Exercise Immunology Meets MiRNAs

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ABSTRACT

A large body of evidence indicates modified expression of protein-coding genes in response to different kinds of physical activity. Recent years have exposed another level of regulation of cellular processes mediated by non-coding RNAs. MicroRNAs (miRNAs) are one of the largest families of non-coding RNAs. MiRNAs mediate post-transcriptional regulation of gene expression. The amount of data supporting the key role of miRNAs in the adaptation of the immune and other body systems to exercise steadily grows. MiRNAs change their expression profiles after exercise and seem to be involved in regulation of exercise-responsive genes in immune and other cell types. Here we discuss existing data and future directions in the field.

Key words: exercise, immunology, miRNA, non-coding RNA, inflammation

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INTRODUCTION

The adaptation to exercise affects virtually all body systems. The immune system is among the systems most responsive to exercise. The regulation of a plethora of physiological processes is now known to be mediated by non-coding RNAs, in particular, miRNAs. These small ~22 nt RNAs are involved in post-transcriptional regulation of gene expression. Estimates are, that more than half of the human protein-coding genes are under miRNA regulation (40), which means that miRNAs are involved in nearly all major mechanisms controlling body processes.

A large amount of recent data demonstrates that miRNAs are essential for the normal activity and development of the immune system (reviewed in (15, 69, 83, 90, 119); see below). Likewise, rapidly increasing evidence indicates a role of miRNAs in the function of skeletal muscle, cardiovascular system, and other body systems. These findings promoted the research on the role of miRNAs in the adaptation to exercise. The field is young: 69 out of 76 publications on "miRNAs and exercise" found in Pubmed in August 2013 were published since 2010. However, the data obtained in this recently started trend suggest that miRNAs play a key role in mechanisms controlling the adaptation to exercise. Exercise rapidly changes the cellular levels of many miRNAs in the immune system, skeletal muscle, and cardiovascular system. These changes modulate the expression of target genes, driving short-term and long-term adaptations.

This review discusses current data on the role of miRNAs in the body adaptation to exercise with particular attention to the immune system and miRNAs in the bloodstream since they are likely involved in post-transcriptional regulation of gene expression in the cells of the immune system.

MiRNA Biogenesis and Function

MiRNAs are key factors of gene expression that regulate a variety of processes including development, cell proliferation, differentiation, apoptosis, and different metabolic pathways. The number of described miRNAs steadily grows, and the MiRBase currently includes more than 2500 human miRNAs (release 20;(58)). MiRNA genes are located in introns of protein-coding genes, introns and exons of non-coding RNA genes, and occasionally in exons of protein-coding genes (19, 38). They are transcribed by RNA polymerase II (67, 137), although certain miRNA genes downstream of Alu repeats are transcribed by RNA polymerase III (17). The primary transcripts (called pri-miRNAs) contain a ~70 nt hairpin (called pre-miRNA), which includes the miRNA sequence. Pri-miRNA is recognized by the 'microprocessor'complex composed of RNase III Drosha and RNA-binding protein DGCR8, which processes it into pre-miRNA (38, 66, 92). Certain pre-miRNAs localized within short introns are processed by splicing machinery, where the spliceosome and debranching enzyme function as the microprocessor. Such miRNAs are known as miRtrons (107, 136). Surprisingly, miRNAs can be derived from other small RNAs, e.g. tRNAs, Y RNAs, and small nucleolar RNAs (snoRNAs) (30, 78, 97, 115). The transcription and processing of miRNAs are controlled by a variety of cofactors (38, 59).

Exportin 5 mediates pre-miRNA export to the cytoplasm (145), where they are further processed by another RNase III, Dicer, in a complex with the RNA-

binding protein TRBP. The resulting ~22 nt double-stranded molecules associate with one of the Ago family proteins, the main component of the RNA-induced silencing complex (RISC). One of the duplex strands is degraded and the mature miRNA in the RISC complex can interact with the complementary target mRNA (38). Such interaction represses the translation or initiates mRNA deadenylation and degradation (47). MiRNAs also have non-canonical functions: upregulation of mRNA translation (44, 93, 130), transcriptional gene silencing (13, 53), and even signal transduction through the binding to Toll-like receptors, which makes miRNAs a kind of hormones ((31, 32, 68), see below, c-miRNAs.). The biogenesis, functions, and regulation of miRNA activity have been reviewed in detail elsewhere (38, 47, 59, 92, 94).

Short length and imperfect complementarity with mRNA allow each miRNA to have hundreds of targets (10). MiRNA binding sites can be found in the 3'-UTR and occasionally in the 5'-UTR (65, 77, 93) as well as in the coding regions of mRNAs (39). An mRNA can have several binding sites for the same or different miRNAs, which allows complex translational regulation (40).

The majority of miRNAs are expressed in a broad range of tissues; however, some miRNAs demonstrate pronounced tissue-specific profiles (62). At the same time, each cell type has a specific miRNA expression profile. For instance, different leukocyte lineages demonstrate different miRNA expression profiles (11, 63, 98, 134); moreover, several miRNAs specific for the skeletal muscle are even considered as a special subset of miRNAs termed 'myomiRs' (54).

MiRNAs can be found not only in the cell but also in various body fluids such as plasma/serum, milk, saliva, and urine. Such miRNAs proved resistant to nucleases and were called circulating miRNAs (c-miRNAs). They have been identified in apoptotic bodies, shedding vesicles, and exosomes as well as in complex with high-density lipoprotein (57, 126). However, a big part of c-miRNAs can be found in the vesicle-free fraction in complex with Ago proteins (7, 127). There is conflicting information on the proportion of free versus vesicle-bound miRNAs (41, 127). The mechanisms of c-miRNAs emergence and their possible physiological role still remain matters of speculation (16). *In vitro* experiments demonstrated that exosomes secreted by one cell type can be absorbed by other cell types, where the exosomal miRNAs can modulate gene expression (126). This proposes c-miRNAs as a new type of messengers that allow distant cells to communicate in the body. This is particularly significant for immune cells since most of them continuously interact with body fluids.

C-miRNAs proved to be good markers for many pathologies and physiological conditions (16). In particular, plasma levels of certain c-miRNAs change after physical activity (8, 9, 110, 128). This is of both, fundamental and practical interest and can be used for functional assessment and injury diagnosis of organs after exercise of different intensities (see below, c-miRNAs). Thus, c-miRNA monitoring can be widely used in sports medicine.

MiRNAs and Immune System

The amount of data on the role of miRNAs in immune functions rapidly grows (1199 out of 1549 publications on "miRNAs and immune system" found in Pubmed in August 2013 were published since 2010). To date, the function of dozens of miRNAs has been determined and their key role in the regulation of

immune system development and function has been established. Below we describe the major progress in the field, while the interested reader can consult specific reviews (15, 37, 69, 83, 90, 119).

The critical importance of miRNAs for the immune function was demonstrated in a series of works in which miRNA biogenesis was affected. For instance, a specific deletion of Dicer in a T-cell lineage impaired T cell development and T helper differentiation and induced autoimmune diseases (71, 85, 148). The ablation of Dicer in early B cell progenitors suppressed the transition from pro-B to pre-B cells (56). Specific deletion of Dicer in activated B cells induced multiple abnormalities in the immune response to pathogen and impaired germinal center B-cell formation (141). Natural killer (NK) cells with Dicer deletion demonstrated impaired maturation, survival, turnover, and other defects (14, 120). Thus, miRNAs are required for normal development and function of the immune system.

