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Review Article

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Epigenetic regulation on gene expression induced by physical exercise

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Abstract

It is well established that physical exercise modulates the function of many physiological systems, such as the musculoskeletal, the cardiovascular and the nervous system, by inducing various adaptations to the increased mechanical load and/or metabolic stress of exercise. Many of these changes occur through epigenetic alterations to DNA, such as histone modifications, DNA methylations, expression of microRNAs and changes of the chromatin structure. All these epigenetic alterations may have clinical relevance, thus playing an important role in the prevention and confrontation of neurophysiological disorders, metabolic syndrome, cardiovascular diseases and cancer. Herein we review the known epigenetic modifications induced by physical exercise in various physiological systems and pathologies, and discuss their potential clinical implications.

Keywords: Acetylation, DNA Methylation, Gene Silencing, Histone Modification, microRNAs

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[1]. 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)[2].

Histone modifications are post-translational alterations on the lysine-rich tail region of histones, especially of H3 and H4 histones. Histone acyltransferases (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[3,4], while the balance between HATs and HDACs determines the level of histone acetylation and, eventually, the level of transcription[4,5]. 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[6,7].

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[8]. 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[6,9], thus preventing the gene transcription.

Another mechanism of epigenetic regulation is mediated by miRNAs. MiRNAs are a group of small noncoding RNA mole-

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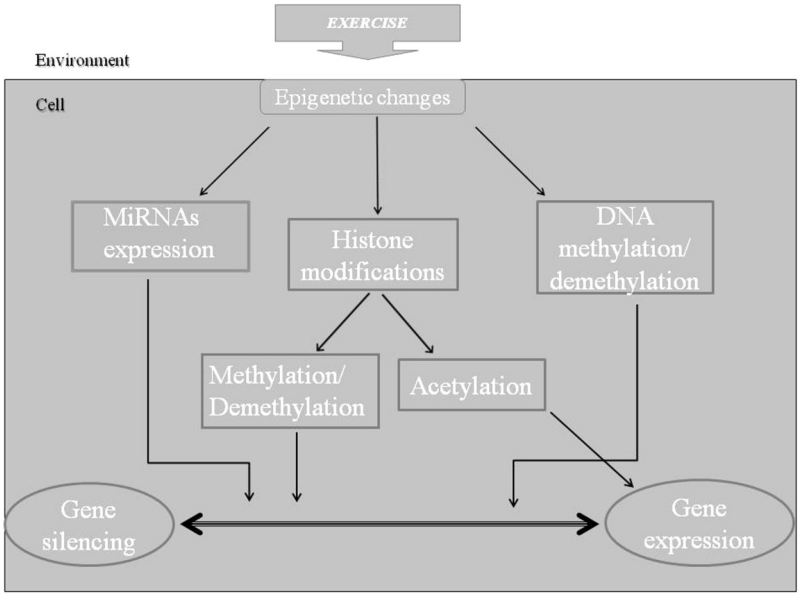

Figure 1. An overview of the possible epigenetic changes induced by exercise.

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-1a, TFAM, PPAR-δ, 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-γ), coactivator 1 alpha (PGC-1a), 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-δ), 3 hours after exercise. Similar results have been also observed in *ex vivo* models[1,8].

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+}/Calmodoulin-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 coactivators in mammalian skeletal muscle. Specifically for the mitochondrial genes, it has been suggested that AMPK acti-

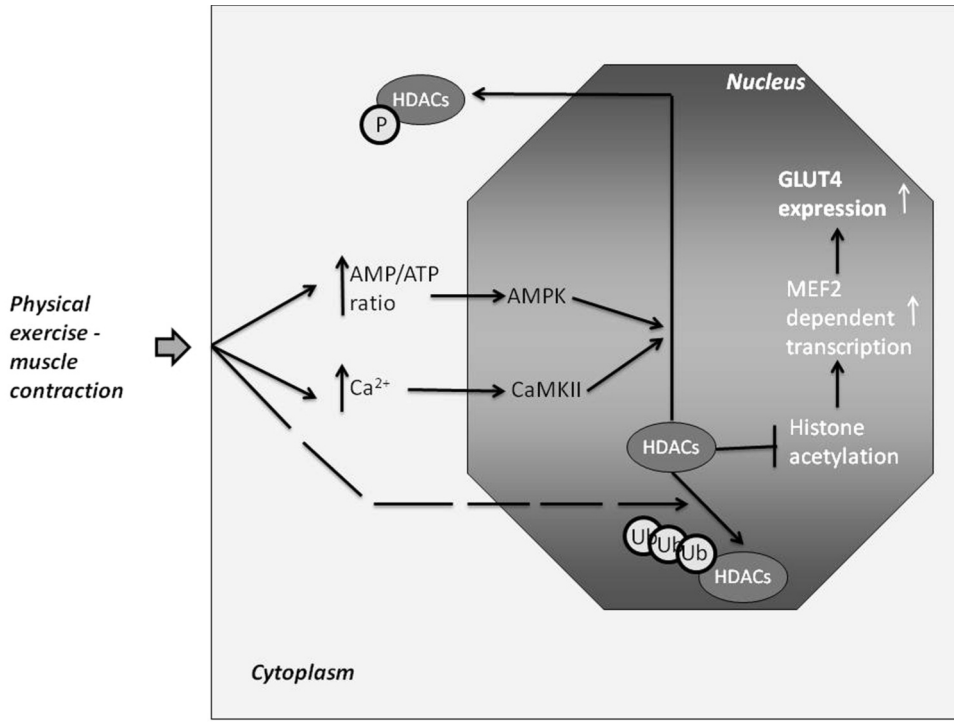

Figure 2. Histone modifications regulate glucose transporter type 4 (GLUT4) expression in response to exercise. AMPK: 5' AMP-dependent protein kinase; CaMKII: Ca2+/calmodulin-dependent protein kinases II; HDAC: histone deacetylase; MEF2: myocyte-specific enhancer factor-2; Ub: ubiquitin-binding domain.

