Cellular Physiology and Biochemistry

Cell Physiol Biochem 2016;39:2381-2397
DOI: 10.1159/000452507
Published online: November 11, 2016
Accepted: September 12, 2016

Review

Regulation of Gene Expression by Exercise-Related Micrornas

Laureane Nunes Masi[a] Tamires Duarte Afonso Serdan[a]
Adriana Cristina Levada-Pires[a] Elaine Hatanaka[a] Leonardo dos Reis Silveira[b]
Maria Fernanda Cury-Boaventura[a] Tania Cristina Pithon-Curi[a] Rui Curi[c]
Renata Gorjão[a] Sandro Massao Hirabara[a,c]

[a]Institute of Physical Activity Sciences and Sports, Cruzeiro do Sul University, Sao Paulo, SP, [b]Institute of Biology, University of Campinas, Campinas, SP, [c]Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, SP, Brazil

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, differenciation, survival, and death. These molecules have also been proposed to be potential biomarkers and/or therapeutical 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.

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

Sandro Massao Hirabara
Cruzeiro do Sul University, Rua Galvao Bueno, 868, Liberdade, Sao Paulo, SP, CEP: 01506-000 (Brasil)
Tel. +55-11-3385-3103, E-Mail sandro.hirabara@cruzeirodosul.edu.br

of sophisticated technologies on global and specific miR analysis allowed to determine the role of these molecules in different processes, including grownth and development; energy metabolism; insulin secretion; immune function; miocardium remodeling; proliferation; differenciation; survival, and cell death. In addition, miRs have been proposed to be potential biomarkers and/or therapeutical 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 bring 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

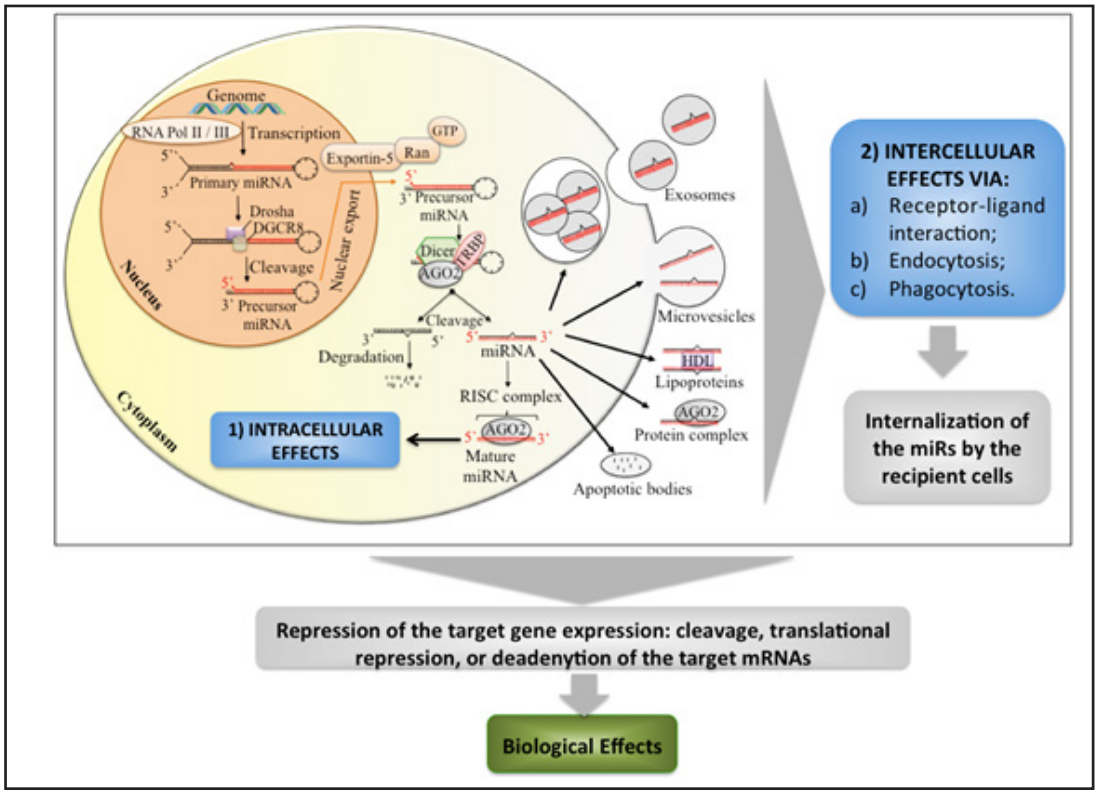

Fig. 1. Canonical pathway of miR biogenesis, secretion and effects. Pri-miRs are transcribed by RNA polymerase II in the nucleus to pre-miRs after been processed by the microprocessor complex consisted of Drosha and DGCR8. Pre-miRs are exported from the nucleus to the cytoplasm by exportin-5 and processed 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 microvesicules, 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, futher 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 (PPARγ) and adipocyte protein (Ap2)), fatty acid metabolism (PPARα, 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 PPARγ 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 pathaway 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 phosphotidyinositol 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 practiciser 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-1α 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-1α, 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 chacacterization 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 bellow.

