From the Department of Clinical Physiology,
Molecular Medicine and Surgery,
Karolinska Institutet, Stockholm, Sweden

IMPACT OF CHRONIC KIDNEY DISEASE ON THE CARDIOVASCULAR SYSTEM

STUDIES IN NON-DIALYSIS PATIENTS AND HEALTHY PEOPLE

Anna Asp

Karolinska Institutet

Stockholm 2018

Cover image: “Le Coeur” by Henri Matisse

All previously published papers were reproduced with permission from the publisher.
Published by Karolinska Institutet.
Printed by Åtta.45 Tryckeri AB, Järfälla

ISBN 978-91-7831-069-2

Impact of Chronic Kidney Disease on the Cardiovascular System

Studies in non-dialysis patients and healthy people

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Anna Asp

Principal Supervisor:

Associate Professor Maria J Eriksson
Karolinska Institutet
Department of Molecular Medicine and Surgery
Division of Clinical Physiology

Co-supervisors:

Anette Rickenlund, Ph.D.
Karolinska Institutet
Department of Molecular Medicine and Surgery
Division of Clinical Physiology

Professor Kenneth Caidahl
Karolinska Institutet
Department of Molecular Medicine and Surgery
Division of Clinical Physiology

Associate Professor Britta Hylander
Karolinska Institutet
Department of Medicine
Division of Nephrology

Opponent:

Professor Eva Nylander
Linköping University
Department of Clinical Physiology
Division of Cardiovascular Medicine

Examination Board:

Associate Professor Kjell Karp
Umeå University
Department of Surgical and Perioperative Sciences
Unit of Clinical Physiology

Associate Professor Torbjörn Linde
Uppsala University
Department of Medical Sciences
Division of Renal Medicine

Associate Professor Agnes Modin
Karolinska Institutet
Department of Molecular Medicine and Surgery
Clinical Physiology Södersjukhuset

Till Måns

“There´s more to the picture than meets the eye”

Neil Young

TACK!

På många sätt kan principen ingen nämnd och ingen glömd vara den bästa i ett sådant här avsnitt. Det går inte att nämna alla som på sitt sätt har bidragit till den här avhandlingen, listan skulle förmodligen aldrig ta slut. Det finns med all säkerhet också personer som har haft stor betydelse för arbetets framåtskridande vid olika tidpunkter i processen, men som i skrivande stund inte kommer upp i mitt huvud. Så du som har bidragit till den här avhandlingen men inte blivit nämnd – tack för din insats och din hjälp.

Det finns dock finns några personer som har haft avgörande betydelse för tillblivelsen och slutresultatet av den här avhandlingen, och som jag särskilt vill tacka.

Maria Eriksson, min huvudhandledare, som varit med från ax till limpa. Tack för att du introducerade mig för forskningen och för alla dina insatser genom åren. När du satte en vetenskaplig artikel i min hand en av mina första dagar på Fysiologiska kliniken på Karolinska Universitetssjukhuset – för att ha som underlag för ett kliniskt utlåtande – då förstod jag att jag var på rätt plats och hade träffat rätt person. Tack för resan vi har gjort tillsammans.

Kenneth Caidahl, bihandledare, som har varit till ovärderlig hjälp med sin stora erfarenhet och skarpa blick för detaljer i vetenskapliga texter, och som också har en förmåga att samla ihop forskningsgruppen för att under trevliga former diskutera och presentera vetenskapliga erfarenheter och resultat.

Anette Rickenlund, bihandledare, vars stora kunskap inom klinisk fysiologi och – inom ramen för den här avhandlingen – särskilt arbetsfysiologi – har varit av största vikt för avhandlingens framåtskridande och slutförande.

Britta Hylander, bihandledare, som var med och initierade PROGRESS-projektet, långt före jag kom in i bilden, och som bidragit både med kunskap om PROGRESS-projektet och expertis inom njurmedicin. Tack också för din uppmuntran i olika skeden av arbetet med avhandlingen.

Lena Persson, min mentor. Tack för all stöd och uppmuntran som du har gett mig under åren. Allt ifrån tips på de bästa KI-kurserna till djuplodande diskussioner om vetenskap och allt däremellan.

Eva Wallén-Nielsen, för professionellt och skickligt utförande av ultraljudsundersökningarna av deltagarna i PROGRESS-projektet. Din insats har varit ovärderlig.

Eva Linder-Klingsell, som jag aldrig hann träffa, men vars omfattande arbete och ultraljudsundersökningar inom PROGRESS-projektet var mitt underlag för fortsatt forskning och har varit av avgörande betydelse för projektet.

Agneta Aspegren-Pagels, som ständigt varit behjälplig och tillgänglig med detaljerad information om patienter och försökspersoner inom PROGRESS. Tack för all din hjälp genom åren!

Carin Wallquist, medförfattare och ”med-doktorand”. Det har varit ett stöd att ”kampera ihop” med dig inom PROGRESS-projektet. Tack för att du delat med dig av din njurmedicinska kunskap (och databas!) och all din uppmuntran.

Helena Wallin, medförfattare och ”med-doktorand”. Ditt slit med det tredje delarbetet har varit imponerande. Dina kunskaper inom arbetsfysiologi och det stora jobb du lagt ned är en viktig del av den här avhandlingen.

Tack till medförfattarna Stefan Jacobson och Eva Jansson, som bidragit med expertis och uppmuntran.

Flemming Larsen, min tidigare chef – tack vare dig började jag jobba på Fysiologiska kliniken på Karolinska Universitetssjukhuset och kunde därmed ta första stegen inom forskningen. Tack för din uppmuntran, fina personlighet och humor.

Elisabeth Berg och Eva Hagel, för ovärderlig kompetens och professionell hjälp med statistiken i studierna.

Ann-Britt Wikström på Institutionen för molekylär medicin och kirurgi, för din otroliga hjälpsamhet och för att du har svar på alla frågor.

Carola Steenberg – du får det svåra att verka enkelt. Din vänlighet och effektivitet är ren magi.

Alla medarbetare på Fysiologiska kliniken på Karolinska Universitetssjukhuset i Solna. Tack för all er hjälp, vänlighet och uppmuntran.

Alla tidigare och nuvarande medarbetare och doktorander i forskningsgruppen på Fysiologiska kliniken på Karolinska Universitetssjukhuset i Solna – för stöd, hjälp, uppmuntran och trevligt umgänge.

Alla medarbetare på Karolinska Institutet och Karolinska Universitetssjukhuset i Solna och Huddinge, som svarat på frågor om stort och smått under alla år.

Slitvargarna på ”OnLine English”, som med ofattbar snabbhet och noggrannhet lyckas detektera och skriva om oändligt många felaktigheter i mina ofullkomliga texter. / The staff at ”OnLine English”, for excellent linguistic revisions. Any language errors are entirely mine due to some late changes.

Tack till Moderna Museet i Stockholm, för förmedlande av omslagsbilden.

Min familj och mina brorsbarn. Heidi och Bob – jag älskar er och önskar er all lycka i era framtida liv, vilken väg ni än väljer.

Måns, som har lärt mig att ingenting varar för evigt. Du finns alltid i mitt hjärta.

Slutligen vill jag tacka de personer vars bidrag är det viktigaste för studierna och slutsatserna som förhoppningsvis kan dras från den här avhandlingen. Alla patienter och friska försökspersoner som ställt upp i PROGRESS-studien och bidragit till att föra forskningen framåt – utan er skulle inga av dessa studier ha kunnat genomföras.

Tack!

SAMMANFATTNING PÅ SVENSKA

Bakgrund:

Patienter med kronisk njursvikt – "chronic kidney disease" (CKD) – har högre dödlighet och incidens av hjärtkärlsjukdom jämfört med njurfriska. Även lindrigt nedsatt njurfunktion är en underskattad riskfaktor för kardiovaskulär sjukdom. De bakomliggande orsakerna till de ökade kardiovaskulära riskerna hos individer med nedsatt njurfunktion är sannolikt multifaktoriell och en kombination av traditionella riskfaktorer (som till exempel ålder, hypertoni, rökning och diabetes) och riskfaktorer specifika för njursjukdom – som till exempel sekundär hyperparathyreoidism, rubbad calcium-fosfat metabolism och kronisk inflammation. Studier har visat att bland annat ökad kärlstyvhet och förekomst av vänsterkammarhypertrofi är associerat med sämre prognos och ökad risk för hjärtkärlsjukdom hos patienter med nedsatt njurfunktion och dialysberoende patienter. Dock är kunskapen om de exakta mekanismerna begränsade, och det finns ett behov av ökad kunskap om associationen mellan lindrigt till måttligt nedsatt njurfunktion och kardiovaskulär sjukdom. De flesta studier har hittills fokuserat på patienter med uttalad nedsatt njurfunktion och dialysberoende patienter. Man har även studerat individer med lindrigt till måttligt nedsatt njurfunktion, men i mindre utsträckning.

Syftet med studierna i denna avhandling var att med icke-invasiva metoder (arbetsprov samt ultraljudsundersökning av hjärta och halskärl) detektera tidiga strukturella och funktionella hjärtkärlförändringar hos individer med icke-dialysberoende CKD, med särskilt fokus på patienter med lindrigt till måttligt nedsatt njurfunktion.

Studierna som ingår i denna avhandling baseras på ett större forskningsprojekt, PROGRESS 2002, som är ett samarbete mellan Fysiologkliniken och Njurmedicinska kliniken på Karolinska Universitetssjukhuset i Solna. Inom projektet har patienter med lindrig till måttligt nedsatt njurfunktion och uttalat nedsatt njurfunktion samt friska försökspersoner följts under fem års tid.

Patienter och försökspersoner:

Rekryteringen av ännu inte dialysberoende patienter med nedsatt njurfunktion av olika grad, och friska kontrollpersoner började 2002. Svensktalande individer mellan 18–62 år inkluderades. Njursjuka patienter med känd kranskärlssjukdom eller stroke exkluderades. Patienter rekryterades konsekutivt i samband med besök på njurmedicinska mottagningen, och delades upp i två grupper utifrån njurfunktion vid baslinjeundersökningen, mätt som glomerulär filtrationshastighet (GFR) med iohexolclearance: patienter med kraftigt nedsatt njurfunktion (49 patienter med GFR <20 ml/min/1.73 m^2, motsvarande CKD stadium 4–5) och lindrigt till måttligt nedsatt njurfunktion (54 patienter med GFR 50-70 ml/min/1.73 m^2, motsvarande CKD stadium 2–3). Även 54 friska försökspersoner (GFR ≥80 ml/min/1.73 m^2) som var ålder- och könsmatchade till gruppen med CKD 2–3, rekryterades som

kontrollgrupp. Patienter med CKD 2–3 och kontrollgruppen följdes under 5 år. Exklusionskriterier för samtliga deltagare var njurtransplantation, njurdonator eller blodsmitta. För friska försökspersoner dessutom känd hjärtsjukdom, diabetes eller pågående behandling mot hypertoni, hyperlipidemi eller annan kronisk sjukdom. Patienter med CKD 2–3 exkluderades från fortsatt uppföljning i samband med terminal behandling som dialys eller transplantation.

Metodik:

Vid inklusion genomgick samtliga njurpatienter och försökspersoner en klinisk undersökning, biokemiska analyser, 24-timmars blodtrycksmätning, ankeltrycksmätning med beräkning av ankel-arm-index (AAI), arbetsprov och ultraljudsundersökning av hjärta (ekokardiografi) och halskärl (ultraljudsundersökning av karotisartärer - arteria carotis communis [CCA]). Hos patienterna med CKD 2–3 och kontrollgruppen upprepades dessa undersökningar år 3 och år 5. Klinisk undersökning med bland annat blodtrycksmätning utfördes på Njurmedicinska kliniken och arbetsprov och ultraljudsundersökningarna utfördes på Fysiologkliniken. Jämförelser mellan patienterna med CKD och kontrollgruppen analyserades avseende vänsterkammares struktur och funktion, blodtryck vid vila och arbete, samt analyser av kärlens struktur och funktion. Graden av hjärtkärlförändringar korrelerades till riskfaktorer som ålder, rökning, hypertoni, blodfetter samt markörer av inflammation.

Studie I-IV:

Studie I: I den första artikeln analyserade vi data från ekokardiografi-undersökningarna vid baslinjen i de tre grupperna. Undersökningarna omfattade bedömning av hjärtats vänster kammare, avseende förekomst av eventuell vänsterkammarhypertrofi och bedömning av vänsterkammarfunktion, inklusive kvoten E/e´ – ett mått som används för uppskattning av fyllnadstryck och därmed relaxationsförmåga (diastolisk funktion) i vänster kammare.

Resultat: Patienter med CKD hade högre kvot E/e´ jämfört med kontrollpersoner (E/e´: kontrollpersoner 5.00 ± 1.23 mot CKD 4–5 6.36 ± 1.71, $P < 0.001$ och mot CKD 2–3 5.69 ± 1.47, $P = 0.05$), indikerande tecken på förändrad diastolisk vänsterkammarfunktion hos patienterna med CKD. Prevalensen av vänsterkammarhypertrofi var högre hos patienterna med CKD jämfört med kontrollpersonerna (kontrollpersoner 13% mot CKD 4–5 37%, $P = 0.006$ och mot CKD 2–3 30%, $P = 0.03$).

Konklusion: Tidiga förändringar i hjärtats struktur och funktion kan observeras hos patienter med lindrigt till måttligt nedsatt njurfunktion jämfört med friska försökspersoner, indikerande att hos CKD patienter kan tecken på hjärtengagemang påvisas i ett tidigt skede, något som kan vara en tidig markör för hjärtsjukdom.

Studie II: I den andra artikeln analyserades baslinjedata från ultraljudsundersökningarna av karotisartärerna i de tre grupperna, med bedömning av kärldiameter, väggtjocklek (intima-media tjocklek) och kärlfunktion, inklusive ”pressure-strain elastic modulus” (*Ep*), som är ett mått på kärlstyvhet.

Resultat: CCA diametern var större hos patienterna med CKD 4–5 jämfört med patienter med CKD 2–3 och kontrollpersoner (CKD 4–5, 6.50 ± 0.79 mm mot CKD 2–3, 6.08 ± 0.56 mm, P = 0.003; och mot kontrollpersoner 5.97 ± 0.53 mm, P < 0.001). Ingen signifikant skillnad i CCA diameter påvisades mellan CKD 2–3 och friska kontrollpersoner. I multivariabel analys var systoliskt blodtryck en viktig faktor för skillnaden i CCA diameter mellan CKD 4–5 och de andra grupperna, och i den justerade analysen kvarstod skillnaden i CCA diameter mellan grupperna endast vid högre åldrar. Det var ingen signifikant skillnad i intima-media tjocklek mellan grupperna. *Ep* var högre hos CKD 4–5 jämfört med kontrollpersonerna (P = 0.006).

Konklusion: Patienter med lindrigt till måttligt nedsatt njurfunktion uppvisade inga signifikanta skillnader avseende kärlförändringar (”arterial remodeling”) eller kärlfunktion jämfört med friska försökspersoner. Endast hos patienter med kraftigt nedsatt njurfunktion sågs tecken till förändrad kärlstruktur (vid högre åldrar) och funktion, vilket delvis kunde förklaras av högre blodtryck hos de sjukaste patienterna.

Studie III: I den tredje delstudien analyserade vi baslinjedata från arbetsprov i de tre grupperna. Fynden från arbetsprov korrelerades till biokemiska markörer avseende hjärt- och njurfunktion, samt inflammation.

Resultat: Patienter med CKD 2–3 hade signifikant lägre arbetsförmåga jämfört med friska kontrollpersoner (185 ± 59 watt mot 221 ± 60 watt, P = 0.004), men högre jämfört med patienter med CKD 4–5 (150 ± 54 watt). Maximal hjärtfrekvens (HR) var lägre hos patienter med CKD 2–3 jämfört med kontrollpersonerna (161 ± 24 slag/min mot 177 ± 11 slag/min, P = 0.001), och ytterligare lägre maximal HR sågs hos patienter med CKD 4–5 (144 ± 31 slag/min). En signifikant skillnad kvarstod även efter korrigering för behandling med betablockad.