To date, miRNA expression profiles are available for many cell types of innate and adaptive immune system: monocytes/macrophages, dendritic cells, neutrophils, eosinophils, NK cells, and different T and B cell types (2, 11, 33, 34, 63, 74, 82, 98, 106) Out of more than 2500 described human miRNAs (58), 150-600 species can be usually identified in each cell type of the immune system, 20 or 30 of which are the most abundant (11, 74, 82, 135). Immune cells have specific miRNA expression profiles, which can change during cellular development (11, 82, 106); and the concentrations of the same miRNA species at different differentiation stages can vary by a factor of 1000 (55). Interestingly, new miRNAs are still being described in the immune system (11, 34, 98).

Simultaneously with the large-scale transcriptome studies, individual miRNA species are being actively explored with focus on their function in specific cell types of the immune system (reviewed in (15, 72, 117)). MiRNAs proved to be involved in a variety of cellular pathways so that they seem to mediate all significant events in immune cells. For instance, miRNAs control the differentiation of naive T-helper cells into Th1, Th2, Th17, T-regulatory, and follicular helper T cells (T_{fh} cells) (114). In particular, the normal differentiation of T_{fh} cells requires the miRNA cluster miR-17-92 (12, 51). MiRNAs also mediate the maintenance of naive T-helper cells in undifferentiated state. MiR-125b is more abundant in naive T-helper cells than in other T cells and suppresses the expression of genes underlying the differentiation of naive T-helper cells (namely, interferon-γ, interleukin (IL)-2 receptor-β, IL-10 receptor-α, and transcriptional repressor Blimp-1), and thus maintains the undifferentiated state of these cells (106).

Interestingly, miRNA processing can change as the immune cells develop. For instance, the sequences of mature miRNAs can be shifted by one or two nucleotides at different stages of T cell development. In particular, the nucleotide sequence of miR-17 expressed in DN3 thymocytes corresponds to the canonical one, while the same miRNA expressed in DN4 and DP thymocytes is shifted 3' by one nucleotide. Cells of all other stages expressed miR-17 that was shifted 3' by two nucleotides. These shifts indicate different processing of pre-miR-17 in the course of T cell development (55). Since the seed region (the region completely complementary to the mRNA target nucleotides 2 to 7/8) (10) is short, even a single-nucleotide shift changes two thirds of the predicted miR-17 targets and the seed sequence becomes identical to that of miR-302a or miR-106a (55).

Thus, alternative miRNA processing during T cell development contributes extra variation to the regulation of miRNA targets.

The range of biochemical pathways controlled by miRNAs in immune cells is particularly wide. For instance, miRNAs control antigen presentation (e.g., miR-148/152 (75)), T-cell receptor signaling (e.g., miR-181a (70)), Toll-like receptor signaling (e.g., let-7e (4)) and cytokine production (e.g., miR-146a (43)). MiRNAs are required for normal proliferation of activated lymphocytes; for instance, miR-182 promotes clonal expansion of activated T helper cells (118) and miR-181b decreases excessive DNA damage accompanying somatic hypermutation and class switch recombination in activated B cells, thus preventing their malignant transformation (25). MiRNAs are involved in both inflammatory and anti-inflammatory responses (90).

Notably, cellular miRNAs can have an effect on the viral life cycle through the regulation of viral genome replication, while viral miRNAs in turn can have an effect on the host cell (95). Different immune abnormalities, in particular, malignant and autoimmune diseases, demonstrate altered miRNA profiles, which can point to the contribution of miRNAs to the development of these diseases (26, 96).

MiRNAs and Response of Peripheral Blood Leukocytes to Exercise

Publications from two groups have demonstrated that acute exercise changes the expression profile of many miRNAs in circulating leukocytes (100, 101, 102, 125). In a series of studies Dr. Dan Cooper and coworkers used miRNA microarrays to study the changes in miRNA expression in untrained subjects immediately after brief bouts of heavy exercise in circulating neutrophils (102), peripheral blood mononuclear cells (PMBCs: T, B, and NK cells and monocytes) (101), and circulating NK cells alone (100). In all cases, expression profiles changed for 20-40 miRNAs (Table S1). Considering that leukocytes express several hundreds of miRNA species (11, 74, 82, 135), this finding supports the specificity of the observed response. Note that most of these miRNAs are not among the most abundant species in neutrophils and PMBCs (88, 131, 134, 135). The authors also linked their miRNA data to corresponding exercise-induced mRNAs by identifying potential mRNA targets for each miRNA and selecting those which were also changed. Resulting biochemical pathways are considered candidates for being under the control of exercise-induced miRNAs. In neutrophils they include ubiquitin-mediated proteolysis, Jak-STAT signaling, and Hedgehog signaling. All these pathways mediate inflammatory response (102). Twelve pathways have been identified in PBMCs including TGF-β signaling and MAPK signaling (101). Exercise-activated pathways in NK cells are predominantly associated with cancer and cell communication: p53 signaling, melanoma, glioma, and prostate cancer, as well as adherens junction and focal adhesion (100).

Unfortunately, parallel expression data on miRNA and mRNA were only available in the NK cell study, while the data for neutrophils and PBMCs were obtained in separate experiments, which can compromise the authenticity of the identified miRNA-mRNA pairs. Moreover, different experimental procedures (ten 2-minute bouts of cycle ergometer exercise for miRNA assay and 30 minutes of constant cycle ergometer exercise for mRNA assay) were used in the study on neutrophils, and the experiments with PBMCs were carried out on individuals of

different age (men with a mean age of 22 years and late pubertal boys with a mean age of 17 years). Nevertheless, in sum, these findings are at least compatible with the assumption that many exercise-induced mRNA changes are under the control of miRNAs.

Attempting to increase the depth of understanding, our group analyzed whole blood samples taken from highly trained athletes after a 30-minute treadmill test at 80% maximal oxygen uptake (moderate test, MT) (125). The whole blood approach allows fast, precise timing and minimizes artifacts. Samples were analyzed for miRNA and mRNA before and immediately after exercise, as well as 30 minutes, and 60 minutes into recovery. This allowed us to identify four dynamically regulated networks with four differentially expressed miRNAs and their validated mRNA targets. All of them displayed anti-correlated expression profiles for both, immediate post-exercise time point and recovery period. These miRNAs included miR-21 and its targets TGFBR3, PDGFD, and PPM1L; miR-24-2 and its targets MYC and KCNJ2; miR-27a and its target ST3GAL6; as well as miR-181a and its targets ROPN1L and SLC37A3 (125). All target genes are involved in processes highly relevant to exercise response including immune function, apoptosis, membrane traffic of proteins, and transcription regulation. These data are in good accordance with the findings by Cooper's group and support the assumption that miRNAs regulate key pathways of the immune response to exercise.

