Circulating microRNA as regulators of the molecular response in exercise in healthy people

Manuel Fernández-Sanjurjo[1], David de Gonzalo-Calvo[2], Sergio Díez-Robles[1], Alberto Dávalos[3], Eduardo Iglesias-Gutiérrez[1]

[1]Departamento de Biología Funcional (Área de Fisiología), Universidad de Oviedo, Asturias. [2]Centro de Investigación Cardiovascular (CSIC-ICCC), Instituto de Investigación Biomédica (IIB Sant Pau), Barcelona. [3]Grupo de Patologías del Metabolismo Lipídico y Nutrición (DISLIPID), IMDEA Alimentación, CEI UAM+CSIC, Madrid.

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Summary

Circulating microRNAs (c-miRNAs) are cell-to-cell communicators implicated in the regulation of molecular responses with strong potential in exercise and practical implications in health. Despite this fact, the number of papers published on this topic is scarce and with inconsistent results. Thus, the aim of this review was to summarize the information available, to analyze the heterogeneity of the results and to identify which are the future perspectives in this field of research.

The results of the studies included in this revision clearly show that acute exercise and training induce a response in c-miRNA profile. This response depends on the model, intensity and dose of exercise. However, there are some questions which must be answered: what are the secretory organs or tissues, the mechanisms of transport, and the tissue and gene targets. A number of differences between studies in the methodologies used (detection technique, number of c-miRNAs analyzed, normalization strategy), in the experimental design (sampling points) and in the characteristics of the participants (aging, exercise background, dietary intake) makes it difficult to establish direct comparisons and to draw firm conclusions. Finally, this role of exercise as c-miRNA profile modulator, could be considered a valuable alternative to upcoming pharmacological and nutritional interventions based on miRNAs. Moreover, the validation of c-miRNAs as biomarkers of exercise will allow the development of more specific recommendations, using training as a therapeutic and preventive tool, and exploring the maximal limits for a safe and healthy exercise.

Key words:
Circulating microRNAs.
Acute exercise.
Training. Exercise biomarkers.

MicroRNA circulantes como reguladores de la respuesta molecular al ejercicio en personas sanas

Resumen

Los microRNAs circulantes (c-miRNAs) son reguladores de la expresión génica y mediadores de la comunicación intercelular, con un gran potencial como coordinadores de la respuesta molecular al ejercicio y, por tanto, con eventuales implicaciones prácticas para la salud y el rendimiento. Sin embargo, su respuesta al ejercicio agudo y al entrenamiento en personas sanas es poco conocida, principalmente porque hasta el momento se ha publicado un número reducido de artículos, con resultados dispares. El objetivo de esta revisión es agrupar y sintetizar el conocimiento disponible, analizar las causas de esta heterogeneidad en los resultados e identificar las principales perspectivas de futuro en esta área.

Los resultados de los trabajos incluidos en esta revisión muestran que el ejercicio agudo y el entrenamiento inducen una respuesta en el perfil de c-miRNAs influida por el modelo, duración, intensidad y dosis de ejercicio. Queda pendiente, no obstante, conocer su origen, forma de transporte, destino, así como validar sus dianas génicas. Sin embargo, estos estudios muestran entre sí numerosas diferencias metodológicas (técnica de detección, número y tipo de c-miRNAs analizados, estrategia de normalización), en el diseño experimental (puntos de muestreo) y en las características de los sujetos (edad, historial de entrenamiento), que hace difícil, tanto establecer comparaciones directas entre ellos, como extraer conclusiones generales sólidas. Finalmente, este papel del ejercicio, como modulador del perfil de c-miRNAs, podría constituir una alternativa viable y coadyuvante a las terapias farmacológicas y dietéticas basadas en miRNAs que actualmente se encuentran en desarrollo. Además, su validación como biomarcadores de ejercicio podría contribuir al desarrollo de recomendaciones de ejercicio más precisas, a optimizar su aplicación como herramienta preventiva o terapéutica y a explorar los límites máximos del ejercicio saludable.

Palabras clave:
MicroRNAs circulantes.
Ejercicio agudo.
Entrenamiento.
Biomarcadores de ejercicio.

Correspondence: Eduardo Iglesias-Gutiérrez
E-mail: iglesiaseduardo@uniovi.es

Introduction

The regular practice of exercise constitutes one of the key determinants of health, and is firmly associated with a reduced risk of mortality for all causes, as well as a lesser incidence of a multitude of pathologies that are highly prevalent in developed countries, such as cardiovascular disease, stroke, metabolic syndrome, type-2 diabetes, colon and breast cancer, depression and the risk of falling[1-3].

Physical activity is intrinsic to our evolution[1,4]. Over time, the human species has maintained an extremely active lifestyle, which contrasts with the sedentary lifestyle that has become established in today's society, both in terms of the low frequency with which exercise is carried out, as well as the less physical component of the majority of working activities. As such, according to data from the Eurobarometer 2010 regarding sports and physical activity[5], 42% of the Spanish population claim that they never do exercise or sports, and 19% claim that they do so 1 to 3 times a month or less. Along the same line, data from the sports habit Survey in Spain 2015[6] reveal that 46.5% of the Spanish population over 15 years of age has not practised any sport in the past year and over 26% of those that practised sport did so less than once a month. For this reason, physical inactivity is a key problem to public health and promoting regular exercise should be an essential part of health intervention across all levels.

The beneficial effect of exercise on organic health is also systematic and is not limited to the most actively involved tissues and organs in the generation of movement[4,7]. In this respect, in 1961 Goldstein described how muscle cells contribute to the humoral regulation of glucose homeostasis during exercise[8]. From this point, countless studies have been carried out that highlight that the response to acute exercise and training involves a complex cross-communication between tissues, and has profound effects on gene expression[7,9,10]. In this way the repair of exercise-induced damage, the recovery and the physiological and metabolic adaptations[2,11] are coordinated. These latter ones are responsible for the systematic effects of exercise on the health[4,12,13]. For this reason the study of the molecular response to exercise has emerged in recent years as an essential tool for understanding how this response is integrated and how it relates to the state of health, allowing new potential mechanisms involved in the processes of illness to be discovered, as well as new therapeutic targets. Having a detailed understanding of the molecular response to exercise is therefore essential in optimising the recommendations for exercise and in exploring the maximum limits of healthy exercise.