Konklusion: Våra resultat med lägre arbetsförmåga och långsammare hjärtfrekvenssvar under och efter arbete hos patienter med lindrigt till måttligt nedsatt njurfunktion jämfört med friska kontrollpersoner indikerar att lindrigt till måttligt nedsatt njurfunktion är associerat med markörer på ökad kardiovaskulär risk.

Studie IV: I den fjärde delstudien analyserade vi associationen mellan blodtryck, biomarkörer och tidiga hjärtkärlförändringar över tid hos en välbehandlad patientgrupp med lindrig till måttlig CKD jämfört med friska försökspersoner. I studien undersöktes olika blodtrycksparametrar, samt hjärtat och kärlens struktur och funktion vid baslinjeundersökningen, samt vid 3- respektive 5-års uppföljning.

Resultat: Systoliskt blodtryck, mätt som medelvärde vid 24-timmars blodtrycksmätning, ökade något över tid hos de friska försökspersonerna, men inte hos CKD patienterna. CCA diametern ökade signifikant under uppföljning hos kontrollerna, men inte hos CKD patienterna (förändring mellan baslinjeundersökningen och år 5 i kontroller: +0.154 mm [95% konfidensintervall: 0.043-0.265], P = 0.001 och hos patienter med CKD: +0.061 mm [-0.043-0.165], P = 0.274). AAI ökade signifikant över tid hos CKD patienterna men inte hos

försökspersonerna (förändring mellan baslinjeundersökningen och år 5 hos kontroller: -0.001 [-0.045-0.044], P = 0.998 och hos CKD-patienter: +0.074 [0.031-0.117], P <0.001). *Ep* ökade inte signifikant över tid varken hos njurpatienter eller friska försökspersoner.

Konklusion: I denna 5-åriga uppföljningsstudie ökade kärldiameter något över tid hos friska försökspersoner med inte hos patienter med lindrig till måttlig CKD, troligen på grund av välkontrollerat blodtryck hos njurpatienterna. AAI ökade mer hos njurpatienterna än hos försökspersonerna, vilket skulle kunna indikera ökande kärlstyvhet hos njurpatienterna; dock bekräftades inte detta fynd av mätning av kärlstyvhet i CCA (beräkning av *Ep*), som inte ökade över tid varken hos njurpatienter eller friska försökspersoner.

Sammanfattning:

Hos patienter med lindrigt till måttligt nedsatt njurfunktion, kunde förändringar i systolisk och diastolisk vänsterkammarfunktion observeras jämfört med friska försökspersoner, och patienter med kronisk njursvikt hade högre förekomst av vänsterkammarhypertrofi jämfört med försökspersonerna, indikerande att hjärtförändringar börjar tidigt hos patienter med kronisk njursvikt. Dessutom observerades en gradvis försämring av arbetsförmåga hos icke-dialysberoende njurpatienter, vilket i regressionsanalyser huvudsakligen associerades med faktorer av betydelse för syreupptagningsförmåga; maximal HR, hemoglobinnivå och slagvolym. Det fanns dock inga signifikanta skillnader i kärlstruktur eller kärlfunktion hos patienter med lindrig till måttlig kronisk njursvikt jämfört med friska försökspersoner. Endast patienter med avancerad grad av kronisk njursvikt visade tecken på förändringar av kärlens struktur och funktion, med systoliskt blodtryck och ålder som viktiga bidragande faktorer. I linje med detta visade uppföljningsstudien av blodtryck och hjärtkärlförändringar hos patienter med lindrig till måttlig kronisk njursvikt jämfört med friska kontroller att bägge grupperna var relativt stabila över tiden. Endast små skillnader kunde observeras mellan grupperna avseende blodtryck och hjärtkärlförändringar över tid, och förändringar i systoliskt blodtryck och kärldiameter var till och med något mer uttalade hos de friska försökspersonerna, indikerande att god blodtryckskontroll hos patienter med lindrig till måttlig kronisk njursvikt skulle kunna sakta ned utvecklingen av hjärtkärlförändringar.

ABSTRACT

Background

Cardiac and arterial remodeling and stiffening occur in end-stage renal disease. The presence of cardiovascular (CV) alterations in earlier-stage chronic kidney disease (CKD) is less well studied. We evaluated CV structure and function in patients with mild-to-moderate CKD (stages 2–3) compared with healthy people and patients with advanced CKD (stages 4–5). This thesis is based on a prospective study, PROGRESS 2002, which is a collaborative project between the Department of Renal Medicine and the Department of Clinical Physiology at the Karolinska University Hospital, Solna, Sweden.

Aims

The aim of this thesis was to study early cardiac and vascular alterations in non-dialysis CKD, with special interest in patients with mild-to-moderate CKD. Aerobic exercise capacity, changes in blood pressure (BP) at rest and during exercise, and arterial and cardiac remodeling and function were assessed to improve understanding of the pathophysiology of CV involvement in renal disease.

Methods and results

In **Study I**, left ventricular (LV) mass index (LVMI) and systolic and diastolic function were evaluated using transthoracic echocardiography, including tissue Doppler imaging, in 103 patients with CKD (stages 2–3 and 4–5) and 53 healthy controls. The peak systolic myocardial velocity (s′), early diastolic myocardial velocity (e′), and early transmitral diastolic flow velocity (E) were measured, and E/e′ was calculated.

CKD patients had a higher mean E/e′ and lower longitudinal systolic function, as assessed by atrioventricular plane displacement and s′, than the controls. The prevalence of LV hypertrophy (LVH) was higher in CKD patients than in controls.

In **Study II**, vascular structure and function were studied using carotid ultrasound in 103 non-dialysis CKD patients (stages 2–3 and 4–5) and 54 healthy controls. Carotid intima–media thickness (CIMT) and common carotid artery (CCA) diameter were measured. Strain, stiffness, and the pressure–strain elastic modulus (*Ep*) of the right CCA were calculated.

CCA diameter did not differ significantly between CKD 2–3 patients and controls. CCA diameter was larger in CKD 4–5 patients than in CKD 2–3 patients and controls (CKD 4–5, 6.50 ± 0.79 mm versus CKD 2–3, 6.08 ± 0.56 mm, P = 0.003; and versus controls, 5.97 ± 0.53 mm, P < 0.001). However, after adjustment, the difference in CCA diameter was significant only for older patients and was dependent on systolic blood pressure (SBP). CIMT, strain, and stiffness did not differ significantly between groups, but *Ep* was higher in CKD 4–5 patients than in controls (P = 0.006).

In **Study III**, aerobic exercise capacity was studied in 99 patients with non-dialysis CKD (stages 2–3 and 4–5) and 54 healthy controls. Peak workload, as a measure of aerobic exercise capacity, and peak heart rate (peak HR) were measured during a maximal exercise test on a cycle ergometer. Cardiac and vascular ultrasound examinations were performed, and muscular function, haemoglobin level, and self-reported physical activity were assessed.

Peak workload, peak HR, and haemoglobin level were significantly lower in CKD 2–3 patients than in controls and were lower in CKD 4–5 than in CKD 2–3 patients. Multiple regression analysis showed that peak workload was strongly associated with systemic oxygen delivery factors, as indicated by stroke volume, peak HR, and haemoglobin level; together with age, sex, and height^2, these factors explained approximately 80% of individual variation in workload in CKD patients, with peak HR contributing most to the variation. Self-reported physical activity level was also an independent determinant of peak workload.

In **Study IV**, 54 patients with CKD stages 2–3 and 54 healthy controls were included and followed for 5 years. Renal function, ambulatory BP monitoring, measurement of ankle–brachial index (ABI), and carotid and cardiac ultrasound examinations were performed at baseline and after 3 and 5 years. CIMT, CCA diameter, elastic properties of the CCA (measured as *Ep*), and LVMI were evaluated.

In the CKD patients, average 24 h SBP and CCA diameter did not increase significantly from the baseline and to year 5, but these both increased in the controls over the same time. The ABI increased significantly between the baseline and year 5 in the CKD patients but not in the controls. LVMI increased significantly between the baseline and year 5 in both groups, but the change over time did not differ significantly between patients and controls. *Ep* did not change over time in either group.

In summary

Alterations in systolic and diastolic myocardial function were seen in patients with mild-to-moderate CKD compared with controls. The prevalence of LVH was higher in CKD patients than in controls, indicating that cardiac involvement starts early in CKD. These studies suggest that there is a gradual deterioration of aerobic exercise capacity in people with mild-to-severe non-dialysis CKD, which is associated mainly with oxygen delivery factors such as peak HR, haemoglobin level, and stroke volume. However, there were no significant differences in carotid artery structure or function in patients with mild-to-moderate CKD compared with healthy subjects. Only patients with advanced CKD and older patients showed signs of arterial remodeling, and SBP and age were important contributing factors to this remodeling. Consistent with this observation, a follow-up study of BP and CV changes in patients with mild-to-moderate CKD compared with healthy controls showed that these factors were relatively stable over time in both groups. Only small differences in BP and CV changes between groups were observed over time, and the changes in SBP and CCA diameter were slightly more pronounced in the controls. These findings suggest that good control of BP in patients with mild-to-moderate CKD might slow the progression of CV changes.

LIST OF SCIENTIFIC PAPERS

I. **Asp AM**, Wallquist C, Rickenlund A, Hylander B, Jacobson SH, Caidahl K, Eriksson MJ. Cardiac remodelling and functional alterations in mild-to-moderate renal dysfunction: comparison with healthy subjects. Clin Physiol Funct Imaging. 2015 May;35(3):223–230. doi: 10.1111/cpf.12154.

II. **Asp AM**, Wallquist C, Rickenlund A, Hylander B, Jacobson SH, Caidahl K, Eriksson MJ. Aspects of carotid structure and function in health and different stages of chronic kidney disease. Clin Physiol Funct Imaging. 2018 May;38(3):402–408. doi: 10.1111/cpf.12429.

III. Wallin H, **Asp AM**, Wallquist C, Jansson E, Caidahl K, Hylander B, Jacobson SH, Rickenlund A, Eriksson MJ. Gradual reduction in exercise capacity in chronic kidney disease is associated with systemic oxygen delivery factors: a cross-sectional study. Submitted.

IV. **Asp AM**, Wallquist C, Rickenlund A, Hylander B, Jacobson SH, Caidahl K, Eriksson MJ. Blood pressure and cardiovascular changes in mild-to-moderate chronic kidney disease and healthy subjects: a 5-year follow-up study. Manuscript.

CONTENTS

LIST OF ABBREVIATIONS

A	Flow velocity during atrial contraction
a′	Late diastolic myocardial velocity
ABI	Ankle–brachial index
ABPM	Ambulatory blood pressure monitoring
ACE	Angiotensin converting enzyme
ANOVA	Analysis of variance
ASE	American Society of Echocardiography
AV	Atrioventricular
BP	Blood pressure
BSA	Body surface area
CCA	Common carotid artery
CIMT	Carotid intima–media thickness
CKD	Chronic kidney disease
CKD–EPI	Chronic Kidney Disease–Epidemiology Collaboration
CV	Cardiovascular
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
E	Early transmitral diastolic flow velocity
e′	Early diastolic myocardial velocity
ECG	Electrocardiogram
EF	Ejection fraction
eGFR	Estimated glomerular filtration rate
Ep	Carotid pressure–strain elastic modulus
ESRD	End-stage renal disease
GFR	Glomerular filtration rate
HDL	High-density lipoprotein

HR	Heart rate
hs-CRP	High-sensitivity C-reactive protein
IM	Intima–media
KDIGO	Kidney Disease: Improving Global Outcomes
KDOQI	Kidney Disease Outcomes Quality Initiative
LADd	Left atrial end-systolic diameter
LDL	Low-density lipoprotein
LV	Left ventricular
LVEF	Left ventricular ejection fraction
LVH	Left ventricular hypertrophy
LVIDd	Left ventricular end-diastolic internal diameter
LVM	Left ventricular mass
LVMI	Left ventricular mass index
NS	Not significant
NKF	National Kidney Foundation
PWTd	End-diastolic LV posterior wall thickness
s′	Peak systolic myocardial velocity
SBP	Systolic blood pressure
SD	Standard deviation
SV	Stroke volume
SWTd	End-diastolic interventricular septal wall thickness
TDI	Tissue Doppler imaging
VO_2peak	Peak oxygen uptake
W	Watt
2D	Two-dimensional

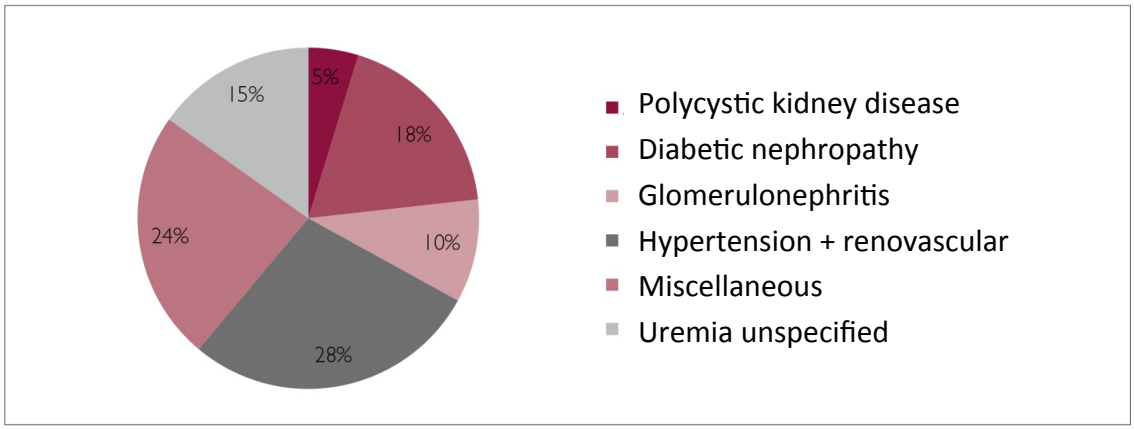
CHAPTER 1

Introduction

Epidemiology and etiology of chronic kidney disease

Chronic kidney disease (CKD) is common, especially in an ageing population. In a large population based study, the prevalence of CKD between 2006 and 2011 was about 6% of the adult population in the Stockholm region in Sweden, and the prevalence was higher (28%) among the elderly (aged >75 years). These figures are consistent with those from other developed countries.[1] The etiology of CKD varies, with diabetic nephropathy, glomerulonephritis and hypertension/nephrosclerosis as the most common underlying causes. Figure 1 shows the etiology of CKD in Sweden according to the Swedish Renal Registry (Yearly report 2017).[2]

Figure 1. Etiology of CKD in the Swedish Renal Registry (2017).



Adapted from the Swedish Renal Registry (Svenskt Njurregister), 2017.

Definition and classification of chronic kidney disease

According to Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group, CKD is defined as "abnormalities of kidney structure or function, present for >3 months, with implications for health".[3] In 2002, the National kidney foundation and the Kidney Disease Outcomes Quality Initiative (NKF/KDOQI) published a classification of CKD, with an updated version in 2013 (Table 1).[3,4] The classification is based on glomerular filtration rate (GFR), and describes 5 stages, where stage 3 is subdivided into 3a and 3b. Without evidence of kidney damage, stage 1 and 2 do not fulfill the criteria for CKD.

Table 1. Stages of CKD related to GFR.

Stage*	GFR, ml/min/1.73 m^2	Kidney damage**	Dysfunction
1	≥90	+	Normal
2	60–89	+	Mild
3a	45–59	+	Mild to moderate
3b	30–44	+	Moderate to severe
4	15–29	+	Severe
5***	<15	+	Kidney failure

* KDOQI Clinical Practice Guidelines, Am J Kidney Dis 2002, 2013.

