Left ventricular systolic deformation in subclinical metabolic cardiomyopathies

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15. Papers I – III
1. Scientific environment

This thesis came together as a result of national and international collaboration. It is based on clinical studies carried out at the Department of Clinical Science, University of Bergen and Department of Paediatrics and Cardiology, Haukeland University Hospital in Bergen, Norway, and at the Institute of Medicine, Oslo University and Department of Paediatric Medicine, Oslo University Hospital, Rikshospitalet, in Oslo, Norway and at the Department of Cardiovascular Medicine, Queen Elizabeth Hospital, University of Birmingham, in Birmingham, United Kingdom. Most data were collected during a three-year scholarship (2005–2008), including a one-year research visit to the University of Birmingham, UK (2005–2006). The work was funded by a research fellowship from Western Norway Regional Health Authority (2005-2008) and my co-workers received grants from the foundation of Renée and Bredo Grimsgaard and Oslo Red Cross.

During this work collaboration with the following three research groups has been essential:

**Research group for congenital cardiovascular physiology**

I have been a member of this research group since 2001. It focuses on cardiopulmonary physiology in children and adults with congenital heart diseases. During late 90’s and beginning of the new century this group established a complete cardiopulmonary exercise lab suitable for both children and adults located in the Children’s department, under the leadership of my main supervisor Professor Gottfried Greve. He introduced me to exercise testing and the difficult process of interpreting exercise data in children and adults with congenital heart
disease. This, together with his in-depth knowledge of cardiomyopathies and my special interest in echocardiography led to the planning of the present thesis. I want to thank Gottfried for supervising me during these years, for his good friendship, for introducing me to our good colleagues in Birmingham, for sharing with me his hemodynamic understanding and for creating an inspiring working environment. Although it has been challenging we have kept our enthusiasm and positive attitude.

At present I am in charge of a transition process that will take the exercise lab into brand new and expanded areas opening in 2014/2015, also including the possibility of exercise echocardiography and in-house exercise interventional trials (swimming pool and big gym). This will probably become my main arena for future research.

The Bergen Heart in Hypertension group

This group is led by my co-supervisor Professor Eva Gerdts. I worked together with this group in 2003/2004, which also marked my introduction to strain echocardiography, both Doppler and speckle strain echocardiography. I worked together with Einar Davidsen (PhD, consultant cardiologist) under supervision of Professor Gerdts, on two projects. The first project evaluated cardiac function in patients with haemochromatosis, using conventional echocardiography and tissue Doppler imaging. The second project was an interventional trial looking at myocardial deformation before and after pyridostigmine in patients with myasthenia gravis, using strain echocardiography. This work was very important for the planning and implementation of my thesis. I want to express my gratitude to Professor Eva Gerdts for her constant support and excellent academic skills throughout all these years. In particular I want to thank her for her positive and structured supervision during finalizing of this thesis.
Haukeland University Hospital competence group on Fabry disease

This group was established in 2003 as a result of a new enzyme replacement therapy era within treatment of Fabry disease. It was a joint session between the Centre of Medical Genetics and Molecular Medicine (Professor Gunnar Houge), Department of nephrology (Professor Einar Svarstad), Department of Paediatrics (consultant paediatric nephrologist, Camilla Tøndel) and the Departments of Heart Disease and Paediatrics (myself, representing both the adult and paediatric cardiology section at the hospital). Establishing long-term follow-up protocols, a patient database and international collaboration were among the first tasks for the group. The group meets regularly to discuss single patients, protocols, latest scientific news and ongoing research projects within the group and arranges also an annual national meeting on diagnosis and treatment of Fabry disease in Norway. So far more than 20 peer review articles have been published from our group. Future research will focus on renal pathology and renal and cardiac markers that may suggest early treatment with enzyme replacement therapy. I want to thank all the members of the group for many important discussions, quite a few good laughs and for your constant focus on best clinical practice. It is a pleasure being part of this group.
2. Acknowledgements

It is a pleasure for me to acknowledge the contribution of many persons to this thesis. I could not imagine completing the work without their help. My sincere thanks go to:

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set standards for me, as to how one should conduct ones work. No one mentioned, no one forgotten.

My two daughters Vesna and Vilde, both following my footsteps into medicine, my son Sjur, who would rather follow the footsteps of Lionel Messi, our dog Luna who happily would follow in anyone footsteps and Nala, that lazy cat; thanks for all your comfort, support and understanding.

Lastly, Dagunn, my beautiful wife; my one and only and my daily reminder that this thesis does not tell the entire truth about the heart – thank you.
3. Abstract

Cardiovascular disease is a significant contributor to morbidity and mortality in patients with inherited metabolic disorders or chronic kidney disease. Conventional echocardiography typically identifies cardiac involvement at a more established stage of the disease. Strain echocardiography, which assesses the deformation of the myocardium, has the potential for early detection of subclinical myocardial dysfunction.

This thesis consists of 3 studies of left ventricular myocardial deformation in patients with diseases causing metabolic myocardial alterations, associated with development of cardiomyopathy. In study 1, Doppler strain echocardiography was performed in 40 patients with Stage II and III chronic kidney disease. In study 2, speckle strain echocardiography was performed in 68 patients who underwent renal transplantation in childhood, and in study 3, speckle strain echocardiography was performed in 38 patients with Fabry disease. In all studies, the ability of strain echocardiography to detect subclinical cardiac dysfunction not detected by conventional echocardiography was studied.

As demonstrated by the results of this thesis, echocardiography, using Doppler or speckle strain, detected impaired left ventricular long axis function in the studied patient groups. In particular, left ventricular longitudinal strain was reduced, while ejection fraction, measured by conventional echocardiography, was generally preserved in patients with early-stage chronic kidney disease and in patients with mild Fabry disease compared to healthy subjects. Furthermore, having metabolic disease was associated with lower left ventricular systolic strain independent of left ventricular mass. In patients who underwent childhood renal transplantation, hypertension was common and a main covariate of left ventricular diastolic
dysfunction. In contrast, left ventricular systolic deformation was comparable between patients and healthy subjects.

In conclusion, Doppler or speckle strain echocardiography, may detect impaired myocardial function in patients with diseases causing metabolic myocardial alterations, in spite of normal findings on conventional echocardiography and without clinical evidence of heart disease.
4. List of papers

**Paper I**

**Paper II**

**Paper III**
5. Abbreviations

CKD  Chronic kidney disease
CV   Cardiovascular
GFR  Glomerular filtration rate
GL-3 Globotriaosylceramide
LV   Left ventricular
6. Introduction

6.1 Echocardiography as a tool for detecting subclinical cardiac involvement in metabolic and renal diseases

Cardiovascular (CV) disease has long been recognised as a significant contributor to morbidity and mortality in children and adults with systemic diseases such as inherited metabolic disorders(1-3) and chronic kidney disease(4). The first signs of cardiac involvement can be difficult to discover since most patients remain asymptomatic for a shorter or longer period. Furthermore, it is well documented that commonly used measures of left ventricular (LV) function like ejection fraction are insensitive to early changes in myocardial function and actually may remain within normal range despite significant changes in LV structure and myocardial function(5, 6). Assessment of myocardial deformation by Doppler or speckle strain echocardiography are two novel methods suitable to detect subtle changes in myocardial function(7-9)(Figure 1). This “asymptomatic window” may represent an important time for early intervention to prevent or delay further progression to symptomatic cardiac disease(10).
Figure 1. Typical example of regional LV myocardial deformation by speckle strain echocardiography from apical 4-chamber view. The analyses provide time curves of normal LV regional longitudinal strain in a healthy subject (panel I) and reduced strain, in an asymptomatic Fabry patient (panel II). Colour coding in Figure tables reflect the respective LV segments.