Adaptive molecular responses to exercise are in great measure determined by the alteration of gene expression[4]. The precise mechanisms by which the expression of the genes involved in the molecular response to exercise is regulated continue to be fundamentally unknown[7,14,15]. Epigenetic regulation, which not only includes DNA methylation[16] but also histone modifications[17] or the expression of microRNAs (miRNAs)[18], seems to play a particularly important role. In this context, and considering the systematic nature of the response to exercise, there is a strong need to assess the response and function of new mediators of intercellular communication such as miRNAs, in particular, circulating miRNAs (c-miRNAs)[19].

Till now, only a limited number of articles have been published regarding the effect of acute exercise and training on the profile of c-miRNAs, with mixed results, which makes it difficult to reach general conclusions that enable the role of these gene expression regulators to be determined in the molecular response to exercise, nor their eventual practical implications on health and performance. For this reason, the aim of this review is to group together and synthesise the currently available knowledge on this topic, to analyse the causes of this heterogeneity in the results and to identify the main future perspective.

Material and method

Bibliographic search engines such as PubMed *(US National Library of Medicine National Institutes of Health),* Scopus and Science Direct were used, using different combinations of key words of the following terms: *circulating, microRNA, miRNA, miR, exercise, physical activity, training, acute exercise and nutrition.* From the articles selected in this way, those excluded were: a) reviews; b) those that only analysed miRNAs in tissues and not circulating miRNAs; c) those that only analysed them in non-human species. 44 articles were identified, from which, considering the previous criteria, 16 were eventually included.

Based on the list of bibliographic references of the articles selected, additional articles were identified that had also been consulted to perform this review.

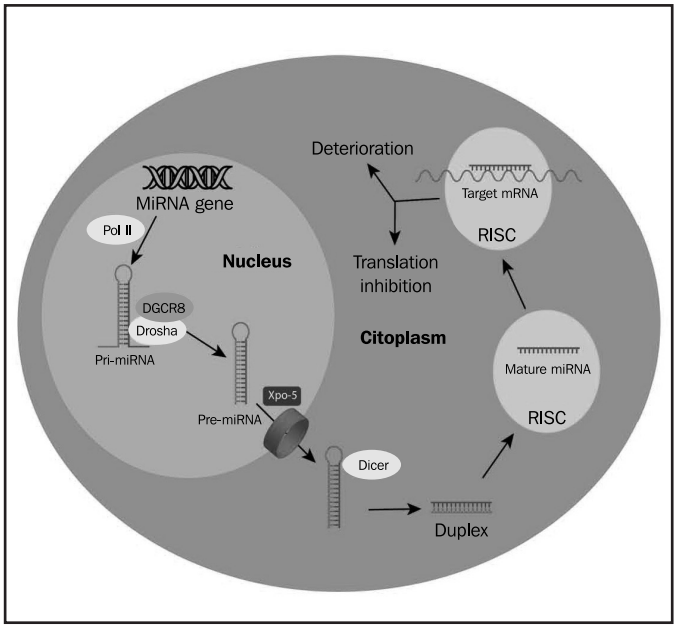
What are microRNAs?

miRNAs are small, non-codified RNA molecules (19-25 nucleotides), which participate in the epigenetic regulation on a post-transcriptional level, acting either by blocking the translation of the messenger RNA (mRNA) or by deteriorating the mRNA, in both cases reducing the protein expression[20]. The importance of their role in regulating gene expression is clear in that the total number of miRNAs expressed in humans - over 2,600 according to miRBase, the main miRNAs database[21] - targets approximately 60% of the coding genome sequences[22], playing a fundamental role in development, the maintenance of homeostasis and the response to physiological and physio-pathological stress[23], including exercise.

The genes that contain miRNAs may be located in intergenic zones where the regulation of their expression is produced by their own elements, or in intronic or exonic regions, where the miRNA expression is closely linked to the expression of the gene in question[24].

The biogenesis of the miRNAs starts in the cellular nucleus and ends in the cytoplasm (Figure 1). In the nucleus the polymerase II RNA generates a long transcript of hundreds of nucleotides, called

Figure 1. Biogenesis of miRNA. miRNAs are processed in the nucleus and the cytoplasm by enzymes with RNAsa III activity, Drosha and Dicer respectively, to generate the mature product which acts on its target mRNA(s), inhibiting their translation or causing their deterioration[25,28].

