Universiteit Antwerpen

This item is the archived peer-reviewed author-version of:

Impaired vascular function contributes to exercise intolerance in chronic kidney disease

Reference:

Van Craenenbroeck Amaryllis, van Craenenbroeck Emeline, Van Ackeren Katrijn, Hoymans Vicky, Verpooten Gert A., Vrints Christiaan, Couttenye Marie Madeleine.- Impaired vascular function contributes to exercise intolerance in chronic kidney disease Nephrology, dialysis, transplantation - ISSN 0931-0509 - 310:12(2016), p. 2064-2072
Full text (Publishers DOI): http://dx.doi.org/doi:10.1093/NDT/GFW303
To cite this reference: http://hdl.handle.net/10067/1399260151162165141

uantwerpen.be

Institutional repository IRUA

IMPAIRED VASCULAR FUNCTION CONTRIBUTES TO EXERCISE INTOLERANCE IN CHRONIC KIDNEY DISEASE

Amaryllis H Van Craenenbroeck[1,3,4]; Emeline M Van Craenenbroeck[2,3]; Katrijn Van Ackeren[3]; Vicky Y Hoymans[3]; Gert A Verpooten[4]; Christiaan J Vrints[2,3]; Marie M Couttenye[1]

[1]*Department of Nephrology, Antwerp University Hospital, Edegem, Belgium*

[2]*Department of Cardiology, Antwerp University Hospital, Edegem, Belgium*

[3]*Laboratory for Molecular and Cellular Cardiology, University of Antwerp, Edegem, Belgium*

[4]*Laboratory of Experimental Medicine and Pediatrics, University of Antwerp, Edegem, Belgium*

Corresponding author:

Amaryllis Van Craenenbroeck, MD PhD

Department of Nephrology, Antwerp University Hospital

Wilrijkstraat 10, 2650 Edegem, Belgium

tel: ++ 32 3 821 38 72

fax: ++ 32 3 829 01 00

amaryllis.vancraenenbroeck@uantwerpen.be

ABSTRACT

Background. Exercise intolerance is an important feature in patients with CKD and is prognostic for both increased morbidity and mortality. Little is known about underlying mechanisms in predialysis CKD. This study aimed to gain more insight in the role of vascular dysfunction in the exercise intolerance of predialysis CKD. In addition, vascular-related microRNA – as epigenetic regulators of exercise capacity – were analysed.

Methods. Sixty-three patients with CKD stage 1-5 and 18 healthy controls were included. Peak oxygen consumption (VO_2 peak) was determined by cardiorespiratory exercise testing, endothelial function by flow-mediated dilation (FMD) and arterial stiffness by carotid-femoral pulse wave velocity (PWV). Plasma miRNA levels (miR-21, miR-126, miR-146a, miR-150 and miR-210) were quantified by qRT-PCR.

Results. Peak oxygen consumption was already impaired in mild CKD (stage 1-3A) and significantly correlated with eGFR ($r=0.525$, $p<0.001$). Likewise, both FMD and PWV were significantly correlated with eGFR (respectively $r=0.319$, $p=0.007$ and $r=-0.365$, $p=0.001$). In multiple regression analysis, PWV remained one of the strongest independent determinants of VO_2 peak (ß=-0.301, $p=0.01$). Of the studied miRNA, circulating levels of miR-146a and miR-150 correlated with eGFR, PWV and VO_2peak, but the association with the latter was lost when correcting for PWV.

Conclusions. Arterial stiffness contributes to the observed reduced aerobic capacity in predialysis CKD, independent of age, hemoglobin levels and endothelial function, and represents a promising therapeutic target for improving exercise capacity in this population. Future work is required to get more insight in both cause and effect of the

observation that higher circulating levels of miR-146a and miR-150 are associated with impaired renal function and increased arterial stiffness.

KEY WORDS

Arterial stiffness, chronic kidney disease, endothelial dysfunction, exercise intolerance, microRNA

SUMMARY

Exercise intolerance is an integral feature of CKD, with a debilitating impact on quality of life, morbidity and mortality. This study offers novel insights in the underlying mechanisms and suggests that arterial stiffness is a promising new therapeutic target for improving exercise capacity in this population. Circulating miR-146a and miR-150 may be involved in the process of arterial stiffness, but further research is warranted to elucidate their origin and pathophysiological role.

INTRODUCTION

Cardiovascular disease is the main cause of morbidity and mortality in patients with chronic kidney disease (CKD) [1]. The risk of death, cardiovascular events and hospitalization sharply rises when the estimated glomerular filtration rate (eGFR) drops below 45ml/min/1.73m^2, with even a higher risk for cardiovascular events than progression to end stage renal disease in these patients [2]. Exercise intolerance is an important feature in patients with CKD [3], and its value as a prognostic factor for both increased morbidity and mortality is well established [4-6]. Reduced physical fitness results in the inability to perform activities in daily life and occupational tasks, with huge effects on quality of life [7]. In addition, the vulnerable CKD patient will become prone to a sedentary lifestyle, being trapped in a vicious circle of fatigue and reduced physical functioning [8].

Peak oxygen consumption (VO_2peak), the gold standard to assess exercise capacity, is determined by the product of cardiac output and arteriovenous oxygen difference. Exercise intolerance could be explained by the variables that influence these factors. Hence, both oxygen delivery mechanisms (cardiac output, peripheral vascular function, erythrocyte count) as well as oxygen utilizing factors (skeletal muscle) possibly contribute to reduced exercise capacity. In the setting of CKD, the presence of uremic myopathy and anemia are well-known determinants of exercise intolerance [9, 10]. The contribution of vascular dysfunction, characterized by endothelial dysfunction and arterial stiffness, has been largely overlooked, despite the fact that they are attractive therapeutic targets to improve exercise intolerance. Moreover, both endothelial dysfunction and arterial stiffness are directly related to increased cardiovascular mortality in CKD [11, 12].

MicroRNAs (miRNAs) are endogenous, non-coding single-stranded RNAs, which repress gene expression at the post-transcriptional level [13, 14]. MiRNAs that are detected in the circulation are released following cell death or injury, but are also actively secreted and carry genetic information from one cell to another, thereby functioning as critical regulators of cellular crosstalk [15]. As such, they are involved in a variety of cardiovascular functions, but their role in CKD-associated vascular disease is only beginning to emerge [14, 16]. Recently, Bye et al reported that certain miR are strongly related to aerobic capacity in healthy subjects [17]. Studying vascular-related miR in exercise intolerant CKD patients is promising as it could lead to the identification of biomarkers and novel therapeutic targets.

