Introduction

It is well known that physical exercise is an important contributing factor to a better quality of life via the changes that it causes to the function of various physiological systems. Recently, many of these changes were attributed to epigenetic alterations that are induced by exercise, thus altering the expression level of various genes. In general, epigenetics are changes occurring in the DNA or the chromatin’s structure that can influence the transcription of several genes independently of their primary sequences (Figure 1). The most common epigenetic changes induced by exercise are histone modifications, such as methylation and acetylation, DNA methylation, and expression of different types of microRNAs (miRNAs).

Histone modifications are post-translational alterations on the lysine-rich tail region of histones, especially of H3 and H4 histones. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) are enzymes that regulate DNA acetylation, with HATs adding acetyl groups and HDACs removing them from DNA. In general, histone lysine acetylation is a reversible process which is associated with the transcriptional activation, while the balance between HATs and HDACs determines the level of histone acetylation and, eventually, the level of transcription. Although there is little evidence regarding the histone methylation, however, it is known that it is a reversible process that occurs through histone methyltransferases (HMTs), which are enzymes that add methyl groups to lysine tail regions of histones. Other enzymes that were recently found, such as peptidylarginine deiminase 4 (PADI4), lysine-specific demethylase 1 (LSD1) and Jumonji C-domain-containing histone demethylase (JHDM), remove the methyl groups.

DNA methylation is also a reversible epigenetic process which is catalyzed by a family of DNA methyltransferases (DNMTs). These enzymes add a methyl group, through a covalent modification, primarily on CpG dinucleotides. CpG dinucleotides are frequently found in clusters, called CpG islands, however most of the DNAs methylation occurs at CpG island shores, which are sequences close to CpG islands. This usually results in gene silencing, either through a direct effect on transcription factor(s) or through recruitment of methyl-CpG binding domain (MBD) proteins, which interact with and activate HDACs, and convert the chromatin to a repressive state, thus preventing the gene transcription.

Another mechanism of epigenetic regulation is mediated by miRNAs. MiRNAs are a group of small noncoding RNA mole-
cules, about 22 nucleotides in length, and they generally function to mitigate or silence protein translation, often acting as subtle regulators\(^{10,11}\). MiRNAs are also known to play a role in DNA methylation\(^{12}\) and chromatin remodeling\(^{13}\). It should be noted that a single miRNA may regulate a high number of target genes, sometimes up to thousands. Recently, miRNAs have been suggested as key regulatory molecules of the immune functions\(^{11,14,15}\) and the effectiveness of the immune response\(^{10}\), as well as important contributing factors to myocardium remodeling\(^{16,17}\).

Epigenetic alterations induced by the “eustress” or “good stress” of physical exercise have a positive impact on various biological functions\(^{18}\), thus the known exercise-induced epigenetic regulations in different physiological systems and pathophysiological mechanisms, as well as their potential clinical implications, are discussed in the following sections of this review. First, focus will be driven on specific epigenetic regulations of metabolic and inflammatory processes; Then, epigenetic mechanisms and effects of physical exercise on important pathologies such as cancer and aging are discussed; Lastly, the existing evidence for the role of epigenetic alterations in the function of the central nervous system and the cardiovascular system are reviewed.

**Epigenetic regulations of metabolic processes induced by exercise**

It is well established that physical exercise causes alterations in the expression of human skeletal muscle genes, as a mechanism of adaptation not only to the mechanical load but also to the metabolic stress of exercise. Many of those changes in gene expression can occur through epigenetic regulations which are induced by exercise and are related to metabolic processes\(^{19-21}\).

In general, acute exercise causes hypomethylation of the whole genome in the skeletal muscle cells of sedentary people. Although this hypomethylation is mainly related to promoters of metabolic genes (e.g., PGC-1\(\alpha\), TFAM, PPAR-\(\delta\), PDK4, citrate synthase) and results in increased gene expression, however the transcription of muscle-specific transcription factors, such as MyoD1 and myocyte-specific enhancer factor (MEF) 2A, does not change both on human and mouse models\(^1\). Moreover, the promoter demethylation and the activation of associated genes depend on the intensity of the exercise; high intensity exercise causes a reduction in the promoter methylation of genes such as peroxisome proliferator-activated receptor gamma (PPAR-\(\gamma\)), coactivator 1 alpha (PGC-1\(\alpha\)), transcription factor A mitochondrial (TFAM), pyruvate dehydrogenase lipoamide kinase isozyme 4 (PDK4) and MEF2A, immediately after exercise, as well as a reduction in the promoter’s methylation of peroxisome proliferator-activated receptor delta (PPAR-\(\delta\)), 3 hours after exercise. Similar results have been also observed in *ex vivo* models\(^{19}\).