The number of differentially expressed miRNAs was higher in the studies by Cooper's group (several dozens) as compared to our study (five) (125). Possible reasons for this discrepancy are numerous, ranging from different numbers of subjects, different exercise procedures, different microarray systems, different cell populations and work up procedures to different fitness level of subjects. Indeed, the microarray used by our group contained four times less miRNAs, but, nevertheless, did contain two thirds of those identified by Cooper's group. Some other reasons also need to be discussed in more details.

Use of whole blood may mask mild expression changes in minor leukocyte populations like NK cells, and can also cause changes through cellular shifts, including subpopulations (125). On the other hand, analysis in isolated cell populations (100, 101, 102) is influenced by manipulation and time delay inherent to the sorting procedure. While this may still suggest that true exercise-induced miRNA changes do exist, we have to acknowledge that even within isolated cell populations, shifts in subpopulations do occur (also discussed in (100, 101, 102)) and may be responsible for a substantial part of the expression changes observed. At present this question cannot be unequivocally answered. We do however believe that the dynamic regulation of mRNA-miRNA pairs as shown in (125) would be hard to explain by a cellular shift. But finally, no matter, if shifts in leukocyte populations / subpopulations are involved or not, miRNA (and of course mRNA) expression data do mirror the actual activation status of the peripheral blood and therefore deliver valuable biological information.

While exercise procedures differed between the two groups (100, 101, 102, 125), the total duration (30 minutes) and intensity (80 vs. 76-77% of VO_2 max) were somewhat comparable. However, the highly trained athletes investigated by our group did not cross the anaerobic threshold while the less trained probands investigated by Cooper's group, did. Crossing the individual anaerobic threshold

(IAT) is associated with major physical stress, which might require the induction of more and different miRNA species than work just below the threshold. Trying to get more pertinent information on this question we decided to perform an additional ramp test to exhaustion (RTE, as described in (109); duration ~15 minutes/including 4-5 minutes above IAT) with the same athletes that had performed the 30-minute moderate test (MT) (125). Whole blood samples collected before and immediately after the test were analyzed for miRNA expression (as described in (109)). Results are given in Table 1 together with results from the MT (recalculated for 2 time points, disregarding recovery, in order to create optimal comparability between our data sets and the results described by Cooper`s group).

Table 1. MiRNAs differentially expressed in whole blood leukocytes before and after exercise tests. MiRNA species with a 1.5-fold or greater expression difference are boldfaced

MiRNA name and fold change[1]	Experiment participants	Exercise type	Time points of blood sampling
mir-24 ↑2.0 **mir-27a ↑1.5** **mir-181b ↑1.5** mir-23a ↑1.3	8 highly trained male athletes with a mean age of 21.7±2.6 years	Moderate test: 30 min treadmill running at 80% VO2 max (as described in (109, 125).	Immediately before and after exercise test
mir-181a ↑1.5 **mir-181b ↑1.5** **mir-101 ↓1.5** mir-142 ↓1.4 mir-29a ↓1.4 mir-124 ↓1.3 mir-29c ↓1.3 mir-223 ↓1.2 mir-30d ↑1.2 mir-130b ↓1.2		Ramp test to exhaustion: 15 min treadmill test with an incremental step protocol until exhaustion (as described in (109)).	

[1] Only the miRNA genes with significant differential expression are listed (false discovery rate < 0.05). The miRNA set for the MT is not identical to that published previously (125) since the samples collected 30 and 60 min after exercise were excluded.

Indeed, expression of a greater number of miRNA species was altered in RTE than in MT. To our knowledge, this is the first report that miRNAs in peripheral leukocytes can change in such a short time following any external stimulus. Numbers of expressed miRNAs in RTE were, however not excessive and clearly less than those identified in the studies by Cooper's group (which also included work above IAT). We think that, apart from crossing the anaerobic threshold, the mere fact that our group investigated highly trained athletes may also make a difference. Trained athletes are known to be able to regulate their body functions more efficiently than non-athletes, be it above or below IAT. They may therefore generally need less miRNAs to be induced than non-athletes.

One more observation we made when analyzing our results may be of interest: three of the four miRNAs found elevated after MT, namely, mir-23a, mir-24, and mir-27a, belong to the same cluster. They are processed from a common precursor and often coregulated (22). The members of this cluster are involved in a plethora of biological processes including haematopoiesis, angiogenesis, cell proliferation, and cardiac hypertrophy (reviewed in (22)). Analysis of their target gene set suggests the involvement of this cluster in several immune-related pathways, e.g. T-cell receptor signaling, and TGF-beta pathways (22). It will be very interesting to unveil the function of this cluster in directing the exercise response in peripheral blood leukocytes.

The sets of differentially expressed miRNAs differ between leukocyte types; however, certain miRNAs proved common for two or three sets (Table 2). Notice that the direction of changes can be opposite in some cases. For instance, miR-223 level increased in neutrophils but decreased in NK cells (Table 2). This could indicate different interpretation of the same external signal by different cell types either upregulating or downregulating the same gene; however, as in other cases, shifts in subpopulations cannot be excluded with certainty.

It is of interest that quite some exercise-induced miRNAs identified in leukocytes demonstrated a similar response in the skeletal muscle, heart, or plasma(Table 2). Moreover, only a few out of hundreds of miRNA species coincided in all microarray studies, which might indicate a critical role for them in adaptation to exercise. For instance, Keller et al. identified 21 miRNA species expression of which changed after exercise in muscle (52), and seven of them demonstrated differential expression in leukocytes, too (Table 2). Although the direction of changes was different in some cases and the sets of their targets likely differ in leukocytes and muscle, these miRNAs can be assumed to be involved in the universal adaptive response to exercise.

Data on the role of individual miRNAs in specific immune functions are increasing but still vastly fragmentary. Thus, a comprehensive discussion of possible roles of indentified miRNAs is difficult. Still, miRNAs of the miR-181 family - miR181a and miR181b - may be worthwhile discussing in more detail, since their differential expression was observed in all our tests in athletes as well as in two out of three tests in non-athletes (101, 102), (Table 2).

Since miR-181 expression was affected in different leukocyte types irrespective of the exercise intensity and training level (Table 2), its involvement in the regulation of some fundamental adaptive changes in the immune system seems likely. MiR-181a suppresses the inflammatory response induced by oxidized low-density lipoprotein in dendritic cells (139), and miR-181b suppresses the NF-kappa B-mediated inflammatory response in endothelial cells *in vivo* (121). On the other hand, Xie et al. demonstrated increased levels of miR-181a in whole blood leukocytes during the early inflammatory response, and proposed that miR-181a upregulation can compensatorily limit hyperinflammatory reactions (140). In a similar way, exercise-induced miR-181 may be interpreted as compensatory anti-inflammatory reaction to primary inflammatory stimuli caused by exercise. Indeed, exercise-induced immune reactions used to be viewed as primary inflammatory reactions followed by anti-inflammatory counter-reactions. This has recently been challenged (oral communication, ISEI meeting, Newcastle, Australia, 2013, and Asghar Abbasi, *Brain Behavior and Immunity*, in press).

Table 2. Overview of miRNAs changed in two or more cell types after exercise. Human, mouse, and rat miRNAs are prefixed with hsa-, mmu-, and rno-, respectively, following the nomenclature adopted in miRBase (58). WBL - whole blood leukocytes, FC - fold change.

