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Review Article

Circulating microRNAs fluctuations in exercise-induced cardiac remodeling: A systematic review

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Abstract: MicroRNAs (miRNAs) are small non-coding RNAs that participate in gene expression regulation. It has been observed that circulating levels of miRNAs may fluctuate during exercise, showing numerous cardiac biological and physiological effects such as structural and functional adaptations. We aimed to provide an overview of the currently available information concerning the role of circulating miRNAs in cardiovascular adaptations in response to acute and/or chronic exercise training. Relevant studies published were searched in three databases: PubMed, Web of Science and Scopus. A combination of the following keywords was used: ("microRNA" OR "miRNA" OR "miR" AND "exercise" OR "training" OR "physical activity") AND "(heart hypertrophy" OR "cardiac remodeling" OR "cardiac muscle mass" OR "cardiac hypertrophy"). Only experimental studies, written in English and conducted in healthy individuals were included. Five articles met the inclusion criteria and were finally included in this systematic review after reviewing both title, abstract and full-text. A total of thirty-six circulating cardiac-related miRNAs were analyzed, but only five of them (miR-1, miR-133a, miR-146a, miR-206 and miR-221) were directly associated with cardiac adaptations parameters, while two of them (miR-1 and miR-133a) were related to cardiac hypertrophy. Most of them were upregulated immediately after a marathon and returned to basal levels at longer times. Therefore, we conclude that, although evidence is still limited, and long-term studies are needed to obtain more robust evidence, exercise is more likely to affect circulating cardiac-related miRNAs levels.

Keywords: Cardiac hypertrophy, miRNA, exercise adaptations, biomarker

Introduction

Physical exercise is considered an effective non-pharmacologic strategy to prevent cardiovascular diseases [1], and its prescription is recommended by the guidelines of reference organizations, like the American College of Sports Medicine or the American Heart Association [2]. Physical exercise can restore myocardial function, improve maximal oxygen consumption (VO_{2max}) and endothelial cell function, left ventricular (LV) systolic and diastolic function, increase cardiac mass, develop new blood vessels, and decrease collagen content and fibrosis [3-6]. Exercise-based cardiac rehabilitation is then effective at all stages of cardiovascular disease treatment, and counteracts declined cardiac function to a certain extent in injured as well as in aging hearts [7-9]. Moreover, exercise-modulated gene expression and cell signaling may protect the heart from further injuries and continuous maladaptive remodeling processes [10].

A specific RNA-based therapy using microRNAs (miRNA) has gained attention during last decade, due to its connection with cardiac physiology and pathology [11, 12]. miRNAs are small non-coding RNAs, typically composed by 18-25 nucleotides, that regulate gene expression, either by inhibiting messenger RNAs translation or impairing mRNA stability. Cur-

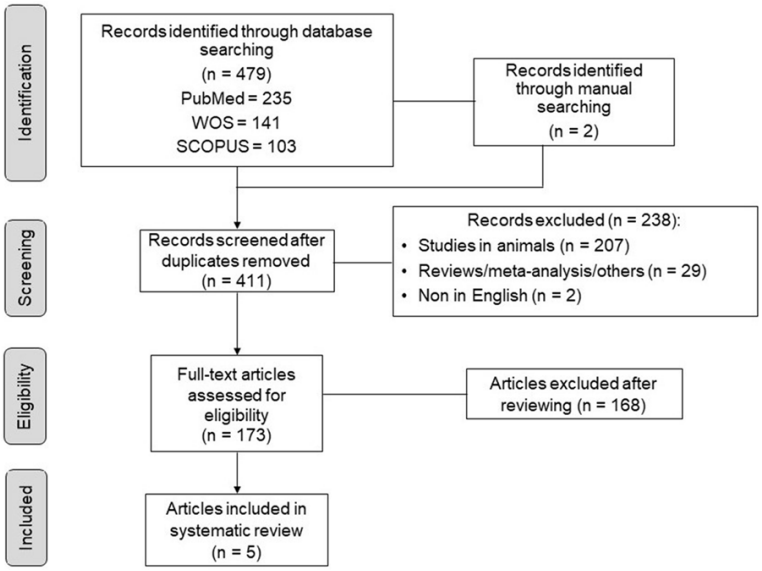

Figure 1. Flow chart of the systematic literature review.

