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**POTENCIJALNE IMPLIKACIJE EPIGENETSKE MODIFIKACIJE INDUKOVANIH VEŽBANJEM**

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The potential implications of exercise-induced epigenetic modifications

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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 the potential to act as a trigger for chemical changes that activate or silence genes and so affect the phenotype. 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 their metabolism and homeostasis. In contrast to some other environmental influences, exercise generates positive epigenetic changes that may be contributing factor to a improved 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 candidate genes and their gene expression. However, 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 "biological counterpart to the holy grail," arguing that the genetic composition are responsible for a large number of individual variations in the physical performance. But is quite clear that this molecular information however acts dynamically in relation to the environment and these epigenetic shifts in response to the exercise worthwhile because it 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 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.
epigenetic changes are not determined by the genetic code and occur in DNA or chromatin’s structure and may affect the transcription of certain genes regardless of their primary sequence. Enhanced levels of gene transcripts can in this manner affect the synthesis and degradation of protein components directly altering their normal function by changing availability of a substrate, or through an indirect mechanism that conduct to the altered expression of growth factors, receptors and to the altered activity at gene promoters resulting in long-term functional and structural remodeling. The most common epigenetic changes induced by exercise are histone modifications, like methylation and acetylation, DNA methylation and expression of different types of microRNAs (miRNAs)

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 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 protein levels of genes that regulate mitochondrial function, including PGC-1α, mitochondrial transcription factor A (TFAM), peroxisome proliferator-activated receptor δ (PPAR-δ), pyruvate dehydrogenase kinase isoenzyme 4 (PDK4), etc. Using human isolated contracting muscle and cultured myotubes, Barres et al. demonstrated that acute exercise changes the promoter methylation of responsive genes, indicating the DNA hypomethylation as an early event in contraction-induced gene expression. However, it has been shown that acute exercise has a dose-dependent influence on DNA methylation and requires a certain intensity of exercise that initiates DNA methylation of responsible genes. Interestingly, high-intensity exercise notably reduced promoter methylation of the following factors: PGC-1α, TFAM, 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 explains or by hydroxylation of the methyl group (5-hydroxyl methyl), which is an intermediate for demethylation or from a loss of methyl groups.

Another epigenetic event that regulates gene expression is histone post-translational modifications (PTMs). Histone modifications include a number of various posttranslational modifications to the lysine rich tail regions of histones, in particular H3 and H4.
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 regulate genes that comprise peroxisome proliferator activated receptor gamma coactivator α (PGC-α), carnitinepalmitoyltransferase 1 (CPT-1), medium chain acyl-CoA dehydrogenase (MCAD), hexokinase II (HKII), glycogen phosphorylase, and ATP synthase β.

It is still not entirely clear about ubiquitination as a potential modifications that may be part of the exercise adaptation. Potthoff and coworkers have studied this issue in an animal model and found that ubiquitin-mediated proteosomal degradation of HDACs in the adaptive response to exercise pointing out that this proteosomal degradation can take part in the adaptive response to repeated exercise bouts.

MiRNAs are a group of short (20-24 nucleotide) endogenous posttranscriptional regulators that are capable to block the translation of protein-coding genes. They become more relevant in the regulation of cell- and tissue-specific gene expression including a role as potential biomarkers for physiological and pathological conditions. Packed in exosome vesicles, miRNAs are released to the circulation by nearly all cell types, including skeletal muscle. The 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”. More recent studies of Nielsen et al. determined that endurance exercise and resistance training induce changes in the miRNA human plasma signature. Studies have shown that these changes are dynamic during the short period in acute exercises and during the long periods with strenuous exercise. Another study of Davidsen et al. 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 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) member of the HIF family of transcriptional activators
which are essential for maintaining O₂ homeostasis, switch 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. HIF1α regulates gene expression through hypoxia response elements (HRE) present in the promoter regions of target genes. This binding can be affected through DNA methylation and histone modification, which may maintain a favorable chromatin conformation around HRE sites. In the presence of oxygen, HIF1α is regulated through hydroxylation, ubiquitination, and degradation by prolyl hydroxylase enzymes (PHD). In the absence of oxygen, this is inhibited which allows for 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.