In addition, exercise can lead to changes in the action of cytosolic messengers such as Ca\(^{2+}\) and AMP, both in humans and mice, which result in the activation of signaling cascades and eventually to alterations in gene transcription. These alterations occur through the activation of Ca\(^{2+}/\)calmodulin-dependent protein kinase (CaMK) and AMP-dependent protein kinase (AMPK)\(^{22}\). AMPK can change the expression of genes, such as the glucose transporter type 4 (GLUT4) and mitochondrial genes, by activating cellular transcription factors and co-activators in mammalian skeletal muscle. Specifically for the mitochondrial genes, it has been suggested that AMPK acti-
vates the PGC-1α co-activator, which increases the expression of other transcription factors that, in turn, lead to the transcriptional changes. Moreover, over-expression of PGC-1α and nuclear respiratory factor 1 (NRF-1) appears to increase the expression of the GLUT4 and the activity of MEF2 in mice implying that AMPK could increase the expression of GLUT4 protein through PGC-1α pathway.

Nevertheless, the expression of GLUT4 can also be increased through other pathways (Figure 2). Specifically in human skeletal muscle, the class IIa HDACs, which consist of HDAC4,5,7 and 9, are highly expressed and regulated by neuromuscular activity, while their action, particularly at the promoter’s region, is reduced by exercise. These regulations occur through an ubiquitin-mediated proteasomal degradation, and through phosphorylation by CaMKII, AMPK, or protein kinase D (PKD) which leads to the exit of HDACs from the nucleus (Figure 2). The class IIa HDACs can interact with MEF2 and repress MEF2-dependent transcription, by creating a complex containing HDAC3, which removes acetyl groups. In this way, HDACs regulate the expression of oxidative genes, which is increased after exercise. In particular, the HDAC5 can regulate the expression of GLUT4 in skeletal muscle. HDAC5 interacts with MEF2 resulting in a deacetylation of GLUT4 which, in turn, reduces its expression at rest. However, following acute exercise, AMPK phosphorylates HDAC5, causing its dissociation from MEF2. This dissociation enables MEF2 to interact with co-activators such as PPAR-γ, PPARG1a and HATs, acetylating GLUT4 and, thus, increasing its expression. The action of MEF2 can also be regulated by CaMK after acute exercise, through a mechanism that also includes acetylation of GLUT4 and influences the binding of MEF2 at the promoter of this gene. The regulation of MEF2 during endurance exercise was found to be independent of sex. Moreover, HDACs can regulate the expression of PGC-1α, which is increased after exercise in an intensity-dependent manner, and is a key factor in the human muscle adaptation to exercise.

Such exercise-induced genetic modifications could have clinical implications. Specifically, in type 2 diabetic patients, PPAR-γ and PGC-1α are hypermethylated in human skeletal muscle. This hypermethylation has been correlated with reduced mRNA expression of PGC-1α and mitochondrial DNA. Thus, exercise may have a beneficial effect on the prevention and confrontation of type 2 diabetes and other metabolic disorders through the afore-mentioned epigenetic mechanisms, since it can increase not only the expression of GLUT4 in muscle, but also the hypomethylation of PPAR-γ and PGC-1α.

Epigenetic alterations can also regulate the transcription of myosin heavy chain genes (MHCs). In particular, acetylation and methylation of histone H3 at specific states is related to a differential expression of I MHC, Ix MHC and IIb MHC genes.

Figure 2. Histone modifications regulate glucose transporter type 4 (GLUT4) expression in response to exercise. AMPK: 5’AMP-dependent protein kinase; CaMKII: Ca2+/calmodulin-dependent protein kinases II; HDAC: histone deacetylase; MEF2: myocyte-specific enhancer factor-2; Ub: ubiquitin-binding domain.
in mouse soleus muscle following reduced muscular activity (muscle deloading), as a result of changes in the chromatin structure\textsuperscript{56,67}. Moreover, HDAC5 has been found to increase the number of type I oxidative fibers following exercise in mice\textsuperscript{26}. Also, it has been shown that the percentage of type I muscle fibers and the maximal aerobic capacity (VO\textsubscript{2} max) in humans are positively correlated with the expression of the acetylspherase MYST4 (monocytic leukemia zinc finger protein-related factor)\textsuperscript{49}, a HAT that regulates the expression of Runt-domain transcription factor (RUNX2)\textsuperscript{49}, which is involved in osteoblast differentiation and bone formation\textsuperscript{50}.

