual. That means the epigenome in the adaptive response of the environmental sensitivity can adjust the metabolism and homeostasis. In contrast to some other environmental influences, exercise generates positive epigenetic changes that may be a contributing factor to improving health and better quality of life. Identification of the genetic background and the genetic determinants of variability in response to exercise is always a complex matter and sometimes exceeds the limits of known candidates genes and their gene expression. However, the individual molecular pathways information in the field of sports performance is still of paramount importance and it is one of the surest indicators of the direction and the framework needs to go. Sports scientists sometimes refer to the genetic basis of physical performance as a „biological counterpart to the holy grail“, arguing that a genetic composition is responsible for a large number of individual variations in the physical performance [2]. But, it is quite clear that this molecular information acts dynamically in relation to the environment and these epigenetic shifts in response to the exercise are worthwhile because they can be used in some trials to improve health. So, the main goal is to translate the obtained changes in the desired metabolic response and to put that initial molecular signature to practical use.

Epigenetic mechanisms: interface between gene expression and environmental cues

In recent times, environmental factors are increasingly marked as important in determining the final phenotype. In this context, regular physical activity is recognized as available and convenient component that has epigenetic capacity with many positive implications on health. The unique plasticity of skeletal muscle and the specificity of its response to homeostatic perturbation enable the integration of a set of changes within the physiological stimuli in the phenotypic response. Improving the sports performance through training is achieved as a result of the transition of gene expression to generate changes in the composition and function of skeletal muscle as well as in other tissues. These epigenetic changes are not determined by the genetic code and occur in the DNA or chromatin's structure and may affect the transcription of certain genes regardless of their primary sequence. The enhanced levels of gene transcripts can, in this manner, affect the synthesis and degradation of protein components by directly altering their normal function through changing the availability of substrate, or through an indirect mechanism

Correspondence to: Biljana Vitošević, University of Kosovska Mitrovica, Faculty for Sport and Physical Education, Dositeja Obradovića nn, 38218 Leposavić, Serbia. E-mail: home.vitosevic@gmail.com

that conduct the altered expression of growth factors, receptors and to the altered activity at gene promoters resulting in the long-term functional and structural remodeling [3, 4]. The most common epigenetic changes induced by exercise are the histone modifications, like methylation and acetylation, DNA methylation and expression of different types of microRNAs (miRNAs) [5].

What type of epigenetic mechanisms will prevail in the metabolic processes of muscle cells depends on the type, intensity, duration and frequency of exercise stimulus. The most common changes occur within the mitochondrial biogenesis and bioenergetics through different metabolic pathways of muscle fibers. As a consistent feature in many studies, the acute or long-term exercise impacts DNA methylation in a gene-specific mode. It has been reported that exercise increases the expression of many messenger RNA (mRNA) and the protein levels of genes that regulate mitochondrial function, including peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC-1α), mitochondrial transcription factor A (TFAM), peroxisome proliferator-activated receptor δ (PPAR-δ), pyruvate dehydrogenase kinase isoenzyme 4 (PDK4), etc. [6, 7]. Using the human isolated contracting muscle and cultured myotubes, Barres et al. [8] demonstrated that the acute exercise changes the promoter methylation of responsive genes, indicating DNA hypomethylation as an early event in the contraction-induced gene expression. However, it was shown that the acute exercise had a dose-dependent influence on DNA methylation and required a certain intensity of exercise that initiated DNA methylation of responsible genes. Interestingly, a high-intensity exercise notably reduced promoter methylation of the following factors: PGC-1a, TFAM, myocyte-specific enhancer factor 2A (MEF2A), and PDK4 immediately after exercise, whereas PPAR-δ methylation was decreased 3 hours after exercise, so that the mechanism responsible for this exercise-induced demethylation was explained either by hydroxylation of the methyl group (5-hydroxyl methyl), which is an intermediate for demethylation, or by a loss of methyl groups [9, 10].