6.2 Left ventricular function in chronic kidney disease

The Kidney Disease Outcomes Quality Initiative of the National Kidney Foundation defines chronic kidney disease (CKD) as either kidney damage (proteinuria) or a decreased kidney glomerular filtration rate (GFR) of less than 60 ml/min/1.73 m² persisting for at least 3 months(11). CKD is stratified into five stages: stage I (GFR >90ml/min/1.73m²), stage II (GFR 60-90 ml/min/1.73 m²), stage III (GFR 30-59 ml/min/1.73 m²), stage IV (GFR 15-29 ml/min/1.73 m²) and stage V (GFR <15 ml/min/1.73m², also called end-stage CKD)(12). The classification stages have been applied to adults and children with CKD including renal allograft recipients(12, 13).

CV disease is the leading cause of death in patients with CKD(4). Abnormalities of LV structure and function are found in up to 75% of patients with end-stage CKD (14, 15) and is associated with an impaired prognosis(16). Although the pathogenesis of LV hypertrophy in CKD is considered multifactorial,
hypertension, alterations of fluid and electrolyte balance and anaemia are identified as the major determinants of LV hypertrophy in CKD patients(17, 18). The prevalence of early stages CKD is much higher than the prevalence of end-stage CKD(19). Indeed, patients with CKD are 5-10 times more likely to die before reaching end-stage CKD because the risk of a CV disease is twice as common and advances at twice the rate compared to those without CKD(20, 21). Early detection and, thereby, adequate treatment of LV dysfunction and LV hypertrophy could potentially yield an improvement in the adverse CV outcomes of CKD patients(22). Due to the complex post-processing of Doppler strain echocardiography, reports on myocardial deformation in CKD have been few until now. However, recent studies have showed that Doppler strain echocardiography, measuring tissue velocity, strain and strain rate, is useful in end-stage CKD to identify LV abnormalities before changes in conventional indices of LV function, such as ejection fraction, occur(23). Furthermore, Rakhit DJ and co-workers demonstrated that reduced strain and strain rate were associated with adverse outcome in stage IV and V CKD and that these subclinical cardiac abnormalities could be improved by renal transplantation, but progressed under continuous dialysis(24). For early stage CKD however, no data on strain echocardiography is available.

6.3 Cardiac function after renal transplantation in childhood

The annual incidence of renal replacement therapy among European children (< 15 years old) was 6.5 per million age related population in 2007(25). During 2009 a total of 292 persons were renal transplanted in Norway. Eight were less than 15 years old, comprising 2.7% of total renal transplantations (www.nephro.no). Survivors of childhood renal transplantation have more than a 10-fold increased risk of CV death compared to the general population(26). The classical risk factors include hyperlipidemia, hypertension and insulin resistance. The single most important factor is hypertension and associated vascular damage and LV
remodeling(27). Research on the complex mechanisms of ‘non-classic’ (uremic) CV risk factors, has led to a better understanding of the precursors of the vasculopathy that occurs early in paediatric CKD. Dysregulations in the calcium-phosphate metabolism and vitamin D axis including secondary hyperparathyroidism, are believed to be the driving force of vascular damage leading to media calcification in the arteries(28, 29). Vascular calcification and the ensuing vascular stiffness starts to develop in early stages of CKD, increases with time in dialysis and is not reversed by renal transplantation in the short term(29, 30). In paediatric patients with CKD, uremic associated factors are regarded as the main contributors to cardiovascular morbidity and mortality rather than atherosclerosis(31).

Successful renal transplantation corrects many of the metabolic abnormalities associated with end-staged CKD, but comorbidities related to the immunosuppressive treatment, persistence of renal insufficiency and consequences of a chronic disorder per se may considerably influence CV health in the post-transplantation patients.

Renal transplanted children and children with CKD have been classified in the highest risk stratum for future CV disease along with children with diabetes type I and homozygous familial hypercholesterolemia(32). Focus has therefore turned towards preventive strategies in reducing the CV risk burden to lower CV morbidity and mortality in adulthood. LV hypertrophy is a predictor of mortality among adults(33) and LV hypertrophy is the most prevalent cardiac abnormality after renal transplantation in childhood(34). One preventive strategy could therefore be to introduce sensitive screening tools for early detection of LV myocardial dysfunction. Early detection may lead to early intervention and better treatment and sensitive tools are important to evaluate the effect of early intervention. Indeed, it has been demonstrated that tissue Doppler imaging(35) and
speckle strain echocardiography(36, 37) are more sensitive than ejection fraction and fractional shortening in the detection of subtle changes in LV contractile function. Furthermore, in asymptomatic patients with early stage CKD(38) or in paediatric patients shortly after renal transplantation(39), global systolic strain was reduced on strain echocardiography while ejection fraction and fractional shortening were normal on conventional echocardiography. However, studies on LV systolic function using speckle strain echocardiography long-term after childhood renal transplantation have so far not been published.

### 6.4 The heart in Fabry disease

Fabry disease is a progressive, X-linked inherited disorder of glycosphingolipid metabolism due to deficient or lack of lysosomal alfa-galactosidase A activity. This results in accumulation of globotriaosylceramide (GL-3) and related glycosphingolipids (galabiosylceramide) within lysosomes. The lysosomes are ubiquitous sub cellular organelles in a variety of cell types, including cardiomyocytes and cardiac fibroblasts and capillary endothelial and renal cells(40). Lysosomes function as the digestive system of the cell, containing an array of enzymes capable of breaking down all types of biological polymers – proteins, nucleic acids, carbohydrates and lipids. The typical clinical feature of Fabry cardiac disease is LV hypertrophy. Although GL-3 accumulation does increase heart mass, studies have shown that GL-3 is responsible for only 1–3% of excessive LV mass in Fabry disease(41, 42). This indicates that LV hypertrophy may not arise solely as a direct consequence of GL-3 infiltration. One potential mechanism could be disruption of myocardial architecture by the lipid deposits leading to myofibrillar disarray similar to what is seen in familial hypertrophic cardiomyopathy(43, 44). However, this phenomenon is not very prominent in Fabry disease(45). Another hypothesis is that GL-3 accumulation causes disturbances in respiratory-chain enzyme activity within the mitochondrial metabolism, leading to reduced levels of creatinine phosphatase, adenosine
diphosphate, and adenosine triphosphate (46-48). An increase in trophic factors, such as lyso-Gb3 (49), or the alteration of cellular adhesion molecules in vascular endothelial cells, inducing coronary small-vessel disease (50), may also have a causative role in the development of CV disease in patients with Fabry disease.