**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 short time to longer periods and concluded that some methylation marks show considerable variation over time, while others are highly stable. In general, these processes are partly reversible, so that for example histone modifications are in a continual state of change, whereas DNA methylation is considered more stable and longer term. However, the variations of methylation levels have a diverse range and are greatly affected by the gene structures and its genomic location. Epigenetic stability is defined as the persistence of modifications in gene expression and/or epigenetic marks that influence gene expression and such stability can exist at different temporal scales. It remains unclear whether the adaptive value of stable and unstable or transient epigenetic changes may cause long-term changes in phenotype. On the other hand it is clear that the nature of the environmental impact that generates the epigenetic change is the most critical factor for epigenetic
stability. In support, recent advances in molecular biology report that epigenetic alterations induced by environmental stressors, can create a persistent memory of the received signal called epigenetic memory. Interestingly, it is proposed that each of these 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 attempting to explain the molecular and epigenetic mechanisms of skeletal muscle memory in humans introduced the term “epi-memory” studying human skeletal muscle cells isolated from different generational populations. They shown that muscle cells had a morphological memory and can retain molecular information of the acute early lifespan in different signalling proteins and that cells possess the ability of retaining elevated methylation for at least thirty cellular divisions. They further compare this type of muscle memory with the motor learning in which learning of motor skills incorporates specific templates of movement through repetition. This implies that their understanding, confirmation and refinement of epigenetic modifications can help in the 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 of 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 valuable facts and to reinforce them in healthcare practice. Exercise is one of those external factors that can modify the expression of genes and that 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 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 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 adipose tissue 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 increased levels of DNA methylation and synchronous
decrease in mRNA expression in adipose tissue in response to the exercise intervention, as well as increased lipogenesis\textsuperscript{27}. Also, this study establish the connection between differential DNA methylation and mRNA expression in response to exercise, thereby they confirmed the relationship between methylation and altered metabolism through gene expression. These results may be of clinical significance and HDAC inhibitors perhaps can be applied in the treatment of obesity and T2D\textsuperscript{28}. Similarly, Wang et al. \textsuperscript{29} are by examination of DNA methylation of peripheral blood leukocytes between obese adolescent and lean controls 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 obesity, energy metabolism and epigenetic modifications and support the fact that exercise induces the expression of a number of genes that regulate glucose uptake in skeletal muscle, including GLUT isoform 4 (GLUT4), which increased expression is further regulated by the transcription factor MEF2 (myocyte enhancer factor 2) and with coactivator protein PPARGC1A \textsuperscript{30}. In addition, increasing in PGC1 expression generated by exercise is an important element for the improvement of insulin sensitivity in skeletal muscle not only by increasing of glucose transporter expression (GLUT4) but also by increasing in mitochondria density and considered that exercise attenuates epigenetic modifications at PGC1 and can lead to inhibition or delay of type 2 diabetes onset\textsuperscript{31}.

Attempting to identify the epigenetic patterns which may predispose to type 2 diabetes (T2D), Nitert et al. \textsuperscript{32} demonstrated that exercise in people with type 2 (T2D) for 6 months consisting of endurance exercise of moderate intensity, is associated with epigenetic changes, citing the example of decreased DNA methylation of two key transcription factors involved in the glucose uptake in muscle and respiratory metabolism (RUNX1 and MEF2A). They further reported 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 men with 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 of an organism. Accordingly, one of the exercise intensity benefits for positive epigenetic changes in terms of mitochondrial biogenesis were shown by Edgett et al. which concluded that intensity-dependent increases in PGC-1α mRNA following submaximal exercise are mainly due to increases in muscle induction. Furthermore, the blunted response of PGC-1α mRNA expression following supramaximal exercise may imply that signalling mediated activation of PGC-1α may also be blunted. According to extensive interventional studies of Voisin et al., genes whose methylation levels changes significantly after exercise in humans include genes involved in particular cellular metabolic states (including PGC-1α, GLUD1, PDK-4, PPAR-d, TFAM, ADIPOR1, ADIPOR2 and BDKRB2), muscle growth (MEF2A), haematopoiesis (RUNX1) and inflammation (ASC).

