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VASCULAR FUNCTION IN CHRONIC KIDNEY DISEASE AND IN RENOVASCULAR DISEASE

by

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To my family

ABSTRACT

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VASCULAR FUNCTION IN CHRONIC KIDNEY DISEASE AND IN RENOVASCULAR DISEASE

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Cardiovascular mortality is 15 to 30 times higher in patients with chronic kidney disease than in the age-adjusted general population. Even minor renal dysfunction predicts cardiovascular events and death in the general population. In patients with atherosclerotic renovascular disease the annual cardiovascular event and death rate is even higher. The abnormalities in coronary and peripheral artery function in the different stages of chronic kidney disease and in renovascular disease are still poorly understood, nor have the cardiac effects of renal artery revascularization been well characterized, although considered to be beneficial.

This study was conducted to characterize myocardial perfusion and peripheral endothelial function in patients with chronic kidney disease and in patients with atherosclerotic renovascular disease. Myocardial perfusion was measured with positron emission tomography (PET) and peripheral endothelial function with brachial artery flow-mediated dilatation.

It has been suggested that the poor renal outcomes after the renal artery revascularization could be due to damage in the stenotic kidney parenchyma; especially the reduction in the microvascular density, changes mainly evident at the cortical level which controls almost 80% of the total renal blood flow. This study was also performed to measure the effect of renal artery stenosis revascularization on renal perfusion in patients with renovascular disease. In order to do that a PET-based method for quantification of renal perfusion was developed.

The coronary flow reserve of patients with chronic kidney disease was similar to the coronary flow reserve of healthy controls. In renovascular disease the coronary flow reserve was, however, markedly reduced. Flow-mediated dilatation of brachial artery was decreased in patients with chronic kidney disease compared to healthy controls, and even more so in patients with renovascular disease. After renal artery stenosis revascularization, coronary vascular function and renal perfusion did not improve in patients with atherosclerotic renovascular disease, but in patients with bilateral renal artery stenosis, flow-mediated dilatation improved.

Chronic kidney disease does not significantly affect coronary vascular function. On the contrary, coronary vascular function was severely deteriorated in patients with atherosclerotic renovascular disease, possibly because of diffuse coronary artery disease and/or diffuse microvascular disease. The peripheral endothelial function was disturbed in patients with chronic kidney disease and even more so in patient with atherosclerotic renovascular disease. Renal artery stenosis dilatation does not seem to offer any benefits over medical treatment in patients with renovascular disease, since revascularization does not improve coronary vascular function or renal perfusion.

Keywords: chronic kidney disease, atherosclerotic renovascular disease, renal artery stenosis, myocardial perfusion, positron emission tomography, peripheral endothelial function, renal perfusion

TIIVISTELMÄ

Niina Koivuviita

VASKULAARIFUNKTIO KROONISESSA MUNUAISTEN VAJAATOIMINNASSA JA RENOVASKULAARITAUDISSA

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Kuolleisuus sydän- ja verisuonisairauksiin kroonista munuaisten vajaatoimintaa sairastavilla potilailla on 15 – 30 -kertainen taustaväestöön verrattuna. On osoitettu, että jo lieväkin munuaisten vajaatoiminta ennustaa sydän- ja verisuonitauti tapahtumia ja kuolleisuutta väestössä. Ateroskleroottista renovaskulaaritautia sairastavilla vuotuinen kuolleisuus sydän- ja verisuonisairauksiin on vielä suurempi. Sepelvaltimoiden ja ääreisverenkierron toiminnasta on vain vähän tutkimustietoa niin munuaisten vajaatoimintaa kuin renovaskulaaritautiakin sairastavilla. Myöskään munuaisvaltimoahtauman laajentamisen aiheuttamia muutoksia sydämen ja ääreisverenkierron toimintaan ei ole juuri tutkittu.

Tämän tutkimuksen tarkoituksena oli ensisijaisesti selvittää sydämen verenvirtausta, sepelvaltimoiden toiminnallista kapasiteettia eli sepelvaltimoiden virtausreserviä ja ääreisverenkierron toiminnan muutoksia niin kroonista munuaisten vajaatoimintaa sairastavilla kuin ateroskleroottista renovaskulaaritautia sairastavilla potilailla. Tutkimusmenetelminä käytettiin positroniemissiotomografiaa (PET) sydämen verenvirtauksen määrittämiseksi sekä ultraäänitutkimusta perifeerisen ääreisverenkierron toiminnan tutkimiseksi.

Viime vuosina on arveltu, että kliinisten tutkimusten huonot tulokset munuaisvaltimoahtauman laajennuksen jälkeisesti liittyisivät munuaisten pienten verisuonten toiminnallisiin ja rakenteellisiin muutoksiin munuaisvaltimoahtauman vaikutuksesta. Muutoksia on havaittu erityisesti munuaisten kuorikerroksessa, joka käyttää noin 80 % munuaiseen ohjautuvasta verenvirtauksesta. Tutkimuksen toisena tavoitteena oli mitata mahdollisia muutoksia munuaisten verenvirtauksessa munuaisvaltimoahtauman laajentamisen jälkeisesti. Tätä varten kehitimme PET -metodin munuaisten verenvirtauksen määrittämiseksi.

Sepelvaltimoiden virtausreservi kroonista munuaisten vajaatoimintaa sairastavilla ei eronnut tilastollisesti merkitsevästi terveiden verrokeiden virtausreservistä. Sen sijaan renovaskulaaritautipotilaiden virtausreservi oli vaikea-asteisesti huonontunut. Olkavarsivaltimon laajenemiskapasitetti oli huonontunut munuaisten vajaatoimintaa sairastavilla terveisiin verrattuna ja renovaskulaaritautipotilailla vielä enemmän. Munuaisvaltimoahtauman laajentamisen jälkeisesti renovaskulaaritautipotilaiden sepelvaltimoiden funktiossa tai munuaisten verenvirtauksessa ei havaittu parantumista. Kuitenkin molemminpuolisesta munuaisvaltimoahtaumasta kärsivillä ääreisverenkierron toiminta parantui tilastollisesti merkitsevästi toimenpiteen jälkeen.

Johtopäätöksinä voidaan todeta, että munuaisten vajaatoiminta sinällään ei näyttäisi merkitsevästi huonontavan sepelvaltimoiden virtausreserviä. Sen sijaan renovaskulaaritautia sairastavilla virtausreservi on huono, todennäköisesti joko subkliinisen sepelvaltimotaudin ja/tai mikrovaskulaaritaudin aiheuttamana. Ääreisverenkierron toiminta sen sijaan oli huonontunut molemmissa potilasryhmissä, renovaskulaaritautipotilailla vielä enemmän. Munuaisvaltimoahtauman laajentaminen ei näytä tarjoavan hyötyä lääkehoitoon verrattuna, sillä toimenpiteen jälkeisesti ei havaittu muutoksia sydämen virtausreservissä sen paremmin kuin munuaisten verenvirtauksessakaan.

Avainsanat: krooninen munuaisten vajaatoiminta, renovaskulaaritauti, sydänlihaksen verenvirtaus, positroniemissiotomografia, ääreisverenkierron toiminta, munuaisvaltimoahtauma, munuaisten verenvirtaus

TABLE OF CONTENTS

ABBREVIATIONS

ACE	Angiotensin converting enzyme
ADMA	Asymmetric dimethylarginine
ARVD	Atherosclerotic renovascular disease
BFM	Basis function method
BNP	Brain natriuretic peptide
BP	Blood pressure
CAD	Coronary artery disase
CAC	Coronary artery calcium
CFR	Coronary flow reserve
CKD	Chronic kidney disease
CRP	C-reactive protein
CT	Computed tomography
CTA	Computed tomographic angiography
CV	Cardiovascular
DBP	Diastolic blood pressure
ECG	Electrocardiogram
EF	Ejection fraction
ESRD	End-stage renal disease
FFR	Fractional flow reserve
FGF-23	Fibroblast growth factor 23
FMD	Flow-mediated dilatation
GFR	Glomerular filtration rate
Hb	Hemoglobin
$H_2{}^{15}O$	^{15}O-labeled water
HD	Hemodialysis
HDL	High-density lipoprotein
HR	Heart rate
LA	Left atrium
LDL	Low-density lipoprotein
LV	Left ventricle
LVH	Left ventricle hypertrophy
LVM	Left ventricle mass
LVMI	Left ventricle mass index
MAP	Mean arterial pressure
MBF	Myocardial blood flow
MRA	Magnetic resonance angiography
MRI	Magnetic resonance imaging
MSNA	Muscle sympathetic nerve activity
NLF	Nonlinear least-squares fitting
NO	Nitric oxide

NSF	Nephrogenic systemic fibrosis
PAH	P-aminohippurate
PET	Positron emission tomography
PTH	Parathyroid hormone
RAS	Renal artery stenosis
RBF	Renal blood flow
RFR	Renal flow reserve
RI	Resistive index
ROI	Region of interest
RPF	Renal plasma flow
RPP	Rate pressure product
SBP	Systolic blood pressure
SD	Standard deviation
SPECT	Singel-photon emission tomography
TAC	Time activity curve
Tnt	Cardiac troponin t

LIST OF ORIGINAL PUBLICATIONS

This dissertation is based on the following original publications, which are referred to in the text by the corresponding Roman numerals.

I Koivuviita N, Tertti R, Järvisalo M, Pietilä M, Hannukainen J, Sundell J, Nuutila P, Knuuti J and Metsärinne K. Increased basal myocardial perfusion in patients with chronic kidney disease without symptomatic coronary artery disease. *Nephrology Dialysis Transplantation* 2009 Sep; 24(9):2773-9.

II Koivuviita N, Tertti R, Luotolahti M, Raitakari O, Vahlberg T, Nuutila P, Knuuti J and Metsärinne. The effect of revascularization of atherosclerotic renal artery stenosis on coronary flow reserve and peripheral endothelial function. *Nephron Clinical Practice* 2010 Dec 24; 118(3):c241-c248.

III Kudomi N, Koivuviita N, Liukko KE, Oikonen VJ, Tolvanen T, Iida H, Tertti R, Metsärinne K, Iozzo P, Nuutila P. Parametric renal blood flow imaging using [15O]H2O and PET. *European Journal of Nuclear Medicine and Molecular Imaging* 2009 Apr; 36(4):683-91.

IV Koivuviita N, Liukko K, Kudomi N, Oikonen V, Tertti R, Manner I, Vahlberg T, Nuutila P and Metsärinne K. The effect of revascularization of renal artery stenosis on renal perfusion in patients with atherosclerotic renovascular disease**.** *Submitted.*

1 INTRODUCTION

Cardiovascular (CV) mortality is 15- to 30- times higher in patients with chronic kidney disease (CKD) than the age-adjusted CV mortality in the general population (Parfrey and Foley 1999). It is well established that even minor renal dysfunction predicts CV events and CV death in the general population (Go et al. 2004). Although the CV risk factors in CKD are poorly defined, the increased risk of CV disease may be due to both traditional and non-traditional risk factors associated with kidney dysfunction, including hypertension, diabetes and inflammation (Muntner et al. 2005, Sarnak and Levey 2000). One of the principal pathophysiological mechanisms involved may be endothelial dysfunction, which has been documented in CKD patients by direct measurements, such as flow-mediated vasodilatation (FMD) (Yilmaz et al. 2006). Despite the evidence of a higher occurrence of coronary atherosclerosis in patients with CKD compared to the general population (Khalique et al. 2007, Goodman et al. 2000), there is a lack of quantitative perfusion and perfusion reserve studies in CKD patients.

In recent years, it has become apparent that also patients with atherosclerotic renovascular disease (ARVD) have an increased cardiovascular event rate and decreased survival, the rate of death being about 8 to 16% per year (Kalra et al. 2005, Wheatley et al. 2009). In spite of greatly increased cardiovascular morbidity in ARVD patients, cardiac morphology and function have not been well characterized. ARVD is associated with left ventricular hypertrophy (LVH) and left ventricular diastolic dysfunction, both of which are associated with increased mortality and CV events (Wright et al. 2005). It has been shown that LVH associates with impairment of microvascular function and coronary flow reserve (CFR) in patients with hypertension (Antony et al. 1993, Treasure et al. 1993).

The increase in the prevalence of ARVD has lead to a dramatic increase in the use of percutaneous transluminal renal angioplasty during the past 20 years (Textor, Lerman and McKusick 2009a). Still, the results of the studies on renal functional or CV outcomes after the revascularization procedures have been very variable (Farmer et al. 1998, Bonelli et al. 1995, Bax et al. 2009). A few clinical studies have suggested that renal artery stenosis (RAS) revascularization might prevent progressive LVH in patients with ARVD (Wright et al. 2009, Zeller et al. 2007, Symonides et al. 1999, Corriere et al. 2009). Although suggested to be beneficial, the cardiac effects of renal artery revascularization have not been well characterized.

More recently it has been suggested that the poor outcomes of renal function after revascularization of RAS could be attributable to the damage in the stenotic kidney parenchyma, especially the reduction of microvascular density (Chade and Kelsen 2010, Wheatley et al. 2009), changes mainly evident at the cortical level which controls almost 80 % of the total renal perfusion. However, the measurement of renal perfusion in humans is difficult to obtain noninvasively. Furthermore, it still remains unclear what constitutes a functionally significant RAS lesion, and equally importantly, uncertainty

still remains regarding which patients and which RAS lesion or RAS kidneys should be treated with revascularization.

Positron emission tomography (PET) with $H_2{}^{15}O$ is currently considered to be the gold standard of noninvasive assessment of myocardial perfusion and CFR (Kaufmann and Camici 2005). PET is also one of the few techniques that allows quantification of renal perfusion (Alpert et al. 2002). The purpose of this study was, first, to characterize myocardial perfusion and perfusion reserve in patients with CKD and with ARVD. The second aim was to study the effect of renal artery stenosis revascularization on these variables in patients with ARVD. Healthy volunteers served as a control group for the CKD patients. PET was used for these studies. Another aim was to quantify renal perfusion in patients with ARVD and to detect the possible changes in it after revascularization. In order to do that, the PET –method was first evolved with healthy volunteers. Peripheral endothelial function was examined with flow-mediated dilatation of the brachial artery in all patient groups.

2 REVIEW OF THE LITERATURE

2.1 Chronic kidney disease

2.1.1 Defining chronic kidney disease

In 2002, the Kidney Disease Outcomes Quality Initiative published a CKD classification based on five categories of estimated glomerular filtration rate (GFR) (Table 2.1) (Foundation 2002).

Table 2.1. Classification of chronic kidney disease

Stage	Description	GFR (ml/min/1.73 m^2)
1	Kidney damage with normal or increased GFR	≥ 90
2	Kidney damage with mildly reduced GFR	60-89
3	Moderately reduced GFR	30-59
4	Severely reduced GFR	15-29
5	Kidney failure	< 15 or dialysis

GFR=glomerular filtration rate

CKD is defined either as kidney damage or as GFR < 60 ml/min for > 3 months. Kidney damage is defined as pathological abnormalities or markers of damage, including abnormalities in blood or urine tests or imaging studies. GFR is calculated from the value of the concentration of creatinine in the serum using an equation validated by the Modification of Diet in Renal Disease study; the inputs into the equation, in addition to serum creatinine, are age, gender, race and a calibration factor for serum creatinine (Levey et al. 1999). For stages 1 and 2, kidney damage is defined by a spot urine albumin-to-creatinine ratio > 0.3 on two measurements.

2.1.2 Cardiovascular disease in chronic kidney disease

The increased cardiovascular (CV) mortality in patients with renal failure was at first demonstrated among dialysis patients. CV mortality rates are 10- to 100-fold higher among dialysis patients than in the general population (Figure 2.1) (Foley, Parfrey and Sarnak 1998). For example, the risk of death of a 30-year-old dialysis patient is similar to that of an 80-year-old patient in the general population. The epidemiology of CV disease in the population of dialysis patients differs from that in the general population; therefore, death from heart failure and sudden death are both more common among dialysis patients than is fatal myocardial infarction or stroke (Baigent, Burbury and Wheeler 2000).

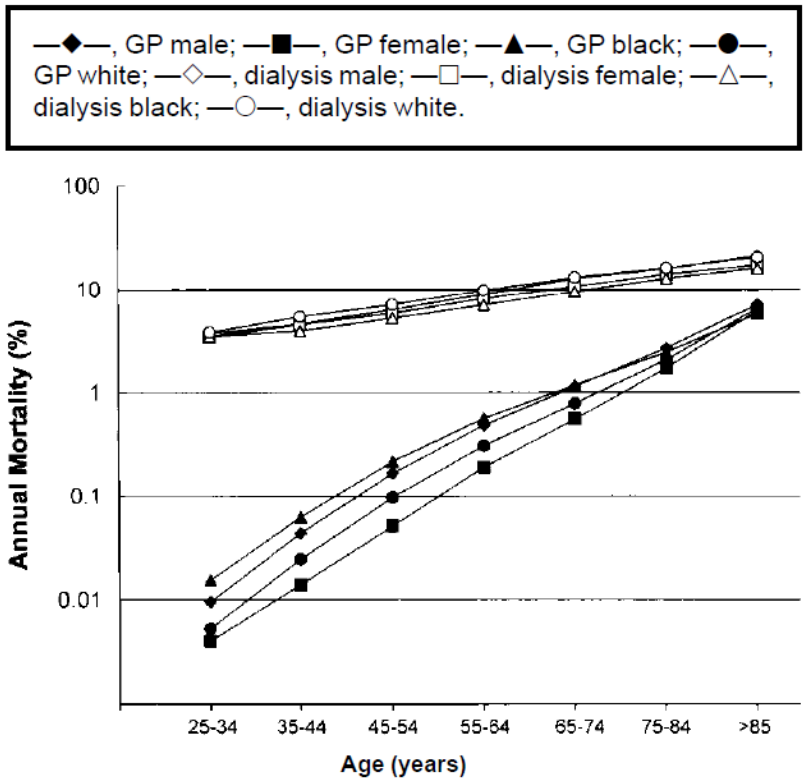

Figure 2.1. Cardiovascular disease mortality defined by death due to arrhytmias, cardiomyopathy, cardiac arrest, myocardial infarction, atherosclerotic heart disease, and pulmonary edema in the general population (GP) compared to end stage renal disease population treated by dialysis. Data from United States Renal Data System. Data is stratified by age, race, and gender. (Foley et al. 1998). Reproduced with the permission of Elsevier.

The association between earlier stages of CKD and excessive mortality was initially reported in a number of population surveys (Jones et al. 1998). The landmark study was conducted among an insured United States population within the Kaiser Permanente health-care programme in the San Francisco area (Go et al. 2004). Over 1.1 million people were followed-up for 2.8 years. The study demonstrated that the risk of CV events rose sharply for subjects with the estimated GFR of less than 45 ml per minute (Figure 2.2). In fact, the risk of death or a CV event is substantially higher than the risk of progressing to end stage renal disease (ESRD). During follow-up, about 3000 patients started dialysis treatment, when at the same time over 50 000 deaths and nearly 140 000 CV events were recorded. Even stage 1 CKD (kidney damage with normal estimated GFR but with overt proteinuria or microalbuminuria) is an established risk factor for CV morbidity and mortality. Even levels of urinary albumin excretion below the accepted threshold for microalbuminuria are associated with an increased risk compared to albumin-free urine (Arnlöv et al. 2005). Once evident, there is a graded relation between proteinuria and CV risk.

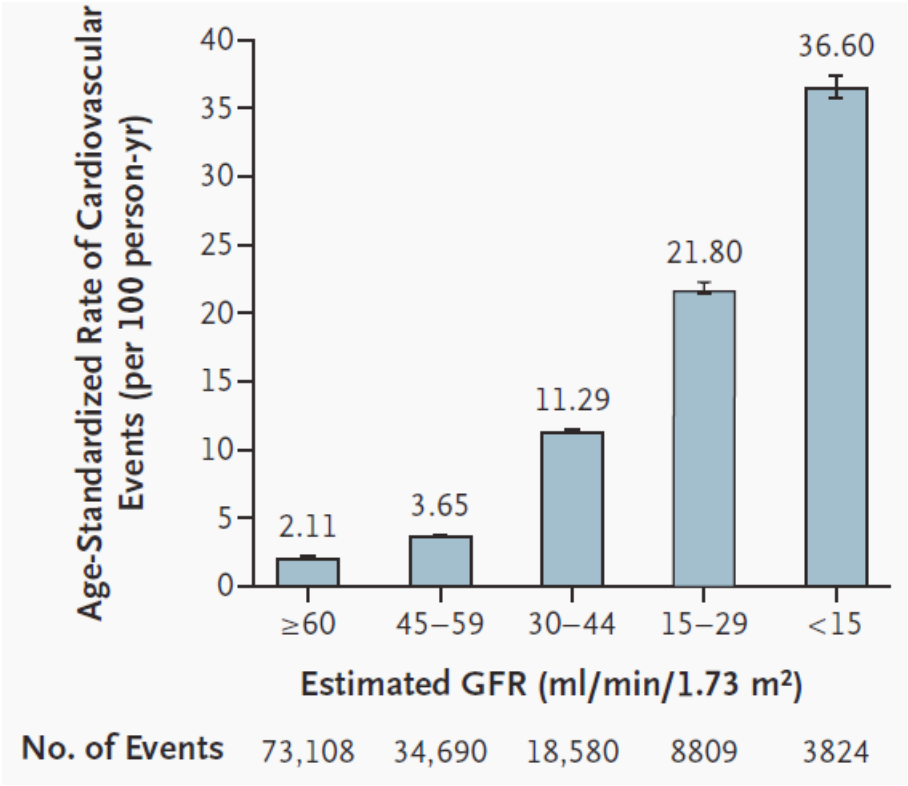

Figure 2.2. Age-standardized rates of cardiovascular events, according to the estimated glomerular filtration rate (GFR) among 1 120 295 ambulatory adults. A cardiovascular event was defined as hospitalization for coronary heart disease, heart failure, ischemic stroke, and peripheral arterial disease (Go et al. 2004). Reproduced with the permission of the Massachusetts Medical Society.

The mechanism related to the increased CV risk associated with CKD is not fully understood. However, there are several suspected reasons. First, CKD may be a marker of the severity of underlying CV disease and thus identify patients at risk of future events. Second, CKD populations exhibit a high prevalence of traditional cardiovascular risk factors. Third, patients with CKD may be less likely to receive beneficial therapies or more likely to experience toxicity from such treatments. Finally, CKD may mediate an increased risk of CV events through non-traditional risk factors. None of these exclude the others (Figure 2.3).

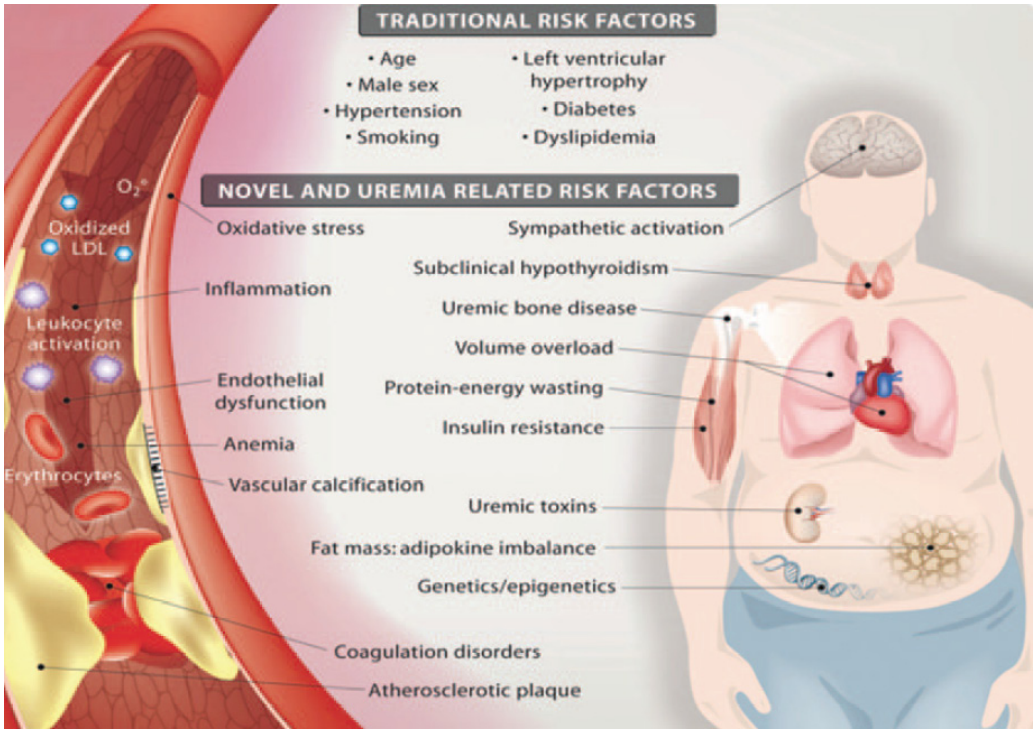

Figure 2.3. Schematic presentation of traditional and non-traditional (or uremia related) cardiovascular risk factors in chronic kidney disease (Stenvinkel et al. 2008). Reproduced with the permission of the American Society of Nephrology.

2.1.2.1 Chronic kidney disease as a marker of severity of vascular disease

Data from cardiac catheterization studies indicate that patients with CKD have more severe coronary lesions than those with normal kidney function. Furthermore, the risk of significant coronary obstruction and lesion complexity increases progressively with decreasing eGFR (Kilickesmez et al. 2010).

2.1.2.2 Traditional risk factors

The prevalence of the traditionalrisk factors, such as age, dyslipidemia, hypertension, diabetes and smoking is higher among people with CKD than among those with normal kidney function; for example, the prevalence of hypertension was about 70 % in the CKD population participating in the third National Health and Nutrition Evaluation Study (NHANES III) (Coresh et al. 2001). Still, the burden of these risk factors varies considerably by patient characteristics, e.g., degree of renal dysfunction and etiology of kidney disease. However, some risk factors for that have been accepted in the general population may have reverse epidemiology in CKD, e.g., the body mass index of patients on dialysis correlates inversely with CV morbidity and mortality. Also, the association between serum cholesterol and mortality in dialysis patients is U-shaped as is the association between hypertension and mortality (Degoulet et al. 1982, Port et al. 1999).

The common occurrence of persistent inflammation and / or protein-energy wasting in advanced CDK seems largely to account for the seemingly paradoxical association between traditional risk factors and CV outcome in this patient group. Furthermore, the recent statin trials in patients with ESRD ended up in negative results (Fellström et al. 2009, Wanner et al. 2005). This has forwarded the concept that non-traditional, or uremia related, risk factors may play a more important role in the pathophysiology of CV disease in individuals with CKD than in those without CKD.

2.1.2.3 Non-traditional risk factors

Endothelial dysfunction

See section 2.8.1, page 49.

Oxidative stress

Oxidative stress, defined as the imbalance between generation of oxidant compounds and antioxidant defence mechanisms, leads to oxidation of lipids, carbohydrates and proteins. Reactive oxygen species deplete nitric oxide (NO) reserves, stimulate vascular smooth muscle proliferation and migration, and stimulate the generation of proinflammatory cytokines (Roberts et al. 2006). Increased levels of oxidative stress markers are present in the plasma of CKD patients. Oxidative stress seems to occur early in CKD evolution, as demonstrated by identification of oxidative markers in CKD stage 3 patients (Oberg et al. 2004). In patients with CKD, increased oxidative stress may be caused by deficiency of antioxidants, advanced age, diabetes, inflammation, and exposure to bioincompatible dialysis membranes and fluids. Moreover, dialysis treatment seems ineffective in the correction of oxidation stress (Pupim et al. 2004). There are only a few small, prospective, epidemiological studies that have evaluated the association between surrogate markers of oxidative stress and CV disease in CKD patients (Descamps-Latscha et al. 2005), and only a few regarding the impact of intervention with antioxidant treatments aimed at reducing CV disease in CKD patients (Boaz et al. 2000). Thus, the causal relationship between oxidative stress and CV disease in CKD patients has not yet been firmly establishes.

Inflammation

The association between CKD and different markers of inflammation, such as C-reactive protein (CRP), interleukin 6, tumor necrosis factor α and fibrinogen, suggest that CKD is a chronic, low-grade inflammatory process. Elevated levels of CRP, interleukin 6 and fibrinogen are independent predictors of CV outcomes in patients with CKD (Stenvinkel et al. 2008). Although the precise mechanisms that contribute to the high prevalence of inflammation in CKD are unknown, reactive oxygen species may certainly be involved. Oxidative stress can activate transcriptor factors, such as NF-κβ which regulates inflammatory mediator gene expression. Both dialysis-related and dialysis-

unrelated factors may contribute to the high prevalence of inflammation in CKD patients (Nanayakkara and Gaillard 2010).

Anemia

Patients with an estimated GFR < 60 ml/min are much more likely to have anemia than patients with a higher GFR and the prevalence and severity of anemia increase with declining renal function (Astor et al. 2002) Anemia in CKD is linked to LVH (Levin et al. 1999). Studies in the first decade of 2000 have indicated that anemia in patients with CKD may predispose to ischemic heart disease, heart failure and premature death (Sarnak et al. 2002). However, randomized controlled trials on anemia correction in CKD patients, e.g., the Correction of Hemoglobin and Outcomes in Renal Insufficiency (CHOIR) trial (Singh et al. 2006) and the Trial to Reduce Cardiovascular Events with Aranesp Therapy (TREAT) (Pfeffer et al. 2009) have only added to the confusion. Correction of anemia with erythropoiesis-stimulating agents (ESAs) is associated with an increased, not a decreased, risk of mortality and/or CV complications. Thus, although it is clear that anemia is associated with increased morbidity and mortality, the beneficial effect of treatment of anemia using ESAs on mortality in CKD is currently doubtful.

Vascular calcification

Abnormal serum calcium, phosphorus, parathyroid hormone (PTH) and vitamin D sterol levels are prominent mineral disturbances related to CKD. Regulation of phosphorus excretion by the kidney is the key mechanism for maintenance of the normal phosphate balance. Most patients develop hyperphosphatemia in CKD stages 4 and 5, despite a progressive elevation of PTH and fibroblast growth factor-23 (FGF-23) levels which promote phosphaturia and suppress the 1α-hydroxylase activity which reduces the 1,25-dihydroxyvitamin D levels. As the number of viable nephrons decreases in CKD, in spite of high FGF-23 levels, the net phosphate excretion does not increase sufficiently. A high phosphate level in combination with a reduced level of 1,25-dihydroxyvitamin D leads to secondary hyperparathyroidism.

Since the initial reports by Lowrie et al. (Lowrie and Lew 1990) and Block et al. (Block et al. 1998) on an association between hyperphosphatemia and the relative risk of mortality in chronic hemodialysis patients, a large number of observational studies subsequently confirmed the finding of a U curve-like association in patients with CKD (Kestenbaum et al. 2005, Tentori et al. 2008). Similar associations have now been reported for the general population with no evidence of renal function impairment (Dhingra et al. 2007, Foley et al. 2008). Also an association between early atherosclerosis and serum phosphorus have been identified, both in subjects with normal kidney function (Foley et al. 2009) and in chronic hemodialysis patients (Ishimura et al. 2005). It is, however, worth noting that the widely publicized independent association between increased serum phosphate levels and adverse clinical outcomes comes from heavily adjusted multivariate models (Block et al. 1998) and the relation between phosphate and mortality is far less consistent

in view of the unadjusted analyses reported in these same studies. In the future, FGF-23 may be a more sensitive marker than phosphorus for increased CV disease risk. Increased FGF-23 levels are strongly associated with an enhanced risk of mortality in incident hemodialysis patients (Gutiérrez et al. 2008). The study involved 400 patients and a linear, dose-dependent relationship between increasing FGF-23 levels and mortality was reported: the highest FGF-23 quartile achieving nearly a 600 % higher risk than the lowest FGF-23 quartile. FGF-23 was more predictive of mortality than the serum phosphorus concentration, which was associated with only a 20 % greater risk for the upper versus the lower quartiles. Furthermore, the association between FGF-23 and mortality was strongest in the group of patients with a serum phosphate level of less than 1.8 mmol/l, a population for which phosphorus binding agents are not recommended nor approved.

In CKD, as with age and diabetes, vascular calcification occurs more commonly in the media of the vascular wall than in the intima. However, it has been shown that also media calcification is associated with CV morbidity and mortality (Lehto et al. 1996). Clinically, arterial calcification is detected mainly through multislice computed tomography that uses electrocardiographic gating to allow heart imaging only in diastole to avoid motion artefacts. This permits quantification of calcification in the coronary arteries. This technique has shown that patients on hemodialysis have more extensive coronary artery calcification (CAC) than patients with normal kidney function (Chertow et al. 2002, Goodman et al. 2000). Furthermore, in patients with stage 4 CKD CAC score is already higher than in patients without CKD, and CAC is gradually increasing towards ESRD and dialysis (Block et al. 2007, Russo et al. 2007). In incident dialysis patients followed for 5 years a CAC score more than 400 was associated with increased mortality (Block et al. 2007). More importantly, observational and experimental data suggest a direct relationship between serum phosphorus and CAC and with increased mortality (Block et al. 2007, Goodman et al. 2000). These findings allow the use of CAC as a surrogate marker for CV disease.

Vascular calcification is an active and biologically regulated process that shares many of the the characteristics of bone formation and repair. Because vascular calcification may result from an imbalance between promoters and inhibitors, the concentrations of calcium and phosphorus are not the only factors influencing ectopic ossification in CKD (Stenvinkel et al. 2008). In order to make vascular calcification happen, vascular smooth muscle cells (VSMCs) need to transform into chondrocyte-like or osteoblast-like cells. Increased phosphate concentrations, as well as uremia, aging, diabetes and inflammation stimulate VSMCs through active processes (Nikolov et al. 2009). Calcification inhibitors can inhibit this physicochemical component of calcification. To date, the best studied circulating inhibitor of vascular calcification is fetuin-A. Low levels of fetuin-A are associated with increased CV mortality in CKD stage 5 (Ketteler et al. 2003). Matrix Gla protein, pyrophosphate and osteopontin are also local inhibitors of calcification. The importance of calcification inhibitors is shown by the profound phenotype and site specificity of vascular calcification that occur in mice with null mutations, suggesting

that, as for bone, calcification would proceed unabated unless regulated by these inhibitors (Moe and Chen 2008) (Figure 2.4).

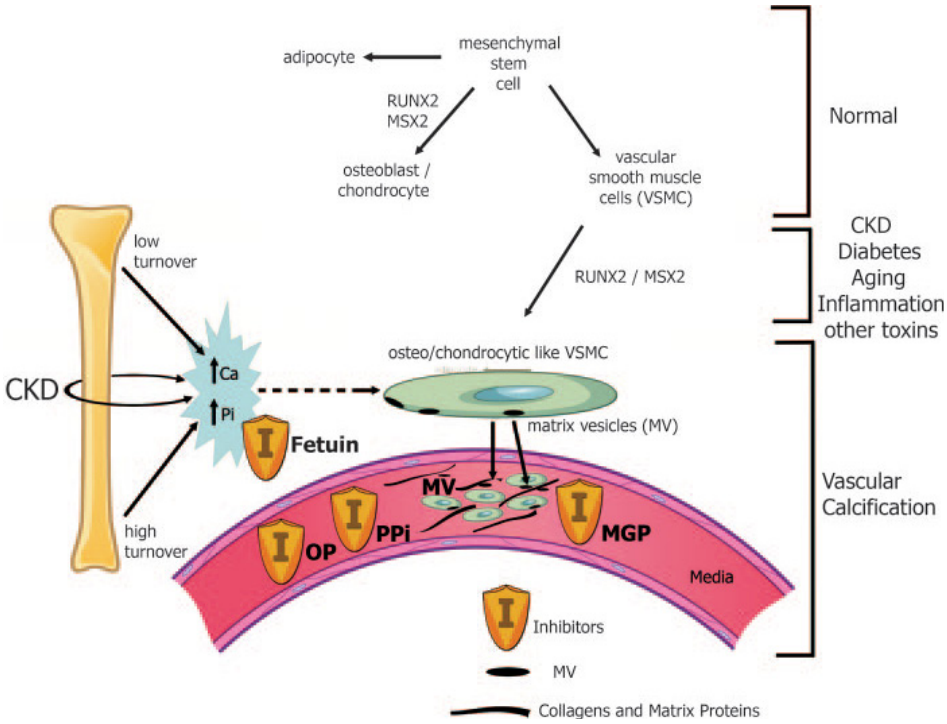

Figure 2.4. Vascular calcification in chronic kidney disease (Moe and Chen 2008) (I=inhibitors, PPi=pyrophosphate, MGP=matrix Gla protein, OP=osteopontin). Reproduced with the permission of the American Society of Nephrology.