** According to blood or urine test or imaging studies.

*** ESRD, end-stage renal disease.

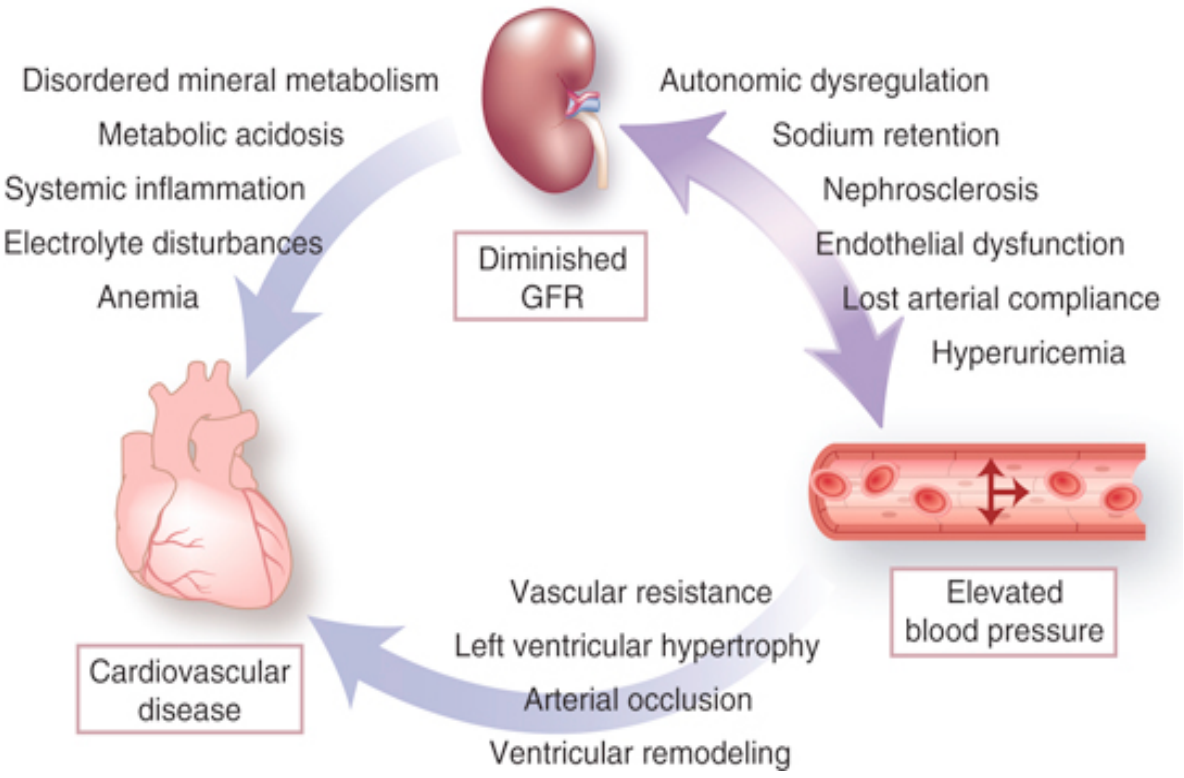
Cardiovascular disease in chronic kidney disease

Patients with CKD have an increased risk of cardiovascular (CV) disease (CVD) and all-cause mortality,[5-7] and this risk becomes evident as the glomerular filtration rate (GFR) decreases below 60 ml/min/1.73 m^2.[5] However, inconsistent results have been reported for the association between mild-to-moderate CKD and CV risk.[7-10]

Mechanisms underlying the association between decreased renal function and CVD are still incompletely understood. It has been suggested that hypertension contributes to cardiac damage in CKD through induction of left ventricular (LV) hypertrophy (LVH),[11] and that the prevalence of LVH increases with declining renal function.[12-15] However, data on the

prevalence of LVH in the early stages of kidney disease are conflicting.[16,17] CKD patients are exposed to coronary ischemia as a result of a reduction in coronary reserve and capillary density.[18] Large artery stiffness is an independent risk factor for all-cause and CV mortality in both the general and renal disease population.[19] Figure 2 summarizes different factors and pathways causing CV complications.

Figure 2. Joint impact of CKD and hypertension on the CV system.



Reprinted from *Kidney International*., 2010; vol 77, Middleton et al., Hypertension, chronic kidney disease, and the development of cardiovascular risk: a joint primacy, Pages No. 753–755, Copyright 2018, with permission from Elsevier.

Most of the prevalence and prognostic studies in CKD have been performed in patients with end-stage renal disease (ESRD). The association between mildly reduced GFR and CVD has been studied, but much less.[8,20] The relationships between biomarkers and the risk profile of patients with mild-to-moderate CKD and the prevalence of increased carotid intima–media thickness (CIMT), arterial stiffness, and variables of cardiac function have not been well characterized. Exercise tolerance and the blood pressure (BP) response to exercise have been studied infrequently non-dialysis CKD patients.

Studying biomarkers in relation to cardiac and vascular function in patients with mild-to-moderate CKD may increase understanding of initial pathways of the disease and the significance for its progression. Both traditional risk factors, such as age and hypertension and non-traditional factors, such as anemia, chronic inflammation, oxidative stress, and abnormalities in mineral metabolism, play a role in the pathogenesis of CVD in CKD. Despite growing recognition of the frequent presentation of combined cardiac and renal dysfunction, the "cardio–renal syndrome", the extent of CV abnormalities and underlying pathophysiology are not completely understood, especially in patients with mild-to-moderate renal dysfunction. Inflammation is an acknowledged part of the pathophysiological process of atherosclerosis,[21,22] and increased levels of various inflammatory markers are characteristic in both CVD and CKD.[23]

Left ventricular mass and function

The increased CV risk in CKD patients is associated with cardiac remodeling, and the development of LVH.[12,24-26] In non-dialysis CKD patients, LVH was found to be the strongest predictor of progression to ESRD or death.[27] In a large cross-sectional study, the prevalence of LVH in non-dialysis CKD patients ranged from 32% in patients with estimated GFR (eGFR) ≥60 ml/min/1.73 m^2 to 75% in patients with an eGFR <30 ml/min/1.73 m^2.[28]

Reduced kidney function is also a risk factor for the development of heart failure.[29,30] Mild renal dysfunction has been shown to be a strong predictor of congestive heart failure with preserved LV ejection fraction (LVEF).[31] Diastolic myocardial function was reported being worse in patients with CKD than in hypertensive patients with normal renal function, especially in those with more advanced stages of CKD.[13] Left atrial size is influenced by impaired LV filling and has been shown to be a predictor of mortality in ESRD patients with LVH.[32] In ESRD, the risk of CV events was found to be highest in patients with both LVH and reduced LV function.[33] However, results showing an association between a decline in renal function and LV function are inconsistent.[13,14,28,34-37]

Global systolic LV function is conventionally measured as LVEF, which is calculated from simplistic models using diastolic and systolic dimensions or volumes.[38] In the last decade, the interaction between the complicated structure and orientation of myocardial fibers and contractile LV function has been clarified.[39] Longitudinal contraction of the left ventricle can be measured by atrioventricular (AV) plane displacement with the use of M-mode and/or

systolic myocardial velocity using tissue Doppler imaging (TDI). Evaluation of diastolic LV function has also been influenced by the development of TDI by adding variables calculated from transmitral flow and diastolic myocardial velocities.

Although changes in LV geometry have been demonstrated in patients with CKD, the association between renal function and impaired cardiac function has not been established, especially when traditional echocardiographic methods are used.[28] TDI can provide a quantitative evaluation of myocardial function and has an advantage over conventional echocardiography in diagnosing subclinical alterations in systolic and diastolic LV function.[40,41] In patients with advanced CKD, subclinical diastolic LV dysfunction, as assessed by TDI, has been found to be associated with adverse outcome.[42,43] TDI has been shown to be a more sensitive tool than conventional echocardiography for the detection of impaired diastolic function in CKD patients.[34,44] Only a few studies have evaluated systolic function in CKD when using TDI.[35,37,44,45]

Exercise capacity in chronic kidney disease

Patients with ESRD have reduced exercise tolerance and suffer from physical inactivity. Exercise capacity, measured as peak work capacity or peak oxygen uptake (VO_2peak), is impaired in ESRD patients.[46-51] Exercise capacity, measured as VO_2peak, is a strong predictor of survival in ESRD.[52]

The mechanisms underlying reduced exercise capacity in CKD patients are multifactorial and not fully understood. Contributing factors include congestive heart failure,[53] physical inactivity,[54,55] and abnormal neurocirculatory control and hemodynamic responses during exercise.[56]

Exercise capacity and BP response to exercise in non-dialysis-dependent CKD patients have only been little studied. Faria et al. showed that, compared with healthy controls, non-dialysis patients with CKD stages 3–5 had lower maximal and submaximal exercise tolerance, measured in a cardiopulmonary exercise test (ergometric treadmill, ramp protocol) and 6-minute walk test, respectively.[57]

Heart rate (HR) recovery after exercise has been shown to be a predictor of CV risk.[58-60] In a cohort of CKD patients with IgA nephropathy, reduced HR recovery was associated with decreased eGFR. Patients with eGFR <60 ml/min/1.73 m^2 had a reduced HR recovery compared with patients and controls with higher eGFR.[61]

It has been reported that aerobic exercise capacity is associated with an inflammatory state in CKD patients, independent of the presence of diabetes.[62]

CKD and anemia have been shown to be independently associated with reduced physical function and exercise capacity in patients with coronary artery disease, and that these effects were additive.[63]

Carotid intima–media thickness and vascular function

Vascular calcification is common in CKD and can occur in the intima and/or media of the arterial wall in various vascular beds, leading to arterial stiffness (arteriosclerosis) and calcified occlusive lesions (atherosclerosis).[64] In CKD patients and the general population, elevated serum phosphate levels have been correlated with increased risk of CV and all-cause mortality, and play a role in vascular calcification. Hyperphosphatemia promotes vascular calcification, partly by inducing vascular smooth muscle transformation into osteoblastlike cells.[65]

CIMT as measured by carotid ultrasound, is an accepted marker to predict CVD.[66] In the general population, increased CIMT predicts adverse CV events.[67] CIMT has been shown to be an independent predictor of CV mortality in dialysis patients,[68] and a predictor of CVD in non-dialysis CKD patients.[69,70] CIMT has also been demonstrated to be associated with inflammation in non-dialysis CKD patients.[71] It has been reported that decreased kidney function is associated with a faster increase in CIMT.[72] In CKD stages 4 and 5, arterial stiffness is an independent predictor of CV events.[73]

There is need of better understanding of the prevalence of subclinical atherosclerosis in earlier stages of CKD. Non-invasive techniques, such as carotid ultrasound, can be used to evaluate the atherosclerotic burden in CKD patients without a previous CV event. Arterial stiffness assessment using carotid ultrasound examination may help in describing the early phases of vascular wall remodeling in subclinical vascular disease.

CHAPTER 2

Aims

The overall aim of this thesis was to compare the pathophysiological changes in the CV system between patients with non-dialysis CKD at different stages and healthy controls. Of special interest was to identify clinically relevant abnormalities of early cardiac and vascular alterations in mild-to-moderate CKD, which may have clinical implications for patient management and risk stratification. It was hypothesized that patients with mild-to-moderate CKD would show signs of early CV abnormalities, as measured using different non-invasive techniques. The specific aims of the studies were:

Study I

To investigate whether cardiac structure and function differ between patients with mild-to-moderate CKD, those with advanced CKD, and healthy controls.

Study II

To evaluate vascular structure and function in patients with mild-to-moderate and advanced CKD in comparison with healthy controls.

Study III

To assess aerobic exercise capacity in patients with mild-to-moderate or advanced CKD, to compare this with exercise capacity in healthy controls, and to identify factors associated with of exercise capacity in CKD patients.

Study IV

To study BP and CV changes over time in patients with mild-to-moderate CKD compared with healthy controls.

CHAPTER 3

Methods

The PROGRESS 2002 study

This thesis is based on the single-center, prospective observational cohort study PROGRESS 2002. The study is a collaboration between the Department of Renal Medicine and the Department of Clinical Physiology at the Karolinska University Hospital, Solna, Sweden.

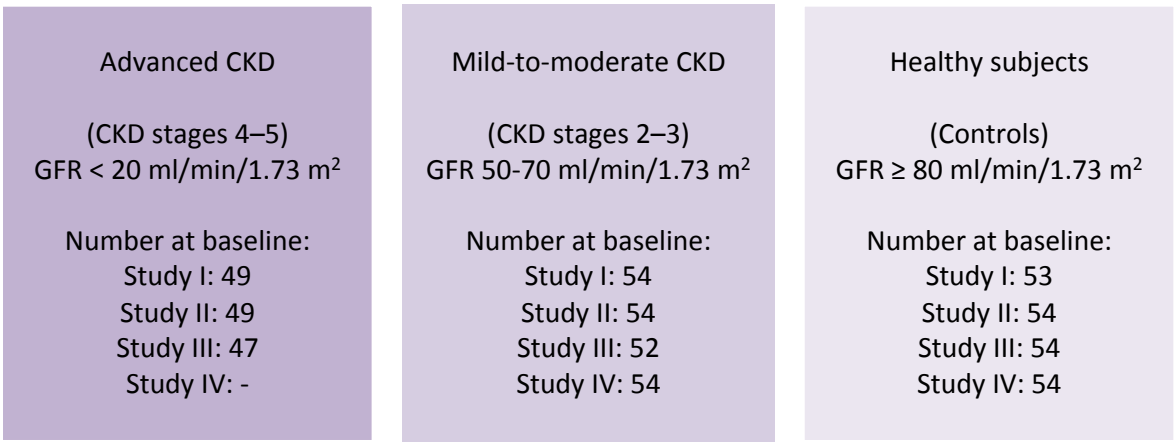
Patients and control subjects

Enrolment of patients with CKD of different severity levels and healthy controls started in 2002 and was completed in 2009. Swedish-speaking people aged 18–62 years were included. CKD patients were divided into two groups: mild-to-moderate CKD (54 patients with GFR 50–70 ml/min/1.73 m^2, corresponding to CKD stages 2–3) and advanced CKD (49 patients with GFR <20 ml/min/1.73 m^2, corresponding to CKD stages 4–5). Patients with known current malignancy were excluded. A group of 54 healthy controls (54 controls with GFR ≥80 ml/min/1.73 m^2), matched for age, sex and living area to the group with mild-to-moderate CKD was recruited. Of these, 31 were recruited by excerpt from the Swedish Total Population Register. This method of recruiting was later replaced, and 23 healthy controls were recruited by advertisement at the Karolinska University Hospital web site. People who were interested in participating underwent an interview about their health history and medication. Measurement of GFR by iohexol clearance was performed in all CKD patients and controls at baseline. The inclusion criteria for the controls were: GFR ≥80 ml/min/1.73 m^2; absence of kidney disease, CVD, and diabetes; and not taking any medication on an ongoing basis.

The exclusion criteria for all participants were kidney transplantation, kidney donation, or the presence of blood-transmitted disease. Patients with mild-to-moderate CKD and healthy controls were followed for 5 years. Patients in the mild-to-moderate CKD group were excluded from the further follow-up if they had undergone terminal treatment such as hemo- or peritoneal dialysis, or kidney transplantation. All follow-up visits of the participants were completed by the end of 2014.

In the studies of this thesis, the number of participants varied slightly at baseline due to the specific study design and methodology of each study (Figure 3).

Figure 3. Number of CKD patients and controls at baseline.



Ethical considerations

The study protocol was reviewed and approved by the Local Ethics Committee at Karolinska Institutet (Reference number: 02-052) and all participants gave their written informed consent.

The study protocol approved included fasting blood samples for the analysis of biomarkers, tests for aerobic work capacity and muscle strength, ambulatory BP monitoring (ABPM), cardiac and carotid ultrasound. Ultrasound investigations of the heart and carotid arteries are non-invasive investigations that involve no radiation and have no known risks.

Ethical permits

1. Dnr 02-052. KI research committee Nord at Karolinska University Hospital processed application at the committee meeting 2002-02-04.

Title: Factors with impact on progression of renal failure.

APPROVED 2002-05-24

2. Dnr 02-052. Additional application 2003-03-24.

Title: Factors with impact on progression of renal failure.

COMPLETION APPROVED 2003-04-02.

Measurement and estimation of glomerular filtration rate (Studies I-IV)

Iohexol clearance was used to measure GFR at the baseline in CKD patients and controls.[74] At the follow-up, eGFR was calculated using the Chronic Kidney Disease–Epidemiology Collaboration (CKD–EPI) equation.[75] The CKD–EPI equation is based on BSA, serum creatinine concentration, age, sex, and ethnicity, and was chosen because it has been shown to provide a more precise estimate of filtration capacity in the range observed in people with mild CKD.