Moreover, another epigenetic regulatory mechanism which is involved in skeletal muscle physiology includes miRNAs. As it has been already mentioned, miRNAs are tissue specific molecules that have the tendency to silence protein translation and decrease genes transcription. Particularly in muscle cells, the miRNAs (myomiRNAs) contribute to the myocyte proliferation and differentiation, the determination of muscle fiber types, and to muscle hypertrophy and atrophy, while their deregulation is typical in muscle diseases and dysfunction\textsuperscript{51}. The regulation of the myomiRNAs is controlled by various transcription factors, such as the key myogenic regulatory factors (MRFs), which include MyoD1 and myogenin, MEF2, serum response factor (SRF) and myocardin-related transcription factor-A (MRTF-A)\textsuperscript{52}. Apart from myomiRNAs, there are also “circulating” miRNAs (c-miRNAs) in the plasma, which mediate many physiological processes such as angiogenesis, inflammation, skeletal and cardiac muscle contractility and ischemia adaptations. Some of these miRNAs can be altered by acute exhaustive aerobic exercise (miR-21 and miR-221), or by sustained aerobic exercise training (miR-20a), or even by both types of exercise (miR-146a and miR-222), others remain unchanged (miR-133a, miR-210, miR-328) by aerobic exercise, and others (miR-133) can change through resistance exercise while remain unchanged following aerobic exercise\textsuperscript{53,54}.

Aerobic exercise has been shown to cause mainly a reduction in the expression of various types of miRNAs in human skeletal muscle, 22% of which target genes that regulate transcription and 16% target genes that are involved in muscle metabolism, especially in oxidative phosphorylation\textsuperscript{55}. Thus, the decrease in miRNAs expression causes an increase in the expression of mitochondrial and lipid oxidation enzymes, without affecting the amount of the mRNA of metabolic genes. Also, four miRNAs that are down regulated by endurance (aerobic) exercise target the genes RUNX1, PAX3 and SOX9, which may be modulators of the muscle adaptations induced by aerobic exercise\textsuperscript{56}. In addition, miRNAs in skeletal muscle may play a role in the regulation of muscle cell size after resistance exercise and ingestion of essential amino acids that stimulate the anabolic process, although such a role is still undefined. Nevertheless, it has been shown that such anabolic stimulus changes the expression of different types of miRNAs and those changes differ between young and old men\textsuperscript{56}. Furthermore, endurance exercise has been shown to alter the expression of various types of miRNAs in mice, which play a key role in the remodeling and maintenance of skeletal muscle mass. Specifically, these endurance exercise-induced alterations in miRNAs expression modulate the expression of key genes, such as PGC-1a and PDK4, without affecting the expression of cytoplastic or nuclear complexes\textsuperscript{57,58}, and also affect the process of angiogenesis which naturally occurs in skeletal muscle after physical exercise training\textsuperscript{59}. Similarly in humans, an acute bout of endurance exercise has been shown to increase the expression of myomiRNAs that target genes which participate in TGF-β, MAPK and other signaling pathways, while a 12-week endurance training program surprisingly resulted in a decrease of all myomiRNAs\textsuperscript{60}.

### Exercise-induced epigenetic regulation of inflammatory processes

It is well known that exercise is associated with inflammatory responses\textsuperscript{51-67}. Apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) is a mediator of the cytosol-type inflammatory signaling pathway\textsuperscript{56,65}. It activates procaspase-1\textsuperscript{66} and promotes the activation of interleukins\textsuperscript{67,68}, ultimately leading to the initiation of innate immunity. The transcriptional status of ASC gene is regulated by epigenetic mechanisms. Specifically, the methylation of its CpG island surrounding exon 1 is inversely correlated with ASC protein expression\textsuperscript{69,70}. It has been shown that chronic moderate exercise up-regulates the methylation status of ASC, resulting in a decreased activity of the gene in human monocytes\textsuperscript{71} and, thus, preventing the activation of inflammatory cytokines, such as interleukins and tumor necrosis factors (TNF)\textsuperscript{72}. Thus, exercise can protect the cell from an inflammatory environment, which could favor carcinogenesis or the development of several age-related diseases, as discussed in the following sections.

