

Review

Regulation of Gene Expression by Exercise-Related Micrnas

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Key Words

miRs • Exosomes • Gene expression • Skeletal muscle • Endurance • Muscle strength • Leukocyte function • Heart

Abstract

Gene expression control by microRNAs (miRs) is an important mechanism for maintenance of cellular homeostasis in physiological and pathological conditions as well as in response to different *stimuli* including nutritional factors and exercise. MiRs are involved in regulation of several processes such as growth and development, fuel metabolism, insulin secretion, immune function, miocardium remodeling, cell proliferation, differentiation, survival, and death. These molecules have also been proposed to be potential biomarkers and/or therapeutic targets in obesity, type 2 *diabetes mellitus*, cardiovascular diseases, metabolic syndrome, and cancer. MiRs are released by most cells and potentially act on intercellular communication to borderer or distant cells. Various studies have been performed to elucidate the involvement of miRs in exercise-induced effects. The aims of this review are: 1) to bring up the main advances for the comprehension of the mechanisms of action of miRs; 2) to present the main results on miR involvement in physical exercise; 3) to discuss the physiological effects of miRs modified by exercise. The state of the art and the perspectives on miRs associated with physical exercise will be presented. Thus, this review is important for updating recent advances and driving further strategies and studies on the exercise-related miR research.

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Introduction

MicroRNAs (miRs) have been extensively studied in various animal and human models during different physiological and pathological conditions, in order to comprehend their function and participation in the regulation in different gene expressions [1]. The development

of sophisticated technologies on global and specific miR analysis allowed to determine the role of these molecules in different processes, including growth and development; energy metabolism; insulin secretion; immune function; myocardium remodeling; proliferation; differentiation; survival, and cell death. In addition, miRs have been proposed to be potential biomarkers and/or therapeutic targets of several diseases, such as obesity, type 2 *diabetes mellitus*, cardiovascular diseases, metabolic syndrome, and cancer.

In relation to physical exercise, several studies have also been performed to elucidate the function of miRs in the exercise-induced acute and/or chronic effects. However, the involved mechanisms are not fully elucidated yet. The aim of this review is: 1) to bring up the main advances for the comprehension of the effects and mechanisms of action of miRs; 2) to present the main results on miR involvement in physical exercise; 3) to discuss the physiological effects of miRs modified by physical exercise.

Overview on microRNAs (miRs)

Definition, synthesis, and mechanisms of action of miRs

MiRs were discovered in 1993 at the nematode *Caenorhabditis elegans* [2]. Control of gene expression by miRs has been associated with physiological and pathological conditions and in response to nutritional factors and physical activity [1, 3, 4]. Over 2,500 miRs have already been identified to regulate the synthesis of more than 60% of total proteins in human cells [5]. These polynucleotides comprise a single strand and non-coding small RNA class (between 18 and 24 nucleotides). Each miR controls expression of hundreds to millions mRNAs through RNA-specific sequence base pairing.

According to miR-mRNA pairing studies, about 20-30% of the genes are potential miR targets, considering that usually many mRNAs are regulated by a single miR and various miRs regulate a single gene expression [6-8]. The amount of miR in each cell varies from approximately 500 to more than 10,000 copies, depending on the cell type, the specific miR, and the stimulatory factors [9]. MiRs are found in a wide variety of species such as virus, nematodes, fishes, birds, mammals, and plants [10]. MiR encoding usually occurs in intronic regions and it is generally associated with expression of their respective target-gene and common regulatory factors. A small fraction of miRs is co-expressed through their own transcription factors [9, 11]. In humans, miRs have been related to several biological processes, including development and growth, energetic metabolism, maintenance and function of pancreatic β cells, immune function, inflammation, insulin sensitivity, myocardial remodeling and cell death, survival, proliferation and differentiation [12-14]. Moreover, miRs have been associated with a wide range of diseases such as *diabetes mellitus*, obesity, cardiovascular diseases, cancer, and metabolic syndrome [15-21].

The synthesis of miRs begins in cell nucleus where RNA polymerase II synthesizes the primary miR (pri-miR), containing several hairpins [22, 23]. The pri-miR is processed by the endonuclease activity of the RNase III enzymatic complex (Drosha)/ double-stranded binding protein (DGCR8) to generate the miR precursor (pre-miR), containing around 70 basepairs (bp). In the next step, the pre-miR is translocated to the cytoplasm through nuclear pore by the exportin-5, a nuclear Ran-GTP dependent export carrier [24]. In the cytoplasm, pre-miR is cleaved by the endoribonuclease Dicer, a RNase III, resulting in formation of the double stranded miR. The double stranded miR is then processed by the RNA-induced silencing complex (RISC), which contains argonaute protein [25] and GW182, and generates mature miR (Fig. 1). The binding of miRs to their targets occurs by base-pairing (complementary base pairing), generally on the 3'UTR mRNA target region, although some miRs can bind to other coding region. The complementarity of only seven basepairs is sufficient for regulation of mRNA expression *in vivo* [26, 27].

