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The influence of lifestyle factors on microRNA expression and signal pathways: a review

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1 **The influenceeffect of lifestyle factors on microRNA expression and signal pathways: a review**

2 **Abstract**

3 The term "lifestyle" includes different factors that contribute to the maintenance of a good health
4 status. Increasing evidences suggest that lifestyle factors may influence epigenetic mechanisms, such
5 as microRNAs (miRNAs) expression. The dysregulation of miRNAs can modify the expression of
6 genes and molecular pathways that may lead to functional alterations. This review summarizes human
7 studies highlighting that diet, physical activity, smoking, and alcohol consumption may affect the
8 miRNA machinery and several biological functions. Most miRNAs are involved in molecular
9 pathways that influence inflammation, cell cycle regulation, and carcinogenesis resulting in the onset
10 or progression of pathological conditions. Investigating these interactions will be pivotal for
11 understanding the etiology of pathologic processes, the potential new treatment strategies, and for
12 preventing diseases.

13

14 **Keywords:** microRNA, lifestyle, diet, physical activity, smoking, alcohol consumption, signal
15 pathways, health

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18 **Introduction**

19 MicroRNAs (miRNAs) are a class of non-coding RNA, about 22 nucleotides in length[1], which
20 regulate gene expression through post-transcriptional silencing or activation processes[2]. These
21 molecules, detected in cells, tissues, organs, and in a variety of body fluids, like serum/plasma, saliva,
22 urine, etc.[3,4], are involved in many aspects of biology, such as intercellular communication, cell
23 differentiation, embryogenesis, developmental timings, organogenesis, metabolism, and
24 apoptosis[1,5–7]. Recent analysis suggests that, through multiple transcript targets, miRNAs are
25 associated with several molecular pathways and transcriptional factors[8,9]. Consequently, miRNA
26 dysregulation can lead to the disruption of these signaling molecules or pathways, resulting in the
27 onset of non-communicable diseases (NCDs)[10].

28 Accumulating evidence suggests that lifestyle factors and environmental exposures may impact
29 epigenetic mechanisms, such as miRNA expression[11,12]. According to the Thesaurus of
30 Psychological Index Terms, the term “lifestyle” refers to “typical way of life or manner of living
31 characteristic of an individual or group”[13], including different factors such as diet, physical activity,
32 smoking, and alcohol consumption.

33 Since it is evident that an unhealthy lifestyle may influence the risk of pathological conditions[14,15],
34 there is a need to understand the exact biological mechanism underlying miRNA alterations related
35 to adopted lifestyles by individuals in order to clarify their potential involvement in this context.

36 The aim of this review is to provide a summary of the human studies investigating the influence of
37 each single lifestyle factor (diet, physical activity, alcohol consumption, and smoking) on miRNAs
38 expression and their altered molecular targets that may be involved in pathogenic processes. In this
39 work, miRNA nomenclature is reported adopting the latest version used in *miRbase* [16,17] and
40 miRNA target analysis is classified according to miRTarBase[18].

41 **The influenceeffects of dietary factors**

42 There is much evidence that deficiency or augmented intake of nutrients can have effects on human
43 health[19,20] and regulate the occurrence of genetic damages[21]. Table 1 lists the studies that have

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investigated the modulation of the expression of miRNAs by dietary factors and also includes sample type, study design, analysis of miRNA targets, and biological function.

Several authors have investigated the association ~~effect~~ of specific dietary profiles with~~on~~ the expression of miRNAs. Tarallo et al.[22] compared the association between dietary habits and the expression of miRNAs in both plasma and stool samples of healthy subjects following a vegan, vegetarian, or omnivorous diet. Among the seven analyzed miRNAs, the only statistically significant one was the miRNA-92a-3p that increased in both samples of subjects consuming a vegan diet compared to the other two dietary groups. It was observed that miRNA-92a-3p could be involved in immune function, and it was found overexpressed in several types of cancers[23], therefore, following a vegan diet could contribute to an increased risk of cancer, probably due to the omission of animal derivates. Moreover, miRNA-92a-3p belongs to the miR-17-92 cluster that was also investigated in the study of Humphreys et al.[24], in which the authors carried out a randomized controlled cross-over study examining the association~~effect~~ of a diet rich in lean red meat (LRM) (known to increases colorectal cancer risk) or in LRM plus resistant starch (RS) with~~on~~ miRNAs expression. The results displayed the up-regulation of miR-19a-3p and miR-19b-3p in the group of healthy volunteers consuming the LRM diet, while their levels along with those of other miRNAs of the cluster were lower with the supplementation of RS. ~~, and the down-regulation of miR-17-5p, miR-19a-3p, miR-19b-3p, miR-20a-5p, and miR-92a-3p in the group consuming LRM plus RS diet.~~ The increased miRNA levels in the LRM diet corresponded with increased cell proliferation, and a reduction in their putative gene target expression, including a cell-cycle inhibitor that was cyclin-dependent kinase inhibitor 1A (CDKN1A). Probably, the addition of RS could ameliorate the risk associated with LRM diet, increasing butyrate levels in the colorectum. ~~The decreased levels of miR-17-92 cluster in individuals consuming LRM plus RS suggested a protective effect of RS in the colorectal cancer risk, also underlined through the functional experiment for target genes prediction that showed the association of decreased level of the cell-cycle inhibitor, cyclin-dependent kinase inhibitor 1A (CDKN1A), to the increased level of miR-17-92 cluster with the LRM diet.~~

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70 Another study investigating the possible role ~~effect~~ of RS in ~~the prevention of~~ colorectal cancer was
71 conducted by Malcomson et al.[25]. In their randomized controlled study, colorectal tissue samples
72 of healthy participants were examined to investigate miRNAs level in response to supplementation
73 with RS and polydextrose. The analysis revealed the up-regulation of miR-32-5p in the group
74 consuming RS and polydextrose compared with the placebo group. The implication of miR-32-5p in
75 cell cycle regulation and inflammation[26] underlined the possible correlation of butyrate with a pro-
76 inflammatory response resulting in the increased risk of tumor. The study of McCann et al.[27]
77 investigated the association between miRNAs and dietary glycemic load because of its impact on
78 cancer risk. Healthy premenopausal women at risk for breast cancer, following a 12-month low-
79 glycemic-load dietary intervention, were compared to a control group. The results showed the up-
80 regulation of let-7b-5p and miR-521 and the down-regulation of miR-130a-3p and miR-663b in the
81 intervention group *versus* the control; therefore a lower glycaemic load, known to have beneficial
82 effects on insulin homeostasis[28], led to these differentially expressed miRNAs associated with
83 cancer-, energy metabolism-, and insulin signaling- related pathways, as observed by the
84 bioinformatics analysis.
85 The positive effects of nuts on cardiovascular diseases (CVD) and metabolic conditions are well
86 recognized[29]. A cross-sectional study was conducted by Ortega et al.[30] in which miRNAs
87 expression was analyzed in healthy sedentary individuals following an 8-week nuts-enriched normo-
88 caloric diet. The results indicated dysregulated levels of some miRNAs, of which, through
89 bioinformatics analysis, the authors showed the link of miR-125a-5p and miR-330-3p with pathways
90 related to inflammation, metabolism, and cancer. This condition could mirror an improved lipid
91 profile of individuals consuming a nuts-enriched diet.
92 Hernández-Alonso et al.[31] examined the profile of seven glucose- and insulin-related miRNAs after
93 consumption of pistachios, already known for their beneficial effects on diabetes[32]. A randomized
94 cross-over study was performed with 49~~54~~ pre-diabetic subjects consuming this type of nutrient
95 followed by an isocaloric control diet for 4 months. Among the analyzed miRNAs, only miR-192-5p

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and miR-375 were significantly down-regulated in subjects consuming pistachios. Target gene analysis for these two miRNAs suggested B-cell lymphoma 2 (BCL2), implicated in the control of mitochondrial pathway of β-cell apoptosis induced by pro-inflammatory cytokines[33], as the most involved gene.

