Plasma levels of microRNA in chronic kidney disease:
patterns in acute and chronic exercise.

Van Craenenbroeck AH1,2,3, Ledeganck KJ3, Van Ackeren K4, Jürgens A3, Hoymans VY1,4,
Fransen E5, Adams V6, De Winter B3, Verpooten GA3, Vrints CJ1,4, Couttenye MM2,
Van Craenenbroeck EM1,4

Author contributions
research idea and study design: AVC, KL, VH, CV, VA, EVC
data analysis/interpretation: AVC, KL, EVC, VH, MC
statistical analysis: AVC, EVC, EF
data acquisition: AVC, KVA, AJ, BDW
supervision or mentorship: EVC, MC, CV, GV, BDW

Author affiliations
1 Laboratory of Cellular and Molecular Cardiology, Department of Cardiology, Antwerp University Hospital, Wilrijkstraat 10, 2650 Edegem, Belgium
2 Department of Nephrology, Antwerp University Hospital, Antwerp, Belgium
3 Laboratory of Experimental Medicine and Pediatrics, University of Antwerp, Antwerp, Belgium
4 Cardiovascular Diseases, Department of Translational Pathophysiological Research, University of Antwerp, Antwerp, Belgium
5 StatUA Center for Statistics, University of Antwerp, Antwerp, Belgium
6 Department of Internal Medicine/Cardiology, University of Leipzig, Leipzig, Germany
Running head

microRNA and exercise in chronic kidney disease

Corresponding author

Amaryllis Van Craenenbroeck
Antwerp University Hospital, Department of Nephrology
Wilrijkstraat 10, 2650 Edegem, Belgium
Tel: ++ 32 3 821 38 72
Fax: ++ 32 3 829 01 00
amaryllis.vancraenenbroeck@uantwerpen.be
ABSTRACT

Introduction Exercise training is an effective way to improve exercise capacity in chronic kidney disease (CKD) but the underlying mechanisms are only partly understood. In healthy subjects, microRNA (miR) are dynamically regulated following exercise and have therefore been suggested as regulators of cardiovascular adaptation to exercise. However, these effects were not studied in CKD before.

Methods The effect of acute exercise (i.e. an acute exercise bout) was assessed in 32 patients with CKD and 12 age- and sex-matched healthy subjects (HS, Study 1). MicroRNA expression in response to chronic exercise (i.e. a 3-months exercise training program) was evaluated in 40 CKD patients (Study 2). In a subgroup of Study 2, the acute-exercise induced effect was evaluated at baseline and at follow-up. Plasma levels of a preselected panel miRNAs, involved in exercise adaptation processes such as angiogenesis (miR-126, miR-210), inflammation (miR-21, miR-146a), hypoxia/ischemia (miR-21, miR-210) and progenitor cells (miR-150), were quantified by RT PCR. Additionally, 7 miRNAs involved in similar biological processes were quantified in the subgroup of Study 2.

Results Baseline, studied miR were comparable in CKD and HS. Following acute exercise, miR-150 levels increased in both CKD (fold change 2.12 ± 0.39, p=0.002 and HS: fold change 2.41 ± 0.48 p=0.018, p for interaction>0.05). miR-146a acutely decreased in CKD (fold change 0.92 ± 0.13, p=0.024), whereas it remained unchanged in HS. Levels of miR-21, miR-126 and miR-210 remained unaltered. Chronic exercise did not elicit a significant change in the studied miR levels. However, an acute exercise-induced decrease in miR-210 was observed in CKD patients, only after training (fold change 0.76 ± 0.15).
Conclusion The differential expression in circulating miRNA in response to acute and chronic exercise, may point towards a physiological role in cardiovascular adaptation to exercise, also in CKD.

NEW & NOTEWORTHY

This study offers novel insights into the role of circulating microRNA in exercise-induced adaptations in chronic kidney disease, which can eventually lead to the identification of targets for effective preventive strategies.

KEY WORDS: chronic kidney disease, microRNA, acute exercise, exercise training
INTRODUCTION

MicroRNAs (miRNA or miR) are short, endogenous, non-coding RNAs that negatively regulate gene expression at the posttranscriptional level. By targeting multiple genes, often involved in related signalling pathways, they are important for various aspects of homeostasis and disease, including cardiovascular and kidney disease [10, 31, 37]. Previously, it has been shown that their presence in plasma could be of great diagnostic and even prognostic value [9]. Apart from being released following cell death or injury, miRNAs can be selectively packaged inside intracellular exosomes, actively secreted into the circulation and transferred to other cells [39]. Therefore, it occurs that miRNAs are important for intercellular crosstalk, modulating protein expression of the recipient cells [13]. Moreover, miRNAs may represent novel therapeutic targets, since there is now ample evidence that miRNA levels in vivo can be regulated by the use of antimiRs [43].