rently, more than 2654 human miRNAs have been characterized (http://www.mirbase.org/), and nearly 60% of all human genes could be a target of miRNAs [13]. Although most miRNAs are present in the cytoplasm, they can translate from the cell to the extracellular environment, so that they can be found in blood, plasma, serum and urine, and thus, are called circulating miRNAs [14]. Then, miRNAs can act as paracrine and endocrine mediators [15] and linked to cell proliferation and apoptosis, immune function and heart physiology [16, 17]. Several animal and human studies have shown altered miRNA levels in cardiac diseases such as hypertrophy, ischemic and dilated cardiomyopathy, aortic stenosis or arrhythmias [18-22]. Cardiovascular miRNAs are differentially expressed with regular physical exercise training, participating in cardiac remodeling [10, 16, 23] and protection against cardiovascular diseases [23, 24] in both human and rodent models. This systematic review aims to provide an overview of the currently available information concerning the role of miRNAs in cardiovascular adaptations in response to acute and/or chronic exercise training.

Materials and methods

Literature search

This systematic review is presented according to the criteria set out in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses recommendations (PRISMA statement) [25]. The search was performed in PubMed, Web of Science and SCOPUS databases using the key-words: ("microRNA" or "miRNA" or "miR") and ("exercise" or "training" OR "physical activity" or "physical exercise") and ("heart hypertrophy" or "cardiac remodeling" or "cardiac muscle mass" or "cardiac hypertrophy"). The filters used in the databases were "humans", "English language" and "articles". All records were reviewed after applying the filters to discard those that did not meet the inclusion criteria. All articles published until October 6st, 2021 were considered. Also, a manual search was made in the bibliography of previous studies and reviews to include other articles related to this review.

Eligibility criteria

Only experimental studies addressing a role for miRNAs in cardiovascular response to exercise training in healthy humans were included. The sample size, subjects' gender and age, and the type of exercise performed, were not considered during the screening. Articles written in languages other than English, studies in which participants suffered from any pathological process or disease, and those carried out in animals' models were excluded. Besides, reviews, meta-analysis, editorials and letters were not considered.

Study selection

The electronic search, carried out according to our predefined criteria, provided 479 studies. Two additional articles were also included after manual search. A total of 481 documents were generated. One hundred and seventy three articles were selected after applying inclusion criteria and removing duplicates. Five articles were finally included in this systematic review after reviewing title and abstract (**Figure 1**).

Results

Studies' characteristics

Experimental studies included middle-aged healthy men participants (39.9±9.31 years), with an average body mass index of 24.7±0.9 kg/m^2. The sample size ranged from 9 [26] to 30 [27] individuals, with a maximum of 95. The athletes studied were resistance runners [26-29], rowers [28], and basketball players [30]. The follow-up period ranged from 3 days [28, 29] to 3 months [26, 31]. Two of five studies were performed in Germany [27, 29], one in United States [28], one in Spain [26], and one in Sanghai [31]. Studies' characteristics and results are summarized in **Table 1**.

All studies analyzed the exercise-induced effects on circulating miRNAs concentrations, as well as whether these fluctuations may be correlated with other physiological variables. Two studies measured cardiac-related miRNAs levels in marathon runners at three time points: before, immediately after and 24 hours after the marathon [28, 29]. Baggish et al. [28] also measured circulating miRNA levels in rowers (n=11) before, immediately after and 24 hours after exercising. De Gonzalo-Calvo et al. [26] measured cardiovascular-related miRNAs in subjects who participated in three different running races distances, i.e., 10-km race, half-marathon and marathon. Blood samples were collected at four time points: before, immediately after, 24 and 72 hours after the run. Finally, the study by Li et al. [31] evaluated circulating miRNAs levels in basketball players and correlated with cardiac variables evaluated by echocardiography.