Proteins are another important nutrient involved mainly in cellular signaling and metabolic functions[34]. Ramzan et al.[35] carried out a randomized study in which a cohort of healthy men followed a diet containing the current recommendation (RDA) or twice the RDA of protein for ten weeks. The results showed a down-regulated profile of miRNAs in response to twice RDA protein diet. In silico analysis identified target genes linked to inflammation, cell proliferation, and apoptosis pathways; the functional analysis on peripheral blood mononuclear cell (PBMC) of these subjects revealed that phosphatase and tensin homolog (PTEN), interleukin-6 (IL-6), tumor necrosis factor alfa (TNF-α), and interleukin-8 (IL-8) were up-regulated in the twice RDA group. The downregulated expression of the analyzed miRNAs could be linked to the increased inflammatory response in the individuals consuming 2RDA.

Vitamin D deficiency is particularly important during the pregnancy due to the adverse health effects both for the mother and fetus[36]. In the case-control study of Enquobahrie et al.[37], pregnant women were divided into two groups: the low plasma vitamin D group (<25.5 ng/ml) and the high plasma vitamin D group (≥31.7 ng/ml). The results showed that early pregnancy maternal plasma vitamin D concentration was associated with differential miRNAs expression and ~~miRNA analysis showed that eleven miRNAs were dysregulated (10 down-regulated and 1 up-regulated) in women with low vitamin D compared with those with high concentrations. A~~ a bioinformatics approach identified the putative gene targets that were involved in cellular morphology, function and maintenance, system development (including the nervous system and skeletal systems), cell-to-cell signaling, cell death, and metabolic and inflammatory processes. ~~Hence, the dysregulation of these miRNAs, associated with low concentration of vitamin D, resulted in pregnancy complications, such as preeclampsia and gestational diabetes.~~

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122 Selenium and coenzyme Q10 intake in the elderly have beneficial effects because of their anti-
123 inflammatory properties[38]. To test the possible association between these substances and ~~how these~~
124 ~~beneficial effects are related to~~ miRNAs, a randomized control trial was done by Alehagen et al.[39].
125 The miRNA analysis indicated a dysregulated expression in the intervention group consuming the
126 intake of selenium and coenzyme Q10 compared to the placebo group. A literature analysis showed
127 the relationship of most of these miRNAs with CVD, oxidative stress, and inflammation[40,41],
128 indicating the positive health effects of these antioxidants on CVD as well as on cancer [42].
129 A possible association between miRNAs and metabolic inflexibility has been investigated in the
130 recent study of Ramzan et al.[43]. miRNAs with metabolic functions were analyzed in healthy weight
131 insulin-sensitive (IS) and overweight insulin-resistant (IR) post-menopausal women consuming a
132 high-carbohydrate meal. Among analyzed miRNAs, only miR-15a-5p and miR-17-5p showed
133 decreased levels ~~significant results~~ in healthy IS women, while remained unaltered in IR group. In
134 silico prediction tools suggested that the targets of these two miRNAs were mainly involved in lipid
135 as well as oxidative metabolism. The functional experiment on the PBMC of these subjects showed
136 a positive correlation with Carnitine Palmitoyltransferase 1A (CPT1A), linked to metabolic
137 homeostasis, and IL-8 gene (implicated in inflammation) only in the healthy weight IS women.~~,~~ This
138 suggest~~s~~ts~~ting~~ a possible implication~~linking~~ of these miRNAs in the development of ~~with~~ metabolic
139 inflexibility in the overweight subjects.~~ at risk of developing chronic metabolic pathologies.~~
140 [Table 1 near here]
141 In the light of the studies included, we have observed that the miR-17 cluster, including miR-19a-3p,
142 miR-19b-3p, and miR-92a-3p, were the most mentioned, but the miRNA profiles showed inconsistent
143 results. The functional validation of the gene target of the cluster displayed a negative correlation
144 only with LRM, indicating that higher fiber diets leading to an increased production of butyrate by
145 fermentation of dietary fiber, were connected with a minor risk of cancer.
146 Another interesting aspect to be considered is that carcinogenic substances such as heavy metals and
147 pesticides can be introduced through food. These contaminants can lead to dysregulation of miRNAs

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and changes in gene regulation explaining the harmful effects of these chemicals on health [12,44–46]. Therefore, more studies should focus on this issue in order to understand the actual contribution of these contaminants through food ingestion in miRNA alteration.

The influence~~effects~~ of physical activity

Physical activity (PA) generally contribute to beneficial health effects on humans, as from the reduction of genotoxic damage [47]. PA includes both endurance/aerobic and strength/resistance exercises. Endurance exercise can determine changes in cardiovascular as well as in the skeletal muscle system and are connected to maximal oxygen consumption and mitochondrial biogenesis. Conversely, strength training can improve muscle metabolism and promote muscle hypertrophy. As a result of prolonged strength exercise, individuals have stronger muscles with less risk of injuries, whereas performing prolonged aerobic training determines better endurance capacity[48].

Table 2 summarizes the studies on the expression of miRNAs in response to acute and chronic exercises.

The influence~~effects~~ of acute exercise

The expression of several miRNAs is altered after acute exercise, defined as a single isolated PA session[49].

Several studies have examined the association~~effect~~ of different endurance exercises with~~on~~ miRNA signatures.

Wahl et al.[50] conducted a study investigating the profile of three vascular miRNAs after high-intensity training, sprint-interval training (SIT), and a high-volume training (HVT) with cycle ergometer in healthy cyclists. MiRNA quantification analysis indicated increased levels of miR-21-5p and miR-126-3p after both SIT and HVT. These two miRNAs were also validated with an *in vitro* experiment that revealed their endothelial origin and their function in intercellular communication.

In the longitudinal study of Baggish et al.[51], the levels of miRNAs related to muscle contractility, angiogenesis, and inflammation were assessed in healthy athletes using a cycle ergometer. The authors found the up-regulation of miR-21-5p, miR-146a-5p, miR-221-3p, and miR-222-3p after a

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174 distinct time of training. Furthermore, miR-146a-5p correlated with peak exercise capacity and
175 cardiorespiratory fitness. A literature analysis showed the relationship of these miRNAs with
176 pathways linked to angiogenesis and inflammation[52]. The expression of miR-486-5p, one of the
177 muscle-specific miRNAs (also called myomiRs), was examined by Aoi et al.[53]. Healthy young
178 individuals performed cycling exercise at 70% maximal oxygen uptake (VO2max) for 60 min. The
179 level of this miRNA was decreased after the exercise compared to the baseline and its reduction ~~.~~
180 ~~Moreover, miR-486-5p~~ was negatively correlated with VO2max.~~,~~ These results suggested
181 ~~underlining~~ that this miRNA intervene in energy~~'s beneficial effect on~~ metabolism during exercise.
182 Other authors have investigated miRNAs change after cycling exercises[54–56] (Table 2).
183 Another type of endurance training that may cause stress and damage muscles is the marathon[57].
184 In another investigation on the myomiRs family, Gomes et al.[58] examined their expression in five
185 well-trained athletes before and after a half-marathon. They observed increased levels of miR-1-3p,
186 miR-133a, and miR-206 after the half-marathon. These miRNAs have muscle functions and may be
187 used as potential biomarkers of muscle damage or adaptation. In the observational study of de
188 Gonzalo-Calvo et al.[59], the profile of inflammation related-miRNAs was assessed in nine subjects
189 performing both a 10-km race and a marathon. The analysis showed that miRNA levels were
190 differentially altered after the two competitions. Functional in silico analysis suggested that the
191 potential targets of the investigated miRNAs included genes linked to inflammation pathways that
192 could lead to immune system diseases and cancer. In another study of Baggish et al.[60], miRNAs
193 with important muscular, inflammatory, and endothelial functions were analyzed in healthy runners,
194 and the results showed high levels of several miRNAs after the marathon. Moreover, a positive
195 correlation was found with biomarkers of skeletal muscle damage, cardiac muscle injury, cardiac
196 tissue stress, and inflammation, suggesting that the marathon race led to these biological alterations.
197 The influence of the marathon race on miRNAs profile was investigated also in other
198 studies[55,61,62] (Table 2).