Ultrasound examinations (Study I-IV)

All echocardiographic and carotid ultrasound examinations were performed by two experienced sonographers using an ultrasound machine with a 4-MHz probe equipped with TDI capabilities (Sequoia 512, Siemens Medical Solutions, Mountain View, CA) and stored digitally on magneto optical discs and on an EchoPAC server (Image Vault 5.0 system, General Electric Company, Horten, Norway).

Transthoracic echocardiography (Studies I, III and IV)

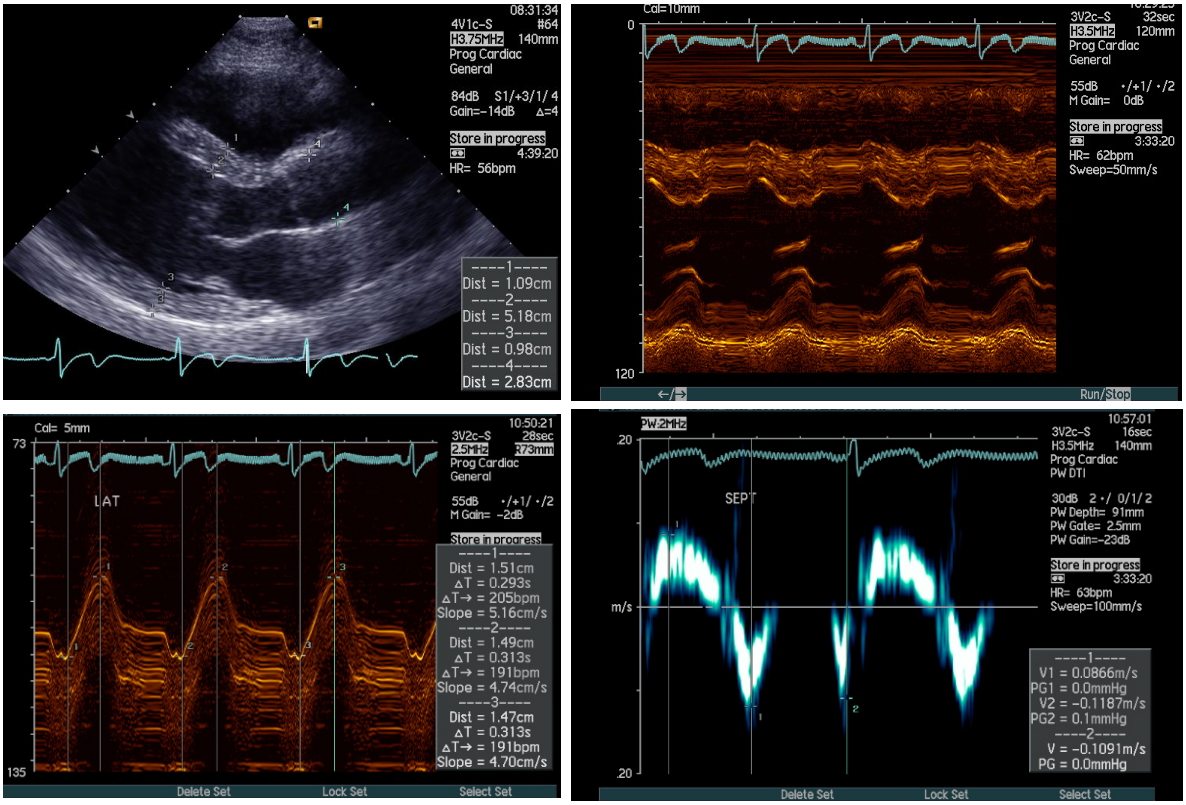
Two-dimensional (2D), M-mode and Doppler echocardiography were acquired according to the guidelines of the American Society of Echocardiography (ASE).[38] Standard echocardiographic 2D images from the parasternal long-axis view were obtained for the measurements of LV dimensions, including LV end-diastolic internal diameter (LVIDd), end-diastolic interventricular septal wall thickness (SWTd), end-diastolic LV posterior wall thickness (PWTd) and left atrial end-systolic diameter (LADs). LV mass (LVM) and LVEF were calculated, preferably from M-mode recordings in the standard parasternal long-axis view or, if that was not possible, from 2D images. LVM was calculated using the formula initially described by Devereux et al. and recommended by the ASE [38,76]:

$$LVM = 0.8 \times (1.04[(LVIDd + PWTd + SWTd)^3 - (LVIDd)^3]) + 0.6 \text{ g}$$

LVM index (LVMI) was calculated as LVM/body surface area (BSA). LVH was defined as LVMI >95 g/m^2 for women and >115 g/m^2 for men, according to ASE recommendations.[38] LVEF was calculated using the Teichholz method.[77] The AV plane displacement was

measured from M-mode recordings at the mitral annulus adjacent to the septal, lateral, anterior and inferior LV wall.[78] The early transmitral diastolic flow velocity (E), E deceleration time, and flow velocity during atrial contraction (A) were recorded using pulsed Doppler, and the E/A ratio was calculated. Figure 4 shows examples of the echocardiographic measurements.

Figure 4. Examples of the echocardiographic measurements.



Upper row: 2D measurements of LV dimension, M-mode measurements for assessing LVM and LVEF. Lower row: M-mode measurements of AV plane displacement in the lateral part of the mitral annulus (LAT), TDI measurements in the septal part close to the mitral annulus (SEPT).

Tissue Doppler imaging

TDI was used to record the early diastolic myocardial velocity (e′), late diastolic myocardial velocity (a′), and peak systolic myocardial velocity (s′) at the septal, lateral, inferior, and anterior basal regions of the LV wall (at end-expiration). The E-wave velocity and e′ obtained at the septal and lateral sites were used to calculate the septal and lateral E/e′ ratios. Both values and the mean E/e′ ratio (mean value of the septal and lateral E/e′ ratios) were used as estimates of the LV filling pressure. The mean of the s′ velocities of the four sites was

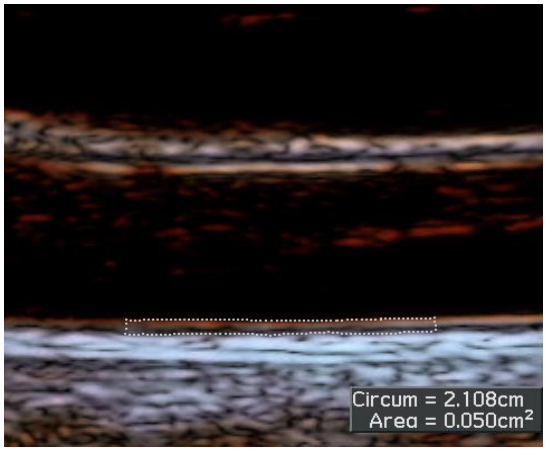
calculated and used for assessing LV systolic function. All TDI variables are presented as the measurement of one cardiac cycle.

Carotid ultrasound (Studies II, III and IV)

The carotid ultrasound examinations included measurements of CIMT and the diameter of the common carotid artery (CCA) and calculations of the elastic properties of the CCA, according to a standardized protocol.[79]

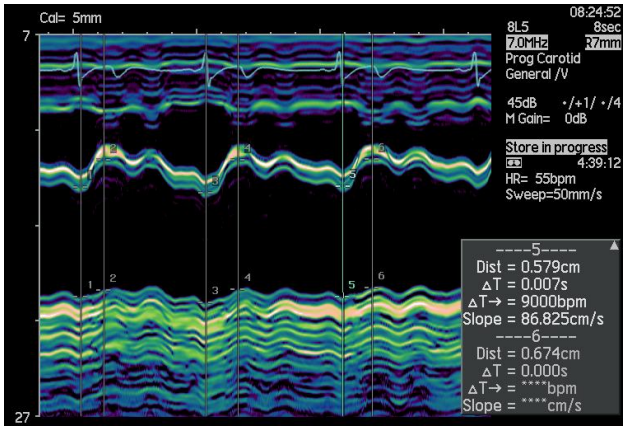
CCA diameter and CIMT were measured just proximal to the bulb on 2D images in the longitudinal plane, captured in the end-diastolic phase, assisted by electrocardiogram (ECG). The CCA diameter was measured from the leading edge of the near wall echogenic intima–media (IM) line to the leading edge of the luminal echo of the far wall. CIMT was measured in the far wall of the CCA and was defined as the distance between the leading edge of the luminal echo and the leading edge of the media/adventitia echo. CIMT was measured as the mean thickness over a length of 1 cm (Figure 5). CCA diameter and CIMT were measured separately for the left and right CCA, and the averages were calculated.

Figure 5. 2D measurements of CCA diameter and CIMT.



An M-mode recording of the right CCA just proximal to the bulb in the longitudinal plane was obtained for measurements of the systolic and diastolic CCA diameters used for calculations of strain, stiffness, and the pressure–strain elastic modulus (*Ep*).

Figure 6. M-mode measurements for calculations of the elastic properties of the CCA.



CCA diameters were measured from the leading edge of the echogenic near-wall IM echo to the leading edge of the far wall (Figure 6). CCA diameters in systole (D_{syst}) and diastole (D_{diast}), the systolic BP (SBP) and the diastolic BP (DBP) (measured in the right arm immediately before and after the M-mode scan) were used in the equations below.

Strain was defined as the amount of deformation relative to the unstressed state (dimensionless), according to the equation:

$$\text{Strain} = (D_{syst} - D_{diast})/D_{diast}.$$

Ep, the pressure–strain elastic modulus, which is one measure of distensibility, was defined as the equation described by Peterson et al.,[80] as below:

$$Ep = K \times (\text{SBP} - \text{DBP})/\text{strain}.$$

$K = 133.3$ and is the factor for converting mmHg to N m^{-2}.

Wall stiffness (dimensionless) was calculated according to the equation by Kawasaki et al.,[81] where ln(SBP/DBP) is the natural logarithm of the ratio of SBP to DBP:

$$\text{Stiffness} = \ln(\text{SBP}/\text{DBP})/\text{strain}.$$

24-hour ambulatory blood pressure monitoring (Study IV)

Ambulatory BP monitoring (ABPM) was performed over 24 h from morning to morning with a cuff of appropriate size placed on the non-dominant arm. BP was measured three times per hour, day and night. The participants were instructed to behave normally but to avoid demanding physical activities during the registration period.

Ankle–brachial index (Study IV)

Ankle–brachial index (ABI) was assessed by duplicate resting measurements of BP in the upper arms and ankles. A Doppler stethoscope was used to measure the SBP in either the posterior tibial or dorsalis pedis artery. The ABI was calculated as the ratio between the SBP in each leg divided by the SBP value of the left and right upper arm, respectively, and the lowest of the four ratios was selected. Normal ABI was defined as between 0.90 and 1.40.[82]

Aerobic exercise capacity (Study III)

A symptom-limited exercise test on a bicycle ergometer was performed according to clinical patient survey practice. The initial workload and workload increase/minute were individualized to achieve symptom limitation within 6–10 min. Participants were encouraged to continue cycling until exhaustion. Aerobic exercise capacity was defined as the peak workload in W, but because different ramps were used, the achieved value was adjusted to 15 W increments for men and 10 W increments for women.[83] Perceived exertion was reported as the highest rating on a predefined scale for leg fatigue, dyspnoea, or general exhaustion as the limiting symptom.[84] Predicted values for expected exercise capacity were derived from a Swedish population study that takes into account age, sex, height, and the workload increment/minute.[85] Resting HR and BP were measured in the supine position before the exercise test. Peak SBP was defined as the last measurement made before the end of exercise. A continuous 12-lead ECG was used to register the HR response and ST-T segments. Predicted peak HR was calculated as 220 minus age.

Muscular function (Study III)

The maximum voluntary isometric contraction was measured using a handheld Takei^{TM} dynamometer to determine handgrip strength. The test was performed with the dominant arm and the participant in a standing position.

Physical activity level (Study III)

Self-reported physical activity level was rated by the participants using a four-point scale modified from the Saltin–Grimby Physical Activity Level Scale,[86] which ranges from regular exercise on at least three occasions per week (Level 1) to mostly sedentary activities, with light exercise for less than 2 hours per week (Level 4).

Biochemical analyses

Fasting blood samples were collected from a peripheral vein in the morning. Plasma and serum samples were centrifuged (20 minutes at 3000 rpm), transferred to aliquots, and stored at –70 °C pending analyses. Routine assays were performed at the Karolinska University Laboratory at the Karolinska University Hospital, Solna, Sweden, which is certified by the Swedish Board for Accreditation and Conformity Assessment (SWEDAC). Lipid profiles were assessed by measuring the concentrations of total cholesterol and high-density lipoprotein (HDL) cholesterol in plasma using enzymatic methods. The concentration of low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula.[87] The plasma concentrations of high-sensitivity CRP (hs-CRP), calcium, and phosphate were measured using routine protocols.

Statistical methods

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 21.0 or 23.0 (IBM Corp. Armonk, NY, USA).

In all studies, the results are presented as number and percentage, mean and standard deviation (SD), or median and interquartile range, as applicable. Groups were compared at baseline using one-way analysis of variance (ANOVA) or the Kruskal–Wallis test if the assumptions of normality and homogeneity were not fulfilled. Group comparisons using one-way ANOVA were followed by a Tukey post hoc test when appropriate. When the Kruskal–Wallis test was used, a Bonferroni post hoc test was performed for the adjustment of P values between groups, when appropriate. For categorical variables, the chi-square test was used. A P value <0.05 for a two-tailed test was considered significant.

Potential correlations between variables were analyzed using Pearson's correlation or Spearman's correlation when the correlation seemed to be nonlinear.

Further specific statistical methods have been used in Studies II, III, and IV, and are described below.

Study II: For CCA diameter, an initial unadjusted analysis was performed to compare CCA diameter between the groups, followed by ANOVA adjusted for the background factors SBP, age, sex, height, and smoking. Interaction effects were tested for the product of group and age, sex, height, SBP, and smoking.

Study III: To identify factors associated with aerobic exercise capacity in CKD patients, the two CKD groups were merged into one group (CKD 2–5). Peak workload was used as the dependent variable in multiple linear regression analyses. Adjustments for age, sex, and height squared were performed in all analyses, followed by four different strategies:

1. The increase in explanatory value (R^2) for peak workload in both CKD 2–5 and controls was tested for single independent variables.

2. Multiple independent variables were added into a model with peak workload as the dependent variable in the CKD 2–5 group. The independent variables included systemic oxygen delivery factors, peripheral factors, and diastolic LV function.

3. Manual forward regression was used to analyze the stepwise increase in explanatory value for peak workload in the CKD 2–5 group by adding the significant independent variables from the regression analysis described above (Strategy 2).

4. Clinical determinants – A regression analysis in the CKD 2–5 group, including variables that could easily be measured in a clinical setting, such as self-reported physical activity level, handgrip strength, and hemoglobin level was performed.

Study IV: Groups were compared at baseline using Student's *t* test or the Mann–Whitney *U* test if the assumptions of normality and homogeneity were not fulfilled. For categorical variables, the chi-square test was used.

Linear mixed models were used to analyze BP and CV changes over time in patients and controls. The initial longitudinal analyses were followed by analyses adjusted for different background factors such as age, sex, and smoking.

To compensate for non-normal distributions, CIMT, LVMI, and hs-CRP were log-transformed (log) when used in the linear mixed-models analyses.

CHAPTER 4

Results

Baseline characteristics

The clinical characteristics of the study participants are summarized in Table 2. The mean age of the participants was 48 years; 60% were men. There were no significant differences between the groups in age, sex, body size, or smoking habits. The CKD groups had similar etiology of CKD and prevalence of diabetes mellitus but differed significantly in their use of medication.

At follow-up (Figure 7) the patients with CKD stages 2–3 and the healthy controls were examined after 3 and 5 years, respectively. Two of the CKD patients died (cancer) before the end of the follow-up period, but none of the patients progressed to renal replacement therapy within the follow-up period of 5 years. However, due to lost to follow-up, a reduced number of participants were seen, especially in the control group.

