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

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Keywords:

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.

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, histórico 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.

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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 falling1-3.4

Physical activity is intrinsic to our evolution1-3. 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 activity2, 42% of the Spanish population claim that they do not 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 20155 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 movement1,2,5. In this respect, in 1961 Goldstein described how muscle cells contribute to the humoral regulation of glucose homeostasis during exercise6. 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 expression7,8,9. In this way the repair of exercise-induced damage, the recovery and the physiological and metabolic adaptations5,10 are coordinated. These latter ones are responsible for the systematic effects of exercise on the health1,3,11. 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 expression11. The precise mechanisms by which the expression of the genes involved in the molecular response to exercise is regulated continue to be fundamentally unknown12,13,14. Epigenetic regulation, which not only includes DNA methylation15 but also histone modifications16 or the expression of microRNAs (miRNAs)17, 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)18.

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 expression20. 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 database21 - targets approximately 60% of the coding genome sequences22, playing a fundamental role in development, the maintenance of homeostasis and the response to physiological and physio-pathological stress23, 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 question24.

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...
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. 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. Once in the cytoplasm, the hairpin breaks by a complex formed by RNAsa III or Dicer and TRPBP (transactivation response RNA-binding protein). This way a double-chained molecule or duplex miRNA is generated, which contains the mature miRNA and its complementary chain. 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. 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, 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 miRNA 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.

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, mainly transported in exosomes or microvesicles.

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.

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.
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: 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. 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. Their use in the clinical practice in the short or long term has been proposed by diverse authors.

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. As such, Bye et al. 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. 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, Claus et al. 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. 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., 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. 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.

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. 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 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. 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. and Nielsen et al. With this miRNA involved in muscle function, hypoxia and inflammation processes, as can be seen in Table 3.

The studies by Wardle et al. and Bye et al. describe the base differences of the c-miRNAs between people that regularly do exercise or athletes 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.

<table>
<thead>
<tr>
<th>Type of exercise</th>
<th>Characteristics of the subjects</th>
<th>microRNAs analysed</th>
<th>Increase</th>
<th>Reduction</th>
<th>Bibliographic reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endurance</strong></td>
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<tr>
<td>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).</td>
<td>10 university rowers (19.1 ± 0.6 years).</td>
<td>miR-20a miR-21 miR-133a miR-146a miR-210 miR-221 miR-222 miR-328</td>
<td>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.</td>
<td>Baggish et al.52</td>
<td></td>
</tr>
<tr>
<td>Exercise on exercise bike. 60 min at 70% of the VO2max</td>
<td>11 untrained males (21.5 ± 4.5 years)</td>
<td>miR-1 miR-133a miR-133b miR-206 miR-208b miR-486 miR-499</td>
<td>Immediately after the exercise: miR-486. The base levels recovered in less than 24 h.</td>
<td>Aoi et al.53</td>
<td></td>
</tr>
<tr>
<td>Exercise on exercise bike. 60 min at 65% of the maximum power</td>
<td>13 trained males (28 ± 8 years).</td>
<td>752 miRNA (miRNome panels).</td>
<td>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.</td>
<td>Nielsen et al.57</td>
<td></td>
</tr>
<tr>
<td>Exercise on exercise bike. 4 h at 70% of the individual anaerobic threshold.</td>
<td>12 trained males (32.4 ± 2.3 years).</td>
<td>miR-126 miR-133</td>
<td>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.</td>
<td>Uhleman et al.56</td>
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<tr>
<td><strong>Marathon</strong></td>
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<tr>
<td>Marathon</td>
<td>14 male endurance runners (42.8 ± 6.0 years).</td>
<td>miR-1 miR-21 miR-133a miR-134 miR-155 miR-206 miR-208b miR-499</td>
<td>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.</td>
<td>Mooren et al.56</td>
<td></td>
</tr>
<tr>
<td>Marathon</td>
<td>21 male marathon runners (51.8 ± 1.4 years)</td>
<td>miR-1 miR-126 miR-133a miR-134 miR-146a miR-208a miR-422b miR-499-5p</td>
<td>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.</td>
<td>Baggish et al.58</td>
<td></td>
</tr>
<tr>
<td>Marathon</td>
<td>22 male marathon runners (56.8 ± 5.2 years)</td>
<td>miR-126 miR-133</td>
<td>Immediately after the exercise: miR-126 and miR-133.</td>
<td>Uhleman et al.56</td>
<td></td>
</tr>
<tr>
<td><strong>Half-marathon</strong></td>
<td>5 amateur runners (31.6 ± 4.4 years).</td>
<td>miR-1 miR-133a miR-206</td>
<td>Immediately after the exercise: miR-1, miR-133a and miR-206.</td>
<td>Gomes et al.59</td>
<td></td>
</tr>
<tr>
<td>10 km race</td>
<td>9 amateur runners (39.1 ± 2.2 years).</td>
<td>106 c-miR related to inflammatory response</td>
<td>Immediately after the race: miR-150</td>
<td>de Gonzalo-Calvo et al.49</td>
<td></td>
</tr>
</tbody>
</table>
## Type of exercise