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With regard to strength/resistance exercise, Cui et al.[63] conducted a randomized study, enrolling three groups of students who underwent strength endurance, muscular hypertrophy, and maximum strength exercise. From an initial miRNAs screening, only 16 were further validated in the three groups. The results showed dysregulated expression of miR-21-5p, miR-133a, miR-133b, miR-181a-5p, miR-206, miR-208b, and miR-532-5p after distinct-time-course exercises. According to the authors, all these dynamic miRNA changes probably depended on the acute resistance training modality or intensity. Moreover, miR-532-5p was positively and negatively correlated with interleukin-10 (IL-10) and insulin-like growth factor-1 (IGF-1), respectively, whereas miR-133a was negatively correlated with cortisol and positively with testosterone/cortisol. The predicted target genes of miRNA-532-5p indicated its involvement in immune response and metabolic processes. Margolis et al.[64] investigated the association between ~~effect~~ ~~of~~ aging and ~~on~~ miRNA profiles in nine young and adult subjects after~~following~~ resistance exercises ~~in nine young and adult subjects~~. The results showed an up-regulation of ten miRNAs in younger subjects compared to adults after 6 hours of exercise. Bioinformatic analysis suggested that these miRNAs were involved in pathways related to hypertrophy, inflammation, and metabolism. Notably, miR-19a-3p, miR-19b-3p, miR-20a-5p, miR-26b-5p, miR-143-3p, and miR-195-5p were positively associated with p-Akt^{Ser473} and p-S6K1^{Thr389}, indicating the absence of an anabolic response in older people in comparison to younger. The association~~effect~~ of strength exercises with~~on~~ miRNA profiles was also evaluated in other investigations[65–67], as reported in Table 2.

It is of note that low aerobic fitness, measured as VO2max, increases the risk of CVD[68]. Bye et al.[69] investigated the profile of miRNAs in healthy subjects with low and high VO2max. The results of miRNAs analysis showed the up-regulation of miR-210 and miR-222-3p in individuals with low VO2max and of miR-21-5p in males with low VO2max. Bioinformatics approaches showed that these miRNAs were linked to pathways involved in angiogenesis, growth and development, immune system, and stress.

The influence~~effects~~ of chronic exercise

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225 Very few studies have examined the association~~effect~~ ~~of~~ between miRNAs expression and ~~after~~
226 chronic exercise, defined as regular PA done over an extended time period[49].
227 Li et al.[54] conducted a cross-sectional study in which miRNA expression was evaluated in
228 basketball players. miRNA analysis in the serum samples showed a decrease of mir-208b and an
229 increase of mir-221-3p level following 3 months of exercise training. Moreover, mir-221-3p was
230 correlated with VO2, peak workload, and creatine kinase, suggesting its potential role in
231 cardiovascular adaptation response to long-term basketball exercise. A literature analysis indicated
232 that miR-208b is involved in pathways linked to CVD, therefore, its down-regulation improves
233 cardiac function after chronic exercise[70]; miR-221-3p is involved in vascular pathways and thus
234 determines an enhanced muscle regeneration and controlled angiogenesis[71]. More recently,
235 Denhman et al.[72] investigated miRNAs change in untrained subjects after ~~the effect of~~ 6-weeks
236 vigorous exercise training ~~on miRNA change in untrained subjects~~. In the first phase, the
237 dysregulation of thirteen miRNAs (one up- and twelve down-regulated) was observed. The
238 expression of six miRNAs was further validated, confirming the same trend of the sequencing.
239 Bioinformatics analysis showed that the predicted pathways, targeted by the 13 differentially
240 regulated miRNAs, were linked to cell cycle, p53, and cancer.
241 Davidsen et al.[73] conducted a study to examine miRNA expression in subjects divided into high
242 and low responders, in terms of gain in lean muscle mass and muscle fiber area, following 12 weeks
243 of resistance training. miRNAs analysis showed the over-expression of miR-451a and the down-
244 regulation of miR-26a-5p, miR-29a-3p, and miR-378a-5p in low responders compared to high
245 responders. In silico analysis suggested that these miRNAs were associated with mammalian target
246 of rapamycin (mTOR) pathway implicated in skeletal muscle hypertrophy. Moreover, a quantitative
247 PCR was performed to examine mRNA expression of IGF-I, vascular endothelial growth factor-A
248 (VEGF-A), and eukaryotic translation initiation factor 4E type 2 (eIF4E2). In particular, only IGF-I
249 showed a significant positive correlation with the down-regulated miRNAs in low responders,
250 suggesting the involvement of IGF-I in muscle hypertrophy through mTOR pathway activation.

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251 [Table 2 near here]

252 With regard to the considered studies both on acute and chronic exercises, we observed that the
253 expression of several miRNAs varies significantly depending on various modes of exercise. Few
254 studies[56,59,63,64,69,73,74] have performed the bioinformatic analysis for the predicted gene
255 targets, and among these, only Davidsen et al.[73] performed the validation and demonstration of the
256 biological function of the miRNA gene target influenced by physical exercise; notably, exercise
257 training induced changes in inflammation, cardiovascular system, cell metabolism, and hypertrophy.
258 Among the most relevant miRNAs, we found the myomiRs 1-3p, 21-5p, 133, and 206 up-regulated
259 in all studies, except for miR-21-5p and miR-133 that were also downregulated in other
260 papers[54,56,63]. This discrepancy probably depends on the type, duration, intensity of exercise, and
261 individuals' fitness status. miR-21-5p was related to angiogenic processes, enhancing the expression
262 of hypoxia-inducible factor-1 (HIF-1α) and VEGF[75]. As previously mentioned, the down-
263 regulation of this miRNA reflected the inflammatory process, in terms of increased apoptosis and
264 hypoxic conditions in skeletal muscle, that occur after physical training. In fact, its predicted targets
265 are transforming growth factor beta (TGF-β) and mitogen-activated protein kinase (MAPK) involved
266 in the control of inflammation[76]. miR-133 was linked to myogenesis and contribute to muscle
267 hypertrophy by removing the inhibitory effect on growth factors and their receptor's
268 transcription[77]. Another most investigated miRNA was miR-146a-5p, which presented increased
269 and reduced expression in response to both endurance[51,54,56,60,74] and resistance exercises,
270 respectively[66,67]. Moreover, it was overexpressed after both acute and chronic exercise[51]. It is
271 reported ~~that~~ as a miRNA ~~was~~ involved in the inflammation and cell cycle by targeting p53, c-myb,
272 TGFβ, and MAPK, and it was also implicated, as for miR-21-5p, in angiogenesis through platelet-
273 derived growth factor receptor (PDGFR) pathway[52].

274 Some miRNAs were correlated with exercise parameters such as peak exercise capacity (VO2max)
275 and cardiorespiratory fitness. In particular, the myomiR-486-5p was negatively correlated with
276 VO2max. The miR-486-5p gene target is PTEN, a negative regulator of phosphoinositide-3-

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kinase/Akt signaling. miR-486-5p can regulate the uptake of glucose in the skeletal muscle through the activation of the insulin pathway and the suppression of PTEN. The uptake of glucose is fundamental for the maintaining of muscle contraction during exercise in subjects with low VO2 max[78].