Cardiovascular-related miRNAs

The selected studies measured a wide range of cardiovascular-related miRNAs, including: miR-1, miR-16-5p, miR-21-5p, miR-25-3p, miR-26a, miR-27a-3p, miR-29a-3p, miR-29b, miR-30a, miR-30b-5p, miR-34a-5p, miR-103a-3p, miR-106b-5p, miR-107, miR-126 (also referred to as miR-126-3p), miR-132-3p, miR-133a, miR-133b, miR-139-5p, miR-142-5p, miR-143-3p, miR-150-5p, miR-195-5p, miR-199a-3p, miR-206, miR-208a, miR-208b, miR-375-5p, miR-378, miR-486, miR-497-5p, miR-499, miR-499-5p, miR-590-5p and miR-940. The different biological and physiological cardiovascular effects elicited by each miRNA is summarized in **Figure 2**.

MiR-1, miR-21-5p, miR-27a-3p, miR-29a-3p, miR-30a, miR-34a-5p, miR-103a-3p, miR-126 (miR-126-3p), miR-133a, miR-142-5p, miR-143-3p, miR-195-5p, miR-199a-3p, miR-208a, miR-208b, miR-499 and miR-499-5p were significantly upregulated immediately after a marathon [26-29]. Most miRNAs returned to baseline levels 24 hours after finishing the marathon except miR-499-5p, that varied individually [28], and miR-1, miR-133a and miR-206, which persisted significantly increased [29]. Clauss et al. [27] reported that miR-26a and miR-29b levels decreased immediately after the marathon and 24 hours after, but only miR-26a showed significant differences, whilst de Gonzalo-Calvo et al. [26] observed that miR-106b-5p and miR-107 were significantly downregulated 24 hours after the marathon. Clauss et al. [27] also compared the difference between elite runners (ER; N=15; ran 73.9±3.9 km per week) and non-elite runners (NER; N=15; 33.9±2.7 km per week), and found that miR-1, miR-30a and miR-133a were significantly increased in ER, whilst miR-26a was decreased in NER. miR-103a-3p and miR-139-5p were significantly downregulated after a 10-km running race, whilst miR-132-3p and miR-150-5p increased immediately after the race. The levels of these miRNAs returned to baseline levels 24 hours later, though miR-590-5p decreased significantly 24 hours after the race [26]. Finally, Li et al. [31] found that miR-208b was decreased and miR-221 was increased after a long period of training (3-month basketball training), whilst miR-221, miR-21, miR-146a, and miR-210 were reduced immediately after exercise.

Association between miRNAs concentrations, cardiac function variables, cardiac biochemical markers and exercise performance

miR-1, miR-133a, miR-146a and miR-221 were associated with cardiac structure, function and biomarkers (**Table 2**). Specifically, miR-1 and miR-133a were negatively correlated with left atrium diameter (LAD) [27], whilst miR-133a were positively correlated with interventricular septum (IVS) [29]. miR-1 was positively correlated with fractional shortening (FS) [29]. Interestingly, miR-1 and miR-133a were

Table 1. Summary of main characteristics of the studies included in the systematic review