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 dfferent 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-1α (PGC-1α) 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-1α 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, VO_2max, 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 animais 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 determinating endurance adaptation. In addition, muscle miR expression profile was greately 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 directely 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 traininig [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_2max, 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_2max, 30 min - 3 per week during 4 weeks	Serum	↓ mir-486	Aerobic capacity
[138]	Healthy individual with low VO_2max	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 egometer exercise at 76% VO_2max	Blood neutrophils (immediately after)	↑ or ↓ 38 miRs	Modulation of neutrophil function
[130]	Healthy elite athletes	10 x 2 min bouts of cycle egometer exercise at 77% VO_2max	Blood NK cells (immediately after)	↑ or ↓ 23 miRs	Modulation of NK cell function
[131]	Healthy elite athletes	10 x 2 min bouts of cycle egometer exercise at 82% VO_2max	Blood PBMC (immediately after)	↓ mir-199a, 130a, 151-5p, 221, 23b	Correlation to risk for atheroscleosis

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-1a)
[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 resitance training, 5 per week, during 12 weeks	Skeletal muscle (48 h after)	↑ mir-451 ↓ mir-26a, 29a, 378	Muscel hypertrophy induced by resistance training
[51]	Healthy and sedentary young men	Aerobic traininig (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_2max
[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-1a, mtTFA, and Foxj3-Mef2c)
[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 C57BL6/J 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

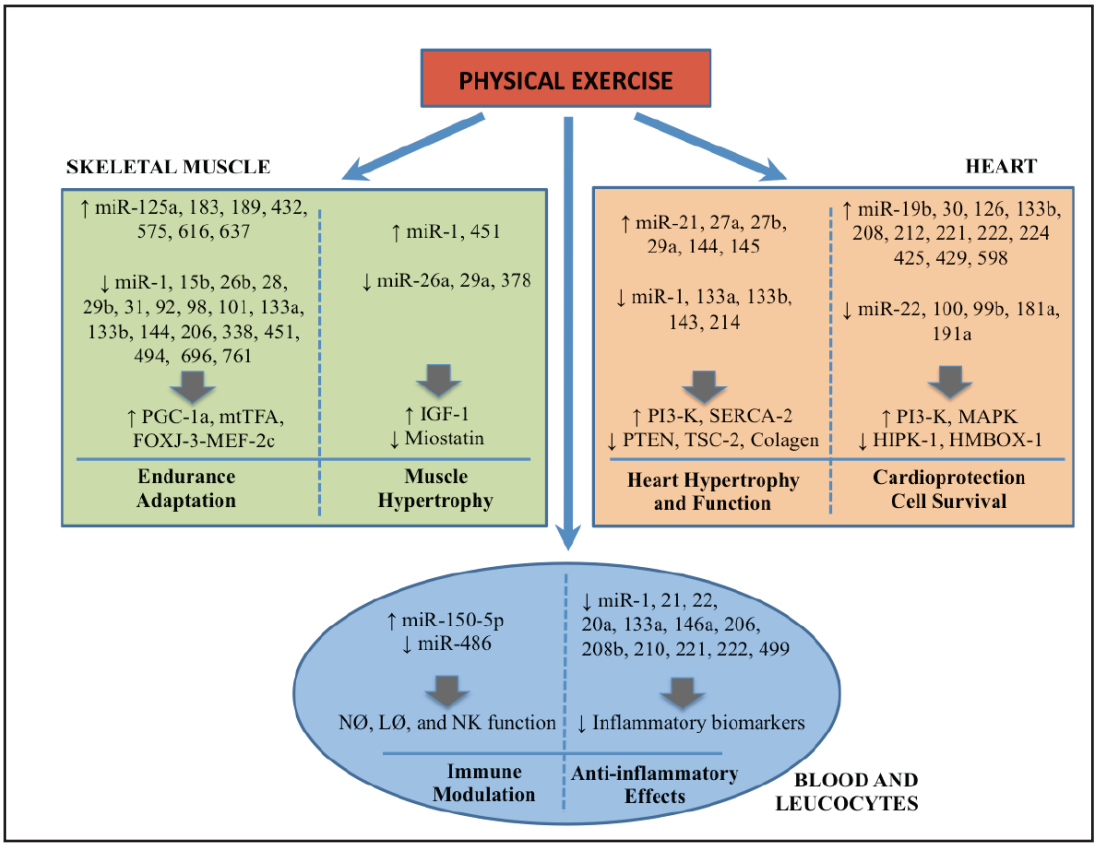

Fig. 2. General effects of physical exercise on miR expression in skeletal muscle, heart, and blood (serum, plasma, and leukocytes), potential target genes and associated physiological adaptations. For details, see the section "*Modulation of miRs expression by physical exercise and their possible effects*". FOX-J3/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 Ca^{2+}-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. Consequenly, 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].

Davidsen 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 McCartthy 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 biobenesis, 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]. Previus studies in C2C12 myoblast cells have demonstrated that miR-1 modulates IGF-1/PI3-K/Akt signaling pathway by directely 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 demonostrated 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 cardic 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 ventricule 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]. Accordantly, 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 ventricule 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 erythematosis, 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% VO_{2max}. 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 KCNJ2; 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 regenation 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 practioners and athletes to improve performance and physical fitness and decrease risk factors for muscle damage. Also, information on miRs will be of great important 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 plataform 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 coachs 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.

Acknowledgements

The authors are grateful to the Prof. Dr. Alice C. Rodrigues for the excellent advices. Our research group is supported by grants from Sao Paulo State Research Foundation (FAPESP), Coordination for the Improvement of Higher Level Personnel (CAPES), National Council for Scientific and Technological Development (CNPq), National Institute of Science and Technology in Obesity and Diabetes (INOD), Dean's Office for Post-Graduate and Research of the Cruzeiro do Sul University, and Dean's Office for Research of the University of Sao Paulo.