Although vascular calcification is associated with an increased risk of CV disease and mortality (Block et al. 2007), there is still no definite proof that reducing vascular ossification improves patient outcome (Block et al. 2005, Suki et al. 2007). Therefore, also vascular calcification should currently be considered as a CV risk marker rather than as an etiological factor in CKD.

Sympathetic hyperactivity

Already in the 1970s increased catecholamine levels, which portray sympathetic activity, were reported in CKD patients. The mechanisms that cause sympathetic hyperactivity arise from the failing kidneys (Klein et al. 2003b). Kim et al. (Kim et al. 1972) showed already in the 1970s that hypertensive and normotensive hemodialysis patients differ with regard to their peripheral vascular resistance and not cardiac output. Importantly, after bilateral nephrectomy blood pressure was reduced by a decrease in vascular resistance, not in cardiac output. Renin secretion is inappropriate in relation to the state of sodium-volume balance in CKD (Klein et al. 2003a). There is clear evidence that high circulating angiotensin II levels can stimulate central sympathetic outflow by

a direct effect on the vasomotor center in the brain stem. This can be quantified in humans as increased muscle sympathetic nerve activity (MSNA) (Klein et al. 2003b). To enhance the vicious circle, renal sympathetic nerve endings release noradrenalin directly on granular juxtaglomerular cells and increase renin release.

There is increasing evidence that sympathetic hyperactivity is harmful. Blood pressure in CKD patients correlates with MSNA. Also, blood pressure reduction following treatment with chronic angiotensin converting enzyme (ACE) inhibitors or angiotensin II receptor antagonists correlates with a reduction of the MSNA (Klein et al. 2003b). This data supports the concept that sympathetic hyperactivity contributes to the hypertension in CKD patients.

The sympathetic nervous system may also be important for determining the rate of progression of kidney function in CKD patients. Catecholamines have direct effects that may promote renal damage, including proliferative effects mediated by beta-adrenoceptors on the function of podocytes (Neumann et al. 2004).

In essential hypertension, indices of sympathetic activity are related to LVH. CKD patients exhibit, as well, a positive relationship between left ventricular mass (LVM) and MSNA (Siddiqi et al. 2010).

2.1.3 Cardiac structure and function in chronic kidney disease

LVH is the most common cardiac alteration in CKD, present already in patients with moderate CKD, and prevalence increasing with decreasing kidney function (27 % in CKD 2, 30 % in CKD 3, 45 % in CKD 4 and 70 % in CKD 5) (Levin et al. 1999, Shlipak et al. 2005). The development of LVH is associated with hypertension, anemia and poor control of extracellular fluid volume. The presence of LVH is a strong predictor of adverse outcomes, independent of conventional risk factors (Foley et al. 1995). LV diastolic dysfunction is also frequent among CKD patients and is associated with the risk to develop heart failure and with mortality. Impairment of diastolic function in patients with CKD may occur very early, even in the absence of LVH (Nardi et al. 2007).

2.2 Atherosclerotic renovascular disease

2.2.1 Prevalence, natural history and prognosis

Narrowing of the lumen of the renal artery is termed renal artery stenosis (RAS). Atherosclerosis accounts for 90 % of cases of RAS. It is usually caused by plaque formation in the aortic wall with progression into the renal artery lumen: this gives rise to the typical appearance of the ostial location of the lesion which is usually eccentric. In advanced cases, segmental and diffuse intrarenal atherosclerosis may also be observed, particularly in patients with ischemic nephropathy. Approximately 30 % of patients have bilateral lesions, and 25 % present with at least one completely occluded renal artery.

Atherosclerotic renovascular disease (ARVD) is being recognized with increasing frequency. In a recent study of over 16 000 000 patients over 65 years in the United States (Kalra et al. 2010), the incidence of ARVD was 3.09 per 1000 patient-years over a 13 -year period. The likelihood of a diagnosis of ARVD had tripled toward the end of the study period compared to 1992 when the study began. It is possible, although not likely, that the prevalence is truly increasing, since the population ages and survival from other vascular diseases, including stroke and myocardial infarction, is improving. However, diagnostic modalities for ARVD have improved highly, techniques such as ultrasound, computed tomography, and magnetic resonance imaging, facilitating noninvasive ARVD detection.

Prevalence in general population

Data on the prevalence of ARVD in the general population is scarce. There is only one study which has evaluated the occurance of hemodynamically significant RAS (> 60 % lumen occlusion based on Doppler flow study) in the United States population over 65 years old. 870 patients were studied, and 6.8 % had RAS (Edwards et al. 2005). It was more common in men than in women (9.1 versus 5.5 %, P = 0.053) and related to age, HDL cholesterol and systolic blood pressure.

Prevalence in risk groups

The fact that ARVD increases with age was first demonstrated in postmorten studies undertaken almost a half a century ago (Schwartz and White 1964). In a more recent study based on color duplex sonography in 269 patients, the prevalence of RAS in the age groups 50-59, 60-69, and > 70 years was 11 %, 18 %, and 23 %, respectively (Coen et al. 2003).

No angiographic study has determined the prevalence of RAS in the general hypertensive population. In patients with clinical characteristics suggestive of renovascular hypertension (such as severe hypertension, therapy-resistant hypertension, onset of hypertension on a young age or recent onset of hypertension) six angiographic studies have been performed (Emovon et al. 1996, Horvath et al. 1982, Kalra et al. 1990, Svetkey et al. 1991, van Jaarsveld et al. 2001, Vasbinder et al. 2004). The prevalence of RAS in these studies ranged from 10.4 to 20.3 %.

The prevalence of ARVD is also high among patients with type 2 diabetes. A meta-analysis of 3 prevalence studies that examined the occurance of RAS in various risk groups found a pooled prevalence rate of 20.0 % (95 % confidence interval, 15.4 % - 25.5 %) in patients with diabetes mellitus and coexistent hypertension (de Mast and Beutler 2009). Conversely, epidemiologic and interventional studies in ARVD patients typically show that 30 % of these populations have diabetes (Kalra et al. 2005, Losito et al. 2005).

Smoking is also a risk factor for ARVD. In one interventional study, 44 % of patients were smokers (Plouin et al. 1998).

There are no reports on the association between ARVD prevalence and hyperlipidemia. However, the lipoprotein profile in ARVD patients mirrors the one in other severe vascular diseases; apolipoprotein A1 is very low (Scoble et al. 1999).

Prevalence in patients with atherosclerosis

ARVD is often identified incidentally in patients who have other vascular diseases. Up to 11 % of patients referred to cardiologists predominantly with angina or myocardial infarction have significant unilateral RAS and 4 % bilateral RAS higher than or equal to 50 %, when assessed by abdominal aortography and coronary angiography (Cohen et al. 2005). In the most recent coronary angiography study of 650 patients selective renal arteriography was used and over 50 % RAS was detected in 14.5 % of the patients, 3.1 % bilateral. The prevalence of RAS is strongly correlated with the number of affected coronary vessels and with the estimated GFR (Ollivier et al. 2009) (Figure 2.5)**.** Prospective observational studies have shown that the presence and severity of RAS have a negative impact on survival in such patients with coronary artery disease (CAD) (Conlon et al. 2001).

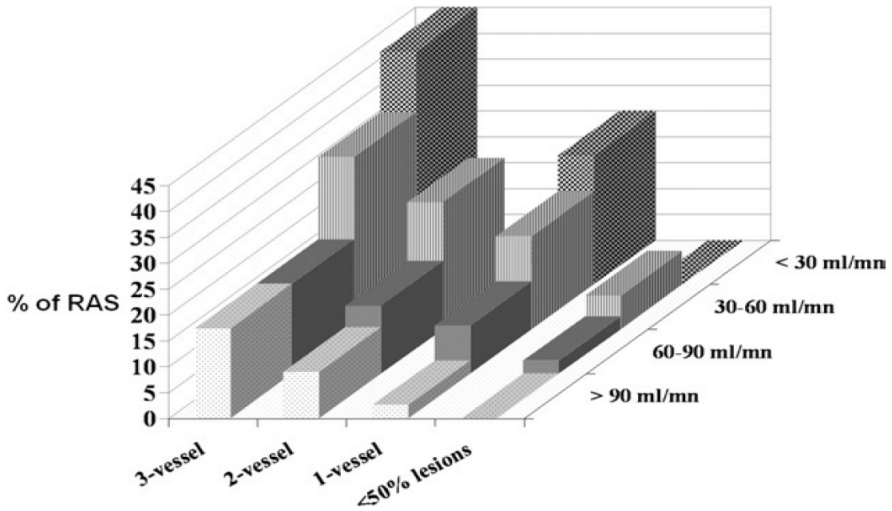

Figure 2.5. Percentage of renal artery stenosis as a function of severity of renal failure and number of coronary artery lesions. (Ollivier et al. 2009). Reproduced with the permission of Elsevier.

Among patients diagnosed with peripheral vascular disease, the prevalence of RAS is even higher than in CAD patients. In a meta-analysis covering 12 studies, which included a total number of 2871 patients, the prevalence of RAS varied from 12.0 to 45.5 % and the pooled prevalence was 25.3 % (95 % CI 23.6 – 27.0 %) (de Mast and Beutler 2009).

The presence of RAS in hypertensive patients with stroke and/or carotid stenoses is well established. In postmortem series of almost 350 patients with brain infarctions RAS (>

75 %) was recorded in 10 % of patients. RAS was more likely in patients with carotid artery stenosis (Kuroda et al. 2000). In a Japanese study involving over 700 patients, 22.2 % had both carotid artery stenosis and RAS on angiography (Kawarada et al. 2007). Conversely, in a prospective study of high-risk patients, carotid disease was found in 46 % of those with RAS but only in 12 % without ARVD (Louie et al. 1994).

Overall, ARVD mirrors the extent and severity of atherosclerosis elsewhere in the arterial vasculature.

Prevalence in patients with end-stage renal failure

In the United States, the incidence of prior ARVD in patients starting dialysis rose from 7.1 % in 1996 to 11.2 % in 2001 according to Medicare claims (Guo et al. 2007). However, ARVD was listed as the primary cause of renal failure only in half of the patients who entered dialysis treatment. Thus, some of the cases represent incidental ARVD, which is not a pathophysiologically important cause of ESRD, but rather a comorbid condition.

Natural history and prognosis

Like atherosclerosis in other vascular beds, ARVD is a progressive disease. Among patients with atherosclerotic RAS, progressive stenosis was reported in 51 % of renal arteries five years after diagnosis (Caps et al. 1998a, Caps et al. 1998b), 3 – 16 % of the renal arteries became totally occluded, and renal atrophy (defined as > 1 cm shrinkage in bipolar length) developed in 20 % of patients with RAS of more then 60 % as opposed to 5.5 % in kidneys with normal renal arteries (Caps et al. 1998a, Zierler et al. 1994). Atrophy was associated with an increase in serum creatinine, which emphasizes the role of renal shrinkage as renal impairment progresses. In the era of statin therapy the risk of progression seems less (Cheung et al. 2007).

Data concerning impairment of renal function over time is limited. In the Revascularization versus medical therapy for renal-artery stenosis (ASTRAL) study (Wheatley et al. 2009) patients on medical therapy had a mean increase in the creatinine concentration of about 10 % from baseline during the first year of follow-up, but only by a further 1 % during the succeeding 3 years. In the large Medicare study on ARVD (Guo et al. 2007), the annual rate of ESRD was 2.9 % per year. In the older Medicare cohort, the annual mortality rate was 16.3 %, nearly 6 times greater than the risk of developing ESRD, and significantly greater than the 6.4 % annual mortality of the non-ARVD population (Kalra et al. 2005). The overall annual mortality in the ASTRAL study was clearly less, 8 %, which compares favorable with the mortality of the 2000 to 2001 cohort of Medicare patients described above (Kalra et al. 2005). In ARVD patients who reach ESRD, the annual mortality rate approaches 33 %. In particular, cardiovascular mortality among patients on dialysis is higher than for any other cause for ESRD, even diabetes (Guo et al. 2007).

There are at least two factors that may contribute to decreased survival in patients with RAS: 1) As the severity of RAS increases, survival decreases at 2-year survival rates of 96 % in patients with unilateral RAS, 74 % in patients with bilateral RAS and 47 % in patients with stenosis or occlusion to a solitary functioning kidney (Connolly et al. 1994). In a cohort of nearly 4000 patients who underwent aortography at the same time with cardiac catheterization, the 4-year survival was 57 % among patients with ≥ 75 % stenosis of at least one renal artery compared to 89 % among those with < 75 % stenosis (Conlon et al. 2001). The impact of RAS on survival remained robust regardless of how the coronary artery disease was treated. 2) As renal function declines, survival declines (Go et al. 2004), see section 2.1.

2.2.2 Clinical presentation of atherosclerotic renovascular disease

Atherosclerotic renal artery stenosis produces a spectrum of clinical syndromes ranging from incidental lesions to advanced disease. RAS may occur alone (isolated anatomical RAS) or in combination with hypertension (renovascular or essential hypertension), renal insufficiency (ischemic nephropathy) or both.

2.2.2.1 Renovascular hypertension

Over 90 % of ARVD patients are hypertensive. It is often questionable, however, whether a given RAS lesion actually causes hypertension. Essential hypertension may be the primary contributor to the development of ARVD rather than its result, as suggested by the fact that hypertension usually persists despite successful revascularization. Hypertension is typically treatment resistant, and is characterized by severe systolic hypertension and low diastolic pressure.

Pathophysiology of renovascular hypertension

Already in the 1930s Goldblatt et al (Goldblatt et al. 1934) demonstrated that reduction of perfusion to the kidney produced sustained elevation of arterial pressure. A decrease in renal perfusion pressure activates the renin-angiotensin system, which leads to the release of renin and the production of angiotensin II. This has direct effects on sodium excretion, sympathetic nerve activity, intrarenal prostaglandin concentrations and nitric oxide production (Basso and Terragno 2001). After the first orally active angiotensin converting enzyme (ACE) inhibitor, captopril was introduced, experimental studies confirmed that 2-kidney-1-clip renovascular hypertension in the rat could be prevented by blocking this system (DeForrest et al. 1982). Recent studies in knockout mice without angiotensin type 1 receptors confirm that renal artery clipping requires these receptors to produce 2-kidney-1-clip hypertension (analogous to unilateral renovascular hypertension in humans) (Cervenka et al. 2002). Recent studies on kidney transplantation from angiotensin 1 receptor-deficient mice indicate independent roles for kidney and systemic receptors in this process (Crowley et al. 2005)

In unilateral RAS, the rise in blood pressure (BP) is associated with elevated plasma renin activity. A certain degree of vascular occlusion seems to be required after which renin release is activated, as confirmed by recent human studies using low-profile pressure wires (De Bruyne et al. 2006). The nonstenotic contralateral kidney responds to higher BP with pressure natriuresis; this lowers the blood pressure as sodium is excreted. However, the fall in BP reduces perfusion, again, to the stenotic kidney, which drives the release of renin and elevates angiotensin II levels. This type of renovascular hypertension is angiotensin II dependent and characterized by elevated plasma renin activity (Brunner et al. 1971).

In contrast, experimental 1-kidney-1-clip (corresponding in humans to bilateral RAS or stenosis to a solitary functioning kidney) reflects a balance between angiotensin II dependent and volume dependent mechanisms (Gavras et al. 1973). In the absence of a normoperfused contralateral kidney, 1-kidney-1-clip hypertension cannot excrete sodium in response to the rise in arterial pressure from angiotensin II. Sodium and water retention lead to volume expansion, which now is the primary mechanism for hypertension. This expansion suppresses plasma renin activity and lowers angiotensin II levels. Hence, plasma renin activity is normal, and BP does not fall with angiotensin receptor blockade (Gavras et al. 1975).

The dominant role of the renin-angiotensin system appears to be transient during the initiation of renovascular hypertension. Experimental models lose the absolute dependence upon angiotensin within a few weeks, and activation of oxidative pathways develops (Welch et al. 2003). Studies with human volunteers confirm the activation of oxidative stress pathways in renovascular hypertension that can return towards normal with successful revascularization (Parildar et al. 2003). Thus, activation of the renin-angiotensin system appears capable of activating additional systems that sustain elevated vasoconstrictor tone and arterial pressure.

Experimental studies in a swine model of renal artery stenosis suggest that there are important interactions between early atherosclerosis (induced by cholesterol feeding) and renovascular hypertension. The data demonstrates that the combination of hypercholesterolemia and RAS amplifies activation of mechanism that promotes renal vascular, glomerular and tubulointerstitial injury compared to RAS alone, despite similar hemodynamics (Chade et al. 2003).

2.2.2.2 Chronic kidney disease

The commonest nephrologic presentation of ARVD in nephrology clinics is asymptomatic CKD. Hypertension (rather than specific ischemia resulting from severe narrowing of the RAS lesion) may be crucial in the pathogenesis of CKD. ARVD is probably more often an association with, rather than the cause of CKD. This is supported by histological studies showing a pattern of non-specific intrarenal injury hard to distinguish from hypertensive damage (Wright et al. 2001).

Pathophysiology of ischemic nephropathy

Although the term ischemic nephropathy suggests that impairment of renal function beyond occlusive disease of the main renal arteries results from hypoxia, the contribution of true hypoxia to renal damage in RAS has been controversial. Of all organs of the human body, the kidneys have one of the highest supplies of oxygen, so that less than 10 % of the delivered oxygen is needed to maintain renal metabolic needs (Brezis et al. 1984). However, studies with pigs with a graded decrease in renal blood flow showed that regional medullary (more than cortical) renal tissue oxygenation declined and this finding supports the notion that there is regional tissue hypoxia (Warner et al. 2009). There are several reasons for possible heterogeneous intrarenal hypoxia: renal arteriovenous oxygen shunting within preglomerular arterial vessels (as much as 50 % of inflowing oxygen may diffuse directly to postglomerular vein) which might prevent production of reactive oxygen species in what would otherwise become a hyperoxic microenvironment (O'Connor et al. 2006); tubular work, which modulates renal oxygen consumption; tubular hypertrophy or atrophy, which affects O_2 consumption (Evans et al. 2008); and decreased efficiency of tubular reabsorption due to microvascular dysfunction and rarefaction (Zhu et al. 2004), inflammation and fibrosis in the stenotic kidney (Warner et al. 2009).

The severity of histopathological damage is an important determinant of renal outcomes in patients with atherosclerotic nephropathy, regardless of RAS (Wright et al. 2001). Glomerulosclerosis is a relatively late event in human RAS and is often linked to long duration, pre-existing injury and exacerbated by comorbid conditions, such as hypertension, dyslipidemia and atherosclerosis. Glomerular lesions occur only with severe stenosis nearing cortical infarction (Lerman et al. 1999). The earliest pathological feature in renal ischemia is a tubulointerstitial injury (Greco and Breyer 1997). There might exist an imbalance between synthesis and degradation of extracellular matrix favoring tubular injury, matrix accumulation and interstitial fibrosis in atherosclerotic RAS (Lerman, Textor and Grande 2009).

In addition to tubulointerstitial injury, patients with atherosclerosis exhibit intrarenal microvascular disease leading to vascular rarefaction (Textor 2004). Prolonged periods of reversible vasoconstriction can cause lasting changes in the microcirculation. A decrease in oxygen supply up-regulates the expression of hypoxia-inducible factor (HIF)-1α, and, in turn, of vascular endothelial growth factor and this induces compensatory new vessel formation. However, prolonged or severely increased oxidative stress may destabilize HIF-1α protein and thereby interfere with tissue repair (Lerman et al. 2009). Thus, chronic hypoxia itself may restrict compensatory angiogenesis (Lerman et al. 2009). Indeed, in an experimental model of atherosclerotic renovascular disease a decreased spatial density of microvessels (Zhu et al. 2004) was detected in stenotic kidneys, and it could be improved by pro-angiogenic intervention (Chade et al. 2009).

In addition to hypoperfusion, ARVD involves cardiovascular risk factors which activate mechanisms that further aggravate renal functional impairment and tissue injury.

Hypercholesterolemia increases the availability of oxidized low-density lipoprotein (ox-LDL), which is cytotoxic to renal mesangial, epithelial and endothelial cells. Ox-LDL accelerates the development of fibrosis by increasing intracellular and extracellular matrix protein synthesis. Furthermore, angiotensin II stimulates both LDL oxidation and ox-LDL uptake by up-regulating the expression it its receptor LOX-1 (Lerman et al. 2009). Renal microvessels are particularly sensitive to ox-LDL, which, in addition to low shear stress and ischemic insults, could lead to endothelial dysfunction preceding the actual histopathological changes.

2.2.2.3 Acute kidney injury

Acute kidney injury may follow ARVD for several reasons:

1) Acute renal artery occlusion
2) Bilateral critical RAS
3) In association with accelerated-phase hypertension
4) Cholesterol atheroembolism either spontaneously or as a consequence of angiographic procedures
5) Radiocontrast injury
6) As a consequence of use of medicines that block renin angiotensin system

2.2.3 Screening for atherosclerotic renovascular disease

Proposed clinical indications for screening for atherosclerotic RAS are (Hirsch et al. 2006):

1. Onset of severe hypertension at > 55 years of age
2. Accelerated, malignant or treatment-resistant hypertension
3. Worsening of renal function after administration of an ACE inhibitor
4. Unexplained atrophic kidney or a difference in size of > 15 mm between kidneys
5. Sudden, unexplained pulmonary edema
6. Unexplained renal dysfunction, including patients starting renal replacement therapy

However, there is no evidence that the outcome of the patients treated with revascularization according to these indications is any better than of patients treated conservatively.

2.2.4 Evaluation of atherosclerotic renovascular disease

RAS may be identified by several imaging modalities, even when it is unrelated to the patient's hypertension or ischemic nephropathy. The degree of the renal artery obstruction that causes significant hemodynamic changes of renal perfusion has been one topic of debate. Based on the flow studies with latex casts to induce luminal occlusion or on studies on the magnitude of renin release related to the poststenotic gradient, it has been implied that definitive hemodynamic effects develop only after a luminal occlusion of 75 % to 85 % (Textor et al. 2009b). In experimental RAS in pigs, however, it has been

shown that renal perfusion gradually decreases in relation with RAS already from 20 % of renal artery occlusion (Warner et al. 2009). In addition, the results of studies on the outcomes of renal or cardiovascular function after revascularization have been very variable (Farmer et al. 1998, Bonelli et al. 1995, Bax et al. 2009). It has recently been suggested that the poor outcomes after revascularization of renal artery stenosis could be attributable to be the damage to the stenotic kidney parenchyma, especially the reduction of microvascular density (Chade and Kelsen 2010, Wheatley et al. 2009). These changes are mainly seen in the cortex which controls almost 80 % of the total renal blood flow. Thus it remains unclear what actually constitutes a functionally significant RAS lesion. There is also uncertainty as to which patients and which RAS lesion or RAS kidneys should be treated with revascularization.

Catheter angiography is the gold standard for the diagnosis of RAS (Kim et al. 1991). It offers the highest spatial and temporal resolution of the anatomy of the stenoses of the main renal artery and its branches. The main advantages of catheter angiography are the possibility to make immediate interventions in the same session as the diagnostic arteriography and functional assessment of the hemodynamic significance of the RAS by translesional pressure measurement. A mean pressure gradient of > 10 % across the lesion is considered to be hemodynamically significant (De Bruyne et al. 2006, Drieghe et al. 2008). However, factors such as cardiac output, systemic blood pressure and the vasodilatory state of the renal microvasculature affect the pressure gradient. Thus, stenoses of similar severity may demonstrate varying pressure gradients and it is unknown if a translesion pressure gradient across an atherosclerotic RAS predicts the clinical outcome of renal artery stenting (Carr et al. 2010). Also the *fractional flow reserve* (FFR) can be measured during catheter angiography when a nonobstructive pressure wire is used. FFR measurements are performed after induction of maximal hyperemia with intrarenal papaverine. It has been shown that an abnormal FFR of less than 0.8 predicts a positive response to renal artery intervention with a blood pressure improvement after stent placement in patients with moderate unilateral RAS (Mitchell et al. 2007). As an invasive test, catheter angiography has the highest risks associated with iodinated contrast agents, ionizing radiation, atheroembolic disease, bleeding, dissection, thrombosis and arterial injury. It is also the most expensive, both in terms on financial cost and the amount of time required. Therefore, catheter angiography is not suitable as a screening technique.

Ultrasound is an ideal screening test for RAS because it is noninvasive, is non-inonizing, has low cost and renal failure and contrast allergy do not constitute contraindications. Although ultrasound (US) is sensitive, the accuracy is highly operator-dependent, and ranges from 60 % to more than 90 %. The latter figure concerns kidneys that are easy to visualize and study with US, e.g., transplanted kidneys. RAS is inferred when the flow velocity in the main renal artery exceeds 2.5 m/s (Baxter et al. 1995). RAS is also possible if a parvus-tardus waveform of the intrarenal arteries is seen. This is reflected as spectral broadening of the arterial waveform, retarded acceleration of less than 3.0 m/s^2 and increased acceleration time above 0.08 to 0.10 seconds. However, these findings may

be less specific than the peak systolic velocity in the main renal artery and should ideally be used to support the diagnosis based on peak systolic velocity (Richardson et al. 2000). The resistive index [1-(end diastolic volume (EDV)/peak systolic velocity (PSV)] has been used to differentiate the kidneys / patients who could benefit from the revascularization procedure. However, the study results regarding the value of this index are conflicting (Crutchley et al. 2009, Radermacher et al. 2001). Accessory renal arteries cannot generally be adequately examined or even identified on US. This problem combined with limited visualization of the renal vasculature due to abdominal gas or fat increases the rate of technical failure compared to other modalities. The asymmetry between left and right kidney dimensions assessed by US seems to have poor specificity for ARVD. In a study with 238 patients, over 11 mm of asymmetry of the renal dimensions was detected in 75 % of patients with ARVD, but also in 44 % of patients with no ARVD (Coen et al. 2003).

Intravascular Doppler. One study has used the intravascular Doppler methodology in patients with ARVD. Mounier-Vehier and colleagues demonstrated that the renal flow reserve was impaired in RAS kidneys and that it improved after the revascularization (Mounier-Vehier et al. 2004). This is an invasive procedure which is not generally applicable for clinical practice.

Computed tomographic angiography (CTA). The median sensitivity and specificity with regard to a diagnosis of RAS compared to conventional catheter angiography are 94 % and 93 % for CTA (Zhang et al. 2009). The major limitation of CTAis that the technique can provide only an anatomic but not a physiologic assessment of the stenosis. CTA also requires significant amounts of potentially nephrotoxic iodinated contrast medium and exposes patients to relatively high doses of ionizing radiation. In addition, the presence and degree of RAS can often be obscured by heavily calcified vessels.

Electron-beam computed tomography has been used for noninvasive quantification of single-kidney perfusion in patients with ARVD (Lerman et al. 1996b), and more extensively in animal models of renovascular hypertension (Chade et al. 2002, Chade et al. 2009, Chade and Kelsen 2010).

Magnetic resonance imaging. When compared to conventional catheter angiography, 3-dimensional gadolinium magnetic resonance angiography (MRA) is accurate for diagnosing RAS. The median sensitivity and specificity compared to conventional catheter angiography are 92 % % and 94 % without contrast and 96 % and 93 % with contrast (Zhang et al. 2009). MRA has the distinct advantage of providing a functional assessment of blood flow and organ function. Combining luminal imaging with functional pulse sequences may offer more comprehensive evaluation of the kidneys without markedly increasing scanning time or cost. Thus, MRA can become a highly effective screening method for renovascular disease.

Post-stenotic dilatation of more than 20 % is often seen in connection with moderate and severe renal artery stenoses. In the most severe stenoses that are nearly occlusive and do not allow jet-flow through the lumen post-stenotic dilatation is reduced.

Three-dimensional phase-contrast MRA has the potential for assessing pressure gradients. However, as the capacity of grading stenoses is based on colours of flowing blood, the underestimation of mild stenoses and overestimation of severe stenoses is considered a disadvantage of three-dimensional phase-contrast MRA.

Gadolinium extraction decreases in kidneys with RAS. Combining renal artery flow measurements with an additional pulse sequence to measure gadolinium concentration in the renal artery (input) and renal vein (output) permits calculation of the gadolinium clearance rate for each kidney (Niendorf et al. 1998). Because gadolinium is filtered but not excreted or reabsorbed, this corresponds directly with creatinine clearance and the GFR can be estimated. Quantitative perfusion measurements of the kidney have been introduced, which offer an independent measure of parenchymal blood flow to the renal cortex (Attenberger et al. 2010, Schoenberg et al. 2003). Thus far there are no widely accepted values for MR-based quantification of renal perfusion parameters.

Blood oxygen-level-dependent (BOLD) MRI detects changes in tissue deoxyhemoglobin during maneuvers that affect oxygen consumption. The technique has been used to detect hypoxia in kidneys with RAS, both in humans and animals (Textor et al. 2008, Rognant et al. 2010). In these studies no hypoxia was detected in the kidneys downstream of renal artery stenosis, suggesting that atrophy could be induced by other factors.

Impact of nephrogenic systemic fibrosis

Nephrogenic systemic fibrosis (NSF) was first recognized in the USA in 1997. NSF is an idiopathic skin condition characterized by thickening and hardening of the skin of the extremities and sometimes of the trunk, sometime leading to contractures and immobility and to death (Deo, Fogel and Cowper 2007). In 2006 a pivotal study from Austria linked gadolinium exposure to NSF (Grobner 2006). Until 2009 there were 250 confirmed cases of NSF (Grobner 2006). NSF occurs only in patients with renal impairment. European authorities have advised that gadolinium should be avoiding in patients with a GFR < 30 ml/min. However, a critical review of the literature indicates that only patients with severe kidney impairment, < 15 ml/min, are at high risk. Almost 90 % of reported cases were dialysis-dependent patients (Chrysochou et al. 2009). The most recent data of patients screened and entered into the multicenter ASTRAL trial confirm that the incidence of NSF among patients with a mean GFR of 40 ml/min was very low (0.06 %) (Chrysochou et al. 2009).

Captopril renography is now rarely used to detect functionally significant RAS, as its diagnostic sensitivity and specificity are close to 50 % in patients with CKD (Textor et al. 2009b). Captopril renography is inferior to CTA and MRA in detecting RAS according to a meta-analysis (Vasbinder et al. 2001).

Biomarkers. Renin and brain natriuretic peptide (BNP) are markers of renal ischemia. Unfortunately, plasma renin is not a reliable test in clinical practice as many antihypertensive drugs affect its measurements. Patients with bilateral renal artery disease or a single

functioning kidney will have suppressed renin levels owing to volume expansion (Olin 2002). As a result, the positive and negative predictive values of peripheral renin measurements are reported in the 35 % to 60 % range, which is not useful in many cases (Gerber et al. 1994). The same problems afflict renal vein renin measurements, and are not recommended as a part of regular diagnostic evaluation (Textor et al. 2009b).

BNP is a neurohormone released from the ventricular myocardium. It promotes diuresis, natriuresis, arterial vasodilatation and antagonizes renin. BNP predicts improvement in blood pressure control after renal artery revascularization (Silva et al. 2005).

2.2.5 Treatment of atherosclerotic renovascular disease

Currently, the optimal management of ARVD is unknown. During the past 70 years, advances in imaging and interventional methods make detection, monitoring, and revascularization more feasible than ever before. As a result, nearly 3- to 4-fold more endovascular procedures are being performed in the USA than a decade ago (Textor et al. 2009b). Unfortunately, the benefits of revascularization procedure have been ambiguous. It is recognized that only a fraction of patients treated with renal revascularization have improved blood pressure levels or reduced medication requirements, and kidney function after revascularization infrequently improves and sometimes declines. At the same time, advances in medical therapy have dramatically improved ability to lower blood pressure and reduced the medical risk as compared to before.

2.2.5.1 Revascularization in the treatment of atherosclerotic renovascular disease

Renal revascularization is performed for approximately 16 % of newly diagnosed patients of ARVD in the United States, and endovascular procedures account for over 95 % of all these interventions (Kalra et al. 2005). Angioplasty with stent insertion, or primary stenting, is preferred to angioplasty alone, since stenting provides better arterial patency and markedly lower restenosis rates (van de Ven et al. 1999). The use of drug-eluting stents (Granillo et al. 2005) does not seem to provide any additional advantage.

Atheroembolic disease is a major concern when the abdominal aorta is manipulated. Renal artery manipulation is a predictor of serious embolic events (Scolari et al. 2003). The true benefit of distal embolic protection devices, originally designed for coronary and cerebrovascular applications, is interesting but unproven (Cooper et al. 2008). Furthermore, development of renal embolic events can occur days and even weeks after vessel manipulation, and this makes temporary protection of doubtful value (Textor et al. 2009a). Nor should it be overlooked that even in skilled hands renal endovascular intervention is by no means risk-free, as about 3 % of patients will experience a major vascular complication and over 10 % a less serious adverse events such as contrast-related acute renal injury or major groin hematoma (Leertouwer et al. 2000).

There are certain uniformly accepted indications for renal revascularization. These include: prevention or treatment of life-threatening flash pulmonary edema (Bloch

et al. 1999, Pickering et al. 1988), relief of critical stenosis to preserve renal mass (controversial), acute occlusion resulting in acute renal failure (Harden et al. 1997) and resistant renovascular hypertension (Goldsmith, Reidy and Scoble 2000). Outside these indications, the outcomes following revascularization are variable in terms of hypertension control and, especially, renal functional.

To date, there have been two randomized clinical trials (RCTs) comparing surgical treatment and medical management, and 6 RCTs comparing renal revascularization and medical management in a mix of ARVD patients with hypertension and renal function varying from normal to moderate CKD (Table 2.2). Taken together, current data do not support the concept that there would be any major benefit from renal artery inteventions to most patients with ARVD. Much of this data comes form the ASTRAL study, the largest study so far, and this study has been criticized for many reasons. The majority of participating centers (65%) included only one patient per year, and patients were included only when the treating physician was unsure whether or not the patient would benefit from intervention. Information on the course of patients who were treated with angioplasty outside the study is not available. In addition, 25 % of patients had normal renal function, and 40 % had RAS < 70 % (Alfke and Radermacher 2010).