MicroRNAs contribute to both development and progression of chronic kidney disease (CKD), and might add to the increased cardiovascular risk in these patients [24, 37]. The high prevalence of cardiovascular disease in CKD is the result of a complex interplay of several mechanisms, including endothelial dysfunction and vascular calcification [11]. Exercise training is an effective way to reduce this cardiovascular risk and to improve exercise capacity, also in CKD patients [15]. The analysis of miRNAs can improve our understanding of adaptations to exercise and training at the gene expression level in CKD.

In this regard, previous work has already demonstrated that both brief exercise and exercise training transiently or adaptively change several circulating miRNAs in athletes and healthy volunteers [3, 6]. However, little is known about baseline values and variation in miRNA levels following exercise in CKD patients. We tested the hypothesis that plasma profiles of specific miRNAs, involved in angiogenesis (miR-126, miR-210), inflammation (miR-21,
miR-146a), hypoxia/ischemia adaptation (miR-21, miR210) and progenitor cell biology (miR-150) are affected following both acute and chronic exercise in CKD.

The aims of the present study were 1) to examine the effects of acute exercise (i.e. an acute exercise bout) on plasma levels of miR-21, miR-126, miR-146a, miR-150 and miR-210 in patients with CKD compared to healthy controls and 2) to evaluate whether plasma levels of miR-21, miR-126, miR-146a, miR-150 and miR-210 at rest and after acute exercise are influenced by chronic exercise (i.e. an aerobic exercise training program of 3 months).

Additionally, the effect of chronic exercise on the acute exercise-induced response of 7 other miRNAs involved in angiogenesis (miR-17, miR-92a, miR-130, miR-24), hypoxia/ischemia adaptation (miR-24) and vascular calcification in CKD (miR-125b, miR-145 and miR-155) was studied in a subgroup of patients.
METHODS

Study population and design

The effect of acute exercise on plasma miR levels (Study 1) was studied in 32 ambulatory patients with CKD stage 1-5. Twelve healthy subjects (HS), without relevant medical history or pharmacological treatment, served as controls. All participants were called in for a cardiopulmonary exercise test (CPET), and refrained from excessive physical exertion 24 hours prior to the test. Venous blood was sampled immediately before and 10 minutes after peak exercise.

Next, the effect of chronic exercise on plasma miR levels (Study 2) was studied in 40 ambulatory patients with CKD stage 3-4. They were randomized into a 12-week aerobic training program (ET; n=19) or usual care (UC; n=21) as part of a single center randomized controlled trial [41]. A CPET was performed at baseline and after 12 weeks, and blood was sampled before the CPET. In a subgroup of patients (ET: n=13, UC: n=9), venous blood was sampled both at rest and 10 minutes after peak exercise, baseline and at 12 weeks follow-up (Figure 1).

For both studies, diagnosis of CKD was based on the estimated glomerular filtration rate (eGFR) using the CKD-EPI formula [17] and/or the presence of kidney injury as recommended by the National Kidney Foundation’s KDOQI guidelines [1]. The following exclusion criteria were applied: history of overt cardiovascular disease, pregnancy, renal replacement therapy, age <18 years, treatment with immunosuppressive or oral anticoagulation therapy and active malignant disease. The studies were approved by the ethics committee of the Antwerp University Hospital and conformed to the principles outlined in the declaration of Helsinki. Inclusion took place between April 2012 and July 2014. Written informed consent was obtained from all participants.
Acute exercise: cardiopulmonary exercise testing (CPET)

A maximal symptom-limited CPET was performed on a bicycle ergometer (Cardiovit CS-200 Ergo-Spiro, Schiller AG, Baar, Switzerland). An individualized ramp protocol, starting with either 20 or 40 Watt and an incremental load of 10 or 20 Watt per minute, was chosen to ensure an optimal exercise duration of 8-10 minutes. Twelve-lead ECG was recorded continuously and blood pressure was measured every 2 minutes. Breath-by-breath gas exchange measurements allowed on-line determination of ventilation (VE), oxygen uptake (VO₂) and carbon dioxide production (VCO₂). Peak oxygen consumption (VO₂peak) was determined as the highest attained VO₂ during the final 30 seconds of exercise. VO₂peak and maximal workload were also expressed as a percentage of the predicted value (% Predicted VO₂peak, % Predicted Wattmax), according to the nomogram of Hansen and Wasserman [12]. Subjects were encouraged to exercise upon exhaustion, according to the respiratory exchange ratio (RER) and identification of the anaerobic threshold (AT, V-slope method).

