Thieme

Sprint Interval Training Decreases Circulating MicroRNAs Important for Muscle Development

Authors
Joshua Denham[1], Adrian Gray[1], John Scott-Hamilton[2], Amanda D. Hagstrom[1]

Affiliations
1 School of Science and Technology, University of New England, Armidale, Australia
2 School of Health, University of New England, Armidale, Australia

Key words
epigenetics, non-coding RNA, HIIT, cardiorespiratory fitness, exercise, myomiR

accepted after revision 28.09.2017

Bibliography
DOI https://doi.org/10.1055/s-0043-120763
Published online: 10.11.2017
Int J Sports Med 2018; 39: 67–72

ISSN 0172-4622

Correspondence
Dr. Joshua Denham, PhD
University of New England
School of Science and Technology
Booloominbah Drive
2351, Armidale
Australia
Tel.: +61/267/735 860, Fax: +61/267/735 860
jdenham2@une.edu.au

ABSTRACT

Small non-coding RNAs, such as microRNAs (miRNAs), have emerged as powerful post-transcriptional regulators of gene expression that play important roles in many developmental and biological processes. In this study, we assessed the abundance of circulating microRNAs important for skeletal muscle and heart adaptations to exercise (miR-1, miR-133a, miR-133b and miR-486), following acute exercise and short-term sprint interval training (SIT). Twenty-eight individuals completed four all-out efforts on a cycle ergometer, and donated blood before and 30 min after the cessation of exercise. A subset of 10 untrained men completed 4-6 efforts of SIT, three times a week for 6 weeks, and donated resting blood samples before and after the intervention. MiRNA TaqMan qPCR was performed and whilst no changes were observed after a single session of SIT (all $p > 0.05$), the 6-wk SIT intervention significantly reduced the whole blood content of all four miRNAs (mean fold-changes: 0.37–0.48, all $p < 0.01$). Our data suggests that circulating miRNAs are responsive to short-term SIT and could have roles in SIT-induced health and performance adaptations. Further work is required to establish whether circulating miRNAs could serve as biomarkers for predicting exercise training responses and monitoring exercise interventions.

Introduction

Small non-coding RNAs, such as microRNAs (miRNAs), have emerged as powerful post-transcriptional regulators of gene expression that play important roles in many developmental and biological processes [1, 51]. MiRNAs are genetically conserved, small RNA molecules (18–25 nucleotides) that fine-tune gene expression by binding to the untranslated regions (UTR) of their mRNA targets. Although miRNAs normally reduce protein abundance through translational repression or mRNA degradation [28], they can also facilitate translation [42, 52]. Furthermore, miRNAs are implicated in evolution as miRNA diversity is related to species complexity [6, 7, 24]. MiRNA-mRNA targeting has a profound influence on signaling pathways to the fact that one miRNA can have hundreds of mRNA targets [23, 33, 35].

While many miRNA are ubiquitously expressed, some are particularly abundant in certain tissues [34] and are implicated in pathogenesis of disease, including cardiovascular disease [5, 53], type 2 diabetes [21, 22, 31] and cancers [29, 36]. Circulating miRNAs isolated from peripheral blood samples are a mixture of those generated by immune cells or others that originate from surrounding organs. Since circulating miRNAs are stabilized and transported to other tissues by exosomes and argonaute proteins [2, 57], miRNAs isolated from blood samples are proposed as biomarkers of biological aging and age-related disease [30, 41, 49].

Given that exercise is an effective lifestyle strategy to reduce the risk and prevent the progression of many age-related diseases and accelerated aging, it is posited that the mechanism could involve miRNAs [15, 48]. Circulating miRNAs are vulnerable to single bouts of aerobic and resistance exercise in numerous tissues, including brain, skeletal muscle, heart and blood [15, 45]. Specific miRNAs are correlated to important fitness and performance adaptations to exercise. For example, resting serum (miR-21 and miR-210) [11] and whole blood (miR-1 and miR-486)[18] miRNAs are inversely and positively correlated to $\dot{V}O_{2max}$, respectively. In both studies, the aforementioned miRNAs were differentially expressed in individuals with relatively low $\dot{V}O_{2max}$ compared to their fitter peers [11, 18]. MiRNAs can also distinguish between high and low responders in exercise-adaptations (weight loss, improvements in $\dot{V}O_{2max}$, skeletal muscle hypertrophy) after aerobic [3, 44] and resistance [14, 40] exercise training interventions.