Disclosure Statement

The authors declare they have no competing interests.

References

1 Xu T, Liu Q, Yao J, Dai Y, Wang H, Xiao J: Circulating microRNAs in response to exercise. Scand J Med Sci Sports 2015;25:e149-154.
2 Lee RC, Feinbaum RL, Ambros V: The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 1993;75:843-854.
3 Nielsen S, Akerstrom T, Rinnov A, Yfanti C, Scheele C, Pedersen BK, Laye MJ: The miRNA plasma signature in response to acute aerobic exercise and endurance training. PLoS One 2014;9:e87308.
4 Sawada S, Kon M, Wada S, Ushida T, Suzuki K, Akimoto T: Profiling of circulating microRNAs after a bout of acute resistance exercise in humans. PLoS One 2013;8:e70823.

5 Londin E, Loher P, Rigoutsos I: Reply to Backes and Keller: Identification of novel tissue-specific and primate-specific human microRNAs. Proc Natl Acad Sci U S A 2015;112:E2851.
6 Lindow M, Gorodkin J: Principles and limitations of computational microRNA gene and target finding. DNA Cell Biol 2007;26:339-351.
7 Baek D, Villen J, Shin C, Camargo FD, Gygi SP, Bartel DP: The impact of microRNAs on protein output. Nature 2008;455:64-71.
8 Ha TY: MicroRNAs in Human Diseases: From Cancer to Cardiovascular Disease. Immune Netw 2011;11:135-154.
9 Liang H, Li WH: MicroRNA regulation of human protein protein interaction network. Rna 2007;13:1402-1408.
10 Tili E, Michaille JJ, Calin GA: Expression and function of micro-RNAs in immune cells during normal or disease state. Int J Med Sci 2008;5:73-79.
11 Saini HK, Griffiths-Jones S, Enright AJ: Genomic analysis of human microRNA transcripts. Proc Natl Acad Sci U S A 2007;104:17719-17724.
12 Bushati N, Cohen SM: microRNA functions. Annu Rev Cell Dev Biol 2007;23:175-205.
13 Ma X, Becker Buscaglia LE, Barker JR, Li Y: MicroRNAs in NF-kappaB signaling. J Mol Cell Biol 2011;3:159-166.
14 Viereck J, Bang C, Foinquinos A, Thum T: Regulatory RNAs and paracrine networks in the heart. Cardiovasc Res 2014;102:290-301.
15 Visone R, Croce CM: MiRNAs and cancer. Am J Pathol 2009;174:1131-1138.
16 Xie H, Sun L, Lodish HF: Targeting microRNAs in obesity. Expert Opin Ther Targets 2009;13:1227-1238.
17 Heneghan HM, Miller N, Kerin MJ: Role of microRNAs in obesity and the metabolic syndrome. Obes Rev 2010;11:354-361.
18 Wang Y, Taniguchi T: MicroRNAs and DNA damage response: implications for cancer therapy. Cell Cycle 2013;12:32-42.
19 Schroen B, Heymans S: Small but smart--microRNAs in the centre of inflammatory processes during cardiovascular diseases, the metabolic syndrome, and ageing. Cardiovasc Res 2012;93:605-613.
20 Vickers KC, Remaley AT: MicroRNAs in atherosclerosis and lipoprotein metabolism. Curr Opin Endocrinol Diabetes Obes 2010;17:150-155.
21 Virtue A, Mai J, Yin Y, Meng S, Tran T, Jiang X, Wang H, Yang XF: Structural evidence of anti-atherogenic microRNAs. Front Biosci (Landmark Ed) 2011;16:3133-3145.
22 Lee Y, Kim M, Han J, Yeom KH, Lee S, Baek SH, Kim VN: MicroRNA genes are transcribed by RNA polymerase II. Embo J 2004;23:4051-4060.
23 Smalheiser NR: EST analyses predict the existence of a population of chimeric microRNA precursor-mRNA transcripts expressed in normal human and mouse tissues. Genome Biol 2003;4:403.
24 Lund E, Guttinger S, Calado A, Dahlberg JE, Kutay U: Nuclear export of microRNA precursors. Science 2004;303:95-98.
25 Ameresekere M, Borg R, Frederick J, Vragovic O, Saia K, Raj A: Somali immigrant women's perceptions of cesarean delivery and patient-provider communication surrounding female circumcision and childbirth in the USA. Int J Gynaecol Obstet 2011;115:227-230.
26 Ameres SL, Hung JH, Xu J, Weng Z, Zamore PD: Target RNA-directed tailing and trimming purifies the sorting of endo-siRNAs between the two Drosophila Argonaute proteins. Rna 2011;17:54-63.
27 Baccarini A, Chauhan H, Gardner TJ, Jayaprakash AD, Sachidanandam R, Brown BD: Kinetic analysis reveals the fate of a microRNA following target regulation in mammalian cells. Curr Biol 2011;21:369-376.
28 Singh J, Nagaraju J: In silico prediction and characterization of microRNAs from red flour beetle (Tribolium castaneum). Insect Mol Biol 2008;17:427-436.
29 Bartel DP: MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004;116:281-297.
30 Hu Z: Insight into microRNA regulation by analyzing the characteristics of their targets in humans. BMC Genomics 2009;10:594.
31 Forman JJ, Legesse-Miller A, Coller HA: A search for conserved sequences in coding regions reveals that the let-7 microRNA targets Dicer within its coding sequence. Proc Natl Acad Sci U S A 2008;105:14879-14884.
32 Kaminska D, Kuulasmaa T, Venesmaa S, Kakela P, Vaittinen M, Pulkkinen L, Paakkonen M, Gylling H, Laakso M, Pihlajamaki J: Adipose tissue TCF7L2 splicing is regulated by weight loss and associates with glucose and fatty acid metabolism. Diabetes 2012;61:2807-2813.