The influenceeffects of smoking

Tobacco smoke, one of the biggest public health threats in the world[79], contains a complex mixture of organic and inorganic chemicals, many of which have inflammatory and carcinogenic properties and may induce different genetic and epigenetic changes[80].

Several studies (reported in Table 3) have been conducted in order to investigate the role of cigarette smoking on miRNA dysregulation and their biological effects.

de Ronde et al.[81] investigated whether monocyte-derived miRNA expression levels can be used to identify smokers with an increased risk of developing CVD. They found that miR-124-3p was up-regulated in monocytes of whole blood of smokers, while its level was lower in former smokers and in nonsmokers, suggesting that the effect of smoking determines only a short-lasting change on miR-124-3p levels is only short-lasting. Flow cytometric analysis revealed that the up-regulation of miR-124-3p was associated with an altered monocyte phenotype, that was CD206. Specifically, further in vitro analysis indicated that the overexpression of this miRNA was associated with the upregulation of the surface marker CD206 in both monocytes and macrophages, contributing to the atherogenic plaque development. These findings suggested that a high level of miR-124-3p resulted in an increased risk of subclinical atherosclerosis and may identify which smoking subjects are susceptible to the atherogenic effects of tobacco smoke. miRNA signature was also analyzed in a case-control study of Badrnya et al.[82] in which miR-29b-3p was up-regulated, and miR-223-3p was down-regulated in young healthy smokers compared to nonsmokers. These miRNAs are implicated in the development of CVD, in particular, miR-29b-3p has a pro-atherogenic activity[83], and miR-223-3p is associated with the risk of myocardial infarction[84]. In the study of Takahashi et al.[85], forty-four plasma miRNAs showed a significant difference between smokers and nonsmokers: 43 were up-

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regulated, and only 1 was down-regulated in smokers. Many of these miRNAs (i.e., miR-223-3p, let-7e-5p, and let-7g-5p) were reported as potential biomarkers of diseases (mostly cancer and CVD) in previous studies[86,87].

In the study of Héliot et al.[88], miRNA analysis showed a clear difference in expression of extracellular vesicles (EVs) between smokers and nonsmokers. To assess the association~~effects~~ of smoking with~~on~~ miRNA and mRNA expression, human bronchial epithelial cells (BEAS-2B) were exposed to the smokers and nonsmokers EVs. let-7e-5p and let-7g-5p significantly decreased after smokers EVs exposure. The down-regulation of these miRNAs was found to be correlated with interleukin 6 receptor (IL-6R) mRNA rise expression, which, in turn, was associated with an increase of IL-6. This inflammatory mediator released in the airway epithelium of smokers may increase the risk of developing chronic obstructive pulmonary disease and lung cancer.

The study of Willinger et al.[89] described miRNA profiles of cigarette smoking in a cohort of about 5000 people and related it to inflammatory biomarkers, gene expression, and pulmonary function. Six miRNAs were found to be dysregulated in the blood of smokers, five of which were downregulated. Bioinformatics analysis showed that these miRNAs might have a potential role in inflammatory responses and immune-related pathways. To explore the functional significance of the cigarette smoking-related miRNAs, the authors evaluated their associations with smoking-related clinical features: miR-25-5p, miR-181a-2-3p, miR-423-5p, and miR-1180 were negatively associated with C-reactive protein. In contrast, miR-1285-3p was positively associated with IL-6. Human lung epithelial cells were then cultured for testing miRNA effects on cytokine expression, and it was observed that miR-1180 reduced the production of granulocyte-macrophage colony-stimulating factor (GM-CSF); miR-1180 and miR-1285-3p increased the levels of chemokine C-X-C motif ligand 5 (CXCL5) and CXCL6. These findings confirmed the hypothesis that miRNAs work as mediators of smoking-induced inflammation and target organ damage.

Metzler-Guillemain et al.[90] assessed the miRNA and mRNA profiles in spermatozoa samples of smokers and nonsmokers. An overall decreased level of miRNAs and mRNA was observed in

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329 smokers. The validation analysis showed that miR-296-5p, miR-520d-3p, and miR-3940-5p were
330 down-regulated in smokers. Some of the putative targets of the down-regulated miRNAs were found
331 to be up-regulated among the detected transcripts, such as polycystic kidney disease 2 (PKD2), which
332 is potentially regulated by miR-520d-3p. This study indicated that tobacco smoke exposure could
333 affect human spermatogenesis. The study by Marczylo et al. [91] presented evidence that cigarette
334 smoke induces differential miRNA expression in the spermatozoa of smokers compared with
335 nonsmokers. Through bioinformatics analysis, it was showed that the dysregulated miRNAs mainly
336 intervene in vital pathways for healthy sperm and normal fetal development, especially cell death and
337 apoptosis. Moreover, these miRNAs were analyzed in relation to their mRNA targets associated with
338 epigenetic processes, of which histone deacetylases (HDAC) and chromobox 7 (CBX7) were the
339 most relevant.

340 Wang et al.[92] investigated the miRNA profile in the small airway epithelium of healthy smoking
341 subjects after smoking cessation, compared to a control group of nonsmokers. After 3 months of
342 smoking cessation, 12 miRNAs (9 validated) did not return to the expression level of healthy
343 nonsmokers. To further assess the role of smoking cessation persistent miRNAs, the functions of their
344 target genes were explored: Wnt/β-catenin signaling, cardiac β-adrenergic signaling, and protein
345 kinase A signaling were found to be the top 3 canonical pathways. In addition, the putative target
346 genes of miR-1246, one of the smoking-persistent-down miRNA, were assessed through an in vitro
347 experiment that ~~to assess the putative target genes of miR-1246, which was a smoking-persistent-~~
348 ~~down miRNA,~~ showed a negative correlation with three genes. Since many of the altered persistent-
349 miRNAs were associated with differentiation, inflammatory diseases, or lung cancer, it is likely that
350 these miRNAs may play a role in the small airway epithelium of smokers in ~~–~~ the subsequent
351 development of these disorders. Other studies[93–96] compared the miRNA profiles between healthy
352 current smokers and nonsmokers and showed differential expression in the two groups (Table 3).
353 Prenatal tobacco smoke exposure and its association with miRNAs was evaluated in the study of
354 Maccani et al.[97], who investigated 25 placenta samples from smoking and non-smoking mothers.

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355 The authors found that maternal cigarette smoking during pregnancy was associated with decreased
356 expression of miR-16-5p, miR-21-5p and miR-146a-5p. Bioinformatics analysis suggested that the
357 predicted targets were involved in cell cycle regulation, growth, immunomodulation and development
358 in the placenta. Changes in these target gene expression might have further effects downstream for
359 both placenta and fetus, resulting in a possible altered fetal programming. The same issue was also
360 addressed in other studies[98,99], as reported in Table 3.
361 [Table 3 near here]
362 Considering these studies, we observed a clear difference between the miRNA signature of smoking
363 subjects and non-smoking ones. Overall, the miRNA expression seems to be mostly down-regulated
364 in smokers. Some studies[85,88,95] showed that let-7 family members were down-regulated among
365 current healthy smokers. These miRNAs are known to be implicated in tumor suppression as they
366 functionally inhibit the mRNAs of well-characterized oncogenes and, initially were identified in
367 human lung cancers[100]. In particular, let-7 expression is reduced in non-small cell lung cancer, and
368 this reduction is correlated with poor prognosis. Some~~Other~~ miRNAs showed a differential direction
369 of expression: miR-21-5p were found to be down-regulated in two studies[93,97] and up-regulated
370 in one[85]; while miR-223-3p was overexpressed in two studies[85,88] and under-expressed in
371 one[82].
372 It is not clear if cigarette smoking induces long-term changes in human miRNA expression or whether
373 these have a transient character. Takahashi et al. [85], for example, observed that the plasma miRNA
374 profiles of subjects who quit smoking during their study then resembled those of the control group.
375 Similarly, Wang et al.[92] observed that most of the dysregulated miRNAs in smokers returned to a
376 normal level after smoking cessation of some of the study participant, however, they highlighted that
377 some miRNAs persisted in an altered expression, suggesting that these molecules may play a role in
378 the subsequent development of inflammatory diseases and lung cancer. Furthermore, the possible
379 association~~effects on~~ between miRNA machinery and ~~due to~~ e-cigarette, an emerging habit mainly
380 among young people, and also the relevance of passive smoking on miRNA alteration in subjects

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381 who do not smoke, could be more deeply investigated as it has been demonstrated that passive
382 smoking causes damage at a cellular level even in children[101].