Many miRNAs highly expressed in myocytes, called myomiRs, are critical for the normal development of the heart and skeletal muscle [38] and are also linked exercise-induced adaptations [25, 47]. Notably, many of the metabolic and performance adaptations to exercise are abolished in miR-133a$^{-/-}$ mice [39]. Given these important functions of myomiRs, we previously analyzed the abundance of circulating miRNAs (miR-1, -133a and -486) in context with acute maximal aerobic exercise and long-term endurance training [18]. Interestingly, relative to healthy controls, the endurance athletes possessed a higher abundance of miR-1 and miR-486 and these miRNAs were positively correlated to $\dot{V}O_{2max}$ [18]. MyomiRs likely have highly specific roles in skeletal and heart muscle adaptations to aerobic and anaerobic exercise, as endurance athletes have differentially expressed blood-based miRNAs compared to strength-trained individuals [27, 55] and discordant miRNA expression patterns are observed after concentric and eccentric exercise [4]. Therefore, we extended our previous findings and examined the influence of acute and six weeks of sprint interval training (SIT) on circulating miRNAs (miR-1, -133a/b and -486). The purpose of this study was to examine the influence of anaerobic exercise on circulating myomiRs. We hypothesized that myomiRs would be dynamically regulated by a single session of exercise and six weeks of SIT.

Materials and Methods

Participants

Twenty-eight apparently healthy men were recruited for this study (N = 28, age (y) 35.5 ± 11.1; body mass (kg) 82.1 ± 14.0; height 179.9 ± 8.6; BMI (kg·m^{-2}) 25.4 ± 3.2). A subset of these participants completed a 6-week SIT intervention (n = 10, age (y) 33.3 ± 10.9; body mass (kg) 90.2 ± 13.5; height (cm) 183.4 ± 6.9; BMI (kg·m^{-2}) 26.8 ± 3.5; $\dot{V}O_{2max}$ (ml·kg^{-1}·min^{-1}) 42.78 ± 6.22). Participants were screened (Pre-exercise Screening System, ESSA) and provided written informed consent. This study was approved by the university's Human Research Ethics Committee (approval number: HE15-294). Our study complied with the ethical standards published most recently by the International Journal of Sports Medicine [26].

Acute sprint interval session

Twenty-eight participants completed a one-off acute sprint interval session consisting of four 30-s maximal 'all out' efforts separated by 4-min rest periods. Participants completed a standardized low intensity warm-up consisting of 5 min of steady state cycling (< 50 % of P_{max}). Participants were instructed to give maximal effort and were verbally encouraged by the supervising exercise physiologist.

6-week sprint interval training (SIT) intervention

A subset of healthy men (n = 10) undertook a 6-wk, three times per week, supervised SIT program. Each session consisted of a brief 5-min warm-up, including steady state cycling (< 50 % of P_{max}) with the initial session prescription consisting of four, 30-s all-out efforts, with 4-min rest between sets. Prescription was progressed by the addition of an extra effort every fortnight, with participants completing six all-out 30-s efforts by the end of the intervention period. Training was performed on the Wattbike ergometer (Wattbike Pro, UK) three times a week on alternate days (e. g., Monday, Wednesday and Friday). The supervising exercise physiologist instructed participants not to pace their efforts and provided verbal encouragement for each effort. Cardiorespiratory fitness was assessed by pulmonary analysis after blood sampling before and two to three days following the SIT intervention. Subjects began cycling at 100 W and the load was increased by 20 W·min^{-1} until volitional exhaustion. Gas (O_2 and CO_2) exchange was monitored using the Oxycon system (Jaeger, Germany) and $\dot{V}O_{2max}$ was determined as the highest O_2 uptake over one minute.