Another epigenetic event that regulates gene expression is the histone post-translational modifications (PTMs). The histone modifications include a number of various posttranslational modifications to the lysine rich tail regions of histones, in particular H3 and H4 [11, 12]. The modifications like phosphorylation, ubiquitination, methylation and acetylation, and their effects on transcription are different. It is known that subfamily of histone deacetylases (HDACs) has an essential role in skeletal muscle physiology and regulates genes that comprise PGC-α, carnitinepalmitoyl transferase 1 (CPT-1), medium chain acyl-CoA dehydrogenase (MCAD), hexokinase II (HKII), glycogen phosphorylase, and ATP synthase β [13].

It is still not entirely clear about ubiquitination as a potential modification that may be part of the exercise adaptation. Potthoff et al. [14] studied this issue using an animal model and found that ubiquitin-mediated proteosomal degradation of HDACs in the adaptive response to exercise, pointing out that this proteosomal degradation could take part in the adaptive response to the repeated exercise bouts.

MiRNAs are a group of short (20–24 nucleotide) endogenous posttranscriptional regulators that are capable of blocking the translation of protein-coding genes [15, 16]. They become more relevant in the regulation of cell- and tissue-specific gene expression including a role as potential biomarkers for the physiological and pathological conditions. Packed in the exosome vesicles, miRNAs are released to the circulation by nearly all cell types, including the skeletal muscles. The relevant literature data show that the most-studied miRNAs are miR-133a/b, miR-206, and miR-1, which are induced during differentiation of myoblasts into myotubes and are collectively referred to as the "myomirs" [17]. More recent studies of Nielsen et al. [18] determined that the endurance exercise and resistance training induce changes in the ci-miRNA human plasma signature. The studies showed that these changes were dynamic during the short period in the acute exercises and during the long periods of strenuous exercise. Another study of Davidsen et al. [19] reports that resistance exercise training leading to hypertrophy of human skeletal muscle is associated with selected changes in miRNA abundance. Their results indicate that miRNAs can play a major role in the phenotypic changes and noticeable intergroup diversity in a response to resistance training.

In addition, there are posttranscriptional changes in the metabolism of carbohydrates and fatty acids that occur immediately after a single bout of exercise as mitochondrial biogenesis which subsequently increase the requirements of oxygen utilization resulting in a drop in intracellular oxygen. Under these conditions of hypoxia, hypoxia-inducible factor 1 (HIF-1) a member of the HIF family of transcriptional activators which are essential for maintaining O2 homeostasis, switchs on the transcription of genes encoding glucose transporters and glycolytic enzymes, acting together with PGC-1α and initiate the mechanism of gene expression that facilitates increased oxygen supply. This complex triggers the transcription of numerous hypoxia-responsive genes of metabolic processes that would be favorable in conditions of reduced oxygen [20]. HIF1α regulates the gene expression through hypoxia response elements (HRE) present in the promoter regions of target genes. This binding can be affected through the DNA methylation and histone modification, which may maintain a favorable chromatin conformation around the HRE sites. In the presence of oxygen, HIF1α is regulated through hydroxylation, ubiquitination, and degradation by prolyl hydroxylase enzymes (PHD) [21]. In the absence of oxygen, this is inhibited which allows for the HIF1α stabilization and activation. For these reasons, HIF can be considered not only an important oxygen sensor, but also an essential regulator of adaptation induced by exercise [22].

Epigenetic stability

Discussing all these exercise-induced epigenetic modifications, the logical question is how much these changes are stable, and what the factors that determine framework of epigenetic stability are. Many have attempted to investigate the stability and inter-individual variation in DNA methylation comparing changes in DNA methylation profiles during a