There are several reasons why there is still continued uncertainty regarding the benefits of renal revascularization (Textor et al. 2009a):

1. Imprecise definition of severity of RAS Inclusion of sub-critical lesion in clinical trials
2. Failure to differentiate the kidneys with potential to recovery
3. Lack of standard method to assess renal hemodynamic/functional reserve
4. Compensatory function of the non-stenotic kidney
5. Complications of the procedure
   5.1 Atheroembolic disease
   5.2 Vessel dissection / occlusion
   5.3 Contrast nephrotoxicity
   5.4 Ischemia / reperfusion injury
6. Advances in medical treatment of hypertension and atherosclerosis
7. Competing risks of comorbid disease
   7.1 Aging population
   7.2 Pre-existing coronary and cerebrovascular disease
   7.3 Renovascular disease as an incidental finding
8. Negative outcomes from randomized trials

2.2.5.2 Medical management in the treatment of atherosclerotic renovascular disease

As patients with ARVD have a very high cardiovascular risk, they need effective preventive medication. A recent study showed that progression or development of RAS lesions was significantly less likely to occur in patients with ARVD on statin therapy than

Table 2.2. Randomized controlled trials comparing medical management to angioplasty ± stent insertion in ARVD

Trial	Year of publication	Patients N	Randomized treatment	Main endpoints	Findings
(Webster et al. 1998)	1998	55	Angioplasty vs. medical treatment	Primary: BP and sCr at 6 months and the change in these from baseline; secondary: major events	No significant difference in BP, GFR or CV events
(Plouin et al. 1998) EMMA	1998	49	Angioplasty (+ stent in two patients) vs. medical treatment	Primary: BP at termination and the change from baseline; secondary: treatment score and the incidence of complication	No significant difference in ambulatory BP. Reduced DDD in angioplasty group
(van Jaarsveld et al. 2000) DRASTIC	2000	106	Angioplasty vs. medical treatment	Primary: BP at 3 and 12 months; secondary: treatment score, sCr, sCr clearance, patency, incidence of complication	No significant difference in BP and sCr or DDD at 1 year. 22 patients in medical arm crossed over to angioplasty on clinical grounds
(van de Ven et al. 1999)	1999	84	Angioplasty vs. angioplasty + stent insertion	Primary: primary success rate of procedure, patency rate at 6 months; secondary: sCr and BP outcomes	Primary success rate: 57 % (angioplasty) vs. 88 % (angioplasty + stent), with better patency rate at 6 months (29 vs. 75 %) and lower restenosis rate (48 vs. 14 %) for the angioplasty + stent group; no difference in BP of GFR
(Bax et al. 2009) STAR	2009	140	Angioplasty + stent vs. medical treatment	Primary: reduction in eGFR > 20% compared to baseline; secondary: changes in BP, safety and CV morbidity and mortality	No significant difference in eGFR, BP or CV events. A number of stent-related complication occurred , including 2 stent-related deaths
(Wheatley et al. 2009) ASTRAL	2009	806	Angioplasty ± stent vs. medical treatment	Primary: Change in 1/sCr over time; secondary: changes in BP, the time to first renal event, the time to first CV event, mortality	No significant difference in primary outcome, BP or CV events.

ARVD=atherosclerotic renovascular disease, BP=blood pressure, GFR=glomerular filtration rate, sCr=serum creatinine, CV=cardiovascular, DDD=daily drug dose

without (Cheung et al. 2007). In an experimental model, simvastatin had renoprotective effects in the ischemic kidney (Chade et al. 2006b). Statins are also safe and effective in preventing cardiovascular events in patients with CKD before renal replacement therapy, including patients with ARVD (Navaneethan et al. 2009).

Opinions differ as to whether blockade of the renin-angiotensin system should be a requirement. There are two different, concordant studies according to which patients treated with these drugs had better survuival than those who were not (Hackam et al. 2008, Losito et al. 2005).

2.2.5.3 Ongoing trials in the treatment of atherosclerotic renovascular disease

The Renal Atherosclerotic Revascularization Evaluation (RAVE) trial is a single-center randomized, parallel group trial that compares angioplasty with best medical management (Tobe et al. 2007). The primary objective of the study is to determine the frequency of progression to a composite endpoint that includes death, dialysis and doubling of serum creatinine among patients with ARVD with a clinical indication for intervention. Medical management will include progressive blood pressure lowering to contemporary targets, statin therapy, aspirin or other antiplatelet therapy, diabetes control, diet, exercise and smoking cessation.

The Randomized Multi-Center Prospective Study Comparing Best Medical Management Versus Best Medical Treatment plus Renal Artery Stenting in Patients with Hemodynamically Atherosclerotic Renal Artery Stenosis (RADAR study) is ongoing in multiple centers in North and South America since late 2009. The primary endpoint is the change in the estimated glomerular filtration rate between the groups during a 12-month follow-up period. Secondary endpoints include technical success, change in renal function, overall adverse clinical events, need for target artery revascularization or target lesion revascularization, change in average systolic and diastolic blood pressure, change in left ventricular mass index estimated by echocardiography, difference in pole to pole kidney length, total antihypertensive regimen and change in New York Heart Association classification.

Stent Revascularization for the Prevention of Cardiovascular and Renal Events among Patients with Renal Artery Stenosis and Systolic Hypertension (CORAL trial) is enthusiastically awaited by the nephrological community (Cooper et al. 2006). In this trial, 1080 patients with atherosclerotic RAS will be randomized to optimal medication plus stenting or to optimal medication alone. The inclusion criteria are based on a stringent definition of atherosclerotic RAS (> 60 % at catheter angiography or > 80 % on CTA of MRA) and either drug-resistant hypertension (defined as a systolic blood pressure >155 mmHg in patients who are treated with two or more antihypertensive drugs) or a GFR < 60 ml/min. Optimal medication for both treatment groups includes a statin and an angiotensin receptor antagonist. The primary end point is survival free from composite cardiovascular and renal events. Patients will be monitored for 3 to 5 years.

2.2.6 Cardiac structure and function in atherosclerotic renovascular disease

Patients with ARVD demonstrate marked abnormalities of cardiac structure and function. These include a significantly higher prevalence of LVH (78 % vs. 46 %), left ventricular (LV) diastolic dysfunction (40 % vs. 12 %) and a greater LV mass index (LVMI) (183 ± 74 vs. 116 ± 33) compared to non-ARVD controls with a similar degree of CKD (36 ± 19 ml/min) and blood pressure (Wright et al. 2005). Only around 5 % of ARVD patients had a normal heart on echocardiography. During longitudinal follow-up, eight patients were managed with RAS revascularization and this gave no significant changes in any biochemical or echocardiographic variables between baseline and at the investigations 1 year later. However, 43 conservatively managed patients had progressive LV dilatation, mainly associated with severe renal impairment at baseline and not with the anatomical severity of RAS (Wright et al. 2009). In another study Corriere et al. (Corriere et al. 2009) performed echocardiography about 7 months after RAS revascularization in 20 patients, and found that there was a decrease in LVM while the diastolic function was largely unchanged. There are several other studies that have described a positive effect of RAS revascularization on cardiac structure (Hegarty et al. 2006, Zeller et al. 2007). In one of the more substantial of these studies (Zeller et al. 2007), 102 patients were treated with RAS revascularization and 101 age-matched hypertensive controls were treated conservatively. LVMI decreased significantly by 10 g/m^2 in the study group after a mean of 24 months of follow-up, while it increased significantly by 9 g/m^2 in the control group (p=0.001). Percutaneous revascularization turned out to be an independent predictor for regression of LVMI even after correction for reduced blood pressure.

Unlike hypertensive patients with LVH with whom longitudinal decreases in LV mass has been associated with a reduction in CV morbidity and mortality (Devereux et al. 2004), there are no studies involving patients with ARVD to indicate whether a decrease in LV mass after revascularization is associated with a similar reduction in CV endpoints. The ASTRAL trial includes a heart substudy where cardiac structural and functional changes are examined echocardiographically (N=100) and with magnetic resonance imaging (N=50). The results are awaited.

A swine model of early renovascular hypertension (12 weeks after placing a local irritant stent in the left renal artery) demonstrated that hypertension caused neovascularization of myocardial microvessels < 200 μm in diameter. These vessels are largely responsible for myocardial vascular resistance. The neovascularisation was more pronounced in the subendocardial myocardium. The changes in myocardial microvessel architecture precede an increase in LV muscle mass (Rodriguez-Porcel et al. 2006).

2.3 Methods to study myocardial vasculature and perfusion

In the healthy heart, normal coronary artery structure and function results in a balance between oxygen supply and oxygen demand despite the wide range of work and energy

production of the heart. At rest 70 – 80 % of the delivered oxygen is extracted by the myocardium, in contrast to only 25 % of other tissues. Under normal conditions, the myocardium accounts for about 12 % of the total body oxygen consumption, although it accounts for only 0.5 % of total body weight. Perfusion of the heart muscle is supplied by the left and right coronary arteries, which arise from the base of the aorta. These two arteries branch and finally form a high density capillary bed within the myocardium with about one capillary for each myofiber (Opie 1998b). In normal myocardium the capillary density is over 2000 capillaries/mm^3.

Large epicardial arteries have a diameter ranging from a few millimeters to approximately 500 μm and are visible at coronary angiography. At present, *coronary angiography* is the gold standard method for establishing the presence, site and severity of macroscopic coronary artery disease (CAD). It is performed by injecting contrast agent into the coronary arteries. Localization of stenosed vessels and the degree of stenosis are usually assessed visually. Prearterioles (diameter from ~500 to ~100 μm) and arterioles (diameter < 100μm) are below the resolution of current angiographic systems and cannot be seen. Taking into account that atherosclerotic disease of the vascular system is a continuum, it is more than probable that early (preclinical) disease affects the coronary microcirculation (small vessels < 300 – 400 μm), also in patients with normal coronary angiograms. Because there is no technique that allows the direct visualization of the coronary microcirculation in vivo, the assessment of the microcirculation relies on the measurement of parameters that reflect the functional state of the myocardium, e.g., absolute myocardial blood flow (MBF) and coronary flow reserve (CFR). CFR is the magnitude of the increase in coronary flow between basal coronary perfusion and maximal coronary vasodilatation. Since flow resistance is primarily determined by the microvasculature, CFR is a measurement of the ability of the microvasculature to respond to a stimulus and therefore presumably of the function of the small vessels. CFR is determined by measuring coronary or myocardial blood flow and by making measurements both at rest (basal flow) and at maximal hyperemia. Maximal hyperemia can be induced with intracoronary or intravenous infusion of adenosine or an intravenous infusion of dipyridamole. MBF and CFR can be measured during the coronary angiography by measuring the dilative capacity of the coronary arteries with *intracoronary Doppler ultrasound* (IVUS) or with thermodilution. All these methods have the drawback of being invasive and therefore they are justified only in patients with CAD or patients with atypical chest pain.

In recent years the development of noninvasive cardiac imaging has progressed rapidly. *Cardiac multidetector computed tomography* is rapidly becoming an integral part of clinical cardiology. Several imaging techniques have been studied aiming at quantification of cardiac perfusion. In addition *nuclear imaging, magnetic resonance imaging and echocardiography* have been investigated and shown promising preliminary quantitative results (Dijkmans et al. 2006, Fritz-Hansen et al. 2008, Schwitter et al. 2001). Currently, the most powerful technique to quantify perfusion noninvasively in the human heart is *positron emission tomography*.

Table 2.3. Clinical techniques for evaluation of functional abnormalities of myocardial perfusion in humans

Assessment of myocardial ischemia
Electrocardiography (stress ECG test)
Positron emission tomography (metabolic tracers)
Magnetic resonance spectroscopy
Transmyocardial metabolic studies
Myocardial perfusion techniques
Myocardial scintigraphy
Positron emission tomography (blood flow tracers)
Magnetic resonance imaging
Contrast echocardiography
Angiographic myocardial blush
Coronary blood flow techiques
Coronary sinus thermodilution
Intracoronary Doppler flow wire
Angiographic frame count
Doppler echocardiography (IVUS)

2.4 Methods to study renal vasculature and perfusion

In the resting adult, the kidneys receive 1.2 – 1.3 l of blood per minute, or just about 20 % of the cardiac output. In contrast to the heart, where 70 – 80 % of the delivered oxygen is extracted in the myocardium already in the rest, the O_2 consumption to O_2 delivery ratio in the kidney is only about 8 % (Brezis et al. 1984). The bulk of this supply is directed to the renal cortex, where it maximizes flow-dependent clearance of wastes.

The renal artery divides into the interlobar arteries after entering the renal sinus. At the junction between cortex and medulla the interlobar arteries divide and pass over into the arcuate arteries which also branch and give rise to the cortical radial arteries (interlobular arteries) that ascend radially through the cortex. No arteries penetrate the medulla (Kriz and Elgar 2003). The afferent arterioles are short, straight branches of the interlobular arteries. Each divides into multiple capillary braches to form the tuft of vessels in the glomerulus. The capillaries coalesce to form the efferent arteriole, which in turn breaks up into capillaries that supply the tubules (peritubular capillaries) before draining into the interlobular veins. As a result, the blood supply of the peritubular capillaries of the cortex and the medulla is exclusively postglomerular (Kriz and Elgar 2003).

Catheter angiography is the gold standard method for establishing the presence, site and severity of macroscopic renal artery disease and stenosis due to atherosclerotic renovascular disease or fibromuscular dysplasia (Kim et al. 1991). It offers the highest spatial and temporal resolution for anatomically visualizing the main renal artery and the branches. There is also a role for diagnostic angiography in the evaluation of medium and large vessel vasculitis and detection of renal infarction (Paul et al. 1965).

Renal plasma flow can be detected by ρ-aminohippurate (PAH), which is almost completely removed from the plasma during its first pass through the kidneys. Therefore renal clearance of PAH has been commonly used as an estimate of renal plasma flow. Renal blood flow (RBF) is obtained by dividing renal plasma flow by (1-hematocrit). The RPF determined by the PAH clearance is termed the effective RPF (ERPF), because part of the renal blood flow perfuses a region which does not contribute to PAH secretion. The PAH clearance is approximately 10 % lower than true RPF. The mean value of ERPF in young adults is about 650 ml/min per 1.73 m^2 for men and about 600 ml/min per 1.73 m^2 for women. Assessment of plasma clearance with a single injection of radioactive materials such as ^{131}I-hippuran or ^{99m}Tc-mercaptoacetyltriglycine (MAG3), is an alternative method for measurement of RBF. None of these methods are capable of single-kidney measurements without ureteral catheterization.

At an early stage of renal disease, RBF may be normal, and impaired renal vascular tone may become evident only as an attenuated response during stimulation with a vasoactive substance or some physiological challenge. An altered functional response may precede structural renal damage and can serve as a marker of abnormal handling of the continuous challenges met by the kidneys. Such impairment has been demonstrated by various models of renal injury, like hypertension, hypercholesterolemia, renal artery stenosis, ischemia and reperfusion, acute renal failure, diabetes and aging. Vasodilating agents allow the examination of renal flow reserve (RFR), which may allow early detection and monitoring of renovascular injury (Blackshear et al. 1987).

Intravascular Doppler ultrasound provides percutaneous single-kidney measurements of blood velocity when an intrarenal Doppler wire is used. Synchronous documentation of the renal arterial diameter makes it possible to calculate RBF (Elkayam et al. 1998). However, in large or more tortuous tubes and at flow rates > 200 ml/min (common in the renal artery), this method may underestimate blood flow. Flow in collateral and accessory renal arteries may also be missed by Doppler measurements obtained within the main renal artery. Despite its potential limitations, this technique is useful for rapid and sequential quantification of RBF. Important studies using the intravascular Doppler technique in humans have shown that intrarenal acetylcholine results in a significant vasolidatory effect on both conductance and resistance renal blood vessels and leads to a marked reduction in renal vascular resistance and to enhanced RBF (Elkayam et al. 1998). Studies with intravascular Doppler have shown that the RFR is less marked than the coronary circulation (Beregi et al. 1998, Houghton, Cerda and Smith 2000). While CFR, i.e., the hyperemic-to-basal blood flow ratio, is 4 or 5, a RFR of > 2.5 is difficult to achieve, possibly because of the lower basal renal vascular resistance compared with coronary vascular resistance.

The xenon (^{133}Xe) washout technique is based on evaluation of the time-activity excretion curve on an externally admistered indicator. This methodology is invasive because it involves injection of the ^{133}Xe directly into renal artery and external

counting with a scintillation probe (Aukland 1980). It is doubtful if the excretion curves give any information on the intrarenal blood flow distribution. However, this method may still be useful for assessing average RBF, which has been empirically shown to be well represented by the initial washout rate (Hollenberg, Mangel and Fung 1976). The mean RBF is 310 to 370 ml/min/100 g of kidney in healthy subjects (Hollenberg et al. 1989). The xenon (^{133}Xe) washout technique has been also used for assessment of RFR with acetylcholine (Hollenberg et al. 1989) and with adenosine (Wierema et al. 1998).

Doppler ultrasound of the kidneys provides a detailed evaluation of the renal vascular anatomy. The main renal arteries can be identified in most patients. There are several ways of using ultrasound to assess renal perfusion. One approach is to measure flow velocities in the main renal artery. Another approach is to image the intrarenal parenchymal arteries, i.e., interlobar or segmental arteries to calculate resistive and pulsatility indexes.

Advances in computed tomography technology that allow spiral multidetector acquisition with 1.25 mm or thinner slices can provide accurate anatomic images of even small renal arteries during the arterial phase of a fast iodinated contrast agent bolus. Compared to conventional catheter angiography, *computed tomographic angiography (CTA)* is less invasive and faster, offers better soft tissue visualization and allow multiplanar imaging of the renal arteries in any obliquity. However, CTA carries the risks of ionizing radiation and of nephrotoxicity related to iodinated contrast agents.

The high spatial and temporal resolution of *electron-beam computed tomography (EBCT)* enables accurate, reproducible and noninvasive quantification of single-kidney volume and cortical, medullary and papillary perfusion in humans (Lerman et al. 1996a). The main limitations of this technique are related to exposure to radiation and to radiographic contrast agents. RFR has been studied with EBCT in animal models, where it has turned out to be useful for detection of impaired vasodilatation in pigs with hypertension and hypercholesterolemia (Feldstein et al. 1999, Rodriguez-Porcel et al. 2001).

Magnetic resonance imaging (MRI) has been used to measure flow through both the main renal artery and the renal parenchyma. MRA provides high-quality noninvasive anatomic images of the renal arteries. It can be performed with or without intravenous contrast administration (Vasbinder et al. 2001). Recording dynamic changes of signal intensity or disappearance rate after administration of gadopentetate dimeglumine has also been used to assess renal function. However, quantification of RBF with standard gadolinium chelates is not reliable, since these contrast agents are immediately filtered when they pass through the glomeruli (Schoenberg et al. 2003). Improvements in assessing new contrast agents, such as ferumoxytol which is an iron oxide compound (Schoenberg et al. 2000), may change the field of renal perfusion studies with MRI. See also section 2.2.4, page 33-34.

Table 2.4. Cortical renal perfusion values in healthy humans with different techniques

Method	Cortex ml/min/g tissue	Reference
EBCT	3.6 – 3.8	(Flickinger et al. 1996, Lerman et al. 1996a)
Dynamic CT	2.5 – 4.7	(Miles 1991, Miles, Hayball and Dixon 1994)
^{133}Xenon washout	1.3 – 4.1	(Porter and Hollenberg 1998, Blaufox et al. 1970, Ladefoged 1966, Rosen et al. 1968)
PET	3.4 – 4.6	(Alpert et al. 2002, Middlekauff et al. 1995, Middlekauff et al. 1997, Nitzsche et al. 1993)
MRI	2.8	(Roberts et al. 1995)

EBCT=electron beam computed tomography, CT=computed tomography, PET=positron emission tomography, MRI=magnetic resonance imaging

2.5 Methods to study endothelial function

Quantitative angiography combined with intracoronary infusion on an endothelin-dependent vasodilator (serotonin or bradykinin) is used for direct quantification of the endothelial function of the coronary arteries. The technique allows construction of dose-response curves for endothelial agonists and antagonists (Widlansky et al. 2003). The change in luminal diameter or coronary blood flow during administration of these drugs is measured using intravascular ultrasound Doppler imaging wires or catheters at the time of coronary angiography.

The first in vivo evidence of an association between coronary atherosclerosis and endothelial dysfunction was demonstrated by Ludmer and colleagues in 1986 (Ludmer et al. 1986). They showed that while acetylcholine caused a dose-dependent dilation of coronary arteries in subjects without coronary disease, patients with CAD exhibited paradoxical vasoconstriction. This indicated impaired endothelium-dependent vasomotion. The vasoconstriction response to acetylcholine in CAD patients can be attributed to direct constricting effects on vascular smooth-muscle. Coronary endothelial dysfunction has since then been shown to be progressive, i.e., vasoconstriction caused by acetylcholine becomes more pronounced in patients with established coronary disease as compared with patients with hypercholesterolemia (Zeiher et al. 1991).

Quantitative angiography is considered to be the gold standard for early detection of endothelial dysfunction in the coronary arteries. However, the main limitation of this direct measurement of coronary endothelial function is the highly invasive nature of the procedure, which makes this method unsuitable for general clinical research and for testing for subclinical atherosclerosis in high-risk subjects.

Flow-mediated dilatation (FMD) of the brachial artery with ultrasound was first described by Celermajer et al. (Celermajer et al. 1992), and this approach has now been used by numerous groups throughout the world to study and monitor endothelial function. The current procedure for measurement of FMD in humans involves ultrasound evaluation of brachial artery dilatation during reactive hyperemia that follows brief compressive

ischemia of the forearm. The FMD response to ischemia-induced reactive hypeaemia is mediated through an increase in the release of endothelial NO provided that the arterial occlusion time is below 5 minutes (Mullen et al. 2001).

Decreased FMD of the brachial artery is associated with cardiovascular risk factors, such as high serum LDL and total cholesterol (Celermajer et al. 1992), low serum HDL cholesterol (Toikka et al. 1999), cigarette smoking (Celermajer et al. 1993), hypertension (Muiesan et al. 1999), and diabetes (Järvisalo et al. 2004). FMD of the brachial artery correlates significantly with coronary endothelial function (Anderson et al. 1995) and with the severity of coronary atherosclerosis (Neunteufl et al. 1997).

Forearm blood flow by strain gauge plethysmography may yield results that are less observer-dependent than ultrasound-based measurements. The basic concept is the same as with the ultrasound-based methods (Hogas et al. 2010).

Applanation tonometry allows noninvasive assessment of quantitative markers of arterial stiffness, such as aortic pulse wave velocity and augmentation index, obtained by pulse-wave analysis. The pulse wave shape provides information about arterial compliance and serves as a basis for calculation of the augmentation index (the ratio between the pulse pressure at the second systolic and first systolic peaks), which is a common measure of arterial stiffness. Changes in the peripheral pressure waveform induced by β_2 adrenoceptor stimulation have been tested for the assessment of global endothelial function (Hayward et al. 2002).

Laser Doppler flowmetry is a new method used to investigate endothelial function at the level of skin microcirculation (finger) during post ischemic or thermal hyperemia or after local application of endothelium-activating substances by iontophoresis. The principle is to measure the Doppler shift, i.e., the change in light frequency when it is reflected by the moving blood cells inside microvessels (Schabauer and Rooke 1994). The interest of the study of skin microcirculation arises from the hypothesis that skin microvascular function mirrors the state of other microcirculation territories, including the coronaries (Jung et al. 2001).

2.6 Positron emission tomography

PET is based on the detection of two photons created in an annihilation reaction between a positron and a tissue electron. The unique advantages of PET over other nuclear methods are the ability to measure the quantity of tracer concentrations in tissue, high spatial and temporal resolution and high sensitivity following the use of multiple detectors (Rahmim and Zaidi 2008).

2.6.1 Myocardial perfusion and positron emission tomography

Several tracers have been used for the measurement of myocardial perfusion with PET: ^{15}O-water (Iida et al. 1988), ^{13}N-ammonia (Hutchins et al. 1990) and the potassium analog

^{82}Rb (Herrero et al. 1990). Currently, ^{15}O-water and ^{13}N-ammonia are the most widely used tracers. ^{15}O-water is a tracer that diffuses freely through the tissues, which renders its kinetics solely flow-related , i.e., there is not effect by metabolism. When ^{15}O-water is used, perfusion is estimated from the tracer's washout from the myocardium. Single-tissue compartment models of an inert, freely diffusible tracer is used for calculation of myocardial blood flow (MBF) by use of ^{15}O-water (Iida et al. 1988). In the calculation of myocardial perfusion, also partial volume effect and spillover from the left ventricular chamber into the myocardial regions must be corrected. The short half-lives of positron emitting tracers provide generally less radiation to the patient than single-photon emission computed tomography (SPECT) tracers.

PET with $H_2{}^{15}O$ or $^{13}NH_3$ is considered to be the noninvasive gold standard for assessment of MBF and CFR (Kaufmann and Camici 2005). MBF and CFR show some degree of spatial heterogeneity on the individual level, but very small temporal heterogeneity, and thus the method is highly repeatable. Reference values are slightly related to gender and age (Uren et al. 1995). The normal basal mean MBF ranges between 0.8 and 1.2 ml/g/min, measured with PET and ^{15}O-water (Bergmann et al. 1989, Chan et al. 1992, Czernin et al. 1993, Merlet et al. 1993). In a recent analysis using ^{15}O-water, the ideal cut-off for absolute stress perfusion was 2.5 ml/g/min, the range from 2.0 to 2.5 being mildly reduced (Nesterov et al. 2009). As regards perfusion reserve, typical values above 2.5 – 2.7 have been regarded as normal. Baseline MBF increases with aging, but since hyperemic MBF decreases simultaneously, aging is associated with a decrease in CFR. Possibly as a result of estrogen on the vascular tone, baseline MBF is higher in females, but CFR is lower (Chareonthaitawee et al. 2001)

2.6.1.1 Coronary artery disease and positron emission tomography

Machac et al. have listed eight studies that compared PET perfusion with coronary angiography, representing a total of nearly 800 patients. The mean sensitivity was 93 % and specificity was 92 % for detection of significant coronary stenoses (Machac 2005).

It has been shown that coronary artery stenosis does not affect the basal MBF, but hyperemic flow is gradually decreasing after 40 % stenosis in coronary artery. Hyperemic flow approaches unity with basal flow when the degree of stenosis is 80 % or greater (Uren et al. 1994). A threshold of pharmacologically induced hyperemic MBF of 2.5 ml/g/min was the most accurate for identification of epicardial lesions of > 50 % diameter stenosis (Nesterov et al. 2009). However, hyperemic MBF may also be diminished due to coronary microvascular dysfunction in patients with or without CAD lesions on coronary angiography but with multiple cardiovascular risk factors. Furthermore, a grey zone exists in patients with epicardial lesions ≥ 50 % and a CFR between 2.0 and 2.5, where the significance of downstream consequences of a focal epicardial lesion may remain uncertain.

While several standard functional imaging techniques, stress magnetic resonance imaging and stress echocardiography are established methods for detecting ischemia, they do not

provide information about coronary anatomy. Hybrid scanners combining PET with high resolution multidetector CT offer the ability to determine the coronary artery calcium (CAC) score, anatomy of coronary arteries and the functional consequences of CAD either at stress or at rest in association with the left ventricular systolic function. All PET systems are currently manufactured as PET-CT scanners. It has recently been shown that coronary calcium scoring and myocardial perfusion PET imaged with a hybrid PET-CT system provide complementary prognostic information. Schenker et al. reported a single cardiac PET-CT study involving 695 patients with an intermediate pre-test likelihood of CAD (Schenker et al. 2008). The authors observed an increasing prevalence of abnormal PET findings with increasing CAC, but, interestingly also observed abnormal perfusion in 16 % of patients with normal CAC. In other words, although increasing CAC content is generally predictive of a higher likelihood of ischemia, its absence does not eliminate the possibility of flow-limiting CAD. Risk-adjusted survival analysis demonstrated a stepwise increase in cardiac events with increasing CAC in patients with and without ischemia on PET. Among patients with normal PET myocardial perfusion imaging, the annualized event rate of patients with no calcium was lower than of patients with high calcium (2.6 % versus 12.3 %), and in patients with ischemia on PET, the annualized event rate of patients with no calcium was also lower than in patients with high calcium (8.2 % versus 22.1 %).

2.6.1.2 Coronary microvascular dysfunction and positron emission tomography

Structural or functional pathophysiological mechanisms cause coronary microvascular dysfunction. Structural abnormalities increase microvascular resistance by reducing the vascular luminal caliber or by reducing the density of microvessels. Vascular luminal caliber may be reduced by intraluminal obstruction (e.g., microemboli), increased vascular wall size (e.g., infiltrative disorders or vascular hypertrophy) or extravascular compression (e.g., ventricular hypertrophy). Functional abnormalities are more complex, as they involve inappropriate vasoconstrictor and/or inadequate vasodilator responses. These may be due to intravascular mechanisms, e.g., endothelial and/or vascular smooth cell dysfunction, or extravascular mechanisms, e.g., autonomic or humoral dysfunction (Beltrame, Crea and Camici 2009).

Cigarette smoking impairs endothelial function of the brachial as well as the coronary arteries (Celermajer et al. 1993, Zeiher, Schächinger and Minners 1995). Asymptomatic smokers with no evidence of CAD have reduced CFR due to lower hyperemia (Kaufmann et al. 2000a). Importantly, smoking cessation seems to normalize the coronary microvascular function already within 1 month (Morita et al. 2006).

Hyperlipidemia. Coronary flow reserve is reduced in asymptomatic subjects with hypercholesterolemia and angiographically normal coronary arteries as shown by impaired flow at maximal vasodilatation (Pitkänen et al. 1996). There is an inverse correlation between cholesterol, both total- and LDL-cholesterol, and CFR (Kaufmann et al. 2000b, Pitkänen et al. 1996).

Diabetes. Most studies have shown decreased coronary vasoreactivity, coronary endothelial dysfunction and impaired CFR in patients with diabetes (Pitkänen et al. 1998, Yokoyama et al. 1997).

Inflammation. Half of all myocardial infarctions and strokes occur among apparently healthy men and women with levels of LDL cholesterol that are below currently recommended thresholds for treatment (Ridker et al. 2008). Clinical studies affirm a correlation between circulating markers of inflammation and a propensity to develop ischemic events. Statin therapy reduces the incidence of first major cardiovascular events, even in apparently healthy people with elevated high sensitivity C-reactive protein levels (Ridker et al. 2005, Ridker et al. 2008).The cellular interactions in atherogenesis are fundamentally no different from those of chronic inflammatory diseases, e.g., rheumatoid arthritis (RA), glomerulosclerosis, pulmonary fibrosis and liver cirrhosis (Ross 1999). Accelerated coronary atherosclerosis and coronary microvascular dysfunction , in the absence of significant CAD have been documented in patients with RA and systemic lupus erythematosus (SLE) (Manzi et al. 1997, del Rincón et al. 2001, Asanuma et al. 2003, Roman et al. 2003), which is a consequence of impaired hyperaemic MBF (Recio-Mayoral et al. 2009).

Hypertension. Coronary microvascular dysfunction in patients with hypertension is not necessarily related to the presence or any degree of left ventricular hypertrophy (Vogt, Motz and Strauer 1992). Even borderline arterial hypertension is associated with alterations in myocardial hemodynamics: the vasodilatory capacity of the coronary arteries is reduced in comparison to healthy controls (Laine et al. 1998). It has been argued that this is a consequence of remodelling of intramural arterioles and interstitial fibrosis, which assumedly leads to a decreased density of vessels in the coronary microvasculature. At the same time, with elevated systolic and diastolic wall stress and impaired relaxation, extravascular compressive forces are increased (Opherk et al. 1984).

The effect of *aging* on the cardiovascular system is similar to that of hypertension. It has been shown that myocardial blood flow during basal conditions and hyperemia are roughly comparable up to 60 years of age (Uren et al. 1995).

The effect of *antihypertensive treatment* on myocardial perfusion has been investigated in a number of studies (Akinboboye, Chou and Bergmann 2002, Motz and Strauer 1996, Parodi et al. 1997). ACE inhibitors, angiotensin receptor blockers and calcium channel blockers improve myocardial perfusion during maximal vasodilatation, while beta blockers may reduce it (Akinboboye et al. 2002, Buus et al. 2004, Higuchi et al. 2007, Motz and Strauer 1996, Naya et al. 2007) .

The complexity of the assessment of coronary microvascular dysfunction is further increased by the presence of *obstructive CAD*. In patients with stable CAD, coronary microvascular dysfunction should be suspected in those whose symptoms are worse than anticipated on the basis of the severity and extent of angiographic findings. In such patients, coronary microvascular function might represent a new therapeutic target (Camici and Crea 2007).

2.6.2 Renal perfusion and positron emission tomography

PET is one of the few techniques capable of quantification of renal blood flow (RBF) and cortical blood flow in vivo (Alpert et al. 2002, Juillard et al. 2002, Nitzsche et al. 1993). Middlekauff et al used PET to show that cortical RBF decreases and renal vascular resistance increases in response to static handgrip exercise and that exogenous adenosine produces reflex renal vasoconstriction (Middlekauff et al. 1995, Middlekauff et al. 1997). The kinetic model of $H_2{}^{15}O$ is based on the assumptions that all activity is extracted by the parenchyme, that extraction is very rapid and that tubular transport has not started or is insignificant at a level that does not influence the calculation of RBF. However, the very short half-life of $H_2{}^{15}O$ makes it mandatory to have a medical cyclotron. PET has also a relatively low spatial resolution limiting measurement of medullary perfusion.

2.7 Myocardial perfusion in chronic kidney disease and atherosclerotic renovascular disease

2.7.1 Myocardial perfusion in chronic kidney disease

Myocardial perfusion has been studied in CKD patients with single photon emission computed tomography (SPECT), with positron emission tomography (PET) and with transthoracic Doppler ultrasound, mainly in transthoracic manner, but also with intracoronary guidewire.

In SPECT studies an abnormal perfusion pattern provides more powerful prognostic data than coronary angiography in the ESRD population (Venkataraman et al. 2008). This seems to be true also in milder stages of CKD. Hakeem et al. (Hakeem et al. 2008) reported an annual cardiac death rate of 9.5 % in patients with eGFR below 60 ml/min and of 4% in patients with eGFR above 60 ml/min; all patients had perfusion defects in SPECT. There was also an inverse correlation between the extent of perfusion abnormalities and GFR, bigger defects were associated with lower GFR. Similar results have been reported in other studies, as well (Hatta, Nishimura and Nishimura 2009). It has also been found that the heart rate response which is blunted to adenosine infusion during stress perfusion in patients with ESRD is associated with increased mortality (Venkataraman et al. 2009). SPECT is, at best, a semiquantitative method and requires visual interpretation. SPECT may miss the diagnosis of significant CAD in patients with balanced ischemia due to significant 3-vessel disease, where perfusion may appear normal or minimally abnormal on perfusion imaging.

In studies using transthoracic Doppler technique, CFR (the ratio of hyperaemic to baseline diastolic peak flow velocities) is lower in patients with ESRD than in healthy controls or in renal transplantation patients, because of blunted hyperaemic flow (Bozbas et al. 2009, Caliskan et al. 2008, Tok et al. 2005), but also because of higher baseline flow (Bozbas et al. 2009, Caliskan et al. 2008, Niizuma et al. 2008). Decreased CFR has also been observed in hypertensive patients with only subclinical renal damage (Bezante

et al. 2009), and in normotensive patients with autosomal dominant polycystic kidney disease with well-preserved renal function (Turkmen et al. 2008).

In a large, intravascular Doppler (IVUS) study (n=605) Chade et al. (Chade et al. 2006a) used intracoronary adenosine to evaluate CFR in patients with normal or mildly diseased coronary arteries. Patients with eGFR below 60 ml/min had significantly lower CFR than patients with normal GFR, 2.6 ± 0.6 vs. 3.0 ± 0.8.

