From the Department of Clinical Sciences, Danderyd Hospital,
Division of Cardiovascular Medicine,

Karolinska Institutet, Stockholm, Sweden

TREATING VASCULAR DYSFUNCTION IN CHRONIC KIDNEY DISEASE: INTERVENTION WITH VITAMIN D

Kristina Lundwall

Karolinska Institutet

Stockholm 2018

Published by Karolinska Institutet.
Printed by E-print 2018

ISBN 987-91-7831-202-3

Treating vascular dysfunction in chronic kidney disease: intervention with vitamin D

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Kristina Lundwall

Principal Supervisor:
Jonas Spaak, Associate Professor
Karolinska Institutet
Department of Clinical Sciences
Danderyd Hospital
Division of Cardiovascular Medicine

Co-supervisors:
Gun Jörneskog, Associate Professor
Karolinska Institutet
Department of Clinical Sciences
Danderyd Hospital
Division of Internal Medicine

Stefan Jacobson, Professor
Karolinska Institutet
Department of Clinical Sciences
Danderyd Hospital
Division of Nephrology

Opponent:
Gunnar Sterner, Associate Professor
Lund University
Department of Clinical Sciences
Skåne University Hospital

Examination Board:
Anders Fernström, Associate Professor
Linköping University
Department of Medical and Health Sciences
Linköping University Hospital

Torbjörn Linde, Associate Professor
Uppsala University,
Department of Medical Sciences
Uppsala University Hospital

Angela Silveira, Associate Professor
Karolinska Institutet
Department of Medicine Solna,
Cardiovascular Medicine

To my mother, my shadow supervisor

Without you there would not have been any PhD degree. You have dedicated ours after ours helping me, sitting beside me, silently ignoring my sometimes very bad temper. You have the ability to irritate me the most by telling me the truth. Thank you.

"Believe in something, even if it means sacrificing everything"
Colin Kaepernick

"Just do it"
Nike

ABSTRACT

Background: Chronic kidney disease (CKD) is common, affecting 10-15% of the population worldwide. It is currently recognised by both cardiologists and nephrologists as a strong risk factor for cardiovascular events and death. During the last decades it has been shown that CKD leads to a state of activated renin angiotensin-aldosterone system and sympathetic nervous system, to endothelial dysfunction, chronic vascular inflammation, mineral bone disorder, and in late stages also to an acidotic and uremic cell milieu. Together these disturbances create an advanced and rapidly progressing vascular disease, leading to vascular stiffening and calcification. CKD patients have chronically low levels of activated vitamin D, a vitamin now regarded as a hormone involved in a wide range of processes in the body. It affects immune cells, leading to a shift towards anti-inflammatory responses, and inhibits the production of oxidants. Vitamin D upregulates the expression of eNOS, a crucial enzyme in endothelial function, and downregulates the expression of renin. Accordingly vitamin D deficiency might affect several of the processes involved in the progressive vascular disease seen in CKD. The aim of this PhD project was to investigate the effects of intervention with vitamin D on measures and markers of vascular function, inflammation, and upstream epigenetic regulation in patients with CKD.

Methods and results: We performed a randomised placebo-controlled double blind trial (RCT) including 36 participants, with non-diabetic CKD stage 3-4. Patients were randomised to intervention for 12 weeks with 1 or 2 μg of paricalcitol, an active vitamin D analogue, or placebo.

In paper I, we investigated physiological measures of macro- and microvascular function as well as muscle sympathetic nerve activity. We found that treatment with 2 μg of paricalcitol attenuated a decline in endothelial function measured by flow mediated vasodilation and iontophoresis by acetylcholine, and that both treated groups showed ameliorated measures of microcirculation, compared to placebo.

In paper II, a milliplex assay was performed to assess cytokine expression before and after intervention. We found that treatment with both 1 and 2 μg of paricalcitol suppressed levels of PDGF and VEGF, cytokines known to be implicated in vascular function and atherosclerosis. We also examined microRNAs, by PCR-techniques, and detected a downregulation of microRNAs 432, 495 and 576, shown to be involved in atherosclerosis, platelet function and inflammation.

In paper III, concentrations of microparticles (MPs), and their expression of the vascular activation and atherosclerotic markers ICAM-1 and VCAM-1 were determined by antibody labelling and flow cytometry. We showed that treatment with paricalcitol induced a decline in the expression of ICAM-1 on MPs compared to placebo. The results from the combined investigation of cell specific MP profiles showed that treatment with 2 μg of paricalcitol resulted in sustained levels of endothelial, platelet and leukocyte MPs, in contrast to the other two groups where levels declined.

Paper IV used meta-analysis techniques to assess the overall effect-size post treatment in flow mediated vasodilation (FMD) after intervention with any vitamin D compound. Inclusion criteria were any stage of chronic kidney disease, and with no restrictions regarding underlying diseases. Four articles fulfilled the criteria, comprising 305 participants. The overall effect size was in favour of treatment with vitamin D. The results were strongest for the study with the youngest population, for treatment with 2 μg of paricalcitol and treatment with cholecalciferol.

Conclusions: In our examined population, vitamin D has positive effects on endothelial macro- and microcirculatory functions, suppress levels of atherosclerotic and inflammatory markers and maintain the production of microparticles, potentially due to a more normally functioning endothelium. Important questions that remain are whether these findings may translate to effects on hard endpoints, in which patient groups, and the optimal timing of initiation of treatment.

LIST OF SCIENTIFIC PAPERS

I. **Lundwall K**, Jörneskog G, Jacobson S H, Spaak J: Paricalcitol, Microvascular and Endothelial Function in Non-Diabetic Chronic Kidney Disease: A Randomized Trial. *American journal of nephrology;* 2015, 42(4):265–273.

II. Mansouri L*, **Lundwall K***, Moshfegh A, Jacobson S H, Lundahl J, Spaak J. Vitamin D receptor activation reduces inflammatory cytokines and plasma MicroRNAs in moderate chronic kidney disease – a randomized trial. *BMC Nephrology;* 2017, 18(161).

III. **Lundwall K**, Mörtberg J, Mobarrez F, Jacobson S H, Jörneskog G, Spaak J. Changes in microparticle profiles by vitamin D receptor activation in chronic kidney disease – a randomized trial. *Submitted.*

IV. **Lundwall K**, Jacobson S H, Jörneskog G, Spaak J. Treating endothelial dysfunction with vitamin D in chronic kidney disease: a meta-analysis. *BMC Nephrology;* 2018, 19(247).

* = shared first authorship

CONTENTS

LIST OF ABBREVIATIONS

CVD	cardiovascular disease
CKD	chronic kidney disease
MDRD	modification of diet in renal disease
NSTEMI	non ST elevation myocardial infarction
PCI	percutaneous coronary intervention
CRS	cardiorenal syndrome
RAAS	renin angiotensin-aldosterone system
SNS	sympathetic nervous system
ROS	reactive oxygen species
PDGF	platelet derived growth factor
TGF-β	transforming growth factor-β
IL	interleukin
TNF-α	tumour necrosis factor-α
NO	nitric oxide
RONS	reactive oxygen and nitrogen species
eNOS	endothelial nitric oxide synthase
PTH	parathyroid hormone
FGF23	fibroblast growth factor23
MBD	mineral bone disorder
VDRAs	vitamin D receptor analogues
VDR	vitamin D receptor
Th	T helper cell
AT1-receptor	angiotensin II receptor-1
RCT	randomised controlled trial
FMD	flow-mediated vasodilation
PWV	pulse wave velocity
PWA	pulse wave analysis
LVM	left ventricular mass
ACh	acetylcholine
SNP	sodium nitroprusside

RHI	reactive hyperaemia index
SEVR	subendocardial viability ratio
LDF	Laser Doppler flowmetry
SCORE	Systematic COronary Risk Evaluation
ICAM-1	intercellular adhesion molecule-1
VCAM-1	vascular endothelial adhesion molecule-1
vWF	von Willebrand factor
E-selectin	endothelial leukocyte adhesion molecule
MP	microparticle
EMP	endothelial microparticle
PMP	platelet microparticle
miR/miRNA	microRNA
eGFR	estimated glomerular filtration rate
ACEi	angiotensin converting enzyme inhibitor
ARB	angiotensin receptor blocker
AIx	augmentation index
CBV	capillary blood cell velocity
MSNA	muscle sympathetic nerve activity
WoS	Web of Science
ANOVA	analysis of variance
MLM	multilevel modelling
STANDmean ES	standardized mean difference effect size
VEGF	vascular endothelial growth factor
IP10	interferon-gamma induced protein 10

1 INTRODUCTION

1.1 THE CARDIORENAL SYNDROME

Cardiovascular disease (CVD) remains the main cause of death worldwide (1, 2). Well established risk factors are hyperlipidaemia, smoking, hypertension and diabetes, although in the last two decades, chronic kidney disease (CKD) has come forth with similar importance (1, 3). CKD including microalbuminuria is also common, affecting 10-13% of the general population (1, 2). The comorbidity of CKD and CVD is nowadays recognised in leading clinical guidelines for both cardiologists (4, 5) and nephrologists (6) (Table 1).

Table 1: *Risk stratification for future CV disease/death. Based on ESC European Guidelines on cardiovascular disease prevention in clinical practices, Piepoli et al-16 (4).*

Low risk	Moderate risk	High risk	Very high risk
SCORE<1%	SCORE ≥1% and <5%	Cholesterol >8 mmol/L (e.g. in familial hypercholesterolaemia) BP >180/110 DM (young people with type 1 DM without major risk factors at low or moderate risk) **Moderate CKD** **(GFR 30–59 mL/min/1.73 m2)** SCORE ≥5% and <10%	Documented CVD DM with target organ damage (e.g. proteinuria) or major risk factor(e.g. smoking, marked hypercholesterolaemia, marked hypertension) **Severe CKD (GFR <30 mL/min/1.73 m2)** SCORE ≥10%

SCORE= a person´s 10 year risk of CV death; BP=blood pressure; CKD=chronic kidney disease; DM diabetes mellitus; CVD=cardiovascular disease.

An eGFR <60 ml/min is known to be associated with cardiovascular death in the general population (7), but a recent meta-analysis of almost 2 million subjects showed that the risk of myocardial infarction started to increase even at a mild renal dysfunction with an eGFR below 90 ml/min (modification of diet in renal disease formula (MDRD)) (8).

Not only do CKD patients carry a high cardiovascular risk, they also have a worse prognosis once they suffer a cardiovascular event (2, 9, 10). This may be due to direct effects caused by the declining renal function and associated vascular disease, but it may also in part be due to that CKD patients are treated differently. Szummer et al (10) showed a correlation between kidney function and the percentage of early revascularization performed in non-ST elevation myocardial infarctions (NSTEMI), with less percutaneous coronary interventions (PCI) with declining kidney function. Several factors may contribute to fewer interventions in CKD, such as difficulties to correctly diagnose NSTEMI in CKD, fear of contrast-induced nephrotoxicity, and challenges with correct dosing.

The connection between heart and kidney disease comprises a wide spectrum of disorders often classified as the cardiorenal syndrome (CRS). The CRS is subgrouped into 5 types

based on primary aetiology (2); here the focus will be on the chronic nephrocardiac type (Table 2).

Table 2: *Subgroups of the cardiorenal syndrome, based on Ronco et al -08, -14 (2, 11).*

Type	Classification
1	Acute cardiorenal. Heart failure leading to acute kidney disease
2	Chronic cardiorenal. Chronic heart failure leading to kidney failure
3	Acute nephrocardiac. AKD leading to acute heart failure (uremic cardiomyopathy, AKD-related)
4	Chronic nephrocardiac. Chronic kidney disease leading to heart disease (left ventricular hypertrophy, left ventricular diastolic dysfunction, chronic ischaemic heart disease due to kidney disease)
5	Secondary: Systemic disease leading to kidney and heart failure (sepsis, vasculitis, DM).

AKD= acute kidney disease; DM=diabetes mellitus.

Several of the mechanisms underlying the chronic nephrocardiac syndrome have been investigated during the last decade, outlining a syndrome of advanced vascular disease and premature vascular ageing. CKD patients suffer from chronic inflammation, endothelial dysfunction, and mineral bone metabolism disorders, leading to vascular calcification and arterial stiffening (1, 2, 12, 13). Studies have also demonstrated an activated sympathetic nervous system (SNS) and activated renin-angiotensin-aldosterone system (RAAS), contributing to the vascular disease (2, 11, 14, 15). In later stages the acidotic and uremic milieu in the cells will accelerate the inflammatory process further. All these changes together create the perfect conditions for atherosclerosis, vascular stiffening and fibrosis (1, 2, 14, 16, 17).

1.2 VASCULAR DYSFUNCTION IN CKD

1.2.1 RAAS and SNS activation

Both the RAAS and the SNS are highly activated in patients with CKD (2, 11, 14, 15). Renin release is stimulated by a decrease in renal perfusion pressure, and by sympathetic nerve stimulation (18). Furthermore, low levels of active vitamin D in CKD cause an upregulation of renin expression (19), and may contribute to the higher blood pressure seen in CKD, although the importance of this mechanism remains controversial (20). The sympathetic over-activation in CKD is likely due to a combination of renal injury, ischemia, chemoreflex dysregulation, and increases in sympathoexcitatory substances (i.e. Angiotensin II) (21).

While these two systems are essential for homeostasis, a chronic activation is deleterious for the kidneys, vessels and the heart (14, 22). Sustained RAAS activation causes sodium and water retention, systemic vasoconstriction, fibrosis and oxidative stress (14, 22). Sustained SNS activation induces reactive oxygen species (ROS) production, reduces β-adrenoreceptor density and sensitivity, causes cardiomyocyte hypertrophy and subsequent heart failure, and vascular hypertrophy (23-25).

Angiotensin II, the potent end product of the RAAS system, is an important link between the RAAS and the activated immune system seen in CKD. Angiotensin II does not only stimulate central sympathetic outflow, but also activates and stimulates monocytes/macrophages, dendritic cells, NK-cell and neutrophils as well as proinflammatory T-cells, and induces reactive oxygen species (ROS) production chemotaxis and proinflammatory cytokine release (26-28). ROS also interplay with SNS, with increased levels by SNS signalling, and at the same time enhance SNS activity by promoting sympathetic outflow (23, 29) (Figure 1).

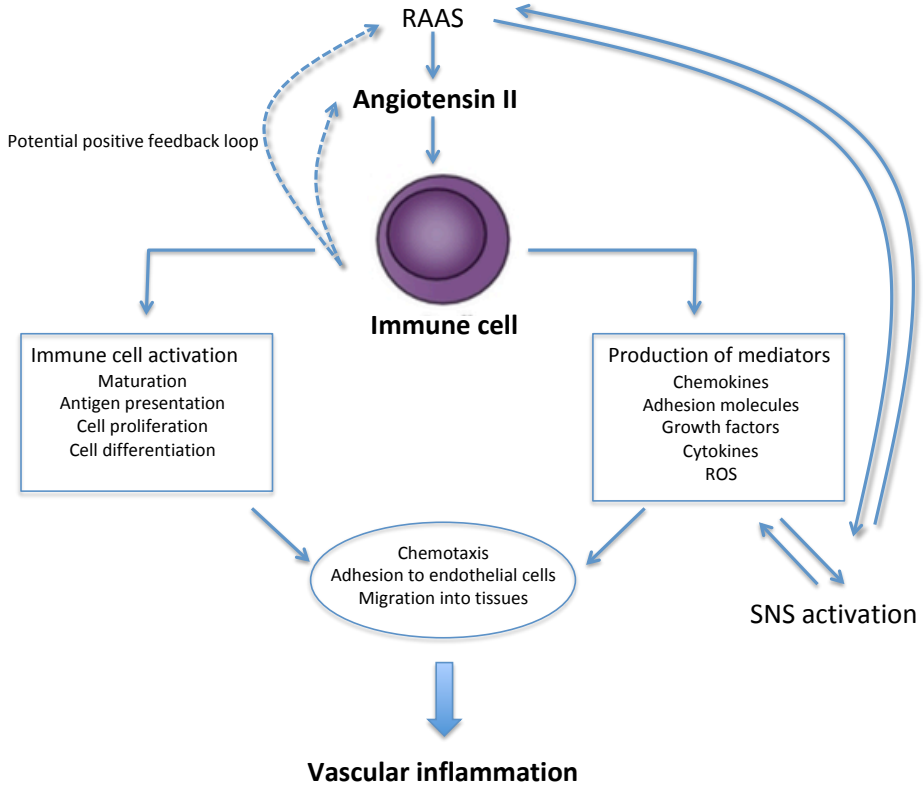


Figure 1: *Angiotensin II, the immune system, and the connection to SNS, leading to vascular inflammation.*

1.2.2 The immune system

The immune cells are currently not only regarded as major players in cancer and autoimmune diseases, but are also central in the development of atherosclerosis and hypertension (28-31). Current research has shown that both the innate and the adaptive immune system are implicated in the process (32, 33). Both these immune systems are further dysregulated in CKD patients, shown by dysfunctional immune cells, by increased levels of proinflammatory

cytokines and acute phase proteins (34-37). The starting point of this activation in CKD patients is not fully understood and probably multifactorial. Hypertensive stimuli and shear stress are thought to lead to the formation of neoantigens presented to T helper cells by dendritic cells (DCs) thereby activating them (28). An activated RAAS and SNS as described above also contribute, and in later stages uremic toxins play an important role as antigens in the body and lead to additional activation of the immune system (2, 16). Many cytokines produced by immune cells are shown to play an active role in the development of kidney injury and disease by enhancing inflammation, fibrosis and proteinuria. Among those are platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF-β), interleukin-1 (IL)-1, IL-6 and tumour necrosis factor-α (TNF-α) (37-41). PDGF and VEGF are also thought to play an active role in the development of atherosclerosis and atherosclerotic plaques (42, 43). ROS, produced by the activated immune cells, are important in the development of endothelial dysfunction, and in keeping the feedback-loop mechanisms of inflammation active.

1.2.3 Endothelial dysfunction

Micro- and macrovascular endothelial dysfunction is evident from the early stages of atherosclerosis, and is a potent independent predictor of cardiovascular risk (17, 31, 44-46). Several studies have described endothelial dysfunction in patients with varying degree of CKD, and it plays an important role in the chronic nephrocardiac syndrome (17, 47-49). The endothelium controls vascular tone, permeability of immune cells and molecules, and haemostasis in the blood vessel. Endothelial cells biosynthesize several vasoactive substances, and in normal homeostasis keep the balance between vasodilatory, anti-inflammatory properties, and vasoconstrictive, proinflammatory properties. One of the most important vasoactive substances produced by the endothelial cells is nitric oxide (NO). NO is essential for a normal endothelial function and inhibits platelet aggregation, transcription of cell adhesion molecules, leukocyte adhesion and growth of vascular smooth muscle cells (50, 51). A central mechanism in the development of endothelial dysfunction is the uncoupling of endothelial NO synthase (eNOS). Reactive oxygen and nitrogen species (ROS/RONS) and NADPH oxidases are believed to play a major role in uncoupling endothelial nitric oxide synthase (eNOS), which turns the enzyme from a protective NO-producing enzyme, to a harmful one, leading to production of very potent oxidants (28, 51).

This loop mechanism created will lead to less NO bioavailability and high levels of ROS/RONS. The endothelial cell balance will shift towards a chronic vasoconstrictive, pro-inflammatory state, with not only activation of endothelial cells, but also activation of immune cells. The activated immune cells will produce more pro-inflammatory cytokines and ROS/RONS, infiltrate the vessel wall, leading to vascular inflammation and dysfunction, and in the end, atherosclerosis (28, 49, 52).

1.2.4 Arterial stiffness and calcification

Arterial calcification and stiffness are important in the development of vascular disease in CKD, where impaired vitamin D metabolism, calcium-phosphate imbalance, and hyperparatyreoidism play important roles (2). Vascular stiffening is mainly the result of endothelial dysfunction, vascular inflammation, and arterial calcification.

All these processes lead to accelerated vascular disease in CKD, with diminished coronary blood flow and risk of myocardial infarction, risk of arrhythmias, of left ventricular hypertrophy and sudden cardiac death (1, 2, 49). The causation and interrelationship of these different aspects of vascular disease in CKD is however debated (12, 13). Vascular inflammation is an early hallmark of vascular disease, and studies have shown that it is followed by, but not coexisting with, calcification and fibrosis (13, 53, 54). Calcification and fibrosis seem to be due to low-grade long standing inflammation with pronounced structural changes (13, 55) and can be seen as the body attempting a healing process, in turn leading to arterial stiffening (13). The preceding endothelial dysfunction on the other hand has a strong inflammatory connection as described above. It is activated by inflammation, but also works as a promoter of the continuous inflammatory process, and as such is an early sign of the vascular disease process (17, 45, 46).