Enzyme replacement therapy with recombinant alfa-galactosidase A has been available since 2001. Biopsy studies have shown that enzyme replacement therapy completely or partially cleared microvascular deposits of GL-3 from the heart of Fabry patients (51). These changes are seen in parallel to a decrease in LV mass and an improvement in myocardial function and exercise capacity (52). However, randomised trials showing that enzyme replacement therapy will prolong life expectancy or reduce major cardiac events such as myocardial infarction, heart failure or cardiac death, are warranted (53). Most centres are reluctant to start high-cost enzyme replacement therapy in asymptomatic patients without evidence of cardiac dysfunction. On the other hand, enzyme replacement therapy seems to be most beneficial in patients with less severe disease (54), with lack of improvement in myocardial function and exercise capacity in patients with end-stage myocardial fibrosis. Timely initiation of therapy is therefore probably of importance, but challenging due to the lack of documented effect on life expectancy and risk for major CV complications.

Tissue Doppler imaging (55, 56) and Doppler strain echocardiography (52, 57) have proven useful to detect early cardiac involvement in patients with Fabry disease and to monitor disease progression and the effect of enzyme replacement therapy. However, these studies are few, probably due to technical challenges and time consuming post-processing. Myocardial deformation using speckle strain echocardiography has not been used previously in Fabry disease. If deformation analysis could improve the detection of subclinical cardiac abnormalities,
independent of LV mass, this could add important information to the understanding of cardiac involvement in Fabry disease.
7. Hypothesis and aim of the thesis

7.1 Hypothesis

- The hypothesis of this project was that strain echocardiography is superior to conventional echocardiography in detecting subclinical cardiac involvement in patients with metabolic diseases.

7.2 Specific aims

- Can Doppler strain echocardiography detect myocardial abnormalities in asymptomatic patients with Stage II or III chronic kidney disease and normal LV systolic function by conventional echocardiography?
- Is LV conventional and speckle strain echocardiography normal in asymptomatic children and young adults who underwent renal transplantation in childhood?
- Can speckle strain echocardiography detect reduced LV systolic deformation in patients with genetically confirmed Fabry disease, independent of LV geometry studied by conventional echocardiography?
8. Methods

8.1 Study populations

8.1.1 Study I

This analysis was a prospectively planned echocardiographic substudy within the first 50 patients with stable Stage II or III CKD, recruited into a single-centre, prospective, double-blind, placebo-controlled, randomised interventional trial of spironolacton compared with placebo (Identifier: NCT00291720, www.clinicaltrials.gov). Patients with diabetes, history of angina, myocardial infarction, heart failure, cerebral or peripheral vascular disease, atrial fibrillation, valvular disease (more than mild regurgitation or any degree of stenosis), uncontrolled hypertension or anaemia with haemoglobin below 12 g/dl were excluded. Ten patients were excluded because of inadequate echocardiographic image quality. All patients had controlled blood pressure with mean daytime 24-hour ambulatory blood pressure less than 130/85 mmHg and established treatment with an angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker. 30 healthy sex- and age-matched volunteers from the study hospital staff served as controls.

8.1.2 Study II

This case control study included 68 paediatric and young adult patients who underwent renal transplantation during childhood (Identifier: NCT01008306, www.clinicaltrials.gov). 34 out of 46 paediatric patients (aged 2 – 17 years) were recruited from the outpatient clinics at Oslo University Hospital (Rikshospitalet), Oslo, Norway and 34 out of 61 adult patients agreed to participate after being identified through the Norwegian Renal Registry (www.nephro.no). Inclusion criteria for both groups were functioning graft >1 year and no clinical sign of cardiac disease. In the adult group 20 patients were excluded due to ongoing
dialysis or <1 year since transplantation (n=6), > 3 transplantations (n=1), orthopaedic restrictions or mental or neurological disorders (n=11), severe heart failure (n=1) and lost from follow-up (n=1). Adult controls were recruited from the hospital blood donor registry. Paediatric controls were recruited from the paediatric outpatient clinic, Haukeland University Hospital, Bergen, among patients that had been referred due to palpitations, syncope or a suspected heart murmur. Exclusion criteria were sign of cardiac disease, major locomotor or musculoskeletal restrictions.

8.1.3 Study III
This was a prospectively planned study of adult patients newly referred to the Department of Heart Disease at Haukeland University Hospital for echocardiography because of genetically confirmed Fabry disease between November 2004 and February 2012. A total of 40 patients were referred during this period. All patients agreed to participate in the study, but one patient was excluded due to lack of written informed consent and another was excluded due to poor acoustic window. Age matched healthy controls were recruited among 19 healthy volunteers from the hospital blood donor registry, recruited as controls for another project in 2004 (also part of study I) and 19 healthy medical students recruited in 2010 and 2011.

All three study protocols were approved by the Local (Study I, South Birmingham, UK) or Regional (Study II, South-east Norway; Study III, West-Norway) Ethics Committee and carried according to the Declaration of Helsinki. Written informed consent was obtained from all patients or their parents if younger than 16 years of age prior to study start as well as from all controls.
8.2 Echocardiography

8.2.1 Study protocol
A Vivid 7 echocardiograph (GE Vingmed Ultrasound, Horten, Norway) equipped with a phased-array M3S or M5S transducer was used for all studies. The patients were examined in left supine position using the apical four- and two-chamber views (all studies), apical three-chamber view (Study I) and parasternal short-axis at the level of the papillary muscle (Study III). Three consecutive heartbeats were recorded at end expiration and stored digitally in raw data format on magnetic optical disks and analysed off-line by one (Study III) or two (Study I and II) independent observers, blinded to the clinical data using EchoPac (GE Vingmed, Horten, Norway) work station.

8.2.2 Conventional echocardiography
Quantitative echocardiography was performed following the Joint European Association of Echocardiography and American Society of Echocardiography guidelines(58). Pulsed-wave tissue Doppler imaging was performed at the mitral annulus from 6 (Study I, apical 4-, 2- and 3-chamber) or 4 (Study II and III, apical 4- and 2-chamber) LV views, reporting the average peak systolic, early diastolic and late diastolic annular myocardial velocity (Figure 2).
LV mass and relative wall thickness were both calculated based on linear measurements of LV chamber diameters and wall thickness in two-dimensional parasternal long-axis view. LV mass was calculated by the Devereux’s equation(59):

\[
LV\ mass\ (g) = 0.8 \times (1.04[(LVIDd + PWDd + IVSDd)^3 / LVIDd]) + 0.6\ g
\]
where LVIDd, PWDd and IVSDd are the end-diastolic diameters of LV internal chamber, posterior wall and septum, respectively. The equation has been validated against autopsy data in patients with various cardiac and non-cardiac pathologies. Relative wall thickness was calculated as the ratio of 2 x PWDd/LVIDd(60). In
both paediatric (study II) and adult patients LV mass was indexed by body surface area. We used in patients less than 17 years of age a LV mass index above 88.9 g/m² to define LV hypertrophy(61). In adult patients we used the prognostically proven cut-off values of above 104 g/m² and 116 g/m² in women and men respectively(62, 63). We used the prognostically validated definition of LV geometry as normal (no LV hypertrophy and normal relative wall thickness), concentric hypertrophy (LV hypertrophy, relative wall thickness ≥0.43), eccentric hypertrophy (LV hypertrophy, normal relative wall thickness) or concentric remodelling (normal LV mass, relative wall thickness ≥0.43)(60).

