Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Physiology

Circulating microRNAs: advances in exercise physiology

Ryan M Sapp and James M Hagberg

Circulating microRNAs (ci-miRs) are post-transcriptional regulators of gene expression released by cells into blood or other biofluids. Acute and chronic exercise have been shown to alter the profile of ci-miRs. The past few years have seen an upsurge in research detailing the exercise responses of ci-miRs and investigating their utility as biomarkers for various conditions. The functions of ci-miRs as paracrine/endocrine mediators of systemic adaptations to exercise training are also strongly suggested, but direct evidence is still lacking. The purpose of this review is to provide an update on recent advancements concerning ci-miRs in the field of exercise physiology.

Address
Department of Kinesiology, School of Public Health, University of Maryland, College Park, MD, USA

Corresponding author: Sapp, Ryan M (rsapp@umd.edu)

Current Opinion in Physiology 2019, **10**:1–9

This review comes from a themed issue on **Exercise physiology**

Edited by **Harry B Rossiter** and **Brian Glancy**

For a complete overview see the Issue and the Editorial

Available online 22nd March 2019

https://doi.org/10.1016/j.cophys.2019.03.004

2468-8673/© 2019 Elsevier Ltd. All rights reserved.

Introduction

MicroRNAs (miRs) are small non-coding RNAs that act as fine-tuning regulators of the transcriptome by repressing the translation of target mRNAs [1]. Cells secrete miRs into circulating blood and other biofluids either actively or passively in response to a range of stimuli. Circulating miRs (ci-miRs) are promising biomarkers of both diseases and exercise responses that may also be missing links in understanding the mediators of systemic adaptations to exercise training. Recently, there has been an upsurge in research on the effects of acute and chronic exercise on ci-miRs. Current focal points and advances in the field are discussed in this review.

Concurrent analyses in skeletal muscle and different circulating fractions

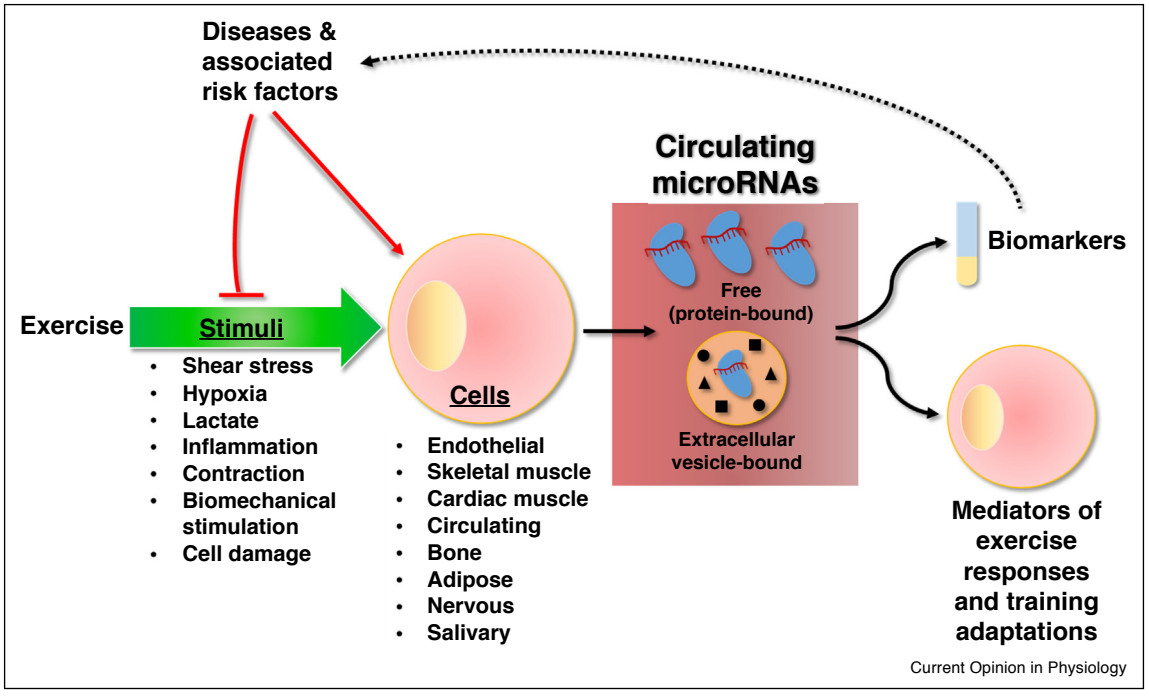
One current hypothesis is that cells release miRs in response to exercise as a means of offloading them in order to allow increased intracellular translation of proteins necessary for adaptation [2•]. This hypothesis is supported by results showing that increased ci-miR expression six hours after a resistance exercise bout correlated positively with intramuscular expression of phosphorylated proteins in the MTORC1 pathway, favoring hypertrophy [3•]. Several of the altered ci-miRs are part of the miR-17∼92 cluster that regulates PTEN, an inhibitor of Akt-mTOR signaling. Additionally, following four weeks of endurance training, mice exhibited increased ci-miR-133a coinciding with decreased skeletal muscle expression of miR-133a and upregulated mRNA expression of its target, serum response factor (SRF) [2•]. Those results also raise the potential of ci-miRs as biomarkers reflecting exercise responses of their source tissue. For example, ci-miRs found to increase in young men following resistance exercise and correlating with hypertrophic responses in muscle were not altered in older men, potentially reflecting resistance to anabolic stimuli with aging [3•].

In contrast, D'Souza *et al.* [4] found that only one (miR-23b-3p) of 38 miRs showed a correlation between plasma and muscle expression at rest, while changes in the two sources with resistance exercise were not related [5]. Of note, miR-133a-3p increased in both muscle and plasma following exercise, though at different time-courses [5]. Further, ci-miRs were found to be poor predictors of muscle size and strength [4]. In another investigation, D'Souza *et al.* [6•] compared exercise-induced changes in miR expression within the circulating exosome fraction, as well as in plasma and muscle. There were differences between fractions relative to which miRs were altered with exercise, as well as time-courses and magnitudes of changes. The results of that study do not support the hypothesis that ci-miRs reflect the exercise-induced offloading of miRs from muscle, as no miRs were decreased in muscle and increased in either circulating fraction. However, it does suggest that miRs are selectively altered and exported in the exosomal or non-encapsulated forms, probably reflecting unique purposes. This supports another hypothesis, that miRs are released by cells to serve as paracrine/endocrine molecules taken up by distant recipient cells, resulting in adaptations. These hypotheses represent two paradigms regarding ci-miRs in the field of exercise physiology, as they are currently regarded as potential biomarkers of exercise responses and paracrine/endocrine mediators of systemic physiological processes (Figure 1).

