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Review

MicroRNAs associated with exercise and diet: a systematic review

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Flowers E, Won GY, Fukuoka Y. MicroRNAs associated with exercise and diet: a systematic review. *Physiol Genomics* 47: 1–11, 2015. First published December 2, 2014; doi:10.1152/physiolgenomics.00095.2014.—MicroRNAs are posttranscriptional regulators of gene expression. MicroRNAs reflect individual biologic adaptation to exposures in the environment. As such, measurement of circulating microRNAs presents an opportunity to evaluate biologic changes associated with behavioral interventions (i.e., exercise, diet) for weight loss. The aim of this study was to perform a systematic review of the literature to summarize what is known about circulating microRNAs associated with exercise, diet, and weight loss. We performed a systematic review of three scientific databases. We included studies reporting on circulating microRNAs associated with exercise, diet, and weight loss in humans. Of 1,219 studies identified in our comprehensive database search, 14 were selected for inclusion. Twelve reported on microRNAs associated with exercise, and two reported on microRNAs associated with diet and weight loss. The majority of studies used a quasiexperimental, cross-sectional design. There were numerous differences in the type and intensity of exercise and dietary interventions, the biologic source of microRNAs, and the methodological approaches used quantitate microRNAs. Data from several studies support an association between circulating microRNAs and exercise. The evidence for an association between circulating microRNAs and diet is weaker because of a small number of studies. Additional research is needed to validate previous observations using methodologically rigorous approaches to microRNA quantitation to determine the specific circulating microRNA signatures associated with behavioral approaches to weight loss. Future directions include longitudinal studies to determine if circulating microRNAs are predictive of response to behavioral interventions.

microRNA; physical activity; weight loss; diet; exercise

OBESITY IS A NATIONAL EPIDEMIC associated with substantially increased risk for numerous diseases (27). Obesity is a complex, multifactorial condition that occurs in the setting of both innate and environmental risk factors (1). Accordingly, there are interindividual differences in the etiology of obesity. In addition to surgery, there are two primary behavioral approaches to weight loss: increased physical activity and caloric restriction (14). Both of these strategies exhibit variable effectiveness in the general population (17). One explanation is inconsistent adherence to diet and physical activity recommendations. Another reason is that individuals differ in their environmental stimuli and underlying biology, and both of these mediate the response to physical activity and diet. Therefore, behavioral approaches to weight loss, although effective, are not fully successful at addressing the problem of obesity.

MicroRNAs (miRNAs) are posttranscriptional regulators of messenger RNA (mRNA). In addition to regulating normal and abnormal physiological processes, epigenetic events are hypothesized to be the mechanism by which adaptation to the environment occurs. Mature miRNAs are 18–24 nucleotides in length. MiRNAs function primarily by binding to complementary region in the 3′-untranslated regions of mRNAs, thereby regulating translation of mRNA to amino acids (20). MiRNA regulation is dynamic. Their effects can be temporary, when the miRNA temporarily binds an mRNA to suppress translation, or permanent, causing degradation of the mRNA strand (32). The exact binding patterns of individual miRNAs and mRNAs are not fully understood. Currently, 2,603 discrete miRNA species are identified in humans (18). A subset of these miRNAs (~300) are detectable in blood from combined cellular and noncellular origins (18). Importantly, while all circulating miRNAs may be useful clinical biomarkers, the origin (i.e., cellular vs. noncellular) is a fundamental consideration to understanding the underlying biological implication of individual miRNAs. Cell-free miRNAs found free, bound to protein complexes, and contained within exosomes, microparticles, and HDL-cholesterol in blood serum/plasma are hypothesized to originate from solid organs (34–36). By contrast, cellular miRNAs found in leukocytes may have a primary function in regulating gene expression in blood cells.

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Prior studies identified blood-based miRNAs as predictors of obesity-related diseases (e.g., Type 2 diabetes, myocardial infarction) (38, 39). In addition, changes in levels of miRNAs correlate with (3, 37) and possibly predict (10) responses to aerobic and endurance exercise training interventions in healthy adults. The identification of miRNAs associated with exercise, diet and weight loss, and response to these lifestyle interventions has three important clinical implications. The first is identification of specific molecular mechanisms underlying responses to diet and weight loss, enabling more tailored interventions for groups or individuals. Second, more precise evaluation of the types, frequency, and duration of interventions to reduce risk while minimizing adverse effects and patient burden (12). Third, improved monitoring of response to weight loss interventions. Research is needed to determine whether miRNAs will be useful for tailoring weight loss treatments in clinical practice. The aim of this study was to systematically review, summarize, and synthesize what is known about circulating miRNAs associated with exercise, diet and/or weight loss in humans. To our knowledge, this is the first systematic review to examine changes in levels of miRNAs in relation to exercise, diet, and weight loss that might capture both beneficial and maladaptive responses. Findings from this review will identify knowledge gaps and facilitate design of tailored exercise, diet, and weight loss intervention studies in the near future.

METHODS

In collaboration with a professional librarian, a comprehensive literature search was performed according to the PRISMA guidelines (24). We sought to identify human studies of blood-based or circulating miRNAs associated with exercise, physical activity, diet, and weight loss in adults. Search limits included peer-reviewed journal articles with full text available, written in English, and published between January 1, 1993 and May 1, 2014. We searched the PubMed, EMBASE, and Web of Science databases. Two searches were performed. One searched for studies reporting on relationships between miRNAs and exercise and/or physical activity. Key search terms included microRNAs, epigenetic, exercise, and related derivatives. The full search parameters are shown in the Appendix. The second search was for studies reporting on relationships between miRNAs and diet and/or weight loss. Key search terms included microRNAs, epigenetics, diet, weight loss, and derivatives. The full search parameters are shown in the Appendix. Articles that did not report on miRNA expression, that reported on noncirculating (e.g., skeletal muscle) miRNAs, and studies not conducted in humans were excluded. References of included studies were reviewed to identify additional studies meeting inclusion and exclusion criteria. Results of the search are shown in Fig. 1. One author (E. Flowers) reviewed abstracts of all articles for inclusion.

A data extraction table was developed to record key information about included studies. One author (E. Flowers) read and extracted data from the included studies. The following study attributes were recorded: research question, study design, time frame, sample, tissue source, intervention, measurement of intervention dose, miRNA quantitation method(s), data normalization method, miRNA(s) measured, miRNA(s) differentially expressed, direction of differential expression, and fold-change when reported (Table 1, Table 2, Table 3). The primary outcome measures were specific miRNAs identified and the direction and magnitude (i.e., fold-change) of differential expression for each miRNA in the experimental compared with control group or condition. Risk of bias within studies was determined by evaluation of the intervention, measurement of the intervention dose, and evaluation of how miRNAs were quantitated, including expression normalization. Risk of bias across studies was evaluated through careful evaluation of the methods and results sections by one reviewer (E. Flowers).