Study	Aim	Methods	Results related with miRNAs
Baggish et al. (2014)	To study whether cardiac-related miRNAs levels are differentially upregulated in response to different exercise protocols (PLS).	*Marathon design (N=21):* Healthy male marathon runners evaluated at three time points: baseline, immediately after and 24 h after a marathon	*Marathon design:* Baseline: miR-1, miR-133a, miR-208a, miR-134, and miR-499-5p: down-expressed miR-146a and miR-126: over-expressed Immediately after the marathon: miR-1, miR-126, miR-133a, miR-134, miR-146a, miR-208a and miR-499-5p: significantly up-regulated 24 h after the marathon: miR-1, miR-126, miR-133a, miR-134, miR-146a, -208a: decreased toward baseline values miR-499-5p: mixture of elevated and non-elevated measurements
		Acute exhaustive exercise design (N=11): Healthy male rowers evaluated at three time points: baseline, immediately after and 1 h after rowing training	*Acute exhaustive exercise design:* Baseline: miR-1: down-expressed miR-208a: undetectable Immediately after exercise: miR-1: No significant alterations miR-208a: undetectable 1 h after exercise: miR-208a: undetectable
Clauss et al. (2016)	To determine the value of miRNAs as potential biomarkers for atrial remodeling in marathon runners (PLS).	Men marathon runners (elite runners (ER, N=15) and non-elite runners (NER, N=15)), exercised during 10-weeks (ER ≥55 km/week; NER ≤40 km/week) before participating in a marathon. Individuals were evaluated at four time points: baseline, after 10-week training program, immediately after and 24 h after the marathon	Immediately after the marathon: miR-30a: significantly increased in both groups miR-1 and miR-133a: non-significantly increased in NER and significantly increased in ER miR-26a: significantly decreased in both groups miR-29b: non-significantly decreased in both groups 24 h after the marathon: miR-30a, miR-1, miR-133a and miR-26a: returned to baseline in both groups miR-29b: non-significantly decreased in both groups
De Gonzalo-Calvo et al. (2018)	To analyze the acute exercise response of miRNAs reported as biomarkers of cardiac disease in highly trained individuals (PLS).	Healthy, highly trained middle-aged amateur subjects (N=9) evaluated at three time points: baseline, immediately after, and 24 h after a 10-km race and a marathon, each separated by one month	*10-km race:* Immediately after the race: miR-103a-3p and miR-139-5p: significantly down-regulated miR-132-3p and miR-150-5p: significantly up-regulated 24 h after the race: miR-103a-3p, miR-103a-3p, miR-132-3p and miR-150-5p and miR-139-5p: returned to baseline levels miR-590-5p: significantly decreased *Marathon race:* Immediately after the race: miR-103a-3p and miR-375-5p: significantly down-regulated miR-21-5p, miR-27a-3p, miR-29a-3p, miR-30a-5p, miR-34a-5p, miR-126-3p, miR-142-5p, miR-143-3p, miR-195-5p and miR-199a-3p: up-regulated 24 h after the race: miR-103a-3p and miR-375-5p: significantly down-regulated miR-21-5p, miR-27a-3p, miR-29a-3p, miR-30a-5p, miR-34a-5p, miR-126-3p, miR-142-5p, miR-143-3p, miR-195-5p and miR-199a-3p: returned to baseline miR-25-3p, miR-29b-3p, miR-30b-5p, miR-106b-5p, miR-107 and miR-497-5p: down-regulated

Li et al. (2018)	To study the effects of acute and long-term exercise on cardiac-related miRNAs concentrations (PLS).	Competitive male basketball players (N=10) evaluated at three time points: baseline, immediately after an exhaustive exercise session and 3-month after basketball season	Immediately after exhaustive exercise: miR-221, miR-21, miR-146a, and miR-210: significantly decreased 3-month after basketball season: miR-208b: significantly decreased miR-221: significantly increased Correlations: miR-146a was correlated with baseline levels of CKMB and Hs-CRP miR-208b was correlated with AT VO_2 at baseline miR-221 was correlated with PW and CK levels after long-term exercise
Mooren et al. (2014)	To investigate the relation between cardiac-related miRNAs with conventional and cardiovascular-related biochemical (PLS).	Male marathon runners (N=14) evaluated at three time points: baseline, immediately after and 24 h after participating in a marathon	Immediately after a marathon: miR-1, miR-133a, miR-206, miR-208b and miR-499: significantly increased 24 h after a marathon: miR-1, miR-133a and miR-206: still significantly increased miR-499 and miR-208b: returned to pre-exercise levels Correlations: miR-133a was positively correlated with IVS miR-1 was negatively correlated with FS miR-1, miR-133a and miR-206 were correlated with VO_{2max}, and V_{IAS}