Ci-miRs are sensitive to different protocols of acute exercise and training

A number of recent studies have assessed the differential responses of ci-miRs to specific exercise protocols. Cycling

Figure 1



Circulating micrornas (ci-miRs) are secreted by various cell types in response to acute and chronic exercise. Exercise stimuli influencing the release of ci-miRs are suggested, but require experimental validation. Diseases and associated risk factors (e.g. aging, obesity) influence the ci-miR profile and responses to exercise. Therefore, exercise-induced ci-miRs are potential biomarkers for athletes, as well as prognostic and diagnostic biomarkers of diseases. Further, they are potential paracrine/endocrine mediators of systemic responses to exercise and adaptations to exercise training.

protocols differing greatly in volume and intensity had contrasting effects on the vascular-related ci-miR-21 and ci-miR-126 [7]. By having men run at varying speeds for a set duration, and varying durations at a set speed, Ramos *et al.* [2•] were able to identify ci-miRs exhibiting dose-dependence to either exercise intensity or duration, as well as those affected at a certain threshold but with no dose-response. Similarly, out of a panel of 74 ci-miRs related to cardiac (patho)physiology, five and 19 ci-miRs were altered in response to a 10 km and marathon race, respectively [8]. Only ci-miR-103a-3p was affected by both exercise bouts, suggesting specific ci-miRs respond to distinct doses of exhaustive exercise [8]. Those results are similar to a previous study by the same authors comparing the responses of inflammation-related ci-miRs, where only ci-miR-150-5p increased immediately after a 10 km race, compared to 12 ci-miRs that increased after a marathon, not including ci-miR-150-5p [9]. Resistance exercise protocols matched for absolute workload but differing in volume, intensity, and rest interval length caused contrasting responses of ci-miRs related to muscle and the vasculature [10]. Effects were seen immediately, one hour, and up to 24 hours after exercise [10].

Schmitz *et al.* [11,12] investigated the effects of different exercise training protocols on resting concentrations of select ci-miRs, as well as the effects of acute bouts of exercise before and after training. Four weeks of high intensity interval (HII) running training protocols differing in intensity, volume, or work/rest interval durations had distinct effects on resting levels of specific ci-miRs. There were also contrasting effects of the different acute protocols, as well as differences in the acute effects of each protocol before compared to after training [11,12]. Multiple studies have found that ci-miRs altered by HII cycling training were unchanged with an acute bout [13,14]. Additionally, there were both similar and unique changes in resting levels of select ci-miRs following 5–8 weeks of explosive as compared to hypertrophic-based resistance training or HII running [15]. Thus, ci-miRs appear to be sensitive to exercise training status, type, dose, intensity, and work/rest interval durations.

The functional relevance of training intensity-dependent changes in ci-miRs has been suggested. In mice, the amount of miR-126 in circulating exosomes originating from circulating angiogenic cells showed dose-dependent increases with training intensity [16•]. Further, the exosomes exerted protective effects on cultured endothelial cells that were greatest from the most highly trained mice and were dependent on transferred miR-126.

Acute and chronic exercise reveal the potential utility of ci-miRs in various conditions

Aging

As mentioned, ci-miRs may reflect changes in skeletal muscle function with aging. Ci-miR-21-5p and ci-miR-146a-5p increased over a ten year period and, combined with age, predicted declines in sprint time and measures of strength in masters athletes [17]. Baseline levels of ci-miR-181a-5p and changes in ci-miR-92a-3p, but not other health/performance measures, predicted changes in walking gait speed following a five month training intervention in older obese individuals [18]. Elevated levels of a set of four muscle-specific ci-miRs were strongly associated with lower rates of whole body protein synthesis in older men [19]. In older, postmenopausal women, specific ci-miRs did not differentiate sarcopenia or osteoporosis status, but were found to correlate with muscular power and bone mineral content [20]. Relatedly, similar ci-miRs associated with bone fracture risk were decreased in young men following HII training and were more sensitive than other biomarkers of bone turnover [21].

Muscle damage and repair

Separate from the idea of selective secretion discussed above, damaged muscle cells passively leak miRs into circulation. Therefore, ci-miRs are promising biomarkers of muscle damage. While classical biomarkers cannot discriminate fiber-type specific damage, Siracusa *et al.* [22•] identified a highly accurate set of three ci-miRs (133b-3p, 206-3p, and 434-3p) able to differentiate slow versus fast skeletal muscle damage. Additionally, these ci-miRs were elevated 12 hours after the muscle-damaging event, which would allow earlier detection of damage compared to some conventional biomarkers [22•]. Such distinct time courses may explain why changes in ci-miR-133a and ci-miR-206 failed to correlate with changes in circulating creatine kinase (CK) or cardiac troponin immediately following a half marathon, despite all markers increasing [23]. These results also do not rule out the possibility that these miRs were secreted actively in response to the exercise bout and suggest that, at least immediately after exercise, they should not be used as markers of exercise-induced muscle damage.

Skeletal muscle-specific miRs were unaltered in extracellular vesicles (EVs) two and 24 hours after muscle damaging exercise, though miR-31 content was decreased at 24 hours post-exercise [24]. Interestingly, miR-31 targets the satellite cell activator Myf5 to promote quiescence. Thus, reduced transport of miR-31 to satellite cells following muscle damage may allow for greater activation and muscle repair, although this is highly speculative and such a mechanism remains to be demonstrated. Ci-miR-29a-3p and ci-miR-495-3p were proposed as potential contributors to muscle repair/recovery, as resting levels in patients with critical limb ischemia due to severe peripheral arterial disease were different from those in elite cyclists specifically in the exercise recovery period, but not at rest [25].