383 **The influenceeffects of alcohol consumption**

384 Excessive alcohol consumption is associated with several diseases, such as obesity, cardiovascular
385 and liver disorders, etc.[102]. In fact, alcohol intake can lead to the dysregulated growth of cells and
386 tissues[103].

387 To date, very few human studies have investigated miRNA profiles after alcohol consumption, as
388 reported in Table 4. miRNAs play a role in alcoholic liver disease, a spectrum of pathologies that can
389 progress to hepatocellular carcinoma[104], and they were suggested to be a master regulator of
390 ethanol-induced multi-organ injury[103]. ten Berg et al.[105] conducted a study in which the serum
391 miRNA profile of young, healthy subjects was evaluated before and after the recreational
392 consumption of alcohol. Thirty miRNAs displayed an up-regulation following alcohol use. In silico
393 analysis showed that the most relevant pathways associated with the 15 largest-fold increase miRNA
394 species were related to carcinogenesis, adrenergic signaling in cardiomyocytes, and the Adenosine
395 Monophosphate activated Protein Kinase (AMPK) signaling pathway. In the study of McCrae et
396 al.[106] addressing the same issue, a small but statistically significant increase of miR-122-5p
397 following the recreational consumption of ethanol by healthy young adults was found. This miRNA
398 is considered a novel liver injury biomarker for use in clinical toxicology[107].

399 It is well known that maternal alcohol exposure during pregnancy contributes to adverse effects on
400 fetal development. Maternal miRNAs could be useful as biomarkers of alcohol consumption and to
401 facilitate the identification of child defects[108]. In the study of Gardiner et al.[109], the expression
402 of miRNAs analyzed in the serum of pregnant women in the alcohol-consuming group compared to
403 controls revealed an increasing level of miR-509-5p, miR-542-3p, and miR-4657 and suppression of
404 miR-602. Functional analysis of putative targets illustrated that the identified miRNAs were involved
405 in biological pathways known to mediate the effect of alcohol.

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406 The study of Balaraman and colleagues[110] showed 11 up-regulated miRNAs in maternal blood
407 serum when the fetus was exposed to alcohol. The common predicted pathways influenced by the
408 detected miRNAs were Ephrin, signal transducer and activator of transcription 3 (STAT3), and
409 epithelial-mesenchymal transition (EMT), that potentially coordinate the regulated signaling network
410 that is critical for placental and fetal growth and maturation. Thus, maternal circulating miRNAs may
411 be used to predict infant outcomes and also as a target for therapeutic intervention.
412 [Table 4 near here]
413 Among the examined studies, the identified miRNAs were very different, and at the moment, none
414 can be considered as a biomarker able to predict the detrimental effects of alcohol. Moreover, the
415 molecular pathways related to alcohol consumption were quite distinct and involved various
416 biological functions, as reported in Table 4. Due to the lack of information, there is the need to
417 elucidate the role of alcohol-sensitive miRNAs that could be implicated in disease development, even
418 during gestation.

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419 **Discussion~~Conclusion and future perspectives~~**

420 To our knowledge, this is the first review to summarize the current literature on the relationship
421 between lifestyle factors and miRNAs. Many studies indicated that unhealthy ~~wrong~~ lifestyles could
422 dysregulate miRNAs expression with implications on the biological pathways altered by these
423 molecules.
424 Most miRNAs detected in the selected studies are involved in several important molecular pathways
425 that influence inflammation, cell cycle regulation, and carcinogenesis, resulting in the onset or
426 progression of pathological conditions.
427 Many other studies investigated the association~~effects of~~ between lifestyle factors and~~on~~ miRNA
428 expression in patients with NCDs, such as CVD, metabolic disorders, respiratory diseases, cancer,
429 etc. However, in this case it is not possible to evaluate whether the miRNA dysregulation occurs
430 before or after the onset of the disease. For this reason, these studies were not included in our review.

431 Taken together, the studies considered in this review highlighted that the expression of some miRNAs
432 in response to a certain lifestyle factor was different, and this may be due to the different sample
433 types, exposure time, and individual and environmental characteristics related to the recruited
434 subjects. In this regard, another aspect that should be considered is endogenous exposure, such as
435 psychological stress, that is correlated with DNA damage that may contribute to the development of
436 various diseases and could modulate the miRNA levels[111]. Discrepancies in miRNAs evaluation
437 could also depend both by the extraction and the detection methodology, suggesting the need for a
438 standardized approach. These technical aspects are very important to directly compare the specific
439 efficiency and accuracy of different methods. Moreover, miRNAs in the blood can be associated with
440 lipoproteins, or contained within exosomes or microvesicles[112], which may complicate miRNAs
441 assessment. In addition, it is noteworthy that most of the examined studies included a small number
442 of participants, didn't adopt standardized intervention protocols, and used observational study
443 designs, which may lead to less coherent results.

444 **Future perspectives**

445 Longitudinal investigations should be conducted to assess the possible long-term effects of behavioral
446 factors on pathogenetic processes regulated by miRNAs, and also rigorous experimental study design
447 is needed to establish the causal relationship between lifestyle factors and miRNAs. Since most of
448 studies adopted in silico analysis, in vitro or functional approaches for the validation of predicted
449 gene targets are mandatory. The biological function of miRNAs should be clarified, above all, to
450 better understanding the etiology of pathologic condition, their potential role as a biomarker of
451 diagnosis and prognosis, their applicability as new treatment strategies, and for preventing the onset
452 of several diseases.
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455 **Executive summary**

456 **Introduction**

457 Lifestyle factors may influence epigenetic mechanisms such as miRNA expression. The
458 dysregulation of miRNAs can modify the expression of genes and molecular pathways that may lead
459 to functional alterations, resulting in the onset of diseases.

460 **The influence~~effects~~ of dietary factors**

461 Dietary factors can lead to different miRNA profiles that are linked to several signal pathways
462 involved in inflammation, metabolism, cell cycle, and cancer. The miR-17 cluster is the most
463 involved.

464 **The influence~~effects~~ of physical activity**

465 The expression of several miRNAs vary significantly depending on various mode of exercises.
466 Exercise training induce changes in inflammation, cardiovascular system, cell metabolism, and
467 hypertrophy. The myomiRs are the most involved in both acute and chronic exercises.

468 **The influence~~effects~~ of smoking**

469 Smoking determines a general down-regulation of miRNA expression. In particular, the most affected
470 miRNAs are the let-7 family members, known to be implicated in tumor suppression. This condition
471 may help explaining the increased risk of lung cancer in smoking subjects.

472 **The influence~~effects~~ of alcohol consumption**

473 Drinking alcohol can modify the expression of miRNAs, but, to date, very few studies investigated
474 this condition. For this reason, there is the need to elucidate the role of alcohol-sensitive miRNAs
475 that could be implicated in diseases development.