Chronic exercise: exercise training

Patients in the ET group underwent a 12-week home-based aerobic training next to their standard therapy (maintenance medication). The program consisted of 4 daily cycling sessions of 10 minutes on magnetically-braked hometrainers (DKN Mag410B, Belgium) using heart rate transmitters (Polar FT7) to obtain the target heart rate (90% of the heart rate at AT). Adherence was monitored monthly by reviewing heart rates during training and by detailed training logs. Patients in the UC group were given standard therapy, without specific instructions on physical activity. In both groups, medical therapy was unchanged during the study period.

Selection of the miR panel
Based on the literature, we initially selected 5 miRs being previously implicated in cellular processes underlying exercise adaptation: miR-21, miR-126, miR-146a, miR-150 and miR-210. Additionally, 7 miRNAs involved in the same relevant processes, were selected (Table 1).

**RNA extraction**
EDTA-treated blood samples were centrifuged within 30 minutes after collection and plasma was immediately stored at -80°C. To avoid technical variability, all samples from a given individual were processed and analyzed in a single batch. Stored plasma was thawed on ice and centrifuged at 4°C for 10 minutes (16000xg). Total RNA, including miRNA, was isolated from 200 µl plasma with the miRNeasy serum/plasma kit (Qiagen, Venlo, the Netherlands). A fixed amount of the synthetic Caenorhabditis elegans-miR-39 (Cel-miR-39) was added to the standard volume of 200 µl plasma, immediately after lysis with Qiazol, to test for sample-to-sample variation in RNA isolation. Total RNA was extracted using chloroform, ethanol and spin column and eluted in 15 µl RNAse-free water.

**Targeted quantification of miR**
Isolated RNA was used for multiplexed reverse transcription of mature miR-21, miR-126, miR-146a, miR-150 and miR-210 into cDNA using specific stem-loop primers (Applied Biosystems). Plasma levels of selected miR were quantified using real-time PCR via TaqMan probes (Applied Biosystems) in a Biorad CFX96 Real-Time PCR system. Exogenously added miR-Cel-39 was used as a spike-in normalisation control. Additional miRNAs were quantified in exactly the same way. All reactions and analyses were performed in duplo. The coefficient of variation (CV) accepted for intra-assay replicates was set at 4%. Ct values were used for relative miR quantification using the delta Ct method. Relative miR levels were expressed as
log (2^{-\Delta CT} \times 100). Fold change of the respective miRs were calculated (relative expression post intervention/relative expression pre intervention).

### Statistical analysis

Continuous data are expressed as mean ± standard deviation (SD) or ± SEM (miR data). Skewed data were logarithmically transformed. Baseline comparisons were performed using independent sample T-test or $X^2$ test. For correlations, Pearson (r) or Spearman’s (rho) correlation coefficients were used.

The response to acute and chronic exercise was assessed by linear mixed model analysis with the miR level as dependent variable and time and group as fixed-effect independent variables. Individual ID was entered as a random intercept, to account for the relatedness between observations within the same individual. To test whether the groups show a different response over time, the significance of the interaction term between time and group was calculated.

All statistical analysis was performed with R, version 3.1.2 (R core team 2014). Linear mixed models were fitted using the add-on package lme4.
RESULTS

Demographic and clinical variables

Demographic and clinical characteristics for Study 1 are shown in Table 2. Patients and HS were matched for age and sex. Severity of CKD ranged from stage 1-5, with the majority in stage 3 (6-22-38-35-9% respectively for CKD stage 1-5). Both HS and CKD patients performed a maximal exercise test, as was objectified by a RER value > 1.15. Aerobic exercise capacity and maximal workload were significantly lower in CKD compared to HS.

Demographic and clinical characteristics for Study 2 are shown in Table 3. Groups were comparable at baseline, including variables of exercise capacity.

Baseline levels of miR in relation to eGFR

All miRs were at a detectable level; baseline plasma levels of all microRNA were comparable between CKD patients and HS in Study 1 (Figure 2). In univariate analysis, a significant correlation was found between miR-146a levels and eGFR, with higher expression levels in more severe renal disease (rho -0.252, p=0.050).

Effect of acute exercise

Following a single exercise bout, the plasma expression level of miR-150 was significantly upregulated in both CKD patients (fold change 2.12 ± 0.39, p=0.002) and in HS (fold change 2.41 ± 0.48, p=0.018). On the other hand, the expression of miR-146a decreased significantly in CKD patients (fold change 0.92 ± 0.13, p=0.024), whereas it remained unchanged in HS (p=0.83) (Figure 3). Plasma levels of miR-21, miR-126 and miR-210 remained unaltered in both groups.
The acute exercise-induced change in miR-150 levels was correlated with VO₂peak (rho 0.326, p=0.043). No correlation was found between VO₂peak and changes in miR146a (rho 0.245, p=0.192) (Figure 4).