Training load and adaptation in athletes

Ci-miRs are altered in athletes in response to soccer-specific or basketball-specific training [26,27]. After three months of basketball training, changes in ci-miR-221 correlated with exercise capacity and serum CK [27], while following two months of soccer training, the exosomal content of ci-miR-29a correlated with estimated VO_2max [26]. Further, the ci-miR response following a tennis match differed between two athletes, while other physiological markers responded similarly, suggesting changes in ci-miR may vary in well-matched athletes exposed to a similar stimulus [28]. Ci-miRs could be used as biomarkers of training load or adaptation in athletes. For example, changes in ci-miRs following resistance exercise correlated with changes in circulating inflammatory markers and hormones, including IL-10, cortisol, testosterone/cortisol ratio, and IGF-1 [10]. Thus, exercise-induced ci-miR responses in athletes could reflect the balance between states of adaptation and overtraining, though this requires further study.

Hicks *et al.* [29] recently used saliva as a source to determine exercise-induced changes to the entire profile of ci-miR and mRNA. Salivary ci-miRs were altered in distance runners following a long training run, as were several mRNAs predicted as targets of the altered ci-miRs. The predicted pathways targeted by the exercise-regulated ci-miRs were involved in metabolism, fluid regulation, and cardiac conduction. A number of ci-miRs additionally correlated with post-exercise heart rate changes, while there were also sex-specific differences. This study provides evidence that salivary ci-miRs are potentially attractive non-invasive biomarkers in exercise physiology.

Obesity

Substantial recent work has assessed the effects of acute and chronic exercise on vascular and inflammation-related ci-miRs in obese individuals. Multiple studies have found higher resting levels of pro-inflammatory ci-miRs in obese individuals compared to healthy weight controls [30,31]. A 30 min bout of moderate intensity running also increased endothelial and inflammation-related ci-miRs to a greater degree in obese individuals [30], while a three month physical activity intervention lowered pro-inflammatory ci-miR-146a-5p to levels comparable to their lean counterparts [31]. Combined use of the baseline level and change in ci-miR-146a-5p was suggested as a predictive biomarker able to identify responders and non-responders to an exercise training intervention [31].

After six weeks of an exercise training and caloric restriction intervention, obese adolescents displayed increased

ci-miR-126 to levels comparable to normal weight controls, which were strongly correlated with changes in BMI, microvascular endothelial function, and circulating NO/ET-1 ratio [32]. Eight weeks of HII training increased microparticle number and content of miR-146a, but not miR-126, in both obese women and normal weight controls [33]. Other miRs related to inflammation and vascular health were increased in one or both groups. While no correlations were found between the microparticle abundance of any miRs and microvascular function, miR-150 was found to correlate with circulating nitrite and advanced oxidation protein products (oxidative stress) [33]. Following gastric bypass surgery, an exercise training intervention altered the expression of ci-miRs related to metabolism and vascular function [34]. Changes in ci-miRs correlated with changes in insulin resistance, body composition, lipids, and measures of β-cell and liver function [34]. Thus, ci-miRs are altered with exercise training in obese individuals and have the potential to act as biomarkers of metabolic, inflammatory, and vascular adaptations to exercise training. The above results also suggest the potential for mechanistic roles of ci-miRs in these processes.

Cardiometabolic diseases

Ci-miRs are proposed as prognostic and diagnostic biomarkers of cardiometabolic diseases. An acute bout of resistance, but not aerobic, exercise increased ci-miR-146a in older diabetic patients, while nondiabetic patients did not respond to either bout [35]. Combining the results of a VO_{2max} test with pre-exercise or post-exercise levels of select ci-miRs allowed the accurate discrimination between patients with coronary artery disease (CAD) and healthy controls [36•]. In contrast to that study which investigated 187 ci-miRs, Hortman *et al.* [37] focused specifically on the exercise-induced changes of three ci-miRs but did not find any clinical value related to the diagnosis of CAD. In pulmonary hypertension patients, six minute walk distance at baseline correlated with the change in a set of ci-miRs with reported roles in muscle function to three weeks of exercise training or one week of nightly supplemental oxygen interventions [38].

Neuromuscular diseases

Individuals with spinal cord injury are at elevated risk for cardiovascular disease (CVD). Athletically active spinal cord-injured individuals displayed a different ci-miR profile than their sedentary counterparts, which was also more similar to able-bodied participants [39]. Several of these ci-miRs correlated with circulating oxidized LDL and carotid intima-media thickness. Thus, ci-miRs could act as biomarkers for CVD risk in spinal cord-injured individuals and may additionally act as underlying regulators of CVD development with chronic inactivity. Amyotrophic lateral sclerosis (ALS) patients display higher levels of skeletal muscle-derived ci-miRs than healthy controls [40]. Six weeks of concurrent aerobic and resistance exercise training effectively lowered muscle-derived ci-miRs in patients with ALS [41].

There are both symptom overlap and problems with currently used questionnaires to diagnose disorders of fatigue/depression, so ci-miRs offer an attractive objective diagnostic alternative. Baraniuk *et al.* [42] performed the first study on the effects of exercise on ci-miRs in cerebrospinal fluid, from patients with Gulf War Illness or Chronic Fatigue Syndrome, as well as healthy controls. There were no differences in baseline levels of ci-miRs between groups, though there were distinct effects of exercise that distinguished each group. These results suggest that exercise-induced cerebrospinal fluid ci-miRs are potential biomarkers able to help diagnose and discriminate between these similar diseases and even separate phenotypes of Gulf War Illness.

Breast cancer

There were no changes in skeletal muscle or cancer-associated ci-miRs following 16 weeks resistance training in breast cancer survivors [43]. However, when separated as responders and non-responders based on strength changes, responders had increased levels of ci-miR-133a-3p and ci-miR-370-3p with training. In another study of breast cancer survivors who underwent a six month exercise and diet intervention, ci-miR-106b-5p, a prognostic marker of breast cancer risk and recurrence [44], was decreased [45].

Ci-miRs as physiological mediators of systemic responses to exercise

The roles of ci-miRs as paracrine/endocrine molecules mediating mechanistic responses to exercise are suggested mostly by correlational relationships with various phenotypes or other molecular markers in previous studies 46] and those discussed above (Table 1). However, direct experimental evidence is lacking. The function of ci-miRs as cell-to-cell messengers is supported by the facts that ci-miRs contained either in EVs or freely bound to a protein carrier (Argonaute 2) are selectively secreted, highly stable, and can be selectively taken up by recipient cells where they are biologically active [47–50,16•]. Still, major hurdles in the field are the unknown cellular origin and destination of ci-miRs. Only a handful of ci-miRs are highly enriched in specific cell types (e.g. endothelium or striated muscle).