short-time to longer periods and concluded that some methylation marks showed considerable variation over time, while others are highly stable [23]. In general, these processes are partly reversible, so that, for example, the histone modifications are in a continual state of change, whereas DNA methylation is considered more stable and long-term. However, the variations of methylation levels have a diverse range and are greatly affected by the gene structures and its genomic location. The epigenetic stability is defined as the persistence of modifications in the gene expression and/or epigenetic marks that influence the gene expression and such stability can exist at different temporal scales [24, 25]. It remains unclear whether the adaptive value of stable and unstable, or transient epigenetic changes may cause the long-term changes in phenotype. On the other hand, it is clear that the nature of the environmental impact that generate the epigenetic change is the most critical factor for the epigenetic stability. In support, recent advances in molecular biology has reported that epigenetic alterations induced by the environmental stressors, can create a persistent memory of the received signal called epigenetic memory. Interestingly, it is proposed that each of that stressors can promote specific alterations to the normal form of DNA methylation- epigenetic footprint, and further cause changes to the gene expression [25]. Sharples et al. [26], in attempt to explain the molecular and epigenetic mechanisms of skeletal muscle memory in humans, introduced the term „epi-memory", studying the human skeletal muscle cells isolated from the different population by generation. They showed that muscle cells had a morphological memory and can retain molecular information of the acute early lifespan in different signaling proteins and that cells possess the ability of retaining elevated methylation for at least thirty cellular divisions. They further compared this type of muscle memory with the motor learning in which learning the motor skills incorporates specific templates of movement through repetition. This implies that their understanding, confirmation and refinement of epigenetic modifications can help in future with targeted therapies, for example, in repairing muscle growth and reducing the loss of muscle mass in the aging process.

The role of epigenetic changes in response to exercise and metabolic disorders

Although the research on molecular genetics of physical exercise and health-related outcomes is still in its infancy, we need to look at the bigger picture, to link all the known and valuable facts as well as to reinforce them in healthcare practice. Exercise is one of those external factors that can modify the expression of genes and that a cascade of epigenetic changes in different tissues can preserve and improve health. So, these epigenetic mechanisms can be used for the purpose of targeted benefits of exercise and can be incorporated in the exercise prescription.

There is no doubt that the physical activity and exercise play a pivotal role in the prevention and treatment of many metabolic disorders. Large part of individual differences in the weight loss response is attributable to genetic and epigenetic factors. Recent studies about the regulation of the epigenome in human adipose tissue show a general increase in the adipose tissue of DNA methylation in response to six months of moderate exercise consisting of spinning and aerobics. Two genes, HDAC4, a histone deacetylase and NCOR2, a nuclear co-repressor, displayed the increased levels of DNA methylation and synchronous decrease in the mRNA expression in the adipose tissue in response to the exercise intervention as well as to increased lipogenesis [27]. Also, this study establish the connection between the differential DNA methylation and mRNA expression in response to exercise, thereby they confirmed the relationship between methylation and altered metabolism through the gene expression. These results may be of clinical significance and the HDAC inhibitors perhaps can be applied in the treatment of obesity and T2D [28]. Similarly, Wang et al. [29] examined DNA methylation of peripheral blood leukocytes between obese adolescent and lean controls and identified two CpG sites in the UBASH3A gene and TRIM3 gene with roles in the immune function that were differentially methylated and that methylation changes may be associated with the pathogenesis of obesity.

Existing data strongly indicate that there is a link between the obesity, energy metabolism and epigenetic modifications and support the fact that the exercises induce the expression of a number of genes that regulate glucose uptake in the skeletal muscle, including GLUT isoform 4 (GLUT4), whose increased expression is further regulated by the transcription factor MEF2 (myocyte enhancer factor 2) and with coactivator protein PPARGC1A [30]. In addition, an increase in the PGC1 expression generated by exercise is an important element for improving the insulin sensitivity in the skeletal muscle not only by increasing the glucose transporter expression (GLUT4) but also by increasing the mitochondria density and it is considered that exercises attenuate the epigenetic modifications at PGC1 and can lead to inhibition, or delay of type 2 diabetes onset [31].