<table>
<thead>
<tr>
<th>Characteristics of the subjects</th>
<th>miRs analysed</th>
<th>Increase</th>
<th>Reduction</th>
<th>Bibliographic reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endurance</strong></td>
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<tr>
<td>Marathon</td>
<td>30 marathon runners, 15 amateur (40.1±1.4 years) and 15 elite (40.0 ± 1.7 years)</td>
<td>miR-1, miR-26a, miR-29b, miR-30a, miR-133a</td>
<td>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.</td>
<td>24 h after the exercise: miR-26a in elite runners.</td>
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<tr>
<td><strong>Strength</strong></td>
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<tr>
<td>Bench press and Leg press. 5 series of 10 repetitions at 70% of 1 MR</td>
<td>12 active males (29.9 ± 1.2 years).</td>
<td>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.</td>
<td>Three days after the exercise: miR-149*.</td>
<td>Three days after the exercise: miR-146a and miR-221.</td>
</tr>
<tr>
<td>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.</td>
<td>11 trained subjects, 4 males and 7 females (37±2 years).</td>
<td>miR-126, miR-133</td>
<td>Immediately after the exercise: miRNA-133. The base levels recovered in less than 1 h.</td>
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</tr>
<tr>
<td><strong>Concentric vs. Eccentric</strong></td>
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<tr>
<td>Uphill and downhill run. 30 min at 1m/s, with 25% inclination and additional load of 12% of the body weight.</td>
<td>9 active males (27-36 years).</td>
<td>miR-1, miR-133a, miR-133b, miR-208a, miR-208b, miR-499</td>
<td>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.</td>
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<tr>
<td><strong>Anaerobic power</strong></td>
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<tr>
<td>Wingate test</td>
<td>18 active males (20.23±0.97 years).</td>
<td>miR-1, miR-16, miR-122, miR-133a, miR-133b, miR-206, miR-499</td>
<td>Immediately after the exercise: miR-1, miR-16, miR-122, miR-133a and miR-133b</td>
<td></td>
</tr>
</tbody>
</table>

### Different methodological approximations and experimental design

Although there are numerous techniques for detecting miRNAs⁶⁰, those most widely used to identify and quantify c-miRNAs in plasma or serum are the massive sequence, microarray and the qRT-PCR⁶¹. Each of these techniques have some differentiated characteristics and some specific advantages and disadvantages⁶²,⁶³ that should be considered depending on the experimental design and the characteristics of the study so that the results are truly informative⁶⁴.

The most widely used technique in the studies that have analysed c-miRNAs in response to exercise have been the qRT-PCR⁴⁴,⁴⁵,⁴⁷-⁴⁹,⁵₂-⁵⁴,⁵⁶-⁵⁹,⁶⁰,⁶¹.

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 VO₂max.