Another question yet to be answered is what stimuli trigger the release of ci-miRs with exercise. Suggested stimuli include laminar shear stress of blood along endothelial cells [7,12,46], hypoxia [7,12], lactate [11], inflammation [30,31], biomechanical stimulation [21], muscle contraction [51], and cell damage [22•,24]. Shear stress, hypoxia, and inflammation have been shown to modulate the cellular release of miRs *in vitro* [52,47,49,53]. It is also currently unknown whether changes in ci-miR are

Table 1

Associations with circulating microRNAs in studies of exercise and fitness

Relevant condition	Ref.	Subjects	Stimulus	Sample type	Circulating microRNAs	Associations
Aging and muscular function	[3•]	Young and older, healthy men	Acute resistance exercise	Serum	(A, B) 19b-3p, 206, 486 (C) 19a-3p, 19b-3p, 20a-5p, 26b-5p, 143-3p, miR-195-5p	(A) age (B) fat-free mass (C) skeletal muscle p-Akt^{Ser473} and p-S6K1^{Thr389}
	[4]	Middle-aged, healthy men	–	EDTA plasma	(A, C) 146a-5p (B, C) 451a (D) 222-3p, 361-5p	(A) age (B) total body lean mass (C) leg lean mass (D) thigh muscle cross-sectional area
	[17]	Masters athletes	10-year follow-up	Serum	(A, B) 21-5p, 146a-5p (C) 146a-5p	(A) knee flexion strength (B) bench press strength (C) 60 m sprint time
	[18]	Sedentary, obese, older men and women	5-month aerobic training	EDTA plasma	BL 181-5p, Δ92a-3p	Δ gait speed
	[19]	Older, overweight men	4 weeks 30% energy restriction diet	Serum	1 + 133a-3p + 133b + 206	Whole body protein synthesis
	[20]	Older, postmenopausal women	–	Serum	(A, B) 125b-5p (C) 21-5p (D) 133a-3p (E) 23a-3p	(A) age (B) jump velocity and power (C) trochanter bone mineral content (D) total body bone mineral content (E) serum TRAP5B
	[21]	Young, healthy men	8 weeks sprint interval training or control	EDTA plasma	(A) 93-5p, 122-5p (B) 100-5p, 122-5p (C) 93-5p, 100-5p, 122-5p, 148-3p	(A) serum sclerostin (B) osteoprotegerin (C) osteocalcin (control group only)
Muscular damage	[22•]	Male and female Wister rats	Muscle crushing injury	EDTA plasma	133b-3p + 206-3p + 434-3p	Damage specific to EDL (fast) or soleus (slow) muscles
Adaptations in athletes	[10]	Young, healthy men	Acute resistance exercise	EDTA plasma	(A) 532 (B) Δ 133a	(A) IGF-1 and IL-10 (B) Δ cortisol and cortisol/testosterone ratio
	[12]	Young, healthy men and women	Acute bout HII exercise and 4 weeks HII training	Whole blood	(A) post-training 222-3p and 29c-3p (B) Δ 222-3p with acute exercise	(A) post-training circulating TGF-β1 (B) Δ running speed at individual 'anaerobic threshold' with training
	[26]	Young, male soccer athletes	2 months soccer training	Serum exosomes	Post-training 29a	Post-training VO_{2max}
	[27]	Young, male basketball athletes	3 months basketball training	Serum	(A) Δ 208b (B) post-training 221	(A) Δ 'anaerobic threshold' VO_2 (B) post-training 'anaerobic threshold', peak workload, and creatine kinase
	[29]	Young, male and female distance runners	~18 km run	Saliva	(A) Δ twenty-six ci-miRs (B) twenty-four ci-miRs (C) twenty-three ci-miRs (D) two ci-miRs	(A) Δ target mRNA(s) (B) Δ heart rate (C) sex (D) age
Obesity	[31]	Middle-aged, obese men and women	3 months exercise training	EDTA plasma	(A) BL 146a-5p (B) post-training 146a-5p	(A) BL total cholesterol and waist circumference (B) post-training age and body weight
	[32]	Obese adolescent boys	6 weeks exercise training and caloric restriction	Serum	Δ 126	Δ BMI, Δ reactive hyperemia index, Δ NO/ET-1
	[33]	Normal weight or obese, young women	8 weeks HII training	Plasma microparticles	(A) 124a (B) 150	(A) HDL, triglycerides, TNF-α (B) nitrites, TBARS, AOPP, adiponectin
	[34]	Obese men and women	6 months exercise training or control following gastric bypass surgery	Heparin plasma	BL: 7, 15a, 106b, 135b, Δ: 7, 15a, 106b, 135b, 122, 206, 149, 221, 34a, 149	Measures of β-cell function and insulin resistance, bone mass, BMI, waist circumference, fat mass, cholesterol, LDL, measures of liver function

Table 1 (*Continued*)

Relevant condition	Ref.	Subjects	Stimulus	Sample type	Circulating microRNAs	Associations
Coronary artery disease	[36•]	Middle-aged to older men and women with or without CAD	VO_{2max} test	EDTA plasma	BL 150-5p + post-exercise 101-3p + 141-3p + 200b-3p	Presence of CAD
Pulmonary hypertension	[38]	Middle-aged to older men and women with pulmonary hypertension	3 weeks exercise training or 1 week nightly oxygen supplementation	Heparin plasma or serum	Δ 22-3p + 21-5p	6-min walk distance
Spinal-cord injury	[39]	Young, active or sedentary spinal cord-injured patients	–	Serum	(A, B) 146a-5p, 328-3p, 191-5p, 103a-3p, 125b-5p, 30b-5p (A) 125a-5p (B) 301a-3p, 766-3p, 28-5p, 146b-5p, 126-5p, 145-5p, 26b-5p, 26a-5p	(A) oxidized LDL (B) carotid intima-media thickness
Disorders of fatigue/ depression	[42]	Men and women with Gulf War Illness	Acute submaximal cycling exercise	Cerebrospinal fluid	Post-exercise 22-3p, 9-3p	START versus STOPP Gulf War Illness phenotypes
Breast cancer	[43]	Breast cancer surviving women	16 weeks resistance training or control	Serum	(A) Δ 133a-3p, 133b-3p (B) Δ 370-3p	(A) Δ Leg press strength (B) Δ non-surgical arm strength
	[45]	Postmenopausal, breast cancer surviving women	–	Serum	191-5p, 17-5p. 103a-3p, 93-5p, 22-3p, 122-5p, 126-3p, 150-5p, 27a-3p, 195-5p, 10a-5p, 30d-5p	Measures of body composition, bone mineral content and density, CRP, IL-6, glucose, insulin, leptin