![Figure 2. Tissue Doppler imaging from apical 4-chamber view. Normal values for systolic (S’), early diastolic (E’) and late diastolic (A’) mitral septal (left panel) and lateral (right panel) annular myocardial velocity.](image)

### 8.2.3 Strain echocardiography

Strain is a measure of tissue deformation and it is defined as the percentage change in length normalised to the original length (Figure 3)(64). The velocity at which this change occurs is the strain rate. During a cardiac cycle the heart shortens and lengthens in the longitudinal direction, it thickens and thins in the radial direction, and it shortens and lengthens in the circumferential direction. In this thesis we investigated LV longitudinal (all studies) and circumferential (study III)
deformation (Figure 4).

![Strain Diagram]

**Figure 3.** Strain is defined as the change in length \((L_1 - L_0)\) normalized to the initial length \(L_0\) of the region of interest. Shortening a myocardial segment of 10 cm to 8 cm, indicate a strain of -20%. No change in length would indicate 0% strain. The time at which this change occurs is the strain rate.
Figure 4. Graphic presentation of myocardial deformation: longitudinal (left panel) and circumferential (right panel). The direction of deformation in systole (shortening) is shown as solid lines and that in diastole (lengthening) as dashed lines.

8.2.3.1 Doppler strain echocardiography
We used Doppler strain echocardiography in Study I to investigate LV longitudinal deformation. Because it requires parallel orientation between the ultrasound beam and the direction of motion, it was only applied in the apical 4-chamber view. We carefully placed the wall under interrogation in the centre of the ultrasound beam and the image sector was narrowed, achieving frame rates of 180 to 220 frames per second. Time of aortic valve closure was obtained from pulsed-wave Doppler at the level of LV outflow tract. We used a sample volume of 6 x 6 mm for tissue velocities and 12 x 8 mm for strain and strain rate (Figure 5). Results are reported as peak systolic strain and strain rate measured at aortic valve closure (end systole) and postsystolic shortening. Furthermore, strain and
strain rate measurements from the septal and lateral basal and midle LV segments were averaged to obtain longitudinal global strain and strain rate.

**Figure 5.** Doppler strain echocardiography. Longitudinal strain curves over multiple heart cycles from the septal basal (yellow) and septal mid (green) segments in (A) patient with CKD and (B) healthy control. Peak strain at the aortic valve closure is significantly reduced in both the basal and mid segments in CKD compared with control subject.

### 8.2.3.2 Speckle strain echocardiography
Speckle strain echocardiography analyses motion by tracking natural acoustic reflections\(^\text{[65]}\). An automatically defined region of interest is divided into blocks in which stable speckle-patterns (“fingerprints”) can be recognized by the software. Each block holds 20 to 40 pixels with variable greyscale intensity, constituting the image. These blocks are tracked consecutively frame to frame and the movement of the blocks are converted into velocity vectors. The change in block-vectors is defined as regional strain. Negative strain is classified as myocardial thickening and represents contraction, and is colour-coded red. Positive strain represents myocardial thinning and reflects relaxation, and is colour-coded blue. The method has been validated against MRI-tagging and sonomicrometry\(^\text{[66, 67]}\) and proved valuable in evaluating LV function in patients with hypertrophic cardiomyopathies\(^\text{[68]}\) and ischemic heart disease\(^\text{[69]}\).
We used this technique in Study II and III to describe LV longitudinal and circumferential peak systolic strain and strain rate by analysing grey-scale images (70) from 6 segments for each view throughout the cardiac cycle: the antero-septal, anterior, lateral, posterior, inferior and septal segments in the parasternal short axis view, lateral and septal basal, middle and apical segments in the apical 4-chamber view and inferior, and anterior basal, middle and apical segments in the apical 2-chamber view (Figure 6). Care was taken to achieve frame rates of at least 50 frames per second by optimizing depth and sector width. Results are reported as the peak systolic strain and strain rate during the whole cardiac cycle. Furthermore, measurements from the individual LV apical and parasternal segments were averaged and reported as LV global longitudinal or circumferential strain and strain rate.

**Figure 6.** Standard division of the LV in six segments when performing regional deformation analysis by speckle strain echocardiography on a parasternal short-axis view (panel I), apical 2-chamber view (panel II) and apical 4-chamber view (panel III). Colour coding in Figure tables reflect the respective LV segments and
the numbers in the table reflect the measured strain in each segment. The lower panels provide time curves of respective LV regional strain. Images are taken on a healthy subject.

8.2.4 Reproducibility
In Study I Doppler derived peak systolic basal tissue velocities, strain and strain rate were analyzed off-line by two independent observers in 10 randomly selected subjects (intraobserver and interobserver variation). In Study II longitudinal global peak systolic strain and strain rate and segmental basal septal and lateral peak systolic strain by speckle strain echocardiography were analyzed in all patients by two independent observers (interobserver variation).

8.3 Statistics
The SPSS statistical computing program versions 14.0 to 17.0 (SPSS Inc., Chicago, Illinois, USA) were used for statistical analysis. Data are presented as mean ± standard deviation for continuous variables and as percentages for categorical variables. The chi-square test was used to compare categorical variables and Student $t$-test to compare continuous variables. Variables not normally distributed were log-transformed before tested in uni- and multivariate analysis. Bivariate correlations were assessed by Pearson’s correlation coefficients for normally distributed data. Multivariate linear regression analysis with an enter procedure and collinearity diagnostic was used to assess independent covariates of segmental and global longitudinal systolic strain and strain rate. Receiver Operating Characteristics curve analysis was used in Study III to identify the cut-off value of LV global longitudinal systolic strain with best sensitivity and specificity in identifying disease. Reproducibility of measurements of systolic tissue velocities and systolic segmental and global longitudinal strain and strain rate were assessed by intraclass correlation coefficients and Bland-Altman limits.
of agreement (71). Two-tailed $p < 0.05$ was considered significant both in univariate and multivariate analyses.
9. **Summary of results**

9.1 **Study I. Subclinical Abnormalities of Left Ventricular Myocardial Deformation in Early-Stage Chronic Kidney Disease: The Precursor of Uremic Cardiomyopathy?**

To determine left ventricular function echocardiography was performed in 24 men and 16 women (mean age 48 ± 11 years) with stage II or III CKD and 30 age and sex-matched healthy subjects. There were no differences in LV ejection fraction or systolic tissue Doppler velocities between patients with CKD and controls. In CKD, mean LV global longitudinal systolic strain (-15% ± 4% vs. -17% ± 3%, P < 0.01) and strain rate were reduced compared to controls (-0.88 ± 0.16 vs. -1.06 ± 0.31, P < 0.05). Regional peak systolic strain was reduced in the basal lateral and the basal and middle septal LV walls. Regional peak systolic strain rate was reduced in the basal and middle lateral and middle septal segments. There was good intra- and interobserver agreement: LV Systolic tissue velocity (r = 0.96; P < 0.001), LV global longitudinal systolic strain rate (r = 0.95, P < 0.001), and strain (r = 0.98, P < 0.001). Bland-Altman limits of agreement for intra- and interobserver variation revealed no systematic bias in differences between measurements with respect to their means: LV systolic tissue velocity 10.4% to -6.9% and 11.8% to – 12.4%, LV global longitudinal systolic strain rate 13.3% to – 12.7% and 19.9% to – 21.6% and strain 15.1% to – 13.5% and 8.5% to – 11.9%.