The inhibition of gene expression by miRs has been classified into two types: translational repression (by binding to the respective mRNA target and, consequently, preventing its translation) and degradation of mRNA targets [2, 28]. In both cases, the

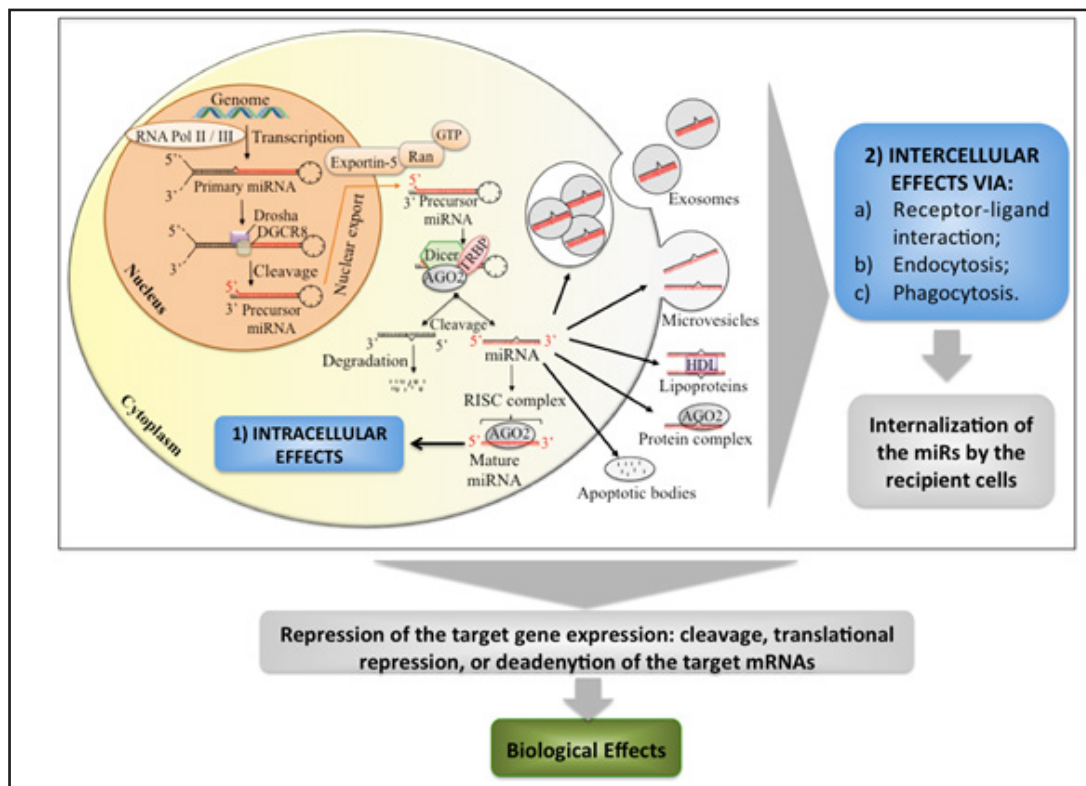


Fig. 1. Canonical pathway of miR biogenesis, secretion and effects. Pri-miRs are transcribed by RNA polymerase II in the nucleus to pre-miRs after been processed by the microprocessor complex consisted of Drosha and DGCR8. Pre-miRs are exported from the nucleus to the cytoplasm by exportin-5 and processed into the miR-miR* duplex by Dicer. After Dicer cleavage, the mature miR is loaded into the effector complex RISC (RNA-induced silencing complex) to produce target mRNA cleavage (perfect complementarity), mRNA repression (imperfect complementarity) or translational repression. miRs can be secreted into the extracellular microenvironment *via* active secretion and exosomes; active secretion *via* microvesicles; lipoprotein complex (such as HDL) and active secretion in protein-miR complex (Ago2). Pri-miRs: Primary miRs; Pol II: RNA Polymerase II; pre-miRs: MiRs precursor; DGCR8: DiGeorge syndrome critical region gene; miR: MicroRNA; RISC: RNA-induced silencing complex; AGO: Argonaute; HDL: High-density lipoprotein; miRs: MicroRNAs; MVB: Multivesicular body.

base pairing between the miR and the untranslated 3' region (3'UTR, seed region) of the target mRNA occurs, resulting in inhibition or degradation of the target mRNA [29]. The translational repression by the miR binding to the mRNA target can occur by three different mechanisms: a) inhibition of translational initiation, b) blocking of the elongation process, and/or c) premature dissociation of mRNA-ribosomal complex, whereas the target-mRNA degradation can occur by: a) removing of the poly-A tail and/or capping region, or b) activation of 5'-3' miR exonuclease activity [30]. Evidence has also been accumulated miRs inhibit translation by binding to coding regions and/or to the 5'UTR [31].

MiRs can be measured in biological fluids, such as blood, serum, milk, plasma, urine, and saliva [32]. The possibility of measuring miRs in plasma and/or other biological fluids, mainly in microvesicles, in response to different *stimuli*, including nutritional factors and physical exercise, opened new perspectives related to the use of these molecules as biomarkers on physiological and/or pathological conditions, consequently, as preventive and/or therapeutic targets of associated diseases [33-35], as well as the discovery of new strategies for modulating the miR expression [36]. However, further studies are still needed

for the complete characterization, involvement, modulation, and function of miRs in different processes and conditions. In the biological fluids, miRs are protected against degradation by association to binding proteins (including argonaute 2 and nucleofosmine 1) [33], high-density lipoproteins (HDL) [37] or microparticles, including exosomes, microvesicles, and apoptotic bodies [38, 39] (Fig. 1).

The exosomes are microvesicles sizing from 30 to 100 nm in diameter, have endocytic origin and are released by exocytosis [40, 41]. These microvesicles act on intercellular communication, transporting macromolecules (such as proteins, lipids, and nucleic acids DNA, mRNAs, and miRs) to borderer or distant cells [42]. Most cells secrete exosomes potentially able to induce paracrine and/or endocrine effects [43, 44]. These microvesicles have been propose to induce intercellular communication via receptor-ligand interaction, endocytosis, and/or phagocytosis, releasing their intravesicular content into different target cells, including several bioactive molecules such as miRs, mRNAs, proteins, and lipids. These molecules potentially modify biological processes and physiological responses of the recipient cells [45-47].