Effect of chronic exercise in CKD patients

After a formal exercise training program, VO₂peak (+ 5.82 ml/kg/min) and maximal workload (+ 37 Watt) improved significantly in the ET group compared to the UC group (p for interaction <0.001)[41]. However, training did not result in a significant change of any of the studied miRs in comparison with the usual care group (p for interaction>0.05, Table 4).

Response to acute exercise following exercise training

To evaluate whether the acute exercise-induced changes in miR are comparable before and after a 12 week training period, a linear mixed model analysis was performed in a subgroup of patients of Study 2 (ET, n=13; UC, n=9). In a second analysis, the response of miR-17, miR-24, miR-92a, miR-125b, miR-130a, miR-145 and miR-155 was also studied in these patients. Figure 5 presents the fold change in response to acute exercise of miR-21, miR-126, miR-146a, miR-150 as well as miR-125b. Only for miR-210, exercise training resulted in a different response to an acute exercise bout. Whereas an acute exercise bout did not elicit an effect in the UC group, a significant decrease in miR-210 levels following acute exercise was observed in ET group after the training program was completed (fold change 0.76 ± 0.15, p for interaction=0.045). Moreover, this acute exercise-induced decrease in miR-210 levels correlated with an increase in VO₂ peak (rho -0.236, p<0.05).

In the studied population, plasma levels of miR-150 and miR-125b significantly increase following an acute exercise bout, both at baseline and follow-up. In contrast with the findings of Study 1, miR-146a did not decrease following acute exercise in the studied subjects.
Plasma levels of miR-17, miR-24, miR-92a, miR-130a, miR-145 and miR-155 remained unaffected by both acute and chronic exercise (data not shown).
This study describes the effect of acute (single exercise bout) and chronic exercise (exercise training) on plasma levels of specific vascular-related miRs in CKD. In addition, the effect of exercise training on the acute exercise-induced response is studied to unravel mechanisms for adaptation to exercise.

Several new findings emerge from this study:

- At baseline, plasma levels of miR-21, miR-126, miR-146a, miR-150 and miR-210 are comparable between CKD patients and healthy subjects. For miR-146a, an inverse correlation was found between plasma levels of miR-146a and eGFR in univariate analysis.

- Acute exercise significantly upregulates plasma levels of miR-150, and this both in CKD and in healthy subjects. Next, in CKD patients only, acute exercise results in a significant decrease in miR-146a. Plasma levels of miR-125b significantly increase following acute exercise, both in sedentary and CKD patients.

- In CKD patients, chronic exercise results in a significant decrease of miR-210 in response to an acute exercise bout.

Dysregulation of miRNA in CKD

To date, limited information is available on plasma levels of miRNA potentially implicated in the pathophysiology of impaired exercise capacity in patients with CKD. The group of Neal and co-workers described reduced levels of total circulating microRNA and five specific miRNAs (including miR-21 and miR-210) in renal failure, at least for patients with end-stage renal disease (eGFR < 15 ml/min/1.73m²)
and for those with CKD stage 4 (eGFR 15-30 ml/min/1.73m²) [23]. Other evidence of lower circulating miR levels in CKD came from the study of Chen et al, who found decreased circulating levels of miR-125b, miR-145 and miR-155 (all affecting vascular smooth muscle cell proliferation) with deterioration of renal function in patients with CKD stage 3-4 (19).

In the present study, the circulating levels of miR-21, miR-126, miR146a, miR-150 and miR-210, were comparable between CKD patients (stage 1-5) and healthy controls. Differences in the studied populations (disease severity and comorbidities) could contribute to this finding. While the group of Neal included a considerable amount of end-stage renal disease patients, the majority of patients in the current study suffered from CKD stage 3-4. Moreover, they report that the decrease in levels of miR-21 and miR-210 takes place at CKD stage 4 at the earliest. Whereas the study of Chen concentrates on the issue of vascular disease in CKD and therefore describes a population with objective CV disease, the present study only investigated patients without cardiovascular disease, which is only a small and very selective subgroup of CKD patients.

In the present study, we focused on vascular-related miRNA since endothelial dysfunction and arterial stiffness are characteristic features of CKD inferring adverse prognosis. We found a relation between worse renal function and higher plasma levels of miR-146a. miR-146a is an inflammation-associated miRNA and can be induced by different pro-inflammatory stimuli, such as IL-1ß, TNF-a and Toll-like receptors (TLR)[30, 34]. IRAK-1 and TRAF6 have been identified as target genes of miR-146a posttranslational repression, proposed as a negative feedback mechanism of TLR and cytokine receptor signaling [34]. As such, the expression of miR-146a might be part
of a mechanism for restraining the excessive production of pro-inflammatory cyto- or chemokines in inflammatory states, such as CKD. Tissue miR-146a is upregulated in various inflammatory human diseases such as rheumatoid arthritis [18, 22, 25] and atherosclerosis [27]. In a murine model of CKD, miR-146a expression in the kidneys has been associated with the development of interstitial lesions and correlated with inflammatory cell infiltration [14]. As such, the potential of miR-146a as disease biomarker in CKD is promising, but should be validated in larger studies.