ARD, Aortic root dimension; AT, Aerobic threshold; BCC, Blood cells count; BMI, Body mass index; CK, Creatine kinase; CK-MB, MB isoform of creatine kinase; CPK, creatine phosphokinase; CRP, C-reactive protein; CT-proAVP, Copeptin; DBP, diastolic blood pressure; DRV, Dimension right ventricle; E/E', ratio between early mitral inflow velocity and mitral annular early diastolic velocity; EDV, End *diastolic* volume; EF, Ejection fraction; ER, elite runners; ESV, End systolic volume; FS, Fraction shortening; h-FABP, Heart tissue fatty acid binding protein; HR, heart rate; Hs, High-sensitivity; IAT, Initial aerobic threshold; IL-6, Interleukin-6; IST, Interventricular septum thickness; LAD, Left atrial dimension; LDH, Lactate dehydrogenase; LVEDD, Left ventricular end-diastolic diameter; LVEDV, Left ventricular end-diastolic volume; LVESD, Left ventricular end-systolic diameter; LVESV, Left ventricular end-systolic volume; LVPWT, Left ventricular posterior wall thickness; miRNAs, microRNAs; MGB, Myoglobin; NER, non-elite runners; NS, no significant; NT-proBNP, Brain natriuretic peptide; PLS, Prospective longitudinal study; PW, Peak workload; PWT, Posterior wall thickness; RBP, Resting blood pressure; RHR, Resting heart rate; RSIAT, running speed at individual anaerobic lactate threshold; RV, Right ventricle; RVDD, Right ventricular diastolic diameter; SBP, systolic blood pressure; TnI, troponin I; TnT, troponin T; V_{IAS}, running speed at individual anaerobic lactate threshold; VO_{2max}, maximum rate of oxygen consumption.

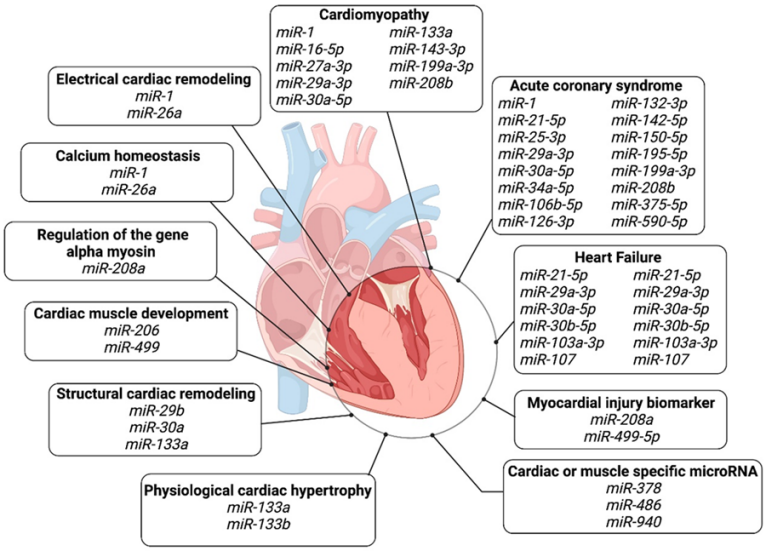

Figure 2. Physiopathological effects of miRNAs in the heart.

also positively correlated with cardiac injury biomarkers such creatine kinase (CK), CK-isoenzyme MB (CK-MB) and cardiac troponin T (cTnT) [27, 29], whilst miR-146a was negatively correlated with CK-MB [31], In ER, circulating peak concentrations of miR-1 and miR-133a were significantly correlated with LAD immediately after and 24 hours after a marathon, while no correlation between circulating miRNA concentrations and LAD was found in NER [27]. Finally, miR-1, miR-133a, miR-206, miR-208b and miR-221 were associated with exercise performance (**Table 3**).

Discussion

MiRNAs regulation through exercise has been reported to promote a number of biological and physiological cardiac adaptations [32]. The findings of this systematic review show that endurance exercise may up and/or downregulate circulating miRNA concentrations. Numerous miRNAs have been analyzed in the included studies, but only a small number of them are consistently modulated by endurance exercise. Interestingly, their targets have been related to cardiac hypertrophy and associated exercise training, suggesting that the exercise-mediated modulation of those microRNAs could contribute to the mechanisms by which exercise regulate cardiac remodeling.