In this observational cross-sectional study we aimed to gain more insight in the determinants of exercise intolerance in patients with CKD, with a focus on vascular dysfunction. Therefore, we first explored whether endothelial dysfunction and arterial stiffness are independent determinants of exercise capacity in CKD. Second, we investigated the relation between circulating levels of vascular-related miRs and both exercise capacity and vascular function in CKD.

MATERIALS AND METHODS

Subjects

Ambulatory patients with the diagnosis of CKD according to the KDOQI guidelines, regardless of severity and cause, were systematically screened for eligibility at the moment of visiting the outpatient clinic of the Antwerp University Hospital. Of the 966 patients assessed in the period between April 2012 and July 2014, 63 patients were included in the study **(Figure 1).** None of them was participating in a formal exercise trial. Diagnosis of CKD was based on the estimated glomerular filtration rate (eGFR) using the CKD-EPI formula [18] and/or the presence of kidney injury as recommended by the National Kidney Foundation's KDOQI guidelines [19]. Patients with a history of overt cardiovascular disease, including coronary artery disease, peripheral vascular disease and cerebrovascular disease, were excluded. Other exclusion criteria were renal replacement therapy, pregnancy, age <18 years, treatment with immunosuppressive or oral anticoagulation therapy and active malignant disease.

Eighteen healthy subjects without relevant medical history, pharmacological treatment and no abnormalities on exercise testing, were asked to participate. The study was approved by the ethics committee of the Antwerp University Hospital, conformed to the principles outlined in the Declaration of Helsinki and all participants gave written informed consent.

Study design

All study participants were called in at the morning for successive blood and urine sampling, vascular assessment, ECG and cardiopulmonary exercise testing (CPET).

Participants were asked to refrain from food, caffeine and excessive physical exertion for 12 hours prior to the study.

In CKD patients, absence of structural heart disease was confirmed by transthoracic echocardiography. Systolic and diastolic function was assessed through measurement of left ventricular ejection fraction (LVEF, modified Simpson rule), left ventricular end-diastolic diameter (LVEDD) and diastolic function (E/é).

Cardiopulmonary exercise test

A symptom-limited cardiopulmonary exercise test (CPET) was performed on a bicycle ergometer (Cardiovit CS-200 Ergo-Spiro, Schiller AG, Baar, Switzerland). A ramp protocol, starting with an equivalent of 20 or 40 Watt and incremental steps equivalent of 10 or 20 Watt/min was used. Twelve-lead ECG was recorded continuously and blood pressure was measured baseline and every 2 minutes. Peak oxygen consumption (VO_2peak) was expressed as the highest attained VO_2 during the final 30 seconds of exercise. Maximal work-economy was defined as maximal workload at VO_2peak (Wattmax/VO_2peak). Online analysis of VE/VO_2 and VE/VCO_2 curves permitted to encourage patients to exercise till exhaustion, confirmed by a respiratory exchange ratio (RER) > 1.10.

Blood sampling and analysis

Serum creatinin, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, iron, total iron binding capacity (TIBC), transferrin saturation and high sensitive C-reactive protein (hs-CRP) were quantified using routine laboratory techniques (Dimension Vista 1500 System, Siemens). Complete blood count was measured on an Advia Haematology Analyzer (ADVIA 2120,

Siemens Healthcare Diagnostics). Plasma samples (EDTA) were immediately stored at -80 °C for later quantification of miR (batch analysis). In all patients, the urine protein-to-creatinin ratio was evaluated in the spot morning urine sample.

Vascular assessments

All measurements were performed between 8:00 and 11:00 am in a quiet room with a stable temperature of 21-24 °C.

Endothelial-dependent vasodilation

Endothelial-dependent vasodilation was assessed by flow-mediated dilation (FMD) of the brachial artery using ultrasound (10 MHz Utrasound Doppler probe, AU5 ultrasound System, Esaote, Biomedica, Genova, Italy) according to the guidelines. [20, 21]. After measuring baseline internal diameter, a pneumatic tourniquet, placed 1-2 cm distal to the elbow, was inflated to 200 mmHg or at least 50 mmHg above peak systolic pressure for 5 minutes. After cuff release, diameter was recorded continuously during 4 minutes and FMD was expressed as % dilation from baseline to maximal post-occlusion diameter. Endothelial-independent vasodilation was measured after sublingual administration of 0.4 mg glyceryl trinitrate (GTN-MD). Off-line analyses were done with FMD-I software by a single trained investigator.

Arterial stiffness

Carotid-femoral pulse wave velocity (PWV) was measured using the SphygmoCor device (AtCor Medical, West Ryde, Australia) according to the current guidelines.[22, 23] The system uses a single high-fidelity applanation tonometer (Millar Instruments, Texas) to obtain a proximal (i.e. carotid) and distal pulse (i.e. femoral) recorded consecutively, and calculates PWV from the transit time between the two arterial sites as PWV = distance (meters)/transit time (seconds). As such, higher PWV values

represent stiffer arteries. All measurements were done in triplicate and were repeated when they did not meet the quality control guidelines, as defined by the manufacturer.

Targeted quantification of miR

A panel of 5 miRNAs, reported in literature to be involved in vascular homeostasis, was designed and included miR-21, miR-126, miR-146a, miR-150 and miR-210 [24]. Stored plasma samples were thawed on ice and centrifuged at 4°C for 10 minutes (16000xg). Total RNA, including miRNA, was isolated from 200 µl EDTA plasma with the miRNeasy serum/plasma kit (Qiagen, Venlo, the Netherlands). To test for sample-to-sample variation in RNA isolation, a fixed amount of the synthetic Cel-miR-39 was added to the sample, immediately after lysis with Qiazol. Total RNA was extracted using chloroform, ethanol and spin column and eluted in 15 µl RNAse-free water. An aliquot of the 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). 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 synthetic miR-Cel-39 was used as a spike-in normalisation control. All reactions and analyses were performed in duplo. The threshold 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 ($2^{-\Delta CT}$*100).