There are two PET-studies on myocardial perfusion in hemodialysis patients. McIntyre et al. (McIntyre et al. 2008) studied four prevalent hemodialysis (HD) patients with no angiographically significant CAD and reported that HD was associated with significant reduction in myocardial blood flow (MBF). There was an improvement in MBF in 85 % of the coronary artery segments demonstrating partial restoration of blood flow after 30 minutes. This finding was corroborated by Dasselaar et al. (Dasselaar et al. 2009) who studied seven non-diabetic HD patients with an uneventful cardiac history. As myocardial perfusion fell already early during HD without significant fluid removal, it seems evident that also other dialysis-associated factors seem to have a role in decreased myocardial perfusion.

2.7.2 Myocardial perfusion in atherosclerotic renovascular disease

A blunted myocardial perfusion reserve before the development of LVH has been demonstrated in a swine model of early renovascular hypertension (12 weeks after placing a local irritant stent in the left renal artery) (Rodriguez-Porcel et al. 2003a, Rodriguez-Porcel et al. 2006).

2.8 Endothelial function in chronic kidney disease and in atherosclerotic renovascular disease

2.8.1 Endothelial function in chronic kidney disease

The endothelium, the largest endocrine organ of the human body, is composed of a single-cell lining covering the internal surface of blood vessels. The endothelium plays a crucial role in regulating vascular tone and structure. It senses mechanical stimuli, such as pressure and shear stress, and hormonal stimuli, e.g., vasoactive substances. In response, it releases agents that regulate vasomotor function, trigger inflammatory processes and affect hemostasis. The endothelium also contributes to mitogenesis, angiogenesis, vascular permeability and fluid balance (Endemann and Schiffrin 2004). Furchgott and Zawadzki demonstrated a significant role for endothelial cells in the maintenance of vasodilatation which opened an avenue of major scientific inquiry in this field (Furchgott and Zawadzki 1980).

Endothelial dysfunction was initially identified as impaired vasodilation to specific stimuli, e.g., acetylcholine or bradykinin. A broader understanding of the term would

include not only reduced vasodilation but also a proinflammatory and prothrombic state associated with dysfunction of the endothelium. Dysfunction of the endothelium has been implicated in the pathophysiology of different forms of cardiovascular disease, including hypertension, coronary artery disease, chronic heart failure, peripheral artery disease, diabetes and chronic renal failure. Recent studies have also shown that both coronary (Halcox et al. 2002) and brachial endothelial dysfunction predict CV events (Gokce et al. 2003, Heitzer et al. 2001, Perticone et al. 2001).

Endothelial function has been assessed primarily in terms of endothelium-dependent vasomotion, largely based on the assumption that impaired endothelium-dependent vasomotion reflects alterations of other functions of the endothelium as well. An important rationale for this approach has been the observation that endothelium-derived nitric oxide (NO), synthesized by endothelial NO synthase (eNOS) from the precursor L-arginine, is not only a major mediator of endothelium-dependent vasodilation but is also critically involved in the regulation of other protective properties of the healthy endothelium (Landmesser, Hornig and Drexler 2004). Reduced NO has often been reported in the presence of impaired endothelial function. It may result from reduced activity of eNOS, from endogenous or exogenous inhibitors or from a reduction of substrate (L-arginine) availability.

Although endothelial dysfunction becomes more prevalent as renal function declines, the exact causes of endothelial dysfunction are not known (Yilmaz et al. 2006, Yilmaz et al. 2008). The level of the inhibitor of eNOS, asymmetrical dimethylarginine (ADMA), which is primarily cleared by kidneys, is elevated in CKD. Elevated ADMA levels predict acute coronary events is the general population (Valkonen et al. 2001). In patients on hemodialysis, ADMA concentrations are associated with higher carotid intima media thickness (IMT) (Nanayakkara et al. 2005) and predict all-cause mortality and CV events (Zoccali et al. 2001). Ravani et al. (Ravani et al. 2005) demonstrated that ADMA is a strong and independent risk marker for progression of CKD.

Increased excretion of albumin into the urine may represent a glomerular reflection of a generalized increase in endothelial permeability (Stehouwer et al. 2004). Although the nature of the link between albuminuria and increased vascular risk may be partly mediated through endothelial dysfunction, persistent low-grade inflammation also seems to play a role in this scenario. Indeed, inflammation is associated with endothelial dysfunction (Stenvinkel et al. 2008) and with albuminuria (Festa et al. 2000). Microalbuminuria is a well established predictor of increased CV risk (Arnlöv et al. 2005). Albuminuria is also a much stronger predictor of future decline of renal function than a slightly reduced GFR (Halbesma et al. 2006).

Nevertheless, a causal relationship between endothelial dysfunction and CV disease in CKD patients remains to be established because pharmacological interventions with statins, vitamin E and homocysteine-lowering therapy aimed at reducing plasma ADMA have not shown survival benefit (Nanayakkara et al. 2009). Until new data becomes

available, endothelial dysfunction should be considered a CV marker rather than an etiological factor of CKD.

2.8.2 Endothelial function in atherosclerotic renovascular disease

There are only a few studies on endothelial dysfunction in patients with renovascular disease. Rizzoni et al. (Rizzoni et al. 1998) reported a similar extent of impairment of endothelial function in patients with primary and with secondary hypertension, as assessed by the vasodilator response to acetylcholine in dissected small resistance arteries of subcutaneous fat. Higashi et al. (Higashi et al. 2002) evaluated the response of forearm blood flow to acetylcholine in 15 subjects with unilateral renovascular disease before and after renal artery revascularization. The response of forearm blood flow to acetylcholine was greater in healthy controls compared to the subjects with renovascular hypertension. However, it increased after successful angioplasty, indicating enhanced endothelial function after revascularization. As the degree of dysfunction is related to the severity of hypertension (Panza et al. 1990), improved blood pressure control could be the explanation. Still, in this study, the change in blood pressure did not correlate with improvement in the response of forearm blood flow to acetylcholine.

The effect of renovascular disease on coronary endothelial function has been studied in a swine model of early renovascular hypertension (12 weeks after placing a local irritant stent in the left renal artery). The coronary endothelial dysfunction seen in renovascular disease was exacerbated by hypercholesterolemia. The effect of combination of renovascular disease and hypercholesterolemia was greater than dysfunction induced by each risk factor alone (Rodriguez-Porcel et al. 2003b).

2.9 Renal perfusion in patients with atherosclerotic renovascular disease

Although RAS is easy to detect with contemporary imaging modalities, the actual changes in renal perfusion of the kidney with RAS are sparsely studied. Furthermore, none of the current imaging modalities are of value as predictors of improved renal function after interventional therapy.

Wierema et al. (Wierema et al. 1998) used the ^{133}Xe washout method in twelve patients with RAS to demonstrate that renal perfusion was 428 ml/min/100 g tissue in essential hypertension patients compared to 343 ml/min/100 g tissue in RAS patients. This method, despite its advantages, cannot be used clinically to study patients with renovascular disease, since it is too invasive.

Computed tomography (CT) has been used to study renal perfusion in humans. Lerman et al. (Lerman et al. 1996b) measured whole kidney, cortical and medullary perfusion with electron-beam computed tomography in 20 patients with atherosclerotic RAS, in 10 patients with fibromuscular dysplasia (FMD) and in 28 patients with essential

hypertension (EH). Cortical perfusion was lower in patients with RAS compared to FMD or EH, 2.44 ± 0.16 ml/min/cc vs. 3.26 ± 0.17 vs. 3.07 ± 0.09. In patients with RAS due to atherosclerosis, the cortical perfusion did not correlate with the degree of RAS. In the study by Paul et al. (Paul et al. 2001), the mean perfusion in unilateral RAS was 3.1 ml/min/mm^3 (range 1.2 - 5.2 ml/min/mm^3), while the perfusion in the contralateral kidney was nearly similar, 3.9 ml/min/mm^3 (mean, range 2.7 - 5.3 ml/min/mm^3). The most recent study by Gloviczki and colleges (Gloviczki et al. 2010) compared renal perfusion in 14 patients with unilateral RAS and in 14 patients with essential hypertension. The cortical renal perfusion was 3.5 ± 0.2 ml/min/ml of tissue in patients with essential hypertension, 2.7 ± 0.3 in stenotic kidneys of patients with RAS and 2.9 ± 0.3 in the contralateral kidneys of patients with RAS. These patients underwent also BOLD magnetic resonance examination to detect any changes in kidney oxygenation. Interestingly, deep medullary and cortical oxygenation did not differ among the patient groups despite reduced cortical perfusion in stenotic kidneys of patients with RAS. Iodinated contras medium is needed for quantification of renal perfusion with CT. This is potentially problematic with atherosclerotic renovascular disease patients, since mostly of them have CKD.

Three different methods for perfusion MRI of the kidney have been described especially for RAS: 1) qualitative assessment of renal perfusion with arterial spin labelling techniques without contrast agents, 2) semiquantitative perfusion measurements with extracellular gadolinium chelates and 3) quantitative assessment of renal perfusion with intravascular contrast agents with absolute parameters of regional renal perfusion (Schoenberg et al. 2006). For clinical routine, the preferred method has been dynamic semiquantitative MR perfusion measurement with gadolinium chelates. However, gadolinium is rapidly and fully filtrated in the glomeruli. So, the semiquantitative perfusion parameters are derived with mathematical fits from the early perfusion phase and the subsequent filtration and excretion phase. In the study by Vallée et al. (Vallée et al. 2000), cortical renal perfusion was 1.09 ± 0.75 ml/min/g tissue in four patients with RAS, 2.20 ± 0.69 ml/min/g tissue in patients with normal renal function and 0.51 ± 0.34 ml/min/g tissue in patients with renal failure (creatinine 316 ± 148 μmol/l). The introduction of nephrogenic systemic fibrosis has made the use of gadolinium more complex in patients with ARVD and CKD. Thus, new intravascular contrast agents are needed, like ultra-small particle iron oxides (USPIOs) for absolute measurements of renal perfusion with reasonable accuracy (Schoenberg et al. 2003). Thus far, none of the USPIO contrast agents has been approved for clinical use.

3 OBJECTIVES OF THE STUDY

The aims of the present study were:

1. To characterize myocardial perfusion, coronary flow reserve and peripheral endothelial function in CKD patients without symptomatic CAD (I)

2. To characterize myocardial perfusion, coronary flow reserve and peripheral endothelial function in ARVD patients and to study the effect of RAS revascularization on these variables (II)

3. To quantify renal perfusion in healthy subjects with PET methodology (III)

4. To characterize renal perfusion in patients with ARVD and to examine any possible changes in renal perfusion after the RAS revascularization (IV)

4 STUDY SUBJECTS AND STUDY DESIGN

4.1 Study subjects

The study included a total of 56 subjects, 19 of whom were atherosclerotic renovascular disease patients, 27 were chronic kidney disease patients and 10 were healthy volunteers. The characteristics of the subjects are shown in Table 4.1.

Table 4.1 Characteristics of the study group

Study	N	Group	Age (y)	eGFR ml/min	BPsyst mmHg	BPdiast mmHg
I	22	CKD patients	54±8	22±16	134±16	82±6
	10	Healthy	60±8	75±6	138±14	82±5
II	19	ARVD patients	69±10	58±22	199±27	90±17
III	6	Healthy	58±5	78±4	136±11	82±4
IV	17	ARVD patients	69±11	56±23	193±26	89±13
	7	CKD patients	72±5	22±12	189±32	81±11
	10	Healthy	60±8	75±6	130±11	79±6

CKD=chronic kidney disease, ARVD=atherosclerotic renovascular disease, eGFR=estimated glomerular filtration rate, BP=blood pressure

All CKD and ARVD patients were recruited from a nephrology outpatient clinic of the Turku University Hospital.

The inclusion criterion for CKD patients in substudies I and IV was CKD stage 3 to 5 without renal replacement therapy. A more detailed description of the CKD patients in substudy I is shown in Table 4.2. The etiology of the CKD in substudy I was polycystic kidney disease in 6 of 22 patients, chronic glomerulonephritis in 8 (3 IgA nephropathy, 1 FSGS, 2 vasculitis, 2 undetermined), chronic interstitial nephritis in 4, post renal obstruction caused by benign prostate hyperplasia in 1, secondary amyloidosis related to juvenile rheumatoid arthritis in 1 and hypertensive nephrosclerosis in 1.

Table 4.2. General characteristics of the subjects in substudy I

	CKD 3 N=5	CKD 4 N=11	CKD 5 N=6	Controls N=10
GFR ml/min	37±7	21±5	12±5	76±5
Age y	54±10	54±10	54±5	60±8
Sex (M/F)	2/3	8/3	3/3	7/3
BP syst mmHg	129±11	140±17	128±16	138±14
BP diast mmHg	81±2	84±6	78±7	82±5
MAP mmHg	97±4	103±7	94±9	101±8
Number of antihypertensive medication	2.0±1.0	2.5±1.2	2.5±1.0	0

CKD=chronic kidney disease, GFR=glomerular filtration rate, BP=blood pressure, MAP=mean arterial pressure

The exclusion criteria for CKD patients in substudy I were any signs or symptoms of cardiovascular disease and diabetes.

The inclusion criteria for ARVD patients in substudies II and IV were RAS over 60 % and planned RAS revascularization.

There is some cross-over of CKD study subjects between substudies I and IV. Partly the same healthy controls were used for study III. Substudies II and IV included same ARVD patients except two ARVD patients who could not participate in substudy IV, one patient due to flash pulmonary edema after a heart study, and the other due to refusal.

All patients and healthy volunteers gave written informed consent. The study was approved by the Ethics Committee of the Turku University Central Hospital and it was conducted in accordance with the Declaration of Helsinki as revised in 1996.

4.2 General study design

The general study outline is presented in Figure 4.1. The PET imaging studies were performed after a 10-hour overnight fast. Alcohol, smoking and caffeine were prohibited for 3 days before assessment. Subjects with symptoms of acute infections within a week prior to or during the study were excluded from the analysis. Peripheral endothelial function determined measuring brachial artery FMD with ultrasound, and echocardiography were measured before the PET study.

In substudy I, all CKD patients were instructed to follow their regular medication regimen on the study day. In substudies II and IV, all patients were instructed to interrupt their antihypertensive medication on the study day and ACEI or ARB medication three days before the study day.

The PET studies were repeated to ARVD patients after RAS revascularization (103 ± 29 days) (II, IV).

Study I

CKD patients underwent only the peripheral endothelial function test and PET of the myocardium. Myocardial perfusion was measured at rest and after dipyridamole infusion with [^{15}O]H2O-PET. Heart rate (HR), blood pressure (BP) and the electrocardiogram (ECG) were monitored throughout the studies. 18 our of 22 CKD patient underwent echocardiography as a part of the clinical treatment protocol before the PET study.

Study II

Myocardial perfusion was measured using [^{15}O]H_2O-PET at rest and after dipyridamole infusion. BP, HR and ECG were monitored throughout the study. FMD and echocardiography was done before the PET study.

Study III

Renal perfusion was measured using [^{15}O]H_2O-PET at rest and 20 minutes after an infusion of enalapril. BP and HR were monitored throughout the study.

Study IV

Renal perfusion was measured using [^{15}O]H_2O-PET. BP and HR were monitored throughout the study.

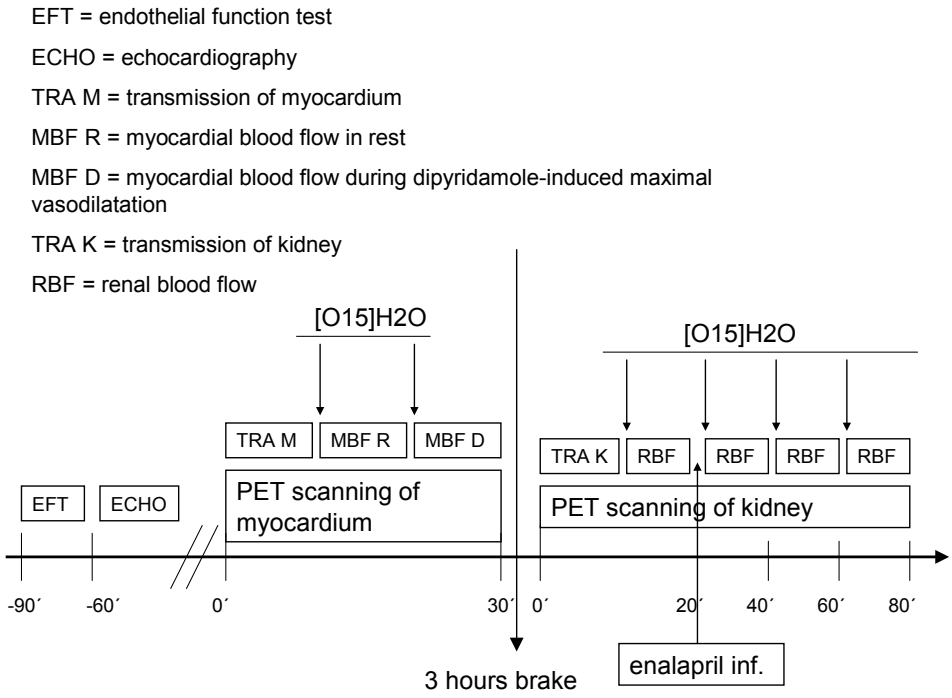

Figure 4.1. General study outline

5 METHODS

5.1 Positron emission tomography

5.1.1 Production of $H_2{}^{15}O$

For production of ^{15}O ($T_{1/2}$=123 s), a low-energy deuteron acceletor Cyclone 3 was used (Ion Beam Application Inc. Louvain-la-Neuve, Belgium). $H_2{}^{15}O$ was produced using a dialysis technique in a continuously working water module (Sipilä et al. 2001).

5.1.2 PET image acquisition

An ECAT 931/08-12 tomograph (Siemens/CTI Inc., Knoxville, TN, USA) with an axial resolution of 6.7 mm and an in-plane resolution of 6.5 mm was used for image acquisition in studies I and II. Correct positioning of the subject was ascertained on a rectilinear transmission scan followed by a five-minute transmission scan with a removable ring source of ^{68}Ge for photon attenuation correction. Special attention was paid to avoid movement of the patient during the study.

In substudies II, III and IV, part of the PET study was carried out using a GE Advance PET scanner (General Electric Medical Systems, Milwaukee, WI, USA) with 35 slices of 4.25 mm thickness. Correct positioning of the subject was ascertained on a rectilinear transmission scan followed by five minutes transmission scan using two rotating rod sources containing $^{68}Ge/^{68}Ga$.

Basal and hyperemic myocardial perfusion. Myocardial perfusion was measured with an intravenous bolus of $H_2{}^{15}O$ (900-1400 MBq); simultaneously with administration of the bolus data acquisition for a 5-min (14x5, 3x10, 3x20, 4x30 seconds) dynamic emission scan was started. After radioactivity decay, a four-minute dipyridamole infusion (0.56mg/kg during a 4-min period) was admistered. Two minutes after the end of the dipyridamole infusion the dynamic imaging study was repeated in the same manner as the first one.

Renal perfusion. Renal perfusion was measured with an intravenous bolus of $H_2{}^{15}O$ (900-1400 MBq); simultaneously data acquisition for a a 4-min (15x4, 5x10 second) dynamic emission scan was started. After radioactivity decay, a 5-minute (0.5-1.0 mg) enalapril infusion was admistered. The perfusion study was repeated in the same manner as the first 20, 40 and 60 minutes after the enalapril infusion.

5.1.3 PET image processing

Reconstruction. All PET data were corrected for dead time, decay and measured photon attenuation. Images with a matrix size on 128 x 128 were produced using the MRP OSEM reconstruction algorithm.

Regions of interest. For the myocardium PET studies, regions of interest (ROIs) were drawn on the left ventricle (LV) myocardium on average over four midventricular transaxial planes covering the septum, the anterior wall, the lateral wall and the whole LV myocardium. The ROIs outlined in the baseline images were copied to the images obtained after dipyridamole administration. For the renal perfusion PET studies, ROIs were drawn for the whole cortical region of the kidneys on summed reconstructed image on an average of six coronal planes, and were copied to the images obtained after enalapril administration.

5.1.4 Calculation of myocardial perfusion

Regional myocardial perfusion (ml/g tissue per min) was calculated according to a single compartment model (Iida et al. 1995). An LV cavity ROI was drawn and used as the input function for determination of the LV time-activity curve (Iida et al. 1992). To enhance the accuracy of the measurements the mean blood flow values at rest and after dipyridamole were calculated and used for further analysis. Myocardial perfusion reserve was defined as the ratio of perfusion after dipyridamole infusion to resting perfusion.

5.1.5 Calculation of renal perfusion

For the calculation of renal perfusion from the PET study, the input function was estimated using average time activity curve (TAC) from descending aorta cavity ROIs (Juillard et al. 2002) drawn on average in three planes.

The delay between renal and aorta TACs was corrected, but due to the large size of aorta, recovery correction was not necessary. Renal perfusion images were generated from the reconstructed dynamic image and the obtained input function by a basis function method assuming a single-tissue compartment model (Kudomi et al. 2009). The renal perfusion was represented by the clearance rate (k_2) multiplied with physiological partition coefficient, i.e., pphys=0.94 ml/g . Mean renal perfusion values were obtained from the renal perfusion images using ROIs drawn on sum images.

5.2 Ultrasound studies

5.2.1 Echocardiography

All measurements were made with the Acuson Sequoia C512 equipment (Siemens, California, USA). The same experienced investigator performed all measurements blinded to the clinical status of the patients. M-mode measurements of the left ventricle (LV) were obtained using guidance by two-dimensional echocardiography at end-systole and end-diastole, as recommended by the American Society of Echocardiography (Sahn et al. 1978). LV mass was calculated as previously described (Devereux et al. 1986). LV mass index was calculated as LV mass in terms of grams divided by body surface area in terms of square meters. LV end-systolic and end-diastolic volumes were measured using

the modified biplane Simpson method in apical four-chamber and two-chamber views (Schiller et al.). The LV ejection fraction (LVEF) was calculated as the ratio between the systolic volume and the end-diastolic volume. In addition, left atrial dimension was measured. All echocardiographic studies were performed after the patient had rested supine for at least 10 minutes. The intraobserver variation of two-dimensional M-mode measurements and of quantitative biplane Simpsons measurements has reported to be approximately from 3 % to 5 %, and from 7 % to 10 % for interobeserver variability (Pietro et al. 1981, Shahgaldi et al. 2009).

5.2.2 Endothelial function test

All studies were performed using an Acuson Sequoia ultrasound machine with an 8/15 MHz linear array transducer. Two experienced vascular sonographers blinded to the clinical and laboratory characteristics of the study subjects performed the scans. The brachial artery diameter was measured from B-mode ultrasound images. In all studies, scans were obtained at rest and during reactive hyperaemia. The subjects laid quietly for 10 minutes before the study at stable room temperature between 20 and 25 ºC. The left brachial artery was scanned longitudinally 2-15 cm above the antecubital crease. Depth and gain settings were optimized. When a satisfactory transducer position was found, the position was marked on the skin and the arm remained in the same position throughout the study. A resting scan was performed and arterial flow velocity was measured using a Doppler signal. Increased flow was then induced by inflation of a pneumatic tourniquet placed around the forearm (distal to scanned part of the artery) to a pressure of 250 mmHg for 4.5 min, followed by pressure release (Järvisalo et al. 2000).

A second scan was taken 30-120 s after cuff deflation. The flow velocity recording was repeated during the first 15 s after the cuff was deflated. All brachial ultrasound scans were recorded on super-VHS videotapes for later analysis. The arterial diameter was measured at a fixed distance from an anatomic marker (e.g. fascial plane) using ultrasonic callipers. Measurements were taken at end-diastole (incident with the R wave on a continuously recorded ECG) from the anterior to the posterior intima lumen interface (i-line). The hyperemic diameter was recorded continuously for 2 min. Maximal FMD was defined as the greatest percentage change relative to the baseline diameter and used for analysis (peak FMD %). The interobserver variation of FMD measurements has reported to be approximately 9% (Järvisalo et al. 1999).

5.3 Biochemical analyses

Plasma creatinine was measured with a specific enzymatic colorimetric assay (Modular P800, Roche Diagnostics GmbH, Mannheim, Germany). The assessment of renal function was based on the estimated glomerular filtration rate (GFR) equation from the modification of diet in renal disease (MDRD) study (Levey et al. 1999). Blood hemoglobin was analyzed with an automated Advia 120 analyzer (Siemens Healtcare Diagnostics) or with a Sysmex XE-2100 analyzer (Sysmex, Roche). Plasma glucose, plasma total

cholesterol, HDL cholesterol and triglycerides were measured with an enzymatic colorimetric assay (Modular P800, Roche Diagnostics GmbH, Mannheim, Germany). The plasma LDL cholesterol concentration was calculated using the Friedewald formula. Also plasma phosphate and total calcium were measured colorimetrically using Modular P800 (Roche Diagnostics GmbH, Mannheim, Germany). Parathyroid hormone (PTH) values were measured with either immunoradiometrically (N-tact PTH IRMA Kit, Incstar Corporation, Stillwater, MN, USA) or immunochemiluminometrically (ECLIA; Modular E170, Roche Diagnostic GmbH, Mannheim, Germany). Plasma B-type N-terminal propeptide (proBNP) was measured with Roche's immunochemiluminometric assay using an automated analyzer (Modular E 170, Roche Diagnostic GmbH, Mannheim, Germany). High-sensitive C-reactive protein was analyzed immunonephelometrically (CardioPhase hsCRP, Dade Behring; Behring Nephelometer II analyzer, Siemens Healtcare Diagnostics, Inc., Siemens Aktiengesellscahft, Munich, Germany) and plasma cardiac troponin t (Tnt) immunochemiluminometrically (ECLIA; Modular E 170, Roche Diagnostics GmbH, Mannheim, Germany).

5.4 Statistical analyses

Values are expressed as mean ± standard deviation. All statistical analyses were performed with the SAS statistical program package, version 9.2 (SAS Institute Inc. Gary, N.C., USA). Statistical significance was inferred at $p < 0.05$.

Study I

The normality of the values of the variables was assessed by Shapiro-Wilk's test. Student's paired t-test was used for normally distributed variables. Groups were compared either with the t-test or Kruskal-Wallis's test, as appropriate.

Study II and IV

The difference between before and after the dilatation measurements was tested with Student's paired t-test or Wilcoxon's signed rank test, as appropriate. Groups were compared with the two-sample t-test or Mann-Whitney's U-test, as appropriate. Correlations were calculated using Pearson's correlation coefficients.

Study III

Student's paired t-test was used for comparison between the physiological states.

6 RESULTS

6.1 Myocardial perfusion, coronary flow reserve and peripheral endothelial function in patients with chronic kidney disease (I)

6.1.1 Myocardial perfusion and coronary flow reserve

Myocardial perfusion corrected for rate pressure product (RPP) was at baseline higher in patients with CKD than in healthy controls. The increase in baseline perfusion was statistically significant as compared to healthy controls, $p < 0.001$, and correlated with eGFR (Spearman's correlation coefficient -0.63, $p = 0.0001$) (Table 6.1, Figure 6.1). The hyperemic myocardial perfusion was not different between the groups and thus CFR was similar in all groups (Table 6.1, Figure 6.2). However, there was a tendency to diminishing CFR as the stage of CKD progressed.

Table 6.1. Myocardial blood flow and perfusion reserve in patients with chronic kidney disease (CKD) and in healthy controls.

Group	N	Blood flow at rest (ml/min/g tissue)	Blood flow at stress (ml/min/g tissue)	Coronary flow Reserve (CFR)
Controls	10	0.87 ± 0.14	2.47 ± 0.85	2.93 ± 1.05
CKD 3	5	1.18 ± 0.18 *	3.30 ± 1.28	3.21 ± 1.31
CKD 4	11	1.25 ± 0.32 *	3.17 ± 1.21	2.67 ± 1.06
CKD 5	6	1.27 ± 0.19 *	2.61 ± 1.04	2.12 ± 0.82

* P < 0.001 Controls vs. CKD 3 to 5, Kruskal-Wallis's test.

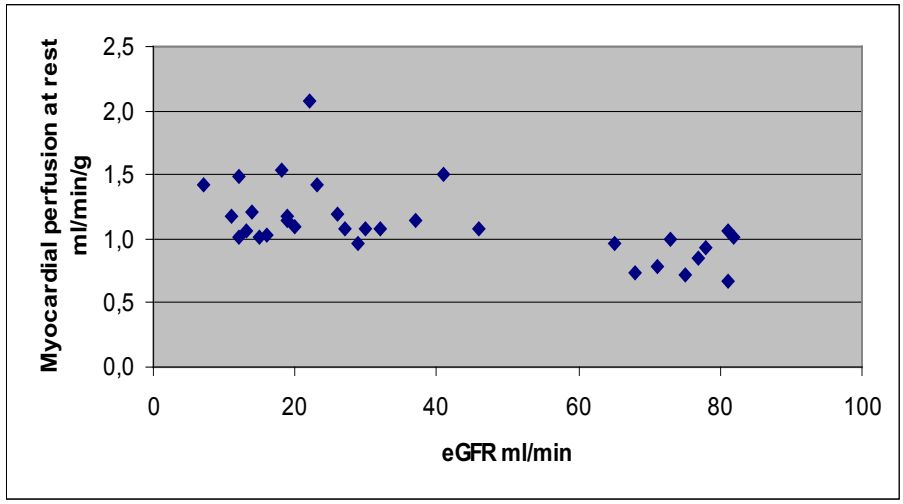

Figure 6.1. Baseline myocardial perfusion (ml/min/g) in relation to estimated glomerular filtration rate (GFR) (ml/min). Spearman's correlation coefficient – 0.63, P=0.0001.

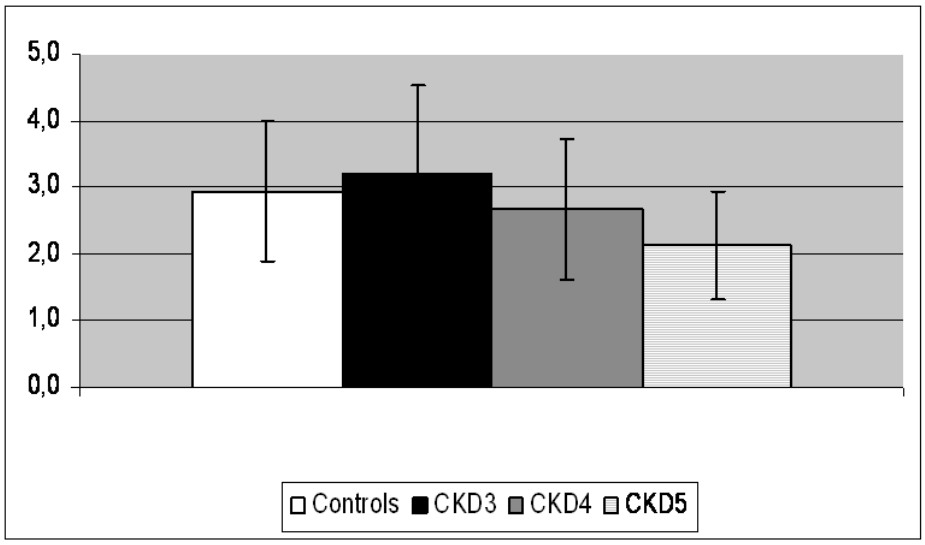

Figure 6.2. Coronary flow reserve in relation to the stage of chronic kidney disease (CKD).

The heart rate (HR) increased significantly at maximal vasodilatation in all CKD patients and controls ($p < 0.05$) (Table 6.2). There was no difference in blood pressure levels between the groups neither at rest nor during maximal vasodilatation (Table 6.2).

Table 6.2. Hemodynamic findings during PET study

Group	N	HR (bpm) at rest	HR (bpm) at max	MAP (mmHg) at rest	MAP (mmHg) at max
Controls	10	57 ± 6	77 ± 12 *	101 ± 8	98 ± 9
CKD 3	5	63 ± 11	84 ± 5 *	97 ± 4	97 ± 3
CKD 4	11	64 ± 10	86 ± 7 *	103 ± 7	102 ± 9
CKD 5	6	63 ± 7	78 ± 10 *	94 ± 9	92 ± 13

* $P < 0.05$ HR at maximal vasodilatation compared to resting values in all groups, Student's paired t-test. PET=positron emission tomography, CKD=chronic kidney disease, HR=heart rate, MAP=mean arterial pressure.

6.1.2 Structural echocardiographic and biochemical variables

Echocardiography was performed on 18 of 22 patients and all controls. The data was not available for four patients, one from the CKD 4 group and three from the CKD 3 group. According to the ECG, none of these four patients had LVH. LVM (pooled CKD 3 to 5 vs. controls, 223 ± 57 vs. 234 ± 52 g, NS) and LVMI (117 ± 22 g/m^2 in controls vs. 114 ± 26 g/m^2 in CKD patients, NS) were similar in patients and controls (Figure 6.3). The left atrium (LA) diameter was 35 ± 3 mm in healthy controls and 39 ± 4 in CKD patients, $p < 0.01$.

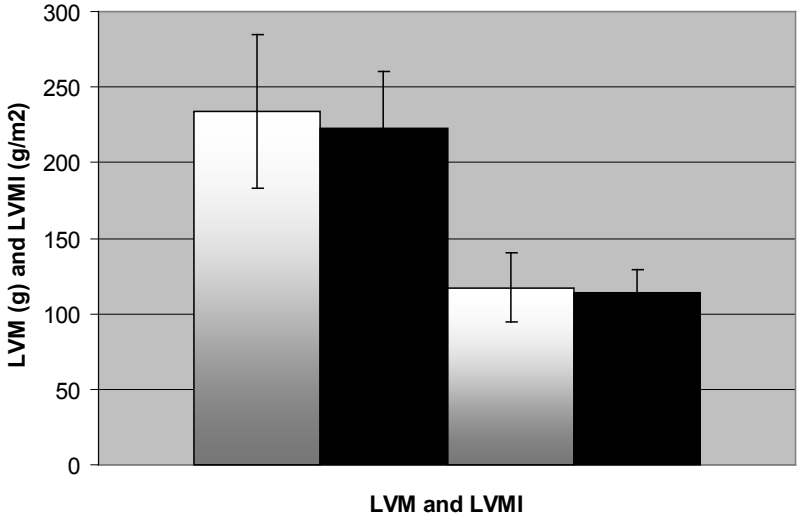

Figure 6.3. Left ventricular mass (LVM) and left ventricular mass index (LVMI) in healthy controls (gray) and in patients with chronic kidney disease (black), difference not statistically significant.

The blood hemoglobin (B-Hb) level was lower in patients with CKD than in controls ($p < 0.05$). The WHO criteria for anemia (B-Hb < 130 g/l in men and < 120 g/l in women) were fulfilled in one patient in the CKD 3 group, in three patients in the CKD 4 group and in all six patients in the CKD 5 group. The hemoglobin value did not correlate with myocardial perfusion values. Only three patients used erythropoetin, one in the CKD 4 group (erythropoetin β 4000 IU per 10 days) and two in the CKD 5 group (one with erythropoetin β 4000 IU per 10 days and the other with darbepoetin 20 μg per week).

The total cholesterol concentration was higher in the CKD 3 group than in the other groups. In the CKD 5 group, HDL cholesterol and total cholesterol were lower ($p < 0.05$) and the use of statins more frequent than in the other groups. The calcium phophorus product was higher in the CKD 5 group (Table 6.3). It did not correlate with myocardial perfusion values.