vates the PGC-1α co-activator, which increases the expression of other transcription factors that, in turn, lead to the transcriptional changes[23]. 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[24,25] 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[5] and regulated by neuromuscular activity[4], while their action, particularly at the promoter's region, is reduced by exercise. These regulations occur through an ubiquitin-mediated proteasomal degradation[26,27], and through phosphorylation by CaMKII[28,29], AMPK[30], or protein kinase D (PKD)[27,31,32], 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[33], by creating a complex containing HDAC3, which removes acetyl groups[34]. In this way, HDACs regulate the expression of oxidative genes[35], 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[30]. 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, PPARGC1a and HATs, acetylating GLUT4 and, thus, increasing its expression[30,36,37], (Figure 2). 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[38,39]. The regulation of MEF2 during endurance exercise was found to be independent of sex[37]. Moreover, HDACs can regulate the expression of PGC-1α, which is increased after exercise in an intensity-dependent manner[40], and is a key factor in the human muscle adaptation to exercise[41].

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[42]. Thus, exercise may have a beneficial effect on the prevention and confrontation of type 2 diabetes and other metabolic disorders[43,44] 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)[45]. In particular, acetylation and methylation of histone H3 at specific states is related to a differential expression of I MHC, IIx MHC and IIb MHC genes

in mouse soleus muscle following reduced muscular activity (muscle deloading), as a result of changes in the chromatin structure[46,47]. Moreover, HDAC5 has been found to increase the number of type I oxidative fibers following exercise in mice[26]. Also, it has been shown that the percentage of type I muscle fibers and the maximal aerobic capacity (VO_2 max) in humans are positively correlated with the expression of the acetyltranspherase MYST4 (monocytic leukemia zinc finger protein-related factor)[48], a HAT that regulates the expression of Runt-domain transcription factor (RUNX2)[49], which is involved in osteoblast differentiation and bone formation[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[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)[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[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[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[55]. 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[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 cytoplasmic or nuclear complexes[57,58], and also affect the process of angiogenesis which naturally occurs in skeletal muscle after physical exercise training[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[60].

Exercise-induced epigenetic regulation of inflammatory processes

It is well known that exercise is associated with inflammatory responses[61-63]. Apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) is a mediator of the cytosol-type inflammatory signaling pathway[64,65]. It activates procaspase-1[66] and promotes the activation of interleukins[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[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 monocytic cells[71] and, thus, preventing the activation of inflammatory cytokines, such as interleukins and tumor necrosis factors (TNF)[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)[73,74]. As far as neutrophils are concerned, Shlomit et al.[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[75]. The second one was the janus kinase-signal transducer and activator of transcription (Jak-STAT) pathway, which is known to modify granulopoeiesis, neutrophil immune function and apoptosis[76,77]. The third one was the Hedgehog pathway, which is thought to have a role in chronic inflammation[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[74]. Some of those pathways play an important role in the regulation of pro- and anti-inflammatory

cytokines during exercise, such as MAPK and TGF pathways[79-81]. Other pathways are known to play a key role in cell communication[82] and in regulating the activation and differentiation of lymphocytes[83], while some others are associated with cancer and are likely to establish a link between physical exercise and cancer prevention[84]. Moreover, changes in individual miRNAs result in multiple effects, such as interactions between different types of miRNAs and a greater T-cell responsiveness along with reduced susceptibility to infection[85,86], regulation of toll-like receptors (TLRs) in monocytes[87,88] and T-regulatory lymphocytes[89], and down-regulation of DNA methylation in CD4+ T-cells[90]. All these effects are induced by exercise and can alter the pathogenesis and progression of diseases, such as systematic lupus erythematosus and rheumatoid arthritis, which are associated with some of the above mentioned epigenetic changes[91].

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[94]. 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[71]. 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 neoplasmatic 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, 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[100,101]. 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[113]. 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[114-116]. Recent studies, reviewed in[106], 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-1a, p53, NF-κB (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[106].

In addition, there are several age-related diseases such as rheumatoid arthritis[117], atherosclerosis[118], and type II diabetes[119] 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[72]. 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[18].

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[120]. In particular, it has been shown that BDNF is associated not only with the effect of exercise on brain plasticity[121-123], but also is involved in neuronal excitability, and particularly in the functions of learning and memory[124-127]. 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[128].

Among the BDNF promoters, the promoter IV is subjected to epigenetic regulation and is related to neuronal activity, learning and memory functions[6]. Methyl-CpG-binding protein (MeCP2) contributes to the gene-silencing effect of DNA methylation[129] and, in the absence of stimulation, occupies a site on the BDNF promoter IV, thus resulting in the repression of BDNF transcription[130]. Neuronal depolarization dissociates MeCP2 from the BDNF promoter IV, resulting in the promoter's demethylation and BDNF transcription, modification and release[131]. This eventually leads to the binding and activation of its tyrosine kinase receptor (TrkB) at both pre- and post-synaptic sites, which, in turn, results in the activation of MAPK cascade[132]. Vaynman et al.[133] 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[133-135]. Vaynman et al.[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/calmodoulin-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.[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.[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[137-139].