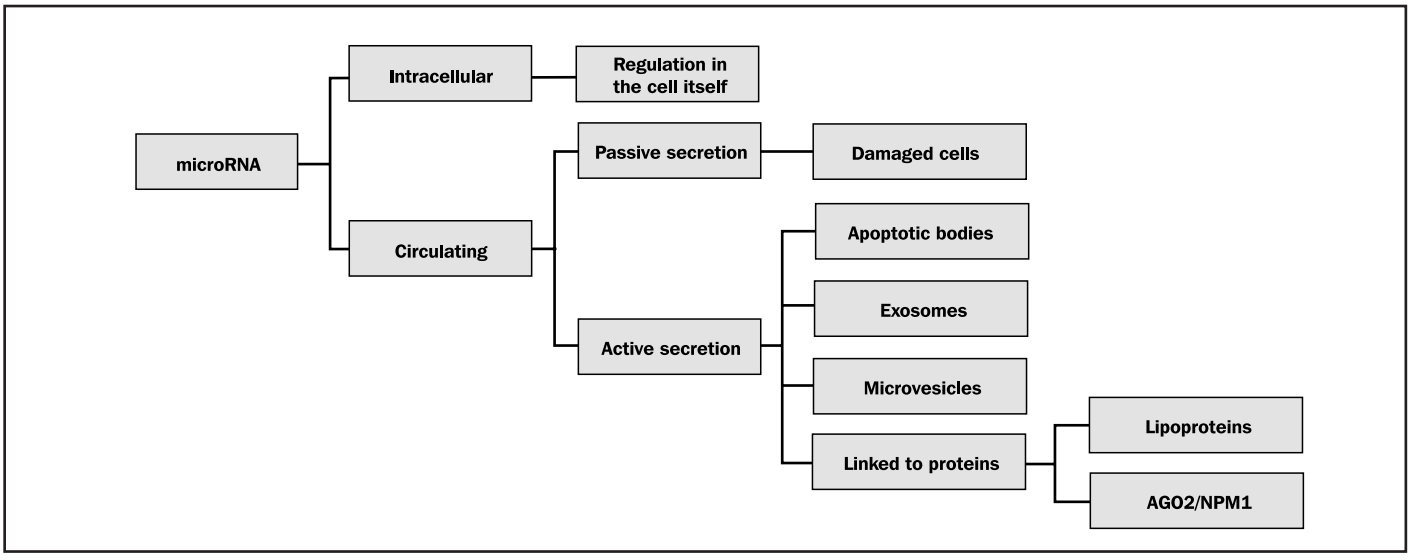


primary microRNA (pri-miR), with secondary structure, which on the extreme 3' of the tail has a chain of polyA and on the extreme 5' has a cap of 7-metyl-guanosine[24]. The pri-miRNA is recognised by a complex formed by the type III ribonuclease and its connective protein DGCR8 (DiGeorge syndrome critical region 8) or Pasha. This complex processes the pri-miR, leading to a second precursor called pre-miR, of some 70 nucleotides, with a secondary structure in the form of a hairpin. This pre-miR is exported towards the cytoplasm via the exportine-5 (Xpo-5) via a mechanism dependent on GTP[25]. Once in the cytoplasm, the hairpin breaks by a complex formed by RNAsa III or Dicer and TRPBP (transactivation response RNA-binding protein)[25]. This way a double-chained molecule or duplex miRNA is generated, which contains the mature miRNA and its complementary chain[26]. The duplex miRNA is de-natured by a helicase that releases the mature miRNA, which then joins to a RNA induced silencing complex (RISC) forming the miRISC complex[26]. The incorporation of one or another chain to the RISC complex depends on the thermodynamic stability of the extreme 5': the least stable is the one that is incorporated[27], even if there are cases in which both strands of the duplex (miR-X-3p or miR-X-5p, where "X" is any miRNA) generate mature miRNAs. Finally, the miRNA guides the RISC complex to complementary places (generally 3'-UTR) in the mRNA, inhibiting its function using different mechanisms, generally depending on the degree of complementarity between the sequences: deterioration of the mRNA if there is total complementarity, or repression of the translation if the complementarity is partial. Given that the majority of the target places in the mRNA only have partial base complementarity with each miRNA, the same miRNA may interact with over 100 different mRNA. Furthermore, each mRNA can contain multiple binding sites for different miRNAs, giving way to a complex network of the gene expression regulation[25, 26].

Circulating microRNAs: intermediaries of intercellular communication

Although miRNAs are intracellular regulators of the gene expression, they have also been detected stably in different bodily fluids (Figure 2), including plasma[29], mainly transported in exosomes or microvesicles[30,31],

Figure 2. Classification of the miRNAs depending on the intracellular or extracellular location in which they can be detected and the way in which they are transported.



associated to proteins (such as Ago2 or Npm1)[32], to lipoproteins[33] and even to apoptotic bodies[25]. This suggests that the c-miRNAs would be secreted in a regulated way and in response to a situation of stress, acting as a genuine intercellular, autocrine, paracrine and endocrine communication system, regulating the gene expression and the phenotype of the receptor cells[34]. They can also be released passively by damaged or necrotic cells[23,25,35].

In 1999 Kopreski *et al*.[36] first described the presence of circulating RNA molecules in serum, that have the capacity to not be deteriorated by the RNAsas present in the plasma. These c-miRNAs were first described in 2008 by Mitchell *et al.*[29] also in the blood flow, though they have later been described in the other bodily fluids[19,37].

Analogically to its intracellular forms, c-miRNAs participate in physiological and adaptive responses, as well as in the start and development of pathological states[38].

Circulating microRNA: are biomarkers useful in the field of exercise?

The release of miRNA into the extracellular environment in response to stress or cell damage opens the door to studies into their potential as biomarkers. In fact, c-miRNAs present the optimum biochemical and physiological properties to constitute excellent biomarkers[39]. i) there are specific c-miRNA profiles for different physiological and pathological situations, which are released from the different cell types involved in the process, ii) their secretion in microvesicles and miRNA-protein complexes gives them great stability in circulation, iii) they display very evolutionary preserved sequences that facilitate their analysis, iv) they enable early detection and the samples have a long average life, v) their determination is performed using relatively economic methods with high sensitivity and specificity, superior to those displayed by current biomarkers based on proteins, via techniques that have already been standardised in clinical laboratories.

Numerous studies have suggested the use of miRNAs as diagnostic, prognostic and therapeutic biomarkers for diverse pathological processes, including cancer, viral infections, nervous system disorders, cardiovascular illness, muscle disorders and diabetes, among others[40]. In some cases, miRNAs seem to present a clinical value that is positioned above the established gold standard, such as, for example, the widely used cardiac biomarkers hs-cTnT and NT-proBNP[41]. Their use in the clinical practice in the short or long term has been proposed by diverse authors[42].

Some studies highlight a relationship between the c-miRNA profile in response to acute exercise and to training and specific adaptations to exercise, which suggests their value as emerging biomarkers in this context[43]. As such, Bye *et al.*[44] observed a base profile of c-miRNAS in healthy adults (40-45 years, men and women), that was different in function to maximum aerobic capacity (VO_2max) and Mooren *et al.*[45] revealed that changes in the plasma concentration of some miRNAs, such as miR-1, miR-133a and miR-206, in response to acute exercise (marathon) revealed a strong correlation with classic performance parameters, such as VO_2max. Moreover, Clauss *et al.*[46] described how the plasma profile of miRNAs related to cardiac remodelling (miR-1, miR-26a, miR-29b, miR-30 and miR-133a) in response to acute exercise (marathon) was different in elite runners as opposed to amateur runners.

On the other hand, Wardle *et al.*[47] observed that the base levels of some c-miRNAs, such as miR-222, miR-21, miR-146a and miR-221 differed between strength athletes (combat sports and weight lifting) and endurance athletes (long-distance running and orientation). Likewise, Banzet *et al*.[48], using a randomised crossed design, described a different profile of some c-miRNAs (miR-1, miR-133a, miR-133b, miR-208a, miR-208b and miR-499) in response to a concentric vs. eccentric exercise and Gonzalo-Calvo *et al*[49] observed that the number, type and kinetics of c-miRNAs related to inflammatory processes differed significantly after lower doses (10 km run) and higher doses (marathon) of acute exercise in active males. These results again highlight c-miRNAs as potential biomarkers in relation to the magnitude of the response to the model and the doses of exercise (regular and acute).

However, the information available until now has not yet allowed for an assessment of their potential use as emerging biomarkers in the context of the exercise. This confirmation should be backed up with a deep knowledge of their response to acute exercise and to training, as well as the influence of confounding factors, such as other biological variables or data processing and normalisation methods[50, 51].

Plasma circulating miRNAs in response to exercise

The effect of acute exercise on the profile of c-miRNAs was first described by Baggish *et al.* in 2011[52]. The limited studies available until now coincide in that acute exercise modifies the profile of c-miRNAs, highlighting their potential as mediators in the acute response mechanisms to exercise, as well as in recovery or adaptation. However, they reveal very heterogeneous results in terms of the number, type and kinetics of appearance and disappearance of plasma c-miRNAs[45,46,48,49,52-59], as can be observed in Table 1, which displays the main characteristics and results of these studies.