Statistical analysis

Data are expressed as mean ± standard deviation (SD). Normality was assessed with one-sample Kolmogorov-Smirnov and logarithmic transformation was applied where

necessary. Differences between the three groups were analyzed using Chi-square test or by one-way ANOVA followed by the Sidak post-hoc test for multiple comparison corrections. Where applicable, comparison between the two CKD groups was done with the Chi-square test or independent samples T-test. Bivariate correlations were measured by Pearson's correlation analysis. Stepwise multiple linear regression analysis was used to evaluate independent determinants of VO_2peak, adjusting for all significant determinants on bivariate correlation analysis.

All tests were two-sided, and a P-value of <0.05 was considered statistically significant. All analyses were performed using PASW Statistics 22.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Demographic and clinical characteristics

Subject characteristics are listed in **Table 1**. Patients were stratified in 2 groups, according to eGFR < or > 45 ml/min/1.73m^2, an established value below which the cardiovascular risk sharply increases [2]. Apart from higher BMI in CKD patients, the presence of traditional risk factors, summarized in the Framingham risk score, was similar between groups, taking into account that 50% of CKD patients were on statin and/or antihypertensive therapy. Etiology of kidney disease differed between groups with unique functional kidney and ADPKD more represented in the group with eGFR>45 ml/min/1.73m^2, whereas in the group with eGFR<45 ml/min/1.73m^2, IgA nephropathy and reflux pathology were the most frequent causes. Parameters of systolic and diastolic function were comparable between groups.

Despite the fact that 19% of the patients with eGFR<45ml/min/1.73m^2 were on erythropoietin substitution and they were non-anemic, their hemoglobin levels were lower compared to the other subjects.

Exercise capacity, peripheral vascular function and circulating microRNA in relation to estimated GFR

Exercise capacity

All patients and healthy subjects performed a maximal exercise test (RER>1.10). Compared to healthy subjects, all CKD patients displayed a significant reduction in VO_2peak, VO_2 at anaerobic threshold and exercise duration and this already in early stages of kidney disease **(Table 2).**

Peripheral vascular function

Flow-mediated dilation, as measure for endothelial-dependent vasodilation, was significantly reduced in patients with eGFR<45ml/min/1.73m^2, compared to healthy subjects **(Table 2).** In these patients, endothelial-independent vasodilation (GTN-MD) was also significantly impaired when compared to healthy subjects. Arterial stiffness, measured by PWV, was significantly higher in patients with eGFR<45 ml/min/1.73m^2. In bivariate analysis, a positive correlation was found between eGFR and both logFMD (r=0.319, p=0.007) and GTN-MD (r=0.407, p=0.001). Estimated GFR was inversely correlated with PWV (r=-0.365, p=0.001).

Circulating miRNA

Of the studied miRNAs, levels of miR-150 were significantly higher in CKD patients compared to healthy subjects, and this both in patients with eGFR < and > 45 ml/min/m^2 **(Table 2).** A significant negative correlation was found with eGFR for both miR-150 and miR-146a (respectively, r=-0.284, p=0.01 and r=-0.253, p=0.03).

Determinants of peak aerobic capacity

Correlation analysis showed that VO_2peak was related to age, eGFR, hemoglobin, FMD and PWV. To identify whether these factors determined VO_2peak independently of each other, a multiple linear regression analysis was performed. **(Table 3).** From these determinants, eGFR and arterial stiffness (PWV) were the sole that were independently associated with VO_2peak.

Circulating microRNA, peak aerobic capacity and PWV

Table 4 gives an overview of the bivariate correlations of circulating microRNA with VO_2peak and PWV, respectively. No association was found with FMD. Both miR-146a and miR-150 positively correlated with PWV **(Figure 2).** For VO_2peak, a

negative correlation was found with circulating levels of miR-146a, miR-150 and miR-210. However, the relation with miR-146a and miR-150 with VO_2peak was not independent of PWV, as became apparent in a multivariable regression model (analysis not shown).

DISCUSSION

The present study investigates whether endothelial dysfunction and arterial stiffness are independent determinants of peak aerobic capacity in CKD patients. In addition, the possible role of vascular-related miRs was evaluated.

Two new findings emerge from this study:

1. Peak aerobic capacity, a strong prognostic factor for increased cardiovascular risk, is impaired already early in the course of CKD. Arterial stiffness and endothelial function both emerge as prominent features in CKD, but only arterial stiffness contributes independently to reduced peak aerobic capacity.
2. The observed covariance of the levels of circulating miR-146a and miR-150 on the one hand, and increased arterial stiffness and low peak aerobic capacity on the other hand, might be a first step in the identification of new biomarkers and/or novel therapeutic targets.

Exercise intolerance: an early and multifactorial feature in chronic kidney disease

Whereas prior studies clearly demonstrated a significantly reduced exercise capacity and increased cardiovascular risk in patients with severe renal impairment (defined as eGFR<45 ml/min/1.73m^2) [3], data on exercise capacity in patients with mild impairment are scarce and often generated in patients with co-existing cardiovascular disease [25]. In this study, by inclusion of patients with CKD stages 1 to 5, we revealed a strong inverse relation between eGFR and peak aerobic capacity and this in patients without established cardiovascular disease.

In the last decades, efforts have been made to unravel the underlying mechanisms for the observed exercise intolerance of CKD patients, which is determined by a myriad

of factors [26] **(Figure 3).** First, impaired physical performance as such is known to induce a vicious circle of fatigue, decreased quality of life and reduction in physical functioning, [8] which is aggravated further by anomalies in different physiological functions. Based on the Fick equation, VO_2peak is the product of cardiac output and arterial-venous oxygen difference. In this regard, researchers mainly focused on oxygen extraction (utilization), and skeletal muscle disorder in particular. Indeed, proximal myopathy of the lower extremities is common in end stage renal disease [27] and subclinical histopathological abnormalities may occur in the pre-dialysis stages [28]. Renal anemia is another factor that contributes to decreased exercise capacity [29]. However, correction of renal anemia by recombinant erythropoietin does not normalize maximal exercise capacity [30-32]. The cardiovascular system is responsible for oxygen delivery to the working muscles, and cardiac output in CKD might be compromised in CKD, both by cardiac autonomic dysfunction and/or systolic/diastolic dysfunction [33, 34]. Next, the capacity of peripheral vessels to dilate in response to increased shear stress during exercise emerged as an important determinant of exercise capacity in several patient groups, such as patients with chronic heart failure (CHF) [35, 36] and coronary artery disease (CAD) [37].