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[121,140-143]. More specifically, Archer et al.[144] have shown that physical exercise alleviates the symptoms of ADHD. It has been previously indicated[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-

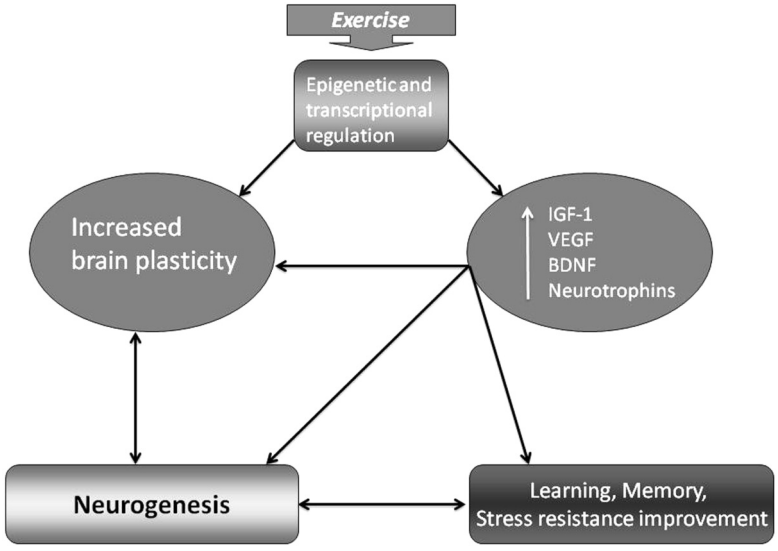

Figure 3. A proposed model for the effect of exercise on molecular, neuroplastic and cognitive patterns through epigenetics. IGF-1: Insulin-like growth factor-1; VEGF: Vascular endothelial growth factor; BDNF: Brain-derived neurotrophic factor.

cise), can partially restore the decreased levels of BDNF, improving both the neurobehavioral deficits and the biomarkers associated with ADHD[144]. With regard to the REM sleep deprivation, Zagaar et al.[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[136], prevent down-regulating changes in the basal and post-stimulation levels of P-CaMKII and BDNF, which are associated with sleep deprivation[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[146]. Indeed, the survival and the integration of the newborn neurons in adult rat brain rely on the good functioning of BDNF/TrkB signaling[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[148]. This hyperacetylation status has been found to be associated with enhanced transcriptional activity[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[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[157], in order to prevent or accelerate recovery in neurodegenerative diseases[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[160], (Figure 3). Collins et al.[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[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[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[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 modulations 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"[17]. 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[173]. 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 (COLIAI and COLIIIAI)[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]. All 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 upregulates 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[176].

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 hopeful 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.

References

1. Barres R, Yan J, Egan B, et al. Acute exercise remodels promoter methylation in human skeletal muscle. Cell Metab 2012;15(3):405-11.
2. A Eccleston ND, C Gunter, B Marte, D Nath. Epigenetics. Nature Insight 2007;447(7143):396-440.
3. Li B, Carey M, Workman JL. The role of chromatin during transcription. Cell 2007;128(4):707-19.
4. McGee SL, Hargreaves M. Histone modifications and exercise adaptations. J Appl Physiol 2011;110(1):258-63.
5. McKinsey TA, Zhang CL, Olson EN. Control of muscle development by dueling HATs and HDACs. Curr Opin Genet Dev 2001;11(5): 497-504.
6. Feng J, Fouse S, Fan G. Epigenetic regulation of neural gene expression and neuronal function. Pediatr Res 2007; 61(5 Pt 2):58R-63R.
7. Rice JC, Allis CD. Histone methylation versus histone acetylation: new insights into epigenetic regulation. Curr Opin Cell Biol 2001;13(3):263-73.
8. Doi A, Park IH, Wen B, et al. Differential methylation of tissue- and cancer-specific CpG island shores distinguishes human induced pluripotent stem cells, embryonic stem cells and fibroblasts. Nat Genet 2009;41(12):1350-3.
9. Phillips T. The role of methylation in gene expression. Nature Education 2008;1(1).
10. Baek D, Villen J, Shin C, et al. The impact of microRNAs on protein output. Nature 2008;455(7209):64-71.
11. Baltimore D, Boldin MP, O'Connell RM, et al. MicroRNAs: new regulators of immune cell development and function. Nat Immunol 2008;9(8):839-45.
12. Duursma AM, Kedde M, Schrier M, et al. miR-148 targets human DNMT3b protein coding region. RNA 2008; 14(5):872-7.
13. Alvarez-Saavedra M, Antoun G, Yanagiya A, et al. miRNA-132 orchestrates chromatin remodeling and translational control of the circadian clock. Hum Mol Genet 20(4):731-51.
14. Lindsay MA. microRNAs and the immune response. Trends Immunol 2008;29(7):343-51.
15. Asirvatham AJ, Magner WJ, Tomasi TB. miRNA regulation of cytokine genes. Cytokine 2009;45(2):58-69.
16. Fernandes T, Hashimoto NY, Magalhaes FC, et al. Aerobic exercise training-induced left ventricular hypertrophy involves regulatory MicroRNAs, decreased angiotensin-converting enzyme-angiotensin ii, and synergistic regulation of angiotensin-converting enzyme 2-angiotensin (1-7). Hypertension 2011;58(2):182-9.
17. Ellison GM, Waring CD, Vicinanza C, Torella D. Physiological cardiac remodelling in response to endurance exercise training: cellular and molecular mechanisms. Heart 2012;98(1):5-10.
18. Sanchis-Gomar F, Garcia-Gimenez JL, Perez-Quilis C, et al. Physical exercise as an epigenetic modulator: Eustress, the "positive stress" as an effector of gene expression. J Strength Cond Res 2012;26(12):3469-3472.
19. Buford TW, Cooke MB, Shelmadine BD, et al. Effects of eccentric treadmill exercise on inflammatory gene expression in human skeletal muscle. Appl Physiol Nutr Metab 2009;34(4):745-753.
20. Harber MP, Konopka AR, Jemiolo B, et al. Muscle protein synthesis and gene expression during recovery from aerobic exercise in the fasted and fed states. Am J Physiol Regul Integr Comp Physiol 2010;299(5):R1254-1262.
21. Drummond MJ, Fujita S, Abe T, et al. Human muscle gene expression following resistance exercise and blood flow restriction. Med Sci Sports Exerc 2008;40(4):691-8.
22. Jorgensen SB, Richter EA, Wojtaszewski JF. Role of AMPK in skeletal muscle metabolic regulation and adaptation in relation to exercise. J Physiol 2006;574(Pt 1): 17-31.
23. Scarpulla RC. Nuclear activators and coactivators in mammalian mitochondrial biogenesis. Biochim Biophys Acta 2002;1576(1-2):1-14.
24. Michael LF, Wu Z, Cheatham RB, et al. Restoration of insulin-sensitive glucose transporter (GLUT4) gene expression in muscle cells by the transcriptional coactivator PGC-1. Proc Natl Acad Sci U S A 2001;98(7):3820-5.
25. Baar K, Song Z, Semenkovich CF, et al. Skeletal muscle overexpression of nuclear respiratory factor 1 increases glucose transport capacity. FASEB J 2003;17(12):1666-73.
26. Potthoff MJ, Wu H, Arnold MA, et al. Histone deacetylase degradation and MEF2 activation promote the formation of slow-twitch myofibers. J Clin Invest 2007; 117(9):2459-67.
27. McGee SL, Fairlie E, Garnham AP, Hargreaves M. Exercise-induced histone modifications in human skeletal muscle. J Physiol 2009;587(Pt 24):5951-8.
28. Backs J, Backs T, Bezprozvannaya S, et al. Histone deacetylase 5 acquires calcium/calmodulin-dependent kinase II responsiveness by oligomerization with histone deacetylase 4. Mol Cell Biol 2008;28(10):3437-45.
29. McKinsey TA, Zhang CL, Lu J, Olson EN. Signal-dependent nuclear export of a histone deacetylase regulates muscle differentiation. Nature 2000;408(6808):106-11.
30. McGee SL, Hargreaves M. Exercise and skeletal muscle glucose transporter 4 expression: molecular mechanisms. Clin Exp Pharmacol Physiol 2006;33(4):395-9.
31. Chang S, Bezprozvannaya S, Li S, Olson EN. An expression screen reveals modulators of class II histone deacetylase phosphorylation. Proc Natl Acad Sci U S A 2005; 102(23):8120-8125.
32. Kim MS, Fielitz J, McAnally J, et al. Protein kinase D1 stimulates MEF2 activity in skeletal muscle and enhances