In addition, it has been shown that exercise can differentially influence the expression patterns of miRNAs in leukocyte subtypes, such as granulocytes and peripheral blood mononuclear cells (PBMCs)\textsuperscript{73,74}. As far as neutrophils are concerned, Shlomit et al.\textsuperscript{73} analyzed neutrophil-specific miRNAs and genes whose expression was significantly altered by aerobic exercise, and identified three pathways in which a connection between miRNAs and gene expression was plausible. The most predominant was the ubiquitin-mediated pathway, which is known to be indispensable in the regulation of immune and inflammatory functions\textsuperscript{75}. The second one was the janus kinase-signal transducer and activator of transcription (Jak-STAT) pathway, which is known to modify granulopoiesis, neutrophil immune function and apoptosis\textsuperscript{76,77}. The third one was the Hedgehog pathway, which is thought to have a role in chronic inflammation\textsuperscript{78}. Taking all the above evidence into consideration, it is suggested that exercise can alter neutrophil function through epigenetic mechanisms.

With respect to PBMCs, in an interesting analysis of the thirty four PBMC-specific microRNAs and genes whose expression was significantly altered by exercise, twelve signaling pathways were identified\textsuperscript{79}. Some of those pathways play an important role in the regulation of pro- and anti-inflammatory...
Epigenetic effects of exercise on cancer

Physical activity is currently suggested as a protective factor against cancer, which lowers the risk of cancer occurrence and mortality\(^{92,93}\). A hypomethylation in repetitive elements in many cancer cells has been reported and it appears to be accompanied by the overall genomic methylation status of the patients\(^{84}\). Physical activity is usually associated with higher levels of global genomic DNA methylation and, thus, it could restore, at least to some extent, the hypomethylated genome in cancer\(^{95}\).

Another underlying mechanism for carcinogenesis is chronic inflammation that can be mediated by ASC protein\(^\text{111}\). As it has been aforementioned, physical exercise decreases the expression of ASC gene through epigenetic mechanisms, and, in turn, the activation of inflammatory cytokines. Thus, exercise can protect the cell from an inflammatory environment which could promote carcinogenesis.

Not only hypomethylation but also hypermethylation has been associated with neoplastic mutations in the genome. Actually, in most types of human neoplasms, a methylation of cytosine in CpG dinucleotides in gene promoters appears to be associated with transcriptional gene silencing\(^{96,97}\). An aberrant DNA methylation may result in silencing of a tumor-suppressor gene, which is a crucial component of the mechanism of carcinogenesis\(^{98}\). CACNA2D3 is a calcium channel related tumor suppressor gene, which the silencing of which has the potential to lead to gastric cancer\(^{98}\). Yuasa et al.\(^{98}\) found that CACNA2D3 methylation was more frequent in patients with no physical activity compared to those with some kind of physical activity, indicating that physical exercise may decrease the methylation status of this particular gene and can have a positive effect against tumorigenesis. L3MBTL1 is another tumor suppressor gene the methylation of which is also inversely correlated with gene expression and is higher in tumors\(^{99}\). Zeng et al.\(^{99}\) observed a decrease in L3MBTL1 methylation after a six month-exercise training that resulted in higher expression of that specific gene, which was associated, possibly in a dose-response manner, with low grade and hormone receptor positive tumors, as well as with low risk of cancer recurrence and breast cancer death. Two other genes with the same characteristics are APC and RASSF1A 100. These particular genes have been associated with breast cancer tumorigenesis and are used as epigenetic markers of breast cancer risk. Coyle et al.\(^{100}\) have provided evidence indicating that physical exercise diminishes or reverses promoter hypermethylation of these tumor suppressor genes in non-malignant breast tissue, allowing their expression.

Furthermore, physical exercise decreases estrogen levels, which have been proposed as inducers of promoter hypermethylation of tumor suppressor genes and are implicated in breast cancer carcinogenesis\(^{100,107}\). Also there is evidence that physical exercise favors the expression of tumor suppressor protein p53, which is down-regulated in many types of cancer, through epigenetic mechanisms including miRNAs\(^{18}\). To conclude, exercise may prevent the progression of carcinogenesis and improve cancer survival through its influence on the epigenetic regulation of either tumor suppressor genes or the inflammatory processes.