Blood sampling and RNA analysis

Fasted venous blood samples were drawn from the antecubital vein into EDTA tubes before and 30 min following the cessation of exercise (for those involved in the single session of SIT trial). The subset of 10 men also donated a blood sample two to four days after their final SIT session (for the 6-wk training intervention). 1 ml of whole blood was washed with an erythrocyte lysis buffer (Qiagen, Australia) and centrifuged at 2500 RPM prior to RNA extraction using the miRVana miRNA Mini kit (ThermoFisher Scientific, Australia), following the manufacturer's guidelines. Total RNA quantity and quality were assessed using the Nanodrop spectrophotometer (ThermoFisher Scientific, Australia) and was stored at –80 °C. Approximately 500 ng of RNA were reverse transcribed using the MicroRNA Reverse Transcription Kit (ThermoFisher Scientific, Australia) and miRNA-specific TaqMan primers (hsa-miR-1-3p, hsa-miR-133a-3p, hsa-miR-133b-3p, hsa-miR-486-5p and snoU6). These miRNAs were selected a priori due to their roles in skeletal and heart muscle development and are implicated in exercise-induced training adaptations [18]. The miRNA abundance was quantified by TaqMan qPCR using experimental layouts outlined previously [18]. All paired samples were run in triplicate with the endogenous control miRNA (snoU6) and negative no-template controls on 384-well plates in the BioRad Thermocycler (CFX96 Touch Real-time PCR Detection System, Bio-Rad). The $2^{-\text{delta-delta}}$ Ct method was used to examine changes in miRNA abundance after exercise and was expressed as fold-change (FC). The average intra-assay co-efficient of variations hsa-miR-1-3p, hsa-miR-133a-3p, hsa-miR-133b-3p, hsa-miR-486-5p and snoU6 triplicates were 0.74 ± 0.34, 0.61 ± 0.32, 0.87 ± 0.43, 0.66 ± 0.41 and 0.72 ± 0.40, respectively.

Statistical analysis

Data were assessed for normality using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Based on our previous findings [18], we calculated that in order to achieve > 80 % power to detect a moderate effect size (d = 0.50) at the alpha level of 0.05, a minimum of 28 individuals was required (G * Power, Version 3.1.9.2). Paired-sample t-tests or Wilcoxon signed rank tests were used to establish statistically significant changes in characteristics and miRNAs after SIT. Significance was set at $p < 0.05$.

Results

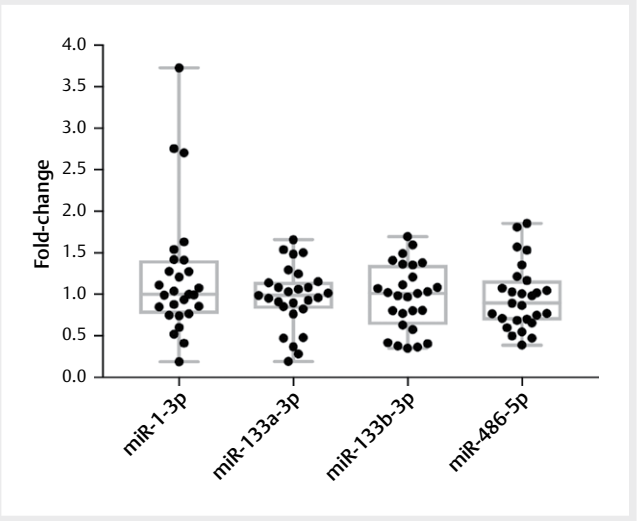
No statistically significant changes were found in whole blood miRNA abundance after a single session of SIT (all $p > 0.05$, ▶ **Fig. 1**). Subjects increased their $\dot{V}O_{2max}$ (8.06 %) and maximum power output in incremental testing (11.11 %) after the 6-wk SIT intervention (both $p < 0.05$). All four miRNAs were markedly reduced following the 6-wk SIT intervention (all $p < 0.01$, ▶ **Fig. 2**).

Discussion

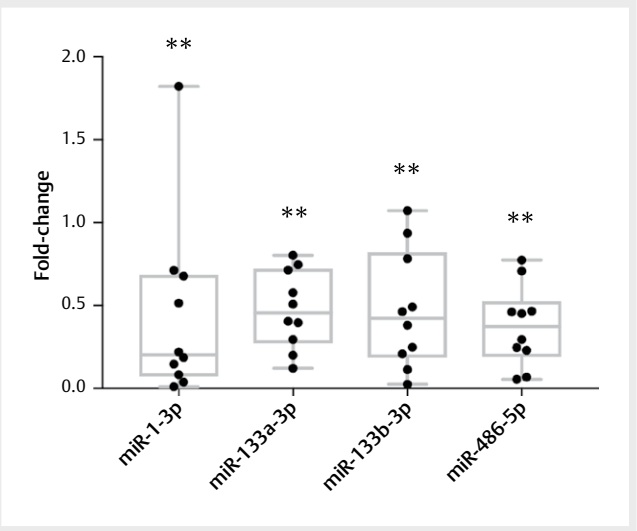
The purpose of this study was to examine the influence of acute and short-term SIT on four miRNAs important for muscle development and linked to exercise-induced adaptations. Although miRNAs were not consistently altered by a single session of SIT, a 6-wk SIT intervention markedly decreased the abundance of miR-1-3p, miR-133a-3p, miR-133b-3p and miR-486-5p in whole blood from men. Considering the significant cardiorespiratory fitness and cycling performance adaptations caused by SIT in the present study, the findings provide evidence that miRNAs may control the expression of genes conducive to health and performance adaptations. They also indicate that whole blood is an appropriate bio-fluid to analyze miRNAs in order to monitor exercise adherence and training-induced physiological adaptations.