33 Turchinovich A, Weiz L, Langheinz A, Burwinkel B: Characterization of extracellular circulating microRNA. Nucleic Acids Res 2011;39:7223-7233.
34 Pritchard CC, Kroh E, Wood B, Arroyo JD, Dougherty KJ, Miyaji MM, Tait JF, Tewari M: Blood cell origin of circulating microRNAs: a cautionary note for cancer biomarker studies. Cancer Prev Res (Phila) 2012;5:492-497.
35 Raitoharju E, Lyytikainen LP, Levula M, Oksala N, Mennander A, Tarkka M, Klopp N, Illig T, Kahonen M, Karhunen PJ, Laaksonen R, Lehtimaki T: miR-21, miR-210, miR-34a, and miR-146a/b are up-regulated in human atherosclerotic plaques in the Tampere Vascular Study. Atherosclerosis 2011;219:211-217.
36 Zhang W, Dolan ME: The emerging role of microRNAs in drug responses. Curr Opin Mol Ther 2010;12:695-702.
37 Mori M, Triboulet R, Mohseni M, Schlegelmilch K, Shrestha K, Camargo FD, Gregory RI: Hippo signaling regulates microprocessor and links cell-density-dependent miRNA biogenesis to cancer. Cell 2014;156:893-906.
38 Zampetaki A, Mayr M: Analytical challenges and technical limitations in assessing circulating miRNAs. Thromb Haemost 2012;108:592-598.
39 Pant S, Hilton H, Burczynski ME: The multifaceted exosome: biogenesis, role in normal and aberrant cellular function, and frontiers for pharmacological and biomarker opportunities. Biochem Pharmacol 2012;83:1484-1494.
40 Simpson RJ, Lim JW, Moritz RL, Mathivanan S: Exosomes: proteomic insights and diagnostic potential. Expert Rev Proteomics 2009;6:267-283.
41 Thery C, Boussac M, Veron P, Ricciardi-Castagnoli P, Raposo G, Garin J, Amigorena S: Proteomic analysis of dendritic cell-derived exosomes: a secreted subcellular compartment distinct from apoptotic vesicles. J Immunol 2001;166:7309-7318.
42 Lee Y, El Andaloussi S, Wood MJ: Exosomes and microvesicles: extracellular vesicles for genetic information transfer and gene therapy. Hum Mol Genet 2012;21:R125-134.
43 Mittelbrunn M, Sanchez-Madrid F: Intercellular communication: diverse structures for exchange of genetic information. Nat Rev Mol Cell Biol 2012;13:328-335.
44 Zomer A, Vendrig T, Hopmans ES, van Eijndhoven M, Middeldorp JM, Pegtel DM: Exosomes: Fit to deliver small RNA. Commun Integr Biol 2010;3:447-450.
45 Abels ER, Breakefield XO: Introduction to Extracellular Vesicles: Biogenesis, RNA Cargo Selection, Content, Release, and Uptake. Cell Mol Neurobiol 2016;36:301-312.
46 Iraci N, Leonardi T, Gessler F, Vega B, Pluchino S: Focus on Extracellular Vesicles: Physiological Role and Signalling Properties of Extracellular Membrane Vesicles. Int J Mol Sci 2016;17:171.
47 Tkach M, Thery C: Communication by Extracellular Vesicles: Where We Are and Where We Need to Go. Cell 2016;164:1226-1232.
48 Raimondo F, Morosi L, Chinello C, Magni F, Pitto M: Advances in membranous vesicle and exosome proteomics improving biological understanding and biomarker discovery. Proteomics 2011;11:709-720.
49 Choi DS, Kim DK, Kim YK, Gho YS: Proteomics, transcriptomics and lipidomics of exosomes and ectosomes. Proteomics 2013;13:1554-1571.
50 Mathivanan S, Fahner CJ, Reid GE, Simpson RJ: ExoCarta 2012: database of exosomal proteins, RNA and lipids. Nucleic Acids Res 2012;40:D1241-1244.
51 Keller P, Vollaard NB, Gustafsson T, Gallagher IJ, Sundberg CJ, Rankinen T, Britton SL, Bouchard C, Koch LG, Timmons JA: A transcriptional map of the impact of endurance exercise training on skeletal muscle phenotype. J Appl Physiol (1985) 2011;110:46-59.
52 Nielsen S, Scheele C, Yfanti C, Akerstrom T, Nielsen AR, Pedersen BK, Laye MJ: Muscle specific microRNAs are regulated by endurance exercise in human skeletal muscle. J Physiol 2010;588:4029-4037.
53 Rankinen T, Bray MS, Hagberg JM, Perusse L, Roth SM, Wolfarth B, Bouchard C: The human gene map for performance and health-related fitness phenotypes: the 2005 update. Med Sci Sports Exerc 2006;38:1863-1888.
54 Tome-Carneiro J, Larrosa M, Yanez-Gascon MJ, Davalos A, Gil-Zamorano J, Gonzalvez M, Garcia-Almagro FJ, Ruiz Ros JA, Tomas-Barberan FA, Espin JC, Garcia-Conesa MT: One-year supplementation with a grape extract containing resveratrol modulates inflammatory-related microRNAs and cytokines expression in peripheral blood mononuclear cells of type 2 diabetes and hypertensive patients with coronary artery disease. Pharmacol Res 2013;72:69-82.