Exercise-regulated epigenetic mechanisms in aging process

Aging is a natural process that is usually associated with numerous pathologies and homeostatic deregulations. It is known that epigenetic mechanisms are involved in the pathogenesis of some of the age-related diseases. Wilson et al.\(^{102}\) and Tra et al.\(^{103}\) have shown that a general demethylation pattern, causing genomic instability, is associated with the aging process. The essential role of microRNAs in the aging process has been also indicated, in regard to the manifestation of many pathological situations\(^{104}\). Furthermore, aging is usually associated with great shortening of telomeres that can lead to cellular damage\(^{105}\). Telomeres are sequences of nucleotides at the ends of chromosomes that protect their integrity and are shortened with each successive cell division\(^{106}\). It has been shown that telomeres are transcribed in order to express non-coding RNAs that may regulate telomere length and chromatin status\(^{107}\), indicating that epigenetic modifications can alter telomeres’ length. There are studies, both in animal models\(^{108,110}\) and in humans\(^{109}\), suggesting that physical exercise is an inducer of telomerase activity and gene transcription, coding for proteins that stabilize telomeres, through epigenetic mechanisms. Further, it has been shown that in some cases physical exercise increases the methylation status of DNA, causes histone modifications and induces the production of miRNAs\(^{2}\). All these effects constitute epigenetic modifications that can restore, to some extent, the deregulation of the right epigenetic pattern during aging process.

Another family of molecules related to aging is sirtuins\(^{111}\). Sirtuins constitute a highly conserved family of proteins with a possible key role in cell survival\(^{112}\), since they are associated with a variety of cellular functions, such as cell cycle regulation, cell survival and life span extension. Sirtuins not only deacetylate histones and several transcriptional regulators in the nucleus, but also modulate specific proteins in the cyto-
plasm and in mitochondria\textsuperscript{137}. It has been shown that Sirt1 is activated by an epigenetic regulatory mechanism including the miRNA miR-134, and is associated with synaptic plasticity and memory formation in mice\textsuperscript{136-138}. Recent studies, reviewed in\textsuperscript{136}, indicate that physical exercise has different effects on Sirt1 activity, depending on the type of exercise and on the part of the animal or human body where the Sirt1 activity was measured. It is supposed that physical exercise regulates, probably through epigenetic mechanisms, the Sirt1 activity which, in turn, regulates important signaling molecules, such as PGC-1\textalpha, p53, NF-\kappaB (nuclear factor kappa-light-chain-enhancer of activated B cells) and other transcriptional factors. All these molecules play a key role in cellular energy metabolism, gene transcription and, consequently, in cell survival. Thus, it has been proposed that exercise could have some beneficial neurophysiological effects and promote successful brain aging, with as less as possible neurodegenerative dysfunctions\textsuperscript{136}.

In addition, there are several age-related diseases such as rheumatoid arthritis\textsuperscript{137}, atherosclerosis\textsuperscript{138}, and type II diabetes\textsuperscript{139} which are associated with chronic inflammation. Chronic exercise training may reduce the expression of pro-inflammatory cytokines through epigenetic modifications and, therefore, help against chronic inflammatory diseases\textsuperscript{140}. Lastly, although aging is usually related to increased frailty, as a result of the aging of muscles, however, epigenetic mechanisms induced by exercise regulate the expression of myogenic regulatory factors, such as Myogenin, MyoD, Myf5 and MRF4, which are associated with muscle atrophy prevention and muscle growth\textsuperscript{141}.

**Exercise-induced epigenetic alterations in central nervous system**

Various studies in the last few years have revealed new evidence that strongly indicate an important role of exercise on brain plasticity and cognition. Those effects of exercise are mainly mediated through the actions of brain-derived neurotrophic factor (BDNF), a neurotrophin which is highly expressed in hippocampus and contributes to neuronal development\textsuperscript{142}. In particular, it has been shown that BDNF is associated not only with the effect of exercise on brain plasticity\textsuperscript{141,142}, but also is involved in neuronal excitability, and particularly in the functions of learning and memory\textsuperscript{143-147}. Moreover, it can act as a mediator between metabolism and brain plasticity, because it is regulated by protein molecules, such as AMPK, which have been shown to be up-regulated by physical exercise in rats\textsuperscript{128}.