Most selected studies have shown that circulating levels of miR-1 and miR-133a increased after a marathon and returned at basal levels 24 hours later. Circulating levels of both microRNAs were related to cardiac parameters of exercise performance. Both microRNAs belong to the same cluster and are muscle specific (myomirs). They have been suggested to control cardiac polarization through the modulation of β-adrenergic control of calcium channels and to be potential targets to prevent long QT phenotypes [33]. Other study reported decreased levels of both miR-1 and miR-133 in mouse and human models of cardiac hypertrophy and, interestingly, the overexpression *in vitro* of both microRNAs inhibited cardiac hypertrophy [34]. Both microRNAs have been found to be downregulated after exercise in the heart of animal models [35]. Levels of these microRNAs change after resistance exercise in the skeletal muscle, with a different effect of exercise on these microRNAs in old and young people [36, 37]. An acute bout of endurance exercise increased the expression of these microRNAs in the skeletal muscle. However, this effect of acute exercise was not evident after a 12 weeks of endurance training. Endurance training decreased resting levels of these microRNAs. Interestingly, these resting levels returned to basal levels 14 days after training cessation [38]. Russel et al. [39] also reported an increase in these microRNAs in the skeletal muscle following an acute endurance exercise bout and a return of miR-133 to normal levels after 10 days of endurance training. Therefore, the response of circulating miR-1 and miR-133 to acute resistance exercise mimics that of the muscle microRNAs. It has been suggested that these muscle-specific microRNAs are released by damaged myofibers and are, therefore, a biomarker of muscle damage due to an acute exercise [40]. However, a recent study has also shown the presence of microRNAs in muscle-specific exosomes, suggesting that myomirs could be actively secreted by muscle in response to exercise [41].

Circulating levels of miR-29b decreased after a marathon, while circulating levels of miR-29a

Table 2. Association between microRNAs and cardiac structure, function and biomarkers

	Cardiac structure	Cardiac function		Cardiac biomarkers		
	LAD	*IVS*	*FS*	*CK*	*CKMB*	*Troponin T*
miR-1	(-) Correlated in ER [27]	NS	(+) Correlated [29]	(+) Correlated in ER [27]	(+) Correlated in ER [27]	(+) Correlated in NER [27]
miR-133a	(-) Correlated in ER [27]	(+) Correlated [29]	NS	(+) Correlated in ER [27, 29]	(+) Correlated in ER [27]	(+) Correlated in NER [27]
miR-146a	NS	NS	NS	NS	(-) Correlated in AE [30]	NS
miR-221	NS	NS	NS	Association [30]	NS	NS

TAE, acute exercise; CK, creatine kinase; CKMB, MB isoform of CK; ER, elite runners; FS, fraction shortening; IVS, interventricular septum; LAD, Left Atrial Diameter; LTE, long term exercise; NER, non-elite runners; NS, no significant. Time sensitive markers of cardiac muscle response.

Table 3. Association between miRNAs and exercise performance

	Performance parameters			
	VO_{2max}	*AT VO_2*	*V_{ias}*	*Peak workload*
miR-1	(+) Correlated [29]	NS	(+) Correlated [29]	NS
miR-133a	(+) Correlated [29]	NS	(+) Correlated [29]	NS
miR-206	(+) Correlated [29]	NS	(+) Correlated [29]	NS
miR-208b	NS	(-) Correlation after LTE [30]	NS	NS
miR-221	NS	Association [30]	NS	Association [30]

AT VO_{2max}, maximal oxygen uptake at aerobic threshold; LTE, long-term exercise; NS, no significant; V_{IAS}, running speed at individual anaerobic lactate threshold; VO_{2max}, maximum rate of oxygen consumption.

showed a similar pattern that miR-1 and miR-133a. This is consistent with the results obtained by Soci et al. [35] who found an increased level of miR-29a in heart muscle after exercises, while miR-29b slightly decreased in a rat model of cardiac hypertrophy. Cardiac miR-29a level is also enhanced after intermittent aerobic exercise in rats [42]. Circulating miR-29a levels have been found upregulated in patients with pathological cardiac hypertrophy in comparison with healthy controls and, moreover, miR-29a was the only circulating microRNA studied that correlated positively with both cardiac hypertrophy and fibrosis [43]. Interestingly skeletal muscle miR-29a has been found to be differentially expressed after resistance training in responders (non-differentially upregulated) and no responders (downregulated) young men [44]. Indeed, circulating levels of miR-29a-3p have been shown to be differently regulated after exhaustive (upregulated) or non-exhaustive (downregulated) cycling exercise [45]. In this regard, miR-29a levels have been related to muscle recovery after exercise [44] and has been reported to regulate glucose uptake and insulin-mediated glucose metabolism in the skeletal muscle, being, therefore, essential for muscle performance during exhaustive exercise [46]. Therefore, results obtained in our systematic review, together with those reported by others point to circulating miR-29a as a good candidate biomarker of physical activity performance.