BL, baseline; Δ, change in; EDL, extensor digitorum longus; IGF-1, insulin-like growth factor 1; IL, interleukin; HII, high intensity interval exercise; TGF-β1, transforming growth factor beta 1; VO_{2max}, maximal oxygen consumption; BMI, body mass index; NO/ET-1, nitric oxide/endothelin-1; HDL, high density lipoprotein; LDL, low density lipoptrotein; TNF-α, tumor necrosis factor alpha; TBARS, thiobarbituric acid reactive substances; AOPP, advanced oxidation protein products; CAD, coronary artery disease; START, stress test activated reversible tachycardia; STOPP, stress test originated phantom perception; CRP, c-reactive protein.

primarily a result of de novo production or export of stored endogenous miRs. The balance among production, export, uptake, and degradation of ci-miRs must be taken into consideration when attempting to explain their kinetics, and little is understood regarding these aspects.

Conclusions

The literature on ci-miRs in exercise physiology has more than doubled over the past few years. The majority of studies have sought to determine the utility of blood-borne ci-miR responses to acute and chronic exercise as biomarkers. In this context, novel sample types including cerebrospinal fluid and saliva have recently been investigated as ci-miR sources [42,29]. Additionally, innovative methods of ci-miR quantification such as single droplet digital PCR [37] and multiplex via flow cytometry (Firefly) [45] or NanoString nCounter [18] have been employed and carry advantages over real-time PCR [54]. Small RNA sequencing has also been used to study the effects of acute exercise and training on the entire profile of circulating small RNA species [55,14]. Despite promising advances, hesitation has been expressed regarding the utility of ci-miRs as biomarkers, given the current lack of similarities in study designs/methods and reproducibility of findings [56]. These limitations are to be expected in such a nascent field, and we believe that studies over the coming years will clarify issues and begin to reveal utility of exercise-regulated ci-miRs in clinical and sports medicine settings. Specifically, larger-scale, validation studies with longer participant follow-ups will be needed. Sample processing and analysis/normalization will also need to become more standardized before ci-miRs can become reliable biomarkers [57]. Lastly, investigations aimed at elucidating mechanisms underlying ci-miR responses to exercise and the mechanistic roles of these ci-miRs will be crucial in revealing their true purpose and potential.

Conflict of interest statement

Nothing declared.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest

1. Bartel DP: **Metazoan microRNAs**. *Cell* 2018, **173**:20-51 http://dx.doi.org/10.1016/j.cell.2018.03.006.

2. • Ramos AE, Lo C, Estephan LE, Tai YY, Tang Y, Zhao J, Sugahara M, Gorcsan J 3rd, Brown MG, Lieberman DE, Chan SY, Baggish AL: **Specific circulating microRNAs display dose-dependent responses to variable intensity and duration of endurance exercise**. *Am J Physiol Heart Circ Physiol* 2018, **315**:H273-H283 http://dx.doi.org/10.1152/ajpheart.00741.2017.

The exercise dose-dependence or threshold responses of ci-miRs were elicited by having men run at variable speeds and durations. In mice, exercise training increased ci-miR-133a along with a decrease in skeletal muscle expression of miR-133a and increase in target gene expression. These results support cellular release of miRs into circulation as a means of depletion to allow upregulation of intracellular gene expression.

3. • Margolis LM, Lessard SJ, Ezzyat Y, Fielding RA, Rivas DA: **Circulating microrna are predictive of aging and acute adaptive response to resistance exercise in men**. *J Gerontol A Biol Sci Med Sci* 2017, **72**:1319-1326 http://dx.doi.org/10.1093/gerona/glw243.

Ci-miRs upregulated in young men six hours after a resistance exercise bout were not increased in older men. Several effected ci-miRs correlated with skeletal muscle levels of p-Akt and p-S6K1, proteins important in mTORC1 signaling. These results suggest ci-miRs may be involved in anabolic resistance to exercise training that is observed with aging.

4. D'Souza RF, Zeng N, Poppitt SD, Cameron-Smith D, Mitchell CJ: **Circulatory microRNAs are not effective biomarkers of muscle size and function in middle-aged men**. *Am J Physiol Cell Physiol* 2019, **316**:C293-C298 http://dx.doi.org/10.1152/ajpcell.00395.2018.

5. D'Souza RF, Markworth JF, Aasen KMM, Zeng N, Cameron-Smith D, Mitchell CJ: **Acute resistance exercise modulates microRNA expression profiles: combined tissue and circulatory targeted analyses**. *PLoS One* 2017, **12**:e0181594 http://dx.doi.org/10.1371/journal.pone.01815.

6. • D'Souza RF, Woodhead JST, Zeng N, Blenkiron C, Merry TL, Cameron-Smith D, Mitchell CJ: **Circulatory exosomal miRNA following intense exercise is unrelated to muscle and plasma miRNA abundances**. *Am J Physiol Endocrinol Metab* 2018, **315**:E723-E733 http://dx.doi.org/10.1152/ajpendo.00138.2018.

Unique responses of miRs to a high-intensity interval cycling bout were observed between skeletal muscle, circulating in plasma or in exosomes.

7. Wahl P, Wehmeier UF, Jansen FJ, Kilian Y, Bloch W, Werner N, Mester J, Hilberg T: **Acute effects of different exercise protocols on the circulating vascular microRNAs -16, -21, and -126 in trained subjects**. *Front Physiol* 2016, **7**:643 http://dx.doi.org/10.3389/fphys.2016.00643.