MiRNA	Leukocytes, FC				Muscle	C-miRNA
	WBL	Neutr.[1]	PBMC[2]	NK[3]	FC, Muscle name, Ref.	FC, Ref.
hsa-miR-7			↑1.4	↑2.2		
hsa-miR-15a hsa-miR-15b			↑1.3		↓1.6 (Vastus lat.;(52))	
hsa-mir-16 rno-mir-16		↓1.23			↓1.36 (Soleus;(36))	
hsa-miR-21* hsa-miR-21 mmu-mir-21			↑1.5		↑2.3 (Gastrocn.;(5))	↑2.6(8)
hsa-mir-23a mmu-mir-23	↑1.3 MT				↓6.2 (Quadriceps;(108))	
hsa-miR-26a hsa-miR-26b			↑1.2		↓1.8 (Vastus lat.;(24)) ↓1.6 (Vastus lat.;(52))	
hsa-miR-27a rno-mir-27a	↑1.5 MT				↑2.0 (Heart;(35))	
hsa-miR-29a rno-miR-29a hsa-miR-29b hsa-miR-29c rno-mir-29c	↓1.4 RTE ↓1.3 RTE			↑2.0 ↑3.5 ↑2.3	↓2.0 (Vastus lat.;(24)) ↑1.5 (Heart;(116)) ↓1.6 (Vastus lat.;(52)) ↑2.18 (Heart;(116))	
hsa-miR-30d hsa-miR-30e	↑1.2 RTE			↑2.1		
hsa-mir-101	↓1.5 RTE				↓2.0 (Vastus lat.;(52))	
hsa-miR-107 mmu-mir-107		↓1.26			↑1.56 (Quadriceps;(108))	
hsa-miR-125a		↑1.22	↓1.3		↑1.6 (Vastus lat.;(52))	
hsa-miR-126		↓1.53	↓1.3	↓3.2		↑4.0(128)
hsa-miR-130a hsa-mir-130b	↓1.2 RTE	↓1.61	↓1.2	↓2.9		
hsa-miR-142	↓1.4 RTE			↑2.5		
hsa-miR-145		↑1.22	↓1.3			
hsa-miR-151-5p		↓1.60	↓1.3	↓2.8		
hsa-miR-181a hsa-miR-181a2* hsa-mir-181b mmu-mir-181	↑1.5 RTE ↑1.5MT,RTE	↑1.64	↑1.4 ↑2.0 ↑1.7		↑1.37 (Quadriceps;(108))	↑1.5(9)
hsa-miR-199a-3p hsa-miR-199a-5p			↓1.3 ↓1.3	↓3.1 ↓2.9		
hsa-miR-221			↓1.2	↓2.1		↑5.8(8)
hsa-miR-223	↓1.2 RTE	↑1.29		↓2.9		
hsa-miR-338			↑1.4	↑2.2	↓1.6 (Vastus lat.; (52))	
hsa-miR-363		↓1.34	↑1.5	↑2.1		
hsa-mir-451			↓3.8		↓4.0 (Vastus lat.; (52)) ↑4.0 (Vastus lat.; (24))	
hsa-miR-652			↓1.2	↓2.2		

[1]according to (102); [2]according to (101); [3]according to (100)

It was proposed that the reaction to exercise may be more of a direct, preemptive anti-inflammatory reaction induced by IL-6 or other mediators, including miRNAs, and miR-181 is certainly a candidate for playing a critical role in such an immediate anti-inflammatory response to exercise.

MiR-181 expression also increased in muscle after acute endurance exercise (108) and during regeneration (86). MiR-181 is known to repress the repressor (Hox-A11) of MyoD and thus mediates myoblast differentiation and muscle regeneration (86). Finally, miR-181 also shows increased plasma levels following acute exercise (9). Together with its protective, anti-inflammatory impact all this prompts us to propose that miR-181 may have a central, multiple role in the adaptation to exercise.

MiRNA and Skeletal Muscle Response to Exercise

Muscle and vascular function are not the focus of this review. Nevertheless, we like to highlight some current facts and findings in this field, honouring the fact that all exercise-related gene expression probably starts in the muscle.

Although more than 150 miRNA species are expressed in the muscle (81, 122), up to 25% of muscle miRNA population correspond to just a few muscle-specific miRNAs (81) (miR-1, miR-133, miR-206, and miR-499), collectively called myomiRs; myomiRs also include less abundant miR-208 and miR-486 (54). Thus, most studies in the field are focused on the identification of changes in the expression of myomiRs (largely miR-1, miR-133, and miR-206) after various forms of exercise. Indeed, adaptation to exercise proved to affect myomiR levels. Somewhat unexpectedly, changes were, however, only moderate in general and even undetectable in some of the studies (Table S2). There is a trend towards increased levels of miR-1, and miR-133 during acute endurance exercise (89, 108) and decreased levels with chronic endurance exercise (52, 89) or with resistance exercise (27, 80, 84), (Table S2). A central function of myomiRs, in particular miR-1, is obviously long term regulation / adaptation of protein synthesis and muscle size. Validated targets of miR-1 include components of the insulin-like growth factor 1(IGF1) pathway (29), and the decrease of miR-1 with chronic endurance exercise and with both, acute and chronic resistance exercise, is therefore a plausible adaptation to the increased need for protein synthesis and muscle growth/regeneration. As mentioned above, significant changes of myomiRs were not seen in all experiments. In a study with high responders and low responders to resistance exercise (low muscle mass gain), no significant changes in expression of myomiRs was observed. Instead, some non-myomiRs (mir-451↑, miR-378↓, miR-29a↓, and miR-26a↓) were changed in low responders only, possibly in a compensatory effort (24).

Age-related loss of muscle function is accompanied by hampered transition of the miR1 precursor pri-miR-1 to miR-1 and by a failure to modify mature miR1 expression in response to a single bout of resistance exercise in elderly men (27). On the other hand, decreased miR1 expression was demonstrated in elderly men following prolonged resistance training (84). Thus, elderly men may just need more time for adaptive changes to occur. In any case, age-related changes can at least partly be relieved by training.

MyomiRs also control the expression of key myogenic transcription factors and regulators such as Pax3, and Pax7 during satellite cell proliferation and dif-

ferentiation (reviewed in (147)), and injection of myomiRs into injured rat muscle could accelerate regeneration (87). Thus myomiRs seem to have control functions in both, adaptation to exercise and regeneration of muscle.

A row of studies has investigated the consequences of enforced physical inactivity, using different experimental settings. MyomiRs miR-1, miR-133, miR206 (Table S2), mir208b and miR499 and some others were down-regulated in response to inactivity (3, 49, 81, 104), and miR208b and miR499 seem to be involved in the slow (type 1) to fast (type 2) fibre switch which accompanies muscular atrophy (81). Altogether results in this field are inconsistent and sometimes contradictory, likely due to different experimental settings. Spontaneous reinnervation following denervation and replacement of muscle fibres by fibroblasts are some of the problems encountered in those experiments.