RESULTS

A total of 1,219 records were identified through database searches. Despite several iterations of the search parameters, 1,183 manuscripts identified in the search were excluded because of the following primary reasons (Fig. 1): studies reporting the results of studies from in vitro and animal model studies, studies not reporting on circulating miRNA expression, and reviews. The remaining 36 articles were selected for further assessment. Of these, 22 studies did not meet all inclusion and exclusion criteria: 13 were review articles that were excluded but were read to identify additional studies meeting inclusion, two articles described studies of methylation but not miRNA changes, six measured skeletal muscle expression of miRNAs, and one was a cross-sectional observational study of miRNAs associated with obesity but did not evaluate weight loss. Therefore, 14 studies that met all inclusion and exclusion criteria were selected for this systematic

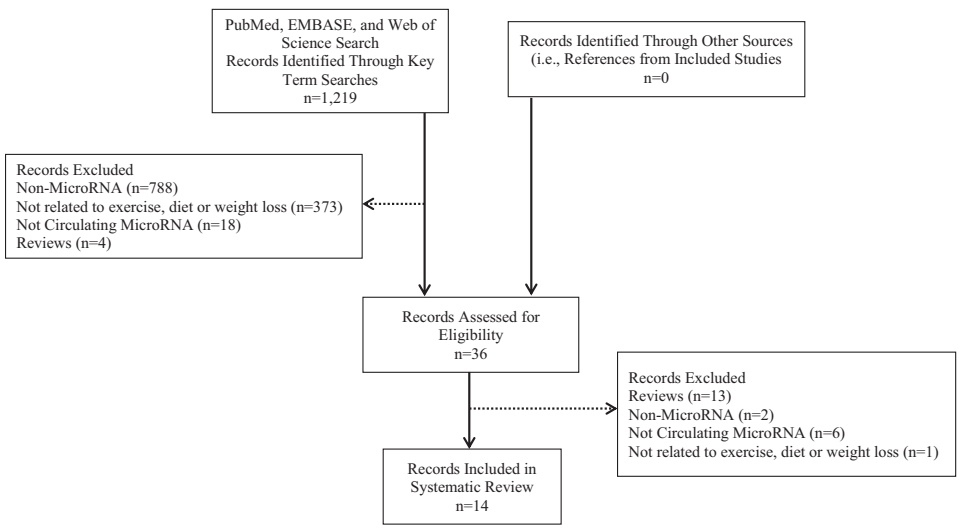

Fig. 1. Selection of studies for inclusion.

Table 1. *Studies included in the review*

Author, Year Country	Research Question	Design	Sample	Tissue Source	Exercise/Diet/Weight Loss Intervention	MicroRNA Quantitation Method	Normalization Method
			Exercise				
Radom-Aizik, 2010 United States	Does neutrophil miR expression change after brief exs?	quasiexperimental: blood collected at baseline and 30 min post-exs	men age 19–30 yr (n = 11); 7 Caucasian; excluded elite athletes	neutrophils isolated from whole blood	30 min cycle ergometry intervals (2 min exs followed by 1 min rest × 10 intervals)	Agilent microarray version 2 (826 miRs); qPCR	standardized 100 nanograms total RNA input
Baggish, 2011 United States	Does expression of circulating miRs change at rest and during exhaustive exs before and after aerobic training?	quasiexperimental: blood collected at rest and after exhaustive exs pre-/post-exs training	student endurance athletes (n = 10)	plasma	90 days rowing training	qPCR	exogenous miR-422b spike-in
Uhlemann, 2014 Germany	What are the effects of exs on miR-126 and endothelium?	quasiexperimental: blood collected pre- and post-each exs	adult men; n = 13 exs test n = 12 4-hour bicycle n = 22 marathon runners n = 11 resistance exs	plasma	four groups: exs test, 70% max on bicycle × 4 h, marathon, resistance exs	qPCR	C-elegans-39 spike in
Aoi, 2013 Japan	Do circulating miRs change in response to acute and chronic exs?	quasiexperimental: blood collected 60 min post-exs test and pre- and post-4 wk intervention	sedentary men (n = 10); mean age 21.5 yr	serum	30 min cycling 3 times per week for 4 wk	qPCR	miR-16 and C elegans-39
Banzet, 2013 Qatar	Are circulating muscle-specific miRs affected by exs and modality?	quasiexperimental: blood collected pre-/post-exs, after 1, 2, and 3 days	men age 27–36 yr (n = 9); BMI 24.6; currently 1–4 h of exs/week	plasma	uphill (concentric) treadmill and downhill (eccentric) walking exs; 6 wk washout	qPCR	geometric mean of miRs-20a, 103, 21, 192, 185 (derived from GENorm)
Bye, 2013 Norway	Which miRs are associated with low vs. high VO_2 max?	cross-sectional: blood collected prior to treadmill test	screening sample: high (n = 12) vs. low (n = 12) $\dot{V}O_{2max}$ matched for other cardiovascular risk factors; validation sample: same criteria (n = 76)	serum	treadmill test	array (720 miRs) qPCR	miR-425
Mooren, 2014 Germany	Are miRs associated with aerobic fitness performance capacity?	quasiexperimental: blood collected 2 days premarathon, immediately post-, and 24 h post-	male marathon runners (n = 14)	plasma	treadmill test; marathon	qPCR	C-elegans-39 spike in
Sawada, 2013 Japan	Are circulating miRs associated with resistance exs?	quasiexperimental: blood collected pre-0 min, 60 min, 1 day, and 3 days post-exs	men not currently engaged in an exs training program (n = 12)	serum	bench press, bilateral leg press	array qPCR	miR-16
Tonevitsky, 2013 Russia	How does miR change during exs?	quasiexperimental: blood collected pre-exs, 30 min and 60 min post-exs	trained male skiiers (n = 8)	whole blood	treadmill test	Affymetrix array (200 miRs)	n/a
Zhou, 2014 China	Does physical activity influence metabolic syndrome risk by influencing miRs?	cross-sectional: self-reported physical activity and miR expression	case-control comparison of individuals with metabolic syndrome (n = 209) compared to controls (n = 234)	serum	metabolic equivalents calculated from physical activity questionnaire	qPCR	C-elegans-39
Baggish, 2014 United States	Are plasma miRs uniquely modulated following sub-max exs?	quasiexperimental: blood collected pre-exs, immediately post- and 24 h post-exs	male marathon runners (n = 24)	plasma	marathon	qPCR	miR-422b