On the other hand, very few studies have analysed the base profile of c-miRNAs in active people compared to sedentary people or in response to long periods of training[44,47,52,53,57]. Table 2 displays the studies in which training interventions have been performed. Despite the three studies being heterogeneous in many aspects, it is clear that miR-21 increases in response to training, as described by Baggish *et al.*[52] and Nielsen *et al*[57]. With this miRNA involved in muscle function, hypoxia and inflammation processes, as can be seen in Table 3.

The studies by Wardle *et al.*[47] and Bye *et al.*[44] describe the base differences of the c-miRNAs between people that regularly do exercise[44] or athletes[47] compared to sedentary people. The results of these studies

Table 1. Studies on the profile of circulating microRNAs in response to acute exercise in healthy people.

Type of exercise	Characteristics of the subjects	miRs analysed	Increase	Reduction	Bibliographic reference
Endurance					
Exercise on exercise bike. Incremental exercise (25W/min) up to exhaustion, before and after a team rowing training period (90 days, 5 km/day, 1-3 hours at 20-24 strokes/min).	10 university rowers (19.1 ± 0.6 years).	miR-20a miR-21 miR-133a miR-146a miR-210 miR-221 miR-222 miR-328	**Pre-training, immediately after the exercise:** miR-21, miR-146a, miR-221 and miR-222. The base levels recovered in less than 1 hour. **Post-training immediately after the exercise:** miR-146a and miR-222.		Baggish *et al*.[52]
Exercise on exercise bike. 60 min at 70% of the VO_2max	11 untrained males (21.5 ± 4.5 years)	miR-1 miR-133a miR-133b miR-206 miR-208b miR-486 miR-499		**Immediately after the exercise:** miR-486. The base levels recovered in less than 24 h.	Aoi *et al*.[53]
Exercise on exercise bike. 60 min at 65% of the maximum power	13 trained males (28 ± 8 years).	752 miRNA (miRNome panels).	**1 h after the exercise:** miR-139-5p, miR-143, miR-223, miR-330-3p, miR-338-3p. **3 h after the exercise:** miR-1.	**Immediately after the exercise:** miR-30b, miR-106a, miR-146, miR-151-3p, miR-151-5p, miR-221, miR-652 and let-7i.	Nielsen *et al.*[57]
Exercise on exercise bike. 4 h at 70% of the individual anaerobic threshold.	12 trained males (32.4 ± 2.3 years).	miR-126 miR-133	**During the exercise, from 30 min after the start of the exercise until the end:** miR-126. The base levels recovered in less than 1 h.		Uhleman *et al*.[56]
Marathon	14 male endurance runners (42.8 ± 6.0 years).	miR-1 miR-21 miR-133a miR-155 miR-206 miR-208b miR-499	**Immediately after the exercise:** miR-1, miR-133a, miR-206, miR-208b and miR-499. The base levels of miR-208b and miR-499 recovered in less than 24 h.		Mooren *et al.*[45]
Marathon	21 male marathon runners (51.8 ± 1.4 years)	miR-1 miR-126 miR-133a miR-134 miR-146a miR-208a miR-422b miR-499-5p	**Immediately after the exercise:** miR-1, miR-126, miR-133a, miR-134, miR-146a, miR-208a and miR-499-5p. The base levels recovered in less than 24 h.		Baggish *et al*.[58]
Marathon	22 male marathon runners (56.8 ± 5.2 years)	miR-126 miR-133	**Immediately after the exercise:** miR-126 and miR-133.		Uhleman *et al*.[56]
Half-marathon	5 amateur runners (31.6 ± 4.4 years).	miR-1 miR-133a miR-206	**Immediately after the exercise:** miR-1, miR-133a and miR-206.		Gomes *et al*.[59]
10 km race	9 amateur runners (39.1 ± 2.2 years).	106 c-miR related to inflammatory response	**Immediately after the race:** miR-150		de Gonzalo-Calvo *et al.*[49]

(keep going)

Type of exercise	Characteristics of the subjects	miRs analysed	Increase	Reduction	Bibliographic reference
Endurance					
Marathon	30 marathon runners, 15 amateur (40.1±1.4 years) and 15 elite (40.0 ± 1.7 years)	miR-1 miR-26a miR-29b miR-30a miR-133a	**Immediately after the exercise:** miR-1, miR-30a and miR-133a, more marked in elite runners. The base levels recovered in less than 24 h.	**24 h after the exercise:** miR-26a in elite runners.	Clauss *et al.*[46]
Strength					
Bench press and Leg press. 5 series of 10 repetitions at 70% of 1 MR	12 active males (29.9 ± 1.2 years).	Microarray and validation with qRT-PCR: miR-149*, miR-1908, miR-20a, miR-21, miR-133a, miR-146a, miR-210, miR-221, miR-222 y miR-328.	**Three days after the exercise:** miR-149*.	**Three days after the exercise:** miR-146a and miR-221.	Sawada *et al.*[55]
Pull-down Machine, Leg press and Butterfly. 3 series of 15 repetitions, with 25% more load in the eccentric phase than in the concentric phase.	11 trained subjects, 4 males and 7 females (37±2 years).	miR-126 miR-133	**Immediately after the exercise:** miRNA-133. The base levels recovered in less than 1 h.		Uhleman *et al.*[56]
Concentric *vs.* Eccentric					
Uphill and downhill run. 30 min at 1m/s, with 25% inclination and additional load of 12% of the body weight.	9 active males (27-36 years).	miR-1 miR-133a miR-133b miR-208a miR-208b miR-499	**2 and/or 6 h after the downhill run:** miR-1, miR-133a, miR-133b and miR-208b. The base levels recovered in less than 24 h. **Immediately after the uphill run:** miR-181b and miR-214. The base levels recovered in less than 2 h.		Banzet *et al.*[48]
Anaerobic power					
Wingate test	18 active males (20.23±0.97 years).	miR-1 miR-16 miR-122 miR-133a miR-133b miR-206 miR-499		**Immediately after the exercise:** miR-1, miR-16, miR-122, miR-133a and miR-133b	Cui *et al.*[54]

are contradictory; whilst with Wardle *et al.*[47] the plasma concentration of miR-21, miR-221, miR-222 and miR-146a reveal higher base levels in young endurance athletes than in sedentary controls. However, in contrast, in a study with healthy adult men and women, Bye *et al.*[44], observed that the base levels of miR-21 and miR-222 were greater in people with a lower VO_2max.