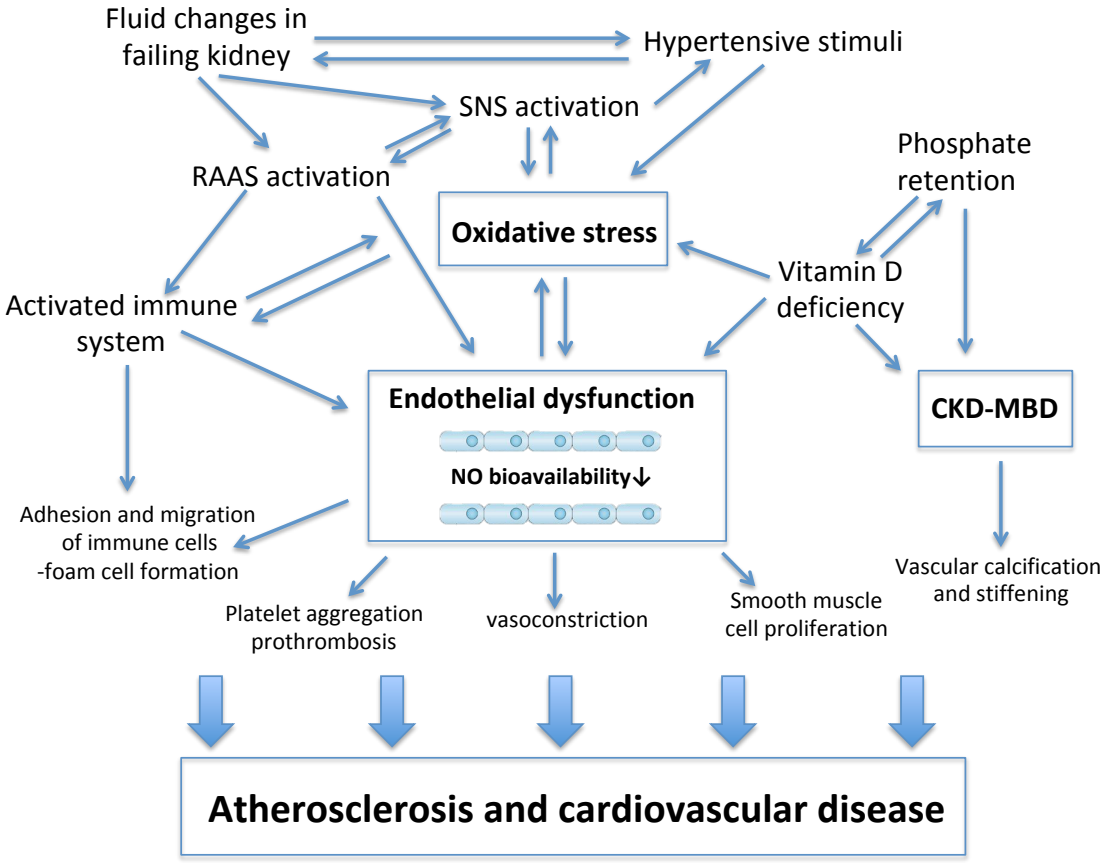


Figure 2: *An illustration of some of the complex mechanisms in CKD leading to CVD. The many feedback-loops in the network further enhance negative effects (also see Figure 1 and text for further details).*

1.3 VITAMIN D AS A TREATMENT OPTION

1.3.1 Vitamin D deficiency and CKD-MBD

Active vitamin D deficiency is common already from early stages of CKD, mainly due to the loss of renal function and availability of 1,25-α-hydroxylase, the vitamin D activating enzyme. Low levels of active vitamin D cause insufficient re- and absorption of calcium from the kidney and gut, which in turn stimulates release of parathyroid hormone (PTH). High levels of PTH stimulate calcium release from skeletal bones, augment absorption of calcium from the kidneys, and increase active vitamin D levels (56). Loss of kidney function also leads to retention of phosphate, which stimulates both PTH- and fibroblast growth factor 23 (FGF23) release, the latter a calcium-phosphate regulating hormone, with anti-vitamin D properties (57, 58). The changes in this axis in CKD are together known as CKD mineral bone disorder (MBD) (59) (Figure 3). There is an ongoing discussion on the importance of these different aspects of CKD MBD, but the regulation of FGF23-, phosphate- and vitamin D levels are all affected early in CKD patients (59).

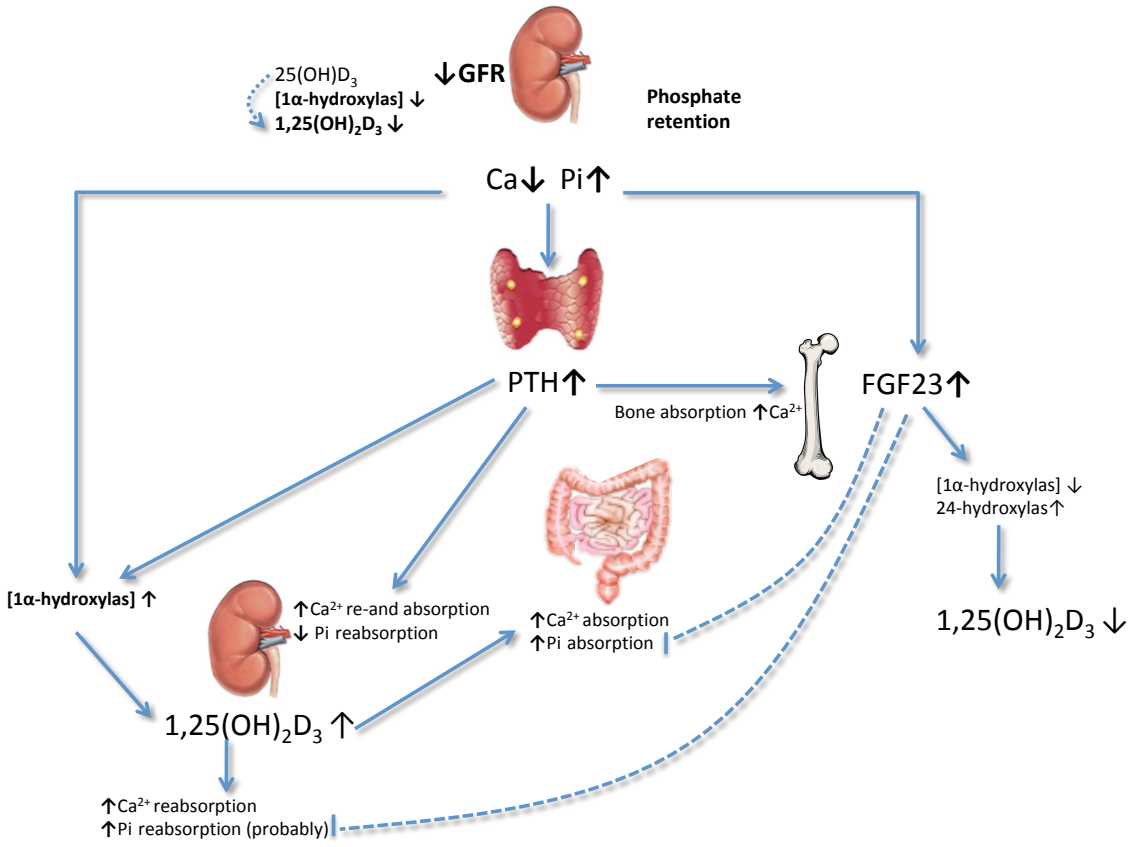


Figure 3: *The CKD-MBD axis. Ca=calcium; Pi=phosphate; PTH= parathyroid hormone; FGF23=fibroblast growth factor 23.*

1.3.2 Vitamin D and CVD

Several observational studies, including all types of populations, have shown a strong association between vitamin D deficiency and an increased risk of cardiovascular events (60-68). The causality of this association is however still debated.

Table 3: *Examples of epidemiological studies on vitamin D and cardiovascular disease.*

Author	Population	Participants	Outcome
Martins - 07	NHANES	15 088	25(OH)D levels correlate to CV risk factors
Wang - 08	Framingham Offspring	1739	25(OH)D deficiency associated with incident CVD
Giovannucci - 08	Health Professionals Follow-up Study	18 225	Low levels of 25(OH)D associated with higher risk of MI
Kendrick - 09	Third National Health and Nutrition Examination Survey	16 603	Strong and independent relationship of 25(OH)D deficiency with prevalent CVD
Semba -10	Prospective cohort study of aging in Tuscany, Italy	1002	Low serum 25(OH)D levels associated with higher risk of all-cause and CVD mortality.
Brondum-Jacobsen -12	Prospective cohort of general Danish population	10 170	Decreasing 25(OH)D levels associated with risk of IHD, MI, and early death
Wang -12	Meta-analysis, association of 25(OH) vitamin D with CVD risk	65 994	Linear inverse association between 25(OH)D and risk of CVD
Brondum-Jacobsen -12	Meta-analysis, association of 25(OH)D, CV risk, MI and early death	82 982	Decreasing 25(OH)D levels associated with risk of IHD, MI, and early death
Schottker -13	German population-based cohort aged 50–74	9758	25(OH)D inversely associated with all-cause, CV, cancer and respiratory mortality
Heidari -15	Community dwelling type 2 DM patients followed to first CHD	2607	25(OH)D deficiency an independent predictor of future CHD events

CVD=cardiovascular disease; MI=myocardial infarction; DM=diabetes mellitus; CHD=coronary heart disease; IHD=ischaemic heart disease.

Vitamin D treatment is an established treatment of hypocalcaemia and secondary hyperparathyreoidism in patients with more advanced kidney failure. However, due to the findings in observational studies, the interest for vitamin D as a possible treatment option to affect cardiovascular risk in earlier stages of CKD has increased.

1.3.3 Treatment or supplementation?

There is an ongoing discussion about how to treat CKD patients with vitamin D deficiency. Some argue that supplementation with the precursors cholecalciferol or ergocalciferol might be preferable over the active hormone calcitriol, or vitamin D receptor analogues (VDRAs, i.e paricalcitol). Many cells of the body are now known not only to have the receptor for

active vitamin D (VDR), but also the 1-α-hydroxylase, and are capable of producing active vitamin D locally. Studies have indicated potential drawbacks with active treatment, such as hypercalcaemia and hyperphosphataemia affecting vascular calcification, induction of adynamic bone disorder and also negative loop back mechanisms with downregulation of 1-α-hydroxylase and upregulation of 24-hydroxylase, an enzyme that degrades active vitamin D (69). Supplementation with the inactive form might give the positive pleiotropic effects of VDR-activation through local production of the active form and thereby activation of the VDR (69). Precursors seem to safely lower PTH and ameliorate 25OHD and $1,25OH_2D_3$-levels, in all CKD stages, though at some less degree than active treatment, and without a rise in calcium and phosphate levels (70, 71). Active treatment on the other hand is correlated to higher calcium and phosphate levels (72-75). In a meta-analysis with both compounds Xu et al (75) did not however see a difference in calcium levels between precursors and active treatment. Some advocate a combination of supplementation with precursors and active treatment (76).

1.3.4 Cellular effects of vitamin D

On the cellular level, it is now clear that vitamin D not only regulates the bone-mineral metabolism, but also displays a wide range of effects in the body. Both the receptor and the Vitamin D activating enzyme are found in many different cell types, among them immune cells, vascular smooth muscle cells and endothelial cells (77-79). In the immune system, vitamin D seems to play a role as a balancing factor between pro- and anti-inflammation (78, 80). It has both antibacterial and proinflammatory effects by inducing production of antimicrobial peptides and bacterial killing by monocytes and macrophages. Vitamin D also mediates anti-inflammatory and anti-fibrotic responses by the suppression of proinflammatory cytokines, induction of anti-inflammatory cytokines, downregulation of the TGF-β pathway and differentiation of T helper (Th) cells towards the less inflammatory Th2 and anti-inflammatory regulatory T cells (74, 81).

In the endothelium, Vitamin D has been shown to decrease the expression of adhesion molecules, decrease levels of oxidative stress, upregulate the enzyme eNOS and thereby induce the production of NO (82-85). Vitamin D is also a negative regulator of the RAAS by a downregulation of renin expressing genes (86, 87), and by antagonistic synergy with the angiotensin II receptor-1 (AT1-receptor) (88-91).

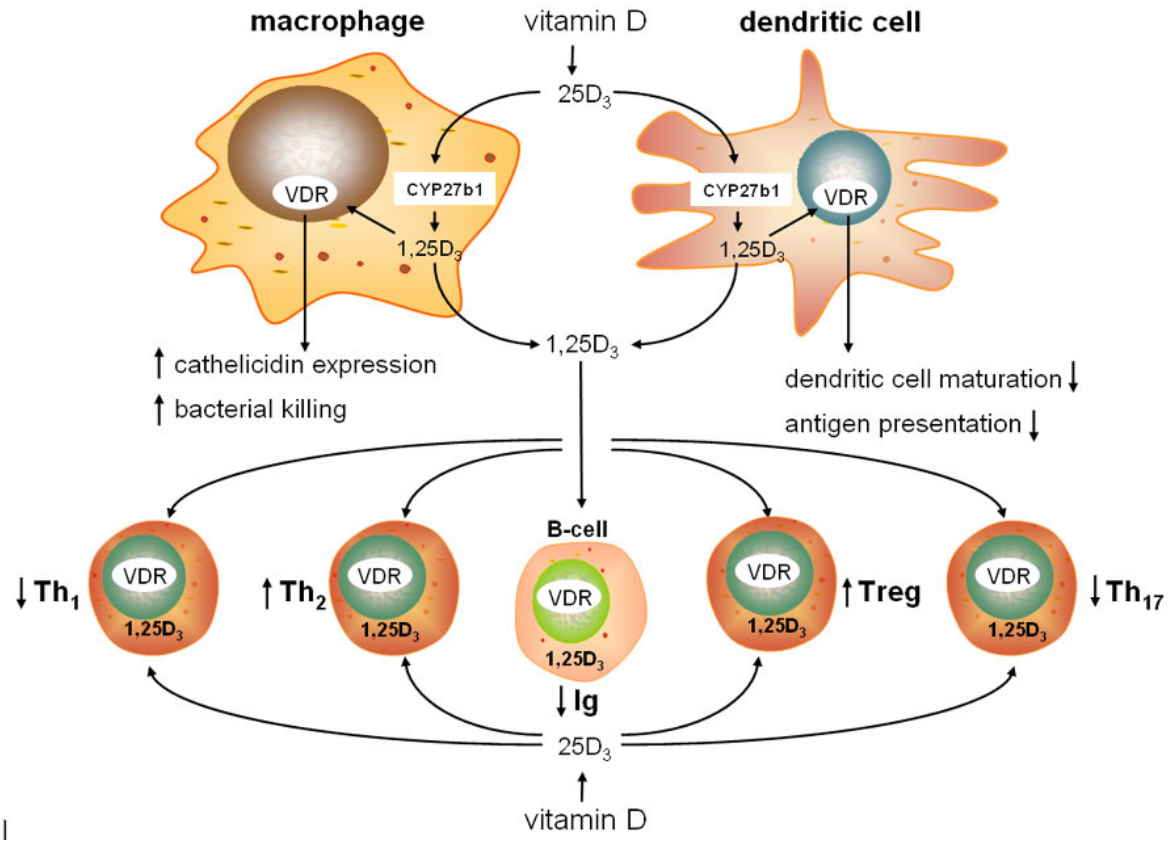


Figure 4: *Vitamin D actions in immune cells. From Hewison et al -09 (80). Published with permission from Springer Nature. VDR=vitamin D receptor; CYP27b1=1-α-hydroxylase; Treg=regulatory T-cell; Th=T helper cell; Ig=immunoglobulin*

1.4 INTERVENTIONS WITH VITAMIN D IN CKD

1.4.1 Studies on markers of inflammation, renal function and glucose metabolism

Three studies between year 2005 and 2010 showed that supplementation with vitamin D and treatment with VDRA in patients with CKD was associated with improved survival and reduced proteinuria (92-94). Meta-analyses on the topic confirm these results, and show that vitamin D affects residual proteinuria in CKD patients, on top of RAAS blockade (72-75). The effects are probably due to the anti-inflammatory actions (74) and the effects on the RAAS described above (86, 87, 91). Glucose metabolism is another interesting area, where one meta-analysis on dialysis patients shows positive effects on glucose control by treatment (95). When it comes to inflammatory markers, a couple of small randomised controlled trials (RCTs) have shown effects on proinflammatory cytokines and CRP (96-99), while Thethi et al (100) failed to show effects on similar parameters.

1.4.2 Studies on hard endpoints are lacking

Large and sufficiently long studies to evaluate hard endpoints are still lacking in this area. In a retrospective trial, Lishmanov et al (101) showed reduced cardiovascular events with vitamin D supplementation in patients with CKD. Meta-analyses on cardiovascular risk and mortality have been performed and show effect of treatment with vitamin D in CKD patients in observational studies (102, 103). One meta-analysis (104) investigated the effect on cardiovascular endpoints in controlled trials, but could not show any benefit of treatment. However, these results may be questioned since none of the included studies had cardiovascular endpoints defined à priori, and the study durations varied from only 3 weeks to 2 years.

1.4.3 Surrogate markers of CV risk

In the absence of hard endpoints, many studies have instead used surrogate markers of cardiovascular risk, such as flow-mediated vasodilation (FMD) and measures of arterial stiffness (pulse wave velocity or pulse wave analysis; PWV/PWA). Whereas PWV/PWA are complex measures of arterial structural changes, and are mainly measures of arterial stiffness (12, 105), FMD is primarily a measure of the capacity of the endothelial cells to produce and respond to NO (106). Still, PWV and FMD are interrelated (107) and both are predictors of cardiovascular risk (17, 45, 46, 108, 109).

1.4.4 Studies on FMD

During the last years a couple of studies have been performed investigating the effects of different vitamin D compounds on FMD (44, 100, 110-113). Most of them showed effects of supplementation or treatment on measures of FMD in CKD patients in stage 3-4 (44, 111-113). Kendrick et al (110) compared the effect of supplementation and active treatment, and did not detect any difference from baseline for the two groups. Neither did Theti et al (100), investigating the effects of paricalcitol in patients with diabetic nephropathy. These studies are systematically reviewed in the present thesis in study IV.

1.4.5 Studies on arterial stiffness and diastolic measures

Left ventricular hypertrophy and diastolic dysfunction is often a result of hypertension and arterial stiffness (114-116). Interventional studies with vitamin D on left ventricular mass

(LVM), diastolic measures and arterial stiffness have, compared to FMD, primarily shown negative results. The PRIMO trial failed to show effects on left ventricular hypertrophy and diastolic function in CKD patients (117). However, secondary and explorative analyses showed less hospitalizations due to cardiovascular events, and a tendency to positive effects on levels of NT-proBNP, and atrial volume indexes. Several studies investigating the effects of vitamin D compounds on measures of PWV and/or PWA in kidney disease patients have been performed during the last decade (44, 82, 111, 112, 118-123). The study durations varied from 8 to 44 weeks, patients were in kidney stage 3-5 and both active treatment and supplementation in different doses were used. Levin et al and Kumar et al (111, 112, 119) showed positive effects of treatment, whereas the rest of the studies failed to show any difference in PWV and/or PWA post treatment.

1.4.6 Other non-invasive methods to assess vascular function

There are other non-invasive methods to assess vascular and endothelial function, such as investigations of skin microvascular reactivity by LDF during iontophoresis with acetylcholine (ACh) and sodium nitroprusside (SNP), laser Doppler fluxmetry (LDF), reactive hyperaemia index (RHI) and subendocardial viability ratio (SEVR). These are less validated as risk markers, but iontophoresis with ACh and SNP have been shown to correlate to CV risk factors (124), and might reflect microvascular dysfunction in the rest of the body (125). However, Jekell et al (126) showed that iontophoresis by ACh (endothelium dependent), LDF and SEVR were not correlated to cardiovascular risk in terms of the Systematic COronary Risk Evaluation (SCORE) estimates in a population of hypertensive patients. This was in contrast to PWV and FMD that both significantly correlated to SCORE. FMD and PWV also correlated significantly to each other, whereas the other measures did not, raising important questions about what these measures exactly stand for in the vasculature. There are not many studies performed on the effects of vitamin D treatment with these measures in CKD patients. Dreyer et al (82) showed positive effects of treatment with ergocalciferol on measures of iontophoresis. Pihlström et al, (123) using active treatment, could not show any effects of intervention on measures of reactive hyperaemia index (RHI).

In conclusion, there are still conflicting results in the field of Vitamin D treatment as a way of affecting measures of cardiovascular risk in CKD. Factors such as study size and duration, study population, and the choice of outcome-measures are of importance to interpret these results.