**Effects of acute exercise**

The dynamic regulation of circulating miRNA in response to a single exercise bout could offer insights into the beneficial effects of exercise training. Originating from endothelial, blood and muscle cells, miRNAs can be released into the circulation - packed into microvesicles or being part of protein complexes- in response to exercise to carry information from one cell to another [2, 26, 46, 49]. As demonstrated in healthy subjects, miRNAs are differentially regulated in response to exercise, which suggests a role for miRNAs in (vascular) adaptation to exercise. The prompt regulation of miRNAs within 10 minutes of maximal exercise might be due to an accelerated posttranscriptional processing of premature miRNA, to an expedited secretion into the circulation or to an aspecific leakage through cell damage [38, 45]. On the other hand, downregulation can be due to uptake of the specific miRNA into recipient cells (eg elicited by exercise-induced leukocytosis) or reduced secretion.

miR-146a is an inflammation-associated miRNA and can be induced by different pro-inflammatory stimuli [30, 34]. We showed that there was an inverse correlation between eGFR and miR-146a levels, with higher levels in more advanced renal
disease. Interestingly, acute exercise resulted in a normalisation of miR-146a levels, at least in Study 1. It is possible that this decrease reflects a swift uptake of miR-146a by monocytes with subsequent downregulation of TLR4 in these recipient cells, but it is clear that this hypothesis needs further exploration [35, 40]. In Study 2, the acute exercise-induced decrease in miR-146a could not be confirmed, possibly due to the lower basal plasma levels of these studied patients.

miR-150 is involved in endothelial progenitor cell biology, and exercise training is known to beneficially affect the mobilization and migration of endothelial progenitor cells (EPC)[28, 42]. We observed a significant upregulation of miR-150 after acute exercise, both in healthy subjects and patients with CKD. It has been shown that microvesicles isolated from plasma of patients with atherosclerosis contain higher levels of miR-150, and that these microvesicles more effectively promote the migration of human dermal microvascular endothelial cells (HMEC-1) than microvesicles from healthy donors [50]. In line, miR-150 was found to support the migration and tube formation ability of EPC in vitro and to enhance their homing ability in vivo in a c-Myb dependent manner [47]. Recently, miR-150 was also found to be a modulator of physiological cardiac hypertrophy in response to exercise [21]. Whether increase of plasma miR-150 levels relates to cardiovascular exercise-induced adaptation, i.e.; left ventricular hypertrophy or the increased mobilization of endothelial progenitor cells, remains to be experimentally validated in an in vivo animal model of physical exercise.

Next, we observed a significant increase in miR-125b levels in CKD patients. Low expression levels of miR-125b (both at the plasma and vascular tissue level) has been
indirectly linked to the process of osteoblastic differentiation of the vascular smooth muscle cells, resulting in vascular calcification in patients with CKD [7]. miR-125b is known to inhibit the Runt-related transcription factor 2 (RUNX2) mediated osteoblastic differentiation of mesenchymal cells, possibly through regulation of nuclear factor kappa beta (NF-kB)[16]. Whether this exercise-induced increase witnesses or mediates the beneficial effect of exercise still needs to be established.

Unlike the observations of Baggish et al (1.89 ± 0.28 fold increase in miR-21 in 10 male athletes immediately post exercise) and Uhlemann et al (2.0 ± 0.2 fold increase in miR-126 in 13 healthy volunteers 5 minutes after exercise), miR-21 and miR-126 were unresponsive to acute exercise in our cohort of 12 healthy subjects and 32 CKD patients [3, 38]. Differences in the exercise protocols, sampling time and participants’ characteristics could possibly account for this discrepancy. Following acute exercise, the level of miR-210 did not change in plasma, which is in accordance with the findings of Baggish et al.[3]

Effects of chronic exercise

Chronic exercise did not result in a significant change in plasma levels of any of the studied miRNAs. However, the miR-210 response to acute exercise was altered in CKD patients who had completed a 3-month exercise training program. Hypoxia-inducible factor 1a (HIF1a) has been identified both as inductor and target of miR-210, suggesting a key role for miR-210 in a negative feedback loop [44]. The HIF signaling cascade mediates the effects of hypoxia at the cellular and tissue level by upregulation of glycolysis enzymes and promotion of VEGF-induced angiogenesis respectively. At the level of the skeletal muscle, an increase in HIF-1a levels is seen
after acute exercise, and this effect is blunted after exercise training [20]. The mirror image happens in the circulation, with an absent response to acute exercise of miR-210 and a significant decrease elicited by an acute exercise bout following training. It is plausible that miR-210 has an essential role in the blunted effect on HIF-1 following training, as the adaptation process could imply rapid uptake by muscle cells of miR-210, leading to a persistent downregulation of HIF-1, also when minimal hypoxic state would be present.