Evidence suggesting miRNAs are crucial to exercise-induced adaptations is accumulating [15, 37, 47, 50]. MyomiRs are particularly responsive to exercise interventions and control pathways conducive to aerobic and anaerobic conditioning [47]. For instance, miR-133a-3p$^{-/-}$ mice exhibit exercise intolerance with a 33 % exercise capacity, reduced mitochondrial content and expression of key metabolic genes in skeletal muscle [39]. Moreover, miR-133a-3p$^{-/-}$ mice do not show significant improvements in the aforementioned parameters after six weeks of treadmill training [39]. Skeletal muscle miR-1-3p and miR-133a-3p are down-regulated during hypertrophy in mice, and miR-1-3p is known to target mRNAs in the insulin-like growth factor-1 signaling cascade [20]. Most findings on endurance and resistance training induced responses indicate miR-1 and miR-133 families are generally down-regulated, possibly to augment protein synthesis for muscle recovery after exercise [20]. Therefore, it is tempting to speculate that the decreased abundance of myomiRs found in the present study could be due to an increase in protein synthesis and other improvements in skeletal muscle metabolism induced by high-intensity exercise (e. g., increased oxidative capacity, insulin sensitivity and mitochondrial biogenesis) [9, 10, 12, 46].

Interestingly, when we used the miRWalk 2.0 database [19] to identify predicted mRNA targets of the myomiRs altered after short-term SIT, we noticed they were predicted to target genes with known roles in aging and other established pathways vital for health and performance adaptations. It is reasonable to suggest



▶ **Fig. 1** Whole blood miRNAs are not regulated by a single session of SIT. No statistically significant changes were observed in whole blood miR-1-3p, miR-133a-3p, miR-133b-3p or miR-486-5p at 30 min post-exercise (mean fold-change ± SE: 1.21 ± 0.15, 0.97 ± 0.07, 0.97 ± 0.08 and 0.96 ± 0.08, respectively, N = 27, all $p > 0.05$). Data are from two-tailed Wilcoxon signed rank tests and are expressed as fold-change ($2^{-\Delta\Delta Ct}$, black dots). The bottom, middle and top lines of the boxes are 25th percentile, median and 75th percentile, respectively, and whiskers indicate minimum and maximum values.



▶ **Fig. 2** Short-term SIT lowers whole blood miRNA abundance. A marked decrease in whole blood miR-1-3p, miR-133a-3p, miR-133b-3p or miR-486-5p was observed in 10 untrained healthy individuals following a 6-wk SIT intervention (mean fold-change ± SE: 0.44 ± 0.17, 0.48 ± 0.07, 0.47 ± 0.11 and 0.37 ± 0.08, respectively, all $p < 0.01$). Data are from two-tailed Wilcoxon signed rank tests and are expressed as fold-change ($2^{-\Delta\Delta Ct}$, black dots). The bottom, middle and top lines of the boxes are 25th percentile, median and 75th percentile, respectively, and whiskers indicate minimum and maximum values. * * $p < 0{,}01$.

that given miR-1-3p (SIRT3, TINF2, BDNF and DRD1), miR-133a/b-3p (TERT, MAPK14 and IL15RA) and miR-486-5p (SIRT1, PTEN and PIK3AP1) were all down-regulated and that their target mRNAs could be up-regulated by SIT. Our findings suggest mRNA targets of the miRNAs altered by SIT – linked to aging and cellular senescence (SIRT1/3, TERT and TINF2) – support other recent evidence indicating high-intensity exercise may attenuate age-related decline in mitochondrial content and lean mass [46]. Rigorous exercise training may attenuate biological aging through telomere and sirtuin signaling pathways, such that endurance athletes exhibit higher sirtuin activity [54], longer telomeres and increased expression of TERT mRNA [17] in leukocytes. The mRNA targets of these miRNAs should be analyzed in context with other markers of cellular aging in future work.