Intriguingly, compared to myomiRs, a number of non-myomiR miRNAs showed much more pronounced differential expression in response to exercise (Table S3). Identified reactive miRNAs include both, species of low (e.g. miR-183 and miR-189 (113)) and high (e.g. miR-23 (113)) abundance. Since they are not muscle-specific, they seem to control vital functions which muscle cells share with some or all other cells. Low-level transcripts are fairly often involved in crucial processes like cell division. Thus this might also be the case for exercise modulated low-level transcripts. On the other hand, non-muscle-specific high abundance miRNAs may well be involved in fast adaptive metabolic reactions to contractile activity. Adaptation of miRNA expression to chronic endurance or resistance training shares some changes and differs in others. Three out of four miRNAs differentially expressed after resistance training, namely miR-26, miR-29, and miR-451 (24) were also changed after endurance training (52). While miR-26 and miR-29 decreased in both, miR-451 was increased after resistance and decreased after endurance exercise. Thus, there are common exercise-related and specific exercise type-related regulations of miR expression, which, together with myomiRs, can form the necessary network to govern adaptation and regeneration.

Exploration of the adaptive significance of individual miRNAs is still in its infancy. Existing data point to a role of miR-696, miR-23 and miR-494 in mitochondrial biogenesis through different pathways (5, 108, 144). PCG1 (Peroxisome proliferator-activated receptor-gamma coactivator-1 alpha) is a critical factor of mitochondrial biogenesis and a predicted target for miR-696 and miR-23 (5, 108). Its regulation by miR-696 was also confirmed by hyperexpression experiments (5). Transcription factors TFA and Foxj3 which also control mitochondrial amplification are validated targets for miR-494 (144).

When looking closer to those miRNAs which show high or very high expression changes due to exercise, we realized that they are often species-specific, taxon-specific or are synthesized via non-canonical ways (unpublished observation). MiR-616 seems to be human/primate-specific while miR-680, miR-696, miR-705, and miR-709 are mouse-specific (MirBase; http://www.mirbase.org, Table S3). MiR-720 is presumably synthesized from a tRNA (111). Recent data indicate that tRNAs can give rise to shorter miRNA-like molecules which are involved in gene silencing and most actively synthesized during stress (115). MiR-680 is encoded in the LTR of mouse retrotransposon ERVB4 (according to the annotation of the genome browser at the University of California,

http://genome.ucsc.edu). The significance of these findings is not clear at present. Possibly it has to do with the need for fast action which is associated with physical activity. It is also possible that the small size of miRNAs makes them a perfect tool of nature to gain species-specific adaptation to exercise out of a pathway which is common to all mammals. In any case, a significant fraction of exercise-associated miRNA expression changes is observed in species-specific or non-canonically synthesized miRNAs.

Chronic exercise is associated with decreased blood pressure, increased capillary numbers and physiological hypertrophy of the heart. Studies on the role of miRNAs in these adaptational processes have just started. All were conducted in rats submitted to 10 weeks of moderate training (swimming) (23, 35, 36, 116). First results indicate massive involvement of miRs in cardiac hypertrophy. 87 of 349 miRs studied were altered, among these, miR-1 and mir-133 (decreased) and members of the miR-29 family (increased) (116). These changes promote muscle fibre growth (myomiRs) and decreased collagen synthesis (miR-29). Increases in miR-27 and miR-126 and decrease in miR-16 may have critical roles in angiogenesis by targeting negative regulators of vascular endothelial growth factor (VEGF) pathway (miR126), and by reducing miR-16 dependent inhibition of angiogenesis (VEGF, its receptor VEGFR2 and FGF receptor 1 are all validated targets of miR16) (23, 36). MiR-27 probably targets angiotensin converting enzyme (ACE), and this may lead to decreased blood pressure (35). Finally, miR-27 also regulates the inflammatory response (20). Altogether, the results in this field need confirmation to prove that they are indeed an adequate mirror of vascularization.

MiRNAs Might Contribute to the Beneficial Effect of Exercise in Different Diseases

Exercise is known to be beneficial in a plethora of diseases. Recent data increasingly indicate the involvement of miRNAs in the beneficial effect of exercise. For instance, increased expression of miR-21 and decreased expression of miR-15a was observed in rats with spinal cord injury after post-injury cycling exercise. It was accompanied by the corresponding changes in the expression of their target genes: the mRNA levels of proapoptotic genes PTEN and PDCD4 decreased, while that of anti-apoptotic factor Bcl-2 increased (73).

Spontaneously hypertensive rats demonstrated increased levels of miR-16 and miR-21 and decreased level of miR-126 in the soleus muscle relative to control. Some of their targets showed anti-correlated expression. Exercise training normalized the expression of these miRNAs to levels similar to controls (36). Exercise training also normalized the expression of certain targets of these miRNAs; for instance, the levels of anti-apoptotic factor Bcl-2 and proangiogenic factor VEGF (targeted by miR-16) increased (36).

Microarray analysis of the whole blood in patients with coronary arterial disease after coronary artery bypass graft surgery and exercise rehabilitation program demonstrated increased expression of miR-92a and miR-92b. At the same time, the mRNA level of the respiratory chain component NDUFA1 and proapoptotic factor CASP3, which are predicted targets of these miRNAs, decreased (124).

Thus, miRNAs are obviously mediators of antiapoptotic and proangiogenic

effects of exercise. At present we do not know how these findings relate to exercise-induced lymphocyte apoptosis.

Circulating MiRNAs and Exercise

Emerging data indicate changes in the blood levels of c-miRNAs after exercise (see Table S4). Uhlemann et al. evaluated human plasma concentrations of miR-126 and miR-133 before and after different exercises and found elevated concentrations of these miRNAs following a marathon (miR-126 and miR-133) and after resistance exercise (miR-133) (128). Banzet et al. likewise found increased levels of miR-133 and other myomiRs (miR-1, miR-208b, and miR-499) in plasma after downhill walking (9), Table S4. Thus, increased plasma levels of these miRNAs may be used as markers for injury of muscle (miR-133) and endothelial cells (miR-126). Mir-133 may serve as a convenient replacement of creatine phosphokinase (CPK), while miR-126 could be the first available marker for endothelial damage (128).

It should be noted that not all published data on the subject are in agreement. For instance, Sawada et al. (110) observed no changes in the serum level of myomiRs in humans after acute resistance exercise, while Uhlemann et al. (128) reported myomiR differential expression after a similar exercise. Different percentage of eccentric load, different time points, and different numbers of probands can explain this inconsistency.

Apparently, c-miRNA concentrations change after exercise not only through cell damage. Changed plasma levels of miR-149*, miR-146a, and miR-221 were demonstrated three days after resistance exercise, while levels of myomiRs remained unaltered (110). Further, increased plasma levels of c-miRNAs miR-181b and miR-214 in absence of elevated myomiRs or CPK were reported immediately after uphill exercise (9). This suggests that the increased levels of these miRNAs resulted from active secretion rather than cell damage. Baggish et al. studied exhaustive exercise tests before and after a 90-day period of rowing training (8). Eight miRNAs involved in angiogenesis, inflammation, muscle contractility, and adaptation to hypoxia were analyzed. Four patterns of c-miRNA response to exercise have been revealed. (i) The levels of miR-146a and miR-222 increased after acute exhaustive exercise before and after sustained exercise training; (ii) miR-21 and miR-221 levels increased after acute exhaustive exercise only before sustained exercise training; (iii) miR-20a level increased after sustained exercise training but not after acute exhaustive exercise; and (iv) miR-133a, miR-328, and miR-220 levels remained unaltered after all tests (8), (Table S4). The unchanged levels of miR-133a likely indicate the absence of muscle damage, while the different patterns of c-miRNA response observed point to the existence of different control mechanisms not associated with cell damage.