The microvesicules present ordinary (example: tetraspanin, TSG101, and alix) and specific (example: mRNAs, miRs, proteins, and lipids) components [48]. Ordinary exosomal proteins are involved in biogenesis, structure, and transport, from these microvesicles, whereas specific components refer to the cellular origin and formation process [49]. Two data base platforms are available for specific exosomal components: *EVpedia* (<http://evpedia.info>) and *ExoCarta* (<http://exocarta.org>). These platforms provide information on mammal cell microvesicules in relation to mRNAs, miRs, proteins, and lipids [50].

The functions of miRs in the specific tissues

The miR-1, miR-133a, miR-133b, miR-206, and miR-499 are highly expressed in the heart and skeletal muscle, whereas miR-208 is specifically expressed in the heart [51-53]. MiR-21 and miR-155 are widely expressed in different tissues being associated with inflammatory response [14, 54]. The miR-143 and miR-103 are involved in the differentiation of 3T3-L1 preadipocytes. In animals fed with a high-fat diet, miR-107 and miR-143 are associated with body weight gain, visceral fat accumulation, plasma levels of leptin and expression of genes related to adipocyte differentiation (peroxisome proliferator-activated receptor gamma (PPAR γ) and adipocyte protein (Ap2)), fatty acid metabolism (PPAR α , carnitine palmitoyltransferase-1B (CPT-1B), and lipoprotein lipase (LPL) activity, whereas miR-221 and -222 area correlated with expression of tumor necrosis factor-alpha (TNF- α) and adiponectin. Let-7, miR-30, miR-103, miR-143, and miR-422b are overexpressed during adipogenesis [55-58]. On the opposite, miR-27a and -27b inhibit adipogenesis by acting on PPAR γ expression as reported in 3T3-L1 adipocytes. MiR-7 has been suggested to be involved in the pathogenesis of myocardial infarction and heart failure [59], miR-155 in the inflammatory process of the atherosclerosis via SOCS-1 activation in macrophages [60], and miR-378 in the cytokine-induced inflammation through SREBP and C/EBP pathway in adipose tissue [61]. MiR-103 and miR-107 negatively regulate insulin sensitivity in liver from animal experimental models and patients with insulin resistance [62, 63], whereas miR-1 has been positively related to the insulin sensitivity and miR-106b, -27a, and -30d negatively to the GLUT-4 expression in skeletal muscle cells from animal and cellular models [64, 65]. MiRs also play an important role in skeletal muscle function [66, 67]. Skeletal muscle cells produce several miRs, named myomiRs, such as miR-1, miR-133a, miR-133b, miR-206, miR-378, miR-499, and miR-208. The PI3-K/Akt/mTOR pathway positively regulates the muscle protein synthesis. Previous studies have been found a positive association between this pathway and miR-486 expression. This miR prevents the repression of phosphatidylinositol 3-kinase (PI3-K)/Akt/mTOR pathway by suppressing the expression of PTEN, an antagonist of PI3-K, leading to stimulation of protein synthesis [68]. In addition, Hitachi et al. [69] demonstrated that myostatin negatively regulates mTOR signaling causing suppression of PI3K/Akt pathway through activation of SMAD-2 pathway and repression of ANK-1 expression, thus inhibiting miR-486 promoter activity. These findings demonstrate the

mechanisms involved in the muscle hypertrophy may also be modulated by miRs, such as miR-486.

Regulation of gene expression by physical exercise

Physical Exercise and Gene Expression

Physical activity is recommended as non-pharmacological therapy in insulin resistance, obesity, heart diseases, type 2 *diabetes mellitus*, and some types of cancer [70-78]. Many beneficial effects of physical exercise have been reported: a) improvement of plasma lipid composition (decreased triglyceride; total cholesterol and LDL-cholesterol; and increased HDL-cholesterol levels), b) amelioration of glucose homeostasis (elevated insulin-independent glucose uptake; glucose transporter-4 (GLUT-4) expression; and insulin sensitivity); c) increased skeletal muscle oxidative capacity (improved biogenesis and mitochondrial metabolism), and d) reduction in inflammation markers [74, 79-83]. Physical exercise modulates expression of several miRs involved in protein synthesis, such as miR-26a, miR-29a, miR-378, miR-451, and miR-696 [84-86]. However, the mechanisms involved are not fully known yet.

The effects of physical exercise are in many cases associated with changes in gene expression. The characteristics of the physical exercise (such as intensity, duration, frequency, modality, trained level), and age of the practicer play a central role in the regulation of gene expression, which can occur by different mechanisms (e.g. generation of second messengers, modulation of myogenic regulatory and epigenetic factors). Generation of second messengers by physical exercise involves activation of specific signaling pathways such as Ca⁺⁺/CaMK, AMP/AMPK, and PKD. Activation of the AMP/AMPK signaling by physical exercise results in expression of GLUT-4 and transcription factors (PGC-1 α and NRF-1), which ones lead to expression of genes involved in biogenesis and mitochondrial oxidative capacity. Concomitant activation of AMP/AMPK pathway, Ca⁺⁺/CaMK and PDK pathways has been related to the decrease in class IIa HDAC activity, resulting in hypomethylation and consequent increase in expression of PGC-1 α , PPAR- γ and MEF-2, factors associated to expressions of GLUT-4 and mitochondrial genes [87, 88]. The activities of MRFs, including MyoD-1, myogenin, MEF-2, SRF and MRTF-A, are also associated to miR expression, such as miR-31, miR-34c, miR-181, miR-206, miR-223, miR-335, miR-449, and, miR-494 [89, 90].