The difficulty in drawing solid conclusions from these studies may be due to different methodologies in the experimental design, in the exercise model or in the characteristics of the subjects (age, training history, etc.) among other aspects, whose influence we will analyse below.

Different methodological approximations and experimental design

Although there are numerous techniques for detecting miRNAs[60], those most widely used to identify and quantify c-miRNAs in plasma or serum are the massive sequence, microarray and the qRT-PCR[60,61]. Each of these techniques have some differentiated characteristics and some specific advantages and disadvantages[62, 63] that should be considered depending on the experimental design and the characteristics of the study so that the results are truly informative[64].

The most widely used technique in the studies that have analysed c-miRNAs in response to exercise have been the qRT-PCR[44,45,47-49,52-54,56-59],

Table 2. Studies on the profile of circulating microRNAs in response to training in healthy people.

Exercise model	Training time	Modified miRs	References
Cycling (exercise bike)	12 weeks 5 times/week 60 min at 65% maxP	**Post-training increase (3-5 days):** miR-342-3p, let-7d, miR-766, miR-25, miR-148a, miR-185, miR-21. **Post-training decrease (3-5 days):** miR-103, miR-107.	Nielsen *et al.*[57]
Cycling (exercise bike)	4 weeks 3 times/week 30 min at 70% VO_2max	**Post-training decrease:** miR-486	Aoi *et al.*[53]
Rowing	90 days training to optimise Performance over 5 km	**Post-training increase:** miR-146a, miR-21, miR-221 and miR-222.	Baggish *et al.*[52]

Table 3. Circulating miRNAs analysed in different publications grouped according to the biological processes in which they are involved.

Metabolic route	Bibliographic reference	miRNAs analysed
Muscle function (cardiac and skeletal)	Baggish *et al.*[52]	miR-21, miR-133a
	Aoi *et al.*[53]	miR-1, miR-133a, miR-133b, miR-206, miR-208b, miR-486, miR-499
	Uhleman *et al.*[56]	miR-133
	Mooren *et al.*[45]	miR-1, miR-133a, miR-206, miR-208b, miR-499
	Baggish *et al.*[58]	miR-1, miR-133a, miR-499-5p, miR-208a
	Gomes *et al.*[59]	miR-1, miR-133a, miR-206
	Clauss *et al.*[46]	miR-1, miR-26a, miR-29b, miR-30a, miR-133a
	Banzet *et al.*[48]	miR-1, miR-133a, miR-133b, miR-208a, miR-208b, miR-499
	Cui *et al.*[54]	miR-1, miR-133a, miR-133b, miR-206, miR-499
Inflammatory Response	Baggish *et al.*[52]	miR-21, miR-146a
	Mooren *et al.*[45]	miR-155, miR-21
	Baggish *et al.*[58]	miR-146a
	de Gonzalo-Calvo *et al.*[49]	106 c-miR related to inflammatory response
Endothelial damage	Uhleman *et al.*[56]	miR-126
	Baggish *et al.*[58]	miR-126
Angiogenesis	Baggish *et al.*[52]	miR-20a, miR-221, miR-222, miR-210, miR-328
Hypoxia	Baggish *et al.*[52]	miR-21, miR-210, miR-146a
Brain tissue	Baggish *et al.*[58]	miR-134
Cellular proliferation	Cui *et al.*[54]	miR-16, miR-122

though Sawada *et al.*[55] chose to use the microarray, with later validation and quantification via qRT-PCR.

The majority of authors have analysed the circulating levels of a selection of one or several miRNAs (typically between 5 and 8), mainly the so-called myomiRs, whose expression is specific to the skeletal and/or cardiac striated muscle: miR-1, miR-133a, miR-133b, miR-206a, miR-208b, miR-486 and miR-499[45,48,53,54,58,59,65]. Other authors, in turn, have accompanied the myomiRs analysis with some miRNAs, previously described as circulating markers of processes directly related to the response to acute exercise, which is displayed in Table 3.

The tissue or cell type from which these c-miRNAs originate is potentially diverse, as suggested by Nielsen *et al.*[57], though it has not been analysed and is unknown. For this reason, considering the systematic nature of the response to acute exercise, this type of analysis provides an incomplete perspective of the response of the c-miRNAs. Moreover, even with these limited approximations, the results obtained are heterogeneous, among other reasons because not all the authors have analysed the same myomiRs and very few have analysed them all. As such, Baggish *et al.*[52] and Sawada *et al.*[55] did not observe changes in the expression of any myomiR in response to different exercise models. On the other hand, Uhleman *et al.*[56], Mooren *et al.*[45], Baggish *et al.*[58], Banzet *et al.*[48] and Clauss *et al.*[46] observe significant post-exercise increases of miR-1, miR-133, miR-206, miR-208b and/or miR-499 and Cui *et al.*[54] only observe a significant post-exercise decrease in some of them. However, the majority coincide in that the response of the circulating myomiRs to acute exercise is not the result of a passive release by the damaged

muscle tissue, as neither the plasma levels or their kinetics reveal correlation with those of classic muscle damage markers, such as the creatine kinase plasma concentration[45,53,56,58,59], therefore it could be a regulated process. However, it is not know how they are secreted nor if they are captured by a tissue. Nor have their gene targets been validated.

Only the study by Gonzalo-Calvo *et al.*[49], in which 106 inflammatory miRNAs were analysed, those by Bye *et al.*[44], Nielsen *et al.*[57] and Dávalos *et al.*[66], in which a commercial panel of over 750 of the miRNAs expressed in different human tissues were used, and that by Sawada *et al.*[55], which used microarray, performed a more extensive approximation. Interestingly enough, none of these authors observed changes in the expression of myomiRs expression in response to acute exercise. Both the bio-informatic and statistical analysis of this data differs notably from the most limited approximations, which makes it difficult to perform direct comparisons between studies.

In this respect, and in terms of the analysis of the qRT-PCR results, the strategy used for the standardisation also varied between the different studies, especially because until now no stable constituent miRNA has been established or validated to regulate the expression of miRNAs, nor within the context of response to exercise[46]. Various authors have decided to regulate a Caenorhabditis elegans miRNA depending on the levels of cel-MIR-39, added externally and in equal quantity to all the samples[45,46,53,55,56,59]. Others, in turn, based on specific software to detect which gene or group of genes is expressed more stably in a set of samples, have used these miRNAs, different in each case, to regulate their qRT-PCR data[47,48]. The choice of an inadequate strategy may skew the capacity to identify differences between the study groups and, clearly, the lack of homogeneity between studies is a major disadvantage when comparing results[51-67]. Although ideally there would be a solid and common regulation strategy, it is clear that the nature of c-miRNA analysis renders this impossible, therefore the ideal option is to identify, validate and use the regulators that best adapt to the specific characteristics of each study[51].