Figure 7. Patients with CKD 2–3 and controls at baseline and follow-up (Study IV)

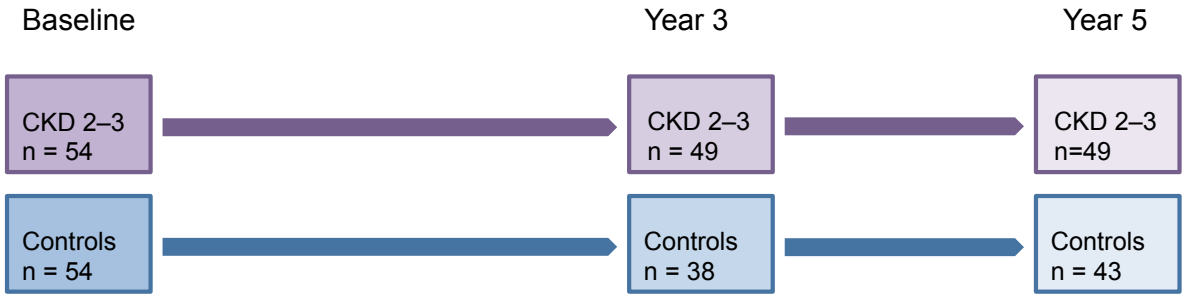


Table 2. Baseline characteristics of CKD patients and controls.

	Controls n = 54	CKD 2–3 n = 54	CKD 4–5 n = 49	P for comparison between groups
GFR, ml/min/1.73 m^2	99.3 ± 12.0	60.1 ± 5.2	15.3 ± 3.9	<0.001 (all)
Age, years	47.5 ± 10.6	46.8 ± 10.8	49.0 ± 11.5	NS
Male, n (%)	33 (61.1)	33 (61.1)	29 (59.2)	NS
Current or past smoker, n (%)	20 (37.7)	27 (50.9)	25 (51.0)	NS
Height, m	1.76 ± 0.09	1.74 ± 0.09	1.73 ± 0.10	NS
BSA, m^2	1.92 ± 0.19	1.92 ± 0.24	1.91 ± 0.23	NS
SBP, mmHg	117 ± 12	123 ± 15	130 ± 20	<0.001‡ 0.067§ 0.090†
DBP, mmHg	73 ± 8.9	77 ± 10	78 ± 10	0.039‡ 0.055† 0.98§
LDL, mmol/l	3.36 ± 1.12	3.15 ± 0.93	2.64 ± 0.93	0.001‡ 0.038§ 0.53†
Diabetes, n (%)	–	11 (20.4)	7 (14.3)	NS
Diagnosis of CKD, n (%)				
Familial/hereditary/ congenital diseases	–	14 (25.9)	13 (26.5)	NS
Primary glomerulonephritis	–	17 (31.5)	12 (24.5)	NS
Secondary glomerular/ systemic disease	–	9 (16.7)	10 (20.4)	NS
Miscellaneous/unknown	–	14 (25.9)	14 (28.6)	NS
Medication, n (%)				
Diuretics	–	12 (22.2)	34 (69.4)	<0.001
ACE inhibitors	–	23 (42.6)	29 (59.2)	NS
Angiotensin II receptor blockers	–	22 (40.7)	23 (46.9)	NS
Beta-blockers	–	11 (20.4)	20 (40.8)	0.024
Calcium channel blockers	–	10 (18.5)	28 (57.1)	<0.001
Statins	–	13 (24.1)	32 (65.3)	<0.001

CKD = chronic kidney disease; n = number; GFR = glomerular filtration rate (measured by iohexol clearance); BSA = body surface area; LDL = low-density lipoprotein cholesterol; ACE = angiotensin converting enzyme; NS = not significant. Values are reported as number (percentage) or mean ± SD. P values were obtained using one-way ANOVA for continuous values or the chi-square test for categorical values. P values for the comparisons between groups are indicated as: ‡ = CKD 4–5 versus controls, † = CKD 2–3 versus controls, § = CKD 4–5 versus CKD 2–3.

Results of the ultrasound measurements at baseline (Studies I and II)

The main results from the cardiac and carotid ultrasound examinations in the three groups (CKD 4–5, CKD 2–3 and controls) at baseline are summarized in Table 3.

Left ventricular structure and function (Study I)

LVIDd did not differ significantly between groups. LVMI differed significantly only between the CKD 4–5 group and controls (P = 0.006). However, the prevalence of LVH was significantly higher in both the CKD 4–5 (37%) and CKD 2–3 (30%) groups than in the controls (13%).

LV radial systolic function, assessed as LVEF calculated using the Teichholz method, did not differ significantly between groups. However, there was a tendency towards lower LVEF in CKD patients compared with controls (controls 66.0% ± 8.5% vs. CKD 2–3 62.5% ± 7.6%, P = 0.09; and vs. CKD 4–5 62.2% ± 9.6%, P = 0.07). In addition, the CKD groups had significantly lower longitudinal systolic contraction, measured as the mean of the four sites of AV plane displacement, compared with the controls. The mean of the systolic TDI velocities of the four sites was also significantly lower in patients than in controls.

Traditional variables of diastolic function, such as left atrial diameter, mitral E/A ratio, and the mitral E deceleration time did not differ between groups. However, the mean E/e′ ratio was significantly higher in CKD patients than in controls, indicating alterations in diastolic function in the CKD patients compared with the controls.

Vascular structure and function (Study II)

CCA diameter and CIMT are presented as the average of the left and right CCA. CCA diameter was significantly larger in the CKD 4–5 group than in the CKD 2–3 group and controls. CIMT did not differ between groups. In the assessment of the elastic properties of the CCA, *Ep* was significantly higher in CKD 4–5 patients than in controls.

A significant linear correlation was found between CCA diameter and age in the CKD 4–5 group (r = 0.44, P = 0.001) but not in the other groups (CKD 2–3 r = 0.18, P = 0.19; controls r = 0.19, P = 0.16). In the total cohort, CCA diameter correlated inversely with GFR (r = –0.31, P < 0.001) and positively with SBP (r = 0.63, P < 0.001).

In the adjusted analysis, ANOVA was used to compare CCA diameter between groups after adjustment for the background factors age, sex, height, smoking, and SBP, and the interaction between group and background factors was also assessed. In the final model, age, sex, SBP, and the interaction between group and age were included. The adjusted R^2 for the model was 0.48. In this final model, CCA diameter was dependent on sex ($P < 0.001$) and SBP ($P < 0.001$), but the effect of the factor "group" (with the three categories: controls, CKD 2–3, and CKD 4–5) on CCA diameter was no longer significant ($P = 0.19$). However, there was a significant interaction between group and age ($P = 0.03$) with a significant effect of age on CCA diameter only in the CKD 4–5 group. These findings indicate that after the adjustments, the difference in CCA diameter between CKD 4–5 and the other groups remained only in older patients.

Table 3. Main results for the echocardiographic and carotid variables at baseline.

	Controls n = 53 (echo) n = 54 (CCA)	CKD 2–3 n = 54	CKD 4–5 n = 49	P for comparison between groups
LADs, cm	3.38 ± 0.48	3.58 ± 0.59	3.64 ± 0.62	NS
LVIDd, cm	4.71 ± 0.49	4.69 ± 0.60	4.65 ± 0.56	NS
SWTd, cm	0.99 ± 0.16	1.09 ± 0.20	1.15 ± 0.23	<0.001‡ 0.02§
PWTd, cm	0.99 ± 0.14	1.02 ± 0.14	1.09 ± 0.19	0.002‡
LVMI, g/m^2	91.2 ± 17.6	99.7 ± 29.6	107.0 ± 27.2	0.006‡
LVH	7 (13)	16 (30)	18 (37)	0.006‡ 0.03§
LVEF Teichholz, %	66.0 ± 8.5	62.5 ± 7.6	62.2 ± 9.6	0.07‡ 0.09§
Mean AV, cm	1.50 ± 0.17	1.39 ± 0.20	1.40 ± 0.21	0.04‡ 0.01§
Mean s′, cm/s	11.5 ± 1.9	10.4 ± 2.1	10.4 ± 2.1	0.03‡ 0.02§
E/A	1.43 ± 0.39	1.33 ± 0.35	1.24 ± 0.49	NS
E deceleration time	206 ± 47	203 ± 40	213 ± 48	NS
Mean E/e′	5.00 ± 1.23	5.69 ± 1.47	6.36 ± 1.71	<0.001‡ 0.05§
CCA diam. average, mm	5.97 ± 0.53	6.08 ± 0.56	6.50 ± 0.79	< 0.001‡ 0.003† 0.61§
CIMT average, mm	0.65 ± 0.13	0.64 ± 0.10	0.63 ± 0.11	NS
Ep CCA right, (N m^{-2}) × 10^4	6.10 (4.77–6.93)	6.48 (5.43–8.10)	7.35 (5.15–9.85)	0.006‡ 0.20§ 0.62†

Values are reported as number (percentage), mean ± SD, or median (interquartile range) for skewed variables.

Abbreviations: CKD = chronic kidney disease; n = number; LADs, left atrial end-systolic diameter; LVIDd, left ventricular end-diastolic dimension; SWTd, wall thickness of interventricular septum; PWTd, posterior wall thickness; LVMI, left ventricular mass/body surface area; LVH, left ventricular hypertrophy; LVEF, left ventricular ejection fraction; AV, atrioventricular plane displacement; E, early transmitral diastolic flow velocity; A, flow velocity during atrial contraction; s′, peak systolic myocardial velocity; mean s′, mean peak systolic myocardial velocity of the septal, lateral, inferior, and anterior part of the mitral annulus; e′, early diastolic myocardial velocity; mean E/e′, mean septal E/e′ and lateral E/e′; CCA = common carotid artery; diam. = diameter; CIMT = carotid intima–media thickness; *Ep* = carotid pressure–strain elastic modulus; NS, not significant.

P values represent post hoc values after one-way ANOVA, the Kruskal–Wallis test, or chi-square test. P values for comparisons between groups are indicated as: ‡ = CKD 4–5 vs. controls, § = CKD 2–3 vs. controls, and † = CKD 4–5 vs. CKD 2–3.

Exercise capacity (Study III)

Exercise capacity and HR response

In the study population, there was an inverse relationship between CKD stage and achieved peak workload as well as peak HR. Peak workload and peak HR were significantly lower in CKD 2–3 patients than in controls, and these values were reduced further in CKD 4–5 patients. The subgroup analysis comparing the CKD patients not taking beta-blockers with controls showed similar patterns. During the exercise test, all participants exercised until near exhaustion, and perceived exertion did not differ between groups (P = 0.5).

Determinants of aerobic exercise capacity in CKD

Tested as single determinants, systemic oxygen delivery factors (stroke volume (SV), peak HR and haemoglobin level), CV factors (including E/e′, LVEF and *Ep*), handgrip strength, and physical activity level were all associated with peak workload in CKD 2–5 patients after adjusting for age, sex, and height squared. Among these, peak HR increased R^2 the most in the CKD patients. Among the systemic oxygen delivery factors, only SV (but not peak HR or hemoglobin level) was associated with peak workload after adjusting for age, sex and height squared. Handgrip strength and physical activity level were also associated with peak workload in the controls.

Tested as multiple determinants, systemic oxygen delivery factors (SV, peak HR and haemoglobin level) contributed significantly to explaining the individual variation in peak workload in CKD 2–5 patients after adjusting for age, sex, and height, but handgrip strength, *Ep* and the LV diastolic function variable E/e′ did not.

The variables SV, peak HR and hemoglobin level were added one by one to a model including age, sex, and height squared. Peak HR resulted in the largest increase in R^2, from 0.60 to 0.79. Age did not remain a significant explanatory factor for peak workload when peak HR was included in the models. Adding physical activity level in the last step slightly increased the R^2.

To control for the use of beta-blockers, a separate multiple regression analysis with age, sex, height squared, SV, peak HR, and hemoglobin level was performed in patients not taking

beta-blockers (n = 65). In this sub-group analysis, peak HR, SV, and haemoglobin level remained as significant explanatory variables for the variation in peak workload.

The regression model including age, sex, height squared, handgrip strength, hemoglobin level and physical activity level produced an R^2 value of 0.72. In this model, age, handgrip strength, physical activity level and hemoglobin level all significantly contributed to explain the variation in peak workload.

Changes in cardiovascular parameters at the follow-up (Study IV)

GFR and hs-CRP concentration

At baseline, GFR measured by iohexol clearance (ml/min/1.73 m^2) was 60.1 ± 5.2 in patients with CKD 2–3 and 99.3 ± 12.0 in controls (P < 0.001). At baseline and follow-up, eGFR was calculated using the CKD–EPI equation in CKD patients and controls, and the changes over time were analyzed using linear mixed models. Both patients and controls exhibited a significant decrease in renal function between the baseline and year 5, but the magnitude of change over time did not differ significantly between groups (P = 0.159). The hs-CRP concentration was higher in CKD patients than in the controls at each time point, but did not change significantly over time in either group.

BP and ultrasound measurements

As measured by ABPM, the average daytime SBP increased slightly but significantly in the controls between the baseline and year 5 but did not change significantly during the same period in CKD 2–3 patients. The change over time differed significantly between the groups (daytime SBP, P = 0.001). After adjustment for the covariates age-at-inclusion, sex, height, and smoking, the increase in daytime SBP in the controls over time remained significant (P = 0.006), and the difference in the change over time between the groups remained significant (P = 0.003).

ABI increased significantly over time in CKD patients but not in controls; the change between the baseline and year 5 was –0.001 [95% confidence interval (CI) –0.045 to 0.044, P = 0.998] in controls and +0.074 (0.031 to 0.117, P < 0.001) in CKD patients.

The CCA diameter increased significantly during follow-up in the controls, but not in the CKD patients; the change between the baseline and year 5 was +0.154 mm (95% CI 0.043 to

0.265, P = 0.001) in controls and +0.061 mm (–0.043 to 0.165, P = 0.274) in CKD patients. After adjustments for age-at-inclusion, sex, height, smoking, and SBP, the increase in CCA diameter over time remained significant in controls (P = 0.003). Sex and SBP were the two covariates that had a significant effect on CCA diameter (P = 0.005 and 0.001, respectively).

CIMT increased significantly between the baseline and year 5 in controls but not in CKD patients, but the difference in the change between the baseline and year 5 was not significant between controls and patients. Vascular function measured as *Ep* did not change during follow-up in patients or controls. LVMI increased significantly between the baseline and year 5 in both groups. However, there were no significant differences between the groups at any time point, and no significant difference between the groups in the change over time.

CHAPTER 5

Discussion

The aim of this thesis was to identify the clinically relevant markers of early cardiac and vascular alterations in non-dialysis CKD patients, with special interest in mild-to-moderate CKD, to improve understanding of the pathophysiology of early CV involvement in renal disease. We hypothesized that even mild-to-moderate CKD patients would show signs of early CV abnormalities compared with healthy subjects as shown by different non-invasive techniques.

At baseline, cardiac structure and function differed between patients with mild-to-moderate CKD and healthy controls, and the CKD patients had a diminished aerobic exercise capacity. By contrast, vascular structure and function did not differ significantly between patients with mild-to-moderate CKD and healthy controls. These findings suggest that alterations affecting cardiac structure and function, and consequently aerobic exercise capacity, occur earlier than vascular changes in CKD. Only patients with advanced CKD showed signs of altered vascular structure and function at baseline.

At the 5-year follow-up, patients with mild-to-moderate CKD showed modest signs of progression of CV alterations compared with healthy controls. Renal function decreased over the 5 years in both CKD patients and healthy controls, but the change over time did not differ significantly between the groups.

Cardiac structure and function in chronic kidney disease (Studies I and IV)

At baseline, the prevalence of LVH was higher in patients with mild-to-moderate or advanced CKD than in controls. Using TDI, we found significant alterations in diastolic and longitudinal systolic LV function in both groups of CKD patients compared with controls. At follow-up, LVMI increased significantly in both mild-to-moderate CKD patients and controls, but the change did not differ significantly between these two groups.