Precisely how the circulating miRNAs were reduced after six weeks of SIT is unclear. Either the endogenous production and transport of these miRNAs from surrounding tissues into circulation was attenuated, recipient tissue uptake was more efficient, or circulating factors that encourage the decay of the miRNAs were up-regulated by SIT. Leukocyte DNA methylation alterations to miRNA genes have led to mature miRNA content changes in previous exercise interventions [16], which could account for the observed changes in the present study. The exact fate and inter-cellular functions of circulating miRNAs in exosomes, argonaute proteins or other proteins regulated by exercise remain incompletely understood.

In contrast to our previous study that found significant decreases in miRNAs (miR-1-3p, miR-133a-3p and miR-486-5p) immediately after maximal aerobic exercise [18], the present study did not reveal any consistent changes in these miRNAs after a single session of SIT. This could be a temporal effect as we chose to analyze the exercise-induced changes to miRNAs 30 min into the recovery phase in the present study, whereas miRNAs were analyzed immediately after maximal exercise testing in the previous investigation [18]. Alternatively, circulating miRNAs may have a unique response to aerobic and anaerobic modes of exercise. Indeed, SIT would rely heavily on anaerobic metabolism during the effort and aerobic metabolism during the rest period when exercise post oxygen consumption is occurring. We have experimentally demonstrated that miRNAs involved in hypoxia pathways (miR-21 and miR-210) are regulated by a single session of SIT and short-term running SIT [16]. Interestingly, others have analyzed the effects of high-intensity interval training (HIIT) and traditional continuous exercise on vascular remodeling miRNA (miR-16 and miR-126) [32] and plasma-based myomiR (miR-1, miR-133a/b and miR-208) [13] abundance, and found similar miRNA responses to both forms of exercise. An explanation for the discrepancy between our findings and previous ones may be the different sample analyzed (whole blood vs plasma), different time-point, other methodological discordances or that SIT may induce a unique miRNA response because it is at highest end of the intensity continuum for aerobic training. Certainly, a time-course analysis of the acute and long-term influences of miRNA responses is required to resolve the issue.

While the majority of studies have measured circulating miRNAs in plasma or serum to examine their utility as exercise biomarkers, whole blood may be a convenient alternative for clear reasons. First, whole blood miRNAs are from surrounding tissues shuttled into plasma, red blood cells and other immune cells, which increases the diversity of miRNAs and enables the concurrent analysis of leukocyte miRNA and the expression of their mRNA targets. Second, whole blood RNA yields (which includes small RNAs – e. g., miRNAs) are much higher than alternative bio-fluids used for biomarker studies (e. g., saliva, urine, plasma or serum). Finally, whole blood analyses circumvent issues regarding reference miRNA selection and the need to use spike-in miRNAs. Therefore, others should consider quantifying whole blood miRNAs for exercise biomarker discovery and move towards standardized techniques that will be important for replication and validation experiments.

A limitation of the study is that we only included a modest number of men (n = 10) in the 6-wk SIT intervention. We did, however, have appropriate power to detect statistically significant changes in miRNAs, but whether women have a similar response to training is unclear. Immune cells also express miRNAs that are known as myomiRs [43]. Therefore, immune fluctuations that occur during the menstrual cycle [8, 56] could influence miRNA responses to exercise training in women. The four myomiRs in this study were chosen a priori as they were regulated by exercise in our previous work [18]. While this is not a major limitation and can be viewed as a strength because it was hypothesis driven, we are planning to analyze the genome-wide response to small non-coding RNAs isolated from whole blood in future experiments. Finally, we did not measure the mRNA targets of the miRNAs and do not know which tissues they may post-transcriptionally regulate to influence exercise physiology. These limitations require addressing in future investigations.

In conclusion, we found a marked reduction in the abundance of miRNAs (miR-1-3p, miR-133a/b-3p and miR-486-5p) in whole blood samples from men after a 6-wk SIT intervention. These findings further support the premise that circulating miRNAs could serve as biomarkers of exercise responses and likely play important roles in health and performance adaptations to exercise. To our surprise, no statistically significant changes were observed in miRNA abundance 30 min after the cessation of a single session of SIT, which could suggest a discordant miRNA response between anaerobic and aerobic exercise and emphasizes that time-course studies are required to address this possibility. Such studies will enable us move towards molecular biomarkers of exercise traits and personalized exercise prescription.

Acknowledgements

This study was financially supported by the School of Science and Technology.

Conflict of Interest

The authors have no conflict of interest to declare.