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478 The authors have no affiliations or financial involvement with any organization or entity with a
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Table 1. The influence~~effects~~ of dietary factors on miRNA expression

Dietary factors	miRNA	miRNA expression	Sample type	Extraction method	Detection method	Study design	Number of subjects	miRNA target analysis	Biological function	Reference
Vegan diet	92a-3p	↑	Plasma and stool	miRvana MiRNA Isolation Kit (Ambion)	qRT-PCR	Cross-sectional	8 vegans 8 vegetarians 8 omnivorous	N/P		[22~~21~~]
LRM diet LRM diet + resistant starch	19a-3p, 19b-3p, 21-5p 21-5p 17-5p, 19a-3p, 19b-3p, 20a-5p, 92a-3p	↑ ↓	Rectal tissue	Trizol (Ambion)	qRT-PCR	Randomized controlled cross-over	10 LRM diet 13 LRM diet + resistant starch	CDKN1A[#] (SEE)	Cell cycle	[24~~3~~]
R~~esistant starch~~ and PD~~polydextrose intake~~	32-5p	↑	Colorectal tissue	miRNeasy Mini Kit (Qiagen)	qRT-PCR	Randomized double-blind controlled	14 cases (RS+PD) 15 controls	N/P		[25~~4~~]
Low glycemic load diet	521, let-7b-5p 130a-3p, 663b	↑ ↓	Serum	miRNeasy Mini kit (Qiagen)	qRT-PCR	Randomized controlled	18 cases (women at risk for breast cancer) 20 controls	Insulin signaling, TGF-β, p53, cytokine-cytokine receptor interaction, cancer-related pathways (WSE)	Metabolism, inflammation, cell and cell interactions	[27~~6~~]
Almonds and walnuts intake	18a-5p, 19b-3p, 106a-5p, 130b-3p, 192-5p, 486-5p, 769-5p 125a-5p, 221-3p, 328, 330-3p	↑ ↓	Plasma	mirVana PARIS Isolation Kit (Applied Biosystems)	qRT-PCR	Cross-sectional	30	Only for miR-125a-5p, -330-3p: MAPK, TGF-β, Wnt, cancer-related pathways (WSE)	Inflammation, metabolism, cancer	[30~~29~~]
Pistachio intake	192-5p, 375	↓	Plasma	mirVana PARIS Isolation Kit (Applied Biosystems)	qRT-PCR	Randomized cross-over	24 pistachio/control sequence 25 control/pistachio sequence	BCL2 (WSE)	Apoptosis	[31~~0~~]
Twice RDA protein diet	23b-3p, 99a-5p, 100-5p, 125b-5p, 203a	↓	Plasma	AllPrep DNA/RNA/miRNA Universal Kit (QIAGEN)	qRT-PCR	Parallel randomized	15 RDA 14 2RDA	PTEN, IL-6, TNF-α, and IL-8 (WSE)	Inflammation, tumor suppressor	[35~~4~~]
Vitamin D	574-5p 92b-3p, 93-5p, 138-5p, 196a-5p, 320d, 423-3p, 484, 573, 589-5p, 601	↑ ↓	Blood	PAXgene Blood RNA Kit (Qiagen)	OneArrayTM miRNA microarray platforms	Case-control	6 High Vitamin D 7 Low Vitamin D	BCL11A, E2F3, IGF-1, NFAT5, PCGF3, RAD23B, SOX4, TNRC6B etc. (WSE)	Cellular morphology, function and maintenance, cell-to-cell signaling, cell death, carbohydrate/lipid metabolism	[37~~6~~]

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Selenium and coenzyme Q intake	101 miRNAs	Dysregulated	Plasma	miRCURYTM RNA isolation kit-biofluids (Exiqon A/S)	qRT-PCR	Randomized double-blind controlled	25 intake of selenium and coenzyme Q10 25 controls	N/P		[398]
High carbohydrate meal (4 h post-meal)	15a-5p, 17-5p	↓	Plasma	Trizol LS (Thermo Fisher Scientific)	qRT-PCR	Randomized controlled	20 healthy weight IS 20 overweight IR	CPT1A# IL-8# (SEE)	Metabolic homeostasis Inflammation	[432]

BCL11A: B-cell lymphoma 11A; E2F3: E2F transcription factor 3; NFAT5: nuclear factor of activated T-cells 5; PCGF3: polycomb group ring finger 3;
RAD23B: RAD23 Homolog B; SOX4: SRY-Box transcription factor 4; TNRC6B: trinucleotide repeat containing adaptor 6B; -RS: resistant starch; PD: polydextrose intake.
qRT-PCR: quantitative real-time polymerase chain reaction; SEE: strong experimental evidence; WSE: weak in silico evidence.
#these genes are experimentally validated;
↓ / ↑, difference in miRNAs expression, respectively up- and down-expressed;
N/P, not performed.

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Table 2. The influence~~effects~~ of physical activity on miRNA expression

	Physical activity	miRNA	miRNA expression	Sample type	Extraction method	Detection method	Study design	Number of subjects	miRNA target analysis	Biological function	Reference
Acute	High-volume training and sprint-interval training in cycle ergometer	21-5p, 126-3p	↑	Serum	PeqGold Extraction Kit (PeqLab)	qRT-PCR	Cross-sectional	12 triathletes/cyclists	N/P		[50~~46~~]
Acute	Immediately after the cycle ergometer (in pre-training period)	21-5p, 146a-5p, 221-3p, 222-3p	↑	Plasma	MicroRNA Extraction Kit (Benevbio)	qRT-PCR	Cohort	10 rowers	N/P		[51~~47~~]
	1 h after cycle ergometer	21-5p									
	Immediately after the cycle ergometer (in post-training period)	146a-5p, 222-3p									
Chronic	90 days of rowing training	21-5p, 146a-5p, 221-3p, 222-3p									
Acute Chronic	Exercise in cycle ergometer 4 weeks of cycling	486-5p	↓	Serum	TRIzol LS (Invitrogen)	qRT-PCR	Cross-sectional	11 untrained	N/P		[53~~49~~]
Acute	Cycle ergometer	21-5p, 146a-5p, 210, 221-3p	↓	Serum	mirVana PARIS isolation kit (Ambion)	qRT-PCR	Cross-sectional	10 basketball athletes	N/P		[54~~0~~]
Chronic	3 months of basketball training	221-3p	↑								
		208b	↓								
Acute	Bicycling for 4 h Spiroergometry at 50 W with an increase of 25 W every 2 min	126-3p	↑	Plasma	miRNeasy Mini Kit (Qiagen)	qRT-PCR	Cross-sectional	12 trained 13 trained	N/P		[55~~1~~]
	Marathon	126-3p, 133						22 marathon runners 11 trained			
	Lateral pulldown, leg press and butterfly	133									
Acute	1h after cycle ergometer	139-5p, 143-3p, 223-3p, 330-3p, 338-3p	↑	Plasma	Qiazol lysis reagent (Qiagen)	qRT-PCR	Cross-sectional	13	N/P		[56~~2~~]
	3h after cycle ergometer	1-3p									
	Immediately after cycle ergometer	30b, 106a-5p, 146a-5p, 151-3p, 151-5p, 221-3p, 652-3p, let-7i-5p	↓								