C-miRNA quantification can be used to predict the risk of cardiovascular diseases. Microarray data show that healthy individuals with low maximal oxygen uptake have increased levels of circulating miR-21, miR-210, and miR-222 (18). Low maximal oxygen uptake is indicative of a predisposition to cardiovascular diseases (138); accordingly, the identified miRNAs can serve as convenient noninvasive markers of risk for these diseases (18).

Thus, the first studies in the field demonstrated that exercise-induced changes in plasma/serum levels of c-miRNAs can result from both, cell damage

or independent mechanisms. The contribution of cell damage seems to increase with exercise load (128). Rapid and substantial (several-fold) increase in c-miRNA levels after intense exercise in the absence of tissue damage markers in plasma (8) can indicate the peak release of previously synthesized miRNAs into the bloodstream, while stable changes in c-miRNA levels after long-term exercise training suggest modified basal expression and/or secretion of miRNAs.

Table 3. C-miRNAs described in relation to exercise and possible relevance for the immune system.

MiRNA name, fold change and source		Presumed immune-related effects[a]	Number of hits[b]
miR-1	↑4.0 (9)	Anti-inflamm., anti-astmathic, anti-prolif.(42, 76, 123)	16
miR-20a	↑ 3.0 (8)	Unknown	0
miR-21	↑ 2.6 (8)	Both pro- and anti-inflamm., oncogenic (32, 60, 91, 99, 142)	93
miR-126	↑4.0 (128)	Pro-angiogenic; anti-inflamm., pro-astmathic (pro-Th2) (6, 79, 103, 105)	29
miR-133	↑8.9 (128), ↑4,8 (9), NC (8)	Unknown; found in inflammatory vesicles (46)	2
miR-146a	↑3.0 (8), ↓2.0 (110)	Anti-inflamm.; possible counterpart of mir-21 in regul. of inflamm. (21, 50, 112, 143)	17
miR-149*	↑2.3 (110)	Unknown (rare passenger strand of anti-inflamm. mir-149)	0
miR-181b	↑1,5 (9)	Anti-inflamm., controls autoimmunity (48, 132)	4
miR-208	↑11,5 (9)	Unknown	0
miR-214	↑1,8 (9)	Prolif. stim, pro-oncogen. (105, 146)	6
miR-221	↑ 5.8 (8) ↓ 2.0 (110)	Anti-inflamm., anti-angiogenic (28, 45, 129)	13
miR-222	↑2.4 (8)	Anti-inflamm. (105)	9

[a] as judged from described effects of corresponding cellular miRs (personal interpretation of literature data)

[b] hits found in PubMed search for name of miRNA and inflammation

NC = no change

In most cases, the cell source(s) of c-miRNAs remain unclear. The bulk of miRNAs are expressed in several cell types albeit at different rates (62). For instance, miR-133 is abundant in the skeletal muscle and heart but also detectable in the brain (61) and brown adipose tissue (133). MiR-126, typical of endothelium, is also expressed in the liver, haematopoietic cells, and some other tissues (62). Accordingly, direct identification of the c-miRNA source is nearly impossible except in case of overt cell damage (64). Further studies on the mechanisms of miRNA secretion and the nature of circulating miRNA-containing complexes may help to address these questions.

To date, the effect of altered c-miRNA concentrations on body systems remains largely unclear. Possible effects can be deduced from validated targets of these miRNAs; in the case of exercise, these are largely associated with muscle function, angiogenesis, inflammation, and oxidative stress, i.e., the processes primarily affected by exercise. Here, the question, how circulating miRNAs may find and enter

their target cells, needs to be discussed. Studies mentioned above evaluated c-miRNAs without considering their carrier. The nature of the carrier (high-density lipoprotein-particles, exosomes, apoptotic bodies, shedding vesicles or vesicle-free Ago protein bound miRNAs) may dramatically influence the cell/organ-specific targeting and even biological effects.

To our knowledge, at present, no clear proof is available to show which biological effects can be attributed to exercise-induced c-miRNA expression. In spite of all the imponderabilities mentioned above, it seems, however, reasonable to assume, that they have identical or similar effects as their cellular counterparts. On this basis, Table 3 presents the presumed effects of exercise-induced c-miRNAs as judged from literature data with focus on immune related effects. The majority of exercise-induced miRNAs has predominantly anti-inflammatory effects rather than inflammatory ones. Since exercise-induced gene (mRNA) expression also has a remarkable anti-inflammatory bias (1) this concordance makes likely that the protective generalized reaction of the body to exercise is organized by help of miRNAs or co-organized together with hormones at a very early stage.

Intriguingly, it has recently been shown that c-miRNAs can also induce biological effects without directly interacting with mRNA. Tumour cells can use extracellular miRNAs as ligands to Toll-like receptors (TLR7 in mice and TLR8 in human) of macrophages and thereby modulate the immune response to their favor (31, 32, 68). Although, up to now, this new and exciting hormone-like mechanism has only been demonstrated for cancer and for neurodegenerative processes, it may well be functional under physiological conditions as well. Modulation of immune functions by exercise is one of the fields to be investigated in this context.

Altogether we are convinced that the future will see c-miRNAs as useful markers for exercise-related damage or malfunction and will also expose an important biological role for them in exercise immunology.

Concluding Remarks and Future Directions

Recent data indicate that miRNAs are an essential element in the adaptation of the immune system and other systems to exercise. Progress in the field requires flanking progress in related fields, namely, detailed data on the role of each miRNA in the function of different cell types. Such data are actively generated now, and since miRNAs are a hotspot in molecular biology, further data expansion can be expected in the nearest future. The improvement of technical approaches is also desirable, in particular, rapid sorting of blood cells and preservation of isolated RNA. RT-PCR is widely used in miRNA studies discussed here but it allows only a small number of miRNA species to be detected; in the cases when microarray technology is used, microarray data files are not always made publicly available. Wide application of large-scale screening methods and publication of comprehensive microarray data will undoubtedly accelerate the progress in exercise immunology.

As of now, only limited numbers of exercise physiology studies involving miRNAs have been carried out on human and animal groups of different size, age, and sex, using different exercise designs, all of which makes correct comparison of results and data meta-analysis largely impossible. Small sample size in the

studies and small changes in the studied parameters (often below 50%), coupled with significant individual variation in gene expression levels, open the door to misinterpretations. In this context, the development of uniform study designs by the scientific community is of primary importance.

Other classes of non-coding RNAs can also be expected to mediate the reaction of our immune system to acute or chronic exercise. For instance, well-studied tRNAs unexpectedly proved to mediate stress responses (115), and exercise is of course known to be a kind of stress. Moreover, the involvement of another group of non-coding RNAs, snoRNAs, in the reaction of leukocytes to exercise has recently been demonstrated by our group (109). We expect many more exciting discoveries about the role of miRs & Co in exercise immunology.