Attempting to identify the epigenetic patterns which may predispose to type 2 diabetes (T2D), Nitert et al. [32] demonstrated that exercises lasting for 6 months and consisting of endurance exercise of moderate intensity, in the people with type 2 diabetes (T2D), were associated with the epigenetic changes, citing the example of decreased DNA methylation of two key transcription factors involved in the glucose uptake in the muscle and respiratory metabolism (RUNX1 and MEF2A). They further reported on differential DNA methylation of mitogen-activated protein kinase (MAPK), insulin and calcium signaling genes concluding as possible that the exercise-induced epigenetic modifications reduce the future risk of T2D among the men with the positive family history (FH+).

Other impacts of exercise-induced epigenetic modifications

The impact of exercise-induced epigenetic modifications appears to have multiple influences within all cells in organism. Accordingly, one of the exercise intensity benefits for the positive epigenetic changes in terms of mitochondrial

biogenesis was shown by Edgett et al. [33], who concluded that the intensity-dependent increases in PGC-1α mRNA following submaximal exercise are mainly due to the increases in muscle induction. Furthermore, the blunted response of PGC-1α mRNA expression following the supramaximal exercise may imply that signaling mediated activation of PGC-1a may also be blunted. According to the extensive interventional studies of Voisin et al. [34], the genes whose methylation levels change significantly after exercise in humans includ the genes involved in particular cellular metabolic states (including PGC-1a, GLUD1, PDK-4, PPAR-d, TFAM, ADIPOR1, ADIPOR2 and BDKRB2), muscle growth (MEF2A), hematopoiesis is (RUNX1) and inflammation (ASC).

Various studies have implied that epigenetic mechanisms also play a role in the definition of the onset of age-associated diseases and lifespan potential. Lopez-Otin et al. [35] postulated some hallmarks of aging like genomic instability, telomere attrition, epigenetic alterations, etc., and suggested that exercise can influence, at least partly, most of these hallmarks. The relationship between the epigenetics regulation and aging is complex and controversial, depending on the process hypo- or hypermethylation, on the type of cells, enzymes, but it seems that exercise can promote the protective effects and help to attenuate that age-deregulations [36, 37]. Genomic imprinting is a unique epigenetic phenomenon that summarizes connection of inheritance with the environment and signifies the "genotype-independent parent-of-origin" gene expression. The effect of parental origin refers to the genomic imprint, and methylation is considered the main mechanism by which the expression is modified. Such an expression of different alleles (mother or father) may take place in all cells and tissues, and it is believed that about 1% of the human genome is imprinted. These genes are of major importance in the medical context, regardless of their low percentage. In order to determine the impact of imprinted genes in human skeletal muscle, Brown [38] identified these genes and changes in DNA methylation associated with exercise. An important conclusion of this recent bioinformatics meta-analysis is that the modification of DNA methylation induced by exercise can slow down the aging process, but also to mitigate the occurrence of certain health disorders.

It is a well-established fact that the exercises, due to the increased metabolic demand, are associated with the increased formation of reactive oxygen species (ROS), but regular exercise reduces the prevalence of a wide range of ROS-associated diseases. Furthermore, the effects of exercise attend to be beneficial for the brain function and include the processes of neurogenesis via neurotrophic factors, increased capillarization, decreased oxidative damage and increased proteolytic degradation. It is known that the oxidative modification of DNA could lead to the increased apoptosis and that impaired function could be the major factors related to the brain aging and neurodegenerative diseases [39]. Moreover, the exercise-induced changes increase the resistance against oxidative stress, facilitates recovery from oxidative stress, and attenuates age-associated decline in cognition. In addition, some recent studies suggest a notable role of exercise on brain plasticity and cognitive health through the epigenetic modifications mostly by the action of brain-derived neutrophic factor (BDNF) highly expressed in hippocampus [40].