55 Esau C, Kang X, Peralta E, Hanson E, Marcusson EG, Ravichandran LV, Sun Y, Koo S, Perera RJ, Jain R, Dean NM, Freier SM, Bennett CF, Lollo B, Griffey R: MicroRNA-143 regulates adipocyte differentiation. J Biol Chem 2004;279:52361-52365.
56 Kajimoto K, Naraba H, Iwai N: MicroRNA and 3T3-L1 pre-adipocyte differentiation. Rna 2006;12:1626-1632.
57 Sun T, Fu M, Bookout AL, Kliewer SA, Mangelsdorf DJ: MicroRNA let-7 regulates 3T3-L1 adipogenesis. Mol Endocrinol 2009;23:925-931.
58 Xie H, Lim B, Lodish HF: MicroRNAs induced during adipogenesis that accelerate fat cell development are downregulated in obesity. Diabetes 2009;58:1050-1057.
59 Xu Y, Zhu W, Sun Y, Wang Z, Yuan W, Du Z: Functional Network Analysis Reveals Versatile MicroRNAs in Human Heart. Cell Physiol Biochem 2015;36:1628-1643.
60 Yang Y, Yang L, Liang X, Zhu G: MicroRNA-155 Promotes Atherosclerosis Inflammation via Targeting SOCS1. Cell Physiol Biochem 2015;36:1371-1381.
61 Jiang X, Xue M, Fu Z, Ji C, Guo X, Zhu L, Xu L, Pang L, Xu M, Qu H: Insight into the effects of adipose tissue inflammation factors on miR-378 expression and the underlying mechanism. Cell Physiol Biochem 2014;33:1778-1788.
62 Trajkovski M, Hausser J, Soutschek J, Bhat B, Akin A, Zavolan M, Heim MH, Stoffel M: MicroRNAs 103 and 107 regulate insulin sensitivity. Nature 2011;474:649-653.
63 Dehwah MA, Xu A, Huang Q: MicroRNAs and type 2 diabetes/obesity. J Genet Genomics 2012;39:11-18.
64 Zhou T, Meng X, Che H, Shen N, Xiao D, Song X, Liang M, Fu X, Ju J, Li Y, Xu C, Zhang Y, Wang L: Regulation of Insulin Resistance by Multiple MiRNAs via Targeting the GLUT4 Signalling Pathway. Cell Physiol Biochem 2016;38:2063-2078.
65 Frias Fde T, de Mendonca M, Martins AR, Gindro AF, Cogliati B, Curi R, Rodrigues AC: MyomiRs as Markers of Insulin Resistance and Decreased Myogenesis in Skeletal Muscle of Diet-Induced Obese Mice. Front Endocrinol (Lausanne) 2016;7:76.
66 Kirby TJ, Chaillou T, McCarthy JJ: The role of microRNAs in skeletal muscle health and disease. Front Biosci (Landmark Ed) 2015;20:37-77.
67 McCarthy JJ: microRNA and skeletal muscle function: novel potential roles in exercise, diseases, and aging. Front Physiol 2014;5:290.
68 Winbanks CE, Beyer C, Qian H, Gregorevic P: Transduction of skeletal muscles with common reporter genes can promote muscle fiber degeneration and inflammation. PLoS One 2012;7:e51627.
69 Hitachi K, Nakatani M, Tsuchida K: Myostatin signaling regulates Akt activity via the regulation of miR-486 expression. Int J Biochem Cell Biol 2014;47:93-103.
70 Albright A, Franz M, Hornsby G, Kriska A, Marrero D, Ullrich I, Verity LS: American College of Sports Medicine position stand. Exercise and type 2 diabetes. Med Sci Sports Exerc 2000;32:1345-1360.
71 Holloszy JO: Exercise-induced increase in muscle insulin sensitivity. J Appl Physiol (1985) 2005;99:338-343.
72 Menshikova EV, Ritov VB, Fairfull L, Ferrell RE, Kelley DE, Goodpaster BH: Effects of exercise on mitochondrial content and function in aging human skeletal muscle. J Gerontol A Biol Sci Med Sci 2006;61:534-540.
73 Corcoran MP, Lamon-Fava S, Fielding RA: Skeletal muscle lipid deposition and insulin resistance: effect of dietary fatty acids and exercise. Am J Clin Nutr 2007;85:662-677.
74 Hawley JA, Lessard SJ: Exercise training-induced improvements in insulin action. Acta Physiol (Oxf) 2008;192:127-135.
75 Misigoj-Durakovic M, Durakovic Z: The early prevention of metabolic syndrome by physical exercise. Coll Antropol 2009;33:759-764.
76 Fallucca F, Pozzilli P: Physical exercise, public health and quality of life in diabetes. Diabetes Metab Res Rev 2009;25:S1-3.
77 Colberg SR, Grieco CR: Exercise in the treatment and prevention of diabetes. Curr Sports Med Rep 2009;8:169-175.
78 Donnelly JE, Blair SN, Jakicic JM, Manore MM, Rankin JW, Smith BK: American College of Sports Medicine Position Stand. Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults. Med Sci Sports Exerc 2009;41:459-471.
79 Goodpaster BH, Katsiaras A, Kelley DE: Enhanced fat oxidation through physical activity is associated with improvements in insulin sensitivity in obesity. Diabetes 2003;52:2191-2197.