It is essential to clarify the key factors limiting exercise capacity in this patient group. The focus on vascular dysfunction as potential culprit in exercise intolerance is one of the novelties of this study, as it could become a target for intervention strategies. It appears that reduced exercise capacity is already evident at an early stage of CKD (eGFR > 45ml/min/1.73m^2), and that it can be predicted by the presence of arterial stiffness, even in the absence of established cardiovascular disease. Therefore, tackling arterial stiffness may represent a promising new therapeutic target for improving exercise capacity in these patients.

MiR-146a and miR-150 as circulating markers for increased arterial stiffness

This is the first study to show that high levels of circulating miR-146a and miR-150 are associated with impaired renal function, increased arterial stiffness and lower aerobic capacity. The association with the latter appeared to be not independent of arterial stiffness.

Under physiological as well as stress-dependent conditions, miRNAs are released by different cell types into the circulation, where they are present in a remarkably stable form [38, 39]. Next to active and specific secretion, cell damage resulting in apoptosis may also be a source of circulating miRNAs, leading to a modified transcriptional program when taken up by recipient cells [40]. For example, upon endothelial damage, endothelial apoptotic bodies were shown to carry miR-126, with this raising alarm in recipient adjacent endothelial cells and attracting progenitor cells [41]. Next, higher circulating miRNA levels might reflect aspecific leakage following massive cell damage [42], leakage following higher miR transcription during cellular adaptation, or an accelerated posttranscriptional processing of premature miRNA [43].

Apart from its essential regulatory function in hematopoiesis, miR-150 has a role in progenitor cell mobilisation and migration by targeting CXCR4 (CXC chemokine receptor 4) and c-MYB [44]. It has been implicated in cardiovascular conditions other than CKD. Microvesicles isolated from the plasma of patients with atherosclerosis contained higher levels of miR-150 compared to healthy controls [45]. Recently, a high level of miR-150 was shown to have potential as a prognostic marker of unstable angina [46]; its dysregulation being involved in acute myocardial infarction [47].

In turn, 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) [48]. IRAK-1 and TRAF6 were identified as target genes of miR-146a posttranslational repression, suggesting a negative feedback mechanism of TLR and cytokine receptor signaling [48]. Expression of miR-146a is upregulated in PBMC of patients with CAD [49], in human atherosclerotic plaques [50], and in serum of patients with diabetes mellitus type 2 [51]. Recent *in vitro* evidence shows that miR-146a can be implicated in vascular medial calcification [52], but this has not been studied yet in the setting of CKD.

The observed covariance of the levels of circulating miR-146a and miR-150 on the one hand, and increased arterial stiffness and low peak aerobic capacity on the other hand, might be a first step in the identification of new biomarkers and/or novel therapeutic targets. However, further work is necessary to elucidate the cellular origin and biological processes involved in the release of miR-146a and miR-150 in CKD. Next, their biological effect on target recipient cells (endothelial or vascular smooth muscle cells) should be confirmed before they can be regarded as valuable therapeutic targets.

This study is limited in its observational design to conclude about causal relationships, and future studies are warranted to identify probable targets to adequately reduce cardiovascular complications in CKD.

In conclusion, patients with CKD have reduced exercise capacity, which is strongly associated with the stage of CKD and the presence of arterial stiffness, independent of age, hemoglobin levels and endothelial function. Higher circulating levels of miR-146a and miR-150 are associated with arterial stiffness in CKD. Future research is warranted to elucidate both the origin of these circulating miRNAs, as well as their modulating capacity as part of intercellular communication.

ACKNOWLEDGEMENTS

This manuscript is dedicated to Professor Viviane Conraads (16/07/1963 – 12/12/2013), former leader of our research group. The authors thank Bert Ectors and Miek Meeussen for performing cardiopulmonary exercise tests and Tibor Stoop for performing the vascular function tests.

TRANSPARENCY DECLARATION

The co-authors have all contributed to this manuscript and approve of this submission. The authors declare no conflicts of interest. Neither this manuscript nor substantial parts of it are under consideration for publication elsewhere, been published nor made available elsewhere. Part of this work was presented as an abstract on the 52nd ERA EDTA Congress.

REFERENCES

1. Gansevoort R.T., Correa-Rotter R., Hemmelgarn B.R., et al. Chronic kidney disease and cardiovascular risk: epidemiology, mechanisms, and prevention. Lancet 2013; 382: 339-352
2. Go A.S., Chertow G.M., Fan D., McCulloch C.E., Hsu C.Y. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. The New England journal of medicine 2004; 351: 1296-1305
3. Johansen K.L., Painter P. Exercise in individuals with CKD. Am J Kidney Dis 2012; 59: 126-134
4. Johansen K.L., Kaysen G.A., Dalrymple L.S., et al. Association of physical activity with survival among ambulatory patients on dialysis: the Comprehensive Dialysis Study. Clin J Am Soc Nephrol 2013; 8: 248-253
5. Sietsema K.E., Amato A., Adler S.G., Brass E.P. Exercise capacity as a predictor of survival among ambulatory patients with end-stage renal disease. Kidney Int 2004; 65: 719-724
6. Ting S.M., Iqbal H., Kanji H., et al. Functional cardiovascular reserve predicts survival pre-kidney and post-kidney transplantation. J Am Soc Nephrol 2014; 25: 187-195
7. Heiwe S., Clyne N., Dahlgren M.A. Living with chronic renal failure: patients' experiences of their physical and functional capacity. Physiother Res Int 2003; 8: 167-177
8. Heiwe S., Jacobson S.H. Exercise training for adults with chronic kidney disease. Cochrane Database Syst Rev 2011; CD003236
9. Kosmadakis G.C., Bevington A., Smith A.C., et al. Physical exercise in patients with severe kidney disease. Nephron Clin Pract 2010; 115: c7-c16
10. Nishikawa M., Ishimori N., Takada S., et al. AST-120 ameliorates lowered exercise capacity and mitochondrial biogenesis in the skeletal muscle from mice with chronic kidney disease via reducing oxidative stress. Nephrol Dial Transplant 2015; 30: 934-942
11. Hirata Y., Sugiyama S., Yamamoto E., et al. Endothelial function and cardiovascular events in chronic kidney disease. Int J Cardiol 2014; 173: 481-486
12. London G.M., Blacher J., Pannier B., et al. Arterial wave reflections and survival in end-stage renal failure. Hypertension 2001; 38: 434-438