muscle performance. Mol Cell Biol 2008;28(11):3600-9.
33. Lu J, McKinsey TA, Zhang CL, Olson EN. Regulation of skeletal myogenesis by association of the MEF2 transcription factor with class II histone deacetylases. Mol Cell 2000;6(2):233-44.
34. Fischle W, Dequiedt F, Hendzel MJ, et al. Enzymatic activity associated with class II HDACs is dependent on a multiprotein complex containing HDAC3 and SMRT/N-CoR. Mol Cell 2002;9(1):45-57.
35. Czubryt MP, McAnally J, Fishman GI, Olson EN. Regulation of peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1 alpha) and mitochondrial function by MEF2 and HDAC5. Proc Natl Acad Sci U S A 2003;100(4):1711-6.
36. McGee SL, Sparling D, Olson AL, Hargreaves M. Exercise increases MEF2- and GEF DNA-binding activity in human skeletal muscle. FASEB J 2006;20(2):348-9.
37. Vissing K, McGee SL, Roepstorff C, et al. Effect of sex differences on human MEF2 regulation during endurance exercise. Am J Physiol Endocrinol Metab 2008;294(2): E408-15.
38. Smith JA, Kohn TA, Chetty AK, Ojuka EO. CaMK activation during exercise is required for histone hyperacetylation and MEF2A binding at the MEF2 site on the Glut4 gene. Am J Physiol Endocrinol Metab 2008;295(3): E698-704.
39. Smith JA, Collins M, Grobler LA, et al. Exercise and CaMK activation both increase the binding of MEF2A to the Glut4 promoter in skeletal muscle *in vivo*. Am J Physiol Endocrinol Metab 2007;292(2):E413-20.
40. Egan B, Carson BP, Garcia-Roves PM, et al. Exercise intensity-dependent regulation of peroxisome proliferator-activated receptor coactivator-1 mRNA abundance is associated with differential activation of upstream signalling kinases in human skeletal muscle. J Physiol 2010; 588(Pt 10):1779-90.
41. Olesen J, Kiilerich K, Pilegaard H. PGC-1alpha-mediated adaptations in skeletal muscle. Pflugers Arch 2010; 460(1):153-62.
42. Barres R, Osler ME, Yan J, et al. Non-CpG methylation of the PGC-1alpha promoter through DNMT3B controls mitochondrial density. Cell Metab 2009;10(3):189-198.
43. Ling C, Groop L. Epigenetics: a molecular link between environmental factors and type 2 diabetes. Diabetes 2009; 58(12):2718-25.
44. Barres R, Zierath JR. DNA methylation in metabolic disorders. Am J Clin Nutr 2011;93(4):897S-900.
45. Baar K. Epigenetic control of skeletal muscle fibre type. Acta Physiol (Oxf) 2010;199(4):477-87.
46. Pandorf CE, Haddad F, Wright C, et al. Differential epigenetic modifications of histones at the myosin heavy chain genes in fast and slow skeletal muscle fibers and in response to muscle unloading. Am J Physiol Cell Physiol 2009;297(1):C6-16.
47. Zwetsloot KA, Laye MJ, Booth FW. Novel epigenetic regulation of skeletal muscle myosin heavy chain genes. Focus on "Differential epigenetic modifications of histones at the myosin heavy chain genes in fast and slow skeletal muscle fibers and in response to muscle unloading". Am J Physiol Cell Physiol 2009;297(1):C1-3.
48. Parikh H, Nilsson E, Ling C, et al. Molecular correlates for maximal oxygen uptake and type 1 fibers. Am J Physiol Endocrinol Metab 2008;294(6):E1152-9.
49. Pelletier N, Champagne N, Stifani S, Yang XJ. MOZ and MORF histone acetyltransferases interact with the Runt-domain transcription factor Runx2. Oncogene 2002; 21(17):2729-40.
50. Lian JB, Javed A, Zaidi SK, et al. Regulatory controls for osteoblast growth and differentiation: role of Runx/Cbfa/AML factors. Crit Rev Eukaryot Gene Expr. 2004; 14(1-2): 1-41.
51. Guller I, Russell AP. MicroRNAs in skeletal muscle: their role and regulation in development, disease and function. J Physiol 588(Pt 21):4075-87.
52. Guller I, Russell AP. MicroRNAs in skeletal muscle: their role and regulation in development, disease and function. J Physiol 2010;588(Pt 21):4075-87.
53. Uhlemann M, Mobius-Winkler S, Fikenzer S, et al. Circulating microRNA-126 increases after different forms of endurance exercise in healthy adults. Eur J Prev Cardiol 2012.
54. Baggish AL, Hale A, Weiner RB, et al. Dynamic regulation of circulating microRNA during acute exhaustive exercise and sustained aerobic exercise training. J Physiol 2011;589(Pt 16):3983-94.
55. Keller P, Vollaard NB, Gustafsson T, et al. A transcriptional map of the impact of endurance exercise training on skeletal muscle phenotype. J Appl Physiol 2011; 110(1):46-59.
56. Drummond MJ, McCarthy JJ, Fry CS, et al. Aging differentially affects human skeletal muscle microRNA expression at rest and after an anabolic stimulus of resistance exercise and essential amino acids. Am J Physiol Endocrinol Metab. 2008; 295(6): E1333-1340.
57. Aoi W, Naito Y, Mizushima K, et al. The microRNA miR-696 regulates PGC-1{alpha} in mouse skeletal muscle in response to physical activity. Am J Physiol Endocrinol Metab 2010;298(4):E799-806.
58. Safdar A, Abadi A, Akhtar M, et al. miRNA in the regulation of skeletal muscle adaptation to acute endurance exercise in C57Bl/6J male mice. PLoS One 2009; 4(5):e5610.
59. Fernandes T, Magalhaes FC, Roque FR, et al. Exercise training prevents the microvascular rarefaction in hypertension balancing angiogenic and apoptotic factors: role of microRNAs-16, -21, and -126. Hypertension 2012; 59(2):513-20.
60. Nielsen S, Scheele C, Yfanti C, et al. Muscle specific microRNAs are regulated by endurance exercise in human skeletal muscle. J Physiol 2010;588(Pt 20):4029-4037.
61. Abramson JL, Vaccarino V. Relationship between physical activity and inflammation among apparently healthy