Among the BDNF promoters, the promoter IV is subjected to epigenetic regulation and is related to neuronal activity, learning and memory functions\textsuperscript{148}. Methyl-CpG-binding protein (MeCP2) contributes to the gene-silencing effect of DNA methylation\textsuperscript{139} and, in the absence of stimulation, occupies a site on the BDNF promoter IV, thus resulting in the repression of BDNF transcription\textsuperscript{119}. Neuronal depolarization dissociates MeCP2 from the BDNF promoter IV, resulting in the promoter’s demethylation and BDNF transcription, modification and release\textsuperscript{121}. This eventually leads to the binding and activation of its tyrosine kinase receptor (TrkB) at both pre- and postsynaptic sites, which, in turn, results in the activation of MAPK cascade\textsuperscript{122}. Vaynman et al.\textsuperscript{121} have shown that exercise is likely to establish a positive feedback loop through transcriptional regulation, which results in increasing the mRNA levels of both BDNF and its receptor (TrkB).

Exercise has been shown to induce an increase in BDNF levels in the hippocampus of mouse, a vital area for learning and memory formation\textsuperscript{133-135}. Vaynman et al.\textsuperscript{121} have suggested that the impact of exercise on BDNF, which in turn is associated with hippocampal synaptic plasticity, learning and memory, is mediated by the calcium/calmodulin-dependent protein kinase II (CaMKII) signaling system and by the transcription regulator CAMP response element binding protein (CREB) in rats.

Interestingly, Gomez-Pinilla et al.\textsuperscript{136} showed that physical exercise engages epigenetic mechanisms to promote stable elevations in BDNF expression in rats. Specifically, the finding of that study indicated that exercise reduces methylation of CpG in BDNF promoter IV and affects the MeCP2 level in conjunction with BDNF. Furthermore, it was shown that exercise induces acetylation of histone H3 in the BDNF promoter IV, without changing the acetylation status of total histone H3. However, the acetylation of histone H3 along with a reduction of HDAC5 levels result in the transcription of BDNF gene, indicating that H3 is an important molecule which mediates epigenetic regulations following exercise.

In addition, Gomez-Pinilla et al.\textsuperscript{136} found that exercise elevated the phosphorylation levels of CREB and CaMKII. The activated (phosphorylated) CaMKII accelerates the phosphorylation of CREB, which can recruit CREB-binding protein (CBP). These molecules have strong histone acetylation transferase-promoting activity and, in their turn, activate BDNF transcription. Specifically, CBP functions not only as a molecular scaffold for components of the transcriptional machinery, but it has also the ability to regulate gene expression through its histone acetyltransferase activity, thus inducing chromatin remodeling and activating BDNF transcription. Further, it should be noted that various studies have revealed the importance of the HAT activity of CBP in the transfer of short-term memory to long-term memory in rats, humans and non-human primates\textsuperscript{137,138}.

The above described exercise-induced effects are likely to contribute to the promotion of mental health and resistance to neurological disorders and brain syndromes, since many of them, such as Alzheimer, depression, manic episodes, bipolar disorder, REM sleep deprivation, and attention deficit hyperactivity disorder (ADHD) are caused by the lack of BDNF\textsuperscript{211,140-143}. More specifically, Archer et al.\textsuperscript{144} have shown that physical exercise alleviates the symptoms of ADHD. It has been previously indicated\textsuperscript{136}, that it has a beneficial effect on remodeling the chromatin region which contains BDNF gene, making it accessible to the indispensable transcriptional factors and, thus, inducing the expression of BDNF. In this way, physical exercise, regardless of its type (i.e., endurance or resistance exer-
exercise), can partially restore the decreased levels of BDNF, improving both the neurobehavioral deficits and the biomarkers associated with ADHD\textsuperscript{144}. With regard to the REM sleep deprivation, Zagaar et al.\textsuperscript{145} studied the role of regular physical exercise on cognition in REM sleep deprived mice and found that regular exercise prevents impairments in short-term memory and hippocampal E-LTP caused by sleep deprivation. Thus, exercise-induced compensatory mechanisms, regulated by epigenetic modifications\textsuperscript{136}, prevent down-regulating changes in the basal and post-stimulation levels of P-CaMKII and BDNF, which are associated with sleep deprivation\textsuperscript{145}.