Continued

Table 1.—*Continued*

Author, Year Country	Research Question	Design	Sample	Tissue Source	Exercise/Diet/Weight Loss Intervention	MicroRNA Quantitation Method	Normalization Method
Radom-Aizik, 2014 United States	Does brief exs alter monocyte miR expression?	quasiexperimental: blood collected pre- and post-exs	healthy men (n = 12); BMI 26; mean age 26 yr	monocytes isolated from whole blood	cycle ergometry	Agilent array (961 miRs) qPCR	RNU-44
			Diet/Weight Loss				
Milagro, 2013 Spain	Does miR expression characterize high (≥5% weight loss) vs. low responders to a diet intervention?	quasiexperimental: blood collected at baseline before 8 wk diet intervention	obese women (n = 10)	peripheral blood mononuclear cells	low-calorie (800–880 kcal) diet for 8 wk leading to ≥5% weight loss	SOLiD v4 sequencing qPCR	miR-148a
Ortega, 2013 Spain	Are miRs associated with degree of obesity? Do miRs change after weight loss?	quasiexperimental: blood collected at baseline, 14 wk (diet group), and 1-yr (surgery group)	obese men (n = 32 discovery, n = 80 validation); bariatric surgery patients (n = 22); diet intervention patients (n = 9)	plasma	bariatric surgery; low-calorie (500–1,000 kcal/day deficient) diet for 14 wk resulting in 17% weight loss	Agilent array (n = 754) qPCR	panel of most stably expressed miRs

miR, microRNA; exs, exercise; BMI, body mass index; qPCR, quantitative polymerase chain reaction.

review. Twelve of the 14 included studies evaluated circulating miRNAs associated with exercise (2–5, 8, 25, 29–31, 33, 37, 40). The remaining two studies evaluated miRNAs associated with diet/weight loss (22, 28). None reported on exercise interventions for weight loss in overweight or obese individuals.

MiRNAs Associated With Exercise

Study characteristics. Two general study designs were utilized (Table 1). Studies with a quasiexperimental design evaluated both acute miRNA expression changes following an episode of exercise (2, 4, 5, 8, 25, 29–31, 33) and longitudinal changes in miRNA expression corresponding to prolonged exercise training programs (2, 3). A cross-sectional observational design was used to detect associations with self-reported physical activity (40). The geographic origin and racial makeup of the samples varied. The study samples included relatively healthy adults as opposed to obese individuals. The only sample that included women was the study of self-reported physical activity (40). Five studies quantitated miRNAs from plasma (3–5, 25, 37), four from serum (2, 8, 31, 40), one from neutrophils (30), one from monocytes (29), and one from whole blood (33). Five studies utilized an agnostic approach to detection of miRNAs associated with physical activity by screening a large number of miRNAs with high-throughput quantitation methods (8, 29–31, 33). One study used an Affymetrix array to screen ~200 miRNAs (33). The remainder of the studies used quantitation polymerase chain reaction (qPCR)-based microarrays followed by qPCR validation of individual miRNAs. The studies evaluating individual miRNAs selected based on a priori hypotheses used qPCR-based measurement (2–5, 25). All quantitation approaches have strengths and weaknesses (13). While array-based methods offer high throughput, they have relatively poorer limits of detection for low-abundance miRNAs compared with qPCR or emerging methods, and subsequent qPCR validation is optimal (26). The method of data normalization varied between studies with some using an exogenous spike-in control (2, 3, 25, 40), some using an individual low-variability miRNA (2, 4, 8, 29, 31), and some using a mathematically derived normalization value (i.e., geometric mean of miRNAs with low variability) (5). A consensus on optimal normalization approaches for miRNA analysis has not been established (26).

Intervention characteristics. Ten of 12 studies measured miRNAs associated with cardiorespiratory fitness (2–5, 8, 25, 29, 30, 33, 37), and the remaining two studies measured miRNAs associated with resistance exercise (5, 31). Among the 10 cardiorespiratory fitness studies, six studies measured acute exercise (3, 5, 8, 25, 31, 37), whereas two studies measured prolonged exercise (4, 25) (Table 4). Cardiorespiratory fitness was assessed by cycle ergometry (2, 5, 8, 25, 29, 30, 37), treadmill (33), rowing (3, 4), marathon running (25, 37), and self-report (40). Resistance training included bench press (31), leg press (31, 37), butterfly (37), and lateral pulldowns (37). Studies measured miRNA expression associated with one episode of cardiorespiratory exercise (e.g., 30 min cycle ergometry) (8, 29, 30, 33, 37), endurance exercise (i.e., marathon running) (25, 37), and pre-/postexercise training over weeks to months (2–5, 37). Only one study performed comparisons of miRNA expression associated with all three cardiorespiratory conditions and resistance training (37).

Risk of bias within studies. A summary of the risks of bias for individual studies is shown in Table 5. All but one of the studies described a quasiexperimental study design. The remaining study had a less rigorous cross-sectional design (40). Of the studies evaluating cardiorespiratory fitness, seven per-