9.2 **Study II. Left ventricular function in children and adults after renal transplantation in childhood.**

Conventional and speckle strain echocardiography was performed in 34 paediatric and 34 adult CKD patients aged 3-41 years (mean 20 ± 10). All underwent renal transplantation in childhood median 9.8 years earlier (range 2.0 – 28.4) and compared to 68 age- and sex-matched healthy subjects. Forty-three percent had a
pre-emptive transplant. Of the remaining, 70% received haemodialysis and 30% peritoneal dialysis on average for 6.9 months. Thirty-one percent of paediatric and 35% of adult patients had known hypertension. In renal transplanted patients, multiple regression analysis showed that higher systolic blood pressure was independently associated with higher LV mass index (β 0.618, 95% CI 0.107 - 0.509, P = 0.026) when adjusted for age and diastolic variables. LV global longitudinal systolic strain and strain rate were comparable in patients and controls at the post-transplant follow-up. LV diastolic function, represented by the isovolumic relaxation time and the ratio of early mitral diastolic flow to early diastolic myocardial velocity (E/E’), was impaired in both paediatric patients (69 ± 15 vs. 60 ± 12, P 0.01 and 6.8 ± 1.6 vs. 5.5 ± 0.9, P < 0.01) and adult patients (80 ± 16 vs. 71 ± 11, P 0.02 and 7.3 ± 3.3 vs. 5.2 ± 0.9, P < 0.01) compared to controls. Average peak VO$_2$ was 66% and 85% of expected value in children and adults respectively. In multivariate analysis, systolic blood pressure (β = 0.230, CI -0.000 – 0.008, P = 0.044) and LV diastolic relaxation (β = 0.274, CI -0.001 – 0.006, P = 0.001, respectively) were the main covariates of LV global longitudinal systolic strain rate. The same relations were found for LV global longitudinal systolic strain, but only in a univariate model (β = 0.297, CI 0.002 – 0.006, P = 0.001 and β = 0.349, CI -0.002 – 0.007, P < 0.001). The interobserver agreement was within clinical acceptance: LV global longitudinal systolic strain (r = 0.66; P < 0.01), strain rate (r = 0.90; P < 0.01) and LV basal septal and lateral longitudinal peak systolic strain (r = 0.96 and r = 0.95; both P < 0.01). Bland-Altman limits of agreement revealed no systematic bias in differences between measurements: LV global longitudinal systolic strain (-2.56% to 1.46%), and strain rate (-0.02% to 0.08%) and LV basal septal and lateral longitudinal peak systolic strain (-1.72% to 2.04% and -1.23% to 3.09% respectively).
9.3 **Study III. Speckle strain echocardiography may detect early cardiac involvement in Fabry disease.**

In this study we investigated if speckle strain echocardiography could detect early cardiac involvement in 25 women and 13 men aged 17 - 68 (32 ± 15.9) with genetically confirmed Fabry disease by comparing them to 38 age- and sex-matched healthy subjects. LV hypertrophy was found in 21% of the patients (4 women and 4 men). LV global longitudinal systolic strain was lower in patients compared to controls (-16.3 ± 3.9 vs. -19.5 ± 2.5 %, P = 0.000). LV circumferential deformation was comparable in patients and controls. 28% had Stage II or III chronic kidney disease based on GFR measurements. In multivariate analysis, having Fabry disease (B = 2.457, CI 0.683 – 4.231, P = 0.008) predicted lower LV global longitudinal systolic strain independent of LV mass (B = 0.036, CI 0.023 – 0.050, P = 0.000), diastolic function, GFR, systolic blood pressure and age (multiple R²=0.60, P = 0.000). In receiver operating characteristics curve analysis, LV global longitudinal systolic strain was superior to LV mass in identifying patients with Fabry disease.

9.4 **Feasibility**

We excluded ten patients in Study I and one patient in Study III because of poor image quality. All other patients had sufficient image quality for analysis of strain by Doppler or speckle tracking. However, some segments were not possible to analyse. Summarizing the results of all the patients, the feasibility of longitudinal strain and strain rate by speckle strain echocardiography was 86.3% and 83.6%, respectively and for circumferential strain 84.2%.
10. Discussion

This thesis demonstrates that measurements of longitudinal strain may improve detection of LV systolic dysfunction in patients with diseases causing metabolic myocardial alterations, associated with development of cardiomyopathy. As demonstrated by the results, having Fabry disease was associated with lower LV global longitudinal systolic strain independent of systolic blood pressure, LV mass and age. Furthermore, LV global longitudinal systolic strain was superior to LV mass in identifying patients with Fabry disease. In patients with CKD, LV global longitudinal systolic strain was reduced in patients with early-stage disease, but not in post-transplant follow-up of patients who underwent renal transplantation during childhood. Finally strain echocardiography could be performed with clinical acceptable intra- and inter-observer reproducibility.

10.1 Study population

Differences and similarities between the studied patient populations are presented in Table 1. As pointed out, hypertrophy of cardiomyocytes and some degree of myocardial disarray may be found in the end-stage of the cardiomyopathy both in CKD and in Fabry disease(72-74). In CKD patients this is caused by chronic uraemia and longstanding hypertension. Our data suggest that LV systolic myocardial deformation is less affected in patients who underwent renal transplantation in childhood than in patients with stage II and III CKD (Table 1). In Fabry patients both the accumulation of GL-3 within the heart itself, but also within the kidney, causing progressive renal impairment as in CKD, may contribute to cardiac dysfunction. Of note, in our Fabry population 28% had stage II or III CKD based on GFR. The reduced renal function may influence myocardial function beyond the direct effect of Fabry disease itself, and probably at least in part explains our findings of reduced LV myocardial systolic deformation despite an overall low disease severity score.
Table 1. Characteristics of study population

<table>
<thead>
<tr>
<th>Disease</th>
<th>Pathology</th>
<th>CV manifestation</th>
<th>Echocardiography¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early-stage CKD (n = 40)</td>
<td>Hypertrophic cardiomyocytes², Limited myocyte disarray², Coronary and carotid arteriopathy³, Interstitial fibrosis³</td>
<td>Hypertension, LV hypertrophy, LV myocardial fibrosis</td>
<td>δ LV global longitudinal systolic strain, δ Ejection fraction, LV hypertropy (18%)</td>
</tr>
<tr>
<td>Renal Tx (n = 68)</td>
<td>Hypertrophic cardiomyocytes², Limited myocyte disarray², Coronary and carotid arteriopathy⁴</td>
<td>Hypertension, LV hypertrophy, δ Exercise capacity</td>
<td>δ LV global longitudinal systolic strain, δ Ejection fraction, δ LV systolic myocardial velocity, δ LV diastolic compliance, LV hypertropy (19%)</td>
</tr>
<tr>
<td>Fabry disease (n = 38)</td>
<td>Hypertrophic and vacuolated cardiomyocytes⁵, Limited myocyte disarray⁶, Coronary and carotid arteriopathy⁷, Interstitial fibrosis⁸</td>
<td>Hypertension, LV hypertrophy, LV myocardial fibrosis, Mild valvular insufficiency, PR-interval prolongation</td>
<td>δ LV global longitudinal systolic strain, δ Ejection fraction, δ LV diastolic compliance, LV hypertropy (21%)</td>
</tr>
</tbody>
</table>

CV, cardiovascular; CKD, chronic kidney disease; LV, left ventricular; Tx, transplantation

¹Data from thesis, ²End-stage CKD (myocardial histology of early-stage never described(74), ³(75-77), ⁴(78, 79), ⁵(72, 73), ⁶(72), ⁷(80, 81), ⁸(82, 83)

10.2 Strain echocardiography in subclinical LV dysfunction

When tissue Doppler strain echocardiography was first introduced in the mid 90’s, studies were primarily looking for a new indicator of regional left ventricular contraction and function in ischemic heart disease(84-86). With the growing experience with the method it was tempting to apply the method also to better evaluate global left ventricular contraction and function(87).