Physical exercise modulates expression of myogenic regulatory factors. Increased synthesis and secretion of growth hormone (GH) and insulin growth factor 1 (IGF-1) during physical exercise results in activation of the PI-3K/Akt and SRE/SRF signaling pathways, leading to an increase in mitochondrial density, expression of contractile proteins, and hypertrophy of skeletal muscle. Decrease release of myostatin by physical exercise, mainly strength exercises, by the other hand, attenuates the inhibition of myogenesis in satellite cells, resulting in skeletal muscle mass increase [91].

Physical exercise induces epigenetic changes through several mechanisms including chromatin structure changes (methylation or histone acetylation), DNA methylation, and miR expression. These mechanisms modulate, positively or negatively, expressions of the genes related to different exercise-induced adaptative processes such as: proliferation of precursor cells and differentiation of microtubules, mass maintenance and/or muscle hypertrophy, determination of muscle fiber type, mitochondrial biogenesis and oxidative capacity, and muscle contractility. Aerobic physical exercise negatively modulates expression of various miRs involved in the processes presented above, such as miR-1, miR-26a, miR-29a, miR-378, miR-125a, miR-183, miR-189, miR-432, miR-494, miR-575, miR-616, miR-637, miR-696, and miR-761 [1, 51, 84, 85, 89, 92, 93].

Modulation of miRs expression by physical exercise and associated effects

Studies involving genome wide association, RNA expression profile, and other advanced methodologies have been used for the characterization of miR profile in response to physical

exercise, particularly in the last five years. The main targets evaluated include blood (plasma, serum, or circulating leukocytes) and specific tissues, skeletal muscle, heart, and brain. The main findings described in the literature are in Table 1. Figure 2 summarizes the general alterations in miR expression profile in skeletal muscle, heart, and blood, the possible target genes and the physiological changes observed in different studies and detailed below.

Physical exercise induced-MiRs, muscle hypertrophy, and endurance adaptation

Aerobic physical exercise increases circulating (plasma or serum) levels of mir-1, mir-20a, mir-21, mir-133a, mir-146a, mir-150, mir-206, mir-221, and mir-222, and decreases mir-486 in humans [94-98]. However, studies concerning direct effects of specific plasma or serum miRs on exercise performance and physiological responses are scarce and very limited. Correlative associations and RNA-target prediction findings suggest that the alterations in the miRs listed above are related to the training adaptability to endurance/aerobic capacity and modulation of angiogenesis, inflammation, muscle damage, and skeletal muscle and heart functions in response to exercise.

Involvement of the muscle miRs on the physiological adaptations to exercise training, by the other hand, has been particularly investigated. By increasing or decreasing expression of specific miRs, physical exercise can potentially modulate gene expression profile in many cells and tissues, inducing related-physiological adaptations by different mechanisms. When mice were submitted to endurance training, miR-494 expression is highly downregulated and associated with overexpression of its potential and predicted target genes: mitochondrial transcription factor A (mtTFA) and Forkhead box J-3 (FoxJ-3)/MEF-2c, suggesting an important role of this miR on skeletal muscle adaptation to aerobic training [89]. Other two potential miRs that have been related to the mitochondrial oxidative capacity and biogenesis are miR-696 and miR-761. These two miRs are markedly reduced by endurance training, resulting in elevated peroxisome proliferator-activated receptor-gamma coactivator-1 α (PGC-1 α) expression, mitochondrial oxidative capacity, and biogenesis [1, 84]. Xu et al. [1] proposed that miR-761 acts by inhibiting p38 MAPK signaling pathway, specifically MAPK-activated protein kinase-2 (P-MK2) and activating transcription factor-2 (ATF-2), two downstream kinases of the p38 pathway that have an essential function in increasing the PGC-1 α expression. The involvement of miR-494, miR-696, and miR-761 in the mitochondrial function and biogenesis was confirmed by *in vitro* experiments, using C2C12 myoblast cells with down or overexpression of these miRs [1, 84, 89].

Nielsen et al. [52] demonstrated that four myomiRs are negatively modulated in the human vastus lateralis muscle after 12 weeks of endurance exercise: miR-1, miR-133a, miR-133b, and miR-206. This downregulation was associated with improved endurance capacity, VO₂max, and insulin sensitivity. Studies with predicted target genes appointed out Cdc-42 and Erk-1/2, two proteins involved in the activation of MAPK signaling pathway that controls different processes, including growth, proliferation, and differentiation.