80 Fujii N, Aschenbach WG, Musi N, Hirshman MF, Goodyear LJ: Regulation of glucose transport by the AMP-activated protein kinase. Proc Nutr Soc 2004;63:205-210.
81 McFarlin BK, Flynn MG, Campbell WW, Craig BA, Robinson JP, Stewart LK, Timmerman KL, Coen PM: Physical activity status, but not age, influences inflammatory biomarkers and toll-like receptor 4. J Gerontol A Biol Sci Med Sci 2006;61:388-393.
82 Martin-Cordero L, Garcia JJ, Giraldo E, De la Fuente M, Manso R, Ortega E: Influence of exercise on the circulating levels and macrophage production of IL-1beta and IFNgamma affected by metabolic syndrome: an obese Zucker rat experimental animal model. Eur J Appl Physiol 2009;107:535-543.
83 Toledo FG, Menshikova EV, Ritov VB, Azuma K, Radikova Z, DeLany J, Kelley DE: Effects of physical activity and weight loss on skeletal muscle mitochondria and relationship with glucose control in type 2 diabetes. Diabetes 2007;56:2142-2147.
84 Aoi W, Naito Y, Mizushima K, Takanami Y, Kawai Y, Ichikawa H, Yoshikawa T: The microRNA miR-696 regulates PGC-1{alpha} in mouse skeletal muscle in response to physical activity. Am J Physiol Endocrinol Metab 2010;298:E799-806.
85 Davidsen PK, Gallagher IJ, Hartman JW, Tarnopolsky MA, Dela F, Helge JW, Timmons JA, Phillips SM: High responders to resistance exercise training demonstrate differential regulation of skeletal muscle microRNA expression. J Appl Physiol (1985) 2011;110:309-317.
86 Zacharewicz E, Della Gatta P, Reynolds J, Garnham A, Crowley T, Russell AP, Lamon S: Identification of microRNAs linked to regulators of muscle protein synthesis and regeneration in young and old skeletal muscle. PLoS One 2014;9:e114009.
87 Holloszy JO: Regulation of mitochondrial biogenesis and GLUT4 expression by exercise. Compr Physiol 2011;1:921-940.
88 Little JP, Safdar A, Wilkin GP, Tarnopolsky MA, Gibala MJ: A practical model of low-volume high-intensity interval training induces mitochondrial biogenesis in human skeletal muscle: potential mechanisms. J Physiol 2010;588:1011-1022.
89 Yamamoto H, Morino K, Nishio Y, Ugi S, Yoshizaki T, Kashiwagi A, Maegawa H: MicroRNA-494 regulates mitochondrial biogenesis in skeletal muscle through mitochondrial transcription factor A and Forkhead box j3. Am J Physiol Endocrinol Metab 2012;303:E1419-1427.
90 Yu X, Zuo Q: MicroRNAs in the regeneration of skeletal muscle. Front Biosci (Landmark Ed) 2013;18:608-615.
91 Winbanks CE, Ooi JY, Nguyen SS, McMullen JR, Bernardo BC: MicroRNAs differentially regulated in cardiac and skeletal muscle in health and disease: potential drug targets? Clin Exp Pharmacol Physiol 2014;41:727-737.
92 Mueller WC, Spector Y, Edmonston TB, St Cyr B, Jaeger D, Lass U, Aharonov R, Rosenwald S, Chajut A: Accurate classification of metastatic brain tumors using a novel microRNA-based test. Oncologist 2011;16:165-174.
93 Russell AP, Lamon S, Boon H, Wada S, Guller I, Brown EL, Chibalin AV, Zierath JR, Snow RJ, Stepto N, Wadley GD, Akimoto T: Regulation of miRNAs in human skeletal muscle following acute endurance exercise and short-term endurance training. J Physiol 2013;591:4637-4653.
94 Baggish AL, Hale A, Weiner RB, Lewis GD, Systrom D, Wang F, Wang TJ, Chan SY: Dynamic regulation of circulating microRNA during acute exhaustive exercise and sustained aerobic exercise training. J Physiol 2011;589:3983-3994.
95 Aoi W, Ichikawa H, Mune K, Tanimura Y, Mizushima K, Naito Y, Yoshikawa T: Muscle-enriched microRNA miR-486 decreases in circulation in response to exercise in young men. Front Physiol 2013;4:80.
96 Mooren FC, Viereck J, Kruger K, Thum T: Circulating microRNAs as potential biomarkers of aerobic exercise capacity. Am J Physiol Heart Circ Physiol 2014;306:H557-563.
97 de Gonzalo-Calvo D, Davalos A, Montero A, Garcia-Gonzalez A, Tyshkovska I, Gonzalez-Medina A, Soares SM, Martinez-Camblor P, Casas-Agustench P, Rabadan M, Diaz-Martinez AE, Ubeda N, Iglesias-Gutierrez E: Circulating inflammatory miRNA signature in response to different doses of aerobic exercise. J Appl Physiol (1985) 2015;119:124-134.
98 Wardle SL, Bailey ME, Kilikevicius A, Malkova D, Wilson RH, Venckunas T, Moran CN: Plasma microRNA levels differ between endurance and strength athletes. PLoS One 2015;10:e0122107.
99 Fernandes T, Magalhaes FC, Roque FR, Phillips MI, Oliveira EM: Exercise training prevents the microvascular rarefaction in hypertension balancing angiogenic and apoptotic factors: role of microRNAs-16, -21, and -126. Hypertension 2012;59:513-520.