Global longitudinal systolic strain measured by Doppler or speckle strain echocardiography has proved sensitive to early LV systolic myocardial abnormalities even in patients with normal LV systolic function measured by conventional echocardiographic methods.(5, 6, 9) Strain echocardiography has
been suggested to be particular useful in a number of cardiomyopathies associated with development of LV hypertrophy. This includes the preclinical diagnosis of cardiomyopathies due to non-obstructive hypertrophic cardiomyopathy, Friedreichs ataxia and amyloidosis (37, 88-90). In all these studies there was an association between reduced regional and/or global strain and LV mass. However, it was not studied if reduced strain was predictive of having the disease, independent of LV mass. Indeed abnormal LV geometry is associated with changes in LV regional and global deformation and this can be identified by Doppler or speckle strain echocardiography as shown in our studies. As demonstrated in our studies LV hypertrophy was found in 18% of patients with CKD, in 19% of patients after childhood renal transplantation and in 21% of patients with Fabry disease. Both increased LV mass and high relative wall thickness was associated with reduced global myocardial deformation in our patients in Study III. More important however, is the ability of strain echocardiography to detect LV myocardial dysfunction in asymptomatic patients without LV hypertrophy. In study I and III, LV longitudinal systolic strain was significantly reduced in patients compared to controls, independent of LV mass. Our findings supports previous publications demonstrating lower global strain to be a better marker of LV global dysfunction than LV ejection fraction and fractional shortening in patients with coronary artery disease (91) and our findings suggest that reduction in LV global longitudinal strain is present before LV hypertrophy can be measured and therefore may be superior to LV mass in identification of early subclinical myocardial involvement.

10.3 Cardiac function in chronic kidney disease

Study I is among the first to demonstrate that patients with early-stage CKD have subclinical LV systolic dysfunction with evidence of impaired LV global longitudinal strain on echocardiography. In contrast, a previous report by Hayashi and co-workers found that early diastolic myocardial velocity (E’) was reduced in
patients with severe (Stage IV) CKD only and not in patients with mild/moderate (Stage I–III) CKD(92). Furthermore, the reduction in early diastolic myocardial velocity (E’) in their study was seen both in patients with LV hypertrophy and in those without(92). This is consistent with our finding of comparable LV global longitudinal systolic deformation in patients with and without LV hypertrophy and with and without known hypertension, suggesting that other factors than hypertrophy and hypertension contributes to the myocardial dysfunction. In a study by Nasir et al, Harmonic Phase tagged magnetic resonance imaging showed that circumferential strain and strain rate was reduced in early-stage CKD patients with a creatinine clearance less than 60 mL/min(93). The present results add to this knowledge by demonstrating that LV global longitudinal systolic strain and strain rate were significantly reduced in patients with early-stage CKD compared to healthy subjects, consistent with a hypothesis of subclinical cardiac involvement associated with early-stage CKD. In a 36-months follow-up study of 129 patients with late-stage (Stage IV and V) CKD, Rakhit and co-workers showed that global longitudinal strain measurements worsened in patients on continuous dialysis while it improved in patients undergoing renal transplantation(24). Furthermore, this subclinical LV myocardial dysfunction was associated with adverse outcome, suggesting that the detection of subclinical myocardial dysfunction added incremental value to clinical predictors. Whether this also refers to patients with early-stage CKD need further evaluation in future studies to determine if they are related to the severity of the CKD itself or other factors like LV hypertrophy or systolic blood pressure.

The prognostic value of LV deformation has been studied in patients with heart failure, demonstrating that longitudinal strain measurement is a promising echocardiographic parameter to predict benefit from cardiac resynchronization therapy(94, 95) and it was the strongest predictor for new onset atrial fibrillation in hospitalized patients with heart failure(96). No previous studies have investigated
the prognostic value of strain and strain rate imaging, and there are no studies regarding the effect of intervention on subclinical myocardial abnormalities in early-stage CKD.

We found no difference in global LV systolic myocardial deformation between patients who underwent renal transplantation in childhood and healthy subjects. Also LV ejection fraction and fractional shortening were comparable between patients and controls. We might speculate that this reflects a normalisation of myocardial function after removal of the ‘uraemic state’ before transplantation. Most of our patients had a pre-emptive transplantation with organ from a living donor and mean time on dialysis was only 6.9 months, all of which are favourable in terms of CV prognosis(97, 98). However, mild hypertension was common among our renal transplanted patients. Furthermore, ambulatory blood pressure measurement showed mild hypertension, both in patients with known hypertension and on antihypertensive drugs and in those believed to be normotensive, suggesting an under-diagnosing and under-treatment of hypertension in our cohort. Baltabaeva and co-workers showed that longitudinal peak systolic strain was reduced in the LV basal septal wall, but increased in the LV basal lateral wall in untreated patients with mild to moderately elevated blood pressure(99). They did not report global measurements. In our adult transplanted patients we also found reduced longitudinal peak systolic strain in LV basal septal wall, but with no differences in the LV basal lateral wall compared to healthy subjects. This may be explained by particular high level of wall stress at the LV basal septum due to an increased local radius of curvature compared to the LV free wall(100). The basal septum is therefore often the first region to show changes under the influence of pressure overload. The increase in segmental strain in the LV basal lateral wall may be a compensating mechanism early in hypertension. Our finding of normal global LV myocardial deformation in patients with childhood renal transplantation supports this theory. Chen and co-workers demonstrated reduced regional
longitudinal and circumferential strain rate in hypertensive patients with LV hypertrophy, but normal ejection fraction and fractional shortening measured by conventional echocardiography, suggesting a more severe impairment of LV systolic deformation in patients with combined hypertension and LV hypertrophy(101). In comparison, our renal transplanted patients had a low prevalence of LV hypertrophy (19%) compared to previous reports(102, 103), and this may have contributed to the well-preserved LV systolic function, including normal LV systolic myocardial deformation. Furthermore, it has been suggested that paediatric and adolescent renal recipients have a hyperdynamic circulation, characterised by reduced after-load and sympathetic over-activity(102, 104). This may explain our finding that global systolic myocardial deformation was well preserved and in some wall-segments even increased. In patients with childhood onset CKD it is hypothesised that uremic-associated factors, such as interstitial fibrosis(74) and endothelial dysfunction followed by arterial medial calcification(31), are the main contributors to cardiovascular morbidity and mortality rather than classic atherosclerotic intimal calcification. Abnormal myocardial deformation may reflect cardiomyocyte hypertrophy with disarray and interstitial fibrosis(74, 105). This is commonly seen in patients with familial hypertrophic cardiomyopathy(44) and has also been found on endomyocardial biopsy in patients with end-stage CKD(74)(Table 1).