Table 4: *Interventions with Vitamin D in chronic kidney disease, on surrogate markers of cardiovascular risk. For study populations nr in ( ) are patients analysed.*

Author	Duration	Sample size (nr)	CKD stage	Treatment	Dose	Outcome
Zoccali (-14)	12w	89 (88)	3-4	paricalcitol	2µg daily	FMD
Chitalia (-14)	16 weeks	26	3-4	cholecalciferol	300000IU at baseline, after 8w	FMD, PWV/PWA, ICAM, VCAM, E-selectin, vWF
Theti (-15)	12w	60 (46)	3-4	paricalcitol	1µg daily	FMD
Kumar (-17)	16w	120 (117)	3-4	cholecalciferol	300000IU at baseline, after 8w	FMD, PWV
Kumar (-18)	16w	31	3-4	cholecalciferol	300000IU at baseline, after 8w	FMD, PWV, vWF
Kendrick (-17)	6 months	128 (115)	15-44 eGFR	Cholecalciferol, calcitriol	2000IU, or 0,5µg daily	FMD
Marckmann (-12)	8 weeks	52	HD comp non HD	cholecalciferol	50000IU/w	PWV/PWA, vWF, IL-6, hsCRP
Hewitt (-13)	6 months	60	HD	cholecalciferol	50000IU/w: 8 w, monthly 4m	PWV
Mose (-14)	6 months	64	ESRD	cholecalciferol	3000IU daily	PWV, PWAix, Echo, cBP
Dreyer (-14)	6 months	38	3-4	ergocalciferol	50000IU/w: 1 month, montly: 6m	PWV, iontophoresis LDF,eNOS
Pihlström (-17)	44w	77	renal transplant recipients	paricalcitol	2µg daily	PWV, RHI
Levin (-17)	6 months	119 (87)	15-45 eGFR	Calcifediol, calcitriol	5000IU or 0,5 µg 3 times/w	PWV
Thadhani (-12)	48w	227	15-60 eGFR	paricalcitol	2µg daily	LVMI, echo
Naeini (-17)	6 months	64	ESRD	vitamin D pearls	50000IU/w:3m, every 3:rd w: 3m	VCAM, ICAM

ITT=intention to treat; eGFR=estimated glomerular filtration rate; HD=hemodialysis; ESRD=end stage renal disease; FMD=flow mediated vasodilation; PWV/PWA=pulse wave velocity/analysis; vWF=von willebrand factor; echo=echocardiography; hsCRP=high sensitive CRP; cBP=central blood pressure; LDF=laser Doppler flowmetry: eNOS=endothelial nitric oxide synthase; RHI=reactive hyperemia index; LVMI=left ventricular mass index.

1.4.7 Studies on endothelial markers in CKD

An activated and/or dysfunctional endothelium express activation markers such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), von Willebrand factor (vWF) and endothelial leukocyte adhesion molecule (E-selectin). They play an important role in the recruitment and migration of white blood cells and platelets to the site of inflammation. E-selectin mediates leukocyte rolling, the first step to slow down the flow of the leukocytes at the site of inflammation. ICAM-1 and VCAM-1 then induce leukocyte arrest whereas ICAM-1 initiates the crawling and intracellular transmigration of leukocytes into the vessel wall (127) (Figure 5).

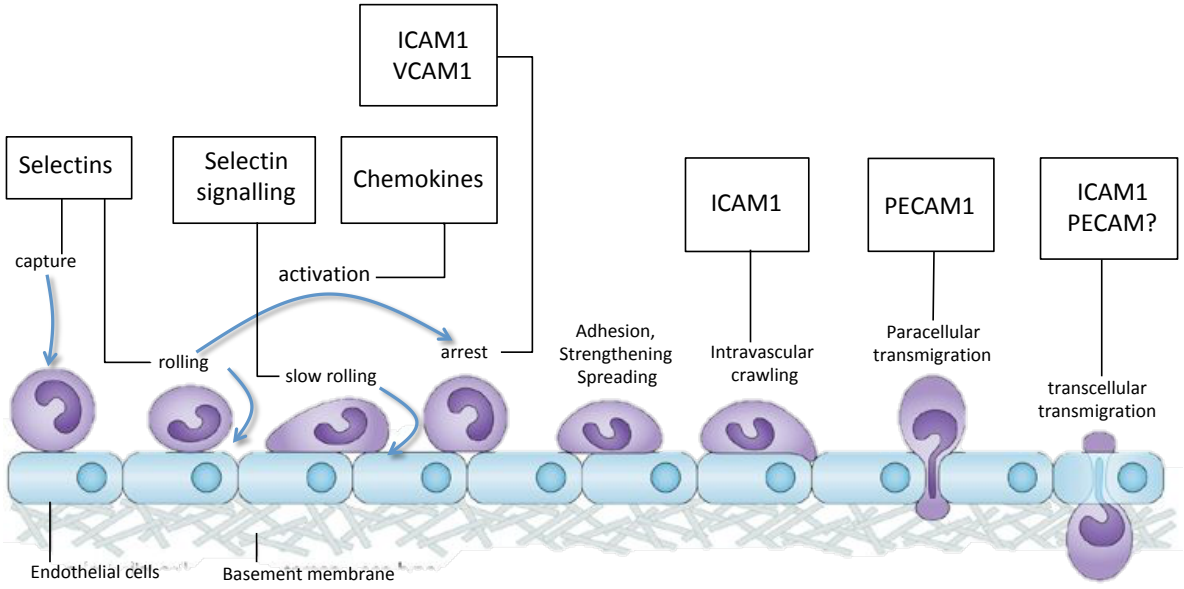


Figure 5: *The interaction of adhesion molecules and leukocytes (simplified). Modified from Ley et al (127). Published with permission from Springer Nature.*

These events play an important role in the atherosclerotic process (128) and in line with this some studies have shown that levels of ICAM-1 correlate to cardiovascular events and death in CKD patients (129-131). VCAM-1 seems to correlate to intima media thickness in HD patients (132) and to measures of dyslipidaemia (130, 131). There are two studies performed showing lower levels of ICAM-1 and VCAM-1 after intervention with vitamin D, interpreted as a less inflammatory and less activated endothelium (44, 133).

1.5 MICROPARTICLES

Microparticles (MPs) are small cell membrane vesicles (100-1000nm) shedded from the parent cells, both during activation of the cell but also increasingly during stress and apoptosis. They are released in response to proinflammatory cytokines, acute phase proteins, ROS, and uremic toxins (134-136). They can be considered cellular biopsies from the tissues they are derived from, and are therefore promising biomarkers for various diseases. Microparticles contain mRNA, microRNA, receptors and proteins, and there are emerging evidence that they are biologically active, affecting cells around them, playing a role in intercellular communication (134-137). Different MPs have been shown to act in the production of proinflammatory cytokines, in promoting vascular inflammation and coagulation and regulating the production of ROS (135, 137).

1.5.1 The importance of subtypes of MPs

Not only their parent cell type but also the mechanism for their release; apoptosis, cell damage or activation, induce different patterns of surface markers, such as CD31, CD62E, CD62P, or CD144 (134, 138, 139). For endothelial microparticles (EMPs), it seems that the subtypes of EMPs reflect different aspects of endothelial function, and might be used to classify the type of endothelial injury occurring (134, 138). In acute coronary syndrome for example, there are mainly high levels of apoptotic ($CD31^+$) EMPs (138, 140, 141) probably reflecting the acute endothelial cell injury due to ischemia. Jimenez et al (138), showed in an in vitro study that when apoptosis was induced in endothelial cells, activation markers on the cells and activation induced EMPs remained stable, while apoptotic markers on the cells and the production of apoptotic EMPs increased rapidly. Dr Lundström et al has also shown interesting results in patients with ischaemic stroke or TIA, where different subtypes of platelet microparticles (PMPs) reflect opposite correlations to prognosis in patients with ischemic stroke.[1] Depending on the cell origin and surface molecules, MPs also seem to exhibit opposite effects on apoptosis, inflammation and oxidative stress (134, 139, 142-145) (Figure 6).

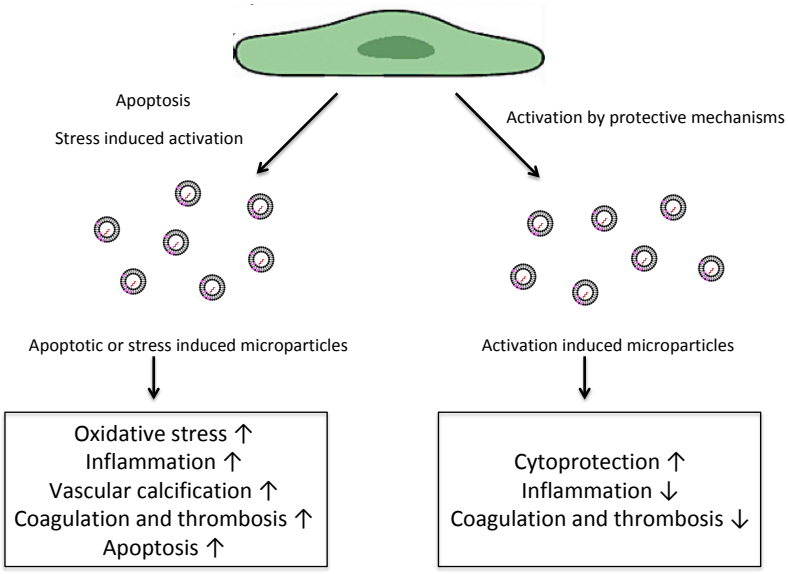


Figure 6: *Different actions of microparticles due to their release mechanisms.*

[1] Published as part of the thesis "Platelet function and thrombin generation in ischemic stroke – clinical correlates and prognostic importance" 2018, by Annika Lundström, part III: Platelet microvesicles are elevated after ischemic stroke or TIA – specific subpopulations have different associations to prognosis

1.5.2 Endothelial microparticles and correlation to future CV risk

EMPs are novel biomarkers of endothelial dysfunction and injury (134, 135, 137, 146, 147). Levels of CD31-positive-CD41-negative ($CD31^+$ $CD41^-$) and CD144-positive ($CD144^+$) EMPs correlate with physiological measurements of vascular function, such as FMD and PWV (148-152). The marked endothelial dysfunction in CKD patients is also reflected in higher levels of $CD144^+$ and $CD31^+CD41^-$EMPs compared to healthy controls (147, 149, 153, 154). $CD144^+$ or $CD31^+CD41^-$ EMPs, and also $CD62E^+$ EMPs (155), may play a predictive role, and high levels correlate with cardiovascular morbidity and mortality in atherosclerosis, kidney failure, pulmonary hypertension and heart failure (146, 148, 155-157) (Table 5).

Table 5: *Observational studies on microparticles in chronic kidney disease and interventional studies in all populations.*

Author	Population	MPs measured	Outcome
MPs in CKD and correlations to risk			
Amabile 2005	44 ESRD, 32 healthy controls	CD31+CD41-, CD144+ EMPs	Correlation to FMD in ESRD
Boulanger 2007	34 HD patients	CD144+, CD31+41- EMPS	Correlation to measures of shear stress in ESRD.
Amabile 2012	81 HD patients	CD31+CD41- EMPs .	Levels predicted all cause and CV mortality in ESRD after 50 months
Dursun 2009	33 pre-HD, 37 HD, 18 healty controls	CD144+, CD146 + EMPs	Higher levels in CKD than healthy controls. PWV correlated to CD144
Faure 2006	45 CKD, 30 HD, 36 healthy controls	CD144+, CD146+EMPs	Higher in CRF and HD patients than in healthy controls
Chen YL 2015	68 CKD-CAD, 10 CAD, 10 healty controls	CD31+42b- EMPs	Higher in patients with CAD than in patients with CAD and CKD.
Trappenburg 2012	27 CKD, 10 healthy controls	CD144+ EMPs, CD41+, CD62P+ PMPs	Higher levels in CKD4 and HD compared to controls
Interventions (all populations)			
Augustine 2014	119 patients referred for stress echo	CD31+41+PMPs, CD31+41-EMPs, erythrocyte MPs	Increasing levels after stress echo in patients with normal examination, but did not change in patients with CVD or pathologic stress echo
Tehrani 2013	20 type 1 DM patients	CD144+ EMPs	Levels tended to increase with Atorvastatin treatment
Mobarrez 2012	19 PAOD patients	CD144+ EMPs	Increasing levels with Atorvastatin treatment
Jia 2017	HUVEC cell line	AnnexinV+ MPs	MP release and superoxide generation significantly inhibited by 1,25(OH)2D3

ESRD=end stage renal disease; HD=hemodialysis; CAD=coronary artery disease; echo=echocardiography; PAOD=peripheral artery occlusive disease; HUVEC=human umbilical vein endothelial cell; CRF=chronic renal failure

1.5.3 Results from interventional studies

The production and thereby the levels of MPs also seem to be dependent on the capability of the cells to become activated. Augustine et al. (158) reported in a study of $CD31^+$ EMP- and PMP-response to dobutamine-stress echocardiography, that patients with signs of coronary disease (wall motion abnormalities) on the examination did not react with elevated $CD31^+$ EMPs or PMPs, whereas patients with a normal stress-test did. These results seem to indicate that a dysfunctional endothelium might be less reactive, not producing more MPs in response to stress or other events in the same way as in healthy controls or treated patients. Mobarrez et al (159) showed similar results in a study on atorvastatin treatment in patients with peripheral artery occlusive disease. Patients treated with atorvastatin produced more EMPs than the placebo group. This was interpreted as a sign of a more reactive and healthy endothelium since atorvastatin has known endothelium-cell protective effects (160). There are to our knowledge no interventional studies performed with vitamin D using MPs as outcome. Two in-vitro studies (83, 85) on endothelial cell lines show lower levels of $CD31^+$ EMPs after vitamin D treatment indicating a protective effect against apoptosis (Table 5).

MPs are clearly affected by kidney disease, and some subtypes might be of use not only as predictors of risk, but also as markers to understand underlying mechanisms. Whether MPs may be used as markers of long-term outcome, is insufficiently studied.

1.6 EPIGENETIC REGULATION, A NEW AND EXPANDING FIELD IN CKD

1.6.1 Epigenetic regulation

CKD have a strong hereditary component, shown in epidemiological studies. Genetic wide association studies have however failed to find strong associations, implying the importance of other factors, such as epigenetic modifications (161, 162). Epigenetic regulation is the heritable interface between the rigid genome and the changing environment. It affects gene expression without changes in the nucleotide sequence, and therefore epigenetic changes are potentially reversible (162). For example, epigenetics are thought to be the reason for the interesting phenomenon “metabolic memory”, described in diabetes, where a period of impaired glucose control will induce cellular changes that will linger on even after periods of excellent treatment (40).

The most commonly discussed epigenetic regulators are microRNAs (miRs/miRNAs), post-translational modifications of nucleosomal histones and DNA methylation (Figure 7).

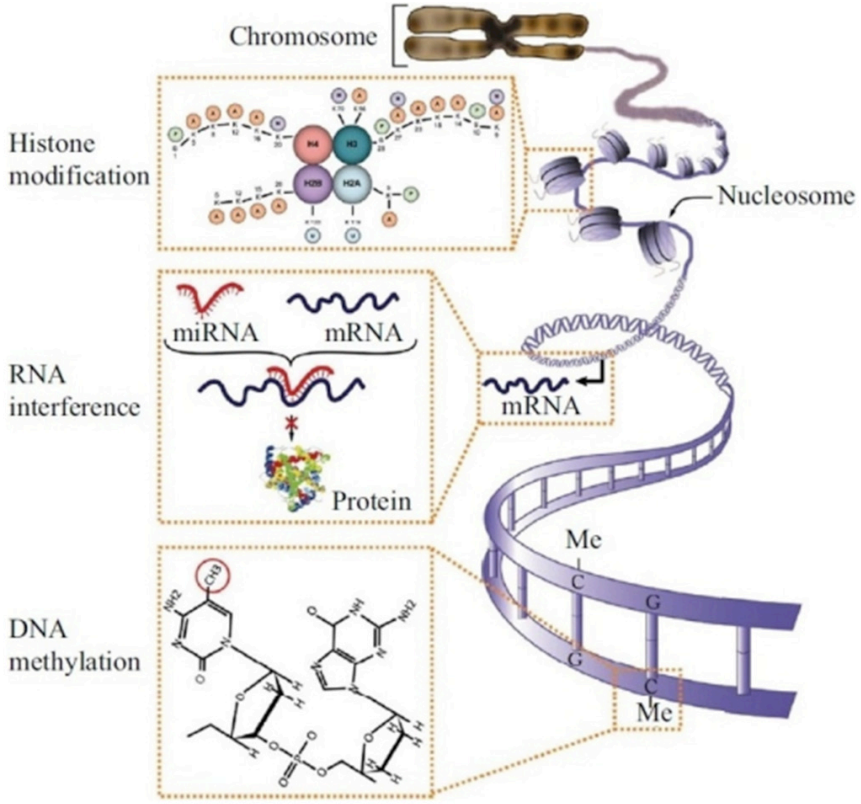


Figure 7: *The most commonly discussed epigenetic regulations. From Kim et al (163). Published in courtesy of the openi agreement: https://creativecommons.org/licenses/by-nc-sa/3.0/legalcode*

1.6.2 microRNAs

miRs are small RNAs (approximately 19-25 nucleotides) that regulate gene expression by binding to mRNAs, resulting in silencing of translation or degradation of that mRNA (40). These miRs can target hundreds of mRNAs, and their effects on gene expression can therefore be substantial (164). Because of this, miRs are not only believed to be future biomarkers for various diseases, but also highly interesting as targets for treatment (40). Several miRs have been associated with inflammation and kidney dysfunction (40, 162). miR-146 and miR-155 are recognized as key players in the immune cell response in chronic inflammation (161). miR-192 is well characterized, and promote fibrosis by upregulation of profibrotic genes, among them the TGF-β-pathway, known to be central in the development of renal fibrosis (162). There are several miRs thought to play a role in normal kidney function, among them the miR-30 family (162). As targets for treatment, there are trials performed on mice, where an anti-miR192 treatment induced changes in the phenotype of the mice with downregulation of mediators of renal fibrosis, and reduced proteinuria (40, 165).

Several miRs are also thought to be implicated in cardiovascular disease (164). For example, downregulation of the miR133 family seems to be implicated in hypertrophy and fibrosis of the heart (164, 166). MiR 495 probably acts in the process of platelet reactivity, and downregulation of miR495 was shown in a mouse model to ameliorate vascular recovery

after ischemia (167, 168). However, here there are conflicting results, where another mouse model has shown that miR495 was downregulated in ischaemic tissue, and showed anti-inflammatory properties (169). An upregulation of a cluster of miRs, among them miR432, seems to be implicated in the atherosclerotic process (170). Upregulation of miR 432, as well as miR 576, also seems to be implicated in the proinflammatory response, a possible pathway of enhancing vascular dysfunction (171, 172).

Epigenetic regulation plays an active role in kidney disease and cardiovascular disease, as in most other diseases, and might be of importance both as predictors of risk, and as possible targets for treatment. For the potential role of epigenetic regulation in interventions there is still very limited knowledge due to the lack of studies in the field.

2 AIMS OF THE THESIS

The overall aim of this thesis was to reach a higher understanding of cardiovascular disease mechanisms and treatment options in chronic kidney disease patients, with focus on the role of vitamin D on different aspects of vascular dysfunction.

The specific aims of the four studies are as follows:

Study I: To investigate if paricalcitol treatment affects measures of muscle sympathetic nerve activity, as well as measures of macro- and microvascular function in moderate CKD.

Study II: To analyse the effect of paricalcitol treatment on the proinflammatory profile and epigenetic modulators, measured by microRNAs, in moderate CKD.

Study III: To assess the effect of paricalcitol treatment on microparticle expression of ICAM-1, and VCAM-1, as well as the effect on the profile of endothelial, platelet and leukocyte microparticles in moderate CKD.

Study IV: To investigate, using overall effect size in a meta-analysis, if there is evidence in existing publications of an effect of vitamin D treatment or supplementation on endothelial function measured by flow-mediated vasodilation.

3 MATERIALS AND METHODS

3.1 STUDY POPULATIONS AND STUDY DESIGN

3.1.1 The SOLID trial; Study I, II and III

Patients (n=36) in study I, II and III were recruited from the Department of Nephrology at Danderyd University Hospital, Stockholm, Sweden, from 2010 to 2013. They were randomised in a double blind manner (12 in each group) to receive 1 µg paricalcitol, 2 µg paricalcitol or placebo. Due to the influence of sun, patients were not recruited during summer. Inclusion criteria were an estimated glomerular filtration rate (eGFR) of 15–59 ml/min/1.73 m^2 calculated from plasma creatinine using the MDRD formula, age >20 years, a plasma PTH level of 3.7–53 pmol/L, a Ca level <2.6 mmol/l, serum albumin >30 g/L and being on stable antihypertensive medication with no change in angiotensin converting enzyme inhibitor (ACEi) or angiotensin receptor blocker (ARB) medication during 2 months before enrolling in the trial. Exclusion criteria were nephrotic syndrome, diabetes mellitus or any treatment with vitamin D or its analogues that could not be discontinued, acute renal failure during the last 3 months, and if expected to need dialysis within 6 months. Patients were also excluded if they had known renal artery stenosis, severe kidney stones, uncontrolled hypertension (repeated measures of a brachial blood pressure >150/100 mm Hg) or other severe disease (active cancer, AIDS/HIV, severe congestive heart failure).

The study protocol was approved by the regional Ethics Committee in Stockholm, and registered on clinicaltrials.gov (SOLID study; NCT01204528). All patients provided written informed consent.