By using human and animal models submitted to endurance training, specifically individuals or animals selected as high responders to aerobic capacity, Keller et al. [51] proposed a "training-responsive transcriptome", in which more than 100 genes are potentially involved in determining endurance adaptation. In addition, muscle miR expression profile was greatly modified by endurance training. Elevation in muscle expression of miR-125a, miR-183, miR-189, miR-432, miR-575, miR-616, and miR-637, and decrease in muscle expression of miR-1, mir-15b, mir-26-b, mir-28, mir-29b, miR-92, mir-98, miR-101, miR-133a, miR-144, miR-206, miR-338, miR-451, miR-455, and miR-589 have been found after the endurance exercise protocol. Interestingly, several of these miRs were directly linked to the regulation of three transcriptional factors, RUNX1, SOX9, and PAX3, which ones are associated with oxygen tension, oxidative stress, and angiogenesis. Using database and biological variation analysis, the authors suggested that the miR expression profile is involved in the training-responsive transcriptome by modulating the RUNX1, SOX9, and PAX3 pathways, resulting in the activation of angiogenesis and tissue developmental networks for adaptation to aerobic training [51]. In addition, Fernandes et al. [99]

Table 1. miRs changed by physical exercise and their functions (Part A, B, C)

A: STUDIES IN BLOOD AND CIRCULATING LEUCOCYTES					
Authors	Experimental model	Exercise protocol	Samples evaluated	Altered miRs	Associated effects
[94]	Endurance athletes	Endurance aerobic rowing exercise training, 1-3 h per day, during 90 day	Plasma	↑ mir-20a, 21, 146a, 221, 222	Modulation of angiogenesis, inflammation, skeletal and cardiac muscle function, and cellular response to hypoxia
[96]	Endurance athletes [21]	Marathon race	Plasma (immediately and 24 h after)	↑ mir-1, 133 ^a and 206 (0 and 24 h) ↑ 208b, 499 (0 h)	Association with VO ₂ max, cardiac function, and muscle damage
[98]	Strength and endurance athletes	At rest, no exercise	Plasma (12 h post-exercise)	Endurance: ↑ mir-222 (vs control) ↑ mir-21, 146a, 221 (vs strength)	Adaptation to performance training
[95]	Sedentary men	Aerobic training (cycling) at 70% VO ₂ max, 30 min - 3 per week during 4 weeks	Serum	↓ mir-486	Aerobic capacity
[138]	Healthy individual with low VO ₂ max	At rest, no exercised	Serum	↑ mir-21, 210, 222	Biomarkers for independent fitness level
[97]	Trained runners	10 Km and marathon races	Serum (immediately after)	10 km: ↑ mir-150-5p Marathon: ↑ 12 miRs	Biomarkers for inflammatory state
[134]	Healthy elite athletes	10 x 2 min bouts of cycle ergometer exercise at 76% VO ₂ max	Blood neutrophils (immediately after)	↑ or ↓ 38 miRs	Modulation of neutrophil function
[130]	Healthy elite athletes	10 x 2 min bouts of cycle ergometer exercise at 77% VO ₂ max	Blood NK cells (immediately after)	↑ or ↓ 23 miRs	Modulation of NK cell function
[131]	Healthy elite athletes	10 x 2 min bouts of cycle ergometer exercise at 82% VO ₂ max	Blood PBMC (immediately after)	↓ mir-199a, 130a, 151-5p, 221, 23b	Correlation to risk for atherosclerosis
B: STUDIES IN SKELETAL MUSCLE					
Authors	Experimental model	Exercise protocol	Samples evaluated	miRs studied	Effects
[84]	C57BL/6 mice	Treadmill running, 5 per week, during 5 weeks	Skeletal muscle (24 h post)	↓ mir-696	↑ mitochondrial biogenesis (↑ PGC-1α)
[52]	Healthy and untrained men	High endurance training by cycle ergometer, 5 per week, during 12 weeks	Skeletal muscle (3 h after)	↓ 1, 133a, 133b, 206	Endurance exercise adaptation
[85]	Healthy recreationally active young men	Rotating resistance training, 5 per week, during 12 weeks	Skeletal muscle (48 h after)	↑ mir-451 ↓ mir-26a, 29a, 378	Muscle hypertrophy induced by resistance training
[51]	Healthy and sedentary young men	Aerobic training (cycling), 4 x 45 min per week, during 6 weeks	Skeletal muscle (24 h after)	↑ mir-125a, 183, 189, 432, 575, 616, 637 ↓ mir-1, 15b, 26b, 28, 29b, 92, 98, 101, 133a, 144, 338, 451, 455, 589	↑ VO ₂ max
[92]	Elderly untrained and healthy subjects	Resistance training or eccentric ergometer training, 2 per week, during 12 weeks	Skeletal muscle (48 to 72 after)	↓ mir-1	↑ muscle growth (↑ IGF-1)
[99]	Male spontaneously hypertensive rats	Swimming training (with 4% load), 1 h per day, 4 per week, during 10 weeks	Skeletal muscle	↑ mir-126 ↓ mir-16, 21	Prevention of microvascular abnormalities (angiogenesis and apoptosis)
[89]	Male C57BL/6j mice	Chronic swimming exercise, 7 x 15 min bouts per day, 1 week	Skeletal muscle	↓ mir-494	↑ mitochondrial biogenesis (↑ PGC-1α, mtTFA, and Foxj3-Mt2c)
[93]	Trained men	Endurance training (cycling): 45 min x 4 day at 75%; 90 min x 2 day at 75%; and 6x5 min x 4 day at 90%	Skeletal muscle	↑ mir-1 ↓ mir-29b, 31	Adaptation to exercise endurance training
[1]	Male Kunming mice	Aerobic exercise training (treadmill), 5 per week, 4 weeks	Skeletal muscle (24 h after)	↓ mir-761	↑ Mitochondrial biogenesis
C: STUDIES IN HEART					
Authors	Experimental model	Exercise protocol	Samples evaluated	miRs studied	Effects
[109]	Female Wistar rats	Swimming training (low and high volume), 1-3 h per day, 5 per week, during 10 weeks	Heart	↑ mir-27a, 27b ↓ mir-143	Heart hypertrophy (target-genes related to the cardiac renin angiotensin system)
[110]	Female Wistar rats	Swimming training (low and high volume), 1-3 h per day, 5 per week, during 10 weeks	Heart	↑ mir-29a ↓ mir-1, 133a, 133b	Cardiac hypertrophy induced by aerobic training (↓ collagen deposition and ↑ ventricle compliance)
[116]	Female Wistar rats	Swimming training (with 5% load), 5 per week, during 10 weeks	Heart	↑ mir-126	↑ Angiogenesis and apoptosis (↓ SPREAD and PI3-K)
[111]	Female Wistar rats	Swimming training (with 5% load), 1 h per day, 5 per week, during 8 weeks	Heart	↑ mir-21, 144, 145 ↓ mir-124	↑ Heart hypertrophy (↑ PI3-ka; ↓ PTEN and TSC2)
[112]	Male C57BL/6j mice	Aerobic swimming training or voluntary wheel exercise	Heart (24 h after)	↑ mir-222	↑ Heart growth and cell proliferation, cardioprotection (↓ HIPK1 and HMBOX1)
[121]	Male Wistar rats	4 x 12 repetitions of leg flexion (80% 1RM), during 8 weeks	Heart (24 h after)	↓ mir-214	↑ Heart contraction/relaxation, hypertrophy (↑ SERCA2)
[117]	Wistar rats	2 x 90 min swimming training per day, during 8 weeks	Heart	↑ mir-19b, 30, 133b, 208b ↓ mir-99b, 100, 191a, 22, 181a	Modulation of cell proliferation and death (apoptosis) (PI3-K and MAPK)
[118]	Male Wistar rats (heart failure by aortic stenosis surgery)	Aerobic exercise training (treadmill), 5 per week, 10 weeks	Heart	↑ mir-598, 429, 224, 425, 221	↑ Cardioprotection