Another area, where physical exercise has positive effects by up-regulating the BDNF levels, is neurogenesis. Data from a genetically modified mouse model indicated a strong association of BDNF with the epigenetic mechanisms by which exercise stimulates adult neurogenesis\textsuperscript{146}. Indeed, the survival and the integration of the newborn neurons in adult rat brain rely on the good functioning of BDNF/TrkB signaling\textsuperscript{147}. In this context, the positive impact of exercise on neurogenesis may be beneficial against various neurodegenerative disorders, such as Alzheimer’s disease.

Apart from up-regulating BDNF, exercise can also alter the activity of hippocampus by changing the HAT/HDAC ratio. As it has been shown in mice, exercise reduces HDAC activity and increases HAT activity in the hippocampus, thus increasing the HAT/HDAC ratio\textsuperscript{148}. This hyperacetylation status has been found to be associated with enhanced transcriptional activity\textsuperscript{4,27,149,155}. Also, there is evidence supporting that a loss of neuronal acetylation is associated with neurodegeneration, since under neurodegenerative conditions, there is a decrease of histone acetylation levels in mice\textsuperscript{156}. The loss of CBP-HAT activity results in a cascade of events towards neurodegeneration. Thus, the HAT/HDAC balance is disturbed in favor of HDAC availability and enzymatic function. In that context, exercise, which induces histone acetylation and restores HAT/HDAC balance, has been regarded as an important strategy in neuroprotection and memory function\textsuperscript{157}, in order to prevent or accelerate recovery in neurodegenerative diseases\textsuperscript{158,159}.

All the above taken together, it could be suggested that exercise increases synaptic integrity and neuroplasticity in the brain, and simultaneously improves memory, learning and stress responses\textsuperscript{160}. (Figure 3). Collins et al.\textsuperscript{155} have also provided evidence that exercise enhances epigenetic mechanisms and gene expression in dentate gyrus of mice hippocampus, improving cognitive response to psychological stress. This occurs through increased phosphoacetylation of dentate histone H3 and higher c-Fos responses, which are caused by exercise. The phosphoacetylation of H3 and the induction of c-Fos are epigenetic responses that provoke gene expression changes in the dentate gyrus, where some of the neuroplasticity processes take place\textsuperscript{161}. Furthermore, exercise increases the expression of glucocorticoid receptors (GRs) and, thus, enhances the effect of stress-induced elevations of glucocorticoid hormone levels in rodents\textsuperscript{161,162}. Hence, physical exercise causes epigenetic modifications, which regulate the transcriptional mechanisms of several genes in the brain, coordinating the adaptive behavioral responses to stressful events.

**Epigenetic effects of exercise on cardiovascular system**

Physical exercise exerts also a great impact on cardiovascular system\textsuperscript{163}. The molecular mechanisms that promote the necessary cardiovascular adaptations include an increase in free radicals in association with improved antioxidative activ-
ity, alterations in the composition and the architecture of the extracellular matrix, and epigenetic modifications\(^{164}\).

With regard to the epigenetic alterations, there is not enough evidence to establish a direct connection between epigenetic modifications and changes in heart and vessels induced by exercise, however, recent data indicate such a possibility. It has been shown that epigenetic modifications caused by physical exercise regulate the activity of genes which are responsible for the expression of pro-inflammatory cytokines, such as the ASC gene, the methylation of which is increased by exercise\(^{71}\). Epigenetic alterations can also regulate the binding of transcriptional factor NFκB to DNA, which is indispensable for various pro-inflammatory cytokines to be expressed\(^{165}\). The HDACs reinforce the NFκB-DNA binding, while HATs impair it\(^{166}\). Apart from that, transcriptional co-activators, like CREB-binding protein (CBP) and P300-CBP-associated factor (PCAF), can function as HATs and, thus, regulate the expression of pro-inflammatory cytokines\(^{167}\).

All these epigenetic modifications ensure the proper functions at the cellular level, because the inflammatory responses are balanced by the expression of anti-inflammatory genes\(^{168}\). However, it is possible that a deregulation of these epigenetic mechanisms can lead to various cardiovascular diseases, through changes in vessels that can ultimately result in the development of atherosclerosis and stenosis\(^{71,169}\). Deregulation of HAT/HDAC ratio, or of their function, can also lead to modified expression of matrix metalloproteinases (MMPs), which are related to pathological alterations of vascular walls\(^{170}\), to altered proliferation of endothelium myocytes in heart and vessels\(^{171}\), and even to lethal cardiomyopathy\(^{172}\). Regular physical exercise can have a protective role against cardiovascular diseases, by restoring HAT and HDAC activity to the normal condition, and by regulating these epigenetic mechanisms\(^{164}\).