Various studies have implied that epigenetic mechanisms also play a role in definition the onset of age-associated diseases and lifespan potential. Lopez-Otin et al. 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 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 protective effects and help to attenuate that age-deregulations. Genomic imprinting is a unique epigenetic phenomenon that summarizes connection of inheritance with the environment and signifies "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 modifies the expression. 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 et al. have identified these genes and changes in DNA methylation associated with exercise. An important conclusion of this recent bioinformatics meta-analysis is that 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 exercise due to increased metabolic demand associated with 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 brain function and includes processes of neurogenesis via neurotrophic factors, increased capillarization, decreased oxidative damage, and increased proteolytic degradation. It is known that oxidative modification of DNA could lead to increased apoptosis and that impaired function could be major factors related to brain aging and neurodegenerative diseases. Moreover, exercise-induced changes increases the resistance against oxidative stress, facilitates recovery from oxidative stress, and attenuates age-associated decline in cognition. In addition, recent studies suggest a notable role of exercise through epigenetic modifications on brain plasticity and cognition, mostly interfere by the action of brain-derived neurotrophic factor (BDNF) highly expressed in hippocampus.

There is not strong evidence to provide a direct connection between epigenetic modulation and changes in cardiovascular system induced by exercise, but recent data show that chronic moderate exercise performs to mitigate the age-dependent decrease in ASC (apoptosis-associated protein) methylation, indicating suppression of redundancy pro-inflammatory cytokines through just reduction of ASC expression. 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. Keeping in mind fact that physical activity can prevent many pathological epigenetic events through, for example, 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. Many of these key regulators of epigenetic mechanisms are associated with 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, exercise improves the antioxidant capacity and maintains cellular oxidative balance,
molecular structure and architecture of the extracellular matrix through mediating signaling cascades. Precisely in this way epigenetic modulation induced by exercise are significant factors in the modification of the functional genome and heart and vascular beds. Baccarelli et al. in experimental work with animals and human epidemiological studies argue that DNA methylation is appear as a primary regulator of inflammation and marked the homocysteine and C-reactive protein, as well as atherosclerotic changes in peripheral blood leukocytes, as cardiovascular-related biomarkers associated with the DNA methylation. In addition, referring to the epigenetics and the cardiovascular relation, miRNAs contribute to the process of myocardium remodeling through different signaling pathways in condition of hypertrophy and neo-angiogenesis – “the athlete’s heart” and thus protects the heart from fibrosis and pathological hypertrophy. 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 exercise and to incorporate them into improved prevention, risk assessment, risk stratification, and treatment of cardiovascular disorders.

Finally, the most recent tightly controlled and extensive human study has shown that 3 months of endurance training in healthy human volunteers caused substantial DNA methylation changes at about 5000 sites across the genome and powerful gene expression. This study indicates that the numerous changes in methylation were not a random and coincidental effect but more a well-controlled adaptive process that generates in response to endurance exercise. 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 enhancer regions (short regions of DNA which activate gene transcription from a distance) with enhancement for binding motifs 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 epigenetic modifications in response to exercise and there are differences depending on the type, duration and intensity of exercise. Future studies should investigate stability of those exercise-induced DNA methylation changes and the possible
effects of epigenetic alterations on diverse periods of training, as well as exercise program that includes different types of speed and effort.

**Conclusions and future perspectives**

By understanding the epigenetic changes, 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 provides insight into how 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 inflammatory processes, energy expenditure, glucose homoeostasis, so that the molecular events within their physiological processes are a very powerful tool. It is conceivable that these observations and health benefits about epigenetic modification within the different cells and tissues in response to exercise as readily available and efficient form of behavior intervention, could in the future combined into valuable clinical information and used in practice for health improvement.

Asked whether we are ready for a prudent and stepwise implication of proven beneficial exercise-induced epigenetic alterations in clinical practice as part of personalized approach, and if not now, when, the answer is that, based on current knowledge and the impressive development of-omics sciences and systems biology, it may not be so far away.
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