Circulating miR-21 levels were reported to be upregulated immediately after a marathon and returned to basal levels 24 hours after by de Gonzalo-Calvo et al. [26] but were reported to be downregulated after exhaustive exercise in basketball players by Li et al. [31]. Ma et al. [47] investigated the role of miRNAs related with cardiovascular adaptations in female rats subjected to a long-term exercise training protocol and found that miR-21 level was significantly upregulated compared with control group. Palabiyik et al. [48] also found an increase in cardiac miR-21 level in the swimming exercise group rats compared to control group rats. Wahl et al. [49] found that circulating level of miR-21 increased after a high-volume training and a spring-interval training protocol, but not after a high-intensity training protocol. Nielsen et al. [50] found that basal serum miR-21 levels decreased after 12 weeks of endurance training but did not change after and acute exercise bout. Circulating miR-21 levels were also increased immediately after an acute exercise bout in congestive heart failure pa-

tients [51]. Interestingly, Wahl et al. [49] also showed that circulating miR-21 was mainly of endothelial origin. miR-21 is synthesized in response to an inflammatory stimulus and plays a key role in the early resolution of inflammation [52]. It has been suggested that endothelial cells release extracellular vesicles containing miR-21 in response to an inflammatory stimulus. miR-21 contained in these extracellular vesicles promotes an anti-inflammatory response in the immune system [49, 53]. miR-21 has been shown to play a key role in the progression of atherosclerosis and related endothelial damage [54]. Therefore, previous results [50] and ours suggest that circulating miR-21 levels respond differently to different types of exercises and inflammation related to the type of exercise could be the stimulus that release miR-21 from endothelial and cardiac cells.

Finally, we found that circulating levels of miR-27a-3p and miR-143-3p also increased immediately after a marathon and returned to basal levels 24 hours after, but only in one out of the 5 articles selected [26]. Fernandes et al. [55] studied the effect of swimming training in female rats. The authors observed left ventricular hypertrophy after exercise compared to a sedentary group where miR-27a and miR-27b were upregulated in exercised rats while miR-143 was downregulated [35]. Circulating miR-27a levels have been found to decrease after 5 months of aerobic exercise training in obese individuals [56]. More studies are needed to assess the exercise-mediated regulation of circulating miR-27a and miR-143a levels.

Exercise modulates gene expression of certain proteins involved in miRNAs biogenesis such as Drosha, Dicer and Exportin 5 [57]. Likewise, circulating miRNAs mediate different cellular processes, especially regulating myocytes proliferation and differentiation throughout gene modulation [32]. Regarding the molecular mechanism implicated in miRNAs regulation of cardiac remodeling, exercise-regulated microRNAs are involved in molecular pathways related to cardiac remodeling and adaptation, such as the IGF-1/PI3K/AKT/mTOR and AMPK/PGC-1α/PPARα pathways and CDC42 and RhoA molecules [34, 35, 57-59].

It has previously been shown that decreased circulating miR-1 level is associated with increased IGF-1 activity [57, 60]. miR-29a has been also shown to promote pathological cardiac hypertrophy by targeting PTEN and, subsequently activating AKT/mTOR signaling pathway [61]. Upregulation in miR-21, miR-27a, and miR-29, activates PI3K and sarcoplasmic reticulum Ca^{2+} ATPase (SERCA)-2 (improving contractility), PTEN and tuberous sclerosis complex (TSC)-2 whilst downregulation in miR-1, miR-133a, miR-133b and miR-143 shows the same effect on the same targets [17]. RAS gene expression is regulated by miR-27a and miR-27b, that increases angiotensin-converting enzyme (ACE) expression, while *ACE2* gene is a target of miR-143 [35]. On the other hand, Ras GTPase proteins (RasGAP), cycling-dependent kinase 9 (CDK9), Ras homolog enriched in brain (RHEB) and fibronectin, are targets of miR-1 [62]. **Table 4** summarizes the role of specific miRNAs in the expression regulation of proteins implicated in the different signaling pathways associated with cardiac remodeling.