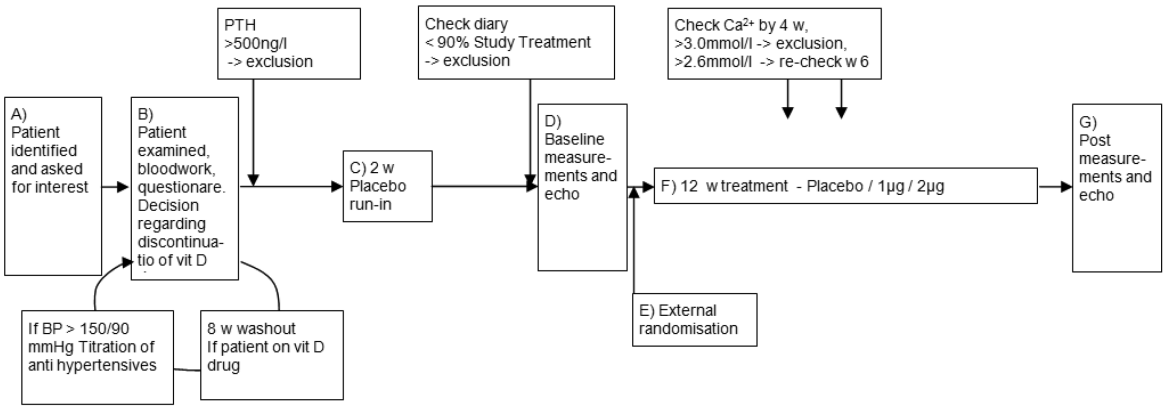


Figure 8: Flow chart of the SOLID trial. echo=echocardiography.

3.1.2 Study IV

Study IV was a meta-analysis investigating the effect of vitamin D on FMD. Inclusion criteria were chronic kidney disease in any stage and with any underlying cause, intervention with any vitamin D compound, a study design with a placebo or non-treatment control group, and outcome in form of flow mediated vasodilation. Exclusion criteria were combination

treatments with vitamin D and calcium, or comparison to other vitamin D compound or calcimimetics, without a non-treatment control group.

3.2 STUDY METHODS

Study I, II, and III started with two weeks of placebo run in, followed by 12 weeks of treatment with 1 or 2 μg paricalcitol, or placebo. Venous blood samples were drawn in the morning, after 12 h fasting and 20 min rest, at baseline and post treatment.

3.2.1 Study I

3.2.1.1 Arterial Stiffness

To assess arterial stiffness we used applanation-tonometry (SphygmoCor, AtCor Pty, NSW, Australia). The equipment was used to acquire a peripheral waveform from which pulse wave velocity (PWV) was measured and pulse wave augmentation index (AIx) was calculated. (105). To determine PWV, two waveforms were sequentially recorded (carotid- radial artery and carotid-femoral artery). The R-wave of a simultaneously recorded ECG was used as a reference frame, and the transit time of the pulse wave was then determined. The distance was measured and the PWV was determined. To determine AIx, a central waveform was assessed by the software, and the augmentation between the first and second systolic pressure peak of the wave-form was calculated. The augmentation was then expressed as a percentage of the total pulse pressure.

3.2.1.2 Large Vessel Endothelial Function

Macrovascular endothelial function was assessed by FMD (106). The brachial artery diameter was measured by a vascular ultrasound device with a 9-MHz linear transducer (Vivid 7 Dimension, GE Medical system, Horten, Norway). Endothelial dependent vasodilation was investigated by inducing ischemic reactive hyperaemia with a pneumatic tourniquet inflated to 250 mm Hg for 5 min. When released, vasodilation was measured as the relative change from rest in brachial artery diameter at 30, 60 and 90s. After 10 min of rest, endothelial independent vasodilation was assessed by the administration of 0.4 mg of sublingual glycerol trinitrate. The relative change from rest in brachial artery diameter was calculated from measurements in diameter 4 min after administration.

3.2.1.3 Skin Microvascular Function – Perfusion Imaging

Skin microvascular function was investigated by laser Doppler perfusion imaging before, during and after iontophoresis with acetylcholine (ACh; Sigma-Aldrich AB, Stockholm, Sweden) and sodium nitroprusside (SNP; Hospira, Inc., Lake For- est, Ill., USA) (173). ACh and SNP, diluted in deionized water, were used to examine endothelium dependent and independent skin microvascular function, respectively. Iontophoresis is a non-invasive technique using a small electric current for drug administration across the skin. Electrode

chambers (LI611 Drug Delivery Electrode Imaging, Perimed, Järfälla, Sweden) were attached to the volar side of the left forearm and filled with either ACh (2%) or SNP (2%). A battery-powered iontophoresis controller (Perilont 382b, Perimed, Järfälla, Sweden) provided a direct current (0.1 mA for 60 s) for drug iontophoresis. ACh was delivered with an anodal and SNP with a cathodal charge. Skin microcirculation was measured by laser Doppler perfusion imaging (Periscan PIM II, Perimed, Järfälla, Sweden) and expressed in arbitrary units (AU). Skin microcirculation was recorded continuously for 10 and 14 minutes after iontophoresis of ACh and SNP, respectively. Peak microvascular flux was determined. At our laboratory, the mean coefficient of variation of peak microvascular flux after iontophoresis of ACh and SNP were 11 and 20%, respectively.

3.2.1.4 Capillary Blood Cell velocity

Blood cell velocity in single nailfold capillaries of the great toe was measured during videophotometric capillaroscopy (174). Capillary blood cell velocity (CBV) was determined by two videophotometric windows positioned along the arterial side of the capillary axis detecting variations in optical density when blood cells and plasma gaps are passing through the capillary. The variations in light intensity are then converted into an electronic signal. Given the distance between the windows and the time delay between similar events in the upstream and downstream windows, CBV can be continuously recorded. CBV was measured during resting condition and during post-occlusive reactive hyperaemia, performed by a small pressure cuff at the proximal phalanx of the great toe. The cuff was inflated to a pressure of 200 mm Hg for 1 min, and peak CBV (mm/s), time to peak CBV (s), as well as relative change from rest (%) were measured. The temperature was continuously recorded during the measurements of CBV We have a good reproducibility in our laboratory (175).

3.2.1.5 Sympathetic Activation

Muscle sympathetic nerve activity (MSNA) was recorded using microneurography by a tungsten microelectrode inserted percutaneously into a muscle fascicle of the peroneal nerve (176). The signal was digitized (Powerlab Neuroamp, ADInstruments, Bella Vista, Australia) and pulse-synchronised bursts were analysed by a blinded investigator. Heart rate was recorded by a standard electrocardiogram, and blood pressure was measured with an automatic cuff. MSNA was quantified as bursts per minute (SNA/min) and as bursts per 100 RR intervals (SNA/RRI).

3.2.2 Study II

3.2.2.1 Characterization of the expression of immune modulators in plasma

Using the Luminex technique, a Milliplex 26-plex (Millipore corp.) was performed to assess the expression of a wide spectrum of cytokines before and after 12 weeks of paricalcitol treatment or placebo. In the Luminex technique, the company prepares microspheres, or beads, numbered to allow differentiation, and then covered in different antigen-specific

antibodies. They are then put together in different patterns allowing a multiple of molecules to be identified in one well simultaneously. The test-sample was then added by us and the expression was quantified by labelled detection antibodies (Figure 9).

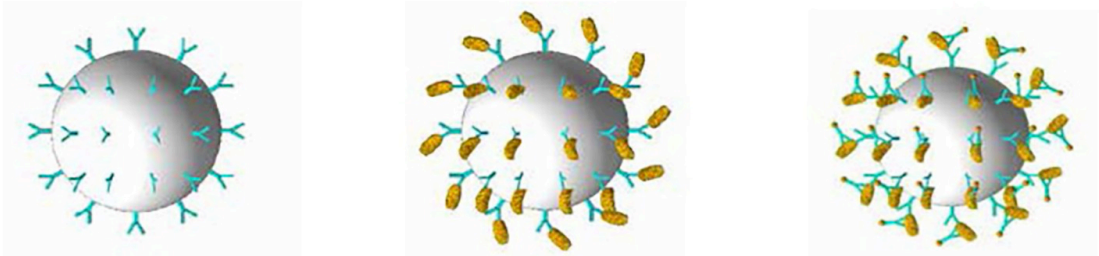


Figure 9: *The Luminex[R] technique. Published with permission from Luminex[R] and ThermoFisher Scientific.*

3.2.2.2 Reverse transcription polymerase chain reaction (RT-qPCR) to assess miRNA expression

We used miRCURY Ready-to-Use PCR Human panel I + II V1.M (EXIQON miRNA qPCR panel) (Figure 10) to assess the possible changes in miRNA expression in plasma. It was a ready to use kit involving a human miRNA panel, with a first step of reverse transcription (RT), of miRNA to DNA, followed by real-time PCR amplification with miRNA specific primers. For the first step, Universal cDNA synthesis kit II (miRCURY LNATM Universal RT microRNA PCR, EXIQON) was used. We then applied miRNA specific primers and ExiLENT SYBR Green master mix kit (miRCURY LNATM Universal RT microRNA PCR, EXIQON) for RT-qPCR. Quality control of the RNA isolation was performed by RNA spike-in (UniSp2, UniSp4, UniSp5), and cDNA synthesis control was assessed by UniSp6 in the RT-reaction. In addition DNA spike in (UniSp3) was added in all samples.

PCR reaction was assessed by MicroAmp^{TM} optical 384-well reaction plates with an ABI 7900 (Life Technology). As the amount of RNA in a sample is too small for exact determination of concentrations, the biofluid input amount in the PCR reaction was used as recommended by the manufacturer.

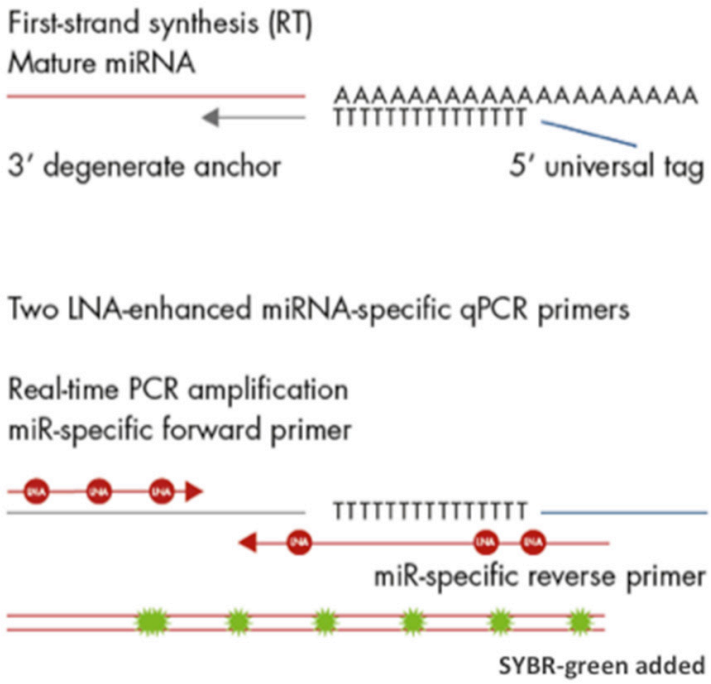


Figure 10: *The miRCURY-technique. First step is the synthesis of cDNA (reverse transcription (RT)) from existing miRNA in the sample. Next steps are amplifications of the cDNA produced in the first step by real-time PCR, using miRNA specific primers, and quantification of the process with help of SYBR-green. Modified from the miRCURY handbook (177). Published with permission from QIAGEN (formerly EXIQON).*

3.2.3 Study III

3.2.3.1 Assessment of microparticle concentrations

As previously described (178), plasma was centrifuged at 2,000 G for 20 min to attain platelet poor plasma, which was then frozen in aliquots at -80 °C until analysis. Platelet poor plasma was thawed and again centrifuged at 2,000 G for 20 min. The supernatant was subsequently centrifuged at 13,000 G for 2 min. 20 µL of the supernatant was incubated in the dark with lactadherin-FITC (BLAC-FITC, Coatech AB, Klädesholmen, Sweden) which binds to phosphatidylserine (PS), together with CD62E-APC (Thermo Fisher Scientific, Waltham, MA, USA), CD41-APC (Beckman Coulter, Brea, CA, USA), CD62P-PE (Thermo Fisher Scientific, Waltham, MA, USA), CD154-APC (Thermo Fisher Scientific, Waltham, MA, USA) CD45-APC (Beckman Coulter, Brea, CA, USA), CD106-PE (VCAM-1, Abcam, Cambridge, UK) and CD54-PE (ICAM-1, Abcam Cambridge, UK). MPs were defined as particles less than 0.9 µm in size, positive to lactadherin. All samples were analysed using a Beckman Coulter Gallios flow cytometer (Beckman Coulter, Brea, CA, USA), and the MP-gate was calibrated using Megamix beads (FSC; 0.5 µm, 0.9 µm and 3.0 µm, BioCytex, Marseille, FR).

3.2.4 Study IV

A systematic literature search of PubMed/Medline, Web of Science (WoS), Embase and Cochrane trials and reviews was performed. Search results were restricted to controlled trials and to the English language. We used the MeSH-terms for vitamin D as well as kidney disease, and all terms listed beneath. The selected articles were coded with a prespecified extraction form including study length, number of participants, vitamin D compound and dosage, age, CKD stage, other treatments and baseline laboratory measurements such as vitamin D status. Methodological study quality and risk of bias were assessed by the Jadad Score, which gives 1-5 points for blinding, randomization and risk of incomplete outcome data (the account of all screened and included patients) (179). Selective outcome reporting was also assessed during the screening and selection process. To assess publication bias, rank correlation and a funnel plot were both performed. We also searched ClinicalTrials.Gov for unpublished articles matching our inclusion criteria.

3.3 STATISTICAL ANALYSES

The SOLID study was a novel hypothesis-testing study, where the study-size was based on a treatment-induced change in MSNA. The intention was to study first non-diabetic CKD, and then diabetic CKD. Whether vitamin D affected MSNA was unknown, and an estimated moderate-large effect size (f=0.36) showed that we needed 36 patients in each group (non-diabetic and diabetic CKD). We included a pre-specified interim analysis after the first group of patients (non-diabetic CKD; n=36). At that interim analysis, we found no significant change in MSNA. Instead of pursuing the hypothesis that MSNA might be affected in diabetic CKD, due to limited resources, we instead decided to turn to secondary explorative outcome measures.

Descriptive statistics were presented as means and proportions. Between group differences in means at baseline were examined by one-way analysis of variance (ANOVA), by the Chi^2-test, or the Fischer´s exact test when appropriate.

In paper I, the effects of intervention (paricalcitol) and time were investigated for vascular function and MSNA by using repeated measures two-way ANOVA as well as by multilevel modelling/mixed model (MLM) to improve the statistical power. We also examined the effects of intervention and time within groups, by paired t-test.

In paper II, non-parametric tests were used, as data was non normally distributed. Using the same patients, changes in cytokine expression was investigated by Kruskal Wallis (pre- and post-measurements separated), followed by Wilcoxon matched-pairs signed rank test for within group comparisons over time and Mann Whitney U-test for two independent group comparisons. For miRNA, the delta change (post - pre measurements) as well as fold change (post/pre measurements) were determined and the between-groups comparison was then

performed by non-parametric Kruskal Wallis and Mann Whitney U-test for two independent group comparisons.

In paper III, changes in microparticle-levels and their expressions in the SOLID patients, was tested using two-way repeated measures ANOVA (ICAM-1, VCAM-1) and, for the profile of subclasses of MPs, two-way repeated measures MANOVA. Post hoc analyses were performed with one-way repeated measures MANOVA (main effect of time) and paired t-test for within group changes.

Paper IV, a meta-analysis of treatment with vitamin D to CKD patients, was analysed as post treatment comparisons, calculating standardized mean difference effect size (STANDmean ES). Weighted standard deviation and then Hedges g were used to calculate effect sizes (STANDmean ES) for each study (180, 181). A positive value indicated an effect in favour of treatment. The overall effect size was then assessed by a fixed effects model (180). A random effects model was also performed as a secondary analysis. I^2 statistics were performed to assess heterogeneity.

Statistical analyses were performed using SPSS software, version 22 and 24 (SPSS Inc., Chicago, IL, USA) and SAS (version 9.3, SAS Institute Inc., 2002–2010).

3.4 ETHICAL CONSIDERATIONS

The SOLID trial was performed in accordance with the declarations of Helsinki. All patients provided and signed written informed consent. The study was registered at ClinicalTrial.Gov as SOLID study; NCT01204528.

The meta-analysis was performed in accordance with the PRIMA guidelines and MOOSE checklist. All included studies assured the use of the declarations of Helsinki and of written informed consent.

4 RESULTS

4.1 THE SOLID STUDY; PAPER I, II AND III

4.1.1 Patient characteristics

Thirty-six patients were included, and 35 completed the trial. The one person who did not complete the trial was in the placebo group, and experienced feelings of dizziness from the study drug (placebo) and did not perform the post-treatment measurements. No major adverse events were found during the study period, and there were no hospitalizations. Baseline characteristics showed that those in the placebo group were slightly older with more previous cardiovascular disease, and a tendency to higher number of patients with polycystic kidney disease as aetiology of CKD.

Table 6: *Baseline characteristics in the SOLID trial as mean and (SD) or nr and (%).*

Characteristics	Placebo (n=12)	Paricalcitol 1µg (n=12)	Paricalcitol 2 µg (n=12)
Age (years)	70.8 (10.0)	66.1 (7.9)	59.1 (11.6)
Sex (% male)	9 (75%)	11 (92%)	8 (67%)
Smokers (current)	1 (8%)	1 (8%)	0
BMI	28.1 (2.4)	26.4 (3.5)	26.8 (2.8)
CKD duration (years)	10.3 (8.8)	5.8 (6.0)	9.7 (10.5)
Cause of CKD			
Hypertension	3 (25%)	4 (33%)	4 (33%)
Polycystic disease	4 (33%)	2 (17%)	1 (8%)
Glomerulonephritis	4 (33%)	3 (25%)	4 (33%)
Other cause	0	2 (17%)	2 (17%)
CVD at inclusion			
Myocardial infarction	3 (25%)	2 (17%)	0
Atrial Fibrillation	1 (8%)	1 (8%)	0
Stroke	3 (25%)	0	0
TIA	1 (8%)	0	0
Heart failure	0	0	1 (8%)
Aortic aneurysm	0	1 (8%)	1 (8%)
Medication			
ACE-i/ARB	11 (92%)	9 (75%)	9 (75%)
β-blockers	6 (50%)	8 (67%)	4 (33%)
Ca flow-inh	10 (83%)	8 (67%)	4 (33%)

BMI=body mass index; CKD=chronic kidney disease; TIA= transient ischaemic attack; ACE-i=angiotensin converting enzyme inhibitor; ARB=angiotensin receptor blocker; Ca-flow-inh=calcium-flow inhibitors. Reprinted in courtesy of American Journal of Nephrology:2015, 42(4):265-73, by permission of S. Karger AG.

The three groups did not differ in eGFR, duration of CKD or serum creatinine levels. They were also well matched in routine biochemistry status, measures of vascular function (FMD and PWV), microcirculatory function except peak CBV (iontophoresis and videophotometric capillaroscopy), and measures of sympathetic nerve activity (MSNA). Measures of cytokine expression and concentrations of microparticles were also well matched between groups at baseline (Table 6).

4.1.2 Routine laboratory findings post treatment

There were no significant changes in albumin, UACR or CRP-levels post treatment. Neither were there any significant changes in phosphate, 25OH-vitamin D, or calcium. However, calcium did change slightly in absolute numbers with highest levels post treatment in the 2 μg treatment group. PTH was significantly suppressed by treatment (two-way ANOVA p=0,02 for treated groups combined, p=0,006 for 3 groups comparison) in a dose dependent manner.

4.2 VASCULAR MEASUREMENTS; PAPER I

There was a significant decrease in FMD across all groups after 3 months study (MLM main effect of time p=0.006). However, when within group changes were examined, the 2 μg treated group showed preserved endothelial function (paired t-test p=0.54) (Figure 11a).

For iontophoresis by acetylcholine the results were similar, with a borderline significant decrease across all groups, (repeated measures ANOVA, main effect of time p=0.06) but preserved function with no significant decrease for the 2 μg treated group (paired t-test, p=0.65) (Figure 11b).

We also performed videophotometric capillaroscopy. There were however several patients where this measure could not be performed. The treated groups were collapsed into one and MLM performed on 26 patients. The results showed borderline significant interaction, with ameliorated microcirculatory function in the treated groups, and declining function in the placebo group, measured as peak CBV (MLM p=0.06). Within group changes also showed ameliorated function for the treated group with borderline significance (paired t-test p=0.05) (Figure 11c).