100 McCarthy JJ, Esser KA: MicroRNA-1 and microRNA-133a expression are decreased during skeletal muscle hypertrophy. J Appl Physiol (1985) 2007;102:306-313.
101 Kirby TJ, McCarthy JJ: MicroRNAs in skeletal muscle biology and exercise adaptation. Free Radic Biol Med 2013;64:95-105.
102 McCarthy JJ: MicroRNA-206: the skeletal muscle-specific myomiR. Biochim Biophys Acta 2008;1779:682-691.
103 Elia L, Contu R, Quintavalle M, Varrone F, Chimenti C, Russo MA, Cimino V, De Marinis L, Frustaci A, Catalucci D, Condorelli G: Reciprocal regulation of microRNA-1 and insulin-like growth factor-1 signal transduction cascade in cardiac and skeletal muscle in physiological and pathological conditions. Circulation 2009;120:2377-2385.
104 Hua Y, Zhang Y, Ren J: IGF-1 deficiency resists cardiac hypertrophy and myocardial contractile dysfunction: role of microRNA-1 and microRNA-133a. J Cell Mol Med 2012;16:83-95.
105 Wang XH: MicroRNA in myogenesis and muscle atrophy. Curr Opin Clin Nutr Metab Care 2013;16:258-266.
106 Bell ML, Buvoli M, Leinwand LA: Uncoupling of expression of an intronic microRNA and its myosin host gene by exon skipping. Mol Cell Biol 2010;30:1937-1945.
107 Callis TE, Pandya K, Seok HY, Tang RH, Tatsuguchi M, Huang ZP, Chen JF, Deng Z, Gunn B, Shumate J, Willis MS, Selzman CH, Wang DZ: MicroRNA-208a is a regulator of cardiac hypertrophy and conduction in mice. J Clin Invest 2009;119:2772-2786.
108 McCarthy JJ: The MyomiR network in skeletal muscle plasticity. Exerc Sport Sci Rev 2011;39:150-154.
109 Fernandes T, Hashimoto NY, Magalhaes FC, Fernandes FB, Casarini DE, Carmona AK, Krieger JE, Phillips MI, Oliveira EM: 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;58:182-189.
110 Soci UP, Fernandes T, Hashimoto NY, Mota GF, Amadeu MA, Rosa KT, Irigoyen MC, Phillips MI, Oliveira EM: MicroRNAs 29 are involved in the improvement of ventricular compliance promoted by aerobic exercise training in rats. Physiol Genomics 2011;43:665-673.
111 Ma Z, Qi J, Meng S, Wen B, Zhang J: Swimming exercise training-induced left ventricular hypertrophy involves microRNAs and synergistic regulation of the PI3K/AKT/mTOR signaling pathway. Eur J Appl Physiol 2013;113:2473-2486.
112 Liu X, Xiao J, Zhu H, Wei X, Platt C, Damilano F, Xiao C, Bezzerides V, Bostrom P, Che L, Zhang C, Spiegelman BM, Rosenzweig A: miR-222 is necessary for exercise-induced cardiac growth and protects against pathological cardiac remodeling. Cell Metab 2015;21:584-595.
113 Melo SF, Fernandes T, Barauna VG, Matos KC, Santos AA, Tucci PJ, Oliveira EM: Expression of MicroRNA-29 and Collagen in Cardiac Muscle after Swimming Training in Myocardial-Infarcted Rats. Cell Physiol Biochem 2014;33:657-669.
114 Lin RC, Weeks KL, Gao XM, Williams RB, Bernardo BC, Kiriazis H, Matthews VB, Woodcock EA, Bouwman RD, Mollica JP, Speirs HJ, Dawes IW, Daly RJ, Shioi T, Izumo S, Febbraio MA, Du XJ, McMullen JR: PI3K(p110 alpha) protects against myocardial infarction-induced heart failure: identification of PI3K-regulated miRNA and mRNA. Arterioscler Thromb Vasc Biol 2010;30:724-732.
115 Wang YS, Zhou J, Hong K, Cheng XS, Li YG: MicroRNA-223 displays a protective role against cardiomyocyte hypertrophy by targeting cardiac troponin I-interacting kinase. Cell Physiol Biochem 2015;35:1546-1556.
116 DA Silva ND J, Fernandes T, Soci UP, Monteiro AW, Phillips MI, EM DEO: Swimming training in rats increases cardiac MicroRNA-126 expression and angiogenesis. Med Sci Sports Exerc 2012;44:1453-1462.
117 Ramasamy S, Velmurugan G, Shanmugha Rajan K, Ramprasath T, Kalpana K: MiRNAs with apoptosis regulating potential are differentially expressed in chronic exercise-induced physiologically hypertrophied hearts. PLoS One 2015;10:e0121401.
118 Souza RW, Fernandez GJ, Cunha JP, Piedade WP, Soares LC, Souza PA, de Campos DH, Okoshi K, Cicogna AC, Dal-Pai-Silva M, Carvalho RF: Regulation of cardiac microRNAs induced by aerobic exercise training during heart failure. Am J Physiol Heart Circ Physiol 2015;309:H1629-1641.
119 Xu Y, Zhu W, Wang Z, Yuan W, Sun Y, Liu H, Du Z: Combinatorial microRNAs suppress hypoxia-induced cardiomyocytes apoptosis. Cell Physiol Biochem 2015;37:921-932.
120 Wang H, Bei Y, Shi J, Xiao J, Kong X: Non-Coding RNAs in Cardiac Aging. Cell Physiol Biochem 2015;36:1679-1687.