410 13. Chen X., Ba Y., Ma L., et al. Characterization of microRNAs in serum: a novel
411 class of biomarkers for diagnosis of cancer and other diseases. Cell Res 2008; 18:
412 997-1006
413 14. Small E.M., Olson E.N. Pervasive roles of microRNAs in cardiovascular biology.
414 Nature 2011; 469: 336-342
415 15. Hergenreider E., Heydt S., Treguer K., et al. Atheroprotective communication
416 between endothelial cells and smooth muscle cells through miRNAs. Nat Cell
417 Biol 2012; 14: 249-256
418 16. Chen N.X., Kiattisunthorn K., O'Neill K.D., et al. Decreased microRNA is
419 involved in the vascular remodeling abnormalities in chronic kidney disease
420 (CKD). PLoS One 2013; 8: e64558
421 17. Bye A., Rosjo H., Aspenes S.T., et al. Circulating microRNAs and aerobic fitness-
422 -the HUNT-Study. PLoS One 2013; 8: e57496
423 18. Levey A.S., Stevens L.A., Schmid C.H., et al. A new equation to estimate
424 glomerular filtration rate. Ann Intern Med 2009; 150: 604-612
425 19. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation,
426 classification, and stratification. Am J Kidney Dis 2002; 39: S1-266
427 20. Corretti M.C., Anderson T.J., Benjamin E.J., et al. Guidelines for the ultrasound
428 assessment of endothelial-dependent flow-mediated vasodilation of the brachial
429 artery: a report of the International Brachial Artery Reactivity Task Force.
430 Journal of the American College of Cardiology 2002; 39: 257-265
431 21. Thijssen D.H., Black M.A., Pyke K.E., et al. Assessment of flow-mediated
432 dilation in humans: a methodological and physiological guideline. Am J Physiol
433 Heart Circ Physiol 2011; 300: H2-12
434 22. Laurent S., Cockcroft J., Van Bortel L., et al. Expert consensus document on
435 arterial stiffness: methodological issues and clinical applications. Eur Heart J
436 2006; 27: 2588-2605
437 23. Determinants of pulse wave velocity in healthy people and in the presence of
438 cardiovascular risk factors: 'establishing normal and reference values'. Eur Heart J
439 2010; 31: 2338-2350
440 24. Van Craenenbroeck A.H., Ledeganck K.J., Van Ackeren K., et al. Plasma levels
441 of microRNA in chronic kidney disease: patterns in acute and chronic exercise.
442 Am J Physiol Heart Circ Physiol 2015; 309: H2008-2016

443 25. McManus D., Shlipak M., Ix J.H., Ali S., Whooley M.A. Association of cystatin
444 C with poor exercise capacity and heart rate recovery: data from the heart and
445 soul study. Am J Kidney Dis 2007; 49: 365-372
446 26. Painter P. Exercise in chronic disease: physiological research needed. Exerc Sport
447 Sci Rev 2008; 36: 83-90
448 27. Johansen K.L., Shubert T., Doyle J., et al. Muscle atrophy in patients receiving
449 hemodialysis: effects on muscle strength, muscle quality, and physical function.
450 Kidney Int 2003; 63: 291-297
451 28. Heiwe S., Clyne N., Tollback A., Borg K. Effects of regular resistance training on
452 muscle histopathology and morphometry in elderly patients with chronic kidney
453 disease. Am J Phys Med Rehabil 2005; 84: 865-874
454 29. Odden M.C., Whooley M.A., Shlipak M.G. Association of chronic kidney disease
455 and anemia with physical capacity: the heart and soul study. J Am Soc Nephrol
456 2004; 15: 2908-2915
457 30. Clyne N., Jogestrand T. Effect of erythropoietin treatment on physical exercise
458 capacity and on renal function in predialytic uremic patients. Nephron 1992; 60:
459 390-396
460 31. Painter P., Moore G., Carlson L., et al. Effects of exercise training plus
461 normalization of hematocrit on exercise capacity and health-related quality of life.
462 Am J Kidney Dis 2002; 39: 257-265
463 32. Macdougall I.C., Lewis N.P., Saunders M.J., et al. Long-term cardiorespiratory
464 effects of amelioration of renal anaemia by erythropoietin. Lancet 1990; 335:
465 489-493
466 33. Klein D.A., Katz D.H., Beussink-Nelson L., et al. Association of Chronic Kidney
467 Disease With Chronotropic Incompetence in Heart Failure With Preserved
468 Ejection Fraction. Am J Cardiol 2015; 116: 1093-1100
469 34. Clyne N., Hellberg M., Kouidi E., Deligiannis A., Hoglund P. Relationship
470 between declining GFR and measures of cardiac and vascular autonomic
471 neuropathy. Nephrology (Carlton) 2015;
472 35. Hambrecht R., Fiehn E., Weigl C., et al. Regular physical exercise corrects
473 endothelial dysfunction and improves exercise capacity in patients with chronic
474 heart failure. Circulation 1998; 98: 2709-2715

475 36. Conraads V.M., Van Craenenbroeck E.M., De Maeyer C., et al. Unraveling new
476 mechanisms of exercise intolerance in chronic heart failure: role of exercise
477 training. Heart Fail Rev 2013; 18: 65-77
478 37. Conraads V.M., Pattyn N., De Maeyer C., et al. Aerobic interval training and
479 continuous training equally improve aerobic exercise capacity in patients with
480 coronary artery disease: The SAINTEX-CAD study. Int J Cardiol 2015; 179:
481 203-210
482 38. Mitchell P.S., Parkin R.K., Kroh E.M., et al. Circulating microRNAs as stable
483 blood-based markers for cancer detection. Proc Natl Acad Sci U S A 2008; 105:
484 10513-10518
485 39. Mause S.F., Weber C. Microparticles: protagonists of a novel communication
486 network for intercellular information exchange. Circ Res 2010; 107: 1047-1057
487 40. Camussi G., Deregibus M.C., Bruno S., et al. Exosome/microvesicle-mediated
488 epigenetic reprogramming of cells. Am J Cancer Res 2011; 1: 98-110
489 41. Zernecke A., Bidzhekov K., Noels H., et al. Delivery of microRNA-126 by
490 apoptotic bodies induces CXCL12-dependent vascular protection. Sci Signal
491 2009; 2: ra81
492 42. Wang K., Zhang S., Marzolf B., et al. Circulating microRNAs, potential
493 biomarkers for drug-induced liver injury. Proc Natl Acad Sci U S A 2009; 106:
494 4402-4407
495 43. Uhlemann M., Mobius-Winkler S., Fikenzer S., et al. Circulating microRNA-126
496 increases after different forms of endurance exercise in healthy adults. Eur J Prev
497 Cardiol 2014; 21: 484-491
498 44. He Y., Jiang X., Chen J. The role of miR-150 in normal and malignant
499 hematopoiesis. Oncogene 2014; 33: 3887-3893
500 45. Zhang Y., Liu D., Chen X., et al. Secreted monocytic miR-150 enhances targeted
501 endothelial cell migration. Mol Cell 2010; 39: 133-144
502 46. Zeller T., Keller T., Ojeda F., et al. Assessment of microRNAs in patients with
503 unstable angina pectoris. Eur Heart J 2014; 35: 2106-2114
504 47. Bostjancic E., Zidar N., Glavac D. MicroRNA microarray expression profiling in
505 human myocardial infarction. Dis Markers 2009; 27: 255-268
506 48. Taganov K.D., Boldin M.P., Chang K.J., Baltimore D. NF-kappaB-dependent
507 induction of microRNA miR-146, an inhibitor targeted to signaling proteins of
508 innate immune responses. Proc Natl Acad Sci U S A 2006; 103: 12481-12486