8. de Gonzalo-Calvo D, Dávalos A, Fernández-Sanjurjo M, Amado-Rodríguez L, Díaz-Coto S, Tomás-Zapico C, Montero A, García-González Á, Llorente-Cortés V, Heras ME *et al.*: **Circulating microRNAs as emerging cardiac biomarkers responsive to acute exercise**. *Int J Cardiol* 2018, **264**:130-136 http://dx.doi.org/10.1016/j.ijcard.2018.02.092.

9. de Gonzalo-Calvo D, Dávalos A, Montero A, García-González Á, Tyshkovska I, González-Medina A, Soares SM, Martínez-Camblor P, Casas-Agustench P, Rabadán M *et al.*: **Circulating inflammatory miRNA signature in response to different doses of aerobic exercise**. *J Appl Physiol* 2015, **119**:124-134 http://dx.doi.org/10.1152/japplphysiol.00077.

10. Cui S, Sun B, Yin X, Guo X, Chao D, Zhang C, Zhang CY, Chen X, Ma J: **Time-course responses of circulating microRNAs to three resistance training protocols in healthy young men**. *Sci Rep* 2017, **7**:2203 http://dx.doi.org/10.1038/s41598-017-02294-y.

11. Schmitz B, Schelleckes K, Nedele J, Thorwesten L, Klose A, Lenders M, Krüger M, Brand E, Brand SM: **Dose-response of high-intensity training (HIT) on atheroprotective miRNA-126 levels**. *Front Physiol* 2017, **8**:349 http://dx.doi.org/10.3389/fphys.2017.00349.

12. Schmitz B, Rolfes F, Schelleckes K, Mewes M, Thorwesten L, Krüger M, Klose A, Brand SM: **Longer work/rest intervals during high-intensity interval training (HIT) lead to elevated levels of miR-222 and miR-29c**. *Front Physiol* 2018, **9**:395 http://dx.doi.org/10.3389/fphys.2018.00395.

13. Denham J, Gray A, Scott-Hamilton J, Hagstrom AD: **Sprint interval training decreases circulating micrornas important for muscle development**. *Int J Sports Med* 2018, **39**:67-72 http://dx.doi.org/10.1055/s-0043-120763.

14. Denham J, Gray AJ, Scott-Hamilton J, Hagstrom AD, Murphy AJ: **Small non-coding RNAs are altered by short-term sprint interval training in men**. *Physiol Rep* 2018, **6**:e13653 http://dx.doi.org/10.14814/phy2.13653.

15. Horak M, Zlamal F, Iliev R, Kucera J, Cacek J, Svobodova L, Hlavonova Z, Kalina T, Slaby O, Bienertova-Vasku J: **Exercise-induced circulating microRNA changes in athletes in various training scenarios**. *PLoS One* 2017, **13**:e0191060 http://dx.doi.org/10.1371/journal.pone.0191060.

16. • Ma C, Wang J, Liu H, Chen Y, Ma X, Chen S, Chen Y, Bihl JI, Yang YI: **Moderate exercise enhances endothelial progenitor**

cell exosomes release and function. *Med Sci Sports Exerc* 2018, **50**:2024-2032.
In mice, exosomes originating from circulating angiogenic cells had training-intensity dependent increases in miR-126 content and protective effects on damaged endothelial cells in vitro that were abolished by inhibiting miR-126. These results suggest a mechanism of vascular protection via transfer of biologically active miR-126 from circulating angiogenic cells to endothelial cells that is improved with training in a dose-dependent fashion.

17. Kangas R, Törmäkangas T, Heinonen A, Alen M, Suominen H, Kovanen V, Laakkonen EK, Korhonen MT: **Declining physical performance associates with serum FasL, miR-21, and miR-146a in aging sprinters**. *Biomed Res Int* 2017, **2017**:8468469 http://dx.doi.org/10.1155/2017/8468469.

18. Zhang T, Brinkley TE, Liu K, Feng X, Marsh AP, Kritchevsky S, Zhou X, Nicklas BJ: **Circulating MiRNAs as biomarkers of gait speed responses to aerobic exercise training in obese older adults**. *Aging (Albany NY)* 2017, **9**:900-913 http://dx.doi.org/10.18632/aging.101199.

19. Margolis LM, Rivas DA, Pasiakos SM, McClung JP, Ceglia L, Fielding RA: **Upregulation of circulating myomiR following short-term energy restriction is inversely associated with whole body protein synthesis**. *Am J Physiol Regul Integr Comp Physiol* 2017, **313**:R298-R304 http://dx.doi.org/10.1152/ajpregu.00054.2017.

20. Chen Z, Bemben MG, Bemben DA: **Bone and muscle specific circulating microRNAs in postmenopausal women based on osteoporosis and sarcopenia status**. *Bone* 2019, **120**:271-278 http://dx.doi.org/10.1016/j.bone.2018.11.001.

21. Sansoni V, Perego S, Vernillo G, Barbuti A, Merati G, La Torre A, Banfi G, Lombardi G: **Effects of repeated sprints training on fracture risk-associated miRNA**. *Oncotarget* 2018, **9**:18029-18040 http://dx.doi.org/10.18632/oncotarget.24707.

22. • Siracusa J, Koulmann N, Sourdrille A, Chapus C, Verret C, Bourdon S, Goriot ME, Banzet S: **Phenotype-specific response of circulating miRNAs provides new biomarkers of slow or fast muscle damage**. *Front Physiol* 2018, **9**:684 http://dx.doi.org/10.3389/fphys.2018.00684.
Extensor digitorum longus (fast) and soleus (slow) muscle differentially express certain miRs. Damage to fast or slow muscle resulted in upregulation of distinct ci-miRs. The combination of ci-miR-133b-3p, ci-miR-206-3p, and ci-miR-434-3p allows the discrimination of damage to slow or fast muscle (receiver operating characteristic (ROC) curve AUC = 0.984).

23. Danese E, Benati M, Sanchis-Gomar F, Tarperi C, Salvagno GL, Paviati E, Montagnana M, Schena F, Lippi G: **Influence of middle-distance running on muscular micro RNAs**. *Scand J Clin Lab Invest* 2018, **78**:165-170 http://dx.doi.org/10.1080/00365513.2018.1426104.