LV remodeling in CKD

We found an increased prevalence of LVH in patients with CKD stages 2–3 (30%) and stages 4–5 (37%) compared with controls (13%); these prevalence rates were similar to those described by others.[12,13] However, a higher prevalence of LVH in CKD patients has been

reported—up to 78% of patients with CKD stages 3–5 and up to 51% of patients with CKD stages 1–2.[17] The reasons for these diverging results may include different treatment strategies in the different patient populations and the use of a different methodology for calculating LVM.[88]

In non-dialysis CKD patients, it has been reported that LVMI increases with declining renal function, and that SBP correlates with LVMI.[13,15,89] The prevalence of LVH was found to be higher in non-dialysis CKD patients with hypertension than in hypertensive patients without CKD.[13] Progression of LVM was observed in a longitudinal study of patients with stage 3 CKD followed for 12 months, despite stable BP and kidney function.[90] In Study IV of this thesis, in which patients with CKD stages 2–3 were followed over 5 years, LVMI increased in patients despite the lack of a significant increase in BP. However, the magnitude of the increase in LVMI did not differ between CKD patients and controls in our study.

LV systolic function in CKD

The association between kidney function and impaired global systolic cardiac function has not been clearly established, especially when traditional echocardiographic methods for the calculation of LVEF have been used.[28] Others have shown that LV myocardial contraction and relaxation seem to be impaired first in the longitudinal direction in subclinical patients with CV risk factors, who may be comparable to the patients studied in this thesis. Despite the impairment of the longitudinal fiber function, the LV pump function (global LVEF) and LV filling may be compensated by preserved circumferential shortening at ventricular systole and three-directional lengthening at atrial systole, respectively.[91] In this thesis (study I), LV radial systolic contraction, assessed as LVEF calculated by the Teichholz method, did not differ significantly between CKD patients and controls. By contrast, both CKD patient groups showed signs of impaired longitudinal systolic contraction with significantly lower systolic myocardial velocity (s′) and lower AV plane displacement than controls. These results are consistent with those of recent studies using a newly developed ultrasound-based strain-imaging technique in terms of the impairment of longitudinal systolic function in CKD patients.[35,37]

LV diastolic function in CKD

Also with regard to diastolic function in CKD, varying results have been reported. A study of the association between kidney function and LV diastolic function using traditional methods found no graded association.[28] The results in this thesis (study I) are consistent with this

earlier study because we did not find any significant differences in the traditional characteristics of transmitral inflow pattern or left atrial size between CKD patients and healthy controls. However, in our study, TDI yielded a significantly higher mitral mean E/e′ ratio in both groups of patients (mild-to-moderate and advanced CKD) than in controls. This finding indicates altered diastolic function, although most of the CKD patients in the cohort had preserved LVEF. These findings are consistent with previous studies showing that TDI is a more sensitive tool than conventional echocardiography for the detection of impaired diastolic function in CKD patients.[34,44]

Vascular structure and function in chronic kidney disease (Studies II and IV)

At baseline, CCA diameter did not differ significantly between patients with mild-to-moderate CKD and well-controlled BP and healthy controls. Only patients with advanced CKD had a significantly larger CCA diameter compared with the other groups, which suggests that vascular remodeling occurs in advanced CKD. However, in the adjusted analysis with CCA diameter as a dependent variable, the difference between patients with advanced CKD and the other groups remained valid only for older patients. The difference in CCA diameter between the advanced CKD group and the other groups could partly be explained by a difference in SBP. CIMT measurement was of limited value as a discriminator between the three groups. Analysis of the elastic properties of the CCA at baseline showed no significant differences between patients with mild-to-moderate CKD and controls, although *Ep* differed significantly between patients with advanced CKD and controls.

At the follow-up, the CCA diameter increased significantly over time only in the controls but not in the patients with mild-to-moderate CKD. By contrast, ABI increased in CKD patients but not in controls, but *Ep* did not change significantly over time in patients or controls.

Vascular remodeling in CKD

In a study of the associations between different vascular biomarkers and prevalent CV events in a population at high CV risk, after adjusting for Framingham risk score, CCA diameter was the only vascular marker associated with CV events.[92] Outward hypertrophic remodeling of large arteries has been described in hypertensive people.[93] However, Briet and co-workers described a phenomenon of maladaptive vascular remodeling in CKD patients that was characterized by an increased arterial diameter that was not completely compensated for by increased wall thickness.[94-96] In their study, they demonstrated a larger CCA diameter but no

significant difference in CIMT between patients with mild-to-moderate CKD and normotensive or hypertensive people without CKD.[94] In study II, we found a similar pattern of no difference in CIMT between CKD patients and controls but a larger CCA diameter in patients with advanced CKD than in controls. The use of statins in CKD patients, which lowers cholesterol concentration, may explain the similar CIMT in CKD patients and controls. In our study and the study of Briet and co-workers, CKD patients had lower cholesterol concentrations than controls, probably because of statin use.

There are also some differences between the results in our study and those reported by Briet and co-workers.[94] In our study, only patients with advanced CKD had a larger CCA diameter than controls. However, mean GFR was higher in patients with mild-to-moderate CKD in our study (60 ± 5 ml/min/1.73 m^2) than in CKD patients in the study by Briet et al. (36 ± 16 ml/min/1.73 m^2). Furthermore, the cohort in our study included a younger CKD population (mean age 48 ± 11 years) than in the study by Briet et al. (mean age of CKD patients 58 ± 15 years). In the adjusted analysis in our study, the difference in CCA diameter between advanced CKD patients and the other groups was valid only for older patients. Therefore, both the degree of renal dysfunction and patient age should be considered when comparing results on arterial remodeling in CKD from different studies.

A follow-up study found an independent relationship between arterial remodeling, CKD progression, and incidence of ESRD.[95] The authors reported that CIMT decreased significantly during follow-up and that this decrease was associated with an increase in carotid internal diameter. Brachial BP remained stable over time in their study. In Study IV of this thesis, patients with mild-to-moderate CKD were followed for 5 years, but no significant increase in CCA diameter was observed in CKD patients. One possible explanation is that BP was well controlled, as evidenced by the lack of a significant increase in average 24 h SBP in CKD patients.

The CIMT measured by carotid ultrasound is a predictor of CVD and is used as a non-invasive surrogate marker for atherosclerosis.[66,67] However, the additional predictive value of CIMT measurements over the Framingham Risk Score in the general population has been questioned recently.[97] In study II of this thesis as well as in some other studies,[94,95,98] measurements of arterial enlargement and stiffness seemed to be more sensitive markers of early CV abnormalities in CKD patients and may show change before conventional surrogate markers such as CIMT.

Arterial stiffness in CKD

In ESRD patients, carotid arterial stiffness is increased and has been shown to predict all-cause and CV mortality.[99,100] Analyses of Framingham Heart Study cohorts showed that arterial stiffness correlated with albuminuria but not with mild-to-moderate CKD.[101] The lack of a significant association between carotid arterial stiffness and mild-to-moderate CKD in Study II of this thesis is consistent with this observation. By contrast, it has been reported that even in people with mild renal insufficiency a lower estimated GFR was associated with greater arterial stiffness.[102] However, that study included an older population (mean age 68 years) than the population in this thesis (mean age 48 years), which may explain to some extent the differences in results between the two studies.

In a follow-up study of non-dialysis patients with CKD stages 3–5, aortic stiffness did not change, but carotid stiffness and Young's elastic modulus increased significantly over time, although BP remained stable.[95] In Study IV of this thesis, SBP as measured by ABPM, and the pressure–strain elastic modulus as measured by *Ep*, remained stable over time in patients with mild-to-moderate CKD.

The ABI is used to assess peripheral artery disease. A population-based study reported that the association between high ABI and mortality was similar to that between low ABI and mortality, which suggests an upper limit of normal ABI of 1.40.[82] A pathologically high ABI is thought to reflect arterial stiffness and vascular aging. Both pathologically low and high ABI has been found to be more common in CKD patients (stage 3 and higher) than in non-CKD controls, and pathologically high ABI was more prevalent in patients with more advanced CKD.[103] In Study IV, ABI increased over time only in patients with mild-to-moderate CKD but not in controls, which may indicate incipient arterial stiffness in CKD patients. However, the values were generally below the upper limit of 1.4 also in the CKD patients, which makes the conclusions of this observation uncertain.

Aerobic exercise capacity in chronic kidney disease (Study III)

Reduced peak HR is a known feature of non-dialysis CKD.[57,104-107] The mechanisms behind the decreased peak HR in CKD are unknown and may involve both cardiac autonomic insufficiency[108] and peripheral factors (e.g., a low muscle mass and/or mitochondrial function, which may reduce oxygen demand leading to a reduction in peak HR), which have been proposed for heart failure.[109] In study III of this thesis, it was shown that aerobic

exercise capacity decreased gradually with CKD stage in the CKD patients. After merging the CKD patients in the PROGRESS cohort into one group, including CKD stages 2–5, regression analyses showed that peak workload in the patients was strongly associated with systemic oxygen delivery factors, such as peak HR, SV, and hemoglobin level, and that peak HR increased the R^2 value most. In the subgroup analysis of CKD patients not taking beta-blockers, peak HR remained strongly related to peak workload. These findings suggest that chronotropic incompetence is an important factor influencing aerobic exercise capacity in CKD patients. The regression models used in study III of this thesis were age adjusted, but age did not remain significant when peak HR was included. This may indicate that peak HR is more strongly related to exercise capacity than is age in non-dialysis CKD patients. The decline in aerobic exercise capacity in healthy people relates primarily to the age-related decline in peak HR.[110] CKD is linked to premature aging processes,[111] but whether reduced peak HR is a characteristic of accelerated aging or other CKD factors are the major cause of chronotropic insufficiency remains to be explained.

Hemoglobin level reflects arterial oxygen content and the capacity for systemic oxygen delivery. The relationship between reduced hemoglobin level and decreased aerobic exercise capacity is well known in association with moderately and severe decreased renal function.[63,107,112,113] In study III, hemoglobin level decreased gradually in parallel with the gradual decrease in exercise capacity in CKD 2–3 and CKD 4–5 patients compared with controls.

CV function influenced peak workload in CKD 2–5 patients. This was evidenced by the associations of LV systolic and diastolic function (E/e′), and vascular function (*Ep*) with peak workload in CKD 2–5 patients when tested as single determinants, after adjustments for age, sex and height squared. In the multivariable analysis of CKD 2–5 patients that included systemic oxygen delivery variables, neither E/e′ nor *Ep* was independently associated with peak workload. Previously, E/e′ and measures of vascular stiffness have been found to be independent determinants of aerobic exercise capacity in non-dialysis CKD patients.[104,105,114] The discrepancies between different studies may reflect the choice of variables included in multivariable analyses. In study III of this thesis, variables directly linked to oxygen delivery, such as SV and peak HR, seem to be markedly stronger determinants of aerobic exercise capacity than measures of LV diastolic function and vascular stiffness. By contrast, self-reported physical activity was significantly associated with peak workload, also after adjusting for other factors. In a separate analysis, variables that may be easily measured in the clinical setting, such as self-reported physical activity level, handgrip strength and

hemoglobin level, along with age, sex and height squared, were independent determinants of aerobic exercise capacity in the CKD patients. Physical activity level has been suggested to reflect peripheral factors, such as local oxygen transport, diffusion, and extraction by mitochondria, and possibly maximal SV.[115,116]

Cardiovascular risk reduction in chronic kidney disease

It seems that established risk factors in the general population, such as hypertension and smoking, also account for much of the increased CV risk in CKD patients, and that non-optimal management of these factors is common in CKD patients.[117] However, CKD specific risk factors, such as mineral bone disorders and anemia, are thought to play an important role in the development of CVD in CKD patients. Nonetheless, few trials on non-traditional risk factors have demonstrated a significant effect on CV events or mortality.[117] Lowering BP has been demonstrated to be effective in reducing CV events in patients with moderate CKD, with no one group of antihypertensive agents offering an advantage over another.[118] However, treatment of hypertension is considered to be one area in which achievement of targets is suboptimal in CKD patients.[117,118] In the studies of this thesis, the mild-to-moderate CKD patients were recruited from a dedicated nephrology clinic and had well-controlled BP over time. The CKD patients were monitored more closely than the controls and received aggressive treatment of BP, hyperlipidemia, and proteinuria.

Measurements of arterial stiffness have been suggested to improve risk prediction above that of the Framingham Risk Score in the general population[119] and might be of value for risk stratification of CKD patients at different stages of the disease, although this remains to be investigated.

In the studies of this thesis, differences in cardiac structure and function and in aerobic exercise capacity were observed between patients with mild-to-moderate CKD and healthy controls. However, vascular structure and function did not differ between these groups at baseline. At follow-up, the patients with mild-to-moderate CKD at baseline showed few CV changes compared with healthy controls.

In summary, the findings of this thesis suggest that it may be possible to slow the progress of CV changes in mild-to-moderate CKD patients by applying an active approach to controlling BP and other risk factors following current guidelines.

With the growing knowledge of the optimal treatment and monitoring of CKD, further prospective studies with larger populations may help improve understanding of the development of CVD in CKD patients in the current clinical context. Longitudinal studies of the mechanisms affecting systemic oxygen delivery in CKD patients are required for a deeper understanding of the multifactorial basis of the reduced aerobic exercise capacity in CKD patients.

CHAPTER 6

Limitations

The groups included were relatively small at baseline (~50 patients per group) and, especially in the control group, the number decreased further at follow-up. However, the use of a group of healthy controls makes the statistical analyses more reliable for detecting early CV changes in the CKD population. Measurement of GFR by iohexol clearance in both CKD patients and controls at baseline provided an optimal base for the classification of CKD stages. To overcome the problem with participants lost to follow-up, analyses were performed using linear mixed models to compensate for missing values.

There were relatively few patients with diabetes in this CKD cohort, which may partly explain the slow progression of CV changes over time. Another limitation is that the duration of comorbid conditions such as diabetes and hypertension could not be determined.

In terms of methodology, at the time of patient inclusion we had no possibility of using speckle tracking analysis in the evaluation of LV deformation or to measure pulse wave velocity to evaluate arterial stiffness. Maximal oxygen uptake was not measured in the assessment of aerobic exercise capacity. However, because of the approximately linear correlation between workload in cycle ergometry and oxygen uptake[120], and the high ratings of perceived exertion indicating near-maximal effort in the patients and controls, the results could be considered valid measurements of aerobic exercise capacity.

CHAPTER 7
Conclusions

In this thesis, the research involving non-dialysis CKD patients and healthy controls describes the characteristics and extent of early structural and functional CV changes in CKD, with special interest in mild-to-moderate CKD and the impact of different stages of CKD on aerobic exercise capacity.

Specific conclusions for studies I-IV:

I. TDI showed alterations in systolic and diastolic myocardial function in patients with even mild-to-moderate CKD compared with healthy controls. The prevalence of LVH increased with increasing severity of CKD. These findings indicate that cardiac involvement is already present in mild-to-moderate CKD and may be a precursor of premature cardiac morbidity.

II. Patients with mild-to-moderate CKD showed no significant differences in carotid artery structure or function compared with healthy controls. Only patients with advanced CKD and older age showed signs of arterial remodeling, as shown by larger CCA diameter compared with patients with mild-to-moderate CKD and healthy controls. These findings suggest that vascular alterations occur in advanced CKD and that SBP and age are important contributing factors. This emphasizes the importance of hypertension control in CKD patients. Measurement of CIMT seems to be of limited value for evaluating vascular abnormalities in patients with CKD at all stages of the disease.

III. Aerobic exercise capacity deteriorates gradually with CKD severity in non-dialysis patients. The reduced exercise capacity in CKD patients was associated primarily with reduced peak HR and hemoglobin level, factors that are important for systemic oxygen delivery. Patients who perform more physical activity have better-maintained aerobic capacity, which may be clinically important by counteracting the risk of increased CV morbidity in CKD patients.

IV. Comparing BP and CV changes in mild-to-moderate CKD patients and healthy controls over time, both groups were relatively stable over a 5-year period. BP and CV variables

changed only slightly over time in both groups, and the changes in SBP and CCA diameter were even slightly more pronounced in the control group. The ABI increased more in mild-to-moderate CKD patients than in controls, which may indicate incipient arterial stiffness in the CKD group. However, carotid distensibility (*Ep*) did not change significantly over time in either group. These findings suggest that good control of BP in mild-to-moderate CKD patients may have beneficial effects by slowing the progression of CV changes.