In CKD, diastolic dysfunction commonly precedes changes in systolic function on conventional echocardiography(106, 107). Our renal transplanted patients and Fabry patients had impaired LV diastolic compliance as also previously reported by others(103, 108). Isovolumic relaxation time and the ratio of early mitral diastolic flow to early diastolic myocardial velocity (E/E’) were significantly higher both in the paediatric patients and adult patients after childhood renal transplantation compared to healthy controls. There are conflicting evidence about improvement in LV diastolic function after renal transplantation(103).
Cyclosporine, used by 47% of our patients, is supposed to contribute to interstitial myocardial fibrosis (109). We did not have cardiac magnetic resonance imaging information of LV myocardial fibrosis in our patients. However, our cohort of renal transplanted patients was fairly young with only short time since transplantation and with well-preserved LV systolic deformation, suggesting that widespread interstitial fibrosis was unlikely. As previously noted, our study showed a high prevalence (33%) of mild hypertension in both children and adults with childhood renal transplantation. It is therefore more likely that the impaired LV diastolic function in our renal transplanted patients can be explained by inadequate blood pressure control rather than by fibrosis caused by cyclosporine treatment. Arterial hypertension is a common consequence after renal transplantation (110). It is linked to an increased risk of CV events, graft failure, proteinuria, and death (111). Diastolic dysfunction is an early finding in mild to moderate hypertension (112) and is found to precede LV hypertrophy in the development of hypertension (113). In our renal transplanted patients impaired LV diastolic relaxation was associated with higher LV mass index in multiple regression analysis ($\beta$ 0.357, CI 0.156, 0.433, $P < 0.001$). Furthermore, previous reports have shown that impaired LV diastolic function identifies hypertensive patients at increased CV risk (114). In our hypertensive renal transplanted patients, higher systolic blood pressure was independently associated with higher LV mass index. This is in accordance with data from Harkel and co-workers who found an association between LV hypertrophy and systolic hypertension in paediatric patients with diastolic dysfunction after renal transplantation (115). In the Losarten Intervention For Endpoint reduction in hypertension (LIFE) Study losartan or atenolol-based antihypertensive treatment in hypertensive patients with LV hypertrophy and LV diastolic dysfunction, resulted in significant improvement in trans-mitral flow patterns but this was not directly associated with reduced CV morbidity and mortality (116). Only patients with in-treatment normal LV filling
had reduced risk for hospitalization for heart failure, emphasising the importance of timely antihypertensive treatment(116).

10.4 Cardiac involvement in Fabry disease
Our study demonstrated that reduced LV global systolic strain is associated with having Fabry disease independent of LV mass. To our knowledge this is the first study to use speckle strain echocardiography in the search for subclinical markers of cardiac involvement in Fabry disease. Speckle strain echocardiography has revealed that LV longitudinal systolic strain in patients with hypertrophic cardiomyopathy is reduced in proportion to patient symptoms(117). Also in our study, abnormal myocardial deformation was confined to the longitudinal LV mechanics. LV longitudinal mechanics predominantly reflect subendocardial myocardial function. This is the most vulnerable component of LV mechanics due to increased degree of shear stress, and the subendocardial myocardial function therefore is likely to deteriorate first(65). It has been shown that circumferential strain, reflecting mid-myocardial and epicardial LV function, remain normal or super-normal, as a compensating mechanism to preserve systolic function, in the early course of hypertrophic cardiomyopathies(117). Depending on extent of myopathy, the extent of compensation offered by circumferential strain in relation to reduction in longitudinal strain in hypertrophic cardiomyopathy may vary(68, 117). Our Fabry patients demonstrated slightly increased LV global systolic circumferential strain and strain rate compared to healthy subjects, supporting the impression of mild Fabry disease with intact circumferential compensating mechanism. Weidemann and co-workers also found abnormal longitudinal strain in patients with Fabry disease using Doppler strain echocardiography(52, 118). In their study they also showed that strain parameters improved after enzyme replacement therapy. As in our study there was a mixture of patients with and without LV hypertrophy. Our finding that reduced global systolic strain was independent of LV mass is important because we need a cardiac marker
independent of LV hypertrophy which can detect cardiac involvement in Fabry patients at an earlier stage. Previous studies have shown an association between the severity of myocardial fibrosis and LV longitudinal strain in Fabry patients(83). This is consistent with data from patients with hypertrophic cardiomyopathy where the amount and location of LV fibrosis and LV end-diastolic wall-thickness were independent predictors of LV longitudinal systolic strain(119). In pressure overload cardiac disease, like hypertension, it is suggested that hypertrophy is induced over the entire ventricle as a reaction to the elevated afterload, until high values of wall stress induces irreversible fibrosis(120). In Fabry disease however, a recent study demonstrated that LV hypertrophy is not obligate in female Fabry patients with LV myocardial fibrosis(57). This may be due to the fact that increased afterload is not the driving force of LV hypertrophy in Fabry disease, but rather the myocardial alterations caused by GL-3 accumulation within the cardiomyocytes.

As demonstrated in study I, early-stage CKD was associated with abnormal LV myocardial deformation. Impaired renal function is common in Fabry patients due to accumulation of globotriaosylceramide (GL-3) within the glomerular endothelial, mesangial and interstitial cells and in the podocytes.(121) Indeed, our data showed that 28% of the patients could be classified as having early-stage (stage II or stage III) CKD based on GFR measurements. Furthermore, a reduced GFR was associated with reduced LV global longitudinal strain \( r = 0.318, P = 0.031 \). We therefore suggest that renal function should be reported when describing LV myocardial deformation in Fabry patients.
Despite the fact that enzyme replacement therapy has been available since 2001 there is no evidence that this has improved life expectancy in Fabry patients. At present non-randomized studies have demonstrated that enzyme replacement therapy have the ability to reduce the amount of GL-3 accumulation in cardiomyocytes(122) and that it can reduce LV hypertrophy and possibly prevent progress in LV fibrosis, particular in patients with mild to moderate cardiac involvement(52). So far no studies have documented effect of treatment on any hard CV endpoints and CV disease remains to be the major cause of death in Fabry disease(2). It has been suggested that the lack of CV prognostic effect may be related to that treatment is started too late(52). At our hospital we start enzyme replacement therapy in patients with significant clinical symptoms or asymptomatic patients with evidence of renal dysfunction measured by GFR or cardiac dysfunction on conventional echocardiography. The restrictive practice is mainly due to the high costs of enzyme replacement therapy, but also to the lack of data on effect of the treatment on CV prognosis. It is challenging to use conventional echocardiography to recognize early cardiac involvement in Fabry disease. Newer modalities may be able to detect subclinical cardiac involvement, without the presence of LV hypertrophy or reduced LV ejection fraction, both more established signs of cardiac damage and advanced disease. This could lead to an earlier diagnosis on an individual base. The benefit of such a strategy, tailoring enzyme replacement therapy, remains to be tested in larger prospective multicentre studies.
11. Limitations

11.1 Study population
When interpreting the results of the studies in this project, it should be kept in mind that they are all observational studies. The true onset of cardiac involvement in a single patient is generally unknown. Therefore our evaluations were performed at different stages of the diseases with potential differences in baseline characteristics, complicating the interpretation of our data. Due to the low prevalence of the diagnoses included in this thesis, the low number of patients included is obviously a limitation to the thesis, and no attempt for power calculations was done. However, this study limitation is difficult to overcome, if not designed as a multi-centre study. Hypertension was seen in all our study populations, and it is difficult to truly adjust for the known influence of hypertension on LV myocardial function in multivariate analysis. Furthermore, given the small size of the study populations, separate analysis within groups of patients with and without hypertension were clearly limited. This was not a study however, of patients with hypertension. Two-thirds of all Fabry patients are female, which explain the male/female ratio in study 3(57). Previous studies have reported gender differences in Fabry disease. Both the extent of cardiac involvement and the time-course have been demonstrated to be different in women compared to men(123, 124). However, our project did not have power to assess gender differences in Fabry disease.