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Table S1. MiRNAs differentially expressed in different leukocyte types before and after exercise test. MiRNA species with a 1.5-fold or greater expression difference are boldfaced.

Leukocyte type	Neutrophils		PBMC		NK cells
MiRNA name and fold change	**hsa-miR-485-3p ↑2.92** **hsa-miR-520d-3p ↑2.82** **hsa-miR-181b ↑1.64** **hsa-miR-130a ↓1.61** **hsa-miR-151-5p ↓1.60** **hsa-miR-1238 ↑1.58** **hsa-miR-193a-3p ↑1.58** **hsa-miR-1225-5p ↑1.55** **hsa-miR-126 ↓1.53** hsa-miR-20a ↓1.24 hsa-miR-106a ↓1.39 hsa-miR-20b ↓1.29 hsa-miR-17 ↓1.40 hsa-miR-93 ↓1.24 hsa-miR-130b ↓1.41 hsa-miR-16 ↓1.23 hsa-let-7i ↓1.25 hsa-miR-107 ↓1.26 hsa-miR-185 ↓1.28 hsa-miR-18a ↓1.35 hsa-miR-18b ↓1.29 hsa-miR-194 ↓1.26 hsa-miR-22 ↓1.28	hsa-miR-363 ↓1.34 hsa-miR-660 ↓1.23 hsa-miR-96 ↓1.28 hsa-miR-125a-5p ↑1.22 hsa-miR-145 ↑1.22 hsa-miR-197 ↑1.38 hsa-miR-212 ↑1.44 hsa-miR-223 ↑1.29 hsa-miR-340* ↑1.25 hsa-miR-365 ↑1.37 hsa-miR-505 ↑1.24 hsa-miR-629* ↑1.44 hsa-miR-638 ↑1.36 hsa-miR-939 ↑1.40 hsa-miR-940 ↑1.39	**hsa-miR-451 ↓3.8** **hsa-miR-181a* ↑2.0** **hsa-miR-181b ↑1.7** **hsa-miR-486-5p ↓1.7** **hsa-miR-363 ↑1.5** **hsa-miR-1225-5p ↑1.5** **hsa-miR-21* ↑1.5** hsa-miR-181a ↑1.4 hsa-miR-181c ↑1.2 hsa-miR-338-3p ↑1.4 hsa-miR-26b ↑1.2 hsa-miR-132 ↑1.3 hsa-miR-15a ↑1.3 hsa-miR-939 ↑1.3 hsa-miR-7 ↑1.4 hsa-miR-140-5p ↑1.3 hsa-miR-940 ↑1.3 hsa-miR-125b ↓1.4 hsa-let-7e ↓1.4 hsa-miR-320 ↓1.2 hsa-miR-151-5p ↓1.3 hsa-miR-31 ↓1.3 hsa-miR-125a-5p ↓1.3	hsa-miR-99b ↓1.4 hsa-miR-652 ↓1.2 hsa-miR-151-3p ↓1.3 hsa-miR-130a ↓1.2 hsa-miR-126 ↓1.3 hsa-miR-199b-3p ↓1.3 hsa-miR-23b ↓1.2 hsa-miR-221 ↓1.2 hsa-miR-199a-5p ↓1.3 hsa-miR-584 ↓1.3 hsa-miR-145 ↓1.3	**hsa-let-7e ↓2.6** **hsa-miR-126 ↓3.2** **hsa-miR-126* ↓2.9** **hsa-miR-130a ↓2.9** **hsa-miR-151-5p ↓2.8** **hsa-miR-199a-3p ↓3.1** **hsa-miR-199a-5p ↓2.9** **hsa-miR-221 ↓2.1** **hsa-miR-223 ↓2.9** **hsa-miR-326 ↓2.3** **hsa-miR-328 ↓2.3** **hsa-miR-652 ↓2.2** **hsa-miR-142-3p ↑3.0** **hsa-miR-142-5p ↑2.5** **hsa-miR-192 ↑2.2** **hsa-miR-29a ↑2.0** **hsa-miR-29b ↑3.5** **hsa-miR-29c ↑2.3** **hsa-miR-30e ↑2.1** **hsa-miR-338-3p ↑2.2** **hsa-miR-363 ↑2.1** **hsa-miR-590-5p ↑2.6** **hsa-miR-7 ↑2.2**
Experiment participants	Eleven healthy men 19–30 years old of average fitness (non-athletes)		Twelve healthy men with a mean age of 22±1 years of average fitness (non-athletes)		Eleven healthy men 20–29 years old of average fitness (non-athletes)
Exercise type	20 min of exercise consisting of 10 2-min bouts of constant work rate cycle ergometry with a 1-min rest interval between each bout (total test time is 30 min) at 76-77% peak VO2				
Time points of blood sampling	Immediately before and after exercise test				
Reference	(102)		(101)		(100)

Table S2. Differential expression of myomiRs in the skeletal muscle after different exercise types and forced immobility.

Exercise type	MiRNA name[1] and fold change			Species	Experiment participants	Muscle studied	Exercise modality	Experiment duration	Time points of expression analysis	Reference
	miR-1	mir-133a	mir-206							
Chronic endurance	↓1.5	↓1.3	↓2.0	Human	10 trained males with a mean age of 30.5 years	Vastus lateralis	Cycle ergometer	12 weeks	Before and 3-5 days after training	(89)
	↓1.6	↓1.9	NC[2]	Human	24 sedentary males with a mean age of 23 years	Vastus lateralis	Cycle ergometer	6 weeks	Before and one day after training	(52)
	NC[3]	NC	NC	Mouse	8-week-old mice	Gastrocnemius	Treadmill running	4 weeks	Experimental and control mice were analyzed one day after experiment	(5)
Acute endurance	↑ 1.3	↑1.4	NC	Human	10 trained males with a mean age of 30.5 years	Vastus lateralis	Cycle ergometer	60 min	Before and immediately after exercise	(89)
	↑1.4	NC	ND	Mouse	7 mice of 4 month old in experimental and control groups each	Quadr. femoris	Treadmill running	90 min	Experimental and control mice were analyzed 3 h after exercise	(108)
Resistance	↓1.7	NC	NC	Human	6 sedentary males with a mean age of 29 years	Vastus lateralis	Leg extension, essential amino acid solution	8 sets of 10 repetitions	Immediately before and 3 h after exercise	(27)
	↓1.3	ND	ND	Human	14 males and 13 females with a mean age of 80.1 years	Vastus lateralis	Resistance exercise or eccentric ergometer sessions	12 weeks	Before and 2-3 days after training	(84)
	NC	NC	NC	Human	17 untrained males of18-30 years old	Vastus lateralis	Rotating split-body resistance training program	12 weeks	Before and 2 days after training	(24)
	↓2.0	↓2.0	NC	Mouse	10-week-old mice	Plantaris	*In vivo* model of muscle hypertrophy	1 week	Experimental and control mice were analyzed after experiment	(80)