References

1. Gasparini A, Evans M, Coresh J, Grams ME, Norin O, Qureshi AR, Runesson B, Barany P, Arnlov J, Jernberg T, Wettermark B, Elinder CG, Carrero JJ. Prevalence and recognition of chronic kidney disease in Stockholm healthcare. *Nephrol Dial Transplant* 2016; **31**(12)**:** 2086-2094.

2. SNR/SRR SNSRR. Årsrapport 2017 [Internet]. Svenskt Njurregister/Swedish Renal Registry SNR/SRR: Jönköping, 2017. https://www.medscinet.net/snr/rapporterdocs/Svenskt%20Njurregister%202017,%20rev%20171114.pdf

3. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO clinical practice guideline for the evaluation and management of chronic kidney disease. Chapter 1: Definition and classification of CKD. *Kidney International Supplements* 2013; **3**(1)**:** 19-62.

4. National Kidney F. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis* 2002; **39**(2 Suppl 1)**:** S1-266.

5. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med* 2004; **351**(13)**:** 1296-1305.

6. Foley RN, Parfrey PS, Sarnak MJ. Clinical epidemiology of cardiovascular disease in chronic renal disease. *Am J Kidney Dis* 1998; **32**(5 Suppl 3)**:** S112-119.

7. Anavekar NS, McMurray JJ, Velazquez EJ, Solomon SD, Kober L, Rouleau JL, White HD, Nordlander R, Maggioni A, Dickstein K, Zelenkofske S, Leimberger JD, Califf RM, Pfeffer MA. Relation between renal dysfunction and cardiovascular outcomes after myocardial infarction. *N Engl J Med* 2004; **351**(13)**:** 1285-1295.

8. Culleton BF, Larson MG, Wilson PW, Evans JC, Parfrey PS, Levy D. Cardiovascular disease and mortality in a community-based cohort with mild renal insufficiency. *Kidney Int* 1999; **56**(6)**:** 2214-2219.

9. Henry RM, Kostense PJ, Bos G, Dekker JM, Nijpels G, Heine RJ, Bouter LM, Stehouwer CD. Mild renal insufficiency is associated with increased cardiovascular mortality: The Hoorn Study. *Kidney Int* 2002; **62**(4)**:** 1402-1407.

10. Hosseinpanah F, Barzin M, Golkashani HA, Nassiri AA, Sheikholeslami F, Azizi F. "Association between moderate renal insufficiency and cardiovascular events in a general population: Tehran lipid and glucose study". *BMC Nephrol* 2012; **13:** 59.

11. Locatelli F, Bommer J, London GM, Martin-Malo A, Wanner C, Yaqoob M, Zoccali C. Cardiovascular disease determinants in chronic renal failure: clinical approach and treatment. *Nephrol Dial Transplant* 2001; **16**(3)**:** 459-468.

12. Levin A, Thompson CR, Ethier J, Carlisle EJ, Tobe S, Mendelssohn D, Burgess E, Jindal K, Barrett B, Singer J, Djurdjev O. Left ventricular mass index increase in

early renal disease: impact of decline in hemoglobin. *Am J Kidney Dis* 1999; **34**(1)**:** 125-134.

13. Nardi E, Palermo A, Mulè G, Cusimano P, Cottone S, Cerasola G. Left ventricular hypertrophy and geometry in hypertensive patients with chronic kidney disease. *J Hypertens* 2009; **27**(3)**:** 633-641.

14. Chen SC, Su HM, Hung CC, Chang JM, Liu WC, Tsai JC, Lin MY, Hwang SJ, Chen HC. Echocardiographic parameters are independently associated with rate of renal function decline and progression to dialysis in patients with chronic kidney disease. *Clin J Am Soc Nephrol* 2011; **6**(12)**:** 2750-2758.

15. Matsumoto M, Io H, Furukawa M, Okumura K, Masuda A, Seto T, Takagi M, Sato M, Nagahama L, Omote K, Hisada A, Horikoshi S, Tomino Y. Risk factors associated with increased left ventricular mass index in chronic kidney disease patients evaluated using echocardiography. *J Nephrol* 2012; **25**(5)**:** 794-801.

16. Henry RM, Kamp O, Kostense PJ, Spijkerman AM, Dekker JM, Nijpels G, Heine RJ, Bouter LM, Stehouwer CD. Mild renal insufficiency is associated with increased left ventricular mass in men, but not in women: an arterial stiffness-related phenomenon--the Hoorn Study. *Kidney Int* 2005; **68**(2)**:** 673-679.

17. Paoletti E, Bellino D, Cassottana P, Rolla D, Cannella G. Left ventricular hypertrophy in nondiabetic predialysis CKD. *Am J Kidney Dis* 2005; **46**(2)**:** 320-327.

18. Amann K, Miltenberger-Miltenyi G, Simonoviciene A, Koch A, Orth S, Ritz E. Remodeling of resistance arteries in renal failure: effect of endothelin receptor blockade. *J Am Soc Nephrol* 2001; **12**(10)**:** 2040-2050.

19. Guerin AP, Pannier B, Metivier F, Marchais SJ, London GM. Assessment and significance of arterial stiffness in patients with chronic kidney disease. *Curr Opin Nephrol Hypertens* 2008; **17**(6)**:** 635-641.

20. Sarnak MJ, Levey AS, Schoolwerth AC, Coresh J, Culleton B, Hamm LL, McCullough PA, Kasiske BL, Kelepouris E, Klag MJ, Parfrey P, Pfeffer M, Raij L, Spinosa DJ, Wilson PW, American Heart Association Councils on Kidney in Cardiovascular Disease HBPRCC, Epidemiology, Prevention. 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)**:** 1050-1065.

21. Ross R. Atherosclerosis--an inflammatory disease. *N Engl J Med* 1999; **340**(2)**:** 115-126.

22. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med* 2005; **352**(16)**:** 1685-1695.

23. Tucker PS, Scanlan AT, Dalbo VJ. Chronic kidney disease influences multiple systems: describing the relationship between oxidative stress, inflammation, kidney damage, and concomitant disease. *Oxid Med Cell Longev* 2015; **2015:** 806358.

24. Foley RN, Parfrey PS, Harnett JD, Kent GM, Martin CJ, Murray DC, Barre PE. Clinical and echocardiographic disease in patients starting end-stage renal disease therapy. *Kidney Int* 1995; **47**(1)**:** 186-192.

25. Middleton RJ, Parfrey PS, Foley RN. Left ventricular hypertrophy in the renal patient. *J Am Soc Nephrol* 2001; **12**(5)**:** 1079-1084.

26. Zoccali C, Benedetto FA, Mallamaci F, Tripepi G, Giacone G, Stancanelli B, Cataliotti A, Malatino LS. Left ventricular mass monitoring in the follow-up of dialysis patients: prognostic value of left ventricular hypertrophy progression. *Kidney Int* 2004; **65**(4)**:** 1492-1498.

27. Paoletti E, Bellino D, Gallina AM, Amidone M, Cassottana P, Cannella G. Is left ventricular hypertrophy a powerful predictor of progression to dialysis in chronic kidney disease? *Nephrol Dial Transplant* 2011; **26**(2)**:** 670-677.

28. Park M, Hsu CY, Li Y, Mishra RK, Keane M, Rosas SE, Dries D, Xie D, Chen J, He J, Anderson A, Go AS, Shlipak MG, Group CRICS. Associations between kidney function and subclinical cardiac abnormalities in CKD. *J Am Soc Nephrol* 2012; **23**(10)**:** 1725-1734.

29. Fried LF, Shlipak MG, Crump C, Bleyer AJ, Gottdiener JS, Kronmal RA, Kuller LH, Newman AB. Renal insufficiency as a predictor of cardiovascular outcomes and mortality in elderly individuals. *J Am Coll Cardiol* 2003; **41**(8)**:** 1364-1372.

30. Kottgen A, Russell SD, Loehr LR, Crainiceanu CM, Rosamond WD, Chang PP, Chambless LE, Coresh J. Reduced kidney function as a risk factor for incident heart failure: the atherosclerosis risk in communities (ARIC) study. *J Am Soc Nephrol* 2007; **18**(4)**:** 1307-1315.

31. Devereux RB, Roman MJ, Liu JE, Welty TK, Lee ET, Rodeheffer R, Fabsitz RR, Howard BV. Congestive heart failure despite normal left ventricular systolic function in a population-based sample: the Strong Heart Study. *Am J Cardiol* 2000; **86**(10)**:** 1090-1096.

32. Patel RK, Jardine AG, Mark PB, Cunningham AF, Steedman T, Powell JR, McQuarrie EP, Stevens KK, Dargie HJ, Jardine AG. Association of left atrial volume with mortality among ESRD patients with left ventricular hypertrophy referred for kidney transplantation. *Am J Kidney Dis* 2010; **55**(6)**:** 1088-1096.

33. Zoccali C, Benedetto FA, Mallamaci F, Tripepi G, Giacone G, Cataliotti A, Seminara G, Stancanelli B, Malatino LS. Prognostic value of echocardiographic indicators of left ventricular systolic function in asymptomatic dialysis patients. *J Am Soc Nephrol* 2004; **15**(4)**:** 1029-1037.

34. de Almeida EA, de Oliveira EI, Lopes JA, Almeida AG, Lopes MG, Prata MM. Diastolic function in several stages of chronic kidney disease in patients with autosomal dominant polycystic kidney disease: a tissue Doppler imaging study. *Kidney Blood Press Res* 2007; **30**(4)**:** 234-239.

35. Edwards NC, Hirth A, Ferro CJ, Townend JN, Steeds RP. Subclinical abnormalities of left ventricular myocardial deformation in early-stage chronic kidney disease: the precursor of uremic cardiomyopathy? *J Am Soc Echocardiogr* 2008; **21**(12)**:** 1293-1298.

36. Hung MJ, Yang NI, Wu IW, Cheng CW, Wu MS, Cherng WJ. Echocardiographic assessment of structural and functional cardiac remodeling in patients with predialysis chronic kidney disease. *Echocardiography* 2010; **27**(6)**:** 621-629.

37. Liu YW, Su CT, Huang YY, Yang CS, Huang JW, Yang MT, Chen JH, Tsai WC. Left ventricular systolic strain in chronic kidney disease and hemodialysis patients. *Am J Nephrol* 2011; **33**(1)**:** 84-90.

38. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise JS, Solomon SD, Spencer KT, Sutton MS, Stewart WJ, Group CQW, Committee ASoEsG, Standards, Echocardiography EAo. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. *J Am Soc Echocardiogr* 2005; **18**(12)**:** 1440-1463.

39. Sengupta PP, Korinek J, Belohlavek M, Narula J, Vannan MA, Jahangir A, Khandheria BK. Left ventricular structure and function: basic science for cardiac imaging. *J Am Coll Cardiol* 2006; **48**(10)**:** 1988-2001.

40. Vinereanu D, Florescu N, Sculthorpe N, Tweddel AC, Stephens MR, Fraser AG. Differentiation between pathologic and physiologic left ventricular hypertrophy by tissue Doppler assessment of long-axis function in patients with hypertrophic cardiomyopathy or systemic hypertension and in athletes. *Am J Cardiol* 2001; **88**(1)**:** 53-58.

41. Derumeaux G, Mulder P, Richard V, Chagraoui A, Nafeh C, Bauer F, Henry JP, Thuillez C. Tissue Doppler imaging differentiates physiological from pathological pressure-overload left ventricular hypertrophy in rats. *Circulation* 2002; **105**(13)**:** 1602-1608.

42. Rakhit DJ, Zhang XH, Leano R, Armstrong KA, Isbel NM, Marwick TH. Prognostic role of subclinical left ventricular abnormalities and impact of transplantation in chronic kidney disease. *Am Heart J* 2007; **153**(4)**:** 656-664.

43. Dogan U, Ozdemir K, Akilli H, Aribas A, Turk S. Evaluation of echocardiographic indices for the prediction of major adverse events during long-term follow-up in chronic hemodialysis patients with normal left ventricular ejection fraction. *Eur Rev Med Pharmacol Sci* 2012; **16**(3)**:** 316-324.

44. Hayashi SY, Rohani M, Lindholm B, Brodin LA, Lind B, Barany P, Alvestrand A, Seeberger A. Left ventricular function in patients with chronic kidney disease evaluated by colour tissue Doppler velocity imaging. *Nephrol Dial Transplant* 2006; **21**(1)**:** 125-132.

45. Gulel O, Soylu K, Yuksel S, Karaoglanoglu M, Cengiz K, Dilek M, Hamiseyev C, Kale A, Arik N. Evidence of left ventricular systolic and diastolic dysfunction by color tissue Doppler imaging despite normal ejection fraction in patients on chronic hemodialysis program. *Echocardiography* 2008; **25**(6)**:** 569-574.

46. Clyne N. Physical working capacity in uremic patients. *Scand J Urol Nephrol* 1996; **30**(4)**:** 247-252.

47. Johansen KL. Physical functioning and exercise capacity in patients on dialysis. *Adv Ren Replace Ther* 1999; **6**(2)**:** 141-148.

48. Kopple JD, Storer T, Casburi R. Impaired exercise capacity and exercise training in maintenance hemodialysis patients. *J Ren Nutr* 2005; **15**(1)**:** 44-48.

49. Moore GE, Brinker KR, Stray-Gundersen J, Mitchell JH. Determinants of VO2peak in patients with end-stage renal disease: on and off dialysis. *Med Sci Sports Exerc* 1993; **25**(1)**:** 18-23.

50. Painter P, Messer-Rehak D, Hanson P, Zimmerman SW, Glass NR. Exercise capacity in hemodialysis, CAPD, and renal transplant patients. *Nephron* 1986; **42**(1)**:** 47-51.

51. Sietsema KE, Hiatt WR, Esler A, Adler S, Amato A, Brass EP. Clinical and demographic predictors of exercise capacity in end-stage renal disease. *Am J Kidney Dis* 2002; **39**(1)**:** 76-85.

52. Sietsema KE, Amato A, Adler SG, Brass EP. Exercise capacity as a predictor of survival among ambulatory patients with end-stage renal disease. *Kidney Int* 2004; **65**(2)**:** 719-724.

53. Silverberg D, Wexler D, Blum M, Schwartz D, Iaina A. The association between congestive heart failure and chronic renal disease. *Curr Opin Nephrol Hypertens* 2004; **13**(2)**:** 163-170.

54. Johansen KL, Chertow GM, Ng AV, Mulligan K, Carey S, Schoenfeld PY, Kent-Braun JA. Physical activity levels in patients on hemodialysis and healthy sedentary controls. *Kidney Int* 2000; **57**(6)**:** 2564-2570.

55. Adams GR, Vaziri ND. Skeletal muscle dysfunction in chronic renal failure: effects of exercise. *Am J Physiol Renal Physiol* 2006; **290**(4)**:** F753-761.

56. Park J, Middlekauff HR. Abnormal neurocirculatory control during exercise in humans with chronic renal failure. *Auton Neurosci* 2015; **188:** 74-81.

57. Faria Rde S, Fernandes N, Lovisi JC, Reboredo Mde M, Marta MS, Pinheiro Bdo V, Bastos MG. Pulmonary function and exercise tolerance are related to disease severity in pre-dialytic patients with chronic kidney disease: a cross-sectional study. *BMC Nephrol* 2013; **14:** 184.

58. Arai Y, Saul JP, Albrecht P, Hartley LH, Lilly LS, Cohen RJ, Colucci WS. Modulation of cardiac autonomic activity during and immediately after exercise. *Am J Physiol* 1989; **256**(1 Pt 2)**:** H132-141.

59. Imai K, Sato H, Hori M, Kusuoka H, Ozaki H, Yokoyama H, Takeda H, Inoue M, Kamada T. Vagally mediated heart rate recovery after exercise is accelerated in athletes but blunted in patients with chronic heart failure. *J Am Coll Cardiol* 1994; **24**(6)**:** 1529-1535.

60. Buchheit M, Gindre C. Cardiac parasympathetic regulation: respective associations with cardiorespiratory fitness and training load. *Am J Physiol Heart Circ Physiol* 2006; **291**(1)**:** H451-458.