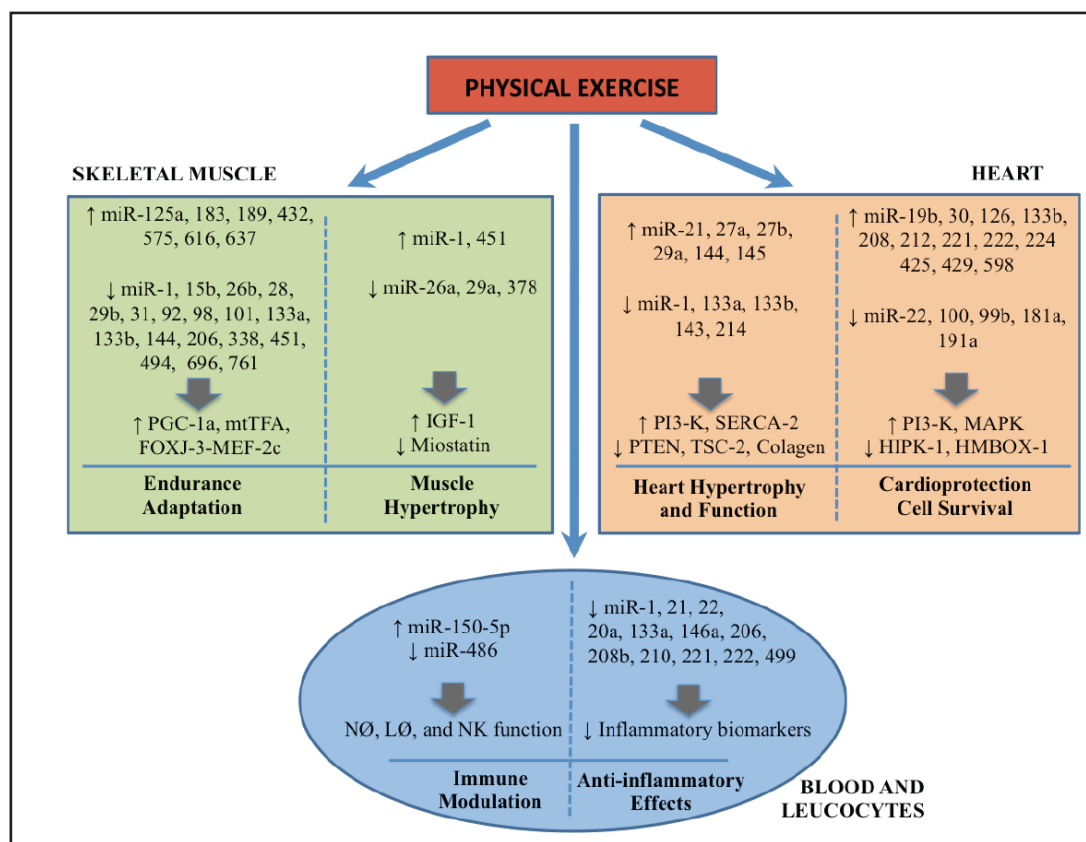


Fig. 2. General effects of physical exercise on miR expression in skeletal muscle, heart, and blood (serum, plasma, and leukocytes), potential target genes and associated physiological adaptations. For details, see the section “Modulation of miRs expression by physical exercise and their possible effects”. FOXJ-3/MEF-2C, forkhead box-J3/myocyte-specific enhancer factor-2C; HIPK-1, homeodomain-interacting protein kinase-1; HMBOX-1, Homeobox containing-1; IGF-1, insulin-like growth factor-1; LØ, lymphocytes; MAPK, mitogen-activated protein kinase; mtTFA, mitochondrial transcription factor A; NØ, neutrophils; NK cells, natural killer cells; PGC-1α, peroxisome proliferator-activated receptor-gamma coactivator-1alpha; PI3-K, phosphatidylinositol-4,5-bisphosphate 3-kinase; PTEN, phosphatase and tensin homolog; SERCA-2, sarco/endoplasmic reticulum Ca²⁺-ATPase; TSC-2, tuberous sclerosis complex-2.

observed, in spontaneously hypertensive mice submitted to aerobic swimming training, an increase of the muscle expression of miR-126 and decrease of the expression of miR-16 and miR-21 associated with improved microvascularization in these animals. Potential targets of these miRs include vascular endothelial growth factor and Bcl-2, which were reduced by the aerobic training. Consequently, a reduction in Bad and an elevation in Bcl-x and endothelial NOS expression was observed and associated with the balance between apoptotic and angiogenic factors, contributing to the normalization of the abnormalities in the spontaneously hypertensive mice [99].