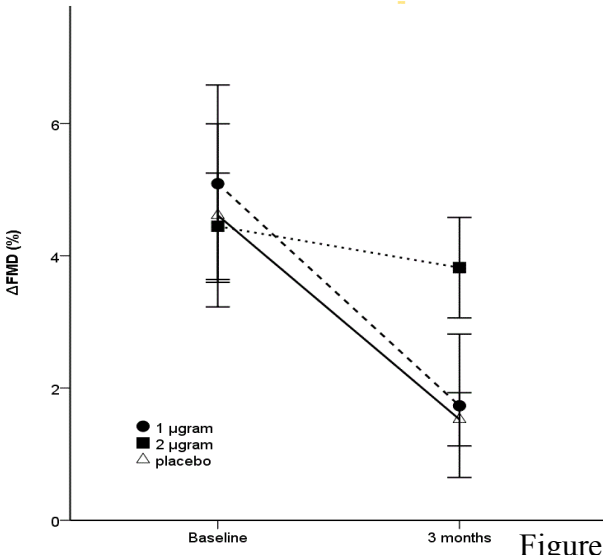


Figure 11a

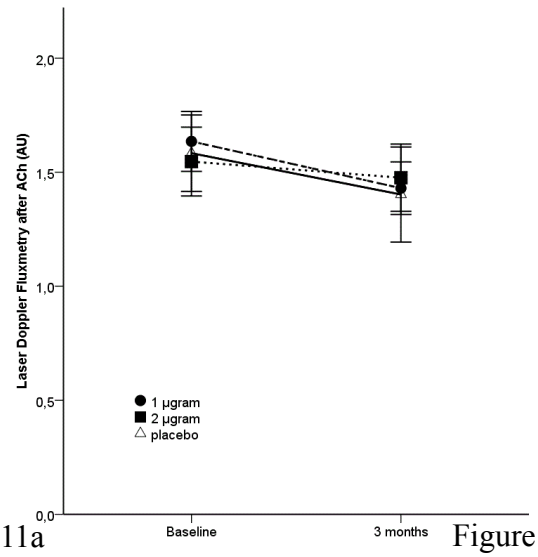


Figure 11b

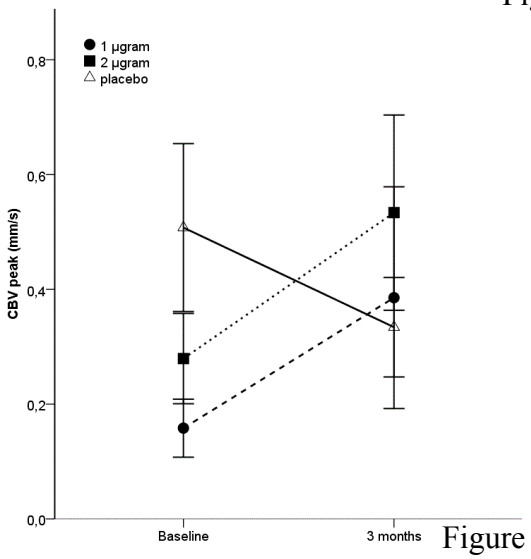


Figure 11c

Figure 11: *Measures of FMD, iontophoresis by acetylcholine and peak capillary blood cell velocity pre- and post treatment. Reprinted in courtesy of American Journal of Nephrology:2015, 42(4):265-73, by permission of S. Karger AG.*

We did not detect any change by intervention and time in PWV, PWAix or MSNA. For MSNA there was only complete data for 24 patients, but with MLM, 11 of those with incomplete data could contribute with values in the analysis.

4.3 PROINFLAMMATORY CYTOKINES AND miRNAs; PAPER II

When comparing pre- and post-measurements of a range of proinflammatory cytokines, VEGF and PDGF were significantly decreased in the two treated groups, but remained unchanged in the placebo group (Wilcoxon matched pairs for within group changes). Levels of interferon gamma induced protein 10 (IP10), a cytokine central in the enhancement of the inflammatory response, were also decreased for treated patients, but only significantly for the 2µg treated group (Wilcoxon matched pairs) (Table 7).

Cytokine	Placebo		p-value	Paricalcitol 1µg		p-value	Paricalcitol 2µg		p-value
	Pre	Post		Pre	Post		Pre	Post	
VEGF	20 (9-36)	18 (13-21)	0.2	20 (15-31)	10 (3-15)	**0.01**	26 (9-60)	10 (7-32)	**0.02**
PDGF	1220 (719-3740)	837 (411-3084)	0.2	1297 (960-1933)	784 (561-915)	**0.005**	2811 (1447-3706)	1234 (1003-2239)	**0.009**
IP-10	342 (169-5167)	332 (71-5091)	0.8	423 (353-2805)	402 (274-614)	0.08	2628 (511- 6200)	492.5 (270-2022)	**0.02**

Table 7: *Concentrations of cytokines (pg/ml) pre- and post intervention, including p-values. Reprinted in courtesy of BMC Nephrology: 2017, 18(161), in line with the open access agreement for Springer Nature.*

The two treatment groups were also collapsed into one, to obtain better power, and we then detected a significant decrease in a wide range of cytokines (Wilcoxon matched pairs), in comparison with placebo where there were no significant changes during the study (Figure 12).

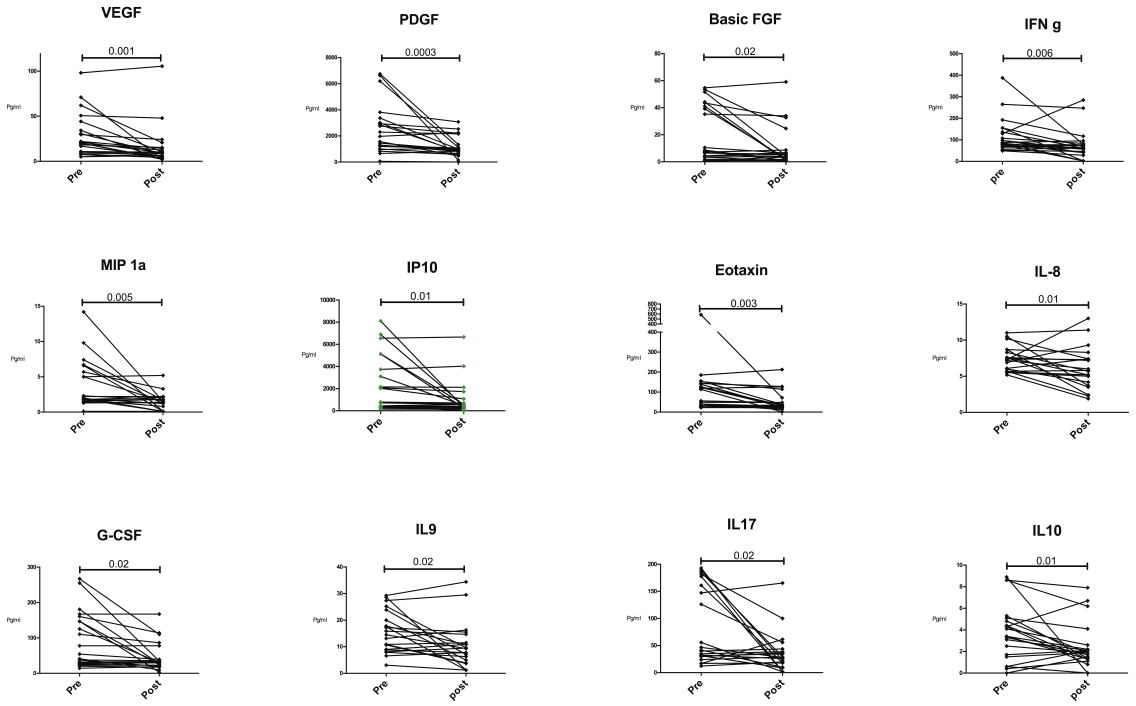


Figure 12: *Changes in cytokine levels pre-post treatment in the collapsed treatment groups. P-values indicated in the figures. There were no significant changes in the placebo group.*

To determine possible changes in miRNAs post treatment, we first performed a pilot study of five randomly selected patients on 2 µg paricalcitol treatment. The top five (p-value and fold change) changed miRNAs were miR 133a, miR432, miR 495, miR 576 and miR 874. These were then analysed in all 36 patients. We chose to include also the five patients in the pilot study in the final analysis to obtain better statistical power. MiR 432, miR 495 and miR 576 were all significantly downregulated by 2µg of paricalcitol treatment (Kruskal-Wallis and post hoc Mann-Whitney U-test comparisons of delta value and fold change) (Figure 13). The effect was similar when collapsing the treatment groups.

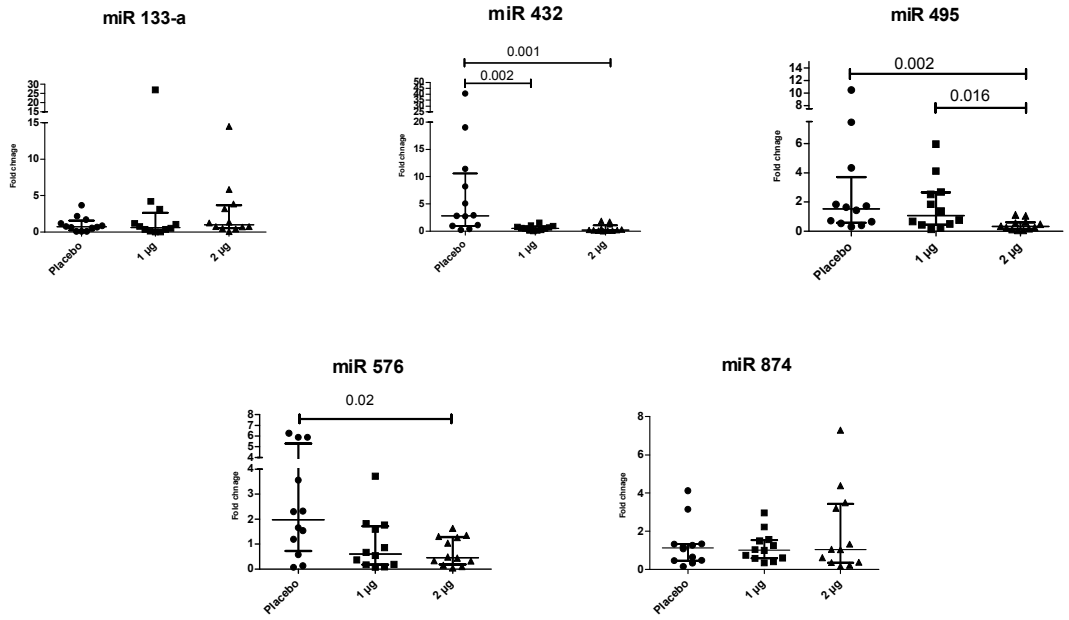


Figure 13: *The five top ranked miRNAs validated in all patients, as fold change (post/pre measurements). Significant change by treatment indicated with p-value. Reprinted in courtesy of BMC Nephrology: 2017, 18(161), in line with the open access agreement for Springer Nature.*

4.4 ENDOTHELIAL MICROPARTICLES AND VASCULAR BIOMARKERS; PAPER III

In this study, we investigated the change in expression of ICAM-1 and VCAM-1 on microparticles as well as the change in total MP profile during intervention.

There was a change by treatment in the expression of ICAM-1 on MPs, seen by a significant interaction (repeated measures two-way ANOVA p=0.04), with augmented levels in the placebo group, and decreased levels in the treated groups (Figure 14a). This was not seen for the expression of VCAM-1 on MPs, where the levels remained stable during intervention (repeated measures two-way ANOVA p=0.52).

To avoid multiple comparisons, a two-way repeated measures MANOVA was used, to investigate the changes in cell-specific MP subtypes ($CD62E^+$ EMPs, $CD41^+$ PMPs, $CD41^+$ $CD62P^+$ PMPs, $CD41^+CD154^+$ PMPs and $CD45^+$ LMPs). There was a significant decrease in all MP subtypes during the study (repeated measures MANOVA, main effect of time p=0.001), with a tendency to interaction between treatment and time (repeated measures two-way MANOVA p=0.08). Post hoc analyses with one way repeated measures MANOVA showed that the findings were due to sustained levels in the 2 µg (p=0.85), and significantly decreasing levels in the 1 µg (p=0.04), and placebo group (p=0.005) Figure 14b).

Focusing on EMPs, further analyses demonstrated that EMP levels were not significantly changed for either the 1 or 2 µg treatment groups during the study (1 µg, paired t-test, p=0.10, and 2 µg, p=0.3), while decreasing in the placebo group (paired t-test, p=0.002). In absolute numbers, a pattern of dose dependent change was observed (Figure 14c).

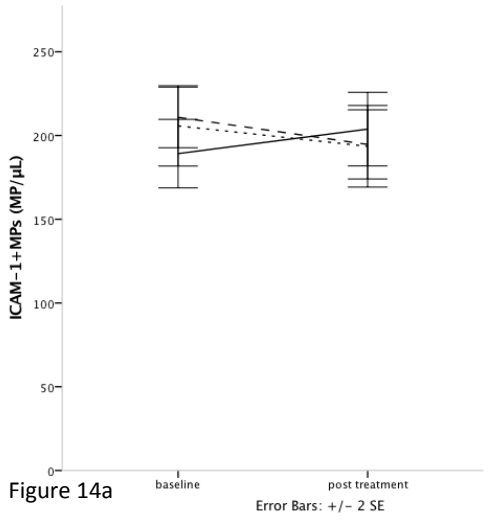
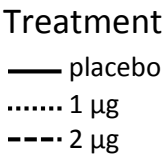


Figure 14a



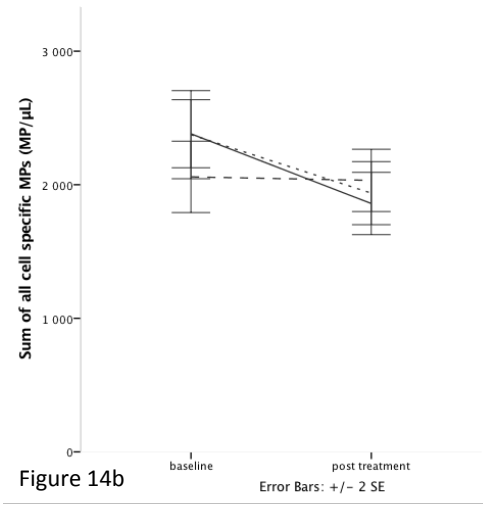


Figure 14b

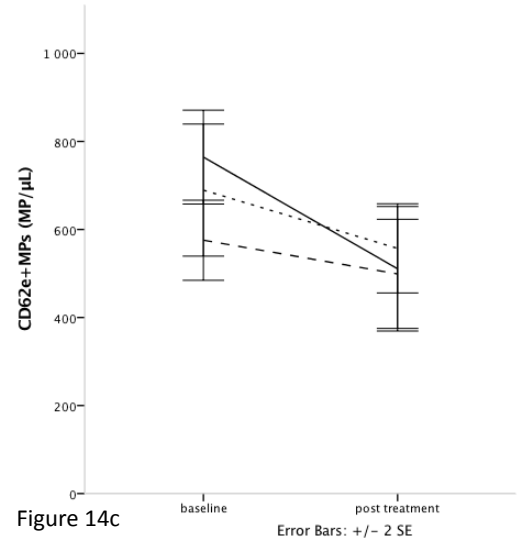


Figure 14c

Figure 14: *Pre and post measurements of microparticles. MP=microparticle; CD62E^{+}MPs=EMPs*

4.5 OVERALL EFFECT SIZE BY PARICALCITOL TREATMENT ON FMD; PAPER IV

4.5.1 Study selection and population

In total, 1.744 articles were found searching the databases. After screening of title and abstract 304 articles remained, and 14 were selected for full review. Of these, four studies met the full inclusion criteria (Figure 15). However, one of the studies comprised two treatment groups (1 and 2 μg of paricalcitol; study one in the present thesis) and after discussion, this study was split in two, using the same placebo group as control, thus resulting in five studies in the final analysis.

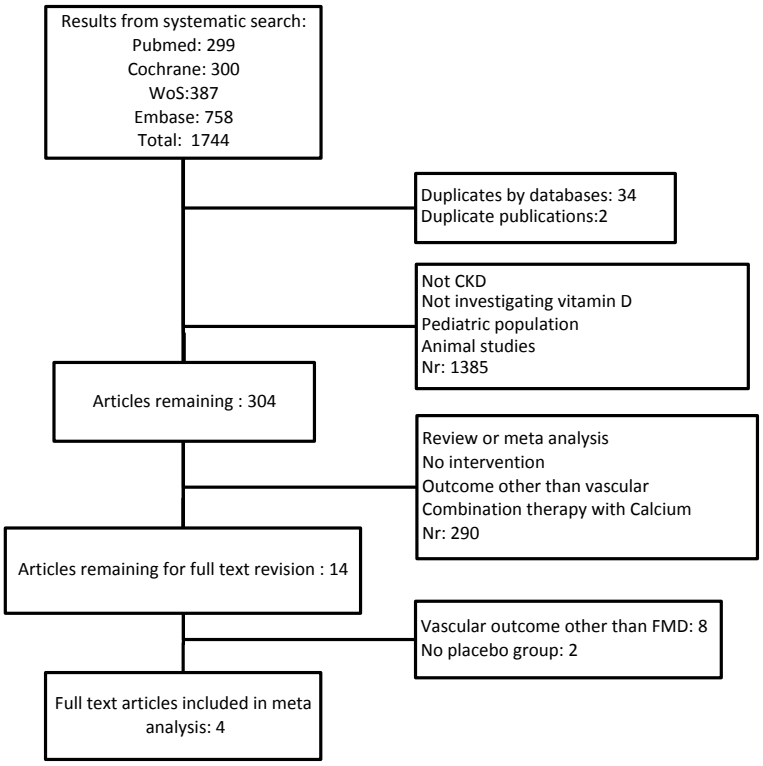


Figure 15: *Flow chart of the selection process. WoS=Web of Science; Reprinted in courtesy of BMC Nephrology: 2018, 19(247), in line with the open access agreement for Springer Nature.*

All included patients were in CKD stage 3-4. Mean age was 59.9 years, ranging from 44-65 years. Study size varied from 24-120 participants, and study duration from 12 to 16 weeks. One study investigated the effect of cholecalciferol in two oral doses of 300 000 IU at baseline and after 8 weeks, while the others used paricalcitol 1 or 2 μg daily (Table 8).

Table 8: Baseline characteristics of the included studies in the meta-analysis.

Author	Zoccali (-14)	Lundwall 2µg (-15)	Lundwall 1µg (-15)	Theti (-15)	Kumar (-17)
Country	Italy	Sweden	Sweden	USA	India
Duration	12w	12w	12w	12w	16w
Sample size (nr)	89, analysis on 88	24, ITT	24, ITT	60, 55 completed, analysis on 46	120, analysis on 117
CKD stage	3-4	3-4	3-4	3-4	3-4
Treatment	paricalcitol	paricalcitol	paricalcitol	paricalcitol	cholecalciferol
Dose	2µg daily	2µg daily	1µg daily	1µg daily	300000IU at baseline and after 8 week
Baseline 25(OH)D (nmol/l)	35.5	65.1	66.7	1.25-OHD: 34.5 (pg/ml)	33.2
Age (mean)	62.5	65.0	68.5	62.5 (median)	44.2
ACEi/ARB (%)	N/A	80.6	80.6	69.1	67.5
Jadad score	4	4	4	3	5
Underlying condition/DM	N/A	Non-diabetic patients	Non-diabetic patients	Diabetic nephropathy	Non-diabetic patients
Outcome	FMD	FMD, PWV, echo, iontophoresis, microcirc	FMD, PWV, echo, iontophoresis, microcirc	FMD	FMD, PWV

ACEi=angiotensin converting enzyme inhibitor; ARB=angiotensin receptor blocker; DM=diabetes mellitus; ITT=intention to treat; FMD=flow mediated vasodilation; PWV=pulse wave velocity; echo=echocardiography. Reprinted in courtesy of BMC Nephrology: 2018, 19(247), in line with the open access agreement for Springer Nature.

4.5.2 Study quality and bias

Both the Jadad score and the Cochrane handbook 5.1 were used to evaluate study quality and risk of biased data. Included studies had Jadad scores from 3 to 5, indicating median to high quality. There were no signs of selective outcome reporting when performing the literature screening and selection. Overall, this indicated a low risk of biased data. Rank correlations and visual inspection of a funnel plot did not indicate publication bias. This conclusion was also strengthened by the search in ClinicalTrials.Gov, where there were no unpublished trials of interest to our inclusion criteria.

4.5.3 FMD outcome

The five included studies comprised 305 patients. Patients were well matched in terms of FMD at baseline in each study. The fixed effects model analysis showed a positive effect of vitamin D treatment on FMD (STANDmean ES 0.78, 95% CI 0.55-1.01) (Figure 16). A random effects model performed as a secondary analysis also showed significantly positive effects of treatment (STANDmean ES 0.67 95% CI 0.06-1.29). The heterogeneity was substantial for the fixed effects model (I^2=84 %), but minimal for the random effects model (I^2=0 %). There were too few studies included to perform a meta-regression to statistically investigate the heterogeneity in the fixed model.