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.
though Sawada et al.\textsuperscript{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\textsuperscript{44,45,48,52,53,54,55,56}. 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.\textsuperscript{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.\textsuperscript{52} and Sawada et al.\textsuperscript{55} did not observe changes in the expression of any myomiR in response to different exercise models. On the other hand, Uhleman et al.\textsuperscript{56}, Mooren et al.\textsuperscript{45}, Baggish et al.\textsuperscript{58}, Banzet et al.\textsuperscript{48} and Clauss et al.\textsuperscript{46} observe significant post-exercise increases of miR-1, miR-133, miR-206, miR-208b and/or miR-499 and Cui et al.\textsuperscript{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

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

<table>
<thead>
<tr>
<th>Exercise model</th>
<th>Training time</th>
<th>Modified miRs</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycling (exercise bike)</td>
<td>12 weeks 5 times/week 60 min at 65% maxP</td>
<td>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.</td>
<td>Nielsen et al.\textsuperscript{57}</td>
</tr>
<tr>
<td>Cycling (exercise bike)</td>
<td>4 weeks 3 times/week 30 min at 70% VO\textsubscript{max}</td>
<td>Post-training decrease: miR-486</td>
<td>Aoi et al.\textsuperscript{53}</td>
</tr>
<tr>
<td>Rowing</td>
<td>90 days training to optimise Performance over 5 km</td>
<td>Post-training increase: miR-146a, miR-21, miR-221 and miR-222.</td>
<td>Baggish et al.\textsuperscript{52}</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Metabolic route</th>
<th>Bibliographic reference</th>
<th>miRNAs analysed</th>
</tr>
</thead>
</table>
| Muscle function (cardiac and skeletal) | Baggish et al.\textsuperscript{52}  
Aoi et al.\textsuperscript{53}  
Uhleman et al.\textsuperscript{56}  
Mooren et al.\textsuperscript{45}  
Baggish et al.\textsuperscript{28}  
Gomes et al.\textsuperscript{59}  
Claus et al.\textsuperscript{46}  
Banzet et al.\textsuperscript{48}  
Cui et al.\textsuperscript{54} | miR-21, miR-133a, miR-133a, miR-133b, miR-206, miR-208b, miR-486, miR-499  
miR-1, miR-133a, miR-133b, miR-206, miR-208b, miR-499  
miR-1, miR-133a, miR-206, miR-208b, miR-499  
miR-1, miR-133a, miR-499-5p, miR-208a  
miR-1, miR-133a, miR-206  
miR-1, miR-26a, miR-29b, miR-30a, miR-133a  
miR-1, miR-133a, miR-133b, miR-208a, miR-208b, miR-499  
miR-1, miR-133a, miR-133b, miR-206, miR-499  |
| Inflammatory Response | Baggish et al.\textsuperscript{52}  
Mooren et al.\textsuperscript{45}  
Baggish et al.\textsuperscript{28}  
de Gonzalo-Calvo et al.\textsuperscript{49} | miR-21, miR-146a, miR-155, miR-21, miR-146a  
106 c-miR related to inflammatory response |
| Endothelial damage | Uhleman et al.\textsuperscript{56}  
Baggish et al.\textsuperscript{28} | miR-126, miR-126 |
| Angiogenesis | Baggish et al.\textsuperscript{52}  
Mooren et al.\textsuperscript{45}  
Baggish et al.\textsuperscript{28}  
de Gonzalo-Calvo et al.\textsuperscript{49} | miR-20a, miR-221, miR-222, miR-210, miR-328 |
| Hypoxia | Baggish et al.\textsuperscript{52} | miR-21, miR-210, miR-146a |
| Brain tissue | Baggish et al.\textsuperscript{28} | miR-134 |
| Cellular proliferation | Cui et al.\textsuperscript{54} | miR-16, miR-122 |
Circulating microRNA as regulators of the molecular response to exercise in healthy people

48, 45, 46, 52, 53, 56, 58, 59, strengh54, 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 marathon45, 58, but in others it was taken one54, two56 or even between two and five days before the test46. 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-miRNAs58-70, 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 plasma71. 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.70 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 observed46, 52, 53, 55.
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

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