In healthy individuals, higher miR-210 levels are directly related to a lower VO$_2$peak [6]. This relation was confirmed in our CKD patients: the higher the acute exercise-induced decrease in miR-210 levels, the higher the improvement in peak oxygen uptake after training. Therefore, adaptive mechanisms to a hypoxic state seem to contribute to the beneficial exercise-induced adaptations, which underlie the increase in aerobic capacity post training.

Study limitations

In the present study, the effects of acute exercise were assessed 10 minutes after peak exercise. Future studies evaluating the time course of miR responses to acute as well as chronic exercise, might shed more light on the beneficial effects of exercise training and point toward novel therapeutic targets in CKD. However, before we can expect implementation on this level, underlying mechanisms have to be clarified, for example by loss-of-function models (antimiR) in a rodent model of physical exercise.

Conclusion

The findings of this study extent the previous observation in healthy volunteers and athletes by showing that circulating miRNA may also play a role in exercise-induced
adaptations in the context of CKD. Our data demonstrate that the expression levels of miR-125b, miR-146a, miR-150 and miR210 change following acute and chronic exercise albeit with a differential profile. miR-150 shows similar changes in CKD patients and healthy controls whereas the change in miR-146a is disease-specific. Moreover, we suggest a role for miR-210 in favorable exercise-induced cardiovascular adaptations. Whether these changes in miR expression patterns underlie a true mechanistic explanation or may serve as a biomarker of cell damage remains to be elucidated in future studies.
ACKNOWLEDGEMENTS

EVC is supported by the Research Foundation – Flanders (FWO) as a senior clinical investigator. No financial disclosures were reported by the other authors of this paper.
REFERENCES


47. Wang W., Li C., Li W., Kong L., Qian A., Hu N., Meng Q., Li X. (2014) MiR-150 enhances the motility of EPCs in vitro and promotes EPCs homing and thrombus resolving in vivo. Thromb Res. 133, 590-598


(A) In Study 1, the effect of acute exercise (CPET) on plasma miR levels was studied in 32 CKD patients and 12 HS. (B) In Study 2, the effect of chronic exercise on plasma miR levels was studied as part of a single center randomized controlled trial. In total, 40 patients with CKD stage 3-4 were involved and measurements took place at randomisation and after 12 weeks of aerobic training (n=19) or usual care (n=21). In a sub study of patients (EX n=13; UC n=9), the effect of chronic exercise on the acute exercise-induced response on plasma miR was evaluated to unravel mechanisms for adaptation to exercise.

CPET= cardiopulmonary exercise testing; HS= healthy subjects; CKD= chronic kidney disease; ET= exercise training; UC= usual care.
Figure 2. Circulating miR at steady state in CKD and healthy subjects (Study 1)

Data in this figure are expressed as the mean logarithm of the relative expression (logRE) of the respective miR ± SEM. Baseline plasma levels of all studied microRNA were comparable between the studied CKD patients (n=32) and healthy subjects (n=12).

HS = healthy subjects; CKD = chronic kidney disease.
Figure 3. Effect of acute exercise on plasma levels of miRNA (Study 1)

Data in this figure are expressed as the mean logarithm of the relative expression (logRE) of the respective miR ± SEM. Plasma levels of miR were quantified immediately before and 10 minutes after peak exercise in CKD patients (n=32) and healthy subjects (n=12). In both CKD patients and HS, plasma levels of miR-150 were significantly upregulated following acute exercise (blue line). The expression of miR-146a decreased significantly in CKD patients (green line) but remained unchanged in HS. Plasma levels of miR-21, miR-126 and miR-210 remained unaltered in both groups.

HS = healthy subjects; CKD = chronic kidney disease.
Figure 4. Relation between VO\textsubscript{2}\text{peak} and acute exercise-induced changes in miR-146a and miR-150 *(Study 1)*

The acute exercise-induced change in miR-150 levels was significantly correlated with miR-150 levels, but not miR-146a levels.
Figure 5. Response to acute exercise: effect of exercise training (Study 2)

Data in this figure are expressed as mean fold change ± SEM. The effect of chronic exercise on the response to an acute exercise bout was evaluated in 22 CKD patients (EX n=13; UC n=9). Only for miR-210, exercise training resulted in a different response to an acute exercise bout (p for interaction <0.05). Indeed, a significant decrease in miR-210 was seen only in the trained patients. Next, the figure shows a significant increase following acute exercise of circulating miR-150 and miR-125b in the studied population.

CKD PR = CKD pre-randomization; ET = exercise training; UC = usual care.
Table 1. Overview of the studied miRNAs.