Davidson et al. [85], by studying low and high responders to resistance exercise training, demonstrated that this type of exercise is able to modulate the expression of specific miRs only in low responsive individual. Resistance training stimulated expression of miR-451 and inhibited expression of miR-26a, miR-29a, and miR-378, this last one was associated with skeletal muscle mass gain. Prediction analysis using the potential target genes of the miRs altered by resistance training appoint out for a compensatory mechanism in low responders, possible to these individuals fail to properly activate muscle growth hypertrophy by mechanical stimulus [85]. Reduction in the miR-1 and miR-133a expression was also observed in the study of McCarthy and Esser [100]. In this study, the authors used a mouse

model of hypertrophy induced by functional overload and associated the decreased miR-1 and miR-133a expression with the improved IGF-1 signaling pathway and muscle growth and hypertrophy. Although the specific function of miR-206 is not understood yet, it has been suggested that this miR is involved in muscle hypertrophy, satellite cell proliferation and differentiation, and fiber muscle phenotype determination, favoring the type I rather than type II phenotype [101, 102].

Mueller et al. [92] reported different adaptative effects when elderly subjects were submitted to two strength-training protocols: conventional resistance exercise training and eccentric ergometer training. After eccentric ergometer training, individuals presented reduced expression of genes related to the mitochondrial function and biogenesis, intramyocellular lipid accumulation, and skeletal muscle remodeling and repair when compared to conventional resistance exercise training. Although these differences, both strength protocols led to a reduction in miR-1 expression, accompanied by elevated IGF-1 expression and skeletal muscle mass gain [92]. Previous studies in C2C12 myoblast cells have demonstrated that miR-1 modulates IGF-1/PI3-K/Akt signaling pathway by directly decreases IGF-1 expression [103]. Interestingly, the inverse is also true, since IGF-1/PI3-K/Akt pathway activation is related to miR-1 downregulation by a mechanism FOXO-dependent [104, 105]. Two potential miRs that can be involved in the regulation of skeletal muscle mass gain comprehend miR-208 and miR-499. Both miRs have been demonstrated to repress the myostatin expression and, consequently, to increase muscle hypertrophy [106-108]. However, further studies are necessary to investigate the participation of these miRs on muscle hypertrophy induced by resistance training.

Physical exercise induced-miRs and cardiac hypertrophy, function, and protection

Aerobic exercise training (swimming or running) in Wistar rats and C57Bl/6J mice for 5 days per week for 8-10 weeks increased expression of miR-21, miR-27a, miR-27b, miR-29a, miR-144, miR-145, and miR-222, and decreased expression of miR-1, miR-124, miR-133a, miR-133b, and miR-143 in the left ventricle which are associated to cardiac hypertrophy and improved heart function in response to exercise [109-112]. Expression of miR-29 (a and c) is increased by swimming training for 10 weeks (one hour per day) and correlated to decreased collagen content (IA and IIIA) in heart from infarcted rats [113]. The physiological cardiac hypertrophy exercise-induced leads to an increase cardiac PI3K activity whereas pathological hypertrophy decreases PI3K activity and causes heart failure [114]. Other potential miR involved in the cardiac hypertrophy is miR-223, since it has been demonstrated that the overexpression of this miR prevents the cardiomyocyte hypertrophy by downregulating cardiac troponin I-interacting kinase expression [115], but this involvement has not been investigated yet.

Aerobic exercise training in Wistar rats also elevated cardiac levels of miR-19b, miR-30, miR-126, miR-133b, miR-208b, miR-221, miR-224, miR-425, miR-429, and miR-598, and reduced levels of miR-22, miR-99b, miR-100, miR-181a, and miR-191a, which are related with cell proliferation/death and cardioprotective effect induced by 5 days per week for 8 to 10 weeks of physical exercise training [112, 116-118]. Accordingly, a combinatorial expression of several miRs has been suggested to determine cardioprotective effects against hypoxia and aging [119, 120]. Resistance physical exercise in Wistar rats decreases expression of miR-214 in the left ventricle that leads to increased SERCA2 expression and improved cardiac contractile function [121]. Although exercise has been reported to protect against cardiac injuries, future research are needed to define the ideal rate, duration and intensity at the physical activity can attenuate cardiovascular disease processes. Furthermore, in order to develop miRNA-based therapy, studies are needed to identify the *in vivo* miRNA on systemic effects instead of focus on site-specific phenotypic effects.