Resting muscle	↓1.1	↓1.1	ND	Human	12 physically active males with a mean age of 26.2 years	Vastus lateralis	Bed rest	7 days	Before and after experiment	(104)
	NC[3]	NC	NC	Mouse	14-week-old mice	Gastrocnemius	Hind limb fixation	5 days	Fixed and free limb muscles	(5)
	NC	NC	NC	Rat	3 6-month-old rats in two experimental and one control groups, each	Soleus	Hind limb suspension	2 and 7 days	Experimental and control rats were analyzed after experiment	(81)
	NC (trend toward a decrease)	NC (trend toward a decrease)	↓2.0	Mouse	4 77-day-old mice in experimental and control groups each	Gastrocnemius	Space flight	~12 days	Experimental and control mice were analyzed ~4 h after landing	(3)
	↓~3.3	↓~3.3	NC	Rat	Adult animals	Soleus	Denervation	4 weeks	Experimental and control rats were analyzed after experiment	(49)

[1]The data on the most often studied myomiRs are presented.
[2]The data on miRNAs with a 1.5-fold or greater expression difference are reported in the original paper.
[3]The data on miRNAs with a twofold or greater expression difference are reported in the original paper.
NC, no change
ND, not determined

Table S3. MiRNA species with most pronounced expression changes in muscle after exercise. Taxon-specific miRNAs are boldfaced.

MiRNA name	mir-183	mir-189	mir-432*	mir-451	mir-589	**mir-616**	mir-21	**mir-696**	**mir-709**	mir-720	mir-23	mir-451	**mir-680**	**mir-696**	**mir-705**	mir-762
Fold change	↑2.0	↑2.0	↑2.3	↓4.0	↓3.5	↑2.0	↑2.3	↓2.9	↓2.3	↓2.0	↓6.2	↑4.0	↑2.3	↑2.1	↑2.1	↑2.1
Reference	(52)						(5)				(108)	(24)	(5)			
Exercise type	Chronic endurance										Acute endurance	Resistance	Immobilization			

Table S4. Differential expression of miRNAs in human plasma and serum after exercise.

MiRNA name	Fold Change	Experiment participants	Exercise type	Time points of expression analysis and c-miRNA source	Refer-ence
miR-1	↑4.0	9 recreationally active males of 27-36 years old	30-min downhill walking	Before and 6 h after exercise; plasma	(9)
miR-20a	↑ 3.0	10 competitive male athletes with a mean age of 19.1±0.6 years	Rowing for 90 days	Before and after 90 days of exercise training; plasma	(8)
miR-21	↑ 1.9	10 competitive male athletes with a mean age of 19.1±0.6 years	Acute endurance exercise (cycle ergometer) **prior** to sustained exercise training	Before and immediately after exercise; plasma	(8)
	↑ 2.6	10 competitive male athletes with a mean age of 19.1±0.6 years	Rowing for 90 days	Before and after 90 days of exercise training; plasma	(8)
miR-126	↑3.4	22 male marathon runners with a mean age of 56.8±5.2 years	Marathon race	Before and immediately after exercise; plasma	(128)
	↑2.0	13 healthy individuals (7 males and 6 females) with a mean age of 30.4±2.0 years	Single symptom-limited exercise test	Before and 5 min after exercise; plasma	(128)
	↑4.0	13 healthy well trained males with a mean age of 32.4±2.3 years	Cycle ergometer for 4 h below the anaerobic threshold	Before and immediately after exercise; plasma	(128)
	NC	11 trained subjects (4 males and 7 females) with a mean age of 37±2 years	Singular resistance training with additional eccentric loads (lat pulldown, leg press and butterfly)	Before and immediately after exercise; plasma	(128)
miR-133	↑8.9	22 male marathon runners with a mean age of 56.8±5.2 years	Marathon race	Before and immediately after exercise; plasma	(128)
	NC	13 healthy individuals (7 males and 6 females) with a mean age of 30.4±2.0 years	Single symptom-limited exercise test	Before and 5 min after test; plasma	(128)
	NC	13 healthy well trained males with a mean age of 32.4±2.3 years	Cycle ergometer for 4 h below the anaerobic threshold	Before and 1 h after exercise; plasma	(128)
	↑2.1	11 trained subjects (4 males and 7 females) with a mean age of 37±2 years	Singular resistance training with additional eccentric loads (lat pulldown, leg press and butterfly)	Before and immediately after exercise; plasma	(128)
	NC	10 competitive male athletes with a mean age of 19.1±0.6 years	Acute endurance and chronic endurance (90 days of rowing) exercises	Before and immediately after exercises; plasma	(8)
	↑4.8	9 recreationally active males of 27-36 years old	30-min downhill walking	Before and 6 h after exercise; plasma	(9)
miR-146a	↑3.0	10 competitive male athletes with a mean age of 19.1±0.6 years	Acute endurance exercise (cycle ergometer) **prior** to 90-day exercise training	Before and immediately after exercise; plasma	(8)
	↑3.0	10 competitive male athletes with a mean age of 19.1±0.6 years	Rowing for 90 days	Before and after 90 days of exercise training; plasma	(8)
	↑7.5	10 competitive male athletes with a mean age of 19.1±0.6 years	Acute endurance exercise (cycle ergometer) **after** 90-day exercise training	Before 90-day exercise training and immediately after acute exercise; plasma	(8)
	↓ 2.0	3 physically active males	Acute resistance exercise (bench press and bilateral leg press)	Before and 3 days after exercise; serum	(110)
miR-149*	↑2.3	3 physically active males	Acute resistance exercise (bench press and bilateral leg press)	Before and 1 day after exercise; serum	(110)

miR-181b	↑1.5	9 recreationally active males of 27-36 years old	30-min uphill walking	Before and immediately after exercise; plasma	(9)
miR-208	↑11.5	9 recreationally active males of 27-36 years old	30-min downhill walking	Before and 6 h after exercise; plasma	(9)
miR-214	↑1.8	9 recreationally active males of 27-36 years old	30-min uphill walking	Before and immediately after exercise; plasma	(9)
miR-221	↑ 3.6	10 competitive male athletes with a mean age of 19.1±0.6 years	Acute endurance exercise (cycle ergometer) **prior** to 90-day exercise training	Before and immediately after exercise; plasma	(8)
	↑ 5.8	10 competitive male athletes with a mean age of 19.1±0.6 years	Rowing for 90 days	Before and after 90 days of exercise training; plasma	(8)
	↓ 2.0	3 physically active males	Acute resistance exercise (bench press and bilateral leg press)	Before and 3 days after exercise; serum	(110)
miR-222	↑2.5	10 competitive male athletes with a mean age of 19.1±0.6 years	Acute endurance exercise (cycle ergometer) **prior** to 90-day exercise training	Before and immediately after exercise; plasma	(8)
	↑2.4	10 competitive male athletes with a mean age of 19.1±0.6 years	Rowing for 90 days	Before and after 90 days of exercise training; plasma	(8)
	↑4.0	10 competitive male athletes with a mean age of 19.1±0.6 years	Acute endurance exercise (cycle ergometer) **after** 90-day exercise training	Before 90-day exercise training and immediately after acute exercise; plasma	(8)

NC, no change

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