509 49. Takahashi Y., Satoh M., Minami Y., et al. Expression of miR-146a/b is associated
510 with the Toll-like receptor 4 signal in coronary artery disease: effect of renin-
511 angiotensin system blockade and statins on miRNA-146a/b and Toll-like receptor
512 4 levels. Clin Sci (Lond) 2010; 119: 395-405
513 50. Raitoharju E., Lyytikainen L.P., Levula M., et al. miR-21, miR-210, miR-34a, and
514 miR-146a/b are up-regulated in human atherosclerotic plaques in the Tampere
515 Vascular Study. Atherosclerosis 2011; 219: 211-217
516 51. Kong L., Zhu J., Han W., et al. Significance of serum microRNAs in pre-diabetes
517 and newly diagnosed type 2 diabetes: a clinical study. Acta Diabetol 2011; 48:
518 61-69
519 52. Leopold J.A. MicroRNAs Regulate Vascular Medial Calcification. Cells 2014; 3:
520 963-980
521
522

TABLES

Table 1. Baseline demographics according to CKD severity

	HS (n=18)	**eGFR>45 ml/min/1.73m^2 (n=32)**	**eGFR<45 ml/min/1.73m^2 (n=31)**	*p-value*
Age (years)	54.9 ± 3.3	47.1 ± 13.9	53.4 ± 14.6	0.06
Sex (M/F)	10/8	16/16	16/15	0.9
SBP (mmHg)	125 ± 19	125 ± 16	126 ± 16	0.9
DBP (mmHg)	80 ± 11	83 ± 13	80 ± 10	0.6
BMI (kg/m^2)	23.8 ± 3.7	26.9 ± 5.1*	28.3 ± 5.4*	0.002
Active smokers	0	1 (3%)	1 (3%)	0.7
Past smoking	4 (22%)	8 (25%)	11 (35%)	0.7
Diabetes mellitus	0	1 (3%)	5 (16%)	0.06
Framingham risk (%)	4.1 ± 3.4	3.5 ± 4.7	5.9 ± 7.4	0.3
Characteristics of kidney disease				
Estimated GFR (ml/min/1.73m^2)	87.9 ± 8.5	63.6 ± 17.6*	24.8 ± 8.9*	<0.001
Urinary protein/creatinin ratio (mg/g creatinin)				<0.001
<30	18 (100%)	4 (12%)	2 (6%)	
30-300	0	25 (78%)	9 (29%)	
>300	0	3 (10%)	20 (65%)	
Etiology				0.02
Reflux nephropathy/lithiasis		2 (6%)	6 (19%)	
Unique functional kidney		10 (32%)	0	
Medication induced		0	1 (3%)	
Diabetic nephropathy	-	1 (3%)	3 (10%)	
Nephrangiosclerosis		8 (25%)	7 (22%)	
IgA nephropathy		2 (6%)	7 (22%)	
ADPKD		7 (22%)	4 (13%)	
FSGS		0	1 (3%)	
ADIKD		1 (3%)	1 (3%)	
Glomerulonephritis		1 (3%)	0	
RTA type 1		0	1 (3%)	

Laboratory measurements				
Total cholesterol (mg/dl)	205 ±41	188 ± 35	184 ± 34	0.1
HDL (mg/dl)	68 ± 19	57 ± 14*	48 ± 12*	<0.001
LDL (mg/dl)	123 ± 40	107 ± 26	106 ± 27	0.1
Triclycerides (mg/dl)	93.5 ± 40.9	123.6 ± 63.3	153.5 ± 69.8*	0.006
Hemoglobin (g/dl)	14.02 ± 1.13	13.87 ± 1.29	12.78 ± 1.19*	<0.001
Serum iron (mg/dl)	113 ± 39	104 ± 35	77 ± 25*	<0.001
TIBC (mg/dl)	334 ± 37	326 ± 51	304 ± 57	0.09
Transferrin saturation (%)	33 ± 10	32 ± 11	26 ± 10*	0.02
Ferritin	151 ± 73	117 ± 93	137 ± 120	0.5
CRP	0.25 ± 0.23	0.25 ± 0.24	0.38 ± 0.33	0.1
hs-CRP (mg/dl)	0.17 ± 0.25	0.21 ± 0.26	0.36 ± 0.34	0.06
Echocardiography				
LVEF (%)	-	65.8 ± 9.2	66.7 ± 10.1	0.7
LVEDD (mm)		45.9 ± 6.4	47.2 ± 7.6	0.5
E/é		10.1 ± 3.2	10.6 ± 2.4	0.5
Medication				
Diuretics	-	5 (16%)	8 (26%)	0.2
ACE-I/ARB		23 (72%)	21 (67%)	0.5
Betablockers		12 (60%)	15 (48%)	0.3
Calcium-channel blockers		10 (31%)	15 (48%)	0.1
EPO		1 (3%)	5 (16%)	0.09
Iron substitution		0	6 (19%)	0.05
ASA		4 (13%)	5 (16%)	0.5
Statin		15 (47%)	16 (52%)	0.5