Table 2. *Summary of findings of miR in response to exercise*

MiR	Author/Date	Direction	Fold Change	Exercise	Tissue
1	Baggish/2014	↑	21.8	marathon	plasma
1	Banzet/2013	↑		downhill vs. uphill treadmill	plasma
1	Mooren/2014	↑		marathon	plasma
1	Mooren/2014	↑		treadmill test	plasma
20a	Baggish/2011	↑	3.0 (ns)	at rest after 90 days exercise training	plasma
21	Baggish/2011	↑	1.9	postacute cycle ergometry	plasma
21	Baggish/2011	↑	2.6	at rest after 90 days exs training	plasma
126	Baggish/2014	↑	1.9	marathon	plasma
126	Uhlemann/2014	↑		four exs conditions	plasma
133a	Baggish/2014	↑	18.5	marathon	plasma
133a	Banzet/2013	↑		downhill vs. uphill treadmill	plasma
133a	Mooren/2014	↑		marathon	plasma
133a	Mooren/2014	↑		treadmill test	plasma
133b	Banzet/2013	↑		downhill vs. uphill treadmill	plasma
146a	Baggish/2011	↑	3	postacute cycle ergometry	plasma
146a	Baggish/2011	↑	3.1	at rest after 90 days exs training	plasma
146a	Baggish/2011	↑	7.5	postacute exs after 90 days training	plasma
146a	Baggish/2014	↑	3.3	marathon	plasma
181b	Banzet/2013	↑		downhill vs. uphill treadmill	plasma
206	Mooren/2014	↑		marathon	plasma
206	Mooren/2014	↑		treadmill test	plasma
208a	Baggish/2014	↑	9.4	marathon	plasma
208b	Banzet/2013	↑		downhill vs. uphill treadmill	plasma
208b	Mooren/2014	↑		marathon	plasma
214	Banzet/2013			downhill vs. uphill treadmill	plasma
221	Baggish/2011	↑	3.6	postacute cycle ergometry	plasma
221	Baggish/2011	↑	5.8	at rest after 90 days exs training	plasma
222	Baggish/2011	↑	2.5	postacute cycle ergometry	plasma
222	Baggish/2011	↑	2.4	at rest after 90 days exs training	plasma
222	Baggish/2011	↑	4	postacute exs after 90 days training	plasma
499	Mooren/2014	↑		marathon	plasma
499-5p 5p	Baggish/2014	↑	2.3	marathon	plasma
499-5p 5p	Banzet/2013	↑		downhill vs. uphill treadmill	plasma
let7d	Bye/2013	↑		treadmill test	serum
21	Bye/2013	↑		treadmill test	serum
21	Bye/2013	↑	ns	treadmill test	serum
29a	Bye/2013	↑		treadmill test	serum
125a	Bye/2013	↑		treadmill test	serum
125a	Bye/2013	↑		treadmill test	serum
126	Zhou/2014	↓		self-reported exs	serum
130a	Zhou/2014	↓		self-reported exs	serum
146a	Sawada/2013	↓		bench press, leg press	serum
149*	Sawada/2013	↑		bench press, leg press	serum
151	Bye/2013	↓		treadmill test	serum
197	Zhou/2014	↑		self-reported exs	serum
210	Bye/2013	↑		treadmill test	serum
210	Bye/2013	↑		treadmill test	serum
210	Bye/2013	↑		treadmill test	serum
221	Sawada/2013	↓		bench press, leg press	serum
222	Bye/2013	↑		treadmill test	serum
486	Aoi/2013	↓		4 wk cycling training	serum
652	Bye/2013	↑	ns	treadmill test	serum
652	Bye/2013	↓		treadmill test	serum
15a	Rasom-Aizik/2014	↑	1.3	cycle ergometry	monocytes
29b	Rasom-Aizik/2014	↑	1.9	cycle ergometry	monocytes
29c	Rasom-Aizik/2014	↑	1.5	cycle ergometry	monocytes
30e	Rasom-Aizik/2014	↑	1.3	cycle ergometry	monocytes
130a	Rasom-Aizik/2014	↓	1.5	cycle ergometry	monocytes
140-5p 5p	Rasom-Aizik/2014	↑	1.3	cycle ergometry	monocytes
151-5p 5p	Rasom-Aizik/2014	↓	1.4	cycle ergometry	monocytes
199a-3p	Rasom-Aizik/2014	↓	1.5	cycle ergometry	monocytes
221	Rasom-Aizik/2014	↓	1.3	cycle ergometry	monocytes
324-5p 3p	Rasom-Aizik/2014	↑	1.4	cycle ergometry	monocytes
338-5p 3p	Rasom-Aizik/2014	↑	1.3	cycle ergometry	monocytes
362-5p 3p	Rasom-Aizik/2014	↑	1.4	cycle ergometry	monocytes
362-5p 5p	Rasom-Aizik/2014	↑	1.3	cycle ergometry	monocytes
532-5p 3p	Rasom-Aizik/2014	↑	1.3	cycle ergometry	monocytes
532-5p 5p	Rasom-Aizik/2014	↑	1.3	cycle ergometry	monocytes
660	Rasom-Aizik/2014	↑	1.4	cycle ergometry	monocytes

Continued

Table 2.—*Continued*

MiR	Author/Date	Direction	Fold Change	Exercise	Tissue
1202	Rasom-Aizik/2014	↑	1.3	cycle ergometry	monocytes
1305	Rasom-Aizik/2014	↑	1.5	cycle ergometry	monocytes
7i	Radom-Aizik/2010	↓	1.3	cycle ergometry	neutrophils
16	Radom-Aizik/2010	↓	1.2	cycle ergometry	neutrophils
17	Radom-Aizik/2010	↓	1.4	cycle ergometry	neutrophils
18a	Radom-Aizik/2010	↓	1.4	cycle ergometry	neutrophils
18b	Radom-Aizik/2010	↓	1.3	cycle ergometry	neutrophils
20a	Radom-Aizik/2010	↓	1.2	cycle ergometry	neutrophils
20b	Radom-Aizik/2010	↓	1.3	cycle ergometry	neutrophils
22	Radom-Aizik/2010	↓	1.3	cycle ergometry	neutrophils
93	Radom-Aizik/2010	↓	1.2	cycle ergometry	neutrophils
96	Radom-Aizik/2010	↓	1.3	cycle ergometry	neutrophils
106a	Radom-Aizik/2010	↓	1.4	cycle ergometry	neutrophils
107	Radom-Aizik/2010	↓	1.3	cycle ergometry	neutrophils
125a-5p	Radom-Aizik/2010	↑	1.2	cycle ergometry	neutrophils
126	Radom-Aizik/2010	↓	1.5	cycle ergometry	neutrophils
130a	Radom-Aizik/2010	↓	1.6	cycle ergometry	neutrophils
130b	Radom-Aizik/2010	↓	1.4	cycle ergometry	neutrophils
145	Radom-Aizik/2010	↑	1.2	cycle ergometry	neutrophils
151-5p 5p	Radom-Aizik/2010	↓	1.6	cycle ergometry	neutrophils
181b	Radom-Aizik/2010	↑	1.6	cycle ergometry	neutrophils
185	Radom-Aizik/2010	↓	1.3	cycle ergometry	neutrophils
193a-3p	Radom-Aizik/2010	↑	1.6	cycle ergometry	neutrophils
194	Radom-Aizik/2010	↓	1.3	cycle ergometry	neutrophils
197	Radom-Aizik/2010	↑	1.4	cycle ergometry	neutrophils
212	Radom-Aizik/2010	↑	1.4	cycle ergometry	neutrophils
223	Radom-Aizik/2010	↑	1.3	cycle ergometry	neutrophils
340*	Radom-Aizik/2010	↑	1.3	cycle ergometry	neutrophils
363	Radom-Aizik/2010	↓	1.3	cycle ergometry	neutrophils
365	Radom-Aizik/2010	↑	1.4	cycle ergometry	neutrophils
485-5p 3p	Radom-Aizik/2010	↑	2.9	cycle ergometry	neutrophils
505	Radom-Aizik/2010	↑	1.2	cycle ergometry	neutrophils
520d-3p	Radom-Aizik/2010	↑	2.8	cycle ergometry	neutrophils
629*	Radom-Aizik/2010	↑	1.4	cycle ergometry	neutrophils
638	Radom-Aizik/2010	↑	1.4	cycle ergometry	neutrophils
660	Radom-Aizik/2010	↓	1.2	cycle ergometry	neutrophils
939	Radom-Aizik/2010	↑	1.4	cycle ergometry	neutrophils
940	Radom-Aizik/2010	↑	1.4	cycle ergometry	neutrophils
1225-5p	Radom-Aizik/2010	↑	1.6	cycle ergometry	neutrophils
1238	Radom-Aizik/2010	↑	1.6	cycle ergometry	neutrophils
21-5p 5p	Tonevitsky/2013	↑		treadmill	whole blood
24-5p 2-5p 5p	Tonevitsky/2013	↑/↓		treadmill	whole blood
27a-5p	Tonevitsky/2013	↑/↓		treadmill	whole blood
181a-5p	Tonevitsky/2013	↑/↓		treadmill	whole blood
181b-5p	Tonevitsky/2013	not shown		treadmill	whole blood

ns, Not significant.