121 Melo SF, Barauna VG, Neves VJ, Fernandes T, Lara Lda S, Mazzotti DR, Oliveira EM: Exercise training restores the cardiac microRNA-1 and -214 levels regulating Ca^{2+} handling after myocardial infarction. BMC Cardiovasc Disord 2015;15:166.
122 Espinoza JL, Takami A, Yoshioka K, Nakata K, Sato T, Kasahara Y, Nakao S: Human microRNA-1245 down-regulates the NKG2D receptor in natural killer cells and impairs NKG2D-mediated functions. Haematologica 2012;97:1295-1303.
123 Makarova JA, Maltseva DV, Galatenko VV, Abbasi A, Maximenko DG, Grigoriev AI, Tonevitsky AG, Northoff H: Exercise immunology meets MiRNAs. Exerc Immunol Rev 2014;20:135-164.
124 Wessner B, Gryadunov-Masutti L, Tschan H, Bachl N, Roth E: Is there a role for microRNAs in exercise immunology? A synopsis of current literature and future developments. Exerc Immunol Rev 2010;16:22-39.
125 Chilton WL, Marques FZ, West J, Kannourakis G, Berzins SP, O'Brien BJ, Charchar FJ: Acute exercise leads to regulation of telomere-associated genes and microRNA expression in immune cells. PLoS One 2014;9:e92088.
126 Belotto MF, Magdalon J, Rodrigues HG, Vinolo MA, Curi R, Pithon-Curi TC, Hatanaka E: Moderate exercise improves leucocyte function and decreases inflammation in diabetes. Clin Exp Immunol 2010;162:237-243.
127 Krause M, Heck TG, Bittencourt A, Scomazzon SP, Newsholme P, Curi R, Homem de Bittencourt PI Jr.: The chaperone balance hypothesis: the importance of the extracellular to intracellular HSP70 ratio to inflammation-driven type 2 diabetes, the effect of exercise, and the implications for clinical management. Mediators Inflamm 2015;2015:249205.
128 Silveira EM, Rodrigues MF, Krause MS, Vianna DR, Almeida BS, Rossato JS, Oliveira LP Jr., Curi R, de Bittencourt PI, Jr.: Acute exercise stimulates macrophage function: possible role of NF-kappaB pathways. Cell Biochem Funct 2007;25:63-73.
129 Gondim OS, de Camargo VT, Gutierrez FA, Martins PF, Passos ME, Momesso CM, Santos VC, Gorjao R, Pithon-Curi TC, Cury-Boaventura MF: Benefits of Regular Exercise on Inflammatory and Cardiovascular Risk Markers in Normal Weight, Overweight and Obese Adults. PLoS One 2015;10:e0140596.
130 Radom-Aizik S, Zaldivar F, Haddad F, Cooper DM: Impact of brief exercise on peripheral blood NK cell gene and microRNA expression in young adults. J Appl Physiol (1985) 2013;114:628-636.
131 Radom-Aizik S, Zaldivar FP Jr., Haddad F, Cooper DM: Impact of brief exercise on circulating monocyte gene and microRNA expression: implications for atherosclerotic vascular disease. Brain Behav Immun 2014;39:121-129.
132 Radom-Aizik S, Zaldivar F, Jr., Leu SY, Adams GR, Oliver S, Cooper DM: Effects of exercise on microRNA expression in young males peripheral blood mononuclear cells. Clin Transl Sci 2012;5:32-38.
133 Dai R, Ahmed SA: MicroRNA, a new paradigm for understanding immunoregulation, inflammation, and autoimmune diseases. Transl Res 2011;157:163-179.
134 Radom-Aizik S, Zaldivar F Jr., Oliver S, Galassetti P, Cooper DM: Evidence for microRNA involvement in exercise-associated neutrophil gene expression changes. J Appl Physiol (1985) 2010;109:252-261.
135 Wu C, Gong Y, Yuan J, Zhang W, Zhao G, Li H, Sun A, Zou Y, Ge J: microRNA-181a represses ox-LDL-stimulated inflammatory response in dendritic cell by targeting c-Fos. J Lipid Res 2012;53:2355-2363.
136 Sun X, Icli B, Wara AK, Belkin N, He S, Kobzik L, Hunninghake GM, Vera MP, Blackwell TS, Baron RM, Feinberg MW: MicroRNA-181b regulates NF-kappaB-mediated vascular inflammation. J Clin Invest 2012;122:1973-1990.
137 Tonevitsky AG, Maltseva DV, Abbasi A, Samatov TR, Sakharov DA, Shkurnikov MU, Lebedev AE, Galatenko VV, Grigoriev AI, Northoff H: Dynamically regulated miRNA-mRNA networks revealed by exercise. BMC Physiol 2013;13:9.
138 Bye A, Rosjo H, Aspenes ST, Condorelli G, Omland T, Wisloff U: Circulating microRNAs and aerobic fitness--the HUNT-Study. PLoS One 2013;8:e57496.
139 Nie M, Liu J, Yang Q, Seok HY, Hu X, Deng ZL, Wang DZ: MicroRNA-155 facilitates skeletal muscle regeneration by balancing pro- and anti-inflammatory macrophages. Cell Death Dis 2016;7:e2261.