High sensitivity CRP was clearly higher in CKD patients as compared to controls, but this trend was not statistically significant (4.3 ± 6.0 vs. 1.2 ± 0.5). It did not correlate with myocardial blood flow. Brain natriuretic peptide (proBNP) levels were higher in CKD patients than in healthy controls and there was statistically significant negative correlation between proBNP levels and eGFR (Spearman correlation coefficient -0.79, $p < 0.0001$).

Table 6.3. Baseline characteristics of the subjects

	CKD 3 N=5	CKD 4 N=11	CKD 5 N=6	Controls N=10
fP-Chol mmol/l	6.1±0.7	5.5±0.8	4.2±0.3 *	5.3±0.9
fP-HDL mmol/l	2.0±0.7	1.5±0.3	1.1±0.4	1.6±0.3
fP-Tg mmol/l	1.7±0.7	1.6±0.5	2.4±1.8	1.1±0.4
fP-LDL mmol/l	3.4±0.4	3.3±0.6	2.3±0.1 *	3.3±0.8
Ca x Pi $mmol^2/l^2$	2.0±0.3	2.4±0.3	3.8±1.3 *	2.3±0.5
B-Hb g/l	128±9	129±21	110±7 *	143±10

* p < 0.05 controls vs. CKD 5, Mann-Whitney's U-test.
CKD=chronic kidney disease, Chol=cholesterol, HDL=high density lipoprotein, LDL=low density lipoprotein, Tg=triglycerides, Ca=calcium, Pi=phosphorus, Hb=hemoglobin

6.1.3 Peripheral endothelial function

The brachial artery diameter at rest was comparable in all groups. The brachial artery FMD response was higher in healthy controls than CKD patients. There were no statistically significant differences between the three CKD groups, although there was a trend towards progressively lower values as the stage of CKD progressed (Table 6.4).

Table 6.4. Brachial artery flow mediated peak dilatation

	Controls	CKD 3	CKD 4	CKD 5
Resting brachial artery diameter (mm)	3.6 ± 0.5	3.3 ± 0.6	3.7 ± 0.6	3.4 ± 1.2
FMD (%)	9.6 ± 2.5	6.0 ± 5.0 *	5.7 ± 4.6 *	5.6 ± 3.2*

* P < 0.03 Controls vs. CKD 3 to 5, Mann-Whitney's U-test.
CKD=chronic kidney disease, FMD=flow-mediated dilatation

6.2 Myocardial perfusion, coronary flow reserve and peripheral endothelial function in patients with atherosclerotic renovascular disease and the effect of renal artery stenosis revascularization (II)

6.2.1 Myocardial perfusion and coronary flow reserve

All 18 ARVD patients had RPP corrected global (whole left ventricle) myocardial perfusion at rest within the normal range. Only one patient of the whole study group had completely normal hyperemic myocardial perfusion. In 11 of the 15 patients studied for CFR, dipyridamole-induced hyperemic myocardial perfusion was globally abnormally low (<2.5 ml/g/min), suggesting diffuse CAD or microvascular disease. Of the 4 patients with normal global CFR, 3 had regional perfusion abnormalities during stress, suggesting obstructive coronary stenosis in the corresponding vessels. Neither stress myocardial

perfusion nor CFR changed after RAS dilatation in the group as a whole (Table 6.5). The basal flow value was decreased after the procedure ($P < 0.05$) only in patients with unilateral RAS. Myocardial perfusion values did not correlate with eGFR, cholesterol values or the calcium-phosphorus product.

Table 6.5. Myocardial perfusion and coronary flow reserve

		Mean myocardial perfusion at rest (ml/min/g tissue)			*Mean myocardial perfusion at stress (ml/min/g tissue)*			*Mean myocardial perfusion reserve*		
Group	**eGFR**	**N**	**Baseline**	**Follow-up**	**N**	**Baseline**	**Follow-up**	**N**	**Baseline**	**Follow-up**
ARVD	58±22	18	1.20±0.35	1.08±0.32	15	2.24±0.73	2.07±0.80	15	2.0±0.6	2.0±0.6
Unilat RAS	62±24	9	1.27±0.39	1.05±0.27*	7	2.26±0.37	2.33±0.69	7	2.0±0.6	2.3±0.7
Bilat RAS	54±21	9	1.14±0.32	1.11±0.36	8	2.23±1.01	1.84±0.85	8	2.1±0.6	1.8±0.4

* $P < 0.05$, Change in myocardial blood flow at rest in unilateral RAS patients, Student's paired t-test.
ARVD=atherosclerotic renovascular disease, RAS=renal artery stenosis, eGFR=estimated glomerular filtration rate.

6.2.2 Peripheral endothelial function

The diameter of the brachial artery at rest was similar at baseline and after revascularization in all patients (Table 6.6). All patients had a low brachial artery FMD response at baseline (3.6 ± 4.1 %), and there was no statistically significant difference between patients with unilateral and bilateral RAS. Revascularization markedly improved FMD in patients with bilateral RAS, but not in patients with unilateral RAS (Table 6.6). There was no statistically significant difference in FMD between diabetic and non-diabetic patients.

Table 6.6. Brachial artery flow mediated dilatation

	ARVD, n=15		**Unilateral RAS n=6**		**Bilateral RAS n=9**	
	Baseline	**Follow-up**	**Baseline**	**Follow-up**	**Baseline**	**Follow-up**
Resting brachial artery diameter, mm	3.8±0.6	3.6±0.7	3.7±0.6	3.7±0.8	3.9±0.6	3.5±0.6
FMD, %	3.6±4.1	6.2±4.7	4.1±2.7	4.2±4.6	3.3±5.0	7.5±4.6 *

* $P < 0.05$, FMD % bilateral RAS, baseline vs. follow-up, Student's paired t-test.
ARVD=atherosclerotic renovascular disease, RAS=renal artery stenosis, FMD=flow-mediated dilatation.

6.2.3 Structural echocardiographic parameters and biochemical variables

LVMI decreased after revascularization ($P < 0.01$). The left ventricular end-diastolic diameter (LVEDD) decreased in patients with bilateral RAS, but not in patients with

unilateral RAS. The left ventricular ejection fraction (LVEF) and the left atrial (LA) diameter did not change statistically significantly after dilatation of RAS (Table 6.7).

Table 6.7. Baseline and follow-up echocardiographic data

	ARVD N=13		Unilateral RAS N=4		Bilateral RAS N=9	
	Baseline	**Follow-up**	**Baseline**	**Follow-up**	**Baseline**	**Follow-up**
LVEDD mm	52±5	51±5	50±6	50±5	53±5	51±6†
LVM g	259±51	236±50*	230±63	219±63	272±43	244±46*
LVMI g/m^2	143±22	130±23*	122±19†	114±20	152±17†	138±22†
EF %	59±9	59±9	66±4	66±5	57±9	56±9
LA mm	46±6	46±7	41±7	42±7	48±5	48±7

* $P < 0.01$ paired t-test, Change in LVM and LVMI in whole group. Change in LVM in bilateral RAS patients. † $P < 0.05$ paired t-test, Change in LVMI and LVEDD in bilateral RAS patients. Unilateral vs. bilateral RAS populations at the baseline, two-sample t-test for LVMI.
ARVD=atherosclerotic renovascular disease, RAS=renal artery stenosis, LVEDD=left ventricular end-diastolic diameter, LVM=left ventricular mass, LVMI=left ventricular mass index, EF=ejection fraction, LA=left atrium

Brain natriuretic peptide (proBNP) levels were higher in patienst with bilateral than unilateral RAS before revascularization ($P < 0.05$). The change in proBNP levels was not statistically significantly different after dilatation of the RAS compared to the baseline. High-sensitive CRP (hsCRP) and cardiac troponin T (TnT) levels were above ther reference values in ARVD patients, but revascularization did not affect these levels (Table 6.8).

6.2.4 Baseline and follow-up demographic and clinical data

The systolic blood pressure was higher in patients with bilateral RAS at baseline than in patients with unilateral RAS ($P < 0.05$). Systolic and mean arterial blood pressures decreased significantly after revascularization in patients with bilateral RAS ($P < 0.05$), whereas the blood pressure of patients with unilateral RAS was unaffected by the revascularization (Table 6.8).

At the baseline, bilateral RAS patients had an average of one medication more than unilateral RAS patients. After the dilatation, the number of antihypertensive medications was same in unilateral RAS patients, while in bilateral RAS patients, there was a nonstatistically significant ($P=0.07$) decrease in the number of antihypertensive medications (Table 6.8).

Estimated GFR remained stable after the dilatation of RAS, both in patients with unilateral and bilateral disease (Table 6.8). The eGFR was significantly lower in the 12 diabetic patients than in the non-diabetic patients, both at the baseline and the follow-up (50 ± 20 vs. 71 ± 20 ml/min, baseline, and 50 ± 18 vs. 77 ± 23 ml/min, follow-up, $P < 0.05$).

Table 6.8. Baseline and follow-up data of the eGFR, blood pressure and biomarkers

	ARVD (N=19)		Unilateral RAS (N=9)		Bilateral RAS (N=10)	
	Baseline	**Follow-up**	**Baseline**	**Follow-up**	**Baseline**	**Follow-up**
eGFR ml/min	58±22	60±24	62±24	65±28	54±21	56±20
sBP mmHg	199±27	192±23	186±16*	195±22	212±29*	191±25†
dBP mmHg	90±17	91±15	85±11	90±16	95±21	91±16
MAP mmHg	128±20	125±16	123±16	125±17	134±22	124±17*
Number of antihypertensive medication	4.3±1.3	3.6±1.4	3.7±1.2	3.7±1.3	4.8±1.2	3.6±1.6
proBNP	2015±3286	1205±1627	505±417*	364±210	3526±4213*	1927±1985
hsCRP	3.7±7.8	5.3±10.3	1.9±1.3	8.6±13.5	5.1±10.4	1.6±1.1
TnT	0.02±0.02	0.02±0.03	0.02±0.03	0.01±0.01	0.02±0.02	0.03±0.04

* $P < 0.05$, unilateral vs. bilateral RAS population at baseline, two-sample t-test for systolic blood pressure and Mann-Whitney's U-test for proBNP. Change in mean arterial blood pressure after dilatation in bilateral RAS population, paired t-test.
† $P < 0.01$, change in systolic blood pressure after dilatation in bilateral RAS population, paired t-test.
ARVD=atherosclerotic renovascular disease, RAS=renal artery stenosis, eGFR=estimated glomerular filtration rate, sBP=systolic blood pressure, dBP=diastolic blood pressure, MAP=mean arterial pressure, proBNP=brain natriuretic peptide, hsCRP=high sensitive C - reactive protein, TnT=cardiac troponin T

6.3 The calculation of renal perfusion with PET and $H_2{}^{15}O$ (III)

The accuracy of the present basis function method (BFM) was consistent with nonlinear least-squares fitting (NLF), i.e., $K_{1,BFM}=0.93K_{1,\ NLF}-0.11$ ml/min/g ($r=0.80$, $p<0.001$), $k_{2,BFM}=0.96k_{2,\ NLF}-0.13$ ml/min/g ($r=0.77$, $p<0.001$) and $V_{A,\ BFM}=0.92V_{A,\ NLF}-0.00$ ml/ml ($r=0.97$, $p<0.001$).

(K_1= uptake rate constant of $H_2{}^{15}O$ of the kidney, ml/min/g, k_2=clearance rate constant of $H_2{}^{15}O$ of the kidney, V_A= activity concentration in the arterial vascular space, ml/ml).

The fitted curve by the present model estimating K_1, k_2 and V_A fitted better than the two other models fixing the partition coefficient of water (p, ml/g) ($=K_1/k_2$) or V_A. Also, the Akaike information criterion (AIC) values from three parameter fitting were the smallest for all subjects except two values for two parameter fitting fixing V_A in patient 2 and fixing p in patient 3, although some AIC values were similar (Table 6.9). These results show that the present method with three parameter fitting is feasible for computing RBF.

Table 6.9. Akaike information criterion (AIC) values for the models

Subject	3-parameters	*p*-fixed	V_A-fixed (0.15)	V_A-ignored
1	484. ± 20.	519. ± 28.	499. ± 15.	494. ± 15.
2	474. ± 9.	486. ± 14.	474. ± 9.	477. ± 8.
3	525. ± 12.	523. ± 8.3.	527. ± 10.	527. ± 7.
4	483. ± 14.	497. ± 21.	501. ± 12.	506. ± 13.
5	497. ± 18.	502. ± 19.	508. ± 32.	499. ± 13.
6	496. ± 11.	507. ± 14.	500. ± 9.	497. ± 9.

3-parameters: K_1, k_2 and V_A were computed, p-fixed: K_1 and V_A was computed with fixing k_2 such that p=K_1/k_2 =0.35 ml/g, V_A-fixed: K_1 and k_2 were computed with fixing V_A at 0.15 ml/g, V_A-ignored: K_1 and k_2 were computed without taking into account V_A.
K_1= uptake rate constant of $H_2{}^{15}O$ of the kidney, ml/min/g, k_2=clearance rate constant of $H_2{}^{15}O$ of the kidney, V_A= activity concentration in the arterial vascular space, ml/ml

Values of K_1, $k_2 \cdot p_{phys}$ and V_A were obtained for the whole renal region and cortical region (Table 7.0). The K_1 values were smaller than $k_2 \cdot p_{phys}$ values and their ratio ranged from 0.35 to 0.45, suggesting that K_1 values underestimated RBF due to the partial volume effect. Both K_1 and $k_2 \cdot p_{phys}$ were not significantly different between the resting and stimulated conditions for the whole renal region and the cortical region, respectively, although the value of V_A was higher under the stimulated conditions than under the basal conditions. The value obtained for GFR was 78±4 ml/min, corresponding to a clearance rate of 0.37 ± 0.02 ml/min/g and to 9.6 % of the k_2 obtained for the cortical region under the normal conditions.

Table 7.0. Values of K_1, $k2 \bullet p_{phys}$ and V_A (n=6) in whole and cortical region calculated by the present method for baseline and enalapril administrated conditions.

	K_1 (ml/min/g)	$k_2 \bullet p_{phys}$ (ml/min/g)	V_A (ml/ml)	*GFR* (ml/min/g)
Whole region				
Baseline	1.09 ± 0.33	3.11 ± 1.48	0.15 ± 0.09	0.35 ± 2 #
Enalapril	1.03 ± 0.44	2.55 ± 1.29	0.16 ± 0.14	
Cortical region				
Baseline	1.57 ± 0.60*	3.64 ± 2.15*	0.18 ± 0.12*	
Enalapril	1.42 ± 0.39*	3.55 ± 1.64*	0.25 ± 0.14*	

No significant difference between baseline and stimulated conditions. * Significant difference between whole and cortical regions. # kidney weight 300 g and cortex ratio 70 % were assumed.
K_1=uptake rate constant of $H_2{}^{15}O$ of the kidney, k_2=clearance rate constant of $H_2{}^{15}O$ of the kidney, V_A= activity concentration in the arterial vascular space, GFR=glomerular filtration rate

6.4 Effect of revascularization of renal arterial stenosis on renal perfusion in patients with atherosclerotic renovascular disease (IV)

6.4.1 Renal perfusion

The mean cortical renal perfusion (both kidneys) in ARVD patients was 1.49 ± 0.5 ml/min/g tissue at baseline. It did not change statistically significantly after RAS revascularization, 1.40 ± 0.5 ml/min/g tissue. For comparison, the cortical flow in healthy volunteers was 1.82 ± 0.3 ml/min/g tissue, and for CKD patients 1.26 ± 0.5 ml/min/g tissue, P=NS, Figure 6.4.

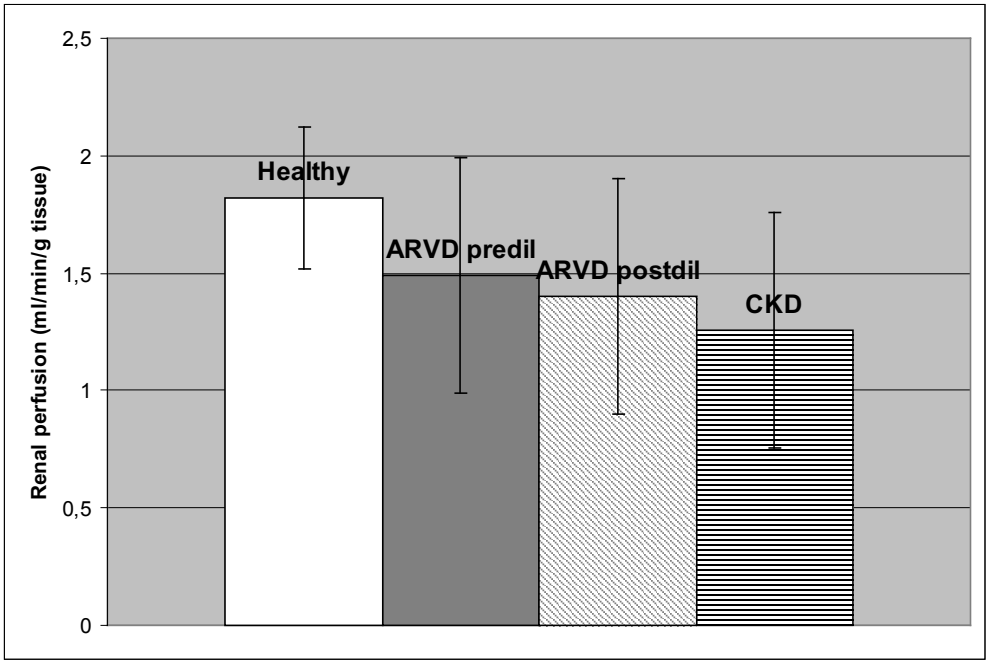

Figure 6.4. Mean cortical renal perfusion (both kidneys) in healthy volunteers, in chronic kidney disease (CKD) patients and in atherosclerotic renovascular disease (ARVD) patients before (predil) and after (postdil) dilatation.

The cortical renal perfusion in stenosed kidneys was highly correlated with the degree of RAS before revascularization, $p < 0.01$ (Figure 6.5). The flow did not correlate with eGFR in ARVD patients. The mean cortical perfusion in the 23 stenosed kidneys was 1.43 ± 0.4 ml/min/g tissue before dilatation and 1.29 ± 0.5 ml/min/g tissue after the RAS revascularization, p=NS (Figure 6.6). In unilateral RAS patients, the mean cortical perfusion in stenosed kidneys was 1.5 ± 0.6 ml/min/g tissue, and in the contralateral kidney 1.8 ± 0.7 ml/min/g tissue, p=NS. After revascularization, there was no statistically significant change in flow values (Figure 6.7).

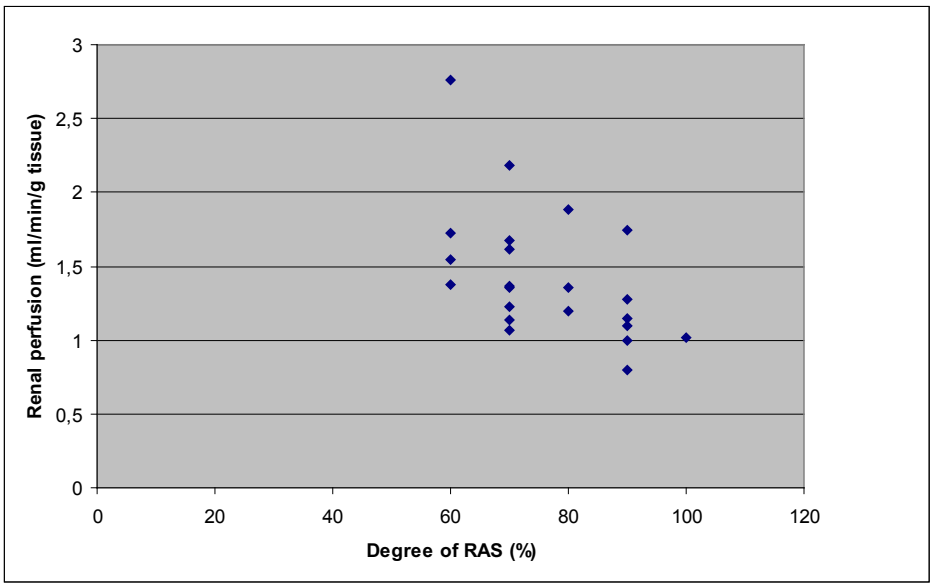

Figure 6.5. Correlation between of renal artery stenosis (RAS) and with renal perfusion in stenosed kidneys, N=23. Spearman's correlation coefficient -0.56, $p < 0.01$.

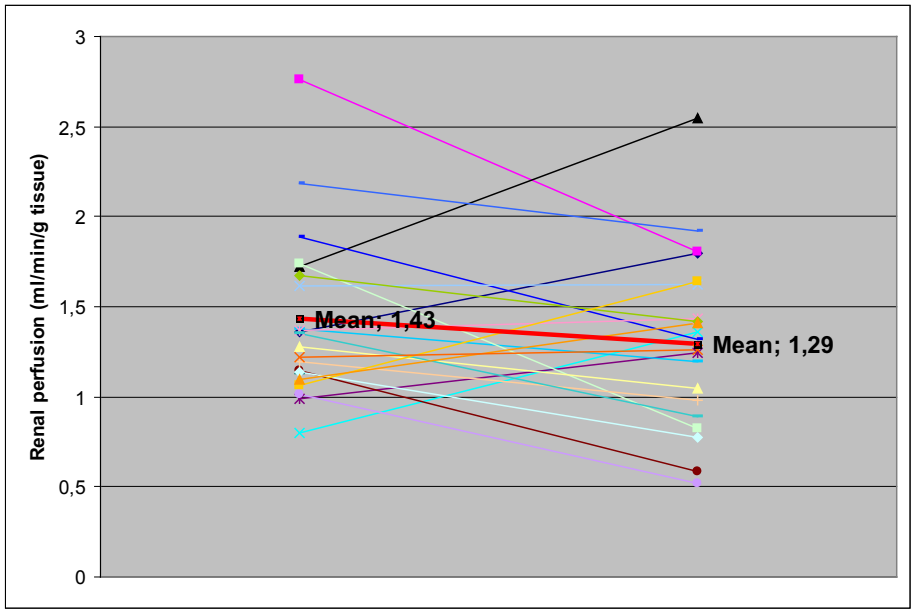

Figure 6.6. Mean cortical perfusion in stenosed kidneys (N=23) before and after renal artery stenosis dilatation, p=NS.

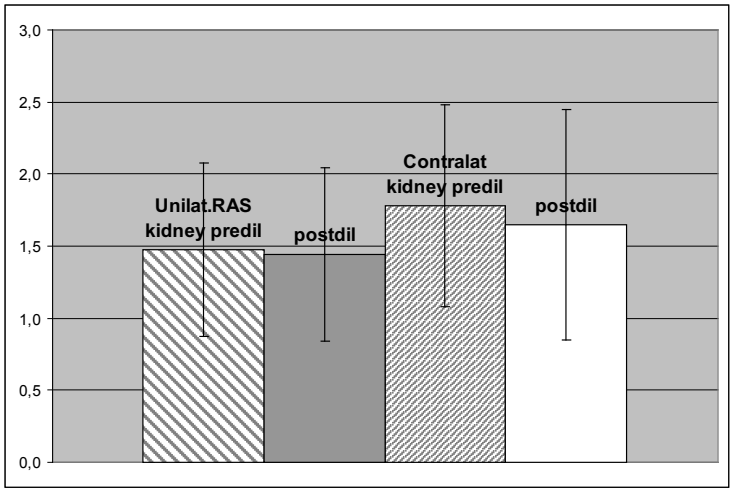

Figure 6.7. Renal cortical perfusion in unilateral renal artery stenosis (RAS) kidneys and in contralateral kidneys (N=8), both before and after RAS dilatation, P=NS.

The cortical renal perfusion was lower in the 9 diabetic patients than in the 8 non-diabetic ARVD patients, both at baseline and after the dilatation (1.28 ± 0.3 vs.1.70 ± 0.5 ml/min/g tissue, baseline, P=0.07 and 1.29 ± 0.3 vs.1.56 ± 0.6 ml/min/g tissue, follow-up, P=0.28). However, the change in flow was not statistically significant. There was no correlation between eGFR and renal perfusion neither among the diabetic nor the non-diabetic patients.

6.4.2 Baseline and follow-up demographic and clinical data

Although the average blood pressure of the ARVD patients did not decrease after revascularization, there was a statistically nonsignificant (P=0.07) trend toward a reduction in the number of antihypertensive drugs required by the patients (Table 7.1).

The estimated GFR remained stable after dilatation (Table 7.1). The eGFR was significantly lower in the 9 diabetic patients than in the 8 non-diabetic patients, both at baseline and follow-up (45 ± 18 vs. 68 ± 22 ml/min, baseline, and 50 ± 22 vs. 70 ± 25 ml/min, follow-up, P < 0.05).

Table 7.1. Baseline and follow-up data of the eGFR, blood pressure, and number of antihypertensive medications

	ARVD (N=17)		CKD (N=7)	Healthy (N=10)
	Baseline	Follow-up	Baseline	Baseline
eGFR ml/min	56±23	59±25	22±12	75±6
sBP mmHg	193±26	185±25	189±32	130±11
dBP mmHg	89±13	91±15	81±11	79±6
Number of antihypertensive medication	4.1±1.2	3.5±1.3	3.1±1.3	0

ARVD=atherosclerotic renovascular disease, CKD=chronic kidney disease, eGFR=estimated glomerular filtration rate, sBP=systolic blood pressure, dBP=diastolic blood pressure

7 DISCUSSION

7.1 Myocardial perfusion and coronary flow reserve in patients with chronic kidney disease and in patients with atherosclerotic renovascular disease (I, II)

7.1.1 Methodological considerations

Myocardial perfusion in CKD patients has been mostly studied with single photon emission computed tomography (SPECT). However, PET offers several advantages over SPECT. At best, SPECT is a semiquantitative method for assessment of regional myocardial perfusion defects. The noninvasive evaluation and quantification of myocardial perfusion and perfusion reserve with PET extends the scope of myocardial perfusion imaging from detection of end-stage, advance and flow-limiting, epicardial CAD to early stages of atherosclerosis or microvascular dysfunction.

The quantification of myocardial perfusion in absolute units has been validated for PET and ^{15}O-water against independent microsphere blood flow measurements in animals over a flow range of 0.5 to 5.0 ml/min/g tissue (Araujo et al. 1991). In humans, ^{15}O-water-PET studies provide similar absolute myocardial perfusion information over a wide range of blood flows (Bol et al. 1993). The use of ^{15}O-water as a tracer is advantageous because it is metabolically inert and freely diffusible across capillary and sarcolemmal membranes. It equilibrates rapidly between the vascular and extravascular spaces and its uptake by the heart does not vary by variations of flow rate. The short half-life (123 s) of ^{15}O-water allows repetitive myocardial perfusion measurements at short intervals.

Coronary flow reserve (CFR), the ratio between myocardial perfusion during near-maximal coronary vasodilatation and basal myocardial perfusion, is an integrated measure of flow through both the large epicardial coronary arteries and the microcirculation. CFR has been proposed as an indirect parameter for evaluation of the function of the coronary circulation. In the absence of coronary stenoses, a reduced CFR reflects dysfunction of the coronary microcirculation. Hyperemic myocardial perfusion can be induced by transient coronary arterial occlusion and reopening, exercise, pacing and by injecting vasodilating agents. Pharmacological stimulation is the most practical method to induce coronary vasodilatation. Depending on the agent used, the flow response may reflect vascular smooth muscle tone, endothelial-mediated relaxation or resistance of microvascular structures. Dipyridamole and adenosine are effective, safe and easy to use, and are therefore the most widely used agents for inducing coronary vasodilatation in noninvasive studies. Dipyridamole increases interstitial adenosine concentrations in vascular smooth muscle by inhibiting the adenosine deaminase activity and thus leads to relaxation of coronary resistance vessels (Opie 1998b). Hyperemia induced by dipyridamole and adenosine has been used as a measure of coronary vasoreactivity. Shear stress induced by increased

flow releases endothelium-derived relaxing factors from endothelial cells (Rubanyi, Romero and Vanhoutte 1986) and this effect is more prominent in vessels with preserved endothelial function. Both adenosine and dipyridamole induced vasodilation have been found to relate to endothelium-dependent vasodilation (Leipert, Becker and Gerlach 1992, Mayhan 1992). Thus, vasodilation induced by both of these agents can be considered as an integrating measure of endothelial function and vascular smooth muscle relaxation.

In resting conditions basal myocardial perfusion is tightly coupled to myocardial work (oxygen consumption). Thus, in myocardial perfusion studies (I, II), basal myocardial perfusion was corrected for rate pressure product (RPP, an index of myocardial work). On the other hand, during dipyridamole-stimulated hyperemia, myocardial perfusion is coupled to blood pressure, and perfusion values were corrected for mean arterial pressure.

7.1.2 Myocardial perfusion and coronary flow reserve in patients with chronic kidney disease (I)

Despite the fact that even mild CKD is a strong cardiovascular risk factor (Go et al. 2004), the abnormalities in coronary function are still poorly understood. The present study examined the effect of uremia on coronary microvascular function. Non-diabetic patients with CKD but without clinical manifestation of coronary artery disease were examined in an effort to isolate the effect of uremia as such on cardiac function. The present study shows that coronary microvascular function was indeed well preserved in these patients, although there was a trend toward lower CFR-values with decreasing GFR.

As CFR is the ratio between myocardial perfusion during hyperemia and perfusion at rest, CFR may decrease either by higher perfusion during rest or by blunted perfusion during hyperemia. In CKD patients, CFR has been mainly studied with transthoracic Doppler by assessment of changes in coronary flow. According to these studies, CFR is lower in ESRD patients due to lower blood flow velocity in hyperemia than in controls (Bozbas et al. 2009, Caliskan et al. 2008, Tok et al. 2005). A similar finding has been reported also in milder stages of CKD (Bezante et al. 2009, Chade et al. 2006a). However, a major limitation of these studies is the fact that the velocity measurements do not take into account the dilatation of coronary vessel during hyperemia. Since even a small change in vessel diameter has profound effects on total flow, this makes the quantification of volume of blood flow impossible. Furthermore, the mass of myocardium supplied by the epicardial artery being measured is another unknown parameter.

This study was the first in CKD patients to examine myocardial perfusion noninvasively and quantitatively with PET. Since then, Charytan and colleges (Charytan, Schelbert and Di Carli 2010) have reported a secondary analysis of RAMPART trial (Relative and Absolute Myocardial Perfusion changes as measured by Positron Emission Tomography to Assess the Effects of ACAT [acyl–coenzyme A:cholesterol acyltransferase] Inhibition: A Double-Blind, Randomized, Controlled, Multicenter Trial), where 435 non-diabetic patients with mild renal insufficiency were studied for CFR with PET. They also reported a correlation between reduced CFR and decreasing GFR. However, statistical

significance was lost with rigorous adjustment. In that study, decreasing CFR was due to both increasing perfusion at rest and decreasing perfusion by adenosine induced hyperemia, although neither trend alone reached statistical significance.

The reasons for impaired coronary reactivity in hyperemia, on the whole, are quite well described. In CKD patients, they relate mainly to traditional CV risk factors, which are common among patients with CKD (Coresh et al. 2001). Smoking (Morita et al. 2006), hypertension, even borderline (Laine et al. 1998), diabetes (Pitkänen et al. 1998), hypercholesterolemia (Pitkänen et al. 1999) and LVH (Antony et al. 1993) all reduce CFR by reducing myocardial perfusion in hyperemia. CAD itself leads to lower perfusion in hyperemia when the epicardial coronary arteries have a greater than 40 % stenosis (Uren et al. 1994). However, the effect of antihypertensive medications on coronary function further complicates the interpretation of the effect of uremia on myocardial perfusion. ACEIs, ARBs and calcium channel blockers improve myocardial perfusion during maximal vasodilatation whereas beta blockers may reduce it (Akinboboye et al. 2002, Buus et al. 2004, Higuchi et al. 2007, Motz and Strauer 1996, Naya et al. 2007).

There are several factors that may explain the tendency towards higher myocardial perfusion at rest in patients with CKD compared to healthy controls. Uremic cardiomyopathy is thought to lead to a myocyte-to-capillary mismatch, i.e., a diminished vascular supply relative to the number and volume of functioning myocytes (Amann et al. 1992, Amann et al. 1998). Anemia is one of the typical findings in CKD patients with GFR under 40 ml/min, and it has been linked to increased resting coronary flow (Abendschein et al. 1982). Patients with CKD and patients on hemodialysis show sustained overactivity of sympathetic nervous system. Although sympathetic overactivity may lead to increased perfusion at rest due to increased cardiac work load, increased coronary vascular resistance in hypertensive patients seems also to ensue (Laine et al. 1998).

Typically CFR values below 2.5 have been regarded as impaired (Nesterov et al. 2009). In our CKD patients, despite the well preserved CFR as a whole, 35 % of patients had CFR below 2.0. In studies with ESRD patients, the proportion has been even higher, from 43 % to 90 % (Bozbas et al. 2009, Caliskan et al. 2008), whereas in patients with milder CKD, the figures have been closer to ours (Bezante et al. 2009). This implies that subclinical CAD is possible, but could not be verified, since we did not perform coronary angiography or coronary CT on our patients. Keeping in mind the results of the PET perfusion studies, it has been shown that individuals with early stages of functional and/or structural alterations of the coronary arteries have increased long-term risk for CV events (Herzog et al. 2009, Schindler et al. 2005).

So, although the current clinical application of myocardial perfusion imaging with PET is aimed at differentiating normal vasomotor stress responses from hemodynamically significant coronary artery lesions and subclinical CAD, this technique may, in the future, help to identify individuals with an intermediate or even low CV risk. If so, these patients could benefit from early initiation of medical preventive therapy or an

intensification of such therapy (Schindler et al. 2010). Myocardial perfusion studies with PET could also prove to be a valuable tool for assessing the functional effectiveness of different treatment strategies, including as complex patients as CKD patients.

7.1.3 Myocardial perfusion and coronary flow reserve in patients with atherosclerotic renovascular disease (II)

The present study is the first and thus far the only one to address the question of myocardial perfusion in patients with ARVD.

Only one patient in the whole study group had normal hyperemic myocardial perfusion. Since the ideal cut-off for normal absolute stress perfusion using ^{15}O-water is 2.5 ml/g/min, (Nesterov et al. 2009), the absolute average value for the whole group of 2.24 ± 0.73 ml/min/g tissue is clearly reduced. It was even lower after the revascularization, 2.07 ± 0.80. However, the rate pressure product normalized myocardial perfusion at rest was in the normal range in all 18 ARVD patients.

As a consequence of decreased MBF at stress, the CFR was remarkably low, 2.0 ± 0.6 both before and after RAS revascularization. When compared to the study in patients with CKD (I), CFR was below 2 in 67 % of ARVD patients vs. 35 % in CKD patients. As CFR is an integrated measure of flow through both the large epicardial coronary arteries and the microcirculation (Kaufmann and Camici 2005), the explanation for low CRF is either microvascular dysfunction or coronary artery stenosis, or both.

As discussed earlier, epicardial coronary artery stenosis affects hyperemic myocardial perfusion once the stenosis of a coronary artery is 40 % or more. Hyperemic flow approaches unity with basal flow when stenosis is 80 % or greater (Uren et al. 1994). Since coronary imaging was not done for our patients, it is difficult to establish if the main cause for the poor CFR values of the ARVD patients is ischemic heart disease or microvascular dysfunction. However, 10 out of 19 patients with ARVD had a previous diagnosis of other manifestations of atherosclerosis than renovascular disease. In addition, in study I, in CKD patients with the same stage of renal impairment (CKD 3) as patients with ARVD, but without atherosclerotic manifestations, the hyperemic flow values were normal. This suggests that although the majority of patients with ARVD were asymptomatic they actually had significant, subclinical coronary artery disease.