formed an objective evaluation of maximal oxygen uptake ($\dot{V}O_{2max}$) (2, 3, 8, 29, 30, 33, 37). One study determined fitness by self-report and conversion to metabolic equivalents (40). An additional limitation of this study is that a validated questionnaire was not used to collect activity data. Of the studies that evaluated resistance training, one used the One Maximum Repetition method to determine individual participants' maximum strength (31). A second used the Borg rating of perceived exertion scale to determine the weight that should be used in the exercise session (37). All studies used microarray followed by qPCR, which are acceptable approaches to miRNA quantitation.

Synthesis of miRNA findings. A total of 70 miRNA isoforms were found to be differentially expressed across 12 exercise studies (Table 2). Fifteen miRNAs or their isoforms were measured in more than one study [i.e., microRNA (miR)-1, miR-20a/b, miR-21, miR-29a/b/c, miR-125a, miR-126, miR-130a/b, miR-133a/b, miR-146a, miR-181a/b, miR-197, miR-208a/b, miR-221, miR-222, miR-499-5p]. In some cases, the direction of expression varied between studies. MiR-126, which has previously been associated with incident Type 2 diabetes (38), was measured in four studies. Two described increased expression in plasma following a marathon (4, 37), resistance training (4), low-intensity 4 h cycling (37), and a cycle ergometer exercise test (37). A study of miRNA expression in neutrophils following a brief cycling exercise intervention (30) found decreased expression of miR-126 as did the self-reported physical activity study (40). MiRNA-126 targets vascular cellular adhesion molecule 1, which is expressed in endothelial cells and implicated in atherosclerosis (19). Additional potentially relevant predicted targets of miRNA-126 include apolipoprotein A-V (APOA5), insulin-like growth factor 1 receptor, insulin receptor substrate 1 (IRS1), and insulin receptor substrate 2 (IRS2) (19).

MiR-221 was measured in four exercise conditions across three studies. Increased expression in plasma was observed

Table 3. *Summary of findings of miR associated with BMI and weight loss*

MiR	Author/Date	Direction	Fold Change	Comparison	Tissue
			BMI		
15a	Ortega/2013	↓		BMI ≥40 kg/m^2 vs. BMI <25 kg/m^2	plasma
130b	Ortega/2013	↓		BMI ≥40 kg/m^2 vs. BMI <25 kg/m^2	plasma
140	Ortega/2013	↑		BMI ≥30 kg/m^2 vs. BMI <25 kg/m^2	plasma
142	Ortega/2013	↑		BMI ≥30 kg/m^2 vs. BMI <25 kg/m^2	plasma
221	Ortega/2013	↓		BMI ≥40 kg/m^2 vs. BMI <25 kg/m^2	plasma
222	Ortega/2013	↑		BMI ≥40 kg/m^2 vs. BMI <25 kg/m^2	plasma
423	Ortega/2013	↓		BMI ≥40 kg/m^2 vs. BMI <25 kg/m^2	plasma
520c	Ortega/2013	↓		BMI ≥40 kg/m^2 vs. BMI <25 kg/m^2	plasma
33b	Milagro/2013	↑		correlation with BMI	monocytes
3615	Milagro/2013	↓		correlation with BMI	monocytes
			Weight Loss		
16	Ortega/2013	↓		post- vs. pregastric bypass surgery	plasma
122	Ortega/2013	↓		post- vs. pregastric bypass surgery	plasma
130b	Ortega/2013	↑		post- vs. pregastric bypass surgery	plasma
140	Ortega/2013	↓		post- vs. pregastric bypass surgery	plasma
142	Ortega/2013	↓		post- vs. pregastric bypass surgery	plasma
221	Ortega/2013	↑		post- vs. pregastric bypass surgery	plasma
27b	Ortega/2013	↓		post- vs. pregastric bypass surgery	plasma
154	Ortega/2013	↓		post- vs. pregastric bypass surgery	plasma
183	Ortega/2013	↑		post- vs. pregastric bypass surgery	plasma
409	Ortega/2013	↓		post- vs. pregastric bypass surgery	plasma
433	Ortega/2013	↓		post- vs. pregastric bypass surgery	plasma
542	Ortega/2013	↑		post- vs. pregastric bypass surgery	plasma
223	Milagro/2013	↑		<5% weight loss after low-calorie diet	monocytes
224	Milagro/2013	↓		<5% weight loss after low-calorie diet	monocytes
376b	Milagro/2013	↓		<5% weight loss after low-calorie diet	monocytes
935	Milagro/2013	↑		<5% weight loss after low-calorie diet	monocytes
4772	Milagro/2013	↑		<5% weight loss after low-calorie diet	monocytes

following cycle ergometry (3) and a 90-day exercise training program (3), whereas decreased expression was reported in monocytes following cycle ergometry (29) and serum following a lower limb resistance exercise (31). Cyclin-dependent kinase inhibitor 1C is a validated target of miR-221 and predicted targets include estrogen receptor 1 (ESR1) and peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PPARGC1A) (19). MiR-652 was measured in serum and initially found to be decreased in individuals with low compared with high $\dot{V}_{O_{2max}}$ ($n = 24$), but no significant differences were identified in a validation set ($n = 76$) (8). There are fewer potentially relevant predicted targets of miR-652 (19). Expression of miR-660 associated with cycle ergometry was measured in two studies, and increased expression was observed in monocytes (29) but decreased expression in neutrophils (30). MiR-660 is predicted to target ESR1; IRS2; nuclear receptor subfamily 3, group C, member 1 (NR3C1); and PPARGC1A (19). MiR-146a was measured in five conditions across three studies. Four conditions (i.e., following cycle ergometry, at rest, and postexercise following a 90-day exercise training program, postmarathon) were associated with increased expression in plasma (3, 4), whereas resistance exercise was associated with decreased expression in serum (31). Predicted targets of miR-146a include IRS2, leptin, protein tyrosine phosphatase, nonreceptor type 1, solute carrier family 6 member 3 (SLC6A3), and steroid-5-alpha reductase, alpha polypeptide 2 (19). Finally, miR-20a and miR-20b were measured in two studies. Both isoforms were decreased in neutrophils following cycle ergometry (30), but increased expression was observed in plasma following a 90-day exercise training program (3). A validated mRNA target of both miR-20a and miR-20b is vascular endothelial growth factor A (VEGFA), which is expressed in endothelial cells, and a potentially relevant predicted target is ABCA1.