Physical exercise induced-miRs and immune function

Physical exercise modulates miRNA expression related to inflammation in different leukocytes. These miRNAs are involved to several inflammatory processes commonly

observed in diseases or infection. Among 2,500 human miRs described, about 150 to 600 species were identified in immune cells, with predominance of 20 to 30 miRs. After a single session of exercise, leukocyte profile in peripheral blood changes causing alterations in expression of several miRs and, consequently, gene expression feature in immunocompetent cells. The miRs control several functions such as antigen presentation (miR-148/152), activation of T cell receptors (miR-181a), *toll-like* receptors (miR-132, let-7e), activation of NK cell receptor (miR-1245), and cytokine production (miR-146a) [122-124]. Physical exercise also modulates to the transcriptional regulation of key genes that could elucidate the mechanism of benefits on health by physical exercise on different immune cells, including neutrophils, macrophages, and lymphocytes [125-129].

Athletes submitted to resistance exercise training exhibit changes in expression of 38 miRs in neutrophils, 23 miRs in natural killer (NK) cells, and 19 miRs in peripheral blood mononuclear cells (PBMC) were found. These changes were associated with altered cell function and increased risk of atherosclerosis [130, 131]. Radom-Aizik et al. [132] studied miR expression modulation in peripheral blood mononuclear cells (PBMC) by brief exercise. In this study, twelve young men performed brief bouts of heavy exercise consisted by 20 minutes of exercise involving ten 2-minute bouts of constant work rate cycle ergometry, with 1-minute rest interval between each bout of exercise. Exercise modulated expression of microRNAs related to inflammatory process such as down-regulation of miRNA-125b, that is decreased by LPS and up-regulation of miR-132, that regulate toll-like receptors (TLRs) in monocytes. MiR-125a and miR-132 are involved with diseases whose pathogenesis is altered by exercise, such as systemic lupus erythematosus, and rheumatoid arthritis [133], showing the importance of these miRNAs expression in inflammatory diseases.

The miRs 181a and 181b were upregulated by brief exercised and may be involved with the monocyte function modulated by physical exercise in patients with asthma [132]. Expression of miR-181 family (miR-181a and miR-181b) is modulated in different types of leukocytes in response to physical exercise [132, 134]. MiR-181 suppresses inflammatory response induced by oxidated low-density lipoprotein in dendritic cells [135] and miR 181b inhibits inflammatory response mediated by nuclear factor-kappa B transcription factor [136]. Thus, increased expression of miR-181 plays a central role for the anti-inflammatory effect promoted by physical exercise.

Radom-Aizik et al. [132] described that in comparison to neutrophils changes, nine microRNAs were modulated in PBMCs, but only six changed in the same course. These observations suggest that exercise differentially modulate miRs in several leukocyte subtypes. Tonevitsky et al. [137] evaluated PBMC in athletes after running in a treadmill at 80% $\text{VO}_{2\text{max}}$. Analysis of miR and mRNA expression was performed before and immediately after exercise, and after recovery periods of 30 or 60 min. Expressions of the following miRs and target mRNAs were changed: miR-21 and their targets TGFBR3, PDGFD and PPM1L; mir-24-2 and MYC and KCN2; mir-27a and ST3GAL6; miR-181a and ROPN1L and SLC37A3. The latter target genes are related to immune function, apoptosis, membrane protein transport, and cytokine transcriptional regulation. MiR-21 is positively correlated to inflammatory process. Bye et al. [138] described that miR-21 was amplified in participants (with 40-45 years) with low aerobic capacity. Furthermore, a significant positive correlation was found between serum levels of miR-21 and C reactive protein (CRP). Increased levels of CRP are reported in individuals with low physical levels and it is correlated with an immune senescence.

Another miR that can be involved in the physical exercise adaptation, specifically skeletal muscle regeneration following muscle injury, is the miR-155. This miR appears to be essential to the balance between pro-inflammatory M1 macrophages and anti-inflammatory M2 macrophages during skeletal muscle regeneration and could be a target molecule to degenerative muscle diseases [139]. However, further studies are necessary to evaluate the involvement of miR-155 during skeletal muscle adaptation in response to physical exercise

Concluding Remarks

Although there is a number of studies on changes in miRs induced by physical exercise, the specific effects of miRs on functions of different cells and tissues, including leukocytes, skeletal muscle, and heart, are still incipient. Studies have been conducted on performance (VO_{2max}), aerobic capacity, inflammation, leukocyte function, skeletal and cardiac muscle hypertrophy, cardioprotection, and muscle and cardiac lesion. The participation of specific miRs in each one of these processes and the mechanisms involved are still under investigation. As described, several studies have been performed to identify miRs as biomarkers of performance, trainability, skeletal muscle injury, heart lesion, and inflammation. Studies are required to provide information on the origin, target cells and tissues, networking, and effects of miRs, which expression is modulated by physical exercise. In the near future, miRs will help physical exercise practitioners and athletes to improve performance and physical fitness and decrease risk factors for muscle damage. Also, information on miRs will be of great importance in the prevention and/or therapy of chronic diseases such as obesity, type 2 diabetes mellitus, cardiovascular diseases, metabolic syndrome, cancer, and autoimmune and inflammatory diseases associated to specific training programs.

The association of specific miRs with changes in cell functions (e.g. leukocytes, cardiomyocytes, and skeletal muscle cells) and disease states is a promising area of investigation. A platform of miRs and associated effects will be of great importance for diagnosis or prognosis of patients/athletes. Blood or tissue biopsy miR typing will then be developed in order to assist athletes and coaches in a more precise and accurate way. Several aspects associated with physical exercise performance and athlete health will be closely followed. The possibilities are many such as to identify: the intensity of undesirable consequences of a training program, beneficial effects of exercise training for a particular patient, the full recovery of the athlete health condition, the individualized exercise prescription.

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Disclosure Statement

The authors declare they have no competing interests.

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