References

[1] Alvarez-Garcia I, Miska EA. MicroRNA functions in animal development and human disease. Development 2005; 132: 4653–4662

[2] Arroyo JD, Chevillet JR, Kroh EM, Ruf IK, Pritchard CC, Gibson DF, Mitchell PS, Bennett CF, Pogosova-Agadjanyan EL, Stirewalt DL, Tait JF, Tewari M. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proc Natl Acad Sci USA 2011; 108: 5003–5008

[3] Baggish AL, Hale A, Weiner RB, Lewis GD, Systrom D, Wang F, Wang TJ, Chan SY. Dynamic regulation of circulating microRNA during acute exhaustive exercise and sustained aerobic exercise training. J Physiol 2011; 589: 3983–3994

[4] Banzet S, Chennaoui M, Girard O, Racinais S, Drogou C, Chalabi H, Koulmann N. Changes in circulating microRNAs levels with exercise modality. J Appl Physiol 2013; 115: 1237–1244

[5] Barwari T, Joshi A, Mayr M. MicroRNAs in Cardiovascular Disease. J Am Coll Cardiol 2016; 68: 2577–2584

[6] Berezikov E. Evolution of microRNA diversity and regulation in animals. Nat Rev Genet 2011; 12: 846–860

[7] Berezikov E, Thuemmler F, van Laake LW, Kondova I, Bontrop R, Cuppen E, Plasterk RH. Diversity of microRNAs in human and chimpanzee brain. Nat Genet 2006; 38: 1375–1377

[8] Bouman A, Heineman MJ, Faas MM. Sex hormones and the immune response in humans. Hum Reprod Update 2005; 11: 411–423

[9] Burgomaster KA, Howarth KR, Phillips SM, Rakobowchuk M, Macdonald MJ, McGee SL, Gibala MJ. Similar metabolic adaptations during exercise after low volume sprint interval and traditional endurance training in humans. J Physiol 2008; 586: 151–160

[10] Burgomaster KA, Hughes SC, Heigenhauser GJ, Bradwell SN, Gibala MJ. Six sessions of sprint interval training increases muscle oxidative potential and cycle endurance capacity in humans. J Appl Physiol 2005; 98: 1985–1990

[11] Bye A, Rosjo H, Aspenes ST, Condorelli G, Omland T, Wisloff U. Circulating microRNAs and aerobic fitness--the HUNT-Study. PLoS One 2013; 8: e57496

[12] Cochran AJ, Percival ME, Tricarico S, Little JP, Cermak N, Gillen JB, Tarnopolsky MA, Gibala MJ. Intermittent and continuous high-intensity exercise training induce similar acute but different chronic muscle adaptations. Exp Physiol 2014; 99: 782–791

[13] Cui SF, Wang C, Yin X, Tian D, Lu QJ, Zhang CY, Chen X, Ma JZ. Similar responses of circulating microRNAs to acute high-intensity interval exercise and vigorous-intensity continuous exercise. Front Physiol 2016; 7: 102

[14] Davidsen PK, Gallagher IJ, Hartman JW, Tarnopolsky MA, Dela F, Helge JW, Timmons JA, Phillips SM. High responders to resistance exercise training demonstrate differential regulation of skeletal muscle microRNA expression. J Appl Physiol 2011; 110: 309–317

[15] Denham J. Exercise and epigenetic inheritance of disease risk. Acta Physiol (Oxf) 2017, doi:10.1111/apha.12881

[16] Denham J, O'Brien BJ, Marques FZ, Charchar FJ. Changes in the leukocyte methylome and its effect on cardiovascular-related genes after exercise. J Appl Physiol 2015; 118: 475–488

[17] Denham J, O'Brien BJ, Prestes PR, Brown NJ, Charchar FJ. Increased expression of telomere-regulating genes in endurance athletes with long leukocyte telomeres. J Appl Physiol 2016; 120: 148–158

[18] Denham J, Prestes PR. Muscle-enriched microRNAs isolated from whole blood are regulated by exercise and are potential biomarkers of cardiorespiratory fitness. Front Genet 2016; 7: 196

[19] Dweep H, Gretz N, Sticht C. miRWalk database for miRNA-target interactions. Methods Mol Biol 2014; 1182: 289–305

[20] Elia L, Contu R, Quintavalle M, Varrone F, Chimenti C, Russo MA, Cimino V, De Marinis L, Frustaci A, Catalucci D, Condorelli G. Reciprocal regulation of microRNA-1 and insulin-like growth factor-1 signal transduction cascade in cardiac and skeletal muscle in physiological and pathological conditions. Circulation 2009; 120: 2377–2385