Risk of bias across studies. Three studies evaluated miRNA expression originating in blood cells (29, 30) or whole blood (33), while the remaining studies reporting on plasma or serum-based miRNA expression, these discrete sources of miRNAs likely reflecting different biological mechanisms. The study design was quasiexperimental in all but one of the studies reporting on miRNA associated with exercise. Five studies used an agnostic approach to detection of differentially expressed miRNAs, which decreases the risk of bias (8, 29–31, 33). However, among these studies, the specific microarray platform and number of miRNA targets included varied. For the remaining studies, an a priori approach for detection of miRNAs with some prior evidence for a role in response to exercise were quantitated. In some cases, individual miRNAs were quantitated and not found to be statistically significant and this was reported (3–5, 25, 40). We cannot determine whether nonsignificant miRNAs were not reported in the remainder of studies that used the a priori approach.

MiRNAs Associated With Diet/Weight Loss

Study characteristics. Two studies evaluated miRNAs associated with weight loss (Table 1) (22, 28). Milagro et al. (22) used next-generation sequencing to detect miRNAs followed

Table 4. *Plasma and serum microRNAs associated with immediate response to acute or prolonged exercise*

MiR	Author/Date	Direction	Exercise
		Acute	
1	Banzet/2013	↑	downhill vs. uphill treadmill
1	Mooren/2014	↑	treadmill test
let7d	Bye/2013	↑	treadmill test
21	Baggish/2011	↑	postacute cycle ergometry
21	Bye/2013	↑	treadmill test
21	Bye/2013	↑	treadmill test
29a	Bye/2013	↑	treadmill test
125a	Bye/2013	↑	treadmill test
125a	Bye/2013	↑	treadmill test
126	Uhlemann/2014	↑	four exercise conditions
133a	Banzet/2013	↑	downhill vs. uphill treadmill
133a	Mooren/2014	↑	treadmill test
133b	Banzet/2013	↑	downhill vs. uphill treadmill
146a	Baggish/2011	↑	postacute cycle ergometry
146a	Sawada/2013	↓	bench press, leg press
149*	Sawada/2013	↑	bench press, leg press
151	Bye/2013	↓	treadmill test
181b	Banzet/2013	↑	downhill vs. uphill treadmill
206	Mooren/2014	↑	treadmill test
208b	Banzet/2013	↑	downhill vs. uphill treadmill
210	Bye/2013	↑	treadmill test
210	Bye/2013	↑	treadmill test
210	Bye/2013	↑	treadmill test
214	Banzet/2013		downhill vs. uphill treadmill
221	Baggish/2011	↑	postacute cycle ergometry
221	Sawada/2013	↓	bench press, leg press
222	Bye/2013	↑	treadmill test
222	Baggish/2011	↑	postacute cycle ergometry
222	Baggish/2011	↑	postacute exercise after 90 days training
499-5p	Banzet/2013	↑	downhill vs. uphill treadmill
652	Bye/2013	↑	treadmill test
652	Bye/2013	↓	treadmill test
		Prolonged	
1	Baggish/2014	↑	marathon
1	Mooren/2014	↑	marathon
126	Baggish/2014	↑	marathon
133a	Baggish/2014	↑	marathon
133a	Mooren/2014	↑	marathon
146a	Baggish/2014	↑	marathon
206	Mooren/2014	↑	marathon
208a	Baggish/2014	↑	marathon
208b	Mooren/2014	↑	marathon
499	Mooren/2014	↑	marathon
499-5p	Baggish/2014	↑	marathon

by qPCR-based validation in peripheral blood mononuclear cells from women participating in a low-calorie diet intervention. A single miRNA (i.e., miR-148a) was used for normalization (22). Ortega et al. (28) used qPCR-based arrays and validation in independent samples to evaluate miRNAs in plasma in men and women who underwent bariatric surgery or a low-calorie diet intervention. A set of stably expressed miRNAs was selected for normalization (28). Both study samples were recruited in Spain.

Intervention characteristics. Milagro et al. (22) reported miRNA expression changes associated with an 8 wk caloric restriction diet (800–1,000 kcal/day) leading to ≥5% decrease in weight. Ortega et al. (28) reported miRNA expression changes associated with Roux en-Y gastric bypass surgery along with a 500–1,000 deficient kcal/day diet for 14 wk resulting in 17% decrease in weight. Neither study incorporated a physical activity component to the intervention. Both studies used acceptable approaches to miRNA quantitation (i.e., microarray followed by qPCR validation).

Risk of bias within studies. The study designs were quasi-experimental. Both studies followed a rigorous protocol for the dietary intervention; however, the exact caloric restriction recommendations differed. Given the length of the intervention, daily caloric intake was not objectively monitored during the study period. Pre- and postintervention weight was assessed by trained study personnel following a protocol.

Synthesis of miRNA findings. Findings from the two studies on miRNAs associated with weight loss are shown in Table 3. Ortega et al. (28) reported the cross-sectional association of miRNA expression with nonobese, obese, and morbidly obese individuals. In the screening and validation samples, 18 miRNAs (i.e., miR-15a, miR-21, miR-122, miR-125b, miR-126, miR-130b, miR-140-5p, miR-142-3p, miR-193a-5p, miR-221, miR-222, miR-423-5p, miR-486-5p, miR-520c-3p, miR-532-5p, miR-590-5p, miR-625, miR-636) were found to be differentially expressed in obese and morbidly obese compared with nonobese (28). To determine whether miRNA expression changes are associated with surgery-induced weight loss, a set of miRNAs that were aberrantly expressed in morbid obesity were evaluated before and after surgery. Ten miRNAs (i.e., miR-21, miR-122, miR-130b, miR-140-5p, miR-142-3p, miR-193a-5p, miR-221, miR-222, miR-423-5p, miR-483-5p) were associated with a 33% decrease in body mass index (BMI) following weight-loss surgery (28). Both studies evaluated miRNA expression associated with diet-induced weight loss. Ortega et al. (28) observed no change in miRNA expression after a 17% decrease in BMI in nine obese participants. Milagro et al. (22) found baseline expression of five miRNAs (i.e., miR-223, miR-224, miR-376b, miR-935, miR-4772) was associated with response (i.e., >5% weight loss) to a low-calorie (800–880 kcal/day) dietary intervention. Predicted targets of the miRNAs identified by Ortega et al. and Milagro et al. include many of the mRNAs targets also identified for miRNAs associated with exercise (i.e., ABCA1, APOA5, CNR1, ESR1, NR3C1, PPARGC1A, SLC2A1, VEGFA) as well as novel mRNAs with biological plausibility (e.g., Fas cell surface death receptor, glycogen synthase 1, interleukin 6 receptor).