Finally, as displayed in Table 1, another of the methodological divergence elements are the different acute exercise models to which the voluntary participants in the various studies were subjected. Although we have grouped them into endurance exercises[45,46,49,52,53,56,58,59], strength[55,56], concentric *vs.* eccentric[48] and maximum anaerobic power[54], the diversity within some groups, in the mode, the duration and the intensity of the exercise, is also important. Even in the studies in which the exercise model was the same, such as those that analysed the acute response to a marathon[45,46,49,56,58], both the characteristics of the subjects (age, gender, years of training), that we will analyse later, as well as the sampling points or the diet control could influence the response observed. In some cases the base sample was extracted just before the start of the marathon[49,56], but in others it was taken one[58], two[45] or even between two and five days before the test[46]. In these cases the differences observed in the expression of c-miRNAs between the base sample and the sample taken after the test do not allow the effect of the exercise to be isolated, due to the potential effect of uncontrolled variability factors, including the most notable one: food intake.

In this respect, there is increasing evidence regarding the influence of dietary components in the expression of miRNAs and in the levels of c-miRNAs[68-70], as well as a new and intriguing relationship between the intake of miRNAs from food sources, their absorption and their appearance in biological fluids such as plasma[71]. Despite this, only the article by Gonzalo-Calvo *et al.*[49] performed a strict control of the food intake before, during and after exercise.

Characteristics of the study subjects

As we mentioned previously, major differences are observed in the characteristics of the subjects included within the different studies, especially in terms of age and sporting experience (Table 1). Whilst in the study by Baggish *et al.*[52] participating university rowers had an average age of 19.1 years, Uhleman *et al.*[56] recruited adult males aged 56.8 years on average, which the authors considered to be "marathon runners" and in the study by Gomes *et al.*[59] the response to a half marathon by obese and overweight amateur runners was analysed, with some participants having less than 6 months experience performing exercise. Both factors could introduce another element of variability that explains the heterogeneity of the response observed.

There is not very much information available regarding the effect of age on the profile of c-miRNAs in humans. In a pioneering study, Noren Hooten *et al.*[72] observed that the expression of miR-151a-5p, miR-181a-5p and miR-1248 was significantly repressed in older men and women (average of 64 years) compared to young people (average 30 years). In turn, Zhang *et al.*[73] suggested that the circulating profiles of miR-29b and miR-92a must change gradually with the ageing process, after observing differences between subjects aged 22, 40, 59 and 70 years on average. For this reason the age difference of the subjects could determine differences, not only in the response to exercise, but as a starting point, in the base levels of some c-miRNAs, introducing a confounding element.

In turn, Baggish *et al.*[58] suggested that systematic training could be associated with base levels of c-miRNAs per se, particularly some myomiRs, in accordance with that observed by Nielsen *et al.*[57] on an intracellular level in muscle-skeletal cells. This could mask the effect of acute exercise on these c-miRNAs and would explain why, in some studies with trained people, changes in the circulating levels of myomiR were not observed[49,52,53,55].

Conclusions

Acute exercise induces a response in the profile of c-miRNAs, which varies depending on the model, intensity or dose of the exercise, and which highlights their potential regulatory role in the systematic processes of recovery and adaptation to the exercise. However, the profiles described in the different studies are highly influenced by confounding

biological, technical and methodological factors. Furthermore, their response during exercise is still unknown.

Finally, the results of the studies included in this review suggest that exercise, as a modulator of the c-miRNAs profile, could constitute a viable and contributing alternative to pharmaceutical and dietary therapies based on miRNAs that are currently under development. In this respect, the therapeutic modulation based on the miRNAs involved in pathological or degenerative processes could imply both the inhibition and the gaining depending on the specific miRNA, and both characteristics have been observed in the response of c-miRNAs to exercise.

Future perspectives

All the studies included in this review have a marked descriptive and associative nature, and aim, therefore, to understand the origin, form of transport, destination and gene targets of the c-miRNAs that respond to exercise. This information is essential in discovering the functional role of these epigenetic regulators in the molecular response to exercise, which, ultimately, coordinates the beneficial effects of exercise on the health.

Their confirmation as biomarkers of exercise could also contribute to the development of more precise or even customised exercise recommendations, their optimisation as a preventive or therapeutic tool, or as a means of exploring the maximum limits of healthy exercise.