[21] Ferland-McCollough D, Ozanne SE, Siddle K, Willis AE, Bushell M. The involvement of microRNAs in Type 2 diabetes. Biochem Soc Trans 2010; 38: 1565–1570

[22] Gallagher IJ, Scheele C, Keller P, Nielsen AR, Remenyi J, Fischer CP, Roder K, Babraj J, Wahlestedt C, Hutvagner G, Pedersen BK, Timmons JA. Integration of microRNA changes in vivo identifies novel molecular features of muscle insulin resistance in type 2 diabetes. Genome Med 2010; 2: 9

[23] German MA, Pillay M, Jeong DH, Hetawal A, Luo S, Janardhanan P, Kannan V, Rymarquis LA, Nobuta K, German R, De Paoli E, Lu C, Schroth G, Meyers BC, Green PJ. Global identification of microRNA-target RNA pairs by parallel analysis of RNA ends. Nat Biotechnol 2008; 26: 941–946

[24] Guerra-Assuncao JA, Enright AJ. Large-scale analysis of microRNA evolution. BMC Genomics 2012; 13: 218

[25] Guller I, Russell AP. MicroRNAs in skeletal muscle: their role and regulation in development, disease and function. J Physiol 2010; 588: 4075–4087

[26] Harriss DJ, Atkinson G. Ethical Standards in Sport and Exercise Science Research: 2016 Update. Int J Sports Med 2015; 36: 1121–1124

[27] Hecksteden A, Leidinger P, Backes C, Rheinheimer S, Pfeiffer M, Ferrauti A, Kellmann M, Sedaghat F, Meder B, Meese E, Meyer T, Keller A. miRNAs and sports: tracking training status and potentially confounding diagnoses. J Transl Med 2016; 14: 219

[28] Huntzinger E, Izaurralde E. Gene silencing by microRNAs: Contributions of translational repression and mRNA decay. Nat Rev Genet 2011; 12: 99–110

[29] Jansson MD, Lund AH. MicroRNA and cancer. Mol Oncol 2012; 6: 590–610

[30] Jung HJ, Suh Y. Circulating miRNAs in ageing and ageing-related diseases. J Genet Genomics 2014; 41: 465–472

[31] Karolina DS, Armugam A, Tavintharan S, Wong MT, Lim SC, Sum CF, Jeyaseelan K. MicroRNA 144 impairs insulin signaling by inhibiting the expression of insulin receptor substrate 1 in type 2 diabetes mellitus. PLoS One 2011; 6: e22839

[32] Kilian Y, Wehmeier UF, Wahl P, Mester J, Hilberg T, Sperlich B. Acute response of circulating vascular regulating micrornas during and after high-intensity and high-volume cycling in children. Front Physiol 2016; 7: 92

[33] Kozomara A, Griffiths-Jones S. miRBase: integrating microRNA annotation and deep-sequencing data. Nucleic Acids Res 2011; 39: D152–D157

[34] Landgraf P, Rusu M, Sheridan R, Sewer A, Iovino N, Aravin A, Pfeffer S, Rice A, Kamphorst AO, Landthaler M, Lin C, Socci ND, Hermida L, Fulci V, Chiaretti S, Foa R, Schliwka J, Fuchs U, Novosel A, Muller RU, Schermer B, Bissels U, Inman J, Phan Q, Chien M, Weir DB, Choksi R, De Vita G, Frezzetti D, Trompeter HI, Hornung V, Teng G, Hartmann G, Palkovits M, Di Lauro R, Wernet P, Macino G, Rogler CE, Nagle JW, Ju J, Papavasiliou FN, Benzing T, Lichter P, Tam W, Brownstein MJ, Bosio A, Borkhardt A, Russo JJ, Sander C, Zavolan M, Tuschl T. A mammalian microRNA expression atlas based on small RNA library sequencing. Cell 2007; 129: 1401–1414

[35] Lim LP, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, Castle J, Bartel DP, Linsley PS, Johnson JM. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature 2005; 433: 769–773

[36] Lin S, Gregory RI. MicroRNA biogenesis pathways in cancer. Nat Rev Cancer 2015; 15: 321–333

[37] Liu X, Xiao J, Zhu H, Wei X, Platt C, Damilano F, Xiao C, Bezzerides V, Bostrom P, Che L, Zhang C, Spiegelman BM, Rosenzweig A. miR-222 is necessary for exercise-induced cardiac growth and protects against pathological cardiac remodeling. Cell Metab 2015; 21: 584–595