The typical causes of impaired coronary microvascular function are discussed above (section 2.6.1.2, page 46-47). In this study II, LVH was present in over 80 % of patients at baseline. All patients were hypertensive, 12 out of 19 had type 2 diabetes, and 26 % of patients were smokers. Although LVMI and systolic blood pressure decreased after revascularization, especially in patients with bilateral renal artery stenosis, there was no improvement in CFR. As the revascularization of RAS did not improve coronary vascular function, impairment of CFR is a consequence of already structural changes of the coronary arteries. However, when looking at the patient population where no changes in treatment occurred other than revascularization of RAS, i.e., patients with unilateral

RAS, CFR did improve slightly, although not statistically significantly so. This was due to both to slightly better perfusion in hyperemia and to slightly decreased flow at rest. In contrats, patients with bilateral RAS and decreased blood pressure the number of antihypertensive drugs was reduced by approximately one, usually a vasodilating agent, calcium antagonist or prazosin. It is known that ACEIs, ARBs and calcium channel blockers improve myocardial perfusion during maximal vasodilatation (Akinboboye et al. 2002, Naya et al. 2007). Thus, it is possible that positive effects of decreased LVM were nullified as vasodilating medication was unintentionally withdrawn.

As the main problem in treating patients with ARVD has been the difficulty to determine the best treatment modality, measuring CFR noninvasively with PET could help to identify the patients at greatest risk for future CV events and also to assess the functional effectiveness of different treatment strategies. Substudy II implies that patients with unilateral RAS perhaps gain more from revascularization than patients with more advanced renovascular disease. However, it could reflect decreased renin angiotensin aldosterone system activation or decreased sympathetic activation in these patients. As a next step it would be valuable to examine the effects of full medical treatment on coronary function in patients with ARVD. The ongoing CORAL trial may shear more light on this matter. The primary end point of CORAL is survival free from composite cardiovascular and renal events (Cooper et al. 2006). The results from two heart substudies of ASTRAL on cardiac structure and function are also awaited (Mistry et al. 2007).

7.2 Peripheral endothelial function in patients with chronic kidney disease and in patients with atherosclerotic renovascular disease (I, II)

7.2.1 Methodological considerations

Brachial artery flow-mediated dilatation (FMD) was measured noninvasively using high-resolution ultrasound as originally described by Celermajer et al. (Celermajer et al. 1992) and used as a marker of systemic arterial endothelial function. FMD involves measurement of the change in the diameter of a conduit artery in response to increased flow, typically induced by a period of ischemia in the distal circulatory bed. The dilatation response to increased blood flow and shear stress is mainly due to increased release of endothelial NO when hyperemia is induced by arterial occlusion of a maximal duration of 5 minutes (Mullen et al. 2001). In the present study, the endothelium-dependent dilatation response was recorded continuously for 120 seconds post-occlusion, as it has previously been shown that in adults the peak FMD is recorded on average at 71 s post-occlusion (Berry, Skyrme-Jones and Meredith 2000). Maximal FMD was defined as the greatest percentage change relative to the baseline diameter and used for further analysis. The interobserver variation of FMD measurements is approximately 9% (Järvisalo et al. 1999).

FMD of brachial artery correlates with the severity of coronary atherosclerosis (Neunteufl et al. 1997), and with coronary endothelial function of the same patients (Anderson et al. 1995).

7.2.2 Peripheral endothelial function in patients with chronic kidney disease (I)

Patients with moderate to severe CKD have significantly impaired peripheral endothelial function, measured as brachial artery FMD compared to healthy controls (I). These results are concordant with previous results of Caglar et al. (Caglar et al. 2008) and with Yilmaz et al. (Yilmaz et al. 2008), who have reported that the degree of endothelial dysfunction correlates with worsening of kidney function – the higher the stage of CKD, the lower the levels of FMD. The FMD values of our patients with different stages of CKD were in the same range as reported in these studies. The absence of a negative relationship between declining GFR and FMD in the present study may be due to a small sample size. Although there was no statistically significant correlation between CFR and the stage of CKD, the trend was similar for FMD, as is to be expected from previous studies confirming the relationship between FMD and coronary endothelial function (Anderson et al. 1995).

Most traditional and non-traditional CV risk factors affect endothelial function. On the other hand, decreasing GFR is associated with an increasing prevalence of CV risk factors. Thus, it is difficult to dissect the effect of uremia itself on peripheral endothelial dysfunction. Indeed, when compared to patients of similar age with essential hypertension as in this study but with no out renal impairment, the FMD values are the same (Benndorf et al. 2007).

7.2.3 Peripheral endothelial function in patients with atherosclerotic renovascular disease (II)

This substudy II is the first study to examine peripheral endothelial function in patients with ARVD with the technique of brachial artery FMD. Compared to patients with CKD but without manifestation of atherosclerosis (I), the baseline FMD values were significantly lower in these patients with ARVD (II). In this manner, coronary endothelial function and peripheral endothelial function seem to go hand in hand, since also the CFR was on average significantly lower in patients with ARVD than in patients with CKD alone. The impact of diabetes must be considered, since 9 out of 15 patients with ARVD had type 2 diabetes. The baseline FMD values of the ARVD patients were close to those previously reported in patients with type 2 diabetes, about 4 % (Meyer et al. 2008).

Only one study has previously examined the effect of RAS dilatation on peripheral endothelial function (Higashi et al. 2002). The blood flow in the brachial artery was measured with an intra-arterial catheter at baseline and after acetylcholine infusion before and after renal artery angioplasty. The response of the blood flow in the forearm to acetylcholine was greater in healthy controls that in patients with renovascular disease at baseline. After RAS revascularization, the response of the forearm blood flow to acetylcholine rose from 19.1 ± 6.5 to 29.5 ± 7.0 (35 % increase) ml per minute per 100 ml in patients with unilateral atherosclerotic RAS (Higashi et al. 2002). In ARVD patients, in substudy II, on the whole FMD increased about 40 % after revascularization,

but the change was not statistically significant. Patients with unilateral RAS had similar FMD-values after revascularization, in contrast to the findings of Higashi and colleges (Higashi et al. 2002). On the other hand, in patients with bilateral RAS, the increase in FMD after revascularization (3.3 ± 5.0 vs. 7.5 ± 4.6) was statistically significant. This may be due to the observed decrement in brachial artery diameter after intervention, possibly brought about by decreased blood pressure and hypervolemia, which enable the peripheral vessels to dilate better upon the stimulation. Although the sample size is small and the standard deviations are large, the observation seems to be logical. The change in FMD in patients with bilateral RAS following revascularization was clear and statistically significant, which was not the case for patients with unilateral RAS.

Patients with unilateral RAS had no change in their blood pressure or in the amount of antihypertensive medication they used (II). In patients with bilateral RAS the number of antihypertensive drugs was reduced with approximately by one, usually a vasodilating agent. It has been proven that vasodilating agents, as well as ACEI or ARBs, improve the FMD capacity at least in essential hypertension (Yilmaz et al. 2010). Despite a reduction in the use of vasodialting agents in patients with bilateral RAS, FMD improved.

7.3 Renal perfusion in healthy volunteers and in patients with atherosclerotic renovascular disease (III, IV)

7.3.1 Methodological considerations

Renal perfusion is an important parameter in the evaluation of the renal function that remains difficult to measure. To date, there is no gold standard method to noninvasively quantify perfusion of a single kidney.

Magnetic resonance imaging (MRI) and computed tomography (CT) provide high spatial and temporal resolution which enables the measurements of cortical as well as medullary renal perfusion. Dynamic MR perfusion imaging is the most popular current method in clinical routine, but this method is associated with a risk of adverse events caused by the contrast agent, gadolinium. Firstly, many patients with ARVD have CKD and eGFR below 30 ml/min. For these patients it is inadvisable to use gadolinium. Secondly, the reported perfusion values are only semiquantitative, since gadolinium is fully filtrated in glomeruli. Partly the same problem is associated with CT and iodinated contrast agents: when eGFR falls below 30 ml/min the risk of contrast-induced nephropathy rises markedly. CT has been extensively used to study renal perfusion, especially in animal models (Chade et al. 2003, Lerman et al. 1999, Rodriguez-Porcel et al. 2001). However, there are only few studies in humans (Gloviczki et al. 2010, Lerman et al. 1996b, Paul et al. 2001).

A benefit of positron emission tomography (PET) studies is their ability to quantify renal perfusion in absolute terms (Alpert et al. 2002, Middlekauff et al. 1997). $H_2{}^{15}O$-PET is sensitive enough to measure even acute dynamic changes in renal perfusion (Alpert et al. 2002, Juillard et al. 2002, Middlekauff et al. 1997). Alpert et al. (Alpert et al. 2002)

also reported the ability of $H_2{}^{15}O$-PET to measure basal renal perfusion in humans with different levels of kidney function. PET measurement of renal cortical perfusion has been validated in animals by the microsphere technique (Juillard et al. 2000, Kuten et al. 1992), and the intraindividual variation of renal perfusion is only 2 % in healthy volunteers (Nitzsche et al. 1993). Renal cortical perfusion PET-values in healthy subjects vary from 2.8 to 4.4 ml/min/g (Alpert et al. 2002, Middlekauff et al. 1997) and in patients with renal failure from 1.01 to 3.2 ml/min/g (Alpert et al. 2002, Juillard et al. 2002). In the present studies (III, IV), the cortical perfusion values that were recorded in healthy subjects and in patients with CKD are in the same range as in these previous studies.

Relatively low spatial resolution and a partial-volume effect due to the complicated cortical structure of the kidney affect the measurements of cortical renal perfusion. These will typically result in underestimation of cortical flow and a blurring of the boundary between cortex and medulla. A cortical ROIs will probably include an unknown admixture of medullary flow. Such effects may account for differences observed between our and previous studies.

Previous studies have calculated renal perfusion from the $H_2{}^{15}O$ uptake rate (K_1 ml/min/g) (Alpert et al. 2002, Juillard et al. 2002), and have assumed that the partition coefficient of water (p) is uniform over the whole region of renal tissue (Alpert et al. 2002). Such assumption may affect the reported values of renal perfusion. Thus, we conducted a study in sex healthy volunteers to generate quantitative K_1, clearance rate ($k_{2)}$ images using $H_2{}^{15}O$ and PET applying basis function computation method (III). From the published values of the water content of tissue (76 %) and blood (81%), the p-value can be physiologically determined as: p_{phys}=0.94 ml/g (Herscovitch and Raichle 1985). The smaller apparent p value might be due to tissue mixture (or partial volume effect) (Blomqvist et al. 1995). The effects of tissue mixture affect mostly K_1 and not the clearance rate (k_2 min^{-1}). Therefore, the clearance rate of $H_2{}^{15}O$ (k_2 min^{-1}) multiplied by p_{phys} could be applied for the blood flow rather than K_1 ml/min/g, when the effect of tissue mixture is not negligible. A simulation study was also performed to evaluate error sensitivities for possible error sources (III). The study showed that the smaller *K1* against *k2·pphys* values suggested that the *K1* values underestimated the absolute RBF value due to the partial volume effect (III). The simulation showed that the delay time and dispersion time constant should be estimated with an accuracy of 2 s (III).This method was further used to study patients with ARVD.

7.3.2 Renal perfusion in patients with atherosclerotic renovascular disease (IV)

There are no previous studies on the change in renal perfusion after revascularization of RAS. In the present study (IV), the effect of RAS revascularization in treatment-resistant hypertensive ARVD patients on renal PET perfusion was analyzed. It was found that dilatation of RAS did not improve the renal perfusion.

The results of this study show a negative correlation between the degree of RAS and the cortical renal perfusion at baseline. There has been much debate about the threshold of

meaningful RAS. Especially the ASTRAL trial was heavily criticized, that it included patients with hemodynamically insignificant RAS. The hemodynamical significance of RAS has been examined with latex casts to induce luminal occlusion or with studies on the magnitude of renin release related to the poststenotic gradient. Definitive hemodynamic effects developed only after luminal occlusion of 75 % to 85 % (Textor et al. 2009b). In experimental RAS in pigs, renal perfusion gradually decreases in correlation with RAS already from 20 % of renal artery occlusion (Warner et al. 2009). In this study (IV), a gradual decrease in the cortical renal perfusion was recorded when the degree of renal arterial stenosis exceeded 60 %

Although less than 10% of blood flow and oxygen delivery are needed to maintain renal metabolism (Brezis et al. 1984), chronic perfusion impairment to the kidneys results experimentally in dowregulation of proangiogenic factors, e.g., vascular endothelial growth factor (VEGF) (Zhu et al. 2004). Furthermore, in the stenotic kidneys microvascular rarefaction develops as the renovascular disease evolves, and the decrease in the number of vessels in stenotic kidneys is associated with a decline in renal perfusion (Chade et al. 2002) . Hypercholesterolemia has also clear additional deleterious effects on renal microvasculature (Chade et al. 2003). Intrarenal administration of VEGF can restore the renal microvascular architecture and normalize renal blood flow and GFR (Iliescu et al. 2010). In an experimental model, the stage of renal microvascular function appeared to be key determinant of the response to revascularization (Chade and Kelsen 2010). Thus, it is likely that intrarenal microvascular disease precedes the onset and represents the silent phase of ischemic renal disease. Persistent, severe damage to the intrarenal microvascular bed may be one reason for the common failure of renal function to improve in patients with ARVD who have undergone successful RAS revascularization (Wheatley et al. 2009).

Like in the heart, where PET perfusion is an integrated measure of blood flow through both the large epicardial coronary arteries and the microcirculation (Kaufmann and Camici 2005), the cortical perfusion in the kidneys is a combination of flow through the renal arteries and the microvasculature. The lack of change in renal cortical PET perfusion after technically successful procedures, as in this study (IV), implicates that there are already structural microvascular changes in patients with ARVD.

As in the heart, where the CFR is prognosticator of CV events, the renal flow reserve (RFR) might provide more information about the state of the renal microvasculature. There are only few studies on RFR in renovascular disease, so far done with invasive methods (Wierema et al. 1998). Since PET allows noninvasive quantification of renal perfusion, PET is a promising tool for future studies on RFR.

Renal perfusion is notoriously heterogeneous (Alpert et al. 2002). In healthy subjects, the distribution of renal cortical perfusion was homogenous, and there was no difference in perfusion between the two kidneys. In contrast, the perfusion maps of patients with CKD and with ARVD showed a heterogeneous pattern of cortical flow. The ROIs were drawn over the whole cortical region, and the mean perfusion value was used for further analyses. As a consequence, it is possible that after revascularization the heterogeneity

could have decreased and also that the less perfused areas had taken their "shear" of the work. Examining this is one the planned future projects.

Clinically, there are similarities between ARVD and stable coronary artery disease. There seems to be no benefit from revascularization of RAS, as detected in large clinical trials (Bax et al. 2009, Wheatley et al. 2009) and in these studies (II, IV) regarding renal and myocardial perfusion. However, there are experimental data on beneficial effects of simvastatin on renal function and renal morphology (Chade et al. 2006b). The future calls for effective methods to evaluate the effects of different treatment strategies, both interventional and medical, for patients with ARVD. In this respect, PET offers a promising, quantitative, low-risk and non-invasive assessment of renal perfusion and also myocardial perfusion in ARVD patients.

7.4 Limitations

The relatively *small sample size* was mainly limited by the study methodology, and it may have affected our results. However, the finding in study I has since been repeated in a much larger patient population by another group (Charytan et al. 2010). The incoming number of ARVD patients per year suitable for revascularization was lower than originally expected. Similarly, same phenomenon was observed in large clinical trials performed (Bax et al. 2009, Wheatley et al. 2009). Due to small sample size the results of the studies II and IV are more hypotheses-generating than definitive, although they are in line with the findings of published clinical trials (Bax et al. 2009, Wheatley et al. 2009).

Study design. Considering the high incidence of CV disease and mortality among patients with CKD and with ARVD, one of the limitations is the uncertainty about the anatomical status of the coronary arteries in our patients in studies I and II. In addition, the lack of an ARVD control group who did not undergo revascularization (II, IV) is problematic, since it is possible that only medically managed patients could have fared even worse. This caveat is nevetrtheless not very likely because of the short follow-up.

Study methodology. Although the contrast in the PET parametrical images may indicate relatively sharp cortical boundary, the accuracy of its location should be interpreted cautiously. Thus, in study IV, it is probable that placement of renal cortical ROIs included an unknown admixture of medullary flow areas. The effect of a partial-volume effect was minimized by calculating the renal perfusion from the clearance rate instead from the uptake rate of $H_2{}^{15}O$.

Generalization of the results. Considering that diabetes is the major cause of CKD in developed countries, the results of the study I are applicable only to a fraction of CKD patients.

8 SUMMARY AND CONCLUSIONS

Study I

CFR is quite well preserved in patients with CKD who do not have clinically evident CAD. There is a trend towards lower CFR with decresing GFR.

Brachial artery endothelial function is impaired in CKD patients compared to healty controls.

Study II

CFR is very low in patients with ARVD. This is due to abnormal myocardial perfusion during hyperemia. CFR is not affected by revascularization of RAS in patients with ARVD.

Brachial artery endothelial function is severely impaired in patients with ARVD. After RAS revascularization, flow-mediated dilatation improves. In the present study the average improvemt of 40% was not statistically significant over the entire group of ARVD patients. Patients with bilateral RAS have statistically significantly improved brachial artery endothelial function after revascularization .

Study III

Renal perfusion values obtained by the $H_2{}^{15}O$-PET are more accurate when perfusion is calculated by the clearance rate of $H_2{}^{15}O$ from the kidney and multiplied with the physiological partition coefficient for water.

Study IV

Mean cortical renal perfusion correlates negatively with the degree of RAS in stenosed kidneys. Perfusion values are not affected by RAS revascularization.

Uremia itself seems to have only a moderate effect on the coronary flow reserve, although the peripheral endothelial function is impaired, as assessed by flow-mediated dilatation of the brachial artery already in patients with stage 3 chronic kidney disease. Thus, it seems that the remarkable deterioration of the coronary flow reserve in patients with atherosclerotic renovascular disease is mainly caused by subclinical coronary artery disease. In this manner, peripheral endothelial function and coronary vascular function go hand-in-hand, since also the peripheral endothelial function was more severely damaged in patients with atherosclerotic renovascular disease than in patients with chronic kidney disease.

The fact that there is no change in coronary flow reserve or in renal perfusion after renal artery revascularization implies that dilatation of renal artery stenosis provides no

advantage over medical treatment in patients with atherosclerotic renovascular disease, in general.

Positron emission tomography is a noninvasive method for quantification of renal and myocardial perfusion, and may in the future become a promising tool for evaluating the effects of different treatment strategies in individual patients with atherosclerotic renovascular disease.

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10 REFERENCES

Abendschein, D., J. Fewell, C. Carlson & E. Rapaport (1982) Myocardial blood flow during acute isovolumic anemia and treadmill exercise in dogs. *J Appl Physiol,* 53**,** 203-6.

Akinboboye, O., R. Chou & S. Bergmann (2002) Augmentation of myocardial blood flow in hypertensive heart disease by angiotensin antagonists: a comparison of lisinopril and losartan. *J Am Coll Cardiol,* 40**,** 703-9.

Alfke, H. & J. Radermacher (2010) Renal artery stenting is no longer indicated after ASTRAL: pros and cons. *Cardiovasc Intervent Radiol,* 33**,** 883-6.

Alpert, N., C. Rabito, D. Correia, J. Babich, B. Littman, R. Tompkins, N. Rubin, R. Rubin & A. Fischman (2002) Mapping of local renal blood flow with PET and H(2)(15)O. *J Nucl Med,* 43**,** 470-5.

Amann, K., M. Breitbach, E. Ritz & G. Mall (1998) Myocyte/capillary mismatch in the heart of uremic patients. *J Am Soc Nephrol,* 9**,** 1018-22.

Amann, K., G. Wiest, G. Zimmer, N. Gretz, E. Ritz & G. Mall (1992) Reduced capillary density in the myocardium of uremic rats--a stereological study. *Kidney Int,* 42**,** 1079-85.

Anderson, T., A. Uehata, M. Gerhard, I. Meredith, S. Knab, D. Delagrange, E. Lieberman, P. Ganz, M. Creager & A. Yeung (1995) Close relation of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol,* 26**,** 1235-41.

Antony, I., A. Nitenberg, J. Foult & E. Aptecar (1993) Coronary vasodilator reserve in untreated and treated hypertensive patients with and without left ventricular hypertrophy. *J Am Coll Cardiol,* 22**,** 514-20.

Araujo, L., A. Lammertsma, C. Rhodes, E. McFalls, H. Iida, E. Rechavia, A. Galassi, R. De Silva, T. Jones & A. Maseri (1991) Noninvasive quantification of regional myocardial blood flow in coronary artery disease with oxygen-15-labeled carbon dioxide inhalation and positron emission tomography. *Circulation,* 83**,** 875-85.

Arnlöv, J., J. Evans, J. Meigs, T. Wang, C. Fox, D. Levy, E. Benjamin, R. D'Agostino & R. Vasan (2005) Low-grade albuminuria and incidence of cardiovascular disease events in nonhypertensive and nondiabetic individuals: the Framingham Heart Study. *Circulation,* 112**,** 969-75.

Asanuma, Y., A. Oeser, A. Shintani, E. Turner, N. Olsen, S. Fazio, M. Linton, P. Raggi & C. Stein (2003) Premature coronary-artery atherosclerosis in systemic lupus erythematosus. *N Engl J Med,* 349**,** 2407-15.

Astor, B., P. Muntner, A. Levin, J. Eustace & J. Coresh (2002) Association of kidney function with anemia: the Third National Health and Nutrition Examination Survey (1988-1994). *Arch Intern Med,* 162**,** 1401-8.

Attenberger, U., S. Sourbron, S. Schoenberg, J. Morelli, T. Leiner, G. Schoeppler, W. Samtleben, K. Birkemeier, C. Glaser, M. Reiser & H. Michaely (2010) Comprehensive MR evaluation of renal disease: added clinical value of quantified renal perfusion values over single MR angiography. *J Magn Reson Imaging,* 31**,** 125-33.

Aukland, K. (1980) Methods for measuring renal blood flow: total flow and regional distribution. *Annu Rev Physiol,* 42**,** 543-55.

Baigent, C., K. Burbury & D. Wheeler (2000) Premature cardiovascular disease in chronic renal failure. *Lancet,* 356**,** 147-52.

Basso, N. & N. Terragno (2001) History about the discovery of the renin-angiotensin system. *Hypertension,* 38**,** 1246-9.

Bax, L., A. Woittiez, H. Kouwenberg, W. Mali, E. Buskens, F. Beek, B. Braam, F. Huysmans, L. Schultze Kool, M. Rutten, C. Doorenbos, J. Aarts, T. Rabelink, P. Plouin, A. Raynaud, G. van Montfrans, J. Reekers, A. van den Meiracker, P. Pattynama, P. van de Ven, D. Vroegindeweij, A. Kroon, M. de Haan, C. Postma & J. Beutler (2009) Stent placement in patients with atherosclerotic renal artery stenosis and impaired renal function: a randomized trial. *Ann Intern Med,* 150**,** 840-8, W150-1.

Baxter, G., H. Ireland, J. Moss, P. Harden, B. Junor, R. Rodger & J. Briggs (1995) Colour Doppler ultrasound in renal transplant artery stenosis: which Doppler index? *Clin Radiol,* 50**,** 618-22.

Beltrame, J., F. Crea & P. Camici (2009) Advances in coronary microvascular dysfunction. *Heart Lung Circ,* 18**,** 19-27.

Benndorf, R., D. Appel, R. Maas, E. Schwedhelm, U. Wenzel & R. Böger (2007) Telmisartan improves endothelial function in patients with essential hypertension. *J Cardiovasc Pharmacol,* 50**,** 367-71.

Beregi, J., A. Lahoche, S. Willoteaux, E. McFadden, R. Bordet, C. Gautier & T. Etchrivi (1998) Renal artery vasomotion: in vivo assessment in the pig with intravascular Doppler. *Fundam Clin Pharmacol,* 12**,** 613-8.

Bergmann, S., P. Herrero, J. Markham, C. Weinheimer & M. Walsh (1989) Noninvasive quantitation of myocardial blood flow in human subjects with oxygen-15-labeled water and positron emission tomography. *J Am Coll Cardiol,* 14**,** 639-52.

Berry, K., R. Skyrme-Jones & I. Meredith (2000) Occlusion cuff position is an important determinant of the time course and magnitude of human brachial artery flow-mediated dilation. *Clin Sci (Lond),* 99**,** 261-7.

Bezante, G., F. Viazzi, G. Leoncini, E. Ratto, N. Conti, M. Balbi, S. Agosti, L. Deferrari, G. Deferrari & R. Pontremoli (2009) Coronary flow reserve is impaired in hypertensive patients with subclinical renal damage. *Am J Hypertens,* 22**,** 191-6.

Blackshear, J., D. Garnic, G. Williams, D. Harrington & N. Hollenberg (1987) Exaggerated renal vasodilator response to calcium entry blockade in first-degree relatives of essential hypertensive subjects. *Hypertension,* 9**,** 384-9.

Blaufox, M., A. Fromowitz, A. Gruskin, C. Meng & M. Elkin (1970) Validation of use of xenon 133 to measure intrarenal distribution of blood flow. *Am J Physiol,* 219**,** 440-4.

Bloch, M., D. Trost, T. Pickering, T. Sos & P. August (1999) Prevention of recurrent pulmonary edema in patients with bilateral renovascular disease through renal artery stent placement. *Am J Hypertens,* 12**,** 1-7.

Block, G., T. Hulbert-Shearon, N. Levin & F. Port (1998) Association of serum phosphorus and calcium x phosphate product with mortality risk in chronic hemodialysis patients: a national study. *Am J Kidney Dis,* 31**,** 607-17.

Block, G., P. Raggi, A. Bellasi, L. Kooienga & D. Spiegel (2007) Mortality effect of coronary calcification and phosphate binder choice in incident hemodialysis patients. *Kidney Int,* 71**,** 438-41.

Block, G., D. Spiegel, J. Ehrlich, R. Mehta, J. Lindbergh, A. Dreisbach & P. Raggi (2005) Effects of sevelamer and calcium on coronary artery calcification in patients new to hemodialysis. *Kidney Int,* 68**,** 1815-24.

Blomqvist, G., A. Lammertsma, B. Mazoyer & K. Wienhard (1995) Effect of tissue heterogeneity on quantification in positron emission tomography. *Eur J Nucl Med,* 22**,** 652-63.

Boaz, M., S. Smetana, T. Weinstein, Z. Matas, U. Gafter, A. Iaina, A. Knecht, Y. Weissgarten, D. Brunner, M. Fainaru & M. Green (2000) Secondary prevention with antioxidants of cardiovascular disease in endstage renal disease (SPACE): randomised placebo-controlled trial. *Lancet,* 356**,** 1213-8.

Bol, A., J. Melin, J. Vanoverschelde, T. Baudhuin, D. Vogelaers, M. De Pauw, C. Michel, A. Luxen, D. Labar & M. Cogneau (1993) Direct comparison of [13N]ammonia and [15O]water estimates of perfusion with quantification of regional myocardial blood flow by microspheres. *Circulation,* 87**,** 512-25.

Bonelli, F., M. McKusick, S. Textor, P. Kos, A. Stanson, C. Johnson, P. n. Sheedy, T. Welch & A. Schirger (1995) Renal artery angioplasty: technical results and clinical outcome in 320 patients. *Mayo Clin Proc,* 70**,** 1041-52.

Bozbas, H., B. Pirat, S. Demirtas, V. Simşek, A. Yildirir, E. Sade, B. Sayin, S. Sezer, H. Karakayali & H. Muderrisoglu (2009) Evaluation of coronary microvascular function in patients with end-stage renal disease, and renal allograft recipients. *Atherosclerosis,* 202**,** 498-504.

Brezis, M., S. Rosen, P. Silva & F. Epstein (1984) Renal ischemia: a new perspective. *Kidney Int,* 26**,** 375-83.

Brunner, H., J. Kirshman, J. Sealey & J. Laragh (1971) Hypertension of renal origin: evidence for two different mechanisms. *Science,* 174**,** 1344-6.

Buus, N., M. Bøttcher, C. Jørgensen, K. Christensen, K. Thygesen, T. Nielsen & M. Mulvany (2004) Myocardial perfusion during long-term angiotensin-converting enzyme inhibition or beta-blockade in patients with essential hypertension. *Hypertension,* 44**,** 465-70.

Caglar, K., M. Yilmaz, M. Saglam, E. Cakir, S. Kilic, A. Sonmez, T. Eyileten, M. Yenicesu, Y. Oguz, M. Tasar, A. Vural, T. Ikizler, P. Stenvinkel & B. Lindholm (2008) Serum Fetuin-A Concentration and Endothelial Dysfunction in Chronic Kidney Disease. *Nephron Clin Pract*.

Caliskan, Y., H. Oflaz, M. Demirturk, H. Yazici, A. Turkmen, A. Cimen, A. Elitok & A. Yildiz (2008) Coronary flow reserve dysfunction in hemodialysis and kidney transplant patients. *Clin Transplant,* 22**,** 785-93.

Camici, P. & F. Crea (2007) Coronary microvascular dysfunction. *N Engl J Med,* 356**,** 830-40.

Caps, M., C. Perissinotto, R. Zierler, N. Polissar, R. Bergelin, M. Tullis, K. Cantwell-Gab, R. Davidson & D. J. Strandness (1998a) Prospective study of atherosclerotic disease progression in the renal artery. *Circulation,* 98**,** 2866-72.

Caps, M., R. Zierler, N. Polissar, R. Bergelin, K. Beach, K. Cantwell-Gab, A. Casadei, R. Davidson & D. J. Strandness (1998b) Risk of atrophy in kidneys with atherosclerotic renal artery stenosis. *Kidney Int,* 53**,** 735-42.

Carr, T. r., S. Sabri, U. Turba, A. Park, W. Saad, J. Angle & A. Matsumoto (2010) Stenting for atherosclerotic renal artery stenosis. *Tech Vasc Interv Radiol,* 13**,** 134-45.

Celermajer, D., K. Sorensen, D. Georgakopoulos, C. Bull, O. Thomas, J. Robinson & J. Deanfield (1993) Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. *Circulation,* 88**,** 2149-55.

Celermajer, D., K. Sorensen, V. Gooch, D. Spiegelhalter, O. Miller, I. Sullivan, J. Lloyd & J. Deanfield (1992) Non-invasive detection of endothelial dysfunction in

children and adults at risk of atherosclerosis. *Lancet,* 340**,** 1111-5.

Cervenka, L., V. Horácek, I. Vanecková, J. Hubácek, M. Oliverio, T. Coffman & L. Navar (2002) Essential role of AT1A receptor in the development of 2K1C hypertension. *Hypertension,* 40**,** 735-41.

Chade, A., D. Brosh, S. Higano, R. Lennon, L. Lerman & A. Lerman (2006a) Mild renal insufficiency is associated with reduced coronary flow in patients with non-obstructive coronary artery disease. *Kidney Int,* 69**,** 266-71.

Chade, A. & S. Kelsen (2010) Renal microvascular disease determines the responses to revascularization in experimental renovascular disease. *Circ Cardiovasc Interv,* 3**,** 376-83.

Chade, A., M. Rodriguez-Porcel, J. Grande, J. Krier, A. Lerman, J. Romero, C. Napoli & L. Lerman (2002) Distinct renal injury in early atherosclerosis and renovascular disease. *Circulation,* 106**,** 1165-71.

Chade, A., M. Rodriguez-Porcel, J. Grande, X. Zhu, V. Sica, C. Napoli, T. Sawamura, S. Textor, A. Lerman & L. Lerman (2003) Mechanisms of renal structural alterations in combined hypercholesterolemia and renal artery stenosis. *Arterioscler Thromb Vasc Biol,* 23**,** 1295-301.

Chade, A., X. Zhu, R. Lavi, J. Krier, S. Pislaru, R. Simari, C. Napoli, A. Lerman & L. Lerman (2009) Endothelial progenitor cells restore renal function in chronic experimental renovascular disease. *Circulation,* 119**,** 547-57.

Chade, A., X. Zhu, O. Mushin, C. Napoli, A. Lerman & L. Lerman (2006b) Simvastatin promotes angiogenesis and prevents microvascular remodeling in chronic renal ischemia. *FASEB J,* 20**,** 1706-8.

Chan, S., R. Brunken, J. Czernin, G. Porenta, W. Kuhle, J. Krivokapich, M. Phelps & H. Schelbert (1992) Comparison of maximal myocardial blood flow during adenosine infusion with that of intravenous dipyridamole in normal men. *J Am Coll Cardiol,* 20**,** 979-85.

Chareonthaitawee, P., P. Kaufmann, O. Rimoldi & P. Camici (2001) Heterogeneity of resting and hyperemic myocardial blood flow in healthy humans. *Cardiovasc Res,* 50**,** 151-61.

Charytan, D., H. Schelbert & M. Di Carli (2010) Coronary Microvascular Function in Early Chronic Kidney Disease. *Circ Cardiovasc Imaging*.

Chertow, G., S. Burke, P. Raggi & T. t. G. W. Group (2002) Sevelamer attenuates the progression of coronary and aortic calcification in hemodialysis patients. *Kidney Int,* 62**,** 245-52.

Cheung, C., A. Patel, N. Shaheen, S. Cain, H. Eddington, J. Hegarty, R. Middleton, A. Cowie, H. Mamtora & P. Kalra (2007) The effects of statins on the progression of atherosclerotic renovascular disease. *Nephron Clin Pract,* 107**,** c35-42.

Chrysochou, C., D. Buckley, P. Dark, A. Cowie & P. Kalra (2009) Gadolinium-enhanced magnetic resonance imaging for renovascular disease and nephrogenic systemic fibrosis: critical review of the literature and UK experience. *J Magn Reson Imaging,* 29**,** 887-94.

Coen, G., S. Calabria, S. Lai, E. Moscaritolo, I. Nofroni, G. Ronga, M. Rossi, G. Ventroni, D. Sardella, M. Ferrannini, A. Zaccaria & R. Cianci (2003) Atherosclerotic ischemic renal disease. Diagnosis and prevalence in an hypertensive and/or uremic elderly population. *BMC Nephrol,* 4**,** 2.

Cohen, M., J. Pascua, M. Garcia-Ben, C. Rojas-Matas, J. Gabay, D. Berrocal, W. Tan, G. Stouffer, M. Montoya, A. Fernandez, M. Halac & L. Grinfeld (2005) A simple prediction rule for significant renal artery stenosis in patients undergoing cardiac catheterization. *Am Heart J,* 150**,** 1204-11.

Conlon, P., M. Little, K. Pieper & D. Mark (2001) Severity of renal vascular disease predicts mortality in patients undergoing coronary angiography. *Kidney Int,* 60**,** 1490-7.

Connolly, J., R. Higgins, H. Walters, A. Mackie, P. Drury, B. Hendry & J. Scoble (1994) Presentation, clinical features and outcome in different patterns of atherosclerotic renovascular disease. *QJM,* 87**,** 413-21.

Cooper, C., S. Haller, W. Colyer, M. Steffes, M. Burket, W. Thomas, R. Safian, B. Reddy, P. Brewster, M. Ankenbrandt, R. Virmani, E. Dippel, K. Rocha-Singh, T. Murphy, D. Kennedy, J. Shapiro, R. D'Agostino, M. Pencina & S. Khuder (2008) Embolic protection and platelet inhibition during renal artery stenting. *Circulation,* 117**,** 2752-60.