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Chronic	12 weeks of cycling training	130a-3p, 107 21-5p, 25-3p, 148a-3p, 185-5p, 342-3p, 766-3p, let-7d-5p	↑ ↓					7			
Acute	Half-marathon	1-3p, 133a, 206	↑	Plasma	miRNeasy Mini Kit (Qiagen)	qRT-PCR	Cross-sectional	5 amateur runners	N/P		[58~~4~~]
Acute	10 km-race Marathon	150-5p let-7d-3p, let-7f-2-3p, 125b-5p, 132-3p, 143-3p, 148a-3p, 223-3p, 223-5p, 29a-3p, 34a-5p, 424-3p, 424-5p	↑	Serum	miRCURY RNA isolation kit (Exiqon)	qRT-PCR	Cross-sectional	9 amateur runners	PI3K-Akt, TGF-β, Wnt, MAPK, VEGF, p53 (WSE)	Inflammation, immune system, cell cycle, apoptosis	[59~~5~~]
Acute	Marathon	1-3p, 126-3p, 133a, 134, 146a-5p, 208a, 499a-5p	↑	Plasma	MicroRNA Assay Kit, Life Technologies	qRT-PCR	Cohort	21 marathon runners	N/P		[60~~56~~]
Acute	Marathon	1-3p, 30a-5p, 133a (in elite) 30a-5p (in amateur) 26a-5p (in elite)	↑ ↓	Plasma	TRIzol LS (Invitrogen)	qRT-PCR	Case-control	30 marathon runners	N/P		[61~~57~~]
Acute	Marathon 24 h post Marathon	1-3p, 133a, 206, 208b, 499a-5p 208b, 499a-5p	↑	Plasma	miRNeasy Mini Kit (Qiagen-Sample & Assay Technologies)	qRT-PCR	Cross-sectional	14 trained endurance runners	N/P		[62~~58~~]
Acute	1h and 24h after strength endurance exercise[(a)] Immediately after and 1h post strength endurance exercise[(a)] 1h after muscular hypertrophy exercise[(a)] 24h after muscular hypertrophy exercise[(a)] Immediately after muscular hypertrophy exercise[(a)] Immediately after maximum strength exercise[(a)]	532-5p 208b 21-5p, 181a-5p, 206 133b 21-5p, 133a 133a	↑ ↓ ↑ ↓	Plasma	TRIzol (Invitrogen)	qRT-PCR	Randomized	45 (3 groups of 15)	Only for miR-532: CDK6, NR2C2, NFKB1, GBP1, NCF2 etc. (WSE)	Carbohydrate and lipid metabolic processes, immune response	[63~~59~~]

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Acute	6h after bilateral knee extension and bilateral leg press	18a-5p, 19a-3p, 19b-3p, 20a-5p, 26b-5p, 143-3p, 195- 5p, 206, 221-3p, 486-5p	↑	Serum	miRNeasy Serum/Plasma Kit (Qiagen)	qRT-PCR	Cross-sectional	18	IGF-1, mTOR, Akt, FOXO, TNF, p53, AMPK, insulin signaling (WSE)	Hypertrophy, inflammation, metabolism, cell cycle, apoptosis	[640]
Acute	High strength knee extensors and flexors training without BFR at 70% of the 1RM Low strength knee extensors and flexors during BFR at 30% of the 1RM	10b-5p, 30a-5p, 139-5p, 143-3p, 195-5p, 197-3p 143-3p	↑ ↓	Plasma	miRNeasy Serum/Plasma Advanced Kit, (Qiagen)	qRT-PCR	Randomized crossover	12	N/P		[651]
Acute	2h after horizontal leg press and seated knee extensions 4h after the exercise 2h and 4h after the exercise 4h after the exercise	133a, 206 146a-5p, 486-5p 23a-3p, 378b 133a, 149-5p	↑ ↓ ↑	Muscle biopsy Plasma	AllPrep DNA/RNA/ miRNA Universal Kit (QIAGEN) Trizol LS (Thermo Fisher Scientific)	qRT-PCR	Clinical trial	9	N/P		[662]
Acute	Bench press and leg press	149-5p 146a-5p, 221-3p	↑ ↓	Serum	mirVana PARIS kit (Ambion)	qRT-PCR	Cross-sectional	12	N/P		[673]
Acute	Aerobic fitness	21-5p, 210, 222-3p	↑	Serum	miRNeasyH Mini Kit (Qiagen)	qRT-PCR	Case-control	38 with High VO2max 38 with Low VO2max	MAPK, TGF-β, B-cell receptor, Wnt, T-cell receptor, mTOR, p53, ErbB, VEGF (WSE)	Angiogenesis, growth and development, immune system, stress	[695]
Chronic	6 weeks of sprint interval training on cycle ergometer	370-3p, 423-5p, 451a, 493-3p, 769-5p, 1301-3p	↓	Blood	miRVana miRNA Mini Kit (Thermofisher Scientific)	qRT-PCR	Cross-sectional	10 untrained	p53, thyroid hormone signaling, focal adhesion (WSE)	Cell cycle, inflammation	[7268]
Chronic	12 weeks of pushing, pulling, and leg training	451a 26a-5p, 29a-3p, 378a-5p	↑ ↓	Vastus lateralis muscle biopsy	TRIzol (Invitrogen)	qRT-PCR	Randomized	56	IGF-I[#] (SEE)	Muscle hypertrophy	[7369]
Acute	Distance running and orienteering	21-5p, 146a-5p, 221-3p, 222-3p	↑	Plasma	miRNeasy Mini Kit (Qiagen)	qRT-PCR	Randomized controlled	10 STR-A 10 END-A 10 controls	p53, c-myb, TGF-β, MAPK, SHP2, PDGFR (WSE)	Cell cycle, inflammation, cancer	[7470]

PI3K-Akt: phosphatidylinositol-3-kinase/protein kinase B; CDK6: cyclin-dependent kinase 6; NR2C2: nuclear receptor subfamily 2 group C member 2; NFKB1: nuclear factor kappa B subunit 1;

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GBP1: guanylate binding protein 1; NCF2: neutrophil cytosolic factor 2; FOXO: forkhead box O; SHP2: Src homology region 2-containing protein tyrosine phosphatase 2; STR-A: strength athletes; END-A: endurance athletes.
qRT-PCR: quantitative real-time polymerase chain reaction; SEE: strong experimental evidence; WSE: weak in silico evidence.
~~# these genes are experimentally validated;~~
↓ / ↑, difference in miRNAs expression, respectively up- and down-expressed;
N/P, not performed;
(a) The protocol consisted of bench press, squat, pulldown, overhead press and standing dumbbell curl performed with different repetitions and intensity among the groups.

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Table 3. The influence~~effects~~ of smoking on miRNA expression

miRNA	miRNA expression	Sample type	Extraction method	Detection method	Study design	Number of subjects	miRNA target analysis	Biological function	Reference
124-3p	↑	Blood and monocytes	RNA 6000 Pico kit and Small RNA kit (Agilent Technologies)	qRT-PCR	Case-control	42 smokers 27 former smokers 69 controls	monocyte marker CD206 (WEE)	Atherogenic effect	[81~~77~~]
29b-3p 223-3p	↑ ↓	PMVs	miRNeasy Minikit (Qiagen)	qRT-PCR	Case-control	20 cases 20 controls	N/P		[82~~78~~]
16-5p, 17-5p, 19a-3p, 19b-3p, 20a-5p, 20b-5p, 21-5p, 24-3p, 25-3p, 26a-5p, 26b-5p, 27a-3p, 29a-3p, 30b-5p, 30c-5p, 92a-3p, 93-5p, 106a-5p, 106b-5p, 126-3p, 126-5p, 185-5p, 186-5p, 191-5p, 195-5p, 199a-3p, 221-3p, 223-3p, 301a-3p, 328, 331-3p, 335-5p, 345-5p, 374a-5p, 374b-5p, 425-5p, 451a, 454-3p, 923 let-7b-5p, let-7d-5p, let-7e-5p, let-7g-5p 188-5p	↑ ↓	Plasma	mirVana PARIS kit (Applied Biosystems)	qRT-PCR	Case-control	11 cases 7 controls	N/P		[85~~1~~]
let-7e-5p, let-7g-5p 26b-5p	↓ ↓	Broncho-alveolar EVs	Small and large RNA kit(NucleoSpin® miRNA, Macherey Nagel)	qRT-PCR	Case-control	10 cases 10 controls	IL-6R# (SEE)	Inflammation	[88~~4~~]
25-5p, 181a-2-3p, 423-5p, 1180, 1285-3p 342-5p	↓ ↑	blood	PAXgene tubes (PreAnalytiX)	qRT-PCR	Case-control	524 smokers 2079 former smokers 2420 controls	CXCL5, CXCL6 (miR-1180, 1285-3p), GM-CSF (miR-1180) (WEE)	Inflammation	[89~~5~~]
296-5p, 520d-3p, 3940-5p	↓	Spermatozoa	RNeasy mini kit (Qiagen)	qRT-PCR	Case-control	8 cases 8 controls	PKD2 (WSE)	Spermatogenesis regulation	[90~~86~~]
509-5p 519d 146b-5p	↑ ↓	Spermatozoa	miRNeasy Mini Kit (Qiagen)	Microarray	Case-control	6 cases 7 controls	CBX7, DNMT1, HDAC2, HDAC5, HDAC11, YY1 CBX2, CBX7, EZH1, HDAC4, RBL2	Cellular proliferation, differentiation, apoptosis, and death	[91~~87~~]