<table>
<thead>
<tr>
<th>microRNA</th>
<th>Biological process</th>
<th>Selected validated target genes</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-21</td>
<td>Inflammation</td>
<td>PTEN, PDC4, BCL-2</td>
<td>[3, 6, 36]</td>
</tr>
<tr>
<td></td>
<td>Apoptosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hypoxia/ischemia adaptation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-126</td>
<td>Angiogenesis</td>
<td>Spred-1, PI3KR2, SDF-1</td>
<td>[8, 38, 48]</td>
</tr>
<tr>
<td>miR-146a</td>
<td>Inflammation</td>
<td>IRAK-1, TRAF6, CXCR4, TLR4</td>
<td>[3, 29, 30, 34, 48]</td>
</tr>
<tr>
<td>miR-150</td>
<td>Hematopoiesis</td>
<td>CXCR4, MYB, FLT3, CBL, EGR2, AKT2 and DKC</td>
<td>[21, 47, 50]</td>
</tr>
<tr>
<td></td>
<td>Progenitor cell mobilisation and migration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-210</td>
<td>Angiogenesis</td>
<td>HIF-1a</td>
<td>[3, 6, 44]</td>
</tr>
<tr>
<td></td>
<td>Hypoxia</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Proliferation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-17</td>
<td>Angiogenesis</td>
<td>E2F1</td>
<td>[4, 5, 33]</td>
</tr>
<tr>
<td></td>
<td>Apoptosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-24</td>
<td>Hypoxia</td>
<td>H2A histone family, member X, heme oxygenase 1</td>
<td>[19]</td>
</tr>
<tr>
<td></td>
<td>Apoptosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-92a</td>
<td>Angiogenesis</td>
<td>integrin subunit 5</td>
<td>[4, 5, 33]</td>
</tr>
<tr>
<td>miR</td>
<td>Function</td>
<td>Gene(s)</td>
<td>Reference(s)</td>
</tr>
<tr>
<td>-----------</td>
<td>---------------------</td>
<td>---------------</td>
<td>--------------</td>
</tr>
<tr>
<td>miR-125b</td>
<td>Vascular calcification</td>
<td>NFkB</td>
<td>[7]</td>
</tr>
<tr>
<td>miR-130a</td>
<td>Angiogenesis</td>
<td>GAX, HoxA5</td>
<td>[32]</td>
</tr>
<tr>
<td>miR-145</td>
<td>Vascular calcification</td>
<td>myocardin</td>
<td>[7]</td>
</tr>
<tr>
<td>miR-155</td>
<td>Vascular calcification</td>
<td>AT1R</td>
<td>[7, 33]</td>
</tr>
</tbody>
</table>
Table 2. Baseline demographic and clinical variables of participants in Study 1

<table>
<thead>
<tr>
<th></th>
<th>CKD (n=32)</th>
<th>HS (n=12)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>49.6 ± 15.3</td>
<td>43.4 ± 4.7</td>
<td>0.179</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>18/14</td>
<td>5/7</td>
<td>0.388</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.7 ± 5.3</td>
<td>23.7 ± 2.2</td>
<td>0.012</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73 m²)</td>
<td>46.2 ± 23.8</td>
<td>101.4 ± 9.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>125 ± 15</td>
<td>125 ± 13</td>
<td>0.935</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>81 ± 12</td>
<td>79 ± 9</td>
<td>0.520</td>
</tr>
<tr>
<td>VO\textsubscript{2} peak (ml/kg/min)</td>
<td>26.1 ± 8.0</td>
<td>38.5 ± 9.8</td>
<td>0.002</td>
</tr>
<tr>
<td>% predicted VO\textsubscript{2} peak</td>
<td>85.2 ± 24.3</td>
<td>109.3 ± 22.9</td>
<td>0.008</td>
</tr>
<tr>
<td>VO\textsubscript{2} at anaerobic threshold (ml/kg/min)</td>
<td>24.3 ± 7.1</td>
<td>32.2 ± 8.9</td>
<td>0.027</td>
</tr>
<tr>
<td>Maximal workload (Watt)</td>
<td>150.4 ± 53.2</td>
<td>259.6 ± 89.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Exercise duration (sec)</td>
<td>398 ± 117</td>
<td>790 ± 238</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Respiratory exchange ratio</td>
<td>1.36 ± 0.11</td>
<td>1.27 ± 0.09</td>
<td>0.021</td>
</tr>
<tr>
<td>Resting heart rate (bpm)</td>
<td>74 ± 12</td>
<td>75 ± 13</td>
<td>0.826</td>
</tr>
<tr>
<td>Peak heart rate (bpm)</td>
<td>154 ± 23</td>
<td>175 ± 11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Medication</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE-I</td>
<td>13 (41)</td>
<td>0</td>
<td>0.007</td>
</tr>
<tr>
<td>ARB</td>
<td>6 (18)</td>
<td>0</td>
<td>0.128</td>
</tr>
<tr>
<td>Statin</td>
<td>13 (41)</td>
<td>0</td>
<td>0.007</td>
</tr>
</tbody>
</table>
Data are mean ± SD or number (percentage). BMI= body mass index; eGFR= estimated glomerular filtration rate; VO_{2\text{peak}}= peak oxygen uptake; ACE-I= angiotensin converting enzyme inhibitor; ARB= angiotensin receptor blocker
### Table 3. Baseline demographic and clinical variables of participants in Study 2