11.2 Echocardiography
Strain echocardiography is highly dependent on good image quality. Artefacts should be avoided to achieve reliable measurements. For this reason, speckle strain echocardiography was performed on a single optimal image from each view, rather than averaging measurements from multiple images with different quality. The feasibility in this work was, however, in accordance with modern
echocardiographic machines, harmonic frequencies and study protocols using Doppler or speckle strain echocardiography(125, 126). The proportion of segments that was possible to analyse was average. Because all segments with visually poor tracking were discarded, time-demanding and subjective evaluation was reduced. In our view, this increases the generalizability of the results. Also, to improve generalizability and reduce subjective evaluation, no adjustments were made to the default settings for strain analyses in EchoPac except from slight adjustments of endocardial outline and width of the region of interest, if the visual tracking was poor for the analysis of strain by speckle tracking. In addition to image quality, Doppler and speckle strain echocardiography are dependent on high frame rate. This was taken into account in this work by obtaining gray-scale images at a frame rate above 50 frames/second and Doppler strain at frame rates above 180 frames/second, achieving average frame rates within clinical acceptance(6, 91). Both Doppler and speckle strain echocardiography are load-dependent measures of LV function and the results reported in this thesis should be interpreted keeping this limitation in mind. However, we examined our patients in a stable condition, verifying the clinical usefulness of the method in this circumstance. The statistically significant change in strain of 2.0% to 3.1% and in strain rate of 0.18% in Study I and III may not be clinically significant and is close to the difference of repeated measures. In any individual patient, abnormalities of more than one parameter of tissue characterization may be required before concluding that there is a clinical significant myocardial dysfunction.

The definition of LV geometry was based on conventional parasternal measures of LV dimensions and wall thicknesses assuming the site of measurements to be representative for the whole ventricle. In spite of its limitations, this method is in accordance with current LV quantification guidelines, and assessment of LV geometry by this method is commonly used clinically, and has proven prognostic value in patients with hypertension(60).
11.3 Others

Information from cardiac magnetic resonance imaging was not included in the present project. We therefore do not know whether participating patients had LV fibrosis in the present study. In a consensus document on Fabry disease from 2006 cardiac magnetic resonance imaging was optional and in particular recommended in patients with LV hypertrophy (127). At our institution cardiac magnetic resonance imaging was included in the routine follow-up of Fabry patients in 2009 from the age of 18. A similar practice should probably be established in patients with CKD.

We did not include cardiac biomarkers in our studies. Cardiac biomarkers have become useful in screening for cardiac dysfunction (128). A combination of cardiac troponin T and C-reactive protein proved useful in the risk stratification of patients with end-stage CKD (129).
12. General conclusions

The following conclusions were found for the three prespecified research questions:

1. Can Doppler derived strain imaging detect myocardial abnormalities in asymptomatic patients with stage II or III chronic kidney disease and normal LV geometry and systolic parameters on conventional echocardiography?
   - In patients with stage II or III chronic kidney disease, LV ejection fraction is generally preserved, while LV longitudinal systolic deformation is abnormal. Therefore, LV myocardial deformation assessment may be used for identifying patients with impaired myocardial function in spite of normal ejection fraction and without clinical evidence of heart disease.

2. Is LV conventional and speckle tracking echocardiography normal in asymptomatic children and young adults who underwent renal transplantation in childhood?
   - Children and adults who underwent renal transplantation in childhood had relatively preserved LV myocardial deformation, despite changes in LV geometry including higher relative wall thickness and LV mass index and systolic blood pressure. LV diastolic function was impaired and was, together with systolic blood pressure, the main covariate for reduced LV systolic deformation.

3. Can speckle tracking echocardiography detect reduced LV systolic deformation in patients with genetically confirmed Fabry disease, independent of LV geometry studied by conventional echocardiography?
In patients with genetically confirmed Fabry disease, reduced LV global longitudinal strain was related to disease severity and higher systolic blood pressure and LV mass. Lower LV global longitudinal strain was closer associated with Fabry disease than LV mass and may be a better marker of early cardiac involvement.
13. Perspectives

13.1 Possible implications for clinical practice

LV hypertrophy is a crude measure of cardiac involvement in asymptomatic cardiomyopathies. Myocardial dysfunction may often be detected by strain echocardiography despite normal findings by conventional echocardiography, including normal LV geometry and ejection fraction. Whether the detection of abnormal deformation may have prognostic importance in patients with CKD or Fabry disease remains to be tested in prospective, longitudinal echocardiographic studies in asymptomatic patients with normal LV geometry and systolic parameters by conventional echocardiography.

Our research group has included speckle strain echocardiography in the echocardiographic protocol of evaluation of cardiac function in patients with high risk of developing CV disease such as patients with CKD, diabetes, familial hypertrophic cardiomyopathy and several forms of metabolic diseases, including Fabry disease. However, while awaiting data on the prognostic role of deformation analysis, this should be used with caution in the individual follow-up of such patients.

13.2 Prospects of future research

Our data provide support for a longitudinal clinical outcome trial to investigate whether abnormal systolic deformation is a marker of adverse prognosis in early-stage CKD and mild Fabry disease. To yield sufficient power for definitive answering the value of using strain echocardiography in prognostic assessment of such patients, multicentre studies must be used. Alternatively, well-designed registries reflecting the multicentre experience in Fabry disease may be used.
Focus should be on recruiting asymptomatic non-treated patients with a confirmed diagnosis and normal findings on conventional echocardiography. In Fabry patients also information about the presence of fibrosis by cardiac magnetic resonance imaging should be included in future studies. The hypothesis should be that abnormal systolic deformation is a precursor of LV hypertrophy in patients with early-stage CKD and mild Fabry disease. A possible substudy could be an interventional arm using neurohormonal blocking agents, which have been shown to be effective in improving the prognosis of other groups of patients with LV systolic dysfunction(130, 131). A substudy investigating men and women separately would be particular interesting in Fabry disease, since previous studies suggest gender specific differences related to the extent of cardiac involvement in women and the time-course of these changes. Such information could allow optimization of management with CV drugs and/or enzyme replacement therapy, increasing the likelihood of long-term improvements in CV outcomes and life expectancy.
14. References


58. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, et al. 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 Dec;18(12):1440-63.


102. El-Husseini AA, Sheashaa HA, Hassan NA, El-Demerdash FM, Sobh MA, Ghoneim MA. Echocardiographic changes and risk factors for left ventricular


ventricular hypertrophy and systemic hypertension (the LIFE Study). Am J Cardiol. 2010 Oct 1;106(7):999-1005.