*Data are mean ± SD or number (percentage). *Different from HS, p<0.05*

HS= healthy subjects; eGFR= estimated glomerular filtration rate; SBP= systolic blood pressure; DBP= diastolic blood pressure; ADPKD= autosomal dominant polycystic kidney disease; FSGS= focal segmental glomerulosclerosis; ADIKD= autosomal dominant interstitial kidney disease; RTA= renal tubular acidosis; HDL= high-density lipoprotein; TIBC= total iron binding capacity; hs-CRP= high sensitivity CRP; LVEF= left ventricular ejection fraction; LVEDD=; LDL= low-density lipoprotein; ACE-I= angiotensin converting enzyme inhibitor; ARB= angiotensin receptor blocker; EPO= erythropoietin; ASA= acetylsalicylic acid

Table 2. Exercise capacity, vascular function assessment and circulating miRNA according to CKD severity

	HS (n=18)	**eGFR>45 ml/min/1.73m^2 (n=32)**	**eGFR<45 ml/min/1.73m^2 (n=31)**	*p-value*
EXERCISE CAPACITY				
VO_2 peak (ml/kg/min)	35.99 ± 6.43	28.13 ± 8.34*	24.05 ± 5.44*	<0.001
VO_2 at AT (ml/kg/min)	30.83 ± 7.33	25.08 ± 7.47*	21.31 ± 3.53*	<0.001
Maximal workload (Watt)	205 ± 60	171 ± 55	141 ± 47*	0.001
Exercise duration (sec)	536 ± 186	430 ± 126*	390 ± 105*	0.004
Resting HR (bpm)	76 ± 16	80 ± 23	74 ± 12	0.4
Peak HR (bpm)	169 ± 14	158 ± 23	147 ± 26*	0.006
Peak SBP (mmHg)	200 ± 23	191 ± 26	190 ± 20	0.3
Peak DBP (mmHg)	86 ± 18	85 ± 15	84 ± 15	0.9
MVV (l/min)	110 ± 17	113 ± 28	102 ± 27	0.2
Ventilatory reserve (%)	71 ± 14	60 ± 24	71 ± 24	0.1
PERIPHERAL VASCULAR FUNCTION				
Clinical parameters				
FMD (%)	6.93 ± 3.46	5.86 ± 3.21	4.93 ± 2.51*	0.03
Baseline diameter (mm)	3.52 ± 0.67	3.48 ± 0.55	3.87 ± 0.73	0.06
Maximal diameter (mm)	3.71 ± 0.68	3.67 ± 0.54	4.03 ± 0.72	0.09
GTN-MD (%)	23.94 ± 7.18	20.94 ± 7.87	16.70 ± 7.17*	0.01
PWV (m/s)	7.78 ± 1.03	8.35 ± 1.66	9.50 ± 2.66*	0.01
CIRCULATING miRNA				
miR21	2.32 ± 0.74	1.71 ± 0.69	2.06 ± 1.25	0.1
miR126	5.29 ± 1.08	5.59 ± 1.46	5.69 ± 1.56	0.7
miR146a	4.29 ± 1.18	4.88 ± 1.40	5.24 ± 1.69	0.1
miR150	4.24 ± 1.03	4.93 ± 1.06*	5.12 ± 1.11*	0.01
miR210	0.16 ± 0.94	0.28 ± 1.25	0.27 ± 1.27	0.7

*Data are mean ± SD or number (percentage). *Different from HS, p<0.05. For FMD, p-value is shown for ANOVA after logarithmic transformation. Levels of circulating miRNA are expressed as the logarithm of the relative expression of the respective miRNA.*

VO_2peak= peak oxygen uptake; VO_2 at AT= oxygen uptake at anaerobic threshold; HR= heart rate; SBP= systolic blood pressure; DBP= diastolic blood pressure; MVV= maximal voluntary ventilation; FMD= flow-mediated dilation; GTN-MD: glyceryl trinitrate mediated dilation; PWV= pulse wave velocity

Table 3. Clinical parameters associated with peak aerobic capacity

	Bivariate correlation		**Multiple regression**	
	Pearson r	*p-value*	ß	*p-value*
Age	-0.285	0.01	-0.172	0.2
eGFR	0.525	<0.001	0.363	0.002
Hemoglobin	0.372	0.001	0.199	0.09
logFMD	0.360	0.003	0.162	0.2
PWV	-0.435	<0.001	-0.301	0.01

eGFR= estimated glomerular filtration rate; FMD= flow-mediated dilation; PWV= pulse wave velocity

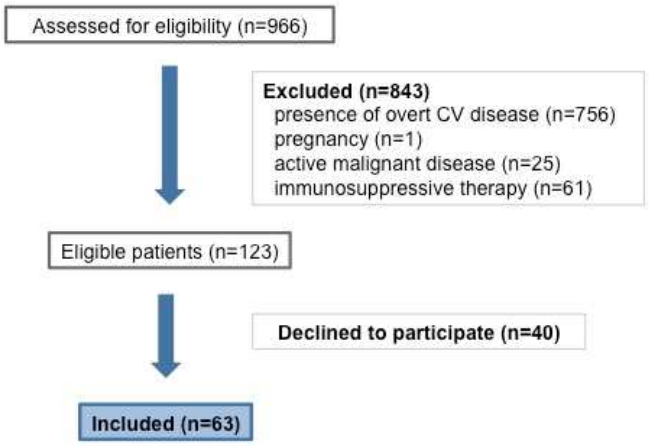
Table 4. Correlation of circulating miR with VO_2peak and PWV

	VO_2peak		**PWV**	
	Pearson r	*p-value*	Pearson r	*p-value*
miR21	-0.006	0.9	0.087	0.4
miR126	-0.227	0.05	0.211	0.07
miR146a	-0.312	0.006	0.286	0.01
miR150	-0.351	0.002	0.262	0.02
miR210	-0.232	0.04	0.179	0.1

Levels of circulating miRNA are expressed as the logarithm of the relative expression of the respective miRNA