61. Kesoi I, Sagi B, Vas T, Kovacs T, Wittmann I, Nagy J. Heart rate recovery after exercise is associated with renal function in patients with a homogenous chronic renal disease. *Nephrol Dial Transplant* 2010; **25**(2)**:** 509-513.

62. Shiraishi FG, Stringuetta Belik F, Oliveira ESVR, Martin LC, Hueb JC, Goncalves Rde S, Caramori JC, Barreti P, Franco RJ. Inflammation, diabetes, and chronic kidney disease: role of aerobic capacity. *Exp Diabetes Res* 2012; **2012:** 750286.

63. Odden MC, Whooley MA, Shlipak MG. Association of chronic kidney disease and anemia with physical capacity: the heart and soul study. *J Am Soc Nephrol* 2004; **15**(11)**:** 2908-2915.

64. London GM, Guerin AP, Marchais SJ, Metivier F, Pannier B, Adda H. Arterial media calcification in end-stage renal disease: impact on all-cause and cardiovascular mortality. *Nephrol Dial Transplant* 2003; **18**(9)**:** 1731-1740.

65. Giachelli CM. The emerging role of phosphate in vascular calcification. *Kidney Int* 2009; **75**(9)**:** 890-897.

66. Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB, Mohler ER, Najjar SS, Rembold CM, Post WS, American Society of Echocardiography Carotid Intima-Media Thickness Task F. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. *J Am Soc Echocardiogr* 2008; **21**(2)**:** 93-111; quiz 189-190.

67. Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzer M. Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. *Circulation* 2007; **115**(4)**:** 459-467.

68. Benedetto FA, Mallamaci F, Tripepi G, Zoccali C. Prognostic value of ultrasonographic measurement of carotid intima media thickness in dialysis patients. *J Am Soc Nephrol* 2001; **12**(11)**:** 2458-2464.

69. Matsushita K, Sang Y, Ballew SH, Shlipak M, Katz R, Rosas SE, Peralta CA, Woodward M, Kramer HJ, Jacobs DR, Sarnak MJ, Coresh J. Subclinical atherosclerosis measures for cardiovascular prediction in CKD. *J Am Soc Nephrol* 2015; **26**(2)**:** 439-447.

70. Szeto CC, Chow KM, Woo KS, Chook P, Ching-Ha Kwan B, Leung CB, Kam-Tao Li P. Carotid intima media thickness predicts cardiovascular diseases in Chinese

predialysis patients with chronic kidney disease. *J Am Soc Nephrol* 2007; **18**(6)**:** 1966-1972.

71. Lemos MM, Jancikic AD, Sanches FM, Christofalo DM, Ajzen SA, Carvalho AB, Draibe SA, Canziani ME. Intima-media thickness is associated with inflammation and traditional cardiovascular risk factors in non-dialysis-dependent patients with chronic kidney disease. *Nephron Clin Pract* 2010; **115**(3)**:** c189-194.

72. Desbien AM, Chonchol M, Gnahn H, Sander D. Kidney function and progression of carotid intima-media thickness in a community study. *Am J Kidney Dis* 2008; **51**(4)**:** 584-593.

73. Zoungas S, Cameron JD, Kerr PG, Wolfe R, Muske C, McNeil JJ, McGrath BP. Association of carotid intima-medial thickness and indices of arterial stiffness with cardiovascular disease outcomes in CKD. *Am J Kidney Dis* 2007; **50**(4)**:** 622-630.

74. Krutzén E, Bäck SE, Nilsson-Ehle I, Nilsson-Ehle P. Plasma clearance of a new contrast agent, iohexol: a method for the assessment of glomerular filtration rate. *J Lab Clin Med* 1984; **104**(6)**:** 955-961.

75. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J, Ckd EPI. A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009; **150**(9)**:** 604-612.

76. Devereux RB, Alonso DR, Lutas EM, Gottlieb GJ, Campo E, Sachs I, Reichek N. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. *Am J Cardiol* 1986; **57**(6)**:** 450-458.

77. Teichholz LE, Kreulen T, Herman MV, Gorlin R. Problems in echocardiographic volume determinations: echocardiographic-angiographic correlations in the presence of absence of asynergy. *Am J Cardiol* 1976; **37**(1)**:** 7-11.

78. Höglund C, Alam M, Thorstrand C. Atrioventricular valve plane displacement in healthy persons. An echocardiographic study. *Acta Med Scand* 1988; **224**(6)**:** 557-562.

79. Jensen-Urstad K, Rosfors S. A methodological study of arterial wall function using ultrasound technique. *Clin Physiol* 1997; **17**(6)**:** 557-567.

80. Peterson LH, Jensen RE, Parnell J. Mechanical Properties of Arteries Invivo. *Circulation Research* 1960; **8**(3)**:** 622-639.

81. Kawasaki T, Sasayama S, Yagi S, Asakawa T, Hirai T. Non-invasive assessment of the age related changes in stiffness of major branches of the human arteries. *Cardiovasc Res* 1987; **21**(9)**:** 678-687.

82. Resnick HE, Lindsay RS, McDermott MM, Devereux RB, Jones KL, Fabsitz RR, Howard BV. Relationship of high and low ankle brachial index to all-cause and cardiovascular disease mortality: the Strong Heart Study. *Circulation* 2004; **109**(6)**:** 733-739.

83. Wallin L, Brudin LH. Physical working capacity determined by different types of bicycle exercise tests. *Clin Physiol* 1988; **8**(5)**:** 529-537.

84. Borg G. Perceived exertion as an indicator of somatic stress. *Scand J Rehabil Med* 1970; **2**(2)**:** 92-98.

85. Brudin L, Jorfeldt L, Pahlm O. Comparison of two commonly used reference materials for exercise bicycle tests with a Swedish clinical database of patients with normal outcome. *Clin Physiol Funct Imaging* 2014; **34**(4)**:** 297-307.

86. Saltin B, Grimby G. Physiological analysis of middle-aged and old former athletes. Comparison with still active athletes of the same ages. *Circulation* 1968; **38**(6)**:** 1104-1115.

87. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; **18**(6)**:** 499-502.

88. Agarwal R. Prevalence of left ventricular hypertrophy: some alternate thoughts. *Am J Kidney Dis* 2005; **46**(6)**:** 1148; author reply 1148-1149.

89. Matsushita K, Kwak L, Sang Y, Ballew SH, Skali H, Shah AM, Coresh J, Solomon S. Kidney Disease Measures and Left Ventricular Structure and Function: The Atherosclerosis Risk in Communities Study. *J Am Heart Assoc* 2017; **6**(9).

90. Seifert ME, de Las Fuentes L, Ginsberg C, Rothstein M, Dietzen DJ, Cheng SC, Ross W, Windus D, Davila-Roman VG, Hruska KA. Left ventricular mass progression despite stable blood pressure and kidney function in stage 3 chronic kidney disease. *Am J Nephrol* 2014; **39**(5)**:** 392-399.

91. Mizuguchi Y, Oishi Y, Miyoshi H, Iuchi A, Nagase N, Oki T. The functional role of longitudinal, circumferential, and radial myocardial deformation for regulating the early impairment of left ventricular contraction and relaxation in patients with cardiovascular risk factors: a study with two-dimensional strain imaging. *J Am Soc Echocardiogr* 2008; **21**(10)**:** 1138-1144.

92. Kozakova M, Morizzo C, La Carrubba S, Fabiani I, Della Latta D, Jamagidze J, Chiappino D, Di Bello V, Palombo C. Associations between common carotid artery diameter, Framingham risk score and cardiovascular events. *Nutr Metab Cardiovasc Dis* 2017; **27**(4)**:** 329-334.

93. Schiffrin EL. The vascular phenotypes in hypertension: Relation with the natural history of hypertension. *J Am Soc Hypertens* 2007; **1**(1)**:** 56-67.

94. Briet M, Bozec E, Laurent S, Fassot C, London GM, Jacquot C, Froissart M, Houillier P, Boutouyrie P. Arterial stiffness and enlargement in mild-to-moderate chronic kidney disease. *Kidney Int* 2006; **69**(2)**:** 350-357.

95. Briet M, Collin C, Karras A, Laurent S, Bozec E, Jacquot C, Stengel B, Houillier P, Froissart M, Boutouyrie P, Nephrotest Study G. Arterial remodeling associates with CKD progression. *J Am Soc Nephrol* 2011; **22**(5)**:** 967-974.

96. Karras A, Haymann JP, Bozec E, Metzger M, Jacquot C, Maruani G, Houillier P, Froissart M, Stengel B, Guardiola P, Laurent S, Boutouyrie P, Briet M, Nephro Test Study G. Large artery stiffening and remodeling are independently associated with all-cause mortality and cardiovascular events in chronic kidney disease. *Hypertension* 2012; **60**(6)**:** 1451-1457.

97. Den Ruijter HM, Peters SA, Anderson TJ, Britton AR, Dekker JM, Eijkemans MJ, Engstrom G, Evans GW, de Graaf J, Grobbee DE, Hedblad B, Hofman A, Holewijn S, Ikeda A, Kavousi M, Kitagawa K, Kitamura A, Koffijberg H, Lonn EM, Lorenz MW, Mathiesen EB, Nijpels G, Okazaki S, O'Leary DH, Polak JF, Price JF, Robertson C, Rembold CM, Rosvall M, Rundek T, Salonen JT, Sitzer M, Stehouwer CD, Witteman JC, Moons KG, Bots ML. Common carotid intima-media thickness measurements in cardiovascular risk prediction: a meta-analysis. *JAMA* 2012; **308**(8)**:** 796-803.

98. Claridge M, Wilmink T, Ferring M, Dasgupta I. Measurement of arterial stiffness in subjects with and without renal disease: Are changes in the vessel wall earlier and more sensitive markers of cardiovascular disease than intima media thickness and pulse pressure? *Indian J Nephrol* 2015; **25**(1)**:** 21-26.

99. Blacher J, Pannier B, Guerin AP, Marchais SJ, Safar ME, London GM. Carotid arterial stiffness as a predictor of cardiovascular and all-cause mortality in end-stage renal disease. *Hypertension* 1998; **32**(3)**:** 570-574.

100. Blacher J, London GM, Safar ME, Mourad JJ. Influence of age and end-stage renal disease on the stiffness of carotid wall material in hypertension. *J Hypertens* 1999; **17**(2)**:** 237-244.

101. Upadhyay A, Hwang SJ, Mitchell GF, Vasan RS, Vita JA, Stantchev PI, Meigs JB, Larson MG, Levy D, Benjamin EJ, Fox CS. Arterial stiffness in mild-to-moderate CKD. *J Am Soc Nephrol* 2009; **20**(9)**:** 2044-2053.

102. Hermans MM, Henry R, Dekker JM, Kooman JP, Kostense PJ, Nijpels G, Heine RJ, Stehouwer CD. Estimated glomerular filtration rate and urinary albumin excretion are independently associated with greater arterial stiffness: the Hoorn Study. *J Am Soc Nephrol* 2007; **18**(6)**:** 1942-1952.

103. Arroyo D, Betriu A, Valls J, Gorriz JL, Pallares V, Abajo M, Gracia M, Valdivielso JM, Fernandez E, Investigators from the Ns. Factors influencing pathological ankle-brachial index values along the chronic kidney disease spectrum: the NEFRONA study. *Nephrol Dial Transplant* 2017; **32**(3)**:** 513-520.

104. Ting SM, Hamborg T, McGregor G, Oxborough D, Lim K, Koganti S, Aldridge N, Imray C, Bland R, Fletcher S, Krishnan NS, Higgins RM, Townend J, Banerjee P, Zehnder D. Reduced Cardiovascular Reserve in Chronic Kidney Failure: A Matched Cohort Study. *Am J Kidney Dis* 2015; **66**(2)**:** 274-284.

105. Howden EJ, Weston K, Leano R, Sharman JE, Marwick TH, Isbel NM, Coombes JS. Cardiorespiratory fitness and cardiovascular burden in chronic kidney disease. *J Sci Med Sport* 2015; **18**(4)**:** 492-497.

106. Nelson A, Otto J, Whittle J, Stephens RC, Martin DS, Prowle JR, Ackland GL. Subclinical cardiopulmonary dysfunction in stage 3 chronic kidney disease. *Open Heart* 2016; **3**(1)**:** e000370.

107. Chinnappa S, White E, Lewis N, Baldo O, Tu YK, Glorieux G, Vanholder R, El Nahas M, Mooney A. Early and asymptomatic cardiac dysfunction in chronic kidney disease. *Nephrol Dial Transplant* 2018; **33**(3)**:** 450-458.

108. Hathaway DK, Cashion AK, Milstead EJ, Winsett RP, Cowan PA, Wicks MN, Gaber AO. Autonomic dysregulation in patients awaiting kidney transplantation. *Am J Kidney Dis* 1998; **32**(2)**:** 221-229.

109. Jondeau G, Katz SD, Zohman L, Goldberger M, McCarthy M, Bourdarias JP, LeJemtel TH. Active skeletal muscle mass and cardiopulmonary reserve. Failure to attain peak aerobic capacity during maximal bicycle exercise in patients with severe congestive heart failure. *Circulation* 1992; **86**(5)**:** 1351-1356.

110. Carrick-Ranson G, Hastings JL, Bhella PS, Shibata S, Fujimoto N, Palmer D, Boyd K, Levine BD. The effect of age-related differences in body size and composition on cardiovascular determinants of VO2max. *J Gerontol A Biol Sci Med Sci* 2013; **68**(5)**:** 608-616.

111. Kooman JP, Kotanko P, Schols AM, Shiels PG, Stenvinkel P. Chronic kidney disease and premature ageing. *Nat Rev Nephrol* 2014; **10**(12)**:** 732-742.

112. Macdougall IC, Lewis NP, Saunders MJ, Cochlin DL, Davies ME, Hutton RD, Fox KA, Coles GA, Williams JD. Long-term cardiorespiratory effects of amelioration of renal anaemia by erythropoietin. *Lancet* 1990; **335**(8688)**:** 489-493.

113. Clyne N, Jogestrand T, Lins LE, Pehrsson SK. Progressive decline in renal function induces a gradual decrease in total hemoglobin and exercise capacity. *Nephron* 1994; **67**(3)**:** 322-326.

114. Van Craenenbroeck AH, Van Craenenbroeck EM, Van Ackeren K, Hoymans VY, Verpooten GA, Vrints CJ, Couttenye MM. Impaired vascular function contributes to exercise intolerance in chronic kidney disease. *Nephrol Dial Transplant* 2016; **31**(12)**:** 2064-2072.

115. Schulman SP, Fleg JL, Goldberg AP, Busby-Whitehead J, Hagberg JM, O'Connor FC, Gerstenblith G, Becker LC, Katzel LI, Lakatta LE, Lakatta EG. Continuum of cardiovascular performance across a broad range of fitness levels in healthy older men. *Circulation* 1996; **94**(3)**:** 359-367.

116. Holloszy JO. Adaptation of Skeletal-Muscle to Endurance Exercise. *Med Sci Sport Exer* 1975; **7**(3)**:** 155-164.

117. Elliott MK, McCaughan JA, Fogarty DG. Do patients with chronic kidney disease get optimal cardiovascular risk reduction? *Curr Opin Nephrol Hypertens* 2014; **23**(3)**:** 267-274.

118. Blood Pressure Lowering Treatment Trialists C, Ninomiya T, Perkovic V, Turnbull F, Neal B, Barzi F, Cass A, Baigent C, Chalmers J, Li N, Woodward M, MacMahon

S. Blood pressure lowering and major cardiovascular events in people with and without chronic kidney disease: meta-analysis of randomised controlled trials. *BMJ* 2013; **347:** f5680.

119. Berard E, Bongard V, Ruidavets JB, Amar J, Ferrieres J. Pulse wave velocity, pulse pressure and number of carotid or femoral plaques improve prediction of cardiovascular death in a population at low risk. *J Hum Hypertens* 2013; **27**(9)**:** 529-534.

120. Åstrand P-O, Rodahl K, Dahl HA, Strømme SB. *Textbook of work physiology : physiological bases of exercise*. Human Kinetics: Champaign, IL, 2003.

Original publications and manuscripts: Studies I-IV