middle-aged and older US adults. Archives of internal medicine 2002;162(11):1286-92.
62. Autenrieth C, Schneider A, Doring A, et al. Association between different domains of physical activity and markers of inflammation. Medicine and science in sports and exercise 2009;41(9):1706-13.
63. Thomas NE, Williams DR. Inflammatory factors, physical activity, and physical fitness in young people. Scandinavian journal of medicine & science in sports 2008; 18(5):543-56.
64. Masumoto J, Taniguchi S, Ayukawa K, et al. ASC, a novel 22-kDa protein, aggregates during apoptosis of human promyelocytic leukemia HL-60 cells. The Journal of biological chemistry 1999;274(48):33835-8.
65. Taniguchi S, Sagara J. Regulatory molecules involved in inflammasome formation with special reference to a key mediator protein, ASC. Seminars in immunopathology. 2007;29(3):231-8.
66. Stehlik C, Lee SH, Dorfleutner A, et al. Apoptosis-associated speck-like protein containing a caspase recruitment domain is a regulator of procaspase-1 activation. J Immunol 2003;171(11):6154-63.
67. Mariathasan S, Monack DM. Inflammasome adaptors and sensors: intracellular regulators of infection and inflammation. Nature reviews Immunology 2007;7(1):31-40.
68. Yamamoto M, Yaginuma K, Tsutsui H, et al. ASC is essential for LPS-induced activation of procaspase-1 independently of TLR-associated signal adaptor molecules. Genes to cells : devoted to molecular & cellular mechanisms 2004;9(11):1055-67.
69. Guan X, Sagara J, Yokoyama T, et al. ASC/TMS1, a caspase-1 activating adaptor, is downregulated by aberrant methylation in human melanoma. International journal of cancer Journal international du cancer 2003;107(2):202-8.
70. Yokoyama T, Sagara J, Guan X, et al. Methylation of ASC/TMS1, a proapoptotic gene responsible for activating procaspase-1, in human colorectal cancer. Cancer letters 2003;202(1):101-8.
71. Nakajima K, Takeoka M, Mori M, et al. Exercise effects on methylation of ASC gene. Int J Sports Med 2010; 31(9):671-5.
72. Taxman DJ, Zhang J, Champagne C, et al. Cutting edge: ASC mediates the induction of multiple cytokines by Porphyromonas gingivalis via caspase-1-dependent and -independent pathways. J Immunol 2006;177(7):4252-6.
73. Radom-Aizik S, Zaldivar F Jr, Oliver S, et al. Evidence for microRNA involvement in exercise-associated neutrophil gene expression changes. J Appl Physiol 2010; 109(1):252-61.
74. Radom-Aizik S, Zaldivar F Jr, Leu SY, et al. Effects of exercise on microRNA expression in young males peripheral blood mononuclear cells. Clinical and translational science 2012;5(1):32-8.
75. Skaug B, Jiang X, Chen ZJ. The role of ubiquitin in NF-kappaB regulatory pathways. Annual review of biochemistry 2009;78:769-96.
76. Fortin CF, Larbi A, Dupuis G, et al. GM-CSF activates the Jak/STAT pathway to rescue polymorphonuclear neutrophils from spontaneous apoptosis in young but not elderly individuals. Biogerontology 2007;8(2):173-87.
77. O'Shea JJ, Murray PJ. Cytokine signaling modules in inflammatory responses. Immunity 2008;28(4):477-87.
78. Benson RA, Lowrey JA, Lamb JR, Howie SE. The Notch and Sonic hedgehog signalling pathways in immunity. Molecular immunology. 2004;41(6-7):715-25.
79. Hoene M, Weigert C. The stress response of the liver to physical exercise. Exercise immunology review 2010; 16:163-83.
80. Matsakas A, Patel K. Skeletal muscle fibre plasticity in response to selected environmental and physiological stimuli. Histology and histopathology 2009;24(5):611-29.
81. Kjaer M, Langberg H, Heinemeier K, et al. From mechanical loading to collagen synthesis, structural changes and function in human tendon. Scandinavian journal of medicine & science in sports 2009;19(4):500-10.
82. Bopp T, Radsak M, Schmitt E, Schild H. New strategies for the manipulation of adaptive immune responses. Cancer immunology, immunotherapy: CII 2010;59(9):1443-8.
83. Oh-hora M. Calcium signaling in the development and function of T-lineage cells. Immunological reviews 2009; 231(1):210-24.
84. Walsh NP, Gleeson M, Shephard RJ, et al. Position statement. Part one: Immune function and exercise. Exercise immunology review 2011;17:6-63.
85. Li QJ, Chau J, Ebert PJ, et al. miR-181a is an intrinsic modulator of T cell sensitivity and selection. Cell 2007; 129(1):147-61.
86. Simpson RJ, Guy K. Coupling aging immunity with a sedentary lifestyle: has the damage already been done? - a mini-review. Gerontology 2010;56(5):449-58.
87. Taganov KD, Boldin MP, Chang KJ, Baltimore D. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proceedings of the National Academy of Sciences of the United States of America 2006;103(33): 12481-6.
88. O'Neill LA, Sheedy FJ, McCoy CE. MicroRNAs: the fine-tuners of Toll-like receptor signalling. Nature reviews Immunology 2011;11(3):163-75.
89. Rouas R, Fayyad-Kazan H, El Zein N, et al. Human natural Treg microRNA signature: role of microRNA-31 and microRNA-21 in FOXP3 expression. European journal of immunology 2009;39(6):1608-18.
90. Pan W, Zhu S, Yuan M, et al. MicroRNA-21 and microRNA-148a contribute to DNA hypomethylation in lupus CD4+ T cells by directly and indirectly targeting DNA methyltransferase 1. J Immunol 2010;184(12): 6773-81.
91. Dai R, Ahmed SA. MicroRNA, a new paradigm for understanding immunoregulation, inflammation, and autoimmune diseases. Translational research : the journal of laboratory and clinical medicine 2011;157(4):163-79.