Cooper, C., T. Murphy, A. Matsumoto, M. Steffes, D. Cohen, M. Jaff, R. Kuntz, K. Jamerson, D. Reid, K. Rosenfield, J. Rundback, R. D'Agostino, W. Henrich & L. Dworkin (2006) Stent revascularization for the prevention of cardiovascular and renal events among patients with renal artery stenosis and systolic hypertension: rationale and design of the CORAL trial. *Am Heart J,* 152**,** 59-66.

Coresh, J., G. Wei, G. McQuillan, F. Brancati, A. Levey, C. Jones & M. Klag (2001) Prevalence of high blood pressure and elevated serum creatinine level in the United States: findings from the third National Health and Nutrition Examination Survey (1988-1994). *Arch Intern Med,* 161**,** 1207-16.

Corriere, M., J. Hoyle, T. Craven, R. J. D'Agostino, M. Edwards, P. Moore & K. Hansen (2009) Changes in Left Ventricular Structure and Function following Renal Artery Revascularization. *Ann Vasc Surg*.

Crowley, S., S. Gurley, M. Oliverio, A. Pazmino, R. Griffiths, P. Flannery, R. Spurney, H. Kim, O. Smithies, T. Le & T. Coffman (2005) Distinct roles for the kidney and systemic tissues in blood pressure regulation by the renin-angiotensin system. *J Clin Invest,* 115**,** 1092-9.

Crutchley, T., J. Pearce, T. Craven, J. Stafford, M. Edwards & K. Hansen (2009) Clinical utility of the resistive index in atherosclerotic renovascular disease. *J Vasc Surg,* 49**,** 148-55, 155.e1-3; discussion 155.

Czernin, J., P. Müller, S. Chan, R. Brunken, G. Porenta, J. Krivokapich, K. Chen, A. Chan, M. Phelps & H. Schelbert (1993) Influence of age and hemodynamics on myocardial blood flow and flow reserve. *Circulation,* 88**,** 62-9.

Dasselaar, J., R. Slart, M. Knip, J. Pruim, R. Tio, C. McIntyre, P. de Jong & C. Franssen (2009) Haemodialysis is associated with a pronounced fall in myocardial perfusion. *Nephrol Dial Transplant,* 24**,** 604-10.

De Bruyne, B., G. Manoharan, N. Pijls, K. Verhamme, J. Madaric, J. Bartunek, M. Vanderheyden & G. Heyndrickx (2006) Assessment of renal artery stenosis severity by pressure gradient measurements. *J Am Coll Cardiol,* 48**,** 1851-5.

de Mast, Q. & J. Beutler (2009) The prevalence of atherosclerotic renal artery stenosis in risk groups: a systematic literature review. *J Hypertens,* 27**,** 1333-40.

DeForrest, J., R. Knappenberger, M. Antonaccio, R. Ferrone & J. Creekmore (1982) Angiotensin II is a necessary component for the development of hypertension in the two kidney, one clip rat. *Am J Cardiol,* 49**,** 1515-7.

Degoulet, P., M. Legrain, I. Réach, F. Aimé, C. Devriés, P. Rojas & C. Jacobs (1982) Mortality risk factors in patients treated by chronic hemodialysis. Report of the Diaphane collaborative study. *Nephron,* 31**,** 103-10.

del Rincón, I., K. Williams, M. Stern, G. Freeman & A. Escalante (2001) High incidence of cardiovascular events in a rheumatoid arthritis cohort not explained by traditional cardiac risk factors. *Arthritis Rheum,* 44**,** 2737-45.

Deo, A., M. Fogel & S. Cowper (2007) Nephrogenic systemic fibrosis: a population study examining the relationship of disease development to gadolinium exposure. *Clin J Am Soc Nephrol,* 2**,** 264-7.

Descamps-Latscha, B., V. Witko-Sarsat, T. Nguyen-Khoa, A. Nguyen, V. Gausson, N. Mothu, G. London & P. Jungers (2005) Advanced oxidation protein products as risk factors for atherosclerotic cardiovascular events in nondiabetic predialysis patients. *Am J Kidney Dis,* 45**,** 39-47.

Devereux, R., D. Alonso, E. Lutas, G. Gottlieb, E. Campo, I. Sachs & N. Reichek (1986) Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. *Am J Cardiol,* 57**,** 450-8.

Devereux, R., K. Wachtell, E. Gerdts, K. Boman, M. Nieminen, V. Papademetriou, J. Rokkedal, K. Harris, P. Aurup & B. Dahlöf (2004) Prognostic significance of left ventricular mass change during treatment of hypertension. *JAMA,* 292**,** 2350-6.

Dhingra, R., L. Sullivan, C. Fox, T. Wang, R. S. D'Agostino, J. Gaziano & R. Vasan (2007) Relations of serum phosphorus and calcium levels to the incidence of cardiovascular disease in the community. *Arch Intern Med,* 167**,** 879-85.

Dijkmans, P., P. Knaapen, G. Sieswerda, E. Aiazian, C. Visser, A. Lammertsma, F. Visser & O. Kamp (2006) Quantification of myocardial perfusion using intravenous myocardial contrast echocardiography in healthy volunteers: comparison with positron emission tomography. *J Am Soc Echocardiogr,* 19**,** 285-93.

Drieghe, B., J. Madaric, G. Sarno, G. Manoharan, J. Bartunek, G. Heyndrickx, N. Pijls & B. De Bruyne (2008) Assessment of renal artery stenosis: side-by-side comparison of angiography and duplex ultrasound with pressure gradient measurements. *Eur Heart J,* 29**,** 517-24.

Edwards, M., T. Craven, G. Burke, R. Dean & K. Hansen (2005) Renovascular disease and the risk of adverse coronary events in the elderly: a prospective, population-based study. *Arch Intern Med,* 165**,** 207-13.

Elkayam, U., A. Mehra, G. Cohen, P. Tummala, I. Karaalp, O. Wani & M. Canetti (1998) Renal circulatory effects of adenosine in patients with chronic heart failure. *J Am Coll Cardiol,* 32**,** 211-5.

Emovon, O., P. Klotman, N. Dunnick, S. Kadir & L. Svetkey (1996) Renovascular hypertension in blacks. *Am J Hypertens,* 9**,** 18-23.

Endemann, D. & E. Schiffrin (2004) Endothelial dysfunction. *J Am Soc Nephrol,* 15**,** 1983-92.

Evans, R., B. Gardiner, D. Smith & P. O'Connor (2008) Intrarenal oxygenation: unique challenges and the biophysical basis of homeostasis. *Am J Physiol Renal Physiol,* 295**,** F1259-70.

Farmer, C., J. Reidy, P. Kalra, G. Cook & J. Scoble (1998) Individual kidney function before and after renal angioplasty. *Lancet,* 352**,** 288-9.

Feldstein, A., J. Krier, M. Sarafov, A. Lerman, P. Best, S. Wilson & L. Lerman (1999) In vivo renal vascular and tubular function in experimental hypercholesterolemia. *Hypertension,* 34**,** 859-64.

Fellström, B., A. Jardine, R. Schmieder, H. Holdaas, K. Bannister, J. Beutler, D. Chae, A. Chevaile, S. Cobbe, C. Grönhagen-Riska, J. De Lima, R. Lins, G. Mayer, A. McMahon, H. Parving, G. Remuzzi, O. Samuelsson, S. Sonkodi, D. Sci, G. Süleymanlar, D. Tsakiris, V. Tesar, V. Todorov, A. Wiecek, R. Wüthrich, M. Gottlow, E. Johnsson, F. Zannad & A. S. Group (2009) Rosuvastatin and cardiovascular events in patients undergoing hemodialysis. *N Engl J Med,* 360**,** 1395-407.

Festa, A., R. D'Agostino, G. Howard, L. Mykkänen, R. Tracy & S. Haffner (2000) Inflammation and microalbuminuria in nondiabetic and type 2 diabetic subjects: The Insulin Resistance Atherosclerosis Study. *Kidney Int,* 58**,** 1703-10.

Flickinger, A., L. Lerman, P. n. Sheedy & S. Turner (1996) The relationship between renal cortical volume and predisposition to hypertension. *Am J Hypertens,* 9**,** 779-86.

Foley, R., A. Collins, C. Herzog, A. Ishani & P. Kalra (2009) Serum phosphorus levels associate with coronary atherosclerosis in young adults. *J Am Soc Nephrol,* 20**,** 397-404.

Foley, R., A. Collins, A. Ishani & P. Kalra (2008) Calcium-phosphate levels and cardiovascular disease in community-dwelling adults: the Atherosclerosis Risk in Communities (ARIC) Study. *Am Heart J,* 156**,** 556-63.

Foley, R., P. Parfrey, J. Harnett, G. Kent, D. Murray & P. Barré (1995) The prognostic importance of left ventricular geometry in uremic cardiomyopathy. *J Am Soc Nephrol,* 5**,** 2024-31.

Foley, R., P. Parfrey & M. Sarnak (1998) Clinical epidemiology of cardiovascular disease in chronic renal disease. *Am J Kidney Dis,* 32**,** S112-9.

Foundation, N. K. (2002) K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis,* 39**,** S1-266.

Fritz-Hansen, T., J. Hove, K. Kofoed, H. Kelbaek & H. Larsson (2008) Quantification of MRI measured myocardial perfusion reserve in healthy humans: a comparison with positron emission tomography. *J Magn Reson Imaging,* 27**,** 818-24.

Furchgott, R. & J. Zawadzki (1980) The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature,* 288**,** 373-6.

Gavras, H., H. Brunner, H. Thurston & J. Laragh (1975) Reciprocation of renin dependency with sodium volume dependency in renal hypertension. *Science,* 188**,** 1316-7.

Gavras, H., H. Brunner, E. Vaughan & J. Laragh (1973) Angiotensin-sodium interaction in blood pressure maintenance of renal hypertensive and normotensive rats. *Science,* 180**,** 1369-71.

Gerber, L., S. Mann, F. Müller, M. Alderman, T. Pickering, J. Sealey & J. Laragh (1994) Response to the captopril test is dependent on baseline renin profile. *J Hypertens,* 12**,** 173-8.

Gloviczki, M., J. Glockner, L. Lerman, M. McKusick, S. Misra, J. Grande & S. Textor (2010) Preserved oxygenation despite reduced blood flow in poststenotic kidneys in human atherosclerotic renal artery stenosis. *Hypertension,* 55**,** 961-6.

Go, A., G. Chertow, D. Fan, C. McCulloch & C. Hsu (2004) Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med,* 351**,** 1296-305.

Gokce, N., J. J. Keaney, L. Hunter, M. Watkins, Z. Nedeljkovic, J. Menzoian & J. Vita (2003) Predictive value of noninvasively determined endothelial dysfunction for long-term cardiovascular events in patients with peripheral vascular disease. *J Am Coll Cardiol,* 41**,** 1769-75.

Goldblatt, H., J. Lynch, R. Hanzal & W. Summerville (1934) STUDIES ON EXPERIMENTAL HYPERTENSION : I. THE PRODUCTION OF PERSISTENT ELEVATION OF SYSTOLIC BLOOD PRESSURE BY MEANS OF RENAL ISCHEMIA. *J Exp Med,* 59**,** 347-379.

Goldsmith, D., J. Reidy & J. Scoble (2000) Renal arterial intervention and angiotensin blockade in atherosclerotic nephropathy. *Am J Kidney Dis,* 36**,** 837-43.

Goodman, W., J. Goldin, B. Kuizon, C. Yoon, B. Gales, D. Sider, Y. Wang, J. Chung, A. Emerick, L. Greaser, R. Elashoff & I. Salusky (2000) Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. *N Engl J Med,* 342**,** 1478-83.

Granillo, G., L. van Dijk, E. McFadden & P. Serruys (2005) Percutaneous radial intervention for complex bilateral renal artery stenosis using paclitaxel eluting stents. *Catheter Cardiovasc Interv,* 64**,** 23-7.

Greco, B. & J. Breyer (1997) Atherosclerotic ischemic renal disease. *Am J Kidney Dis,* 29**,** 167-87.

Grobner, T. (2006) Gadolinium--a specific trigger for the development of nephrogenic fibrosing dermopathy and nephrogenic systemic fibrosis? *Nephrol Dial Transplant*.

Guo, H., P. Kalra, D. Gilbertson, J. Liu, S. Chen, A. Collins & R. Foley (2007) Atherosclerotic renovascular disease in older US patients starting dialysis, 1996 to 2001. *Circulation,* 115**,** 50-8.

Gutiérrez, O., M. Mannstadt, T. Isakova, J. Rauh-Hain, H. Tamez, A. Shah, K. Smith, H. Lee, R. Thadhani, H. Jüppner & M. Wolf (2008) Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. *N Engl J Med,* 359**,** 584-92.

Hackam, D., M. Duong-Hua, M. Mamdani, P. Li, S. Tobe, J. Spence & A. Garg (2008) Angiotensin inhibition in renovascular disease: a population-based cohort study. *Am Heart J,* 156**,** 549-55.

Hakeem, A., S. Bhatti, K. Dillie, J. Cook, Z. Samad, M. Roth-Cline & S. Chang (2008) Predictive value of myocardial perfusion single-photon emission computed tomography and the impact of renal function on cardiac death. *Circulation,* 118**,** 2540-9.

Halbesma, N., D. Kuiken, A. Brantsma, S. Bakker, J. Wetzels, D. De Zeeuw, P. De Jong & R. Gansevoort

(2006) Macroalbuminuria is a better risk marker than low estimated GFR to identify individuals at risk for accelerated GFR loss in population screening. *J Am Soc Nephrol,* 17**,** 2582-90.

Halcox, J., W. Schenke, G. Zalos, R. Mincemoyer, A. Prasad, M. Waclawiw, K. Nour & A. Quyyumi (2002) Prognostic value of coronary vascular endothelial dysfunction. *Circulation,* 106**,** 653-8.

Harden, P., M. MacLeod, R. Rodger, G. Baxter, J. Connell, A. Dominiczak, B. Junor, J. Briggs & J. Moss (1997) Effect of renal-artery stenting on progression of renovascular renal failure. *Lancet,* 349**,** 1133-6.

Hatta, T., S. Nishimura & T. Nishimura (2009) Prognostic risk stratification of myocardial ischaemia evaluated by gated myocardial perfusion SPECT in patients with chronic kidney disease. *Eur J Nucl Med Mol Imaging,* 36**,** 1835-41.

Hayward, C., M. Kraidly, C. Webb & P. Collins (2002) Assessment of endothelial function using peripheral waveform analysis: a clinical application. *J Am Coll Cardiol,* 40**,** 521-8.

Hegarty, J., J. Wright, P. Kalra & P. Kalra (2006) The heart in renovascular disease--an association demanding further investigation. *Int J Cardiol,* 111**,** 339-42.

Heitzer, T., T. Schlinzig, K. Krohn, T. Meinertz & T. Münzel (2001) Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation,* 104**,** 2673-8.

Herrero, P., J. Markham, M. Shelton, C. Weinheimer & S. Bergmann (1990) Noninvasive quantification of regional myocardial perfusion with rubidium-82 and positron emission tomography. Exploration of a mathematical model. *Circulation,* 82**,** 1377-86.

Herscovitch, P. & M. Raichle (1985) What is the correct value for the brain--blood partition coefficient for water? *J Cereb Blood Flow Metab,* 5**,** 65-9.

Herzog, B., L. Husmann, I. Valenta, O. Gaemperli, P. Siegrist, F. Tay, N. Burkhard, C. Wyss & P. Kaufmann (2009) Long-term prognostic value of 13N-ammonia myocardial perfusion positron emission tomography added value of coronary flow reserve. *J Am Coll Cardiol,* 54**,** 150-6.

Higashi, Y., S. Sasaki, K. Nakagawa, H. Matsuura, T. Oshima & K. Chayama (2002) Endothelial function and oxidative stress in renovascular hypertension. *N Engl J Med,* 346**,** 1954-62.

Higuchi, T., C. Abletshauser, S. Nekolla, M. Schwaiger & F. Bengel (2007) Effect of the angiotensin receptor blocker Valsartan on coronary microvascular flow reserve in moderately hypertensive patients with stable coronary artery disease. *Microcirculation,* 14**,** 805-12.

Hirsch, A., Z. Haskal, N. Hertzer, C. Bakal, M. Creager, J. Halperin, L. Hiratzka, W. Murphy, J. Olin, J. Puschett, K. Rosenfield, D. Sacks, J. Stanley, L. J. Taylor, C. White, J. White, R. White, E. Antman, S. J. Smith, C. Adams, J. Anderson, D. Faxon, V. Fuster, R. Gibbons, S. Hunt, A. Jacobs, R. Nishimura, J. Ornato, R. Page, B. Riegel, A. A. f. V. Surgery, S. f. V. Surgery, S. f. C. A. a. Interventions, S. f. V. M. a. Biology, S. o. I. Radiology, A. A. T. F. o. P. G. W. C. t. D. G. f. t. M. o. P. W. P. A. Disease, A. A. o. C. a. P. Rehabilitation, L. National Heart, and Blood Institute, S. f. V. Nursing, T. I.-S. Consensus & V. D. Foundation (2006) ACC/AHA 2005 Practice Guidelines for the management of patients with peripheral arterial disease (lower extremity, renal, mesenteric, and abdominal aortic): a collaborative report from the American Association for Vascular Surgery/Society for Vascular Surgery, Society for Cardiovascular Angiography and Interventions, Society for Vascular Medicine and Biology, Society of Interventional Radiology, and the ACC/AHA Task Force on Practice Guidelines (Writing Committee to Develop Guidelines for the Management of Patients With Peripheral Arterial Disease): endorsed by the American Association of Cardiovascular and Pulmonary Rehabilitation; National Heart, Lung, and Blood Institute; Society for Vascular Nursing; TransAtlantic Inter-Society Consensus; and Vascular Disease Foundation. *Circulation,* 113**,** e463-654.

Hogas, S., L. Voroneanu, D. Serban, L. Segall, M. Hogas, I. Serban & A. Covic (2010) Methods and potential biomarkers for the evaluation of endothelial dysfunction in chronic kidney disease: a critical approach. *J Am Soc Hypertens,* 4**,** 116-27.

Hollenberg, N., R. Mangel & H. Fung (1976) Assessment of intrarenal perfusion with radioxenon: a critical review of analytical factors and their implications in man. *Semin Nucl Med,* 6**,** 193-216.

Hollenberg, N., T. Sandor, E. Holtzman, M. Meyerovitz & D. Harrington (1989) Renal vasomotion in essential hypertension: influence of vasodilators. *Hypertension,* 14**,** 9-13.

Horvath, J., R. Waugh, D. Tiller & G. Duggin (1982) The detection of renovascular hypertension: a study of 490 patients by renal angiography. *Q J Med,* 51**,** 139-46.

Houghton, J., J. Cerda & V. Smith (2000) Basal and reserve renal artery blood flow: effect of endothelium-dependent and -independent vasoactive agonists and radiographic contrast medium in two patients. *J Invasive Cardiol,* 12**,** 211-5.

Hutchins, G., M. Schwaiger, K. Rosenspire, J. Krivokapich, H. Schelbert & D. Kuhl (1990) Noninvasive quantification of regional blood flow in the human heart using N-13 ammonia and dynamic positron emission tomographic imaging. *J Am Coll Cardiol,* 15**,** 1032-42.

Iida, H., I. Kanno, A. Takahashi, S. Miura, M. Murakami, K. Takahashi, Y. Ono, F. Shishido, A. Inugami & N.

Tomura (1988) Measurement of absolute myocardial blood flow with H215O and dynamic positron-emission tomography. Strategy for quantification in relation to the partial-volume effect. *Circulation,* 78**,** 104-15.

Iida, H., C. Rhodes, R. de Silva, L. Araujo, P. Bloomfield, A. Lammertsma & T. Jones (1992) Use of the left ventricular time-activity curve as a noninvasive input function in dynamic oxygen-15-water positron emission tomography. *J Nucl Med,* 33**,** 1669-77.

Iida, H., A. Takahashi, Y. Tamura, Y. Ono & A. Lammertsma (1995) Myocardial blood flow: comparison of oxygen-15-water bolus injection, slow infusion and oxygen-15-carbon dioxide slow inhalation. *J Nucl Med,* 36**,** 78-85.

Iliescu, R., S. Fernandez, S. Kelsen, C. Maric & A. Chade (2010) Role of renal microcirculation in experimental renovascular disease. *Nephrol Dial Transplant,* 25**,** 1079-87.

Ishimura, E., H. Taniwaki, T. Tabata, Y. Tsujimoto, S. Jono, M. Emoto, T. Shoji, M. Inaba, T. Inoue & Y. Nishizawa (2005) Cross-sectional association of serum phosphate with carotid intima-medial thickness in hemodialysis patients. *Am J Kidney Dis,* 45**,** 859-65.

Jones, C., G. McQuillan, J. Kusek, M. Eberhardt, W. Herman, J. Coresh, M. Salive, C. Jones & L. Agodoa (1998) Serum creatinine levels in the US population: third National Health and Nutrition Examination Survey. *Am J Kidney Dis,* 32**,** 992-9.

Juillard, L., M. Janier, D. Fouque, L. Cinotti, N. Maakel, D. Le Bars, P. Barthez, N. Pozet & M. Laville (2002) Dynamic renal blood flow measurement by positron emission tomography in patients with CRF. *Am J Kidney Dis,* 40**,** 947-54.

Juillard, L., M. Janier, D. Fouque, M. Lionnet, D. Le Bars, L. Cinotti, P. Barthez, C. Gharib & M. Laville (2000) Renal blood flow measurement by positron emission tomography using 15O-labeled water. *Kidney Int,* 57**,** 2511-8.

Jung, F., C. Mrowietz, C. Labarrere, S. Schüler & J. Park (2001) Primary cutaneous microangiopathy in heart recipients. *Microvasc Res,* 62**,** 154-63.

Järvisalo, M., L. Jartti, J. Toikka, J. Hartiala, T. Rönnemaa & O. Raitakari (2000) Noninvasive assessment of brachial artery endothelial function with digital ultrasound and 13-MHz scanning frequency: feasibility of measuring the true inner luminal diameter using the intima-lumen interface. *Ultrasound Med Biol,* 26**,** 1257-60.

Järvisalo, M., M. Raitakari, J. Toikka, A. Putto-Laurila, R. Rontu, S. Laine, T. Lehtimäki, T. Rönnemaa, J. Viikari & O. Raitakari (2004) Endothelial dysfunction and increased arterial intima-media thickness in children with type 1 diabetes. *Circulation,* 109**,** 1750-5.

Järvisalo, M., J. Toikka, T. Vasankari, J. Mikkola, J. Viikari, J. Hartiala & O. Raitakari (1999) HMG CoA reductase inhibitors are related to improved systemic endothelial function in coronary artery disease. *Atherosclerosis,* 147**,** 237-42.

Kalra, P., H. Guo, D. Gilbertson, J. Liu, S. Chen, A. Ishani, A. Collins & R. Foley (2010) Atherosclerotic renovascular disease in the United States. *Kidney Int,* 77**,** 37-43.

Kalra, P., H. Guo, A. Kausz, D. Gilbertson, J. Liu, S. Chen, A. Ishani, A. Collins & R. Foley (2005) Atherosclerotic renovascular disease in United States patients aged 67 years or older: risk factors, revascularization, and prognosis. *Kidney Int,* 68**,** 293-301.

Kalra, P., H. Mamtora, A. Holmes & S. Waldek (1990) Renovascular disease and renal complications of angiotensin-converting enzyme inhibitor therapy. *Q J Med,* 77**,** 1013-8.

Kaufmann, P. & P. Camici (2005) Myocardial blood flow measurement by PET: technical aspects and clinical applications. *J Nucl Med,* 46**,** 75-88.

Kaufmann, P., T. Gnecchi-Ruscone, M. di Terlizzi, K. Schäfers, T. Lüscher & P. Camici (2000a) Coronary heart disease in smokers: vitamin C restores coronary microcirculatory function. *Circulation,* 102**,** 1233-8.

Kaufmann, P., T. Gnecchi-Ruscone, K. Schäfers, T. Lüscher & P. Camici (2000b) Low density lipoprotein cholesterol and coronary microvascular dysfunction in hypercholesterolemia. *J Am Coll Cardiol,* 36**,** 103-9.

Kawarada, O., Y. Yokoi, N. Morioka & K. Takemoto (2007) Renal artery stenosis in cardio- and cerebrovascular disease: renal duplex ultrasonography as an initial screening examination. *Circ J,* 71**,** 1942-7.

Kestenbaum, B., J. Sampson, K. Rudser, D. Patterson, S. Seliger, B. Young, D. Sherrard & D. Andress (2005) Serum phosphate levels and mortality risk among people with chronic kidney disease. *J Am Soc Nephrol,* 16**,** 520-8.

Ketteler, M., P. Bongartz, R. Westenfeld, J. Wildberger, A. Mahnken, R. Böhm, T. Metzger, C. Wanner, W. Jahnen-Dechent & J. Floege (2003) Association of low fetuin-A (AHSG) concentrations in serum with cardiovascular mortality in patients on dialysis: a cross-sectional study. *Lancet,* 361**,** 827-33.

Khalique, O., W. Aronow, C. Ahn, M. Mazar, B. Schair, J. Shao & V. Channamsetty (2007) Relation of moderate or severe reduction in glomerular filtration rate to number of coronary arteries narrowed >50-1074451856n patients undergoing coronary angiography for suspected coronary artery disease. *Am J Cardiol,* 100**,** 415-6.

Kilickesmez, K., O. Abaci, B. Okcun, C. Kocas, M. Baskurt, A. Arat, M. Ersanli & T. Gurmen (2010)

Chronic kidney disease as a predictor of coronary lesion morphology. *Angiology,* 61**,** 344-9.

Kim, D., D. Porter, R. Brown, M. Crivello, P. Silva & B. Leeming (1991) Renal artery imaging: a prospective comparison of intra-arterial digital subtraction angiography with conventional angiography. *Angiology,* 42**,** 345-57.

Kim, K., G. Onesti, A. Schwartz, J. Chinitz & C. Swartz (1972) Hemodynamics of hypertension in chronic end-stage renal disease. *Circulation,* 46**,** 456-64.

Klein, I., G. Ligtenberg, J. Neumann, P. Oey, H. Koomans & P. Blankestijn (2003a) Sympathetic nerve activity is inappropriately increased in chronic renal disease. *J Am Soc Nephrol,* 14**,** 3239-44.

Klein, I., G. Ligtenberg, P. Oey, H. Koomans & P. Blankestijn (2003b) Enalapril and losartan reduce sympathetic hyperactivity in patients with chronic renal failure. *J Am Soc Nephrol,* 14**,** 425-30.

Kriz, W. & M. Elgar. 2003. Renal anatomy. In *Comprehensive Clinical Nephrology,* eds. R. J. Johnson & J. Feehally, 1-11. Elsevier Science.

Kudomi, N., N. Koivuviita, K. Liukko, V. Oikonen, T. Tolvanen, H. Iida, R. Tertti, K. Metsärinne, P. Iozzo & P. Nuutila (2009) Parametric renal blood flow imaging using [15O]H2O and PET. *Eur J Nucl Med Mol Imaging,* 36**,** 683-91.

Kuroda, S., N. Nishida, T. Uzu, M. Takeji, M. Nishimura, T. Fujii, S. Nakamura, T. Inenaga, C. Yutani & G. Kimura (2000) Prevalence of renal artery stenosis in autopsy patients with stroke. *Stroke,* 31**,** 61-5.

Kuten, A., H. Roval, L. Griffeth, M. Mintum, C. Perez, T. Wasserman & M. Ter-Pogossian (1992) Positron emission tomography in the study of acute radiation effects on renal blood flow in dogs. *Int Urol Nephrol,* 24**,** 527-9.

Ladefoged, J. (1966) Measurements of the renal blood flow in man with the 133 xenon wash-out technique. A description of the method. *Scand J Clin Lab Invest,* 18**,** 299-315.

Laine, H., O. Raitakari, H. Niinikoski, O. Pitkänen, H. Iida, J. Viikari, P. Nuutila & J. Knuuti (1998) Early impairment of coronary flow reserve in young men with borderline hypertension. *J Am Coll Cardiol,* 32**,** 147-53.

Landmesser, U., B. Hornig & H. Drexler (2004) Endothelial function: a critical determinant in atherosclerosis? *Circulation,* 109**,** II27-33.

Leertouwer, T., E. Gussenhoven, J. Bosch, B. van Jaarsveld, L. van Dijk, J. Deinum & A. Man In 't Veld (2000) Stent placement for renal arterial stenosis: where do we stand? A meta-analysis. *Radiology,* 216**,** 78-85.

Lehto, S., L. Niskanen, M. Suhonen, T. Rönnemaa & M. Laakso (1996) Medial artery calcification. A neglected harbinger of cardiovascular complications in non-insulin-dependent diabetes mellitus. *Arterioscler Thromb Vasc Biol,* 16**,** 978-83.

Leipert, B., B. Becker & E. Gerlach (1992) Different endothelial mechanisms involved in coronary responses to known vasodilators. *Am J Physiol,* 262**,** H1676-83.

Lerman, L., A. Flickinger, P. n. Sheedy & S. Turner (1996a) Reproducibility of human kidney perfusion and volume determinations with electron beam computed tomography. *Invest Radiol,* 31**,** 204-10.

Lerman, L., R. Schwartz, J. Grande, P. Sheedy & J. Romero (1999) Noninvasive evaluation of a novel swine model of renal artery stenosis. *J Am Soc Nephrol,* 10**,** 1455-65.

Lerman, L., S. Taler, S. Textor, P. n. Sheedy, A. Stanson & J. Romero (1996b) Computed tomography-derived intrarenal blood flow in renovascular and essential hypertension. *Kidney Int,* 49**,** 846-54.

Lerman, L., S. Textor & J. Grande (2009) Mechanisms of tissue injury in renal artery stenosis: ischemia and beyond. *Prog Cardiovasc Dis,* 52**,** 196-203.

Levey, A., J. Bosch, J. Lewis, T. Greene, N. Rogers & D. Roth (1999) A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med,* 130**,** 461-70.

Levin, A., C. Thompson, J. Ethier, E. Carlisle, S. Tobe, D. Mendelssohn, E. Burgess, K. Jindal, B. Barrett, J. Singer & O. Djurdjev (1999) Left ventricular mass index increase in early renal disease: impact of decline in hemoglobin. *Am J Kidney Dis,* 34**,** 125-34.

Losito, A., R. Errico, P. Santirosi, T. Lupattelli, G. Scalera & L. Lupattelli (2005) Long-term follow-up of atherosclerotic renovascular disease. Beneficial effect of ACE inhibition. *Nephrol Dial Transplant,* 20**,** 1604-9.

Louie, J., J. Isaacson, R. Zierler, R. Bergelin & D. J. Strandness (1994) Prevalence of carotid and lower extremity arterial disease in patients with renal artery stenosis. *Am J Hypertens,* 7**,** 436-9.

Lowrie, E. & N. Lew (1990) Death risk in hemodialysis patients: the predictive value of commonly measured variables and an evaluation of death rate differences between facilities. *Am J Kidney Dis,* 15**,** 458-82.

Ludmer, P., A. Selwyn, T. Shook, R. Wayne, G. Mudge, R. Alexander & P. Ganz (1986) Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. *N Engl J Med,* 315**,** 1046-51.

Machac, J. (2005) Cardiac positron emission tomography imaging. *Semin Nucl Med,* 35**,** 17-36.

Manzi, S., E. Meilahn, J. Rairie, C. Conte, T. J. Medsger, L. Jansen-McWilliams, R. D'Agostino & L. Kuller (1997) Age-specific incidence rates of myocardial

infarction and angina in women with systemic lupus erythematosus: comparison with the Framingham Study. *Am J Epidemiol,* 145**,** 408-15.

Mayhan, W. (1992) Endothelium-dependent responses of cerebral arterioles to adenosine 5'-diphosphate. *J Vasc Res,* 29**,** 353-8.

McIntyre, C., J. Burton, N. Selby, L. Leccisotti, S. Korsheed, C. Baker & P. Camici (2008) Hemodialysis-induced cardiac dysfunction is associated with an acute reduction in global and segmental myocardial blood flow. *Clin J Am Soc Nephrol,* 3**,** 19-26.

Merlet, P., B. Mazoyer, L. Hittinger, H. Valette, J. Saal, B. Bendriem, B. Crozatier, A. Castaigne, A. Syrota & J. Randé (1993) Assessment of coronary reserve in man: comparison between positron emission tomography with oxygen-15-labeled water and intracoronary Doppler technique. *J Nucl Med,* 34**,** 1899-904.

Meyer, M., D. Lieps, H. Schatz & M. Pfohl (2008) Impaired flow-mediated vasodilation in type 2 diabetes: lack of relation to microvascular dysfunction. *Microvasc Res,* 76**,** 61-5.

Middlekauff, H., E. Nitzsche, M. Hamilton, H. Schelbert, G. Fonarow, J. Moriguchi, A. Hage, S. Saleh & G. Gibbs (1995) Evidence for preserved cardiopulmonary baroreflex control of renal cortical blood flow in humans with advanced heart failure. A positron emission tomography study. *Circulation,* 92**,** 395-401.

Middlekauff, H., E. Nitzsche, A. Nguyen, C. Hoh & G. Gibbs (1997) Modulation of renal cortical blood flow during static exercise in humans. *Circ Res,* 80**,** 62-8.

Miles, K. (1991) Measurement of tissue perfusion by dynamic computed tomography. *Br J Radiol,* 64**,** 409-12.

Miles, K., M. Hayball & A. Dixon (1994) Functional imaging of changes in human intrarenal perfusion using quantitative dynamic computed tomography. *Invest Radiol,* 29**,** 911-4.

Mistry, S., N. Ives, J. Harding, K. Fitzpatrick-Ellis, G. Lipkin, P. Kalra, J. Moss & K. Wheatley (2007) Angioplasty and STent for Renal Artery Lesions (ASTRAL trial): rationale, methods and results so far. *J Hum Hypertens,* 21**,** 511-5.

Mitchell, J., R. Subramanian, C. White, P. Soukas, Y. Almagor, R. Stewart & K. Rosenfield (2007) Predicting blood pressure improvement in hypertensive patients after renal artery stent placement: renal fractional flow reserve. *Catheter Cardiovasc Interv,* 69**,** 685-9.

Moe, S. & N. Chen (2008) Mechanisms of vascular calcification in chronic kidney disease. *J Am Soc Nephrol,* 19**,** 213-6.

Morita, K., T. Tsukamoto, M. Naya, K. Noriyasu, M. Inubushi, T. Shiga, C. Katoh, Y. Kuge, H. Tsutsui & N. Tamaki (2006) Smoking cessation normalizes coronary endothelial vasomotor response assessed with 15O-water and PET in healthy young smokers. *J Nucl Med,* 47**,** 1914-20.

Motz, W. & B. Strauer (1996) Improvement of coronary flow reserve after long-term therapy with enalapril. *Hypertension,* 27**,** 1031-8.

Mounier-Vehier, C., B. Cocheteux, S. Haulon, P. Devos, C. Lions, C. Gautier, A. Carre & J. Beregi (2004) Changes in renal blood flow reserve after angioplasty of renal artery stenosis in hypertensive patients. *Kidney Int,* 65**,** 245-50.

Muiesan, M., M. Salvetti, C. Monteduro, D. Rizzoni, R. Zulli, C. Corbellini, C. Brun & E. Agabiti-Rosei (1999) Effect of treatment on flow-dependent vasodilation of the brachial artery in essential hypertension. *Hypertension,* 33**,** 575-80.