24. Lovett JAC, Durcan PJ, Myburgh KH: **Investigation of circulating extracellular vesicle microRNA following two consecutive bouts of muscle-damaging exercise**. *Front Physiol* 2018, **9**:1149 http://dx.doi.org/10.3389/fphys.2018.01149.

25. Håkansson KEJ, Sollie O, Simons KH, Quax PHA, Jensen J, Nossent AY: **Circulating small non-coding RNAs as biomarkers for recovery after exhaustive or repetitive exercise**. *Front Physiol* 2018, **9**:1136 http://dx.doi.org/10.3389/fphys.2018.01136.

26. Domańska-Senderowska D, Jastrze?bski Z, Kiszałkiewicz J, Brzeziański M, Pastuszak-Lewandoska D, Radzimiński Ł, Brzeziańska-Lasota E, Jegier A: **Expression analysis of selected classes of circulating exosomal miRNAs in soccer players as an indicator of adaptation to physical activity**. *Biol Sport* 2017, **34**:331-338 http://dx.doi.org/10.5114/biolsport.2017.69820.

27. Li Y, Yao M, Zhou Q, Cheng Y, Che L, Xu J, Xiao J, Shen Z, Bei Y: **Dynamic regulation of circulating microRNAs during acute exercise and long-term exercise training in basketball athletes**. *Front Physiol* 2018, **9**:282 http://dx.doi.org/10.3389/fphys.2018.00282.

28. Hoppe MW, Baumgart C, Hilberg T, Freiwald J, Wehmeier UF: **Changes of standard physiological-perceptual markers and circulating microRNAs in response to tennis match-play: a case report of two elite players**. *J Hum Kinet* 2017, **51**:71-81 http://dx.doi.org/10.1515/hukin-2015-0172.

29. Hicks SD, Jacob P, Middleton FA, Perez O, Gagnon Z: **Distance running alters peripheral microRNAs implicated in metabolism, fluid balance, and myosin regulation in a sex-specific manner**. *Physiol Genomics* 2018, **50**:658-667 http://dx.doi.org/10.1152/physiolgenomics.00035.2018.

30. Bao F, Slusher AL, Whitehurst M, Huang CJ: **Circulating microRNAs are upregulated following acute aerobic exercise in obese individuals**. *Physiol Behav* 2018, **197**:15-21 http://dx.doi.org/10.1016/j.physbeh.2018.09.011.

31. Russo A, Bartolini D, Mensà E, Torquato P, Albertini MC, Olivieri F, Testa R, Rossi S, Piroddi M, Cruciani G, De Feo P, Galli F: **Physical activity modulates the overexpression of the inflammatory miR-146a-5p in obese patients**. *IUBMB Life* 2018, **70**:1012-1022 http://dx.doi.org/10.1002/iub.1926.

32. Donghui T, Shuang B, Xulong L, Meng Y, Yujing G, Yujie H, Juan L, Dongsheng Y: **Improvement of microvascular endothelial dysfunction induced by exercise and diet is associated with microRNA-126 in obese adolescents**. *Microvasc Res* 2018, **123**:86-91 http://dx.doi.org/10.1016/j.mvr.2018.10.009.

33. Dimassi S, Karkeni E, Laurent P, Tabka Z, Landrier JF, Riva C: **Microparticle miRNAs as biomarkers of vascular function and inflammation response to aerobic exercise in obesity**. *Obesity (Silver Spring)* 2018, **26**:1584-1593 http://dx.doi.org/10.1002/oby.22298.

34. Nunez Lopez YO, Coen PM, Goodpaster BH, Seyhan AA: **Gastric bypass surgery with exercise alters plasma microRNAs that predict improvements in cardiometabolic risk**. *Int J Obes (Lond)* 2017, **41**:1121-1130 http://dx.doi.org/10.1038/ijo.2017.84.

35. Morais Junior GS, Souza VC, Machado-Silva W, Henriques AD, Melo Alves A, Barbosa Morais D, Nóbrega OT, Brito CJ, Dos Santos Silva RJ: **Acute strength training promotes responses in whole blood circulating levels of miR-146a among older adults with type 2 diabetes mellitus**. *Clin Interv Aging* 2017, **12**:1443-1450 http://dx.doi.org/10.2147/CIA.S141716.

36. • Mayr B, Müller EE, Schäfer C, Droese S, Breitenbach-Koller H, Schönfelder M, Niebauer J: **Exercise responsive micro ribonucleic acids identify patients with coronary artery disease**. *Eur J Prev Cardiol* 2018:2047487318808014 http://dx.doi.org/10.1177/2047487318808014.
The utility of ci-miRs to classify patients with CAD was tested by assessing the responses of 187 ci-miRs to a VO_{2max} test in CAD patients and age/sex-matched healthy individuals. The combined use of VO_{2max}, maximal relative power, baseline ci-miR-150-5p, post-exercise ci-miR-101-3p, ci-miR-141-3p, and ci-miR-200b-3p allowed greater discrimination between groups (receiver operating characteristic (ROC) curve AUC = 0.973) than exercise capacity measures alone.

37. Hortmann M, Walter JE, Benning L, Follo M, Mayr RM, Honegger U, Robinson S, Stallmann D, Duerschmied D, Twerenbold R *et al.*: **Droplet digital PCR of serum miR-499, miR-21 and miR-208a for the detection of functionally relevant coronary artery disease**. *Int J Cardiol* 2019, **275**:129-135 http://dx.doi.org/10.1016/j.ijcard.2018.08.031.

38. Grunig G, Eichstaedt CA, Verweyen J, Durmus N, Saxer S, Krafsur G, Stenmark K, Ulrich S, Grünig E, Pylawka S: **Circulating microRNA markers for pulmonary hypertension in supervised exercise intervention and nightly oxygen intervention**. *Front Physiol* 2018, **9**:955 http://dx.doi.org/10.3389/fphys.2018.00955.

39. Paim LR, Schreiber R, de Rossi G, Matos-Souza JR, Costa E Silva AA, Calegari DR, Cheng S, Marques FZ, Sposito AC, Gorla JI, Cliquet A Jr, Nadruz W Jr: **Circulating microRNAs, vascular risk, and physical activity in spinal cord-injured subjects**. *J Neurotrauma* 2018, **36**:845-852 http://dx.doi.org/10.1089/neu.2018.5880.