<table>
<thead>
<tr>
<th></th>
<th>UC (n=21)</th>
<th>ET (n=19)</th>
<th><em>p</em>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>54.7 ± 14.1</td>
<td>51.5 ± 11.8</td>
<td>0.441</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>10/11</td>
<td>8/11</td>
<td>0.726</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.3 ± 5.8</td>
<td>28.3 ± 6.2</td>
<td>0.965</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73 m²)</td>
<td>42.2 ± 14.9</td>
<td>40.2 ± 15.2</td>
<td>0.665</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>123 ± 16</td>
<td>129 ± 17</td>
<td>0.259</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>79 ± 11</td>
<td>81 ± 13</td>
<td>0.740</td>
</tr>
<tr>
<td>VO₂peak (ml/kg/min)</td>
<td>24.4 ± 6.6</td>
<td>26.4 ± 5.4</td>
<td>0.287</td>
</tr>
<tr>
<td>Medication</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE-I</td>
<td>8 (38)</td>
<td>12 (63)</td>
<td>0.102</td>
</tr>
<tr>
<td>ARB</td>
<td>9 (43)</td>
<td>4 (21)</td>
<td>0.129</td>
</tr>
<tr>
<td>Statin</td>
<td>10 (48)</td>
<td>12 (63)</td>
<td>0.252</td>
</tr>
</tbody>
</table>

Data are mean ± SD or number (percentage). UC = usual care; ET = exercise training; BMI = body mass index; eGFR = estimated glomerular filtration rate; VO₂peak = peak oxygen uptake; ACE-I = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker.
Table 4. Effect of chronic exercise on plasma levels of miRNA

<table>
<thead>
<tr>
<th></th>
<th>ET (n=19)</th>
<th></th>
<th>UC (n=21)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>baseline</td>
<td>3 month</td>
<td>p-value</td>
<td>baseline</td>
<td>3 month</td>
<td>p-value</td>
</tr>
<tr>
<td></td>
<td>follow-up</td>
<td></td>
<td></td>
<td>follow-up</td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR21</td>
<td>2.15 ± 0.22</td>
<td>2.04 ± 0.3</td>
<td>0.538</td>
<td>2.28 ± 0.25</td>
<td>2.18 ± 0.18</td>
<td>0.477</td>
</tr>
<tr>
<td>miR126</td>
<td>5.97 ± 0.33</td>
<td>5.72 ± 0.33</td>
<td>0.472</td>
<td>6.32 ± 0.30</td>
<td>6.06 ± 0.26</td>
<td>0.212</td>
</tr>
<tr>
<td>miR146a</td>
<td>5.53 ± 0.36</td>
<td>4.94 ± 0.41</td>
<td>0.047</td>
<td>5.66 ± 0.33</td>
<td>5.27 ± 0.27</td>
<td>0.113</td>
</tr>
<tr>
<td>miR150</td>
<td>5.40 ± 0.25</td>
<td>5.26 ± 0.32</td>
<td>0.552</td>
<td>5.44 ± 0.23</td>
<td>5.49 ± 0.24</td>
<td>0.842</td>
</tr>
<tr>
<td>miR210</td>
<td>0.90 ± 0.39</td>
<td>0.61 ± 0.29</td>
<td>0.222</td>
<td>0.82 ± 0.32</td>
<td>0.49 ± 0.26</td>
<td>0.233</td>
</tr>
</tbody>
</table>

Data are expressed as the logarithm of the relative expression of the respective miR ± SEM.

ET = exercise training, UC = usual care.
A. CKD stage 1-5

- PRE
- CPET
- POST

Baseline → 3 month follow-up

- HS (n=12); CKD (n=32)

B. CKD stage 3-4

- PRE
- CPET
- POST

Baseline → 3 month follow-up

- ET (n=19); UC (n=21)

- ET (n=13); UC (n=9)
HS

CKD

logRE

logRE

miR-126

miR-150

miR-146a

miR-150

miR-210

PRE

POST
fold change mi-146a

VO_{2\text{peak}}

rho = 0.245
p = 0.192

fold change miR-150

VO_{2\text{peak}}

rho = 0.326
p = 0.043