92. Marmot M AT, Byers T, Chen J, Hirohata T, et al. Food, nutrition, physical activity, and the prevention of cancer: a global perspective. World Cancer Research Fund/American Institute for Cancer Research 2007: 517.
93. Kampert JB, Blair SN, Barlow CE, Kohl HW 3rd. Physical activity, physical fitness, and all-cause and cancer mortality: a prospective study of men and women. Annals of epidemiology 1996;6(5):452-7.
94. Hoffmann MJ, Schulz WA. Causes and consequences of DNA hypomethylation in human cancer. Biochemistry and cell biology = Biochimie et biologie cellulaire 2005; 83(3):296-321.
95. Zhang FF, Cardarelli R, Carroll J, et al. Physical activity and global genomic DNA methylation in a cancer-free population. Epigenetics: official journal of the DNA Methylation Society 2011;6(3):293-9.
96. Esteller M, Corn PG, Baylin SB, Herman JG. A gene hypermethylation profile of human cancer. Cancer research. 2001;61(8):3225-9.
97. Jones PA, Baylin SB. The fundamental role of epigenetic events in cancer. Nature reviews Genetics 2002;3(6):415-28.
98. Yuasa Y, Nagasaki H, Akiyama Y, et al. DNA methylation status is inversely correlated with green tea intake and physical activity in gastric cancer patients. International journal of cancer Journal international du cancer 2009; 124(11):2677-82.
99. Zeng H, Irwin ML, Lu L, et al. Physical activity and breast cancer survival: an epigenetic link through reduced methylation of a tumor suppressor gene L3MBTL1. Breast cancer research and treatment 2012;133(1):127-35.
100. Coyle YM, Xie XJ, Lewis CM, et al. Role of physical activity in modulating breast cancer risk as defined by APC and RASSF1A promoter hypermethylation in nonmalignant breast tissue. Cancer Epidemiol Biomarkers Prev 2007;16(2):192-6.
101. Klein CB, Leszczynska J. Estrogen-induced DNA methylation of E-cadherin and p16 in non-tumor breast cells. . Proc Am Assoc Cancer Res 2005;46:2744.
102. Wilson VL, Smith RA, Ma S, Cutler RG. Genomic 5-methyldeoxycytidine decreases with age. The Journal of biological chemistry 1987;262(21):9948-51.
103. Tra J, Kondo T, Lu Q, et al. Infrequent occurrence of age-dependent changes in CpG island methylation as detected by restriction landmark genome scanning. Mechanisms of ageing and development 2002;123(11):1487-503.
104. Crepaldi L, Riccio A. Chromatin learns to behave. Epigenetics: official journal of the DNA Methylation Society 2009;4(1):23-6.
105. Blackburn EH. Telomere states and cell fates. Nature. 2000;408(6808):53-6.
106. Kaliman P, Parrizas M, Lalanza JF, et al. Neurophysiological and epigenetic effects of physical exercise on the aging process. Ageing research reviews 2011;10(4):475-86.
107. Schoeftner S, Blasco MA. Chromatin regulation and non-coding RNAs at mammalian telomeres. Seminars in cell & developmental biology 2010;21(2):186-93.
108. Werner C, Hanhoun M, Widmann T, et al. Effects of physical exercise on myocardial telomere-regulating proteins, survival pathways, and apoptosis. Journal of the American College of Cardiology 2008;52(6):470-82.
109. Werner C, Furster T, Widmann T, et al. Physical exercise prevents cellular senescence in circulating leukocytes and in the vessel wall. Circulation 2009;120(24):2438-47.
110. Wolf SA, Melnik A, Kempermann G. Physical exercise increases adult neurogenesis and telomerase activity, and improves behavioral deficits in a mouse model of schizophrenia. Brain, behavior, and immunity 2011;25(5):971-80.
111. Longo VD, Kennedy BK. Sirtuins in aging and age-related disease. Cell 2006;126(2):257-68.
112. Pallas M, Verdaguer E, Tajes M, et al. Modulation of sirtuins: new targets for antiageing. Recent patents on CNS drug discovery 2008;3(1):61-9.
113. Houtkooper RH, Pirinen E, Auwerx J. Sirtuins as regulators of metabolism and healthspan. Nature reviews Molecular cell biology 2012;13(4):225-38.
114. Denu JM. Linking chromatin function with metabolic networks: Sir2 family of NAD(+)-dependent deacetylases. Trends in biochemical sciences 2003;28(1):41-8.
115. Guarente L, Kenyon C. Genetic pathways that regulate ageing in model organisms. Nature 2000;408(6809):255-62.
116. Gao J, Wang WY, Mao YW, et al. A novel pathway regulates memory and plasticity via SIRT1 and miR-134. Nature 2010;466(7310):1105-9.
117. Choy EH, Panayi GS. Cytokine pathways and joint inflammation in rheumatoid arthritis. N Engl J Med 2001; 344(12):907-16.
118. Calabro P, Willerson JT, Yeh ET. Inflammatory cytokines stimulated C-reactive protein production by human coronary artery smooth muscle cells. Circulation 2003; 108(16):1930-2.
119. Spranger J, Kroke A, Mohlig M, et al. Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. Diabetes 2003;52(3):812-7.
120. Binder DK, Scharfman HE. Brain-derived neurotrophic factor. Growth Factors 2004;22(3):123-31.
121. Vaynman S, Ying Z, Gomez-Pinilla F. Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. Eur J Neurosci 2004;20(10):2580-90.
122. Vasuta C, Caunt C, James R, et al. Effects of exercise on NMDA receptor subunit contributions to bidirectional synaptic plasticity in the mouse dentate gyrus. Hippocampus 2007;17(12):1201-8.
123. Lista I, Sorrentino G. Biological mechanisms of physical activity in preventing cognitive decline. Cell Mol Neurobiol 2009;30(4):493-503.
124. Martinowich K, Manji H, Lu B. New insights into BDNF function in depression and anxiety. Nat Neurosci 2007; 10(9):1089-93.
125. Berchtold NC, Chinn G, Chou M, et al. Exercise primes a molecular memory for brain-derived neurotrophic factor