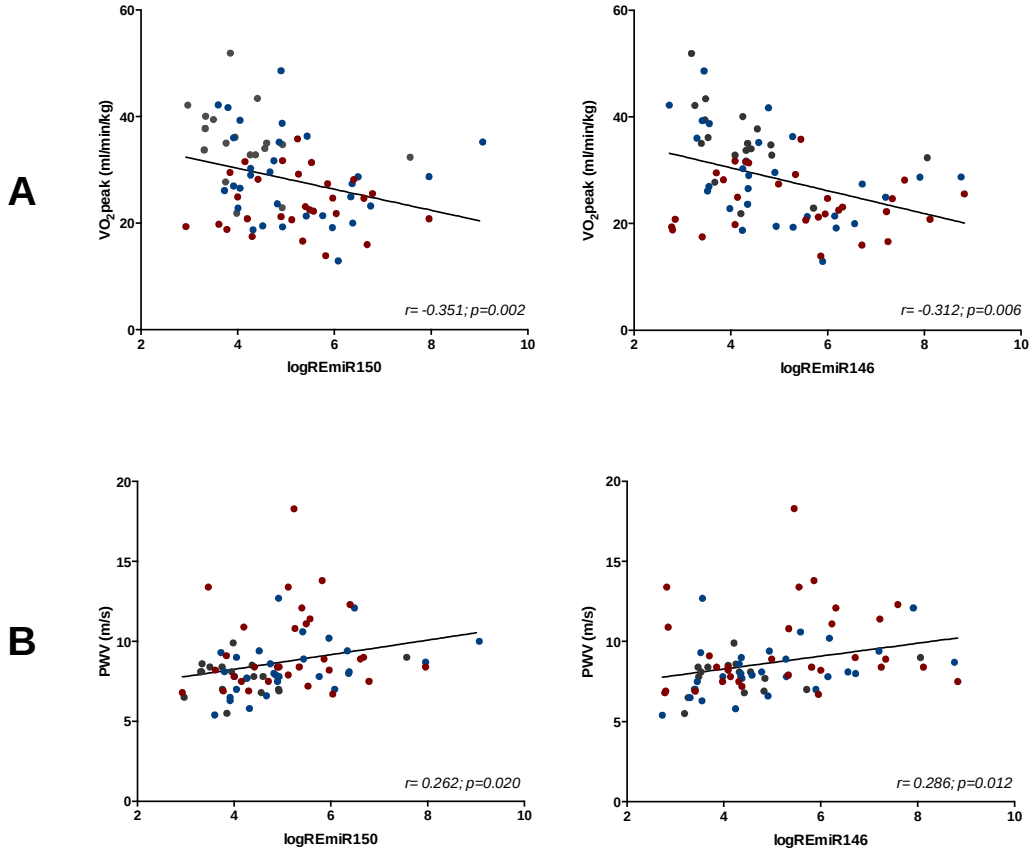
FIGURES

Figure 1: Patient flow



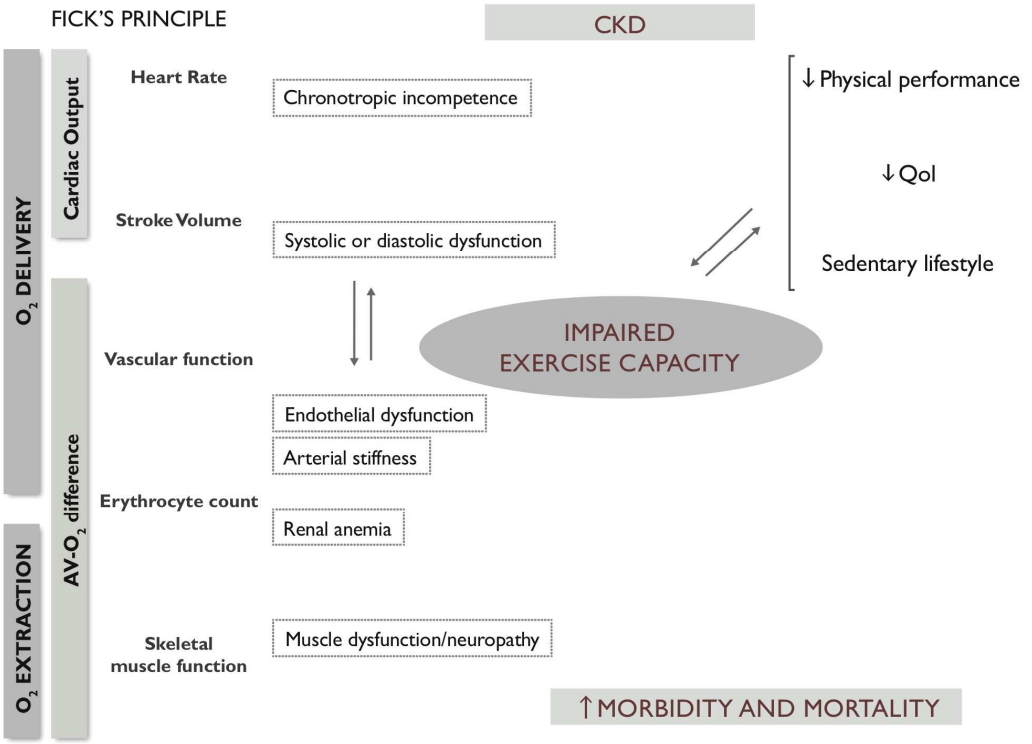
Of the 966 patients assessed in the period between April 2012 and July 2014, only 63 patients were included in the study. 843 subjects were excluded for reasons of not meeting the inclusion criteria, with overt cardiovascular disease counting for 78% of the exclusions. Of the 123 eligible patients, 40 refused to participate and 63 were included in the study after obtaining informed consent.

Figure 2: miRs, PWV and aerobic capacity



Panel A. *Aerobic capacity and miR;* ***Panel B.*** *Pulse wave velocity and miR Grey dots represent healthy subjects; blue dots represent CKD patients with an eGFR>45 ml/min/1.73m^2, red dots CKD patients with an eGFR<45 ml/min/1.73m^2.*

Figure 3: Possible underlying mechanisms of exercise intolerance in CKD



VO_2peak, the gold standard to assess exercise capacity, is determined by the product of cardiac output and arteriovenous oxygen difference. Hence, both oxygen delivery mechanisms (cardiac output, vascular function and erythrocyte count) as well as oxygen extraction mechanisms (skeletal muscle) possibly contribute to the observation of impaired exercise capacity in CKD. Impaired exercise capacity contributes to the increased morbidity and mortality of patients with CKD. It results in the inability to perform activities in daily life and occupational tasks, with huge effects on quality of life. In addition, the vulnerable CKD patient will become prone to a sedentary lifestyle, being trapped in the vicious circle of fatigue and reduced physical functioning, eventually leading to high morbidity and mortality.