Risk of bias across studies. One study evaluated miRNA from blood cells (22), whereas the other study detected miRNAs in plasma (28) (Table 3). As with the studies of miRNA expression associated with exercise, both studies of diet/weight loss and miRNA expression had a quasiexperimental design. Both studies used an agnostic approach to detection of differentially expressed miRNAs, which decreases the risk of bias. However, among these studies, the specific microarray platform and number of miRNA targets included varied.

DISCUSSION

Taken together, the studies reporting miRNA expression associated with exercise support two important future directions. The first is detection of biological pathways implicated with the physiological effects of various modalities of exercise. Several studies identified differential expression of *1*) miRNAs

Table 5. *Evaluation of bias in individual studies*

Author, Year Country	Research Question/Study Design	Possible Sources of Bias
	Exercise	
Radom-Aizik, 2010 United States	Does neutrophil miR expression change after brief exs?	quasiexperimental design
Baggish, 2011 United States	Does expression of circulating miRs change at rest and during exhaustive exs before and after aerobic training?	quasiexperimental design a priori approach to miR detection
Uhlemann, 2012 Germany	What are the effects of exs on miR-126 and endothelium?	quasiexperimental design a priori approach to miR detection
Aoi, 2013 Japan	Do circulating miRs change in response to acute and chronic exs?	quasiexperimental design a priori approach to miR detection
Banzet, 2013 Qatar	Are circulating muscle-specific miRs affected by exs and modality?	quasiexperimental design a priori approach to miR detection
Bye, 2013 Norway	Which miRs are associated with low vs. high $\dot{V}O_{2max}$?	cross-sectional design
Mooren, 2014 Germany	Are miRs associated with aerobic fitness performance capacity?	quasiexperimental design a priori approach to miR detection
Sawada, 2013 Japan	Are circulating miRs associated with resistance exs?	quasiexperimental design
Tonevitsky, 2013 Russia	How does miR change during exs?	quasiexperimental design
Zhou, 2013 China	Does physical activity influence metabolic syndrome risk by influencing miRs?	cross-sectional design subjective quantitation of exercise a priori approach to miR detection
Baggish, 2014 United States	Are plasma miRs uniquely modulated following sub-max-exs?	quasiexperimental design a priori approach to miR detection
Radom-Aizik, 2014 United States	Does brief exs alter monocyte miR expression?	quasiexperimental design
	Diet	
Milagro, 2013 Spain	Does miR expression characterize high (≥5% weight loss) vs. low responders to a diet intervention?	quasiexperimental design dietary intervention
Ortega, 2013 Spain	Are miRs associated with degree of obesity? Do miRs change after weight loss?	quasiexperimental design dietary intervention

associated with cardiorespiratory versus resistance training, *2*) miRNAs associated with acute versus prolonged exercise, and *3*) acute-phase changes in miRNA expression compared with long-term changes after extended exercise training programs. There are numerous known and predicted mRNA targets of the miRNAs that exhibited expression changes in exercise studies, including mRNAs implicated in insulin sensitivity, endothelial cell function, Type 2 diabetes obesity, cardiovascular disease, and cognitive impairment. Functional studies evaluating the relationships between specific miRNAs or clusters of related miRNAs and their mRNA targets can provide information about the physiological effects of various forms of exercise. The second and more clinically relevant implication is miRNAs as biomarkers for prediction of response to exercise interventions. While exercise is widely accepted to have numerous health benefits, there is variability in the intensity and duration of exercise required for improved health outcomes (7, 16). A related implication is monitoring of patient response to exercise interventions. Future studies can begin to investigate whether individual miRNAs and patterns of miRNA expression can be used to specify the type, duration, and intensity of exercise that will be most beneficial on a group or individual level.

Although the number of studies is limited, it appears that miRNAs are also associated with obesity and weight loss. In a cross-sectional analysis, miRNA expression differs between obese and nonobese individuals (28). It is not known whether differences between normal weight, overweight, and obese categories of individuals can also be detected. Significant changes in miRNA expression are associated with surgery-induced weight loss, but the evidence to support similar changes for diet-induced weight loss is weaker (28). However, the findings from Milagro et al. (22) support the possibility that miRNAs might be useful biomarkers for prediction of response to a dietary weight loss intervention. This is an important potential clinical application that merits further investigation. Similar to exercise, dietary approaches to weight loss show a high level of interindividual variability in response. Identification of circulating miRNAs associated with weight loss following dietary changes has the potential to individualize dietary recommendations for maximum response while minimizing patient burden. In addition, improved understanding of the physiological changes associated with obesity and weight loss through identification of which pathways are targeted by circulating miRNAs creates the possibility for new treatment targets. These include mRNAs implicated in Type 2 diabetes, obesity, and cardiovascular disease.

None of the included studies utilized the most rigorous randomized clinical trial study design. The majority of studies reported a quasiexperimental design used to assess changes in miRNA expression pre- and postexercise in the same healthy individual. For the studies of exercise, there was generally a high level of rigor in quantitating the exercise intervention. All but one study (40) reported quantitation of fitness using an objective method (e.g., $\dot{V}O_{2max}$), which decreases possible bias. Similarly, studies of diet and weight loss followed a well-designed protocol for administration of a dietary intervention. However, given the design of the study, precise quantitation of caloric intake was not feasible. For both exercise and diet, possible sources of bias across studies included varying selection of miRNA species measured. Many of the studies used the optimal agnostic approach for detection of differentially expressed miRNAs. Others used a less rigorous a priori approach

to investigate miRNAs with a high probability for differential expression.

There are several possible explanations for discrepant findings between studies of miRNA expression associated with exercise. The first is the type of exercise evaluated. Changes in miRNA expression represent up- or downregulation of specific genes or clusters of related genes in biologic pathways. The physiological changes associated with cardiorespiratory fitness differ from those associated with resistance training (15). For example, miR-146a was increased immediately following acute exercise but decreased following resistance training. Similarly, there are both acute-phase and long-term responses to exercise and exercise training (15). MiRNAs showing differential expression immediately following exercise may differ from those that are altered as a result of long-term training. MiR-20a and miR-20b both decreased following cycle ergometry but increased after completion of a 90-day exercise training program. Another explanation for discrepant findings is the source of the miRNAs. Circulating miRNAs can have a cellular origin, as in the case of the studies that evaluated monocytes (29), neutrophils (30), and whole blood (33). Expression of cellular miRNAs may represent active regulation of genes activated in those cells. By contrast, the origin of miRNAs found in plasma and serum is unknown (6). These miRNAs may reflect changes in regulation of genes associated with any tissue source. For example, during resistance training, skeletal muscle tissue is damaged and myocyte miRNAs may be sloughed into the circulation. Alternatively, miRNAs may be actively released in order to decrease intracellular regulation of specific mRNAs. The study sample sizes varied widely, and small studies may have limited generalizability to a larger and more heterogeneous population. Finally, miRNA data are normalized to avoid bias associated with differences in the amount of tissue used and efficiency of the quantitation method. Consensus on the best method for normalization of miRNA data before analysis has not been determined (9, 11, 21, 23, 26), and different approaches to normalization represent another possible explanation for differences in the direction of effect between studies. Use of a single miRNA normalizer is subject to bias compared with mathematically derived values (e.g., global means, geometric means) and selection of which individual miRNA is used as a normalizer varies across studies.