protein induction in the rat hippocampus. Neuroscience 2005;133(3):853-61.
126. Boulanger LM, Poo MM. Presynaptic depolarization facilitates neurotrophin-induced synaptic potentiation. Nat Neurosci 1999;2(4):346-51.
127. Griffin EW, Bechara RG, Birch AM, Kelly AM. Exercise enhances hippocampal-dependent learning in the rat: evidence for a BDNF-related mechanism. Hippocampus 2009;19(10):973-80.
128. Gomez-Pinilla F, Vaynman S, Ying Z. Brain-derived neurotrophic factor functions as a metabotrophin to mediate the effects of exercise on cognition. Eur J Neurosci 2008; 28(11):2278-87.
129. Chao HT, Zoghbi HY. The yin and yang of MeCP2 phosphorylation. Proc Natl Acad Sci U S A 2009;106(12): 4577-8.
130. Martinowich K, Hattori D, Wu H, et al. DNA methylation-related chromatin remodeling in activity-dependent BDNF gene regulation. Science 2003;302(5646):890-3.
131. Chen WG, Chang Q, Lin Y, et al. Derepression of BDNF transcription involves calcium-dependent phosphorylation of MeCP2. Science 2003;302(5646):885-9.
132. Stephens L, Smrcka A, Cooke FT, et al. A novel phosphoinositide 3 kinase activity in myeloid-derived cells is activated by G protein beta gamma subunits. Cell 1994; 77(1):83-93.
133. Vaynman S, Ying Z, Gomez-Pinilla F. Interplay between brain-derived neurotrophic factor and signal transduction modulators in the regulation of the effects of exercise on synaptic-plasticity. Neuroscience 2003;122(3):647-57.
134. Neeper SA, Gomez-Pinilla F, Choi J, Cotman CW. Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain. Brain Res 1996;726(1-2):49-56.
135. Gomez-Pinilla F, Ying Z, Roy RR, et al. Voluntary exercise induces a BDNF-mediated mechanism that promotes neuroplasticity. J Neurophysiol 2002;88(5):2187-95.
136. Gomez-Pinilla F, Zhuang Y, Feng J, et al. Exercise impacts brain-derived neurotrophic factor plasticity by engaging mechanisms of epigenetic regulation. Eur J Neurosci 2011;33(3):383-90.
137. Bachevalier J. Ontogenetic development of habit and memory formation in primates. Ann N Y Acad Sci 1990; 608:457-77; discussion 477-84.
138. McKee RD, Squire LR. On the development of declarative memory. J Exp Psychol Learn Mem Cogn 1993; 19(2):397-404.
139. Morris RG, Garrud P, Rawlins JN, O'Keefe J. Place navigation impaired in rats with hippocampal lesions. Nature 1982;297(5868):681-3.
140. Caraci F, Copani A, Nicoletti F, Drago F. Depression and Alzheimer's disease: neurobiological links and common pharmacological targets. Eur J Pharmacol 2010;626(1): 64-71.
141. Duman CH, Schlesinger L, Russell DS, Duman RS. Voluntary exercise produces antidepressant and anxiolytic behavioral effects in mice. Brain Res 2008;1199:148-58.
142. Lubin FD, Roth TL, Sweatt JD. Epigenetic regulation of BDNF gene transcription in the consolidation of fear memory. J Neurosci 2008;28(42):10576-86.
143. Tsankova NM, Berton O, Renthal W, et al. Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. Nat Neurosci 2006; 9(4):519-25.
144. Archer T, Kostrzewa RM. Physical exercise alleviates ADHD symptoms: regional deficits and development trajectory. Neurotox Res 2012;21(2):195-209.
145. Zagaar M, Alhaider I, Dao A, et al. The beneficial effects of regular exercise on cognition in REM sleep deprivation: behavioral, electrophysiological and molecular evidence. Neurobiol Dis 2011;45(3):1153-62.
146. Lafenetre P, Leske O, Ma-Hogemeie Z, et al. Exercise can rescue recognition memory impairment in a model with reduced adult hippocampal neurogenesis. Front Behav Neurosci 2010;3:34.
147. Lafenetre P, Leske O, Wahle P, Heumann R. The beneficial effects of physical activity on impaired adult neurogenesis and cognitive performance. Front Neurosci 2011; 5:51.
148. Elsner VR, Lovatel GA, Bertoldi K, et al. Effect of different exercise protocols on histone acetyltransferases and histone deacetylases activities in rat hippocampus. Neuroscience. 2011; 192: 580-587.
149. Kimura A, Matsubara K, Horikoshi M. A decade of histone acetylation: marking eukaryotic chromosomes with specific codes. J Biochem 2005;138(6):647-62.
150. Wade PA. Methyl CpG-binding proteins and transcriptional repression. Bioessays 2001;23(12):1131-7.
151. Rajendrasozhan S, Yang SR, Edirisinghe I, et al. Deacetylases and NF-kappaB in redox regulation of cigarette smoke-induced lung inflammation: epigenetics in pathogenesis of COPD. Antioxid Redox Signal 2008;10(4): 799-811.
152. Strahl BD, Allis CD. The language of covalent histone modifications. Nature 2000;403(6765):41-5.
153. Choi JK, Howe LJ. Histone acetylation: truth of consequences? Biochem Cell Biol 2009;87(1):139-50.
154. Laberge RM, Boissonneault G. On the nature and origin of DNA strand breaks in elongating spermatids. Biol Reprod 2005;73(2):289-96.
155. Collins A, Hill LE, Chandramohan Y, et al. Exercise improves cognitive responses to psychological stress through enhancement of epigenetic mechanisms and gene expression in the dentate gyrus. PLoS One 2009;4(1): e4330.
156. Saha RN, Pahan K. HATs and HDACs in neurodegeneration: a tale of disconcerted acetylation homeostasis. Cell Death Differ 2006;13(4):539-50.
157. Selvi BR, Cassel JC, Kundu TK, Boutillier AL. Tuning acetylation levels with HAT activators: therapeutic strategy in neurodegenerative diseases. Biochim Biophys Acta 2010;1799(10-12):840-53.