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							CBX3, CBX7, DNMT3A, HDAC2, HDAC3, HDAC9, PHC3		
652-3p							CBX4 (WSE)		
133a, 133b, 550a-5p, 634, 1260a, 487b	↑	Small airway epithelium	miRNeasy mini kit (Qiagen)	qRT-PCR	Case-control	10 cases 9 controls	Wnt/β-catenin, cardiac β-adrenergic signaling, PKA (WSE)	Inflammation, cancer, cell differentiation	[92~~88~~]
218-5p, 224-5p, 1246	↓						DYRK1A, GRHL1, GSK3B[#] (SEE)	Inflammation and cancer	
21-5p	↓	Plasma	Exiqon miRCURY biofluids kit (Exiqon)	qRT-PCR	Case-control	39 cases 101 controls	N/P		[93~~89~~]
126-3p	↓	Plasma	miRNeasy serum/plasma cell lysates kit (Qiagen)	qRT-PCR	Case-control	25 cases 25 controls	ErbB, AMPK, mTOR, TNF, VEGF, MAPK, TGF-β signaling pathways (WSE)	Proliferation, inflammation, differentiation, cell growth, and apoptosis	[94~~0~~]
let-7a-5p	↓	Plasma	miRNeasy serum/plasma cell lysates kit (Qiagen)	qRT-PCR	Case-control	21 cases 19 controls	N/P		[95~~1~~]
485-5p 199a-5p	↑	PMBC	TRIzol (Ambion)	Microarray	Clinical trial	12	CYB561D1 FAM178A	Immune response	[96~~2~~]
~~4498~~ 4742-5p 4498	↓						~~CD83, KDM6B,~~ TM2D3, KDM6B, (SSE) CD83(SEE)		
16-5p 21-5p 146a-5p	↓	Placenta	miRNA Isolation Kit (Ambion)	qRT-PCR	Case-control	8 cases 17 controls	BCL2L2, EDA PLAG1, SATB1 TRAF6 (WSE)	Cell cycle regulation, growth, immunomodulation and development	[97~~3~~]
30b-3p, 33b-3p, 129-5p, 138-1-3p, 187-3p, 507, 520b	↑	Umbilical cord blood plasma	miRNeasy Serum/Plasma Kit (Qiagen)	qRT-PCR	Cross-sectional	24	N/P		[98~~4~~]
760	↓								
223-3p	↑	Maternal blood and cord blood	peqGOLD RNAPure (PEQLAB Biotechnologie GmbH)	qRT-PCR	Cohort	441	N/P		[99~~5~~]

DNMT1: DNA methyltransferase 1; HDAC2: histone deacetylase 2; HDAC3: histone deacetylase 3; HDAC4: histone deacetylase 4; HDAC5: histone deacetylase 5; HDAC9: histone deacetylase 9; HDAC11: histone deacetylase 11; YY1: Yin Yang 1; CBX2: chromobox 2; CBX3: chromobox 3; CBX4: chromobox 4; EZH1: enhancer of zeste homolog 1; RBL2: retinoblastoma-like 2; DNMT3A: DNA methyltransferase 3 alpha; PHC3: polyhomeotic homolog 3; PKA: protein kinase A; DYRK1A: dual specificity tyrosine phosphorylation regulated kinase 1A; GRHL1: grainyhead like transcription factor 1; GSK3B: glycogen synthase kinase 3 beta; BCL2L2: BCL2 like 2; EDA: ectodysplasin A; PLAG1: pleomorphic adenoma gene 1; SATB1: SATB homeobox 1; TRAF6: TNF receptor associated factor 6; CYB561D1: cytochrome b561 family member D1; KDM6B: lysine demethylase 6B; TM2D3: TM2 domain containing 3.

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~~# these genes are experimentally validated;~~ qRT-PCR: quantitative real-time polymerase chain reaction; SEE: strong experimental evidence; WEE: weak experimental evidence; SSE: strong in silico evidence; WSE: weak in silico evidence
↓ / ↑, difference in miRNAs expression, respectively up- and down-expressed;
N/P, not performed

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Table 4. The influence~~effects~~ of alcohol consumption on miRNA expression

miRNA	miRNA expression	Sample type	Extraction method	Detection method	Study design	Number of subjects	miRNA target analysis	Biological function	Reference
557, 1247-3p, 3145-5p, 4258, 4433a-3p, 4433b-5p, 4644, 4652-3p, 4739, 4787-5p, 6721-5p, 6763-3p, 6786-5p, 6879-3p	↑	Serum	miRNeasy Serum/Plasma Kit (Qiagen)	Next Generation Sequencing	Cohort	16	AMPK signaling pathway, adrenergic signaling in cardiomyocytes (WSE)	Carcinogenesis, circadian entrainment	[105~~1~~]
122-5p	↑	Serum	miRNeasy kit (Qiagen)	qRT-PCR	Cross-sectional	18	N/P		[106~~2~~]
509-5p	↑	Serum	miRNeasy Serum/Plasma Kit (Qiagen)	qRT-PCR	Case-control	14 cases 16 controls	PTEN, PEDF, HGF, MAPK1, PI3K/Akt signaling	Cell growth, angiogenesis, signal transduction	[109~~5~~]
542-3p							PI3K/Akt signaling, HGF, BDNF, CREB1	Cardiovascular system development and maintenance	
602, 4657	↓						NOS1, HIF1A, HIF3A, RAC1, HSF1 (WSE)	Oxidative stress	
187-5p, 204-5p, 222-5p, 299-3p, 449a, 491-3p, 518f-3p, 519a-3p, 671-5p, 760, 885-3p	↑	Plasma	RNeasy mini kit (Qiagen)	qRT-PCR	Case-control	45 moderate to heavily-exposed mothers 23 low alcohol consuming or unexposed mothers	Ephrin, STAT3, EMT pathways (WSE)	Placental and fetal growth	[110~~06~~]

PEDF: pigment epithelium-derived factor; HGF: hepatocyte growth factor; BDNF: brain-derived neurotrophic factor; CREB1: CAMP responsive element binding protein 1; NOS1: nitric oxide synthase 1; HIF1A: hypoxia inducible factor 1 subunit alpha; HIF3A: hypoxia inducible factor 3 subunit alpha; RAC1: Rac family small GTPase 1; HSF1: heat shock transcription factor 1.
qRT-PCR: quantitative real-time polymerase chain reaction; WSE: weak in silico evidence
↓ / ↑, difference in miRNAs expression, respectively up- and down-expressed;
N/P, not performed.

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