References

1. Booth FW, Lees SJ. Fundamental questions about genes, inactivity, and chronic diseases. *Physiol Genomics.* 2007;28(2):146-57.
2. Fiuza-Luces C, Garatachea N, Berger N a, Lucia A. Exercise is the real polypill. *Physiology (Bethesda).* 2013;28(5):330-58.
3. Li S, Laher I. Exercise Pills: At the Starting Line. *Trends Pharmacol Sci.* 2015;36(12):906-17.
4. Hawley J a, Hargreaves M, Joyner MJ, Zierath JR. Review Integrative Biology of Exercise. *Cell.* 2014;159(4):738-49.
5. Comisión Europea, Dirección General de Educación y Cultura. Special Eurobarometer "Sport and physical activity". Bruselas: TNS Opinion & Social. 2010.
6. Ministerio de Educación, Cultura y Deporte, Subdirección General de Estadística y Estudios, Secretaría General Técnica. Encuesta de hábitos deportivos en España. Madrid: Subdirección General de Documentación y Publicaciones. 2015.
7. Neufer PD, Bamman MM, Muoio DM, Bouchard C, Cooper DM, Goodpaster BH, *et al.* Understanding the Cellular and Molecular Mechanisms of Physical Activity-Induced Health Benefits. *Cell Metab.* 2015;22(1);4-11.
8. Goldstein MD. Humoral nature of the hypoglycemic factor of muscular work. *Diabetes.*1961;10(3):232-34.
9. Coffey VG, Hawley J a. The Molecular Basis of Training Adaptation. *Sport Med.* 2007;37(9):737-63.
10. Egan B, Zierath JR. Exercise metabolism and the molecular regulation of skeletal muscle adaptation. *Cell Metab.* 2013;17(2):162-84.
11. Thompson PD, Crouse SF, Goodpaster B, Kelley D, Moyna N, Pescatello L. The acute versus the chronic response to exercise. *Med Sci Sports Exerc.* 2001;33(6 Suppl):S438-45; discussion S452-3.
12. Lee I. Dose-response relation between physical activity and fitness: Even a little is good; more is better. *JAMA.* 2007;297(19):2137-9.
13. Viña J, Sanchis-Gomar F, Martinez-Bello V, Gomez-Cabrera MC. Exercise acts as a drug; The pharmacological benefits of exercise. *Br J Pharmacol.* 2012;167(1):1-12.
14. Pareja-Galeano H, Sanchis-Gomar F, García-Giménez JL. Physical exercise and epigenetic modulation: Elucidating intricate mechanisms. *Sport Med.* 2014;44(4):429-36.
15. Denham J, Marques FZ, O'Brien BJ, Charchar FJ. Exercise: Putting action into our epigenome. *Sport Med.* 2014;44(2):189-209.
16. Barrès R, Yan J, Egan B, Treebak JT, Rasmussen M, Fritz T, *et al.* Acute exercise remodels promoter methylation in human skeletal muscle. *Cell Metab.* 2012;15(3):405-11.
17. McGee SL, Hargreaves M. AMPK-mediated regulation of transcription in skeletal muscle. *Clin Sci (Lond).* 2010;118(8):507-18.
18. Zacharewicz E, Lamon S, Russell AP. MicroRNAs in skeletal muscle and their regulation with exercise, ageing, and disease. Front Physiol. 2013;4:266.http://dx.doi.org/10.3389/fphys.2013.00266.
19. Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, *et al.* Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. *Cell Res.* 2008;18(10):997-1006.
20. Ebert MS, Sharp P a. Roles for MicroRNAs in conferring robustness to biological processes. *Cell.*; 2012;149(3):505-24.
21. Kozomara A, Griffiths-Jones S. MiRBase: Annotating high confidence microRNAs using deep sequencing data. *Nucleic Acids Res*. 2014;42(D1):68-73.
22. Friedman RC, Farh KKH, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res*. 2009;19(1):92-105.
23. Mendell JT, Olson EN. MicroRNAs in stress signaling and human disease. *Cell.* 2013;148(6):1172-87.
24. Lugo-Trampe Á, Trujillo-Murillo KDC. MicroRNAs: reguladores clave de la expresión génica. *Med Univ.* 2009;11(44):187-92.
25. Chen X, Liang H, Zhang J, Zen K, Zhang CY. Secreted microRNAs: A new form of intercellular communication. *Trends Cell Biol.;* 2012;22(3):125-32.
26. Bartel DP. MicroRNAs: Genomics, Biogenesis, Mechanism, and Function. *Cell.* 2004;116(2):281-97.
27. Khvorova A, Reynolds A, Jayasena SD. Functional siRNAs and miRNAs exhibit strand bias. *Cell.* 2003;115(2):209-16.
28. Castanotto D, Rossi JJ. The promises and pitfalls of RNA-interference-based therapeutics. *Mol Biol.* 2009;457(7228):426-33.
29. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, *et al.* Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci USA.* 2008;105(30):10513-8.
30. Hu G, Drescher KM, Chen XM. Exosomal miRNAs: Biological Properties and Therapeutic Potential. *Front Genet.* 2012;3:56.http://dx.doi.org/10.3389/fgene.2012.00056.
31. Villarroya-Beltri C, Baixauli F, Gutiérrez-Vázquez C, Sánchez-Madrid F, Mittelbrunn M. Sorting it out: Regulation of exosome loading. *Semin Cancer Biol.* 2014;28(1):3-13.
32. Arroyo JD, Chevillet JR, Kroh EM, Ruf IK, Pritchard CC, Gibson DF, *et al.* Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. *Proc Natl Acad Sci.* 2011;108(12):5003-8.
33. Vickers KC, Palmisano BT, Shoucri BM, Shamburek RD, Remaley AT. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. *Nat Cell Biol.* 2011;13(4):423-33.
34. Gupta SK, Bang C, Thum T. Circulating MicroRNAs as biomarkers and potential paracrine mediators of cardiovascular disease. *Circ Cardiovasc Genet.* 2010;3(5):484-8.
35. Etheridge A, Gomes CPC, Pereira RW, Galas D, Wang K. The complexity, function, and applications of RNA in circulation. *Front Genet.* 2013;4:115. http://dx.doi.org/10.3389/fgene.2013.00115.
36. Kopreski MS, Benko FA, Kwak LW, Gocke CD. Detection of tumor messenger RNA in the serum of patients with malignant melanoma. *Clin Cancer Res.* 1999;5(8):1961-5.
37. Weber JA, Baxter DH, Zhang S, Huang DY, How Huang K, Jen Lee M, *et al.* The MicroRNA Spectrum in 12 Body Fluids. *Clin Chem.* 2010;56(11):1733-41.
38. Bang C, Batkai S, Dangwal S, Gupta SK, Foinquinos A, Holzmann A, *et al.* Cardiac fibroblast-derived microRNA passenger strand-enriched exosomes mediate cardiomyocyte hypertrophy. *J Clin Invest.* 2014;124(5):2136-46.
39. Creemers EE, Tijsen AJ, Pinto YM. Circulating MicroRNAs: Novel biomarkers and extracellular communicators in cardiovascular disease? *Circ Res.* 2012;110(3):483-95.
40. Keller A, Meese E. Can circulating miRNAs live up to the promise of being minimal invasive biomarkers in clinical settings? Wiley Interdiscip *Rev RNA.* 2016;7(2):148-56.
41. Wang G-K, Zhu J-Q, Zhang J-T, Li Q, Li Y, He J, *et al.* Circulating microRNA: a novel potential biomarker for early diagnosis of acute myocardial infarction in humans. *Eur Heart J.* 2010;31(6):659-66.
42. Rayner K, Dimmeler S, Calin GA, Thum T, Raizman JE, Diamandis EP. Novel Biomarkers for Acute Myocardial Infarction : Is MicroRNA the New Kid on the Block ? *Clin Chem.* 2014;60(6):812-7.
43. Gomes CP, Kim T-K, Wang K, He Y. The implications on clinical diagnostics of using microRNA-based biomarkers in exercise. *Expert Rev Mol Diagn.* 2015;15(6):1-12.