40. Tasca E, Pegoraro V, Merico A, Angelini C: **Circulating microRNAs as biomarkers of muscle differentiation and atrophy in ALS**. *Clin Neuropathol* 2016, **35**:22-30 http://dx.doi.org/10.5414/NP300889.

41. Pegoraro V, Merico A, Angelini C: **MyomiRNAs dysregulation in ALS rehabilitation**. *Brain Sci* 2019, **9** http://dx.doi.org/10.3390/brainsci9010008 pii: E8.

42. Baraniuk JN, Shivapurkar N: **Exercise induced changes in cerebrospinal fluid miRNAs in Gulf War Illness, Chronic**

Fatigue Syndrome and sedentary control subjects. *Sci Rep* 2017, **7**:15338 http://dx.doi.org/10.1038/s41598-017-15383-9.

43. Hagstrom AD, Denham J: **MicroRNAs in high and low responders to resistance training in breast cancer survivors**. *Int J Sports Med* 2018, **39**:482-489 http://dx.doi.org/10.1055/a-0592-7691.

44. Zheng R, Pan L, Gao J, Ye X, Chen L, Zhang X, Tang W, Zheng W: **Prognostic value of miR-106b expression in breast cancer patients**. *J Surg Res* 2015, **195**:158-165 http://dx.doi.org/10.1016/j.jss.2014.12.035.

45. Adams BD, Arem H, Hubal MJ, Cartmel B, Li F, Harrigan M, Sanft T, Cheng CJ, Pusztai L, Irwin ML: **Exercise and weight loss interventions and miRNA expression in women with breast cancer**. *Breast Cancer Res Treat* 2018, **170**:55-67 http://dx.doi.org/10.1007/s10549-018-4738-6.

46. Sapp RM, Shill DD, Roth SM, Hagberg JM: **Circulating microRNAs in acute and chronic exercise: mere than mere biomarkers**. *J Appl Physiol (1985)* 2017, **122**:702-717 http://dx.doi.org/10.1152/japplphysiol.00982.2016.

47. Zhou J, Li YS, Nguyen P, Wang KC, Weiss A, Kuo YC, Chiu JJ, Shyy JY, Chien S: **Regulation of vascular smooth muscle cell turnover by endothelial cell-secreted microRNA-126: role of shear stress**. *Circ Res* 2013, **113**:40-51 http://dx.doi.org/10.1161/CIRCRESAHA.

48. Jansen F, Yang X, Hoelscher M, Cattelan A, Schmitz T, Proebsting S, Wenzel D, Vosen S, Franklin BS, Fleischmann BK, Nickenig G, Werner N: **Endothelial microparticle-mediated transfer of MicroRNA-126 promotes vascular endothelial cell repair via SPRED1 and is abrogated in glucose-damaged endothelial microparticles**. *Circulation* 2013, **128**:2026-2038 http://dx.doi.org/10.1161/CIRCULATIONAHA.113.001720.

49. Hale A, Lee C, Annis S, Min PK, Pande R, Creager MA, Julian CG, Moore LG, Mitsialis SA, Hwang SJ, Kourembanas S, Chan SY: **An Argonaute 2 switch regulates circulating miR-210 to coordinate hypoxic adaptation across cells**. *Biochim Biophys Acta* 2014, **1843**:2528-2542 http://dx.doi.org/10.1016/j.bbamcr.2014.06.012.

50. Jansen F, Yang X, Baumann K, Przybilla D, Schmitz T, Flender A, Paul K, Alhusseiny A, Nickenig G, Werner N: **Endothelial microparticles reduce ICAM-1 expression in a microRNA-222-dependent mechanism**. *J Cell Mol Med* 2015, **19**:2202-2214 http://dx.doi.org/10.1111/jcmm.12607.

51. Guescini M, Canonico B, Lucertini F, Maggio S, Annibalini G, Barbieri E, Luchetti F, Papa S, Stocchi V: **Muscle releases alpha-sarcoglycan positive extracellular vesicles carrying microRNAs in the bloodstream**. *PLoS One* 2015, **10**:e0125094.

52. Hergenreider E, Heydt S, Tréguer K, Boettger T, Horrevoets AJ, Zeiher AM, Scheffer MP, Frangakis AS, Yin X, Mayr M *et al.*: **Atheroprotective communication between endothelial cells and smooth muscle cells through miRNAs**. *Nat Cell Biol* 2012, **14**:249-256 http://dx.doi.org/10.1038/ncb2441.

53. Alexy T, Rooney K, Weber M, Gray WD, Searles CD: **TNF-α alters the release and transfer of microparticle-encapsulated miRNAs from endothelial cells**. *Physiol Genomics* 2014, **46**:833-840 http://dx.doi.org/10.1152/physiolgenomics.00079.2014.

54. Saliminejad K, Khorram Khorshid HR, Soleymani Fard S, Ghaffari SH: **An overview of microRNAs: biology, functions, therapeutics, and analysis methods**. *J Cell Physiol* 2019, **234**:5451-5465 http://dx.doi.org/10.1002/jcp.27486.

55. Shah R, Yeri A, Das A, Courtright-Lim A, Ziegler O, Gervino E, Ocel J, Quintero-Pinzon P, Wooster L, Bailey CS *et al.*: **Small RNA-seq during acute maximal exercise reveal RNAs involved in vascular inflammation and cardiometabolic health: brief report**. *Am J Physiol Heart Circ Physiol* 2017, **313**:H1162-H1167 http://dx.doi.org/10.1152/ajpheart.00500.2017.

56. Fernández-Sanjurjo M, de Gonzalo-Calvo D, Fernández-García B, Díez-Robles S, Martínez-Canal Á, Olmedillas H, Dávalos A, Iglesias-Gutiérrez E: **Circulating microRNA as emerging biomarkers of exercise**. *Exerc Sport Sci Rev* 2018, **46**:160-171 http://dx.doi.org/10.1249/JES.0000000000000148.

57. Lee I, Baxter D, Lee MY, Scherler K, Wang K: **The importance of standardization on analyzing circulating RNA**. *Mol Diagn Ther* 2017, **21**:259-268 http://dx.doi.org/10.1007/s40291-016-0251-y.