Mullen, M., R. Kharbanda, J. Cross, A. Donald, M. Taylor, P. Vallance, J. Deanfield & R. MacAllister (2001) Heterogenous nature of flow-mediated dilatation in human conduit arteries in vivo: relevance to endothelial dysfunction in hypercholesterolemia. *Circ Res,* 88**,** 145-51.

Muntner, P., J. He, B. Astor, A. Folsom & J. Coresh (2005) Traditional and nontraditional risk factors predict coronary heart disease in chronic kidney disease: results from the atherosclerosis risk in communities study. *J Am Soc Nephrol,* 16**,** 529-38.

Nanayakkara, P. & C. Gaillard (2010) Vascular disease and chronic renal failure: new insights. *Neth J Med,* 68**,** 5-14.

Nanayakkara, P., J. Kiefte-de Jong, P. ter Wee, C. Stehouwer, F. van Ittersum, M. Olthof, T. Teerlink, J. Twisk, C. van Guldener & Y. Smulders (2009) Randomized placebo-controlled trial assessing a treatment strategy consisting of pravastatin, vitamin E, and homocysteine lowering on plasma asymmetric dimethylarginine concentration in mild to moderate CKD. *Am J Kidney Dis,* 53**,** 41-50.

Nanayakkara, P., T. Teerlink, C. Stehouwer, D. Allajar, A. Spijkerman, C. Schalkwijk, P. ter Wee & C. van Guldener (2005) Plasma asymmetric dimethylarginine (ADMA) concentration is independently associated with carotid intima-media thickness and plasma soluble vascular cell adhesion molecule-1 (sVCAM-1) concentration in patients with mild-to-moderate renal failure. *Kidney Int,* 68**,** 2230-6.

Nardi, E., S. Cottone, G. Mulè, A. Palermo, P. Cusimano & G. Cerasola (2007) Influence of chronic renal insufficiency on left ventricular diastolic function in hypertensives without left ventricular hypertrophy. *J Nephrol,* 20**,** 320-8.

Navaneethan, S., F. Pansini, V. Perkovic, C. Manno, F. Pellegrini, D. Johnson, J. Craig & G. Strippoli (2009) HMG CoA reductase inhibitors (statins) for people with chronic kidney disease not requiring dialysis. *Cochrane Database Syst Rev***,** CD007784.

Naya, M., T. Tsukamoto, K. Morita, C. Katoh, T. Furumoto, S. Fujii, N. Tamaki & H. Tsutsui (2007) Olmesartan, but not amlodipine, improves endothelium-dependent coronary dilation in hypertensive patients. *J Am Coll Cardiol,* 50**,** 1144-9.

Nesterov, S., C. Han, M. Mäki, S. Kajander, A. Naum, H. Helenius, I. Lisinen, H. Ukkonen, M. Pietilä, E. Joutsiniemi & J. Knuuti (2009) Myocardial perfusion quantitation with 15O-labelled water PET: high reproducibility of the new cardiac analysis software (Carimas). *Eur J Nucl Med Mol Imaging,* 36**,** 1594-602.

Neumann, J., G. Ligtenberg, I. Klein, H. Koomans & P. Blankestijn (2004) Sympathetic hyperactivity in chronic kidney disease: pathogenesis, clinical relevance, and treatment. *Kidney Int,* 65**,** 1568-76.

Neunteufl, T., R. Katzenschlager, A. Hassan, U. Klaar, S. Schwarzacher, D. Glogar, P. Bauer & F. Weidinger (1997) Systemic endothelial dysfunction is related to the extent and severity of coronary artery disease. *Atherosclerosis,* 129**,** 111-8.

Niendorf, E., T. Grist, F. J. Lee, P. Brazy & G. Santyr (1998) Rapid in vivo measurement of single-kidney extraction fraction and glomerular filtration rate with MR imaging. *Radiology,* 206**,** 791-8.

Niizuma, S., S. Takiuchi, S. Okada, T. Horio, K. Kamide, H. Nakata, F. Yoshihara, S. Nakamura, Y. Kawano, H. Nakahama, Y. Iwanaga & S. Nakatani (2008) Decreased coronary flow reserve in haemodialysis patients. *Nephrol Dial Transplant,* 23**,** 2324-8.

Nikolov, I., A. Mozar, T. Drüeke & Z. Massy (2009) Impact of disturbances of calcium and phosphate metabolism on vascular calcification and clinical outcomes in patients with chronic kidney disease. *Blood Purif,* 27**,** 350-9.

Nitzsche, E., Y. Choi, D. Killion, C. Hoh, R. Hawkins, J. Rosenthal, D. Buxton, S. Huang, M. Phelps & H. Schelbert (1993) Quantification and parametric imaging of renal cortical blood flow in vivo based on Patlak graphical analysis. *Kidney Int,* 44**,** 985-96.

O'Connor, P., W. Anderson, M. Kett & R. Evans (2006) Renal preglomerular arterial-venous O2 shunting is a structural anti-oxidant defence mechanism of the renal cortex. *Clin Exp Pharmacol Physiol,* 33**,** 637-41.

Oberg, B., E. McMenamin, F. Lucas, E. McMonagle, J. Morrow, T. Ikizler & J. Himmelfarb (2004) Increased prevalence of oxidant stress and inflammation in patients with moderate to severe chronic kidney disease. *Kidney Int,* 65**,** 1009-16.

Olin, J. (2002) Atherosclerotic renal artery disease. *Cardiol Clin,* 20**,** 547-62, vi.

Ollivier, R., D. Boulmier, D. Veillard, G. Leurent, S. Mock, M. Bedossa & H. Le Breton (2009) Frequency and predictors of renal artery stenosis in patients with coronary artery disease. *Cardiovasc Revasc Med,* 10**,** 23-9.

Opherk, D., G. Mall, H. Zebe, F. Schwarz, E. Weihe, J. Manthey & W. Kübler (1984) Reduction of coronary reserve: a mechanism for angina pectoris in patients with arterial hypertension and normal coronary arteries. *Circulation,* 69**,** 1-7.

Opie, L. 1998b. Oxygen supply: coronary flow. The Heart Physiology, from Cell to Circulation. 267-293. Lippincott-Raven Publishers.

Panza, J., A. Quyyumi, J. J. Brush & S. Epstein (1990) Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N Engl J Med,* 323**,** 22-7.

Parfrey, P. & R. Foley (1999) The clinical epidemiology of cardiac disease in chronic renal failure. *J Am Soc Nephrol,* 10**,** 1606-15.

Parildar, M., Z. Parildar, I. Oran, C. Kabaroglu, A. Memis & O. Bayindir (2003) Nitric oxide and oxidative stress in atherosclerotic renovascular hypertension: effect of endovascular treatment. *J Vasc Interv Radiol,* 14**,** 887-92.

Parodi, O., D. Neglia, C. Palombo, G. Sambuceti, A. Giorgetti, C. Marabotti, M. Gallopin, I. Simonetti & A. L'Abbate (1997) Comparative effects of enalapril and verapamil on myocardial blood flow in systemic hypertension. *Circulation,* 96**,** 864-73.

Paul, J., P. Ugolini, M. Sapoval, E. Mousseaux & J. Gaux (2001) Unilateral renal artery stenosis: perfusion patterns with electron-beam dynamic CT--preliminary experience. *Radiology,* 221**,** 261-5.

Paul, R. J., A. Ettinger, M. Fainsinger, A. Callow, P. Kahn & L. Inker (1965) Angiographic visualization of renal collateral circulation as a means of detecting and delineating renal ischemia. *Radiology,* 84**,** 1013-21.

Perticone, F., R. Ceravolo, A. Pujia, G. Ventura, S. Iacopino, A. Scozzafava, A. Ferraro, M. Chello, P. Mastroroberto, P. Verdecchia & G. Schillaci (2001) Prognostic significance of endothelial dysfunction in hypertensive patients. *Circulation,* 104**,** 191-6.

Pfeffer, M., E. Burdmann, C. Chen, M. Cooper, D. de Zeeuw, K. Eckardt, J. Feyzi, P. Ivanovich, R. Kewalramani, A. Levey, E. Lewis, J. McGill, J. McMurray, P. Parfrey, H. Parving, G. Remuzzi, A. Singh, S. Solomon, R. Toto & T. Investigators (2009) A trial of darbepoetin alfa in type 2 diabetes and chronic kidney disease. *N Engl J Med,* 361**,** 2019-32.

Pickering, T., L. Herman, R. Devereux, J. Sotelo, G. James, T. Sos, M. Silane & J. Laragh (1988) Recurrent pulmonary oedema in hypertension due to bilateral renal artery stenosis: treatment by angioplasty or surgical revascularisation. *Lancet,* 2**,** 551-2.

Pietro, D. A., A. G. Voelkel, B. J. Ray & A. F. Parisi (1981) Reproducibility of echocardiography. A study evaluating the variability of serial echocardiographic measurements. *Chest,* 79**,** 29-32.

Pitkänen, O., P. Nuutila, O. Raitakari, K. Porkka, H. Iida, I. Nuotio, T. Rönnemaa, J. Viikari, M. Taskinen, C. Ehnholm & J. Knuuti (1999) Coronary flow reserve in young men with familial combined hyperlipidemia. *Circulation,* 99**,** 1678-84.

Pitkänen, O., P. Nuutila, O. Raitakari, T. Rönnemaa, P. Koskinen, H. Iida, T. Lehtimäki, H. Laine, T. Takala, J. Viikari & J. Knuuti (1998) Coronary flow reserve is reduced in young men with IDDM. *Diabetes,* 47**,** 248-54.

Pitkänen, O., O. Raitakari, H. Niinikoski, P. Nuutila, H. Iida, L. Voipio-Pulkki, R. Härkönen, U. Wegelius, T. Rönnemaa, J. Viikari & J. Knuuti (1996) Coronary flow reserve is impaired in young men with familial hypercholesterolemia. *J Am Coll Cardiol,* 28**,** 1705-11.

Plouin, P., G. Chatellier, B. Darné & A. Raynaud (1998) Blood pressure outcome of angioplasty in atherosclerotic renal artery stenosis: a randomized trial. Essai Multicentrique Medicaments vs Angioplastie (EMMA) Study Group. *Hypertension,* 31**,** 823-9.

Port, F., T. Hulbert-Shearon, R. Wolfe, W. Bloembergen, T. Golper, L. Agodoa & E. Young (1999) Predialysis blood pressure and mortality risk in a national sample of maintenance hemodialysis patients. *Am J Kidney Dis,* 33**,** 507-17.

Porter, L. & N. Hollenberg (1998) Obesity, salt intake, and renal perfusion in healthy humans. *Hypertension,* 32**,** 144-8.

Pupim, L., J. Himmelfarb, E. McMonagle, Y. Shyr & T. Ikizler (2004) Influence of initiation of maintenance hemodialysis on biomarkers of inflammation and oxidative stress. *Kidney Int,* 65**,** 2371-9.

Radermacher, J., A. Chavan, J. Bleck, A. Vitzthum, B. Stoess, M. Gebel, M. Galanski, K. Koch & H. Haller (2001) Use of Doppler ultrasonography to predict the outcome of therapy for renal-artery stenosis. *N Engl J Med,* 344**,** 410-7.

Rahmim, A. & H. Zaidi (2008) PET versus SPECT: strengths, limitations and challenges. *Nucl Med Commun,* 29**,** 193-207.

Ravani, P., G. Tripepi, F. Malberti, S. Testa, F. Mallamaci & C. Zoccali (2005) Asymmetrical dimethylarginine predicts progression to dialysis and death in patients with chronic kidney disease: a competing risks modeling approach. *J Am Soc Nephrol,* 16**,** 2449-55.

Recio-Mayoral, A., J. Mason, J. Kaski, M. Rubens, O. Harari & P. Camici (2009) Chronic inflammation and coronary microvascular dysfunction in patients without risk factors for coronary artery disease. *Eur Heart J,* 30**,** 1837-43.

Richardson, D., J. Foster, A. Davison & H. Irving (2000) Parvus tardus waveform suggesting renal artery stenosis-remember the more proximal stenosis. *Nephrol Dial Transplant,* 15**,** 539-43.

Ridker, P., C. Cannon, D. Morrow, N. Rifai, L. Rose, C. McCabe, M. Pfeffer & E. Braunwald (2005) C-reactive protein levels and outcomes after statin therapy. *N Engl J Med,* 352**,** 20-8.

Ridker, P., E. Danielson, F. Fonseca, J. Genest, A. J. Gotto, J. Kastelein, W. Koenig, P. Libby, A. Lorenzatti, J. MacFadyen, B. Nordestgaard, J. Shepherd, J. Willerson & R. Glynn (2008) Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med,* 359**,** 2195-207.

Rizzoni, D., E. Porteri, M. Castellano, G. Bettoni, M. Muiesan, G. Tiberio, S. Giulini, G. Rossi, G. Bernini & E. Agabiti-Rosei (1998) Endothelial dysfunction in hypertension is independent from the etiology and from vascular structure. *Hypertension,* 31**,** 335-41.

Roberts, D., J. Detre, L. Bolinger, E. Insko, R. Lenkinski, M. Pentecost & J. J. Leigh (1995) Renal perfusion in humans: MR imaging with spin tagging of arterial water. *Radiology,* 196**,** 281-6.

Roberts, M., D. Hare, S. Ratnaike & F. Ierino (2006) Cardiovascular biomarkers in CKD: pathophysiology and implications for clinical management of cardiac disease. *Am J Kidney Dis,* 48**,** 341-60.

Rodriguez-Porcel, M., J. Krier, A. Lerman, P. n. Sheedy, J. Romero, C. Napoli & L. Lerman (2001) Combination of hypercholesterolemia and hypertension augments renal function abnormalities. *Hypertension,* 37**,** 774-80.

Rodriguez-Porcel, M., A. Lerman, J. Herrmann, R. Schwartz, T. Sawamura, M. Condorelli, C. Napoli & L. Lerman (2003a) Hypertension exacerbates the effect of hypercholesterolemia on the myocardial microvasculature. *Cardiovasc Res,* 58**,** 213-21.

Rodriguez-Porcel, M., L. Lerman, J. Herrmann, T. Sawamura, C. Napoli & A. Lerman (2003b) Hypercholesterolemia and hypertension have synergistic deleterious effects on coronary endothelial function. *Arterioscler Thromb Vasc Biol,* 23**,** 885-91.

Rodriguez-Porcel, M., X. Zhu, A. Chade, B. Amores-Arriaga, N. Caplice, E. Ritman, A. Lerman & L. Lerman (2006) Functional and structural remodeling of the myocardial microvasculature in early experimental hypertension. *Am J Physiol Heart Circ Physiol,* 290**,** H978-84.

Rognant, N., F. Guebre-Egziabher, J. Bacchetta, M. Janier, B. Hiba, J. Langlois, R. Gadet, M. Laville & L. Juillard (2010) Evolution of renal oxygen content measured by BOLD MRI downstream a chronic renal artery stenosis. *Nephrol Dial Transplant*.

Roman, M., B. Shanker, A. Davis, M. Lockshin, L. Sammaritano, R. Simantov, M. Crow, J. Schwartz, S. Paget, R. Devereux & J. Salmon (2003) Prevalence and correlates of accelerated atherosclerosis in systemic lupus erythematosus. *N Engl J Med,* 349**,** 2399-406.

Rosen, S., N. Hollenberg, J. J. Dealy & J. Merrill (1968) Measurement of the distribution of blood flow in the

human kidney using the intra-arterial injection of 133Xe. Relationship to function in the normal and transplanted kidney. *Clin Sci,* 34**,** 287-302.

Ross, R. (1999) Atherosclerosis--an inflammatory disease. *N Engl J Med,* 340**,** 115-26.

Rubanyi, G., J. Romero & P. Vanhoutte (1986) Flow-induced release of endothelium-derived relaxing factor. *Am J Physiol,* 250**,** H1145-9.

Russo, D., S. Corrao, I. Miranda, C. Ruocco, S. Manzi, R. Elefante, D. Brancaccio, M. Cozzolino, M. Biondi & V. Andreucci (2007) Progression of coronary artery calcification in predialysis patients. *Am J Nephrol,* 27**,** 152-8.

Sahn, D., A. DeMaria, J. Kisslo & A. Weyman (1978) Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. *Circulation,* 58**,** 1072-83.

Sarnak, M. & A. Levey (2000) Cardiovascular disease and chronic renal disease: a new paradigm. *Am J Kidney Dis,* 35**,** S117-31.

Sarnak, M., H. Tighiouart, G. Manjunath, B. MacLeod, J. Griffith, D. Salem & A. Levey (2002) Anemia as a risk factor for cardiovascular disease in The Atherosclerosis Risk in Communities (ARIC) study. *J Am Coll Cardiol,* 40**,** 27-33.

Schabauer, A. & T. Rooke (1994) Cutaneous laser Doppler flowmetry: applications and findings. *Mayo Clin Proc,* 69**,** 564-74.

Schenker, M., S. Dorbala, E. Hong, F. Rybicki, R. Hachamovitch, R. Kwong & M. Di Carli (2008) Interrelation of coronary calcification, myocardial ischemia, and outcomes in patients with intermediate likelihood of coronary artery disease: a combined positron emission tomography/computed tomography study. *Circulation,* 117**,** 1693-700.

Schiller, N., P. Shah, M. Crawford, A. DeMaria, R. Devereux, H. Feigenbaum, H. Gutgesell, N. Reichek, D. Sahn & I. Schnittger Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. *J Am Soc Echocardiogr,* 2**,** 358-67.

Schindler, T., E. Nitzsche, H. Schelbert, M. Olschewski, J. Sayre, M. Mix, I. Brink, X. Zhang, M. Kreissl, N. Magosaki, H. Just & U. Solzbach (2005) Positron emission tomography-measured abnormal responses of myocardial blood flow to sympathetic stimulation are associated with the risk of developing cardiovascular events. *J Am Coll Cardiol,* 45**,** 1505-12.

Schindler, T., H. Schelbert, A. Quercioli & V. Dilsizian (2010) Cardiac PET imaging for the detection and monitoring of coronary artery disease and microvascular health. *JACC Cardiovasc Imaging,* 3**,** 623-40.

Schoenberg, S., S. Aumann, A. Just, M. Bock, M. Knopp, L. Johansson & H. Ahlstrom (2003) Quantification of renal perfusion abnormalities using an intravascular contrast agent (part 2): results in animals and humans with renal artery stenosis. *Magn Reson Med,* 49**,** 288-98.

Schoenberg, S., M. Bock, S. Aumann, A. Just, M. Essig, F. Floemer, M. Knopp & G. van Kaick (2000) [Quantitative recording of renal function with magnetic resonance tomography]. *Radiologe,* 40**,** 925-37.

Schoenberg, S., J. Rieger, H. Michaely, H. Rupprecht, W. Samtleben & M. Reiser (2006) Functional magnetic resonance imaging in renal artery stenosis. *Abdom Imaging,* 31**,** 200-12.

Schwartz, C. & T. White (1964) Stenosis of renal artery: An unselected necropsy study. *Br Med J,* 2**,** 1415-21.

Schwitter, J., D. Nanz, S. Kneifel, K. Bertschinger, M. Büchi, P. Knüsel, B. Marincek, T. Lüscher & G. von Schulthess (2001) Assessment of myocardial perfusion in coronary artery disease by magnetic resonance: a comparison with positron emission tomography and coronary angiography. *Circulation,* 103**,** 2230-5.

Scoble, J., D. de Takats, M. Ostermann, J. Connolly, N. Scott, J. Beeso, K. Poyser, T. Peters & R. Sherwood (1999) Lipid profiles in patients with atherosclerotic renal artery stenosis. *Nephron,* 83**,** 117-21.

Scolari, F., P. Ravani, A. Pola, S. Guerini, R. Zubani, E. Movilli, S. Savoldi, F. Malberti & R. Maiorca (2003) Predictors of renal and patient outcomes in atheroembolic renal disease: a prospective study. *J Am Soc Nephrol,* 14**,** 1584-90.

Shahgaldi, K., P. Gudmundsson, A. Manouras, L. A. Brodin & R. Winter (2009) Visually estimated ejection fraction by two dimensional and triplane echocardiography is closely correlated with quantitative ejection fraction by real-time three dimensional echocardiography. *Cardiovasc Ultrasound,* 7**,** 41.

Shlipak, M., L. Fried, M. Cushman, T. Manolio, D. Peterson, C. Stehman-Breen, A. Bleyer, A. Newman, D. Siscovick & B. Psaty (2005) Cardiovascular mortality risk in chronic kidney disease: comparison of traditional and novel risk factors. *JAMA,* 293**,** 1737-45.

Siddiqi, L., N. Prakken, B. Velthuis, M. Cramer, P. Oey, P. Boer, M. Bots & P. Blankestijn (2010) Sympathetic activity in chronic kidney disease patients is related to left ventricular mass despite antihypertensive treatment. *Nephrol Dial Transplant,* 25**,** 3272-7.

Silva, J., A. Chan, C. White, T. Collins, J. Jenkins, J. Reilly & S. Ramee (2005) Elevated brain natriuretic peptide predicts blood pressure response after stent revascularization in patients with renal artery stenosis. *Circulation,* 111**,** 328-33.

Singh, A., L. Szczech, K. Tang, H. Barnhart, S. Sapp, M. Wolfson, D. Reddan & C. Investigators (2006) Correction of anemia with epoetin alfa in chronic kidney disease. *N Engl J Med,* 355**,** 2085-98.

Sipilä, H., J. Clark, O. Peltola & M. Teräs (2001) An automatic 15OH2O production system for heart and brain studies (abst). *J Labelled Cpd Radiopharm,* 44**,** 1066-1068.

Stehouwer, C., R. Henry, J. Dekker, G. Nijpels, R. Heine & L. Bouter (2004) Microalbuminuria is associated with impaired brachial artery, flow-mediated vasodilation in elderly individuals without and with diabetes: further evidence for a link between microalbuminuria and endothelial dysfunction--the Hoorn Study. *Kidney Int Suppl***,** S42-4.

Stenvinkel, P., J. Carrero, J. Axelsson, B. Lindholm, O. Heimbürger & Z. Massy (2008) Emerging biomarkers for evaluating cardiovascular risk in the chronic kidney disease patient: how do new pieces fit into the uremic puzzle? *Clin J Am Soc Nephrol,* 3**,** 505-21.

Suki, W., R. Zabaneh, J. Cangiano, J. Reed, D. Fischer, L. Garrett, B. Ling, S. Chasan-Taber, M. Dillon, A. Blair & S. Burke (2007) Effects of sevelamer and calcium-based phosphate binders on mortality in hemodialysis patients. *Kidney Int,* 72**,** 1130-7.

Svetkey, L., S. Kadir, N. Dunnick, S. Smith, C. Dunham, M. Lambert & P. Klotman (1991) Similar prevalence of renovascular hypertension in selected blacks and whites. *Hypertension,* 17**,** 678-83.

Symonides, B., J. Chodakowska, A. Januszewicz, M. Lapinski, M. Januszewicz, O. Rowiński, J. Szmidt, A. Kuch-Wocial, M. Kurzyna, G. Małek, H. Berent, C. Szmigielski & W. Januszewicz (1999) Effects of the correction of renal artery stenosis on blood pressure, renal function and left ventricular morphology. *Blood Press,* 8**,** 141-50.

Tentori, F., M. Blayney, J. Albert, B. Gillespie, P. Kerr, J. Bommer, E. Young, T. Akizawa, T. Akiba, R. Pisoni, B. Robinson & F. Port (2008) Mortality risk for dialysis patients with different levels of serum calcium, phosphorus, and PTH: the Dialysis Outcomes and Practice Patterns Study (DOPPS). *Am J Kidney Dis,* 52**,** 519-30.

Textor, S. (2004) Ischemic nephropathy: where are we now? *J Am Soc Nephrol,* 15**,** 1974-82.

Textor, S., J. Glockner, L. Lerman, S. Misra, M. McKusick, S. Riederer, J. Grande, S. Gomez & J. Romero (2008) The use of magnetic resonance to evaluate tissue oxygenation in renal artery stenosis. *J Am Soc Nephrol,* 19**,** 780-8.

Textor, S., L. Lerman & M. McKusick (2009a) The uncertain value of renal artery interventions: where are we now? *JACC Cardiovasc Interv,* 2**,** 175-82.

Textor, S., M. McKusick, S. Misra & J. Glockner (2009b) Timing and selection for renal revascularization in an era of negative trials: what to do? *Prog Cardiovasc Dis,* 52**,** 220-8.

Tobe, S., M. Atri, N. Perkins, R. Pugash & C. Bell (2007) Renal athersosclerotic revascularization evaluation (RAVE study): study protocol of a randomized trial [NCT00127738]. *BMC Nephrol,* 8**,** 4.

Toikka, J., M. Ahotupa, J. Viikari, H. Niinikoski, M. Taskinen, K. Irjala, J. Hartiala & O. Raitakari (1999) Constantly low HDL-cholesterol concentration relates to endothelial dysfunction and increased in vivo LDL-oxidation in healthy young men. *Atherosclerosis,* 147**,** 133-8.

Tok, D., H. Gullu, D. Erdogan, S. Topcu, O. Ciftci, I. Yildirim & H. Muderrisoglu (2005) Impaired coronary flow reserve in hemodialysis patients: a transthoracic Doppler echocardiographic study. *Nephron Clin Pract,* 101**,** c200-6.

Treasure, C., J. Klein, J. Vita, S. Manoukian, G. Renwick, A. Selwyn, P. Ganz & R. Alexander (1993) Hypertension and left ventricular hypertrophy are associated with impaired endothelium-mediated relaxation in human coronary resistance vessels. *Circulation,* 87**,** 86-93.

Turkmen, K., H. Oflaz, B. Uslu, A. Cimen, A. Elitok, E. Kasikcioglu, S. Alisir, F. Tufan, S. Namli, M. Uysal & T. Ecder (2008) Coronary flow velocity reserve and carotid intima media thickness in patients with autosomal dominant polycystic kidney disease: from impaired tubules to impaired carotid and coronary arteries. *Clin J Am Soc Nephrol,* 3**,** 986-91.

Uren, N., P. Camici, J. Melin, A. Bol, B. de Bruyne, J. Radvan, I. Olivotto, S. Rosen, M. Impallomeni & W. Wijns (1995) Effect of aging on myocardial perfusion reserve. *J Nucl Med,* 36**,** 2032-6.

Uren, N., J. Melin, B. De Bruyne, W. Wijns, T. Baudhuin & P. Camici (1994) Relation between myocardial blood flow and the severity of coronary-artery stenosis. *N Engl J Med,* 330**,** 1782-8.

Valkonen, V., H. Päivä, J. Salonen, T. Lakka, T. Lehtimäki, J. Laakso & R. Laaksonen (2001) Risk of acute coronary events and serum concentration of asymmetrical dimethylarginine. *Lancet,* 358**,** 2127-8.

Vallée, J., F. Lazeyras, H. Khan & F. Terrier (2000) Absolute renal blood flow quantification by dynamic MRI and Gd-DTPA. *Eur Radiol,* 10**,** 1245-52.

van de Ven, P., R. Kaatee, J. Beutler, F. Beek, A. Woittiez, E. Buskens, H. Koomans & W. Mali (1999) Arterial stenting and balloon angioplasty in ostial atherosclerotic renovascular disease: a randomised trial. *Lancet,* 353**,** 282-6.

van Jaarsveld, B., P. Krijnen, F. Derkx, J. Deinum, A. Woittiez, C. Postma & M. Schalekamp (2001) Resistance to antihypertensive medication as predictor of renal artery stenosis: comparison of two drug regimens. *J Hum Hypertens,* 15**,** 669-76.

van Jaarsveld, B., P. Krijnen, H. Pieterman, F. Derkx, J. Deinum, C. Postma, A. Dees, A. Woittiez, A. Bartelink, A. Man in 't Veld & M. Schalekamp (2000) The effect of balloon angioplasty on hypertension in atherosclerotic renal-artery stenosis. Dutch Renal Artery Stenosis Intervention Cooperative Study Group. *N Engl J Med,* 342**,** 1007-14.

Vasbinder, G., P. Nelemans, A. Kessels, A. Kroon, P. de Leeuw & J. van Engelshoven (2001) Diagnostic tests for renal artery stenosis in patients suspected of having renovascular hypertension: a meta-analysis. *Ann Intern Med,* 135**,** 401-11.

Vasbinder, G., P. Nelemans, A. Kessels, A. Kroon, J. Maki, T. Leiner, F. Beek, M. Korst, K. Flobbe, M. de Haan, W. van Zwam, C. Postma, M. Hunink, P. de Leeuw & J. van Engelshoven (2004) Accuracy of computed tomographic angiography and magnetic resonance angiography for diagnosing renal artery stenosis. *Ann Intern Med,* 141**,** 674-82; discussion 682.

Venkataraman, R., F. Hage, T. Dorfman, J. Heo, R. Aqel, A. de Mattos & A. Iskandrian (2008) Role of myocardial perfusion imaging in patients with end-stage renal disease undergoing coronary angiography. *Am J Cardiol,* 102**,** 1451-6.

--- (2009) Relation between heart rate response to adenosine and mortality in patients with end-stage renal disease. *Am J Cardiol,* 103**,** 1159-64.

Vogt, M., W. Motz & B. Strauer (1992) Coronary haemodynamics in hypertensive heart disease. *Eur Heart J,* 13 Suppl D**,** 44-9.

Wanner, C., V. Krane, W. März, M. Olschewski, J. Mann, G. Ruf, E. Ritz & G. D. a. D. S. Investigators (2005) Atorvastatin in patients with type 2 diabetes mellitus undergoing hemodialysis. *N Engl J Med,* 353**,** 238-48.

Warner, L., S. Gomez, R. Bolterman, J. Haas, M. Bentley, L. Lerman & J. Romero (2009) Regional decreases in renal oxygenation during graded acute renal arterial stenosis: a case for renal ischemia. *Am J Physiol Regul Integr Comp Physiol,* 296**,** R67-71.

Webster, J., F. Marshall, M. Abdalla, A. Dominiczak, R. Edwards, C. Isles, H. Loose, J. Main, P. Padfield, I. Russell, B. Walker, M. Watson & R. Wilkinson (1998) Randomised comparison of percutaneous angioplasty vs continued medical therapy for hypertensive patients with atheromatous renal artery stenosis. Scottish and Newcastle Renal Artery Stenosis Collaborative Group. *J Hum Hypertens,* 12**,** 329-35.

Welch, W., M. Mendonca, S. Aslam & C. Wilcox (2003) Roles of oxidative stress and AT1 receptors in renal hemodynamics and oxygenation in the postclipped 2K,1C kidney. *Hypertension,* 41**,** 692-6.

Wheatley, K., N. Ives, R. Gray, P. Kalra, J. Moss, C. Baigent, S. Carr, N. Chalmers, D. Eadington, G. Hamilton, G. Lipkin, A. Nicholson & J. Scoble (2009) Revascularization versus medical therapy for renal-artery stenosis. *N Engl J Med,* 361**,** 1953-62.

Widlansky, M., N. Gokce, J. J. Keaney & J. Vita (2003) The clinical implications of endothelial dysfunction. *J Am Coll Cardiol,* 42**,** 1149-60.

Wierema, T., C. Postma, A. Houben, A. Kroon, T. Thien, P. Smits & P. de Leeuw (1998) Adenosine-induced renal vasodilatation is prolonged in renal artery stenosis. *J Hypertens,* 16**,** 2109-12.

Wright, J., A. Duggal, R. Thomas, R. Reeve, I. Roberts & P. Kalra (2001) Clinicopathological correlation in biopsy-proven atherosclerotic nephropathy: implications for renal functional outcome in atherosclerotic renovascular disease. *Nephrol Dial Transplant,* 16**,** 765-70.

Wright, J., A. Shurrab, A. Cooper, P. Kalra, R. Foley & P. Kalra (2005) Left ventricular morphology and function in patients with atherosclerotic renovascular disease. *J Am Soc Nephrol,* 16**,** 2746-53.

--- (2009) Progression of cardiac dysfunction in patients with atherosclerotic renovascular disease. *QJM,* 102**,** 695-704.

Yilmaz, M., J. Carrero, J. Martín-Ventura, A. Sonmez, M. Saglam, T. Celik, H. Yaman, M. Yenicesu, T. Eyileten, J. Moreno, J. Egido & L. Blanco-Colio (2010) Combined therapy with renin-angiotensin system and calcium channel blockers in type 2 diabetic hypertensive patients with proteinuria: effects on soluble TWEAK, PTX3, and flow-mediated dilation. *Clin J Am Soc Nephrol,* 5**,** 1174-81.

Yilmaz, M., M. Saglam, K. Caglar, E. Cakir, A. Sonmez, T. Ozgurtas, A. Aydin, T. Eyileten, O. Ozcan, C. Acikel, M. Tasar, G. Genctoy, K. Erbil, A. Vural & C. Zoccali (2006) The determinants of endothelial dysfunction in CKD: oxidative stress and asymmetric dimethylarginine. *Am J Kidney Dis,* 47**,** 42-50.

Yilmaz, M., M. Saglam, J. Carrero, A. Qureshi, K. Caglar, T. Eyileten, A. Sonmez, E. Cakir, M. Yenicesu, B. Lindholm, P. Stenvinkel & J. Axelsson (2008) Serum visfatin concentration and endothelial dysfunction in chronic kidney disease. *Nephrol Dial Transplant,* 23**,** 959-65.

Yokoyama, I., S. Momomura, T. Ohtake, K. Yonekura, J. Nishikawa, Y. Sasaki & M. Omata (1997) Reduced myocardial flow reserve in non-insulin-dependent diabetes mellitus. *J Am Coll Cardiol,* 30**,** 1472-7.

Zeiher, A., H. Drexler, H. Wollschläger & H. Just (1991) Modulation of coronary vasomotor tone in humans. Progressive endothelial dysfunction with different early stages of coronary atherosclerosis. *Circulation,* 83**,** 391-401.

Zeiher, A., V. Schächinger & J. Minners (1995) Long-term cigarette smoking impairs endothelium-dependent coronary arterial vasodilator function. *Circulation,* 92**,** 1094-100.

Zeller, T., A. Rastan, U. Schwarzwälder, C. Müller, U. Frank, K. Bürgelin, S. Sixt, T. Schwarz, E. Noory & F. Neumann (2007) Regression of left ventricular hypertrophy following stenting of renal artery stenosis. *J Endovasc Ther,* 14**,** 189-97.

Zhang, H., T. Sos, P. Winchester, J. Gao & M. Prince (2009) Renal artery stenosis: imaging options, pitfalls, and concerns. *Prog Cardiovasc Dis,* 52**,** 209-19.

Zhu, X., A. Chade, M. Rodriguez-Porcel, M. Bentley, E. Ritman, A. Lerman & L. Lerman (2004) Cortical microvascular remodeling in the stenotic kidney: role of increased oxidative stress. *Arterioscler Thromb Vasc Biol,* 24**,** 1854-9.

Zierler, R., R. Bergelin, J. Isaacson & D. J. Strandness (1994) Natural history of atherosclerotic renal artery stenosis: a prospective study with duplex ultrasonography. *J Vasc Surg,* 19**,** 250-7; discussion 257-8.

Zoccali, C., S. Bode-Böger, F. Mallamaci, F. Benedetto, G. Tripepi, L. Malatino, A. Cataliotti, I. Bellanuova, I. Fermo, J. Frölich & R. Böger (2001) Plasma concentration of asymmetrical dimethylarginine and mortality in patients with end-stage renal disease: a prospective study. *Lancet,* 358**,** 2113-7.