44. Bye A, Røsjø H, Aspenes ST, Condorelli G, Omland T, Wisløff U. Circulating MicroRNAs and Aerobic Fitness - The HUNT-Study. *PLoS One.* 2013;8(2):e57496.
45. Mooren FC, Viereck J, Kruger K, Thum T. Circulating microRNAs as potential biomarkers of aerobic exercise capacity. *Am J Physiol Circ Physiol.* 2014;306(4):H557-63.
46. Clauss S, Wakili R, Hildebrand B, Kääb S, Hoster E, Klier I, *et al.* MicroRNAs as Biomarkers for Acute Atrial Remodeling in Marathon Runners (The miRathon Study - A Sub-Study of the Munich Marathon Study). *PLoS One.* 2016;11(2):e0148599.
47. Wardle SL, Bailey MES, Kilikevicius A, Malkova D, Wilson RH, Venckunas T, *et al.* Plasma MicroRNA Levels Differ between Endurance and Strength Athletes. *PLoS One.* 2015;10(4):e0122107.
48. Banzet S, Chennaoui M, Girard O, Racinais S, Drogou C, Chalabi H, *et al*. Changes in circulating microRNAs levels with exercise modality. *J Appl Physiol.* 2013;115(9):1237-44.
49. de Gonzalo-Calvo D, Dávalos A, Montero A, García-González Á, Tyshkovska I, González Medina A, *et al.* Circulating inflammatory miRNA signature in response to different doses of aerobic exercise. *J Appl Physiol.* 2015;119(2):124-34.
50. Wang J, Chen J, Sen S. MicroRNA as Biomarkers and Diagnostics. *J Cell Physiol.* 2016;231(1):25-30.
51. Marabita F, de Candia P, Torri A, Tegnér J, Abrignani S, Rossi RL. Normalization of circulating microRNA expression data obtained by quantitative real-time RT-PCR. *Brief Bioinform.* 2016;17(2):204-12.
52. Baggish AL, Hale A, Weiner RB, Lewis GD, Systrom D, Wang F, *et al.* Dynamic regulation of circulating microRNA during acute exhaustive exercise and sustained aerobic exercise training. *J Physiol.* 2011;589(Pt 16):3983-94.
53. Aoi W, Ichikawa H, Mune K, Tanimura Y, Mizushima K, Naito Y, *et al.* Muscle-enriched microRNA miR-486 decreases in circulation in response to exercise in young men. *Front Physiol.* 2013;4:80. http://dx.doi.org/10.3389/fgene.2013.00080.
54. Cui SF, Li W, Niu J, Zhang CY, Chen X, Ma JZ. Acute responses of circulating microRNAs to low-volume sprint interval cycling. *Front Physiol.* 2015;6:311. http://dx.doi.org/10.3389/fgene.2015.000311.
55. 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(7):1-8.
56. Uhlemann M, Möbius-Winkler S, Fikenzer S, Adam J, Redlich M, Möhlenkamp S, *et al.* Circulating microRNA-126 increases after different forms of endurance exercise in healthy adults. *Eur J Prev Cardiol.* 2014;21(4):484-91.
57. Nielsen S, Åkerström T, Rinnov A, Yfanti C, Scheele C, Pedersen BK, *et al*. The miRNA plasma signature in response to acute aerobic exercise and endurance training. *PLoS One.* 2014;9(2):e87308.
58. Baggish AL, Park J, Min P-K, Isaacs S, Parker B a, Thompson PD, *et al.* Rapid upregulation and clearance of distinct circulating microRNAs after prolonged aerobic exercise. *J Appl Physiol.* 2014;116(5):522-31.
59. Gomes CPC, Oliveira-Jr GP, Madrid B, Almeida JA, Franco OL, Pereira RW. Circulating miR-1, miR-133a, and miR-206 levels are increased after a half-marathon run. *Biomarkers.* 2014;19(7):585-9.
60. Tian T, Wang J, Zhou X. A review: microRNA detection methods. *Org Biomol Chem.* 2015;13(8):2226-38.
61. De Planell-Saguer M, Rodicio MC. Detection methods for microRNAs in clinic practice. *Clin Biochem.* 2013;46(10-11):869-78.
62. Tzimagiorgis G, Michailidou EZ, Kritis A, Markopoulos AK, Kouidou S. Recovering circulating extracellular or cell-free RNA from bodily fluids. *Cancer Epidemiol.* 2011;35(6):580-9.
63. Dong H, Lei J, Ding L, Wen Y, Ju H, Zhang X. MicroRNA: Function, Detection, and Bioanalysis. *Chem Rev*. 2013;113(8):6207-33.
64. Huang Y, Zou Q, Wang SP, Tang SM, Zhang GZ, Shen XJ. The discovery approaches and detection methods of microRNAs. *Mol Biol Rep.* 2011;38(6):4125-35.
65. Guller I, Russell AP. MicroRNAs in skeletal muscle: their role and regulation in development, disease and function. *J Physiol.* 2010;588(Pt 21):4075-87.
66. Dávalos A, Úbeda N, Montero A, García-González Á, Ramírez de Molina A, Casas-Agustench P, González-Medina A, Martínez-Camblor P, Rabadán M, Díaz-Martínez E, Iglesias-Gutiérrez E. Exercise dose affects the circulating microRNA profile in response to acute endurance exercise in middle-aged amateur runners. *PLoS one* (comunicación personal).
67. Schwarzenbach H, Da Silva AM, Calin G, Pantel K. Data normalization strategies for microRNA quantification. *Clin Chem.* 2015;61(11):1333-42.
68. Gil-Zamorano J, Martin R, Daimiel L, Richardson K, Giordano E, Nicod N, *et al*. Docosahexaenoic Acid Modulates the Enterocyte Caco-2 Cell Expression of MicroRNAs Involved in Lipid Metabolism. *J Nutr.* 2014;575-85.
69. Tomé-Carneiro J, Larrosa M, Yáñez-Gascón MJ, Dávalos A, Gil-Zamorano J, Gonzálvez M, *et al.* 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.
70. Ross SA, Davis CD. The Emerging Role of microRNAs and Nutrition in Modulating Health and Disease. *Annu Rev Nutr.* 2014;17;34(1):305-36.
71. Baier SR, Nguyen C, Xie F, Wood JR, Zempleni J. MicroRNAs Are Absorbed in Biologically Meaningful Amounts from Nutritionally Relevant Doses of Cow Milk and Affect Gene Expression in Peripheral Blood Mononuclear Cells, HEK-293 Kidney Cell Cultures, and Mouse Livers. *J Nutr.* 2014;144(10):1495-500.
72. Noren Hooten N, Fitzpatrick M, Wood WH, De S, Ejiogu N, Zhang Y, *et al.* Age-related changes in microRNA levels in serum. *Aging (Albany NY).* 2013;5(10):725-40.
73. Zhang H, Yang H, Zhang C, Jing Y, Wang C, Liu C, *et al.* Investigation of MicroRNA Expression in Human Serum During the Aging Process. *J Gerontol A Biol Sci Med Sci.* 2014;70(9):1-8.