There is no strong evidence to provide a direct connection between the epigenetic modulation and changes in cardiovascular system induced by exercise, but recent data show that moderate exercise mitigate the age-dependent decrease in apoptosis associated protein (ASC) methylation, indicating suppression of redundancy pro-inflammatory cytokines through just reduction of ASC expression [41]. These epigenetic modifications just ensure proper function at the cellular level, due to the balance between the inflammatory response and anti-inflammatory genes, so any disruption of these epigenetic mechanisms could lead to the development of atherosclerosis and stenosis [42]. Keeping in mind the fact that physical activity can prevent many pathological epigenetic events, for example, through the increased expression of endothelial growth factor like (VEGF), as well as through the reduction of the many risk factors such as oxidative stress which are held responsible for cardiovascular disorders, many authors point out the role of exercise as a strong regulator of positive epigenetic modification [43–45]. Many of these key regulators of epigenetic mechanisms are associated with the modifications of DNA and histones in endothelial cells, suggesting a direct protective role of physical exercise on endothelial function. It is believed that the role of free radicals in the modulation of extracellular matrix which is regulated by epigenetic mechanisms is very important and that they participate in the development of many pathophysiological processes. In this regard, the exercises improve the antioxidant capacity and maintains cellular oxidative balance, molecular structure and architecture of the extracellular matrix through the mediating signaling cascades. Precisely in this way, the epigenetic modulation induced by exercises is a significant factor in the modification of the functional genome and heart and vascular beds [46]. Baccarelli et al. [47] in an experimental work with animals and humans argued that DNA methylation appears as a primary regulator of inflammation and atherosclerotic changes in peripheral blood leukocytes, and it is connected to several cardiovascular-related biomarkers that include homoczsteine and C-reactive rpotein. In addition, referring to the epigenetics and the cardiovascular relation, miRNAs contribute to the process of myocardium remodeling through the different signaling pathways in condition of hypertrophy and neo-angiogenesis – "the athlete's heart" , and thus protect the heart from fibrosis and pathological hypertrophy [48]. However, although a lot of factors are known and confirmed, further detailed investigations are required to explore other positive effects of epigenetic modulation induced by exercising and to incorporate them into the improved prevention, risk assessment, risk stratification and treatment of cardiovascular disorders.

Finally, the most recent tightly controlled and extensive human study showed that 3 months of endurance training in the healthy human volunteers caused the substantial DNA methylation changes at about 5,000 sites across the genome and powerful gene expression [49]. This study indicates that the

numerous changes in methylation were not a random and coincidental effect but more a well-controlled adaptive process generated as a response to endurance exercises. Thus, the increased methylation seemed to be related to remodeling of the tissue and metabolism, while decreased methylation was related to inflammation, and this can explain the benefits of exercise. DNA methylation was predominantly changed in the enhancer regions (short regions of DNA which activate gene transcription from a distance) with structural improvement for binding of myogenic regulatory factors (MRFs), myocyte enhancer factors (MEFs) and ETS proteins, so it can be assumed that the training-induced integrated epigenetic adjustment contributes to the heterogeneity in individual responses.

All of these data in the literature point out the existence of particular regions in the genome that are sensitive to the epigenetic modifications in response to exercise and there are differences depending on the type, duration and intensity of exercise. Future studies should investigate the stability of those exercise-induced DNA methylation changes and the possible effects of epigenetic alterations in different periods of training, as well as the exercise program that includes different types of speed and effort.

Conclusions and future perspectives

By understanding the epigenetic changes which are important for responses of various phenotypes, it is logical to expect that these valuable facts as part of important biological adaptation can be used to improve the health of individuals. Epigenetics provide a scientific basis for how the training intervention and other external factors can reshape the individual and provide the insight into the way the changes in gene expression through a complex network of coordinated pathways may affect the phenotype. Key epigenetic elements are responsible for regulating adiposity, numerous molecular pathways related to the inflammatory processes, energy expenditure, and glucose homoeostasis, so that the molecular events within their physiological processes are very powerful tool. It is conceivable that these observations and health benefits about the epigenetic modification within the different cells and tissues in response to exercise as readily available and efficient form of behavior intervention, could be combined for the valuable clinical information and used in practice for health improvement in the future.

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