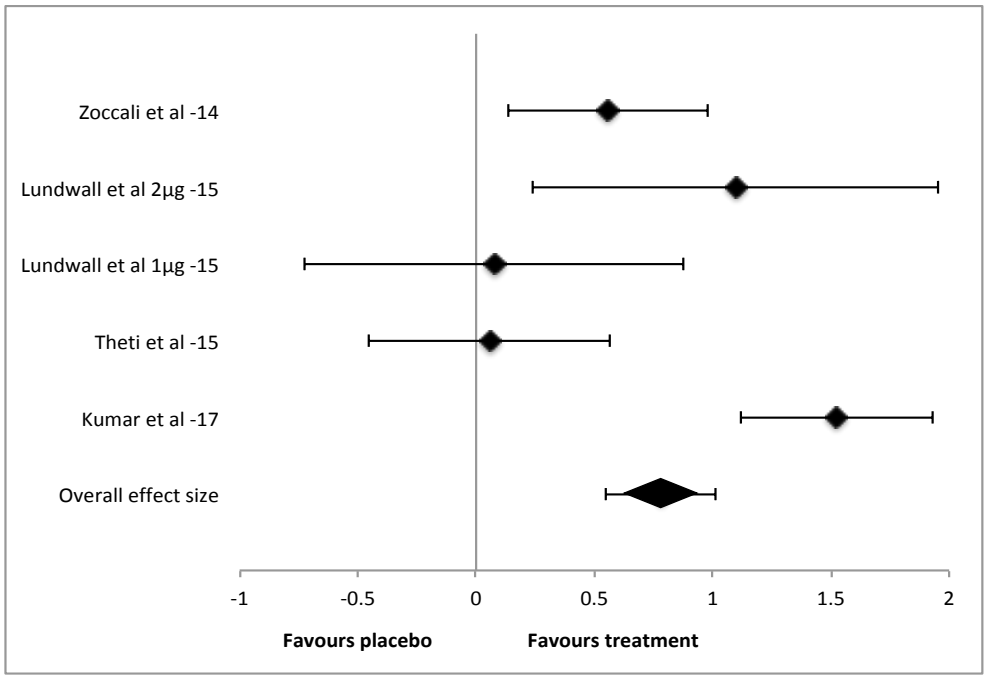


Figure 16: *Forest plot of effect sizes in the included studies. Overall effect size in a fixed effects model. Reprinted in courtesy of BMC Nephrology: 2018, 19(247), in line with the open access agreement for Springer Nature.*

5 GENERAL DISCUSSION

In the SOLID study we show that 2 μg of paricalcitol to patients with moderate CKD preserves macro- and microvascular endothelial function measured by FMD and iontophoresis by acetylcholine, and seems to improve microcirculatory measures, in line with previous findings (44, 82, 111-113). We also show that this population, with few previous cardiovascular events, has a rapid decline in endothelial and microcirculatory functions.

Further investigations in our patients demonstrate that treatment with paricalcitol improves the inflammatory profile, measured by cytokine release, also in line with previous findings in small RCT:s (96-99). The effects were most pronounced for the vascular inflammatory cytokines VEGF and PDGF showing again the positive effects of vitamin D on vascular cells. The upstream regulation, measured by the change in microRNAs, was also changed by treatment, showing lower expression of microRNAs with a connection to atherosclerosis, platelet function and inflammation (167, 168, 170-172). These results, however in a new field, give an indication of the possible upstream epigenetic effects of vitamin D on our laboratory and physiological findings.

To further explore the vascular function in our patients, as well as investigate new possible biomarkers of cardiovascular disease, we assessed the microparticle profile pre- and post-treatment, as well as their expression of vascular activation markers. To our knowledge, no studies have previously investigated the effects of vitamin D on the MP profile in CKD patients. Our results on the microparticle expression on ICAM-1, showing lower expression with treatment, are in line with previous findings (44, 133) on soluble ICAM-1. The assessment of cell-specific MPs shows interesting and intriguing results, with decreasing production of MPs over time in all cells, however preserved by 2 μg of treatment, much like our physiological vascular results of FMD and iontophoresis. These findings are new and demand further research, but might indicate that a dysfunctional vasculature with surrounding cells does not have the ability to produce as much MPs, as healthy cells have. This interpretation is strengthened by a study of Augustine et al (158), showing lower MP levels after a dobutamine stress echocardiography in patients with signs of coronary disease, than in patients with a normal examination.

Treatment with vitamin D in CKD patients seems to lower albuminuria on top of RAAS-blockade (72-75), but the effects on vascular measurements are inconclusive. Most studies on inflammatory markers, iontophoresis and FMD have shown effect of treatment (44, 82, 111-113, 133) but as outlined in the background, most studies using PWV/PWA have failed to show significant changes by treatment. The reasons for this discrepancy in findings might be many, but one important issue may be in the methods of measuring vascular function. PWV/PWA are complex measures of both arterial stiffening and calcification (PWV/PWAix) (12), and beta-2 induced vasodilation (PWA after administration of beta 2-adrenoceptor stimulation) (109), whereas FMD is primarily a measure of the capacity of the endothelial cells to produce and react to NO (106). As such, FMD is mainly a measure of function, not

structure, and is therefore probably an earlier sign of vascular disease, than PWV/PWAix. It is therefore likely easier to affect FMD in short duration studies, since it does not require structural changes to occur. Another important aspect in understanding these results are the cellular effects of vitamin D, with antioxidative, eNOS-upregulating actions, seen in in vitro-studies (44, 83, 85). These are direct pathways for an ameliorated NO-production by the endothelial cells. Even though PWV and FMD are clearly different measures of vascular structure and function, they are interrelated (107, 126) and are both independent predictors of cardiovascular risk (17, 45, 46, 109, 126).

In our last study, we aimed to further understand the diverging results of interventions with vitamin D on vascular function. As all studies performed have been quite small, they all have limited statistical power. We consequently performed a meta-analysis assessing the effect of vitamin D compounds in patients with CKD, and in accordance with the pathways for vitamin D actions, and the short duration of studies, we used FMD as single outcome. Our meta-analysis showed that vitamin D does affect endothelial function in terms of NO production in a positive direction.

The meta-analysis also gives interesting clues to which patients that might benefit the most from Vitamin D treatment. While all patients were in CKD stage 3 to 4, four studies had populations originating from western countries with a mean age of 63.9 years, while the study with the strongest effect size had a population originating from India, with a mean age of 44.2 years (111). It is plausible that the structural vascular changes were not as pronounced when entering the study for these latter patients. Kumar et al also used cholecalciferol, raising questions about the potentially negative effects on calcium- and phosphate metabolism by active vitamin D treatment. Of interest is the sub-study by Zoccali et al (182), on the PENNY trial (113), showing that the effect of paricalcitol on FMD was most pronounced in patients with the lowest rise in phosphate, and abolished in those with the highest rise.

Interventional studies on CKD patients have suffered from inconclusive and negative outcomes in many fields of research. Chronic kidney disease comprises a wide spectrum of underlying disorders, from hypertension, to autoimmune diseases, vasculitis, infections, diabetes type 1 and 2, and the inherited polycystic disease. The CKD diagnosis also includes patients with an almost normal kidney function, through the whole range of renal dysfunction, to dialysis patients with a very complex picture of disturbances due to both their kidney failure and the dialysis per se. When interpreting interventional studies on this group of patients, both the underlying disorder and the stage of kidney disease has to be taken into cautious consideration. In the field of vascular disease, questions have been raised if it is even possible to reverse or ameliorate the advanced vascular disease seen in later stages of chronic kidney disease (12, 13).

The studies performed with vitamin D treatment in CKD patients are too small and of too short duration to answer questions on hard endpoints. PWV/PWA and FMD are both predictors of cardiovascular risk, whereas other measures of vascular function, such as iontophoresis and SEVR are less validated (126). Our meta-analysis shows that there are still

very few patients included in controlled studies investigating these more validated measures of vascular risk. There were no found studies assessing hard endpoints as an *á priori* outcome in our search.

5.1 LIMITATIONS

The major limitations in our RCT are the small number of participants and short duration of intervention. The power calculation was made for a significant change in MSNA, and not for FMD, and therefore we may have a lack of power in the study and a risk of type-2 errors. From this aspect, it might have been wiser to use only a two-group scheme, comparing placebo to treatment, however at the time of the study design it was not known which dose to use. The use of a predictor instead of hard endpoints is another limitation, however closely linked to the short duration, which makes hard endpoints impossible to use.

The meta-analysis has similar limitations with each included study being small and of short duration, and in addition there are few studies performed. It makes the generalizability of our findings limited, but also highlights the need for properly sized studies in the field.

6 CONCLUSIONS

Treatment with vitamin D improves inflammation and preserves measures of endothelial function in patients with CKD stage 3-4. Early intervention, before manifest structural vascular changes have occurred, is probably needed for adequate effects. The question whether to use active treatment or supplementation remains, but some results are in favour of supplementation. Another remaining question is of other aspects of CKD-MBD, where studies indicate that combination therapies, controlling different aspects of the CKD-MBD axis, might be the right way forward. The study lengths are too short and the number of study participants is still too few to answer questions on hard endpoints in this field. However, the lack of evidence on hard endpoints due to these facts, is not to be confused with no evidence, and hopefully the future will give us conclusive answers.

7 FUTURE PERSPECTIVES

We show effects of vitamin D treatment in CKD patients on vascular function and inflammation, but the results in this area are not conclusive, like in many of the research areas of intervention in kidney diseases. In the field of vitamin D and CKD, many questions remain a decade after the pleiotropic effects of vitamin D were first acknowledged.

There are important issues of research methodology in this area. Underlying disease and CKD stage have to be taken into a more appropriate consideration when designing studies. Is it feasible to believe that we can change the late and advanced vascular disease seen in CKD stage 5 and dialysis patients? Would it not be better to lose some of the generalizability in the very broad spectrum of CKD, and instead perform studies on more specified underlying conditions?

Relevant research questions have to be asked and appropriate methods used pertaining to the examined population and the study length. If it is not possible to use hard endpoints, which predictors should be used, and in which populations? Are the outcomes used good enough to answer our questions?

How can we perform larger and longer studies in this area? Here, the usage of already existing registries, registry RCT:s is an interesting field, which gives the possibility to perform larger and longer studies that cost less money and without the need of industry involvement. Here, the Swedish Renal Registry and the SWEDEHEART registry might be of use.

There are also remaining questions regarding the different aspects of CKD-MBD. This axis is brilliantly designed by nature to control itself, and maybe our attempts to correct one side of the axis are too imprecise, tipping the other side over. We need more thorough analyses of our results, to understand why some patients respond to treatment, and some do not. This was elegantly performed by Zoccali et al (182), showing that the effect of paricalcitol on FMD was clearly correlated to the individual rise in phosphate levels in the examined patients. Can we predict which patients that might respond and who will not? Can we from early stages combine treatments affecting the axis to better balance the effects? Here, we have to widen our views and cooperate in the different research fields of CKD-MBD.

8 SVENSK POPULÄRVETENSKAPLIG SAMMANFATTNING

Nedsatt njurfunktion är en av våra vanligaste folksjukdomar och drabbar 10-15 % av världens befolkning. Hjärt-kärlsjukdomar är en annan stor folksjukdom, och är dessutom orsaken till flest dödsfall i världen. De senaste årtiondena har det alltmer uppmärksammats att patienter med njursvikt också i hög utsträckning drabbas av hjärtkärlsjukdom. Det är faktiskt så att de flesta patienter med njursvikt inte dör av sin njursvikt, utan av hjärtkärlsjukdomar som hjärtinfarkt eller stroke. Orsakerna har utforskats och vi har nu en god förståelse av hur njursvikt ger en avancerad och snabbt progredierande kärlsjukdom, med stela, kalkinlagrade blodkärl. Startpunkten är sannolikt en kombination av förändringar i kärlen på grund den minskande njurfunktionen med störningar i vitamin D, kalk och fosfatmetabolismen, aktivering av immunförsvaret med påföljande kronisk inflammation, och dysfunktion i det innersta kärllagret, endotelet.

I detta doktorandprojekt har vårt mål varit att få en bättre förståelse för kopplingen mellan njursvikt och hjärtkärlsjukdom, med fokus på vitamin D-brist, kärldysfunktion och inflammation.

Vitamin D genomgår sitt slutliga aktiverande steg i njuren, vilket medför att njursvikt leder till låga nivåer av aktivt vitamin D. Vitamin D är klassiskt känt som en viktig del i kalciumomsättningen, då det behövs för upptaget av kalcium. Dock har studier de senaste årtiondena visat att vitamin D har betydligt fler funktioner än så. Dess receptor finns spridd i hela kroppen, och aktiveringen sker inte bara i njuren, utan även i andra celler, bland annat immunceller. Vitamin D har en viktig funktion i just immunförsvaret, där det både förbättrar försvaret mot bakterier, men också verkar antiinflammatoriskt, och minskar nivåerna av oxidanter i kroppen.

Det inre kärllagret, endotelet, har en mycket viktig funktion i kärlen då det styr kärlens sammandragning vilket i sin tur påverkar blodtrycket. Endotelet aktiverar och styr också immunceller och blodplättar, och endoteldysfunktion är en av de tidiga hörnstenarna i åderförkalkning, ateroskleros. Endoteldysfunktion har därför också visat sig korrelera till risk för framtida hjärtkärlhändelser. En viktig del i endoteldysfunktion är inflammation orsakat av aktiverade immunceller samt höga nivåer av oxidanter, bildade både från aktiverade immunceller och från endotelet självt. Vitamin D är därför intressant som en möjlig väg att påverka njursviktspatienters förhöjda risk för hjärt-kärlsjukdom, genom att verka antiinflammatorisk och antioxidativt på blodkärlens endotel.

Vi har därför utfört en randomiserad dubbelblind placebokontrollerad studie, på 36 njursviktspatienter utan diabetes, med njursvikt stadium 3-4. De lottades till placebo, eller behandling med 1 eller 2 μg paricalcitol, en aktiv vitamin D-analog. Vi undersökte kärlfunktionsmått, inflammatoriska parametrar, samt epigenetisk påverkan. Vi undersökte också en möjlig ny biomarkör för kärlfunktion, mikropartiklar, före och efter behandling.

Våra patienter var i genomsnitt 65 år gamla, och hade en måttligt nedsatt njurfunktion (medel estimerad njurfunktion GFR 40ml/min). De hade få tidigare hjärtkärlhändelser.

I studie I visade vi att behandling med 2 µg paricalcitol ledde till en bevarad endotelfunktion, medan denna försämrades i placebogruppen, och gruppen som behandlades med 1 µg paricalcitol. Mikrocirkulationen förbättrades för båda behandlingsgrupperna, men försämrades för placebogruppen.

I studie II undersökte vi inflammationsprofilen mätt i form av cytokiner, före och efter behandling. Här såg vi en minskad inflammation av både 1 och 2 µg behandling, dock tydligast visat med minskande nivåer av VEGF och PDGF, två cytokiner direkt kopplade till kärlfunktion och ateroskleros. Här undersöktes också epigenetisk påverkan i form av microRNA, som styr vilka mRNA som kommer att aktiveras och därmed bilda proteiner. Vi visar lägre nivåer av tre microRNA, kopplade till ateroskleros, blodplättsfunktion, och inflammation.

I studie III undersökte vi mikropartiklar, som produceras från celler när dessa är stressade, aktiverade eller döende. Nivåer av endotelmikropartiklar har därför kunnat korreleras till endotelfunktion och även risk för hjärtkärlhändelser. Vi undersökte även mikropartikeluttrycket av ICAM-1 och VCAM-1, vilka är kärldysfunktionsmarkörer. Vi visade att behandling med 1 och 2 µg paricalcitol ledde till minskade nivåer av uttrycket av ICAM-1, medan värdena steg i placebogruppen. Mikropartikelnivåerna sjönk för patienter i placebogruppen och 1 µg-gruppen, men var bevarade för gruppen behandlad med 2 µg. Vi tolkar dessa fynd som ett tecken på att behandling bevarar kärlfunktion och minskar celldöd.

Studie IV var en meta-analys av alla genomförda behandlingsstudier med vitamin D till njursviktspatienter, där utfallet var endotelfunktion mätt med flödesmedierad vasodilatation. Vi hittade fyra publicerade studier, med sammanlagt 305 deltagare. Den sammanlagda effekten av behandling med vitamin D visade på en förbättrad endotelfunktion vid behandling. Vår meta-analys ger också indicier på att det kan vara bättre att behandla från yngre ålder, och tidigare i förloppet av njursvikt.

Detta doktorandprojekt visar att vitamin D har positiva effekter på kärlfunktion och inflammation. Vi behöver dock mer forskning för att kunna välja rätt patienter att behandla och vi bör sannolikt starta behandlingen tidigt, innan kärlsjukdomen hunnit bli alltför uttalad. Vi behöver också förstå hela aterosklerosprocessen vid njursvikt bättre, då det sannolikt krävs behandlingar från flera håll och samarbete inom forskningsfältet för att bättre balansera systemet och minska dessa patienters aggressiva kärlsjukdom.

9 ACKNOWLEDGEMENTS

First of all, I would like to thank the most important persons in this PhD-project, **my 36 patients**. I screened, contacted and included you all personally, and it feels like I know you all. Your interest and kindness during the study have been exceptional. Thank you!

Secondly, I would like to thank my principal supervisor, **Jonas Spaak**. You are a person of extreme assets, working part time in the clinic, driving and evolving the HND-process, and at the same time doing research in a diversity of fields, with many doctoral students. Still, when one asks you a question, you always seem to have an answer and knowledge in that particular field. Amazing.

Gun Jörneskog, my co-supervisor, a great clinician and researcher, you were the one to introduce me to research. I will never forget when you, after a week as your assistant doctor in the endocrinology department, asked me if I was interested in research. Yes I was! I have always trusted you thoroughly, and you have been a safety for me both in good and difficult periods.

Stefan Jacobson, my co-supervisor, with all your knowledge you always make things feel possible and even easy. Also a person with a lot on your hands, you have always been there for discussions, ideas and inspiration. You are a role model, working as a clinician and a professor, showing that this important combination is possible!

Eli Westerlund, my mentor, thank you for all your support and for being my friend. You have been exactly what a mentor should be, someone outside your work that you could confide in, talk to and discuss with, not only about research.

Josefin Mörtberg and **Ladan Mansouri**, co-workers and friends. Ladan, when everything felt quite hopeless you came with you engagement and knowledge, and all of a sudden research felt fun and inspiring again. Thank you for showing me how research can be at it´s best! Josefin, we have supported each other in our similar projects, and I think you can take great credit for me being here now. I hope we can keep on together! **Fariborz Mobarrez**, thank you for all your knowledge and support during the work with my third article. Microparticles are tricky little ones!

To **Karin Malmqvist**, head of the Department of Cardiovascular medicine, **Raffaele Scorza**, acting head of the Department of Cardiovascular medicine, and **Mika Skeppholm**, head of the residents in Cardiology, for making sure that research is prioritized. We have an exceptional research climate in our department.

Viveka Frykman, the head of the residents in cardiology for many years, you impress me, with your clinical skills, your research performance, but most of all with your kindness and possibility to see us all, one at a time.

To all **my colleagues** at the Department of Cardiovascular medicine.

Thomas Kahan, the head of our research group, you are a true scientist AND devoted clinician, and as such an important role model for us younger clinicians involved in research. I sincerely thank you for good ideas, and for our interesting discussions during the years. **Andreas Jekell**, thank you for interesting discussions on methodological issues.

To the Department of Clinical Sciences, especially **Håkan Wallen** and **Nina Ringart**. Nina, what would a PhD student do without you? Fail probably.

Ann-Christin Salomonsson and **Katherina Gatica Aguilera**, I sincerely thank you for all the laboratory work you have assisted me with. None of this had been possible without you!

Our librarian at Danderyd medical library, **Love Strandberg**, you are a great asset for our institution and hospital. Such devotion and helpfulness in things that feels hopelessly difficult for a clinician and PhD-student. Thank you.

Maria and **Jelena**, you keep my body and soul in one part.

Fredrik Schenholm, great thank you for expertise help with my images!

My two best friends, **Hillevi** and **Åsa**. You are a great comfort to me, and I can always rely on you. My medical school girlfriends pack, **Loella**, **Moa**, **Therese** and **Lisa**. When telling you I was scared to death about this, you all stood up, made more or less complicated arrangements and got here to support me. A great thank you! A special thank you to **Loella** for the beautiful front-page illustration.

My mother **Gunilla**, I already dedicated this book to you, and I am very pleased that you in the end also got some credit for the scientific work you have helped me with (see acknowledgements in my last article).

My father **Arne**, who dedicating many years of his life to research on the brain, then himself got Levy Body dementia at the age of 70, a very unfair diagnosis for such an intellectual person. You will not understand what I have performed, but I know you would have been prouder than anyone else.

Martin, my brother, I can always lean on you.

Henrik, my first and true love. They say you should not change the one you love, but you have changed me, into a much happier and better person. We share not only our fantastic children, but also our main interests. I cannot think of anything more fun than skiing and biking with you. You are my best friend.

Sist, minst och ändå störst, mina sötisar, **Eli** och **Ida**. Ni är "the love of my life", vad hade väl världen varit utan er? Ni kommer alltid först, även när mamma skriver avhandling (några Gorby´s-piroger blev det ☺) Kom ihåg att fortsätta vara sådana självsäkra små brudar som ni är nu!

10 REFERENCES

1. Leonard O, Spaak J, Goldsmith D. Regression of vascular calcification in chronic kidney disease - feasible or fantasy? a review of the clinical evidence. British journal of clinical pharmacology. 2013;76(4):560-72.

2. Ronco C, Di Lullo L. Cardiorenal Syndrome. Heart Failure Clinics. 2014;10(2):251-80.

3. Anavekar N, Pfeiffer MA. Cardiovascular risk in chronic kidney disease. Kidney International. 2004;66(supplement 92):S11-S5.

4. Piepoli MF, Hoes AW, Agewall S, al. e. 2016 European Guidelines on cardiovascular disease prevention in clinical practice. European Heart Journal. 2016;37(29):2315-81.