[38] Mitchelson KR, Qin WY. Roles of the canonical myomiRs miR-1, -133 and -206 in cell development and disease. World J Biol Chem 2015; 6: 162–208

[39] Nie Y, Sato Y, Wang C, Yue F, Kuang S, Gavin TP. Impaired exercise tolerance, mitochondrial biogenesis, and muscle fiber maintenance in miR-133a-deficient mice. FASEB J 2016; 30: 3745–3758

[40] Ogasawara R, Akimoto T, Umeno T, Sawada S, Hamaoka T, Fujita S. MicroRNA expression profiling in skeletal muscle reveals different regulatory patterns in high and low responders to resistance training. Physiol Genomics 2016; 48: 320–324

[41] Olivieri F, Capri M, Bonafe M, Morsiani C, Jung HJ, Spazzafumo L, Vina J, Suh Y. Circulating miRNAs and miRNA shuttles as biomarkers: Perspective trajectories of healthy and unhealthy aging. Mech Ageing Dev 2016, doi:10.1016/j.mad.2016.12.004

[42] Orom UA, Nielsen FC, Lund AH. MicroRNA-10a binds the 5'UTR of ribosomal protein mRNAs and enhances their translation. Mol Cell 2008; 30: 460–471

[43] Panwar B, Omenn GS, Guan Y. miRmine: a database of human miRNA expression profiles. Bioinformatics 2017; 33: 1554–1560

[44] Parr EB, Camera DM, Burke LM, Phillips SM, Coffey VG, Hawley JA. Circulating microRNA responses between 'high' and 'low' responders to a 16-wk diet and exercise weight loss intervention. PLoS One 2016; 11: e0152545

[45] Polakovicova M, Musil P, Laczo E, Hamar D, Kyselovic J. Circulating microRNAs as potential biomarkers of exercise response. Int J Mol Sci 2016; 17: E1553

[46] Robinson MM, Dasari S, Konopka AR, Johnson ML, Manjunatha S, Esponda RR, Carter RE, Lanza IR, Nair KS. Enhanced protein translation underlies improved metabolic and physical adaptations to different exercise training modes in young and old humans. Cell Metab 2017; 25: 581–592

[47] Russell AP, Lamon S. Exercise, skeletal muscle and circulating microRNAs. Prog Mol Biol Transl Sci 2015; 135: 471–496

[48] Sapp RM, Shill DD, Roth SM, Hagberg JM. Circulating microRNAs in acute and chronic exercise: More than mere biomarkers. J Appl Physiol 2017; 122: 702–717

[49] Sayed AS, Xia K, Salma U, Yang T, Peng J. Diagnosis, prognosis and therapeutic role of circulating miRNAs in cardiovascular diseases. Heart Lung Circ 2014; 23: 503–510

[50] Soci UP, Fernandes T, Barauna VG, Hashimoto NY, Mota GF, Rosa KT, Irigoyen MC, Phillips MI, de Oliveira EM. Epigenetic control of exercise training-induced cardiac hypertrophy by miR-208. Clin Sci (Lond) 2016, doi:10.1042/CS20160480

[51] Tufekci KU, Meuwissen RL, Genc S. The role of microRNAs in biological processes. Methods Mol Biol 2014; 1107: 15–31

[52] Vasudevan S, Tong Y, Steitz JA. Switching from repression to activation: microRNAs can up-regulate translation. Science 2007; 318: 1931–1934

[53] Vickers KC, Rye KA, Tabet F. MicroRNAs in the onset and development of cardiovascular disease. Clin Sci (Lond) 2014; 126: 183–194

[54] Villanova L, Vernucci E, Pucci B, Pellegrini L, Nebbioso M, Mauri C, Marfe G, Spataro A, Fini M, Banfi G, Russo MA, Tafani M. Influence of age and physical exercise on sirtuin activity in humans. J Biol Regul Homeost Agents 2013; 27: 497–507

[55] Wardle SL, Bailey ME, Kilikevicius A, Malkova D, Wilson RH, Venckunas T, Moran CN. Plasma microRNA levels differ between endurance and strength athletes. PLoS One 2015; 10: e0122107

[56] Yovel G, Shakhar K, Ben-Eliyahu S. The effects of sex, menstrual cycle, and oral contraceptives on the number and activity of natural killer cells. Gynecol Oncol 2001; 81: 254–262

[57] Zhang J, Li S, Li L, Li M, Guo C, Yao J, Mi S. Exosome and exosomal microRNA: Trafficking, sorting, and function. Genomics Proteomics Bioinformatics 2015; 13: 17–24