158. Kramer AF, Hahn S, Cohen NJ, et al. Ageing, fitness and neurocognitive function. Nature 1999;400(6743):418-9.
159. Mattson MP. Neuroprotective signaling and the aging brain: take away my food and let me run. Brain Res 2000;886(1-2):47-53.
160. Fischer A, Sananbenesi F, Wang X, et al. Recovery of learning and memory is associated with chromatin remodelling. Nature. 2007; 447(7141): 178-182.
161. Reul JM, Chandramohan Y. Epigenetic mechanisms in stress-related memory formation. Psychoneuroendocrinology.2007;32(Suppl.1):S21-25.
162. Droste SK, Chandramohan Y, Hill LE, et al. Voluntary exercise impacts on the rat hypothalamic-pituitary-adrenocortical axis mainly at the adrenal level. Neuroendocrinology 2007;86(1):26-37.
163. Golbidi S, Laher I. Exercise and the cardiovascular system. Cardiol Res Pract 2012;2012:210852.
164. Bloch W, Suhr F, Zimmer P. Molecular mechanisms of exercise-induced cardiovascular adaptations. Influence of epigenetics, mechanotransduction and free radicals. Herz 2012;37(5):508-15.
165. Barnes PJ, Karin M. Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. N Engl J Med 1997;336(15):1066-71.
166. Ito K, Hanazawa T, Tomita K, et al. Oxidative stress reduces histone deacetylase 2 activity and enhances IL-8 gene expression: role of tyrosine nitration. Biochem Biophys Res Commun 2004;315(1):240-45.
167. Miao F, Gonzalo IG, Lanting L, Natarajan R. *In vivo* chromatin remodeling events leading to inflammatory gene transcription under diabetic conditions. J Biol Chem. 2004;279(17):18091-7.
168. Ostrowski K, Rohde T, Asp S, et al. Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. J Physiol 1999;515(Pt 1):287-91.
169. McDonald OG, Owens GK. Programming smooth muscle plasticity with chromatin dynamics. Circ Res 2007; 100(10):1428-1441.
170. Galis ZS, Sukhova GK, Lark MW, Libby P. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. J Clin Invest 1994;94(6):2493-503.
171. Puddu GM, Cravero E, Arnone G, et al. Molecular aspects of atherogenesis: new insights and unsolved questions. J Biomed Sci 2005;12(6):839-53.
172. Montgomery RL, Potthoff MJ, Haberland M, et al. Maintenance of cardiac energy metabolism by histone deacetylase 3 in mice. J Clin Invest 2008;118(11):3588-97.
173. Fernandes-Silva MM, Carvalho VO, Guimaraes GV, et al. Physical exercise and microRNAs: new frontiers in heart failure. Arq Bras Cardiol 2012;98(5):459-66.
174. Fernandes T, Soci UP, Oliveira EM. Eccentric and concentric cardiac hypertrophy induced by exercise training: microRNAs and molecular determinants. Braz J Med Biol Res 2011;44(9):836-47.
175. Soci UP, Fernandes T, Hashimoto NY, et al. MicroRNAs 29 are involved in the improvement of ventricular compliance promoted by aerobic exercise training in rats. Physiol Genomics 2011;43(11):665-73.
176. DA Silva ND J, Fernandes T, Soci UP, et al. Swimming training in rats increases cardiac MicroRNA-126 expression and angiogenesis. Med Sci Sports Exerc 2012; 44(8): 1453-62.
177. Bernardo BC, Weeks KL, Pretorius L, McMullen JR. Molecular distinction between physiological and pathological cardiac hypertrophy: experimental findings and therapeutic strategies. Pharmacol Ther 2010; 128(1):191-227.