There are also differences in the findings from the two studies that evaluated miRNA expression associated with diet and weight loss. Namely, one study found no differences in miRNA expression associated with diet-induced weight loss, whereas the other reported five miRNAs changed after weight loss. There was also incomplete overlap in the specific miRNAs differentially expressed following surgery versus diet approaches to weight loss. These differences may be due in part to the study designs. While one study used a pre-/postdesign to determine changes in miRNAs associated with diet-induced weight loss (28), the other evaluated baseline miRNA expression associated with subsequent weight loss (22). Second, one study used a commercially available panel to screen known human miRNAs from plasma (28), while the other used a sequencing method to detect miRNAs in peripheral blood mononuclear cells (22). There may not have been complete overlap in the specific miRNAs screened. Furthermore, the data normalization approach differed between studies. Both studies had small sample sizes that may not be generalizable to larger, more heterogeneous populations.

To develop and validate the current knowledge base regarding circulating miRNAs and exercise and weight loss, numerous future studies are needed. Consensus on the optimal tissue sources and data normalization strategies is needed in order to develop a robust body of literature to support the possibility of clinical applications of miRNAs. Future studies need to continue to carefully describe the origin of miRNAs quantitated in order to understand whether differentially expressed miRNAs are derived directly from blood cells or may have originated from distal tissue sources (e.g., skeletal muscle). Building from consensus on methodological issues, studies are needed to validate which specific miRNAs respond to exercise, $\geq$5% weight loss, and low-calorie diet and determine the time-course of changes in circulating miRNA expression. Additional future directions include studies to determine whether miRNAs are useful clinical biomarkers to predict response to weight loss interventions that incorporate both exercise and diet studies to differentiate miRNA expression changes associated with exercise from those associated with weight loss. In conclusion, circulating miRNAs appear to have an association with exercise and change in response to weight loss. The evidence for an association between circulating microRNAs and low-calorie diet is weaker due to a small number of studies.

APPENDIX: PUBMED SEARCH TERMS

Studies reporting on relationships between miRNAs and exercise and/or physical activity: microRNAs[mh] OR microRNAs[tiab] OR microRNA[tiab] OR miRNA[tiab] OR miRNAs[tiab] OR epigenetic*[ti] OR epigenom*[ti] AND (exercise[mh] OR running[mh] OR walking[mh] OR physical fitness[mh] OR swimming[mh] OR gardening[mh] OR "physical education and training"[mh] OR dancing[mh] OR dance therapy[mh] OR sports[mh] OR yoga[mh] OR fitness centers[mh] OR recreation[mh:noexp] OR "play and playthings"[mh:noexp] OR motor activity[mh:noexp] OR exercise movement techniques[mh:noexp] OR tai ji[mh] OR aerobic*[tiab] OR endurance[tiab] OR exercis*[tiab] OR exertion*[tiab] OR fitness[tiab] OR gym[tiab] OR gyms[tiab] OR gymnasium*[tiab] OR jogging[tiab] OR moderate activ*[tiab] OR vigorous activ*[tiab] OR physical activ*[tiab] OR physical inactiv*[tiab] OR physical train*[tiab] OR pilates[tiab] OR recreation*[tiab] OR running[tiab] OR sedentary[tiab] OR sport*[tiab] OR swim*[tiab] OR walk*[tiab] OR yoga[tiab] OR bicycl*[tiab] OR bike[tiab] OR bikes[tiab] OR biking[tiab] OR rollerblad*[tiab] OR skating[tiab] OR "strength training"[tiab] OR "resilience training"[tiab] OR weight lift*[tiab]) AND english[la] NOT (animals[mh] NOT humans[mh]) NOT (animal*[ti] OR bovine[ti] OR goat*[ti] OR mammal*[ti] OR mice[ti] OR mouse[ti] OR rat[ti] OR rats[ti] OR porcine[ti] OR pig[ti] OR pigs[ti] OR cell line*[ti] OR nonhuman*[ti] OR zebrafish[ti] NOT human*[ti]) NOT (in vitro NOT in vivo) NOT (cancer OR oncology OR metastatic OR metastasis OR tumor OR neoplasm OR HEPG* OR 3T3* OR embryon*).

Studies reporting on relationships between miRs and diet and/or weight loss: microRNAs[mh] OR microRNAs[tiab] OR microRNA [tiab] OR miRNA[tiab] OR miRNAs[tiab] OR epigenetic*[ti] OR epigenom*[ti] AND (body mass index[mh] OR body weight changes[mh:noexp] OR obesity[mh] OR overweight[mh] OR weight loss[mh:noexp] OR weight reduction programs[mh] OR weight change*[tiab] OR weight loss*[tiab] OR weight reduc*[tiab] OR overweight[tiab] OR obesity[tiab] OR obese[tiab] OR obesogen*[tiab] OR diet therapy[mh] OR body mass index*[tiab] OR body mass indic*[tiab] OR diet[tw] OR diets[tiab] OR dietary[tiab] OR dieting[tiab]) AND english[la] NOT (animals[mh] NOT humans[mh]) NOT (animal*[ti] OR bovine[ti] OR goat*[ti] OR mammal*[ti] OR mice[ti] OR mouse[ti] OR rat[ti] OR rats[ti] OR porcine[ti] OR pig[ti] OR pigs[ti] OR cell line*[ti]

OR nonhuman*[ti] OR zebrafish[ti] NOT human*[ti]) NOT (in vitro NOT in vivo) NOT (cancer OR oncology OR metastatic OR metastasis OR tumor OR neoplasm OR HEPG* OR 3T3* OR embryon*).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: E.F. and Y.F. conception and design of research; E.F. analyzed data; E.F. and Y.F. interpreted results of experiments; E.F. prepared figures; E.F. drafted manuscript; E.F. edited and revised manuscript; E.F., G.Y.W., and Y.F. approved final version of manuscript; G.Y.W. performed experiments.

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