5. Sarnak MJ, Levey AS, Schoolwerth AC, Coresh J, Culleton B, Hamm LL, et al. Kidney Disease as a Risk Factor for Development of Cardiovascular Disease: A Statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. Hypertension. 2003;42(5):1050-65.

6. Levey AS, Coresh J, Balk E, Kausz AT, Levin A, Steffes MW, et al. Clinical Guidelines National Kidney Foundation Practice Guidelines for Chronic Kidney Disease: Evaluation, Classificaiton, and Stratification. Annals of Internal Medicine. 2003;139:137-47.

7. Matsushita K, van der Velde M, Astor BC, Woodward M, Levey AS, de Jong PE, et al. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. The Lancet. 2010;375(9731):2073-81.

8. Vashistha V, Lee M, Wu YL, Kaur S, Ovbiagele B. Low glomerular filtration rate and risk of myocardial infarction: A systematic review and meta-analysis. International Journal of Cardiology. 2016;223:401-9.

9. Sederholm Lawesson S, Alfredsson J, Szummer K, Fredrikson M, Swahn E. Prevalence and prognostic impact of chronic kidney disease in STEMI from a gender perspective: data from the SWEDEHEART register, a large Swedish prospective cohort. BMJ open. 2015;5(6):e008188-e.

10. Szummer K, Lundman P, Jacobson SH, Schön S, Lindbäck J, Stenestrand U, et al. Influence of renal function on the effects of early revascularization in non-st-elevation myocardial infarction. Circulation. 2009;120(10):851-8.

11. Ronco C, Haapio M, House Aa, Anavekar N, Bellomo R. Cardiorenal syndrome. Journal of the American College of Cardiology. 2008;52(19):1527-39.

12. Schlieper G, Schurgers L, Brandenburg V, Reutelingsperger C, Floege J. Vascular calcification in chronic kidney disease: An update. Nephrology Dialysis Transplantation. 2016;31(1):31-9.

13. Zoccali C, London G. Con: Vascular calcification is a surrogate marker, but not the cause of ongoing vascular disease, and it is not a treatment target in chronic kidney disease. Nephrology Dialysis Transplantation. 2015;30(3):352-7.

14. Bock JS, Gottlieb SS. Cardiorenal syndrome: New perspectives. Circulation. 2010;121(23):2592-600.

15. Vink EE, de Jager RL, Blankestijn PJ. Sympathetic hyperactivity in chronic kidney disease: pathophysiology and (new) treatment options. Curr Hypertens Rep. 2013;15(2):95-101.

16. Himmelfarb J, Stenvinkel P, Ikizler TA, Hakim RM. Perspectives in renal medicine: The elephant in uremia: Oxidant stress as a unifying concept of cardiovascular disease in uremia. Kidney International. 2002;62(5):1524-38.

17. Stam F, van Guldener C, Becker A, Dekker JM, Heine RJ, Bouter LM, et al. Endothelial dysfunction contributes to renal function-associated cardiovascular mortality in a population with mild renal insufficiency: the Hoorn study. Journal of the American Society of Nephrology : JASN. 2006;17(2):537-45.

18. Siragy HM, Carey RM. Role of the intrarenal renin-angiotensin-aldosterone system in chronic kidney disease. American journal of nephrology. 2010;31(6):541-50.

19. de Borst MH, Vervloet MG, ter Wee PM, Navis G. Cross talk between the renin-angiotensin-aldosterone system and vitamin D-FGF-23-klotho in chronic kidney disease. Journal of the American Society of Nephrology : JASN. 2011;22(9):1603-9.

20. Cremer A, Tambosco C, Corcuff JB, Boulestreau R, Gaillard P, Laine M, et al. Investigating the association of vitamin D with blood pressure and the renin-angiotensin-aldosterone system in hypertensive subjects: a cross-sectional prospective study. J Hum Hypertens. 2018;32(2):114-21.

21. Schlaich M, Socratous F. Sympathetic Activation in Chronic Renal Failure. Journal of the American Society of Nephrology : JASN. 2009;20:933–9.

22. Hadjiphilippou S, Kon SP. Cardiorenal syndrome: review of our current understanding. Journal of the Royal Society of Medicine. 2016;109(1):12-7.

23. Amin JK, Xiao L, Pimental DR, Pagano PJ, Singh K, Sawyer DB, et al. Reactive Oxygen Species Mediate Alpha-adrenergic Receptor-stimulated Hypertrophy in Adult Rat Ventricular Myocytes. Journal of Molecular and Cellular Cardiology. 2001;33(1):131-9.

24. Grassi G, Seravalle G, Mancia G. Sympathetic activation in cardiovascular disease: evidence, clinical impact and therapeutic implications. Eur J Clin Invest. 2015;45(12):1367-75.

25. Li L, Lee EW, Ji H, Zukowska Z. Neuropeptide Y-induced acceleration of postangioplasty occlusion of rat carotid artery. Arteriosclerosis, Thrombosis, and Vascular Biology. 2003;23(7):1204-10.

26. Chang Y, Wei W. Angiotensin II in inflammation, immunity and rheumatoid arthritis. Clin Exp Immunol. 2015;179(2):137-45.

27. Jurewicz M, McDermott DH, Sechler JM, Tinckam K, Takakura A, Carpenter CB, et al. Human T and natural killer cells possess a functional renin-angiotensin system: further mechanisms of angiotensin II-induced inflammation. Journal of the American Society of Nephrology : JASN. 2007;18(4):1093-102.

28. Karbach S, Wenzel P, Waisman A, Munzel T, Daiber A. eNOS uncoupling in cardiovascular diseases-the role of oxidative stress and inflammation. Current pharmaceutical design. 2014;20(22):3579-94.

29. Harrison DG, Guzik TJ, Lob HE, Madhur MS, Marvar PJ, Thabet SR, et al. Inflammation, immunity, and hypertension. Hypertension. 2011;57(2):132-40.

30. Costa C, Incio J, Soares R. Angiogenesis and chronic inflammation: Cause or consequence? Angiogenesis. 2007;10:149-66.

31. Daiber A, Steven S, Weber A, Shuvaev VV, Muzykantov VR, Laher I, et al. Targeting vascular (endothelial) dysfunction. British Journal of Pharmacology. 2016.

32. Barhoumi T, Kasal DA, Li MW, Shbat L, Laurant P, Neves MF, et al. T Regulatory lymphocytes prevent angiotensin II-induced hypertension and vascular injury. Hypertension. 2011;57(3):469-76.

33. Kassan M, Galan M, Partyka M, Trebak M, Matrougui K. Interleukin-10 Released by CD4+CD25+ Natural Regulatory T Cells Improves Microvascular Endothelial Function Through Inhibition of NADPH Oxidase Activity in Hypertensive Mice. Arteriosclerosis, Thrombosis, and Vascular Biology. 2011;31(11):2534-42.

34. Betjes MGH. Immune cell dysfunction and inflammation in end-stage renal disease. Nature Reviews Nephrology. 2013;9(5):255-65.

35. Mansouri L, Paulsson JM, Moshfegh A, Jacobson SH, Lundahl J. Leukocyte Proliferation and Immune Modulator Production in Patients with Chronic Kidney Disease. PloS one. 2013;8(8):1-9.

36. Romão JE, Haiashi AR, Elias RM, Luders C, Ferraboli R, Castro MCM, et al. Positive acute-phase inflammatory markers in different stages of chronic kidney disease. American journal of nephrology. 2006;26(1):59-66.

37. Stenvinkel P, Ketteler M, Johnson RJ, Lindholm B, Pecoits-Filho R, Riella M, et al. IL-10, IL-6, and TNF-alpha: central factors in the altered cytokine network of uremia--the good, the bad, and the ugly. Kidney Int. 2005;67(4):1216-33.

38. Chen S, Jim B, Ziyadeh FN. Diabetic nephropathy and transforming growth factor-beta: transforming our view of glomerulosclerosis and fibrosis build-up. Seminars in nephrology. 2003;23(6):532-43.

39. Floege J, Eitner F, Alpers CE. A New Look at Platelet-Derived Growth Factor in Renal Disease. Journal of the American Society of Nephrology. 2008;19(1):12-23.

40. Kato M, Natarajan R. Diabetic nephropathy--emerging epigenetic mechanisms. Nat Rev Nephrol. 2014;10(9):517-30.

41. Dei Cas A, Gnudi L. VEGF and angiopoietins in diabetic glomerulopathy: how far for a new treatment? Metabolism. 2012;61(12):1666-73.

42. Celletti FL, Waugh JM, Amabile PG, Brendolan A, Hilfiker PR, Dake MD. Vascular endothelial growth factor enhances atherosclerotic plaque progression. Nature medicine. 2001;7(4):425-9.

43. He C, Medley SC, Hu T, Hinsdale ME, Lupu F, Virmani R, et al. PDGFRβ signalling regulates local inflammation and synergizes with hypercholesterolaemia to promote atherosclerosis. Nature Communications. 2015;6:7770.

44. Chitalia N, Ismail T, Tooth L, Boa F, Hampson G, Goldsmith D, et al. Impact of vitamin D supplementation on arterial vasomotion, stiffness and endothelial biomarkers in chronic kidney disease patients. PloS one. 2014;9(3):e91363.

45. Flammer AJ, Anderson T, Celermajer DS, Creager Ma, Deanfield J, Ganz P, et al. The assessment of endothelial function: from research into clinical practice. Circulation. 2012;126(6):753-67.

46. Yeboah J, Folsom AR, Burke GL, Johnson C, Polak JF, Post W, et al. Predictive value of brachial flow-mediated dilation for incident cardiovascular events in a population-based study: the multi-ethnic study of atherosclerosis. Circulation. 2009;120(6):502-9.

47. Bolton CH, Downs LG, Victory JGG, Dwight JF, Tomson CRV, Mackness MI, et al. Endothelial dysfunction in chronic renal failure. roles of lipoprotein oxidation and pro-inflammatory cytokines. Nephrology, dialysis, transplantation. 2001;16:1189-97.

48. Stam F, van Guldener C, Schalkwijk CG, ter Wee PM, Donker AJM, Stehouwer ACD. Impaired renal function is associated with markers of endothelial dysfunction and increased inflammatory activity. Nephrology Dialysis Transplantation. 2003;18(5):892-8.

49. Tomiyama H, Yamashina A. Vascular Dysfunction: A Key Player in Chronic Cardio-renal Syndrome. Internal Medicine. 2015;54(12):1465-72.

50. Marti CN, Gheorghiade M, Kalogeropoulos AP, Georgiopoulou VV, Quyyumi AA, Butler J. Endothelial dysfunction, arterial stiffness, and heart failure. Journal of the American College of Cardiology. 2012;60(16):1455-69.

51. Toda N, Toda H. Coronary hemodynamic regulation by nitric oxide in experimental animals: Recent advances. European Journal of Pharmacology. 2011;667(1-3):41-9.

52. Goligorsky MS. Endothelial cell dysfunction: can´t live with it, how to live without it. Am J Physiol Renal Physiol. 2005;288(10):F871-80.

53. Abdelbaky A, Corsini E, Figueroa AL, Fontanez S, Subramanian S, Ferencik M, et al. Focal arterial inflammation precedes subsequent calcification in the same location: a longitudinal FDG-PET/CT study. Circ Cardiovasc Imaging. 2013;6(5):747-54.

54. Rudd JH, Myers KS, Bansilal S, Machac J, Woodward M, Fuster V, et al. Relationships among regional arterial inflammation, calcification, risk factors, and biomarkers: a prospective fluorodeoxyglucose positron-emission tomography/computed tomography imaging study. Circ Cardiovasc Imaging. 2009;2(2):107-15.

55. Lanzer P, Boehm M, Sorribas V, Thiriet M, Janzen J, Zeller T, et al. Medial vascular calcification revisited: review and perspectives. European Heart Journal. 2014;35(23):1515-25.

56. Blaine J, Chonchol M, Levi M. Renal control of calcium, phosphate, and magnesium homeostasis. Clinical journal of the American Society of Nephrology : CJASN. 2015;10(7):1257-72.

57. Staude H, Jeske S, Schmitz K, Warncke G, Fischer DC. Cardiovascular risk and mineral bone disorder in patients with chronic kidney disease. Kidney & blood pressure research. 2013;37(1):68-83.

58. Öhman K, Larsson TE, Spaak J. Vitamin D-brist vid njursvikt; Riskfaktor för kardiovaskulär sjukdom. Läkartidningen. 2010;107(46):2884-87.

59. Quarles LD. The bone and beyond: 'Dem bones' are made for more than walking. Nature medicine. 2011;17(4):428-30.

60. Brondum-Jacobsen P, Benn M, Jensen GB, Nordestgaard BG. 25-hydroxyvitamin d levels and risk of ischemic heart disease, myocardial infarction, and early

death: population-based study and meta-analyses of 18 and 17 studies. Arterioscler Thromb Vasc Biol. 2012;32(11):2794-802.

61. Giovannucci E, Liu Y. 25-Hydroxyvitamin D and Risk of Myocardial Infarction in Men. Archives of internal medicine. 2008;168(11):1174-80.

62. Heidari B, Nargesi AA, Hafezi-Nejad N, Sheikhbahaei S, Pajouhi A, Nakhjavani M, et al. Assessment of serum 25-hydroxy Vitamin D improves coronary heart disease risk stratification in patients with type 2 diabetes. American Heart Journal. 2015;170(3):573-9.e5.

63. Kendrick J, Targher G, Smits G, Chonchol M. 25-Hydroxyvitamin D deficiency is independently associated with cardiovascular disease in the Third National Health and Nutrition Examination Survey. Atherosclerosis. 2009;205(1):255-60.

64. Martins D, Wolf M, Pan D, Zadshir A, Tareen N. Prevalence of Cardiovascular Risk Factors and the Serum Levels of 25-Hydroxyvitamin D in the United States. Archives of internal medicine. 2007;167(11):1159-65.

65. Schottker B, Haug U, Schomburg L, Kohrle J, Perna L, Muller H, et al. Strong associations of 25-hydroxyvitamin D concentrations with all-cause, cardiovascular, cancer, and respiratory disease mortality in a large cohort study. Am J Clin Nutr. 2013;97(4):782-93.

66. Semba RD, Houston DK, Bandinelli S, Sun K, Cherubini A, Cappola AR, et al. Relationship of 25-hydroxyvitamin D with all-cause and cardiovascular disease mortality in older community-dwelling adults. Eur J Clin Nutr. 2010;64(2):203-9.

67. Wang L, Song Y, Manson JE, Pilz S, Marz W, Michaelsson K, et al. Circulating 25-hydroxy-vitamin D and risk of cardiovascular disease: a meta-analysis of prospective studies. Circ Cardiovasc Qual Outcomes. 2012;5(6):819-29.

68. Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, Lanier K, et al. Vitamin D deficiency and risk of cardiovascular disease. Circulation. 2008;117(4):503-11.

69. Liu W-C, Wu C-C, Hung Y-M, Liao M-T, Shyu J-F, Lin Y-F, et al. Pleiotropic effects of vitamin D in chronic kidney disease. Clinica chimica acta; international journal of clinical chemistry. 2016;453:1-12.

70. Kandula P, Dobre M, Schold JD, Schreiber Jr MJ, Mehrotra R, Navaneethan SD. Vitamin D supplementation in chronic kidney disease: A systematic review and meta-analysis of observational studies and randomized controlled trials. Clinical Journal of the American Society of Nephrology. 2011;6(1):50-62.

71. Westerberg PA, Sterner G, Ljunggren O, Isaksson E, Elvarson F, Dezfoolian H, et al. High doses of cholecalciferol alleviate the progression of hyperparathyroidism in patients with CKD Stages 3-4: results of a 12-week double-blind, randomized, controlled study. Nephrology, dialysis, transplantation. 2018;33(3):466-71.

72. Cheng J, Zhang W, Zhang X, Li X, Chen J. Efficacy and safety of paricalcitol therapy for chronic kidney disease: A meta-analysis. Clinical Journal of the American Society of Nephrology. 2012;7(3):391-400.

73. De Borst MH, Hajhosseiny R, Tamez H, Wenger J, Thadhani R, Goldsmith DJA. Active vitamin D treatment for reduction of residual proteinuria: A systematic review. Journal of the American Society of Nephrology. 2013;24(11):1863-71.

74. Han T, Rong G, Quan D, Shu Y, Liang Z, She N, et al. Meta-analysis: The efficacy and safety of paricalcitol for the treatment of secondary hyperparathyroidism and proteinuria in chronic kidney disease. BioMed Research International. 2013;2013.

75. Xu L, Wan X, Huang Z, Zeng F, Wei G, Fang D, et al. Impact of Vitamin D on Chronic Kidney Diseases in Non-Dialysis Patients: A Meta-Analysis of Randomized Controlled Trials. PloS one. 2013;8(4).

76. Jean G, Souberbielle JC, Chazot C. Vitamin D in chronic kidney disease and dialysis patients. Nutrients. 2017;9(4).

77. Aranow C. Vitamin D and the immune system. Journal of investigative medicine : the official publication of the American Federation for Clinical Research. 2011;59(6):881-6.

78. Hossein-nezhad A, Spira A, Holick MF. Influence of vitamin D status and vitamin D3 supplementation on genome wide expression of white blood cells: a randomized double-blind clinical trial. PloS one. 2013;8(3):e58725-e.

79. Somjen D, Kohen F, Amir-Zaltsman Y, Knoll E, Stern N. Vitamin D analogs modulate the action of gonadal steroids in human vascular cells in vitro. Am J Hypertens. 2000;13(4 Pt 1):396-403.

80. Hewison M. Vitamin D, Immunity and Human Disease. Clinical Reviews in Bone and Mineral Metabolism. 2009;8(1):32-9.

81. Vojinovic J. Vitamin D receptor agonists' anti-inflammatory properties. Annals of the New York Academy of Sciences. 2014;1317(1):47-56.

82. Dreyer G, Tucker AT, Harwood SM, Pearse RM, Raftery MJ, Yaqoob MM. Ergocalciferol and microcirculatory function in chronic kidney disease and concomitant vitamin d deficiency: an exploratory, double blind, randomised controlled trial. PloS one. 2014;9(7):e99461-e.

83. Jia X, Xu J, Gu Y, Gu X, Li W, Wang Y. Vitamin D suppresses oxidative stress-induced microparticle release by human umbilical vein endothelial cells. Biology of Reproduction. 2017;96(September):199-210.

84. Molinari C, Uberti F, Grossini E, Carda S, Invernizzi M, Cisari C. 1α,25-Dihydroxycholecalciferol Induces Nitric Oxide Production in Cultured Endothelial Cells. Cellular Physiology and Biochemistry. 2011:661-8.

85. Xu J, Jia X, Gu Y. Vitamin D Reduces Oxidative Stress – Induced Procaspase-3/ROCK1 Activation and MP Release by Placental Trophoblasts. The Journal of clinical endocrinology and metabolism. 2017;102(6):2100-10.

86. Li Y, Kong J, Wei M, Chen Z, Liu S, Cao L. 1,25 dihydroxyvitamin D3 is a negative regulator of renin angiotensin system. Journal of CLinical Investigation. 2002;110(2):229-38.

87. Yuan W, Pan W, Kong J, Zheng W, Szeto FL, Wong KE, et al. 1,25-Dihydroxyvitamin D 3 Suppresses Renin Gene Transcription by Blocking the Activity of the Cyclic AMP Response Element in the Renin Gene Promoter. Journal of Biological Chemistry. 2007;282(41):29821-30.

88. Forman JP, Williams JS, Fisher NDL. Plasma 25-hydroxyvitamin D and regulation of the renin-angiotensin system in humans. Hypertension. 2010;55(5):1283-8.

89. Gunta SS, Thadhani RI, Mak RH. The effect of vitamin D status on risk factors for cardiovascular disease. Nature reviews Nephrology. 2013;9(June):337-48.

90. Levin A, Li Y. Vitamin D and its analogues : Do they protect against cardiovascular disease in patients with kidney disease ? Kidney international. 2005;68(5):1973-81.

91. Zhang Y, Deb DK, Kong J, Ning G, Wang Y, Li G, et al. Long-term therapeutic effect of vitamin D analog doxercalciferol on diabetic nephropathy: strong synergism with AT1 receptor antagonist. AJP: Renal Physiology. 2009;297(3):F791-F801.

92. Alborzi P, Patel Na, Peterson C, Bills JE, Bekele DM, Bunaye Z, et al. Paricalcitol reduces albuminuria and inflammation in chronic kidney disease: a randomized double-blind pilot trial. Hypertension. 2008;52(2):249-55.

93. de Zeeuw D, Agarwal R, Amdahl M, Audhya P, Coyne D, Garimella T, et al. Selective vitamin D receptor activation with paricalcitol for reduction of albuminuria in patients with type 2 diabetes (VITAL study): a randomised controlled trial. Lancet. 2010;376(9752):1543-51.

94. Teng M, Wolf M, Ofsthun MN, Lazarus JM, Hernán Ma, Camargo Ca, et al. Activated injectable vitamin D and hemodialysis survival: a historical cohort study. Journal of the American Society of Nephrology : JASN. 2005;16(4):1115-25.

95. Sarathy H, Pramanik V, Kahn J, Abramowitz MK, Meier K, Kishore P, et al. The effects of short-term vitamin D supplementation on glucose metabolism in dialysis patients: a systematic review and meta-analysis. International Urology and Nephrology. 2015;47(3):537-49.