Variations in circulating miRNAs levels compared with cardiac markers and physical exercise were also analyzed in two of the included studies. MiR-1 and miR-133 were positively correlated with CK, CK-MB and cTnT in ER [27], whilst miR-146a was negatively correlated with CK-MB levels immediately after an acute exercise bout [31]. On the other hand, VO_{2max} and running speed at individual anaerobic lactate threshold were positively correlated with miR-1, miR-133a and miR-206 [29], while miR-208b was negatively correlated with baseline AT VO_2 [31]. Moreover, miR-221 was significantly correlated with AT VO_2, peak work load and CK after 3-month basketball matches.

The topic of this systematic review is relatively new. Then, large, well-conducted and longitudinal studies are still lacking. Studies carried out in animals can use invasive techniques and these procedures, however, cannot be reasonably used in humans. In addition, all the included studies were performed in male participants, which may implicate a gender bias. Moreover, all individuals were highly-trained athletes, which means that they are not representative enough of the general population. The main issue to consider is the difficulty to find a direct connection between miRNAs variation and cardiac remodeling or adaptations. While some miRNAs levels changed immedi-

Table 4. Regulation of molecules related to cardiac hypertrophy mediated by miRNAs

Molecule cascade	Positive regulation	Negative regulation
IGF-1	-	miR-1 and miR-206
IGF-1R	-	miR-133a/b
PI3K	miR-21, miR-27a, miR-27b, miR-29, miR-144, miR-145 and miR-438	miR-1, miR-124 and miR-125b, miR-133a, miR-133b, miR-143 and miR-214
SERCA-2	miR-21, miR-27a, miR-27b, miR-29, miR-144 and miR-145	miR-1, miR-133a, miR-133b, miR-143 and miR-214
PTEN	miR-1, miR-133a, miR-133b, miR-143 and miR-214	miR-21, miR-438, miR-486, miR-144 and miR-23a
TSC-2	miR-1, miR-133a, miR-133b, miR-143 and miR-214	miR-21, miR-27a, miR-27b, miR-29, miR-144 and miR-145
FOXO	-	miR-486 and miR-23a
mTOR	-	miR-145
PGC-1α	-	miR-696
RAS GAP	-	miR-1
RHEB	-	miR-1
CDK9	-	miR-1

CDK9, Cyclin-dependent kinase 9; FOXO, Forkhead box O; IGF-1, Insulin-like growth factor 1; IGF-R1, Insulin-like growth factor 1 receptor; mTOR, Mechanistic target of rapamycin; PGC-1α, Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PI3K, Phosphoinositide 3-kinase; PTEN, Phosphoinositide-3,4,5-triphosfate 3-phospatase; RAS-GAP, Ras GTPase-accelerating protein; RHEB, Ras homolog enriched in brain; SERCA-2, Sarco/endoplasmic reticulum C^{2+}-ATPase 2; TSC-2, Tuberous sclerosis complex 2.

ately after exercise, cardiac remodeling is mainly a chronic adaptation and its impact on health will likely be observed after years.

Conclusions

Physical exercise promotes health and represents a non-pharmacological strategy to prevent a wide-range of chronic pathologies such as cardiovascular diseases. Novel approaches to determine the exact mechanisms through physical exercise training have emerged. Variations in circulating miRNAs levels have been observed after acute and chronic exercise in endurance runners, basketball players and rowers. Although evidence is still limited, and long-term studies are needed to obtain more robust evidence, exercise is more likely to affect circulating cardiac-related miRNAs levels as well as gene-related expression regulation through different mechanisms. Finally, further investigation is required to better understand the role of miRNAs in cardiac remodeling and cardiovascular adaptations in response to exercise training.

Our systematic review highlights the potential of circulating microRNAs as biomarkers of the impact of exercise on inflammation, cardiac hypertrophy and cardiac adaptation to exercise. miR-1 and miR-133a are muscle-specific microRNAs that could be delivered by the skeletal and cardiac muscle in response to exercise and could contribute to cardiac and muscle adaptation to the exercise, whereas miR-29a could modulate muscle recovery and cardiac hypertrophy. Finally, miR-21 could be released by endothelial and cardiac cells in response to inflammatory signals produced by acute exercise to resolve inflammation in an early phase.

Disclosure of conflict of interest

None.

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