In addition, miRNAs contribute to the process of myocardium remodeling through, as yet, not fully understood mechanism(s). Exercise training causes a non-pathological increase of the myocardial mass, resulting in cardiac hypertrophy and neo-angiogenesis – "the athlete’s heart"\(^{173}\). During the exercise-induced cardiac hypertrophy, new sarcomeres are added both in parallel and in series, increasing the length of the cardiac cells. This results in an increased ventricular stroke volume and cardiac output, which improves aerobic capacity\(^{16}\). It has been shown that aerobic exercise training modulates numerous miRNAs, which in turn regulate their target mRNAs and, thus, provoke the physiological cardiac hypertrophy, through different signaling pathways\(^{175}\). In animal models, aerobic exercise has been shown to cause a decrease in the expression of miRNA-1, -133a and -133b, which provoke an increase in the expression of the Ras homologue gene family-A (RhoA), the cell division control protein 42 (CDC42), the negative elongation factor A (NELFA) protein, and of the Wolf-Hirschhorn syndrome candidate 2 (Whsc2)\(^{174}\). In addition, aerobic exercise causes an increase in the levels of miRNA-29a, -29b and -29c, resulting in decreased expression of collagens I and III (COLIA1 and COLIIIA1)\(^{175}\), an increase in the expression of miRNA-27a and -27b, resulting in decreased levels of angiotensin-converting enzyme 1 (ACE)\(^{16}\), and a decrease in the levels of miRNA-143, which increases the expression of angiotensin-converting enzyme 2 (ACE2)\(^{16}\). The above effects promote the growth and differentiation of cardiac cells, the ventricle compliance, the anti-fibrosis and, eventually, the physiological cardiac hypertrophy\(^{174}\). Moreover, cardiac hypertrophy includes neo-angiogenesis as well and, in animals, it has been proposed that aerobic exercise up-regulates the expression of miRNA-126 which, in turn, decreases the expression of its target mRNAs (PI3KR2 and Spred-1). Thus, aerobic exercise promotes the cardiac angiogenesis through the VEGF pathway and its targets that converge in an increase in the angiogenic pathways MAPK and PI3K/Akt/eNOS\(^{175}\).

It should be noted that the signaling pathways that lead to cardiac hypertrophy and are induced by exercise protect the heart from fibrosis and pathological remodeling, and they are different from those that provoke pathological hypertrophy and may present a different expression pattern of miRNAs\(^{173,174}\). Taking into consideration that cardiac hypertrophy is a major problem in many cardiac diseases, either the enhancement of miRNAs via miRNA-mimics, or the silencing of miRNAs, via miRNA-antagonists, could be regarded as a helpful approach that may help the onset of new therapeutic strategies against cardiac diseases\(^{17,177}\). New data derived from animal models suggest that the targeted regulation of specific miRNAs might be also useful in therapeutic methods against vascular diseases\(^{59}\).

**Conclusions and prospects**

This review provides evidence for the role of epigenetic alterations induced by physical exercise in various physiological systems and pathologies. Those epigenetic modifications are crucial for the activation of signaling cascades associated with genes that regulate metabolism and energy consumption in skeletal muscle. They also regulate numerous molecular pathways related to inflammatory processes. Moreover, some epigenetic modifications that possibly occur due to physical exercise can have a positive effect on restoring the genomic stability in cells with carcinogenesis potential, as well as on partially restoring age deregulated epigenetic patterns. Further insight into the epigenetic mechanisms involved in the aging process and their regulation by physical exercise might reveal ways in which exercise could be used as a preventive and/or complementary therapeutic strategy against age-related diseases. Furthermore, epigenetic alterations have a significant effect on the limbic system and especially on hippocampus, while the cardiovascular system is also affected by epigenetic changes caused by exercise, however, the evidence available for a clear association between them is not robust. It is suggested that exercise-related epigenetic changes could have an important role in preventing and/or confronting various disorders, such as metabolic or neurodegenerative diseases, that are either directly or indirectly associated with deregulation of normal epigenetic procedures and affect many people worldwide. A profound understanding of human epigenetic procedures...
during physical exercise could explain, in a more global and integrated approach, the possible cross talking between cascades which are involved in the regulation of human physiological systems. In this context, exercise remains an essential factor for promoting important biological adaptations that have profound implications for public health.

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