96. Bucharles S, Barberato SH, Stinghen AEM, Gruber B, Piekala L, Dambiski AC, et al. Impact of Cholecalciferol Treatment on Biomarkers of Inflammation and Myocardial Structure in Hemodialysis Patients Without Hyperparathyroidism. Journal of Renal Nutrition. 2012;22(2):284-91.

97. Navarro-González JF, Donate-Correa J, Méndez ML, de Fuentes MM, García-Pérez J, Mora-Fernández C. Anti-inflammatory profile of paricalcitol in hemodialysis patients: a prospective, open-label, pilot study. Journal of clinical pharmacology. 2013;53(4):421-6.

98. Schleithoff SS, Zittermann A, Tenderich G, Berthold HK, Stehle P, Koerfer R. Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial. American Journal of Clinical Nutrition. 2006;83(4):754-9.

99. Shab-Bidar S, Neyestani TR, Djazayery A, Eshraghian M-R, Houshiarrad A, Kalayi A, et al. Improvement of vitamin D status resulted in amelioration of biomarkers of systemic inflammation in the subjects with type 2 diabetes. Diabetes/metabolism research and reviews. 2012;28(5):424-30.

100. Thethi TK, Bajwa MA, Ghanim H, Jo C, Weir M, Gold AB, et al. Effect of paricalcitol on endothelial function and inflammation in type 2 diabetes and chronic kidney disease. Journal of Diabetes and Its Complications. 2015;29:433-7.

101. Lishmanov A, Dorairajan S, Pak Y, Chaudhary K, Chockalingam A. Treatment of 25-OH vitamin D deficiency in older men with chronic kidney disease stages 3 and 4 is associated with reduction in cardiovascular events. American journal of therapeutics. 2013;20(5):480-6.

102. Duranton F, Rodriguez-Ortiz ME, Duny Y, Rodriguez M, Daures JP, Argiles A. Vitamin D treatment and mortality in chronic kidney disease: a systematic review and meta-analysis. American journal of nephrology. 2013;37(3):239-48.

103. Zheng Z, Shi H, Jia J, Li D, Lin S. Vitamin D supplementation and mortality risk in chronic kidney disease: a meta-analysis of 20 observational studies. BMC Nephrol. 2013;14:199.

104. Mann MC, Hobbs AJ, Hemmelgarn BR, Roberts DJ, Ahmed SB, Rabi DM. Effect of oral vitamin D analogs on mortality and cardiovascular outcomes among adults with chronic kidney disease: A meta-analysis. Clinical Kidney Journal. 2015;8(1):41-8.

105. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. European heart journal. 2006;27(21):2588-605.

106. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager Ma, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. Journal of the American College of Cardiology. 2002;39(2):257-65.

107. Soltész P, Dér H, Kerekes G, Szodoray P, Szücs G, Dankó K, et al. A comparative study of arterial stiffness, flow-mediated vasodilation of the brachial artery, and the thickness of the carotid artery intima-media in patients with systemic autoimmune diseases. Clinical Rheumatology. 2009;28(6):655-62.

108. Chowienczyk PJ, Kelly RP, MacCallum H, Millasseau SC, Andersson TLG, Gosling RG, et al. Photoplethysmographic assessment of pulse wave reflection. Journal of the American College of Cardiology. 1999;34(7):2007-14.

109. Wilkinson IB, Hall IR, MacCallum H, Mackenzie IS, McEniery CM, van der Arend BJ, et al. Pulse-wave analysis: clinical evaluation of a noninvasive, widely applicable method for assessing endothelial function. Arteriosclerosis, thrombosis, and vascular biology. 2002;22(1):147-52.

110. Kendrick J, Andrews E, You Z, Moreau K, Nowak KL, Farmer-Bailey H, et al. Cholecalciferol, Calcitriol, and Vascular Function in CKD: A Randomized, Double-Blind Trial. Clinical journal of the American Society of Nephrology : CJASN. 2017;12(9):1438-46.

111. Kumar V, Yadav AK, Lal A, Kumar V, Singhal M, Billot L, et al. A Randomized Trial of Vitamin D Supplementation on Vascular Function in CKD. Journal of the American Society of Nephrology. 2017(8):ASN.2017010003-ASN.

112. Kumar V, Yadav AK, Singhal M, Kumar V, Lal A, Banerjee D, et al. Vascular function and cholecalciferol supplementation in CKD: A self-controlled case series. Journal of Steroid Biochemistry and Molecular Biology. 2018(December 2017).

113. Zoccali C, Curatola G, Panuccio V, Tripepi R, Pizzini P, Versace M, et al. Paricalcitol and Endothelial Function in Chronic Kidney Disease Trial. Hypertension. 2014;64:1005-11.

114. Yucel C, Demir S, Demir M, Tufenk M, Nas K, Molnar F, et al. Left ventricular hypertrophy and arterial stiffness in essential hypertension. Bratislava Medical Journal. 2015;116(12):714-8.

115. Chow B, Rabkin SW. The relationship between arterial stiffness and heart failure with preserved ejection fraction: a systemic meta-analysis. Heart Fail Rev. 2015;20(3):291-303.

116. Zito C, Mohammed M, Todaro MC, Khandheria BK, Cusma-Piccione M, Oreto G, et al. Interplay between arterial stiffness and diastolic function: a marker of ventricular-vascular coupling. Journal of cardiovascular medicine (Hagerstown, Md). 2014;15(11):788-96.

117. Thadhani R, Wenger J, Tamez H, Cannata J, Thompson BT, Andress D, et al. Vitamin D Therapy and Cardiac Structure and Function in Patients With Chronic Kidney Disease. JAMA. 2012;307:674-84.

118. Hewitt NA, O'Connor AA, O'Shaughnessy DV, Elder GJ. Effects of cholecalciferol on functional, biochemical, vascular, and quality of life outcomes in hemodialysis patients. Clinical Journal of the American Society of Nephrology. 2013;8(7):1143-9.

119. Levin A, Tang M, Perry T, Zalunardo N, Beaulieu M, Dubland JA, et al. Randomized Controlled Trial for the Effect of Vitamin D Supplementation on Vascular Stiffness in CKD. CJASN. 2017;12:1-17.

120. Lundwall K, Jörneskog G, Jacobson SH, Spaak J. Paricalcitol, Microvascular and Endothelial Function in Non-Diabetic Chronic Kidney Disease: A Randomized Trial. American journal of nephrology. 2015;42(4):265-73.

121. Marckmann P, Agerskov H, Thineshkumar S, Bladbjerg E-M, Sidelmann JJ, Jespersen J, et al. Randomized controlled trial of cholecalciferol supplementation in chronic kidney disease patients with hypovitaminosis D. Nephrology, dialysis, transplantation. 2012;27(9):3523-31.

122. Mose FH, Vase H, Larsen T, Kancir ASP, Kosierkiewic R, Jonczy B, et al. Cardiovascular effects of cholecalciferol treatment in dialysis patients - A randomized controlled trial. BMC Nephrology. 2014;15(1).

123. Pihlstrøm HK, Gatti F, Hammarström C, Eide IA, Kasprzycka M, Wang J, et al. Early introduction of oral paricalcitol in renal transplant recipients. An open-label randomized study. Transplant International. 2017;30(8):827-40.

124. IJzerman RG, de Jongh RT, Beijk MAM, van Weissenbruch MM, Delemarre-van de Waal HA, Serné EH, et al. Individuals at increased coronary heart disease risk are characterized by an impaired microvascular function in skin. Eur J Clin Invest. 2003;33:536 – 42.

125. Holowatz LA. Commentaries on Viewpoint: The human cutaneous circulation as a model of generalized microvascular function. Journal of Applied Physiology. 2008;105(1):373-.

126. Jekell A, Kalani M, Kahan T. The interrelation of endothelial function and microvascular reactivity in different vascular beds, and risk assessment in hypertension: results from the Doxazosin-ramipril study. Heart Vessels. 2018.

127. Ley K, Laudanna C, Cybulsky MI, Nourshargh S. Getting to the site of inflammation: the leukocyte adhesion cascade updated. Nat Rev Immunol. 2007;7(9):678-89.

128. Libby P. Inflammation in atherosclerosis. Arterioscler Thromb Vasc Biol. 2012;32(9):2045-51.

129. Kocijancic M, Cubranic Z, Vujicic B, Racki S, Dvornik S, Zaputovic L. Soluble intracellular adhesion molecule-1 and omentin-1 as potential biomarkers of subclinical atherosclerosis in hemodialysis patients. Int Urol Nephrol. 2016;48(7):1145-54.

130. Papayianni A, Alexopoulos E, Giamalis P, Gionanlis L, Belechri AM, Koukoudis P, et al. Circulating levels of ICAM-1, VCAM-1, and MCP-1 are increased in haemodialysis patients: association with inflammation, dyslipidaemia, and vascular events. Nephrology, dialysis, transplantation. 2002;17(3):435-41.

131. Stenvinkel P, Lindholm B, Heimburger M, Heimburger O. Elevated serum levels of soluble adhesion molecules predict death in pre-dialysis patients: association with malnutrition, inflammation, and cardiovascular disease. Nephrology, dialysis, transplantation. 2000;15(10):1624-30.

132. Bevc S, Sabic S, Hojs R. Atherosclerosis in hemodialysis patients--the role of microinflammation. Ren Fail. 2008;30(10):1012-6.

133. Naeini A, Moeinzadeh F, Vahdat S, Ahmadi A, Hedayati Z, Shahzeidi S. The effect of Vitamin D administration on intracellular adhesion molecule-1 and vascular cell adhesion molecule-1 levels in hemodialysis patients: A placebo-controlled, double-blinded clinical trial. Journal of Research in Pharmacy Practice. 2017;6(1):16-.

134. Berezin A, Zulli A, Kerrigan S, Petrovic D, Kruzliak P. Predictive role of circulating endothelial-derived microparticles in cardiovascular diseases. Clinical biochemistry. 2015;48(9):562-8.

135. Burger D, Schock S, Thompson CS, Montezano AC, Hakim AM, Touyz RM. Microparticles: biomarkers and beyond. Clinical science (London, England : 1979). 2013;124(7):423-41.

136. Erdbrügger U, Le TH. Extracellular Vesicles in Renal Diseases: More than Novel Biomarkers? Journal of the American Society of Nephrology : JASN. 2016;27(1):12-26.

137. Paudel KR, Panth N, Kim D-W. Circulating Endothelial Microparticles: A Key Hallmark of Atherosclerosis Progression. Scientifica. 2016;2016:8514056-.

138. Jimenez JJ, Jy W, Mauro LM, Soderland C, Horstman LL, Ahn YS. Endothelial cells release phenotypically and quantitatively distinct microparticles in activation and apoptosis. Thrombosis Research. 2003;109(4):175-80.

139. Markiewicz M, Richard E, Marks N, Ludwicka-Bradley A. Impact of endothelial microparticles on coagulation, inflammation, and angiogenesis in age-related vascular diseases. J Aging Res. 2013;2013:734509.

140. Jung C, Sorensson P, Saleh N, Arheden H, Ryden L, Pernow J. Circulating endothelial and platelet derived microparticles reflect the size of myocardium at risk in patients with ST-elevation myocardial infarction. Atherosclerosis. 2012;221(1):226-31.

141. Ye S, Shan X-F, Han W-Q, Zhang Q-R, Gao J, Jin A-P, et al. Microparticles from Patients Undergoing Percutaneous Coronary Intervention Impair Vasodilatation by Uncoupling Endothelial Nitric Oxide Synthase. Shock. 2017;48(2):201-8.

142. Abid Hussein MN, Boing AN, Sturk A, Hau CM, Nieuwland R. Inhibition of microparticle release triggers endothelial cell apoptosis and detachment. Thromb Haemost. 2007;98(5):1096-107.

143. Buendía P, De Oca AM, Madueño JA, Merino A, Martín-Malo A, Aljama P, et al. Endothelial microparticles mediate inflammation-induced vascular calcification. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2015;29(1):173-81.

144. Burger D, Turner M, Munkonda MN, Touyz RM. Endothelial Microparticle-Derived Reactive Oxygen Species: Role in Endothelial Signaling and Vascular Function. Oxid Med Cell Longev. 2016;2016:5047954-.

145. Perez-Casal M, Downey C, Cutillas-Moreno B, Zuzel M, Fukudome K, Toh CH. Microparticle-associated endothelial protein C receptor and the induction of cytoprotective and anti-inflammatory effects. Haematologica. 2009;94(3):387-94.

146. Amabile N, Guérin AP, Tedgui A, Boulanger CM, London GM. Predictive value of circulating endothelial microparticles for cardiovascular mortality in end-stage renal failure: a pilot study. Nephrology, dialysis, transplantation. 2012;27(5):1873-80.

147. Trappenburg MC, Van Schilfgaarde M, Frerichs FCP, Spronk HMH, Ten Cate H, De Fijter CWH, et al. Chronic renal failure is accompanied by endothelial activation and a large increase in microparticle numbers with reduced procoagulant capacity. Nephrology Dialysis Transplantation. 2012;27(4):1446-53.

148. Amabile N, Guerin AP, Leroyer AP, Mallat Z, Nguyen C, Boddaert J, et al. Circulating Endothelial Microparticles Are Associated with Vascular Dysfunction in Patients with End-Stage Renal Failure. Journal of the American Society of Nephrology. 2005;16(11):3381-8.

149. Dursun I. The relationship between circulating endothelial microparticles and arterial stiffness and atherosclerosis in children with chronic kidney disease. Nephrology, dialysis, transplantation. 2009;24:2511–8.

150. Esposito K, Ciotola M, Schisano B, Gualdiero R, Sardelli L, Misso L, et al. Endothelial microparticles correlate with endothelial dysfunction in obese women. Journal of Clinical Endocrinology and Metabolism. 2006;91(9):3676-9.

151. Parker B, Al-Husain A, Pemberton P, Yates AP, Ho P, Gorodkin R, et al. Suppression of inflammation reduces endothelial microparticles in active systemic lupus erythematosus. Annals of the rheumatic diseases. 2014;73(6):1144-50.

152. Wang JM, Su C, Wang Y, Huang YJ, Yang Z, Chen L, et al. Elevated circulating endothelial microparticles and brachial-ankle pulse wave velocity in well-controlled hypertensive patients. Journal of human hypertension. 2009;23(5):307-15.

153. Faure V. Elevation of circulating endothelial microparticles in patients with chronic renal failure. Journal of Thrombosis and Haemostasis. 2006;4:566–73.

154. Soriano S, Carmona A, Triviño F, Rodriguez M, Alvarez-Benito M, Martín-Malo A, et al. Endothelial damage and vascular calcification in patients with chronic kidney disease. American journal of physiology Renal physiology. 2014;307(11):F1302-11.

155. Amabile N, Heiss C, Chang V, Angeli FS, Damon L, Rame EJ, et al. Increased CD62e+ Endothelial Microparticle Levels Predict Poor Outcome in Pulmonary Hypertension Patients. Journal of Heart and Lung Transplantation. 2009;28(10):1081-6.

156. Nozaki T, Sugiyama S, Sugamura K, Ohba K, Matsuzawa Y, Konishi M, et al. Prognostic value of endothelial microparticles in patients with heart failure. European Journal of Heart Failure. 2010;12(11):1223-8.

157. Sinning JM, Losch J, Walenta K, Böhm M, Nickenig G, Werner N. Circulating CD31 +/Annexin V + microparticles correlate with cardiovascular outcomes. European Heart Journal. 2011;32(16):2034-41.

158. Augustine D, Ayers LV, Lima E, Newton L, Lewandowski AJ, Davis EF, et al. Dynamic release and clearance of circulating microparticles during cardiac stress. CircRes. 2014;114(1):109-13.

159. Mobarrez F, Egberg N, Antovic J, Bröijersen A, Jörneskog G, Wallén H. Release of endothelial microparticles in vivo during atorvastatin treatment; A randomized double-blind placebo-controlled study. Thrombosis Research. 2012;129(1):95-7.

160. Steven S, Münzel T, Daiber A. Exploiting the pleiotropic antioxidant effects of established drugs in cardiovascular disease. International Journal of Molecular Sciences. 2015;16(8):18185-223.

161. Bäckdahl L, Bushell A, Beck S. Inflammatory signalling as mediator of epigenetic modulation in tissue-specific chronic inflammation. The International Journal of Biochemistry & Cell Biology. 2009;41:176-84.

162. Wing M, Ramezani A, Hs G, Jm D, Ds R. Epigenetics of progression of chronic kidney disease: fact or fantasy? Seminars in nephrology. 2014;33(4):363-74.

163. Kim GH, Ryan JJ, Marsboom G, Archer SL. Epigenetic mechanisms of pulmonary hypertension. Pulm Circ. 2011;1(3):347-56.

164. Small EM, Frost RJa, Olson EN. MicroRNAs add a new dimension to cardiovascular disease. Circulation. 2010;121(8):1022-32.

165. Putta S, Lanting L, Sun G, Lawson G, Kato M, Natarajan R. Inhibiting microRNA-192 ameliorates renal fibrosis in diabetic nephropathy. Journal of the American Society of Nephrology : JASN. 2012;23(3):458-69.

166. Wang Y, Li M, Xu L, Liu J, Wang D, Li Q, et al. Expression of Bcl-2 and microRNAs in cardiac tissues of patients with dilated cardiomyopathy. Mol Med Rep. 2017;15(1):359-65.

167. Nagalla S, Shaw C, Kong X, Kondkar AA, Edelstein LC, Ma L, et al. Platelet microRNA-mRNA coexpression profiles correlate with platelet reactivity. Blood. 2011;117(19):5189-97.

168. Welten SMJ, Bastiaansen AJNM, De Jong RCM, De Vries MR, Peters EAB, Boonstra MC, et al. Inhibition of 14q32 MicroRNAs miR-329, miR-487b, miR-494, and miR-495 increases neovascularization and blood flow recovery after ischemia. CircRes. 2014;115(8):696-708.

169. Zhou T, Xiang D-K, Li S-N, Yang L-H, Gao L-F, Feng C. MicroRNA-495 Ameliorates Cardiac Microvascular Endothelial Cell Injury and Inflammatory Reaction by Suppressing the NLRP3 Inflammasome Signaling Pathway. Cellular Physiology and Biochemistry. 2018;49(2):798-815.

170. Aavik E, Lumivuori H, Leppänen O, Wirth T, Häkkinen S-K, Bräsen J-H, et al. Global DNA methylation analysis of human atherosclerotic plaques reveals extensive genomic hypomethylation and reactivation at imprinted locus 14q32 involving induction of a miRNA cluster. European heart journal. 2015;36(16):993-1000.

171. Jiang N, Chen W-j, Zhang J-w, Xu C, Zeng X-c, Zhang T, et al. Downregulation of miR-432 activates Wnt/β-catenin signaling and promotes human hepatocellular carcinoma proliferation. Oncotarget. 2015;6(10):7866-79.

172. Ge Y, Zhao K. Serum microRNA expression profile as a biomarker for the diagnosis of pertussis. Mol Biol Rep. 2013;40:1325–32

173. Tehrani S, Mobarrez F, Lins P-E, Adamson U, Wallén HN, Jörneskog G. Impaired endothelium-dependent skin microvascular function during high-dose atorvastatin treatment in patients with type 1 diabetes. Diabetes & vascular disease research. 2013;10(6):483-8.

174. Jörneskog G. Functional microangiopathy in the digital skin of patients with diabetes mellitus. Stockholm: Diss. (sammanfattning). Stockholm : Karol. inst.; 1995.

175. Jörneskog G, Brismar K, Fagrell B. Skin capillary circulation severely impaired in toes of patients with IDDM, with and without late diabetic complications. Diabetologia. 1995;38:474-80.

176. Spaak J, Egri ZJ, Kubo T, Yu E, Ando S-I, Kaneko Y, et al. Muscle sympathetic nerve activity during wakefulness in heart failure patients with and without sleep apnea. Hypertension. 2005;46(6):1327-32.

177. corp. Q. miRCURY® LNA® miRNA PCR Handbook. 2017.

178. Mobarrez F, Vikerfors A, Gustafsson JT, Gunnarsson I, Zickert A, Larsson A, et al. Microparticles in the blood of patients with systemic lupus erythematosus (SLE): phenotypic characterization and clinical associations. Scientific reports. 2016;6.

179. Jadad A, et al. Assessing the quality of reports of randomized clinical trials: is blinding necessary? Controlled Clinical Trials. 17. 1996;17(1):1-12.

180. Borenstein M, Hedges LV, Higgins JPT, Rothstein HR. A basic introduction to fixed-effect and random-effects models for meta-analysis. Research Synthesis Methods. 2010;1(2):97-111.

181. Hesser H, Andersson G. Introduktion till metaanalyser och systematiska översikter: Studentlitteratur; 2015. 1-170 p.

182. Zoccali C, Torino C, Curatola G, Panuccio V, Tripepi R, Pizzini P, et al. Serum phosphate modifies the vascular response to vitamin D receptor activation in chronic kidney disease (CKD) patients. Nutrition, metabolism, and cardiovascular diseases : NMCD. 2016;26(7):581-9.