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The Kidney in Different Stages of the Cardiovascular Continuum

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Abstract

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Patients with chronic kidney disease are at higher risk of developing cardiovascular disease. The complex, interaction between the kidney and the cardiovascular system is incompletely understood, particularly at the early stages of the cardiovascular continuum.

The overall aim of this thesis was to clarify novel aspects of the interplay between the kidney and the cardiovascular system at different stages of the cardiovascular continuum; from risk factors such as insulin resistance, inflammation and oxidative stress, via sub-clinical cardiovascular damage such as endothelial dysfunction and left ventricular dysfunction, to overt cardiovascular death.

This thesis is based on two community-based cohorts of elderly, Uppsala Longitudinal Study of Adult Men (ULSAM) and Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS).

The first study, show that higher insulin sensitivity, measured with euglycemic-hyperinsulinemic clamp technique was associated to improve estimated glomerular filtration rate (eGFR) in participants with normal fasting plasma glucose, normal glucose tolerance and normal eGFR. In longitudinal analyses, higher insulin sensitivity at baseline was associated with lower risk of impaired renal function during follow-up. In the second study, eGFR was inversely associated with different inflammatory markers (C-reactive protein, interleukin-6, serum amyloid A) and positively associated with a marker of oxidative stress (urinary F_2-isoprostanes). In line with this, the urinary albumin/creatinine ratio was positively associated with these inflammatory markers, and negatively associated with oxidative stress.

In study three, higher eGFR was associated with better endothelial function as assessed by the invasive forearm model. Further, in study four, higher eGFR was significantly associated with higher left ventricular systolic function (ejection fraction). The 5th study of the thesis shows that higher urinary albumin excretion rate (UAER) and lower eGFR was independently associated with an increased risk for cardiovascular mortality. Analyses of global model fit, discrimination, calibration, and reclassification suggest that UAER and eGFR add relevant prognostic information beyond established cardiovascular risk factors in participants without prevalent cardiovascular disease.

Conclusion: this thesis show that the interaction between the kidney and the cardiovascular system plays an important role in the development of cardiovascular disease and that this interplay begins at an early asymptomatic stage of the disease process.

Keywords: epidemiology, chronic kidney disease, cystatin C, glomerular filtration rate, albuminuria, euglycemic hyperinsulinemic clamp, insulin sensitivity, inflammation, oxidative stress, endothelial dysfunction and left ventricular dysfunction

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List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. Nerpin E, Risérus U, Ingelsson E, Sundström J, Jobs M, Larsson A Basu S, and Ärnlöv J. Insulin sensitivity measured with euglycemic clamp is independently associated with glomerular filtration rate in a community-based cohort. *Diabetes Care* 2008 Aug;31:1550–1555.

II. Nerpin E, Helmersson-Karlqvist J, Risérus U, Sundström J, Jobs E, Larsson A, Basu S, Ingelsson E, and Ärnlöv J. The association between kidney damage, kidney dysfunction and inflammation and oxidative stress in elderly men. *BMC Res Notes* 2012 Sep;27;5:537.

III. Nerpin E, Ingelsson E, Risérus U, Helmersson-Karlqvist J, Sundström J, Jobs E, Larsson A, Lind L and Ärnlöv J. Association between glomerular filtration rate and endothelial function in an elderly community cohort. *Atherosclerosis* 2012 Sep;224(1):242-6.

IV. Nerpin E, Ingelsson E, Risérus U, Sundström J, Andrén B, Jobs E, Larsson A, Lind L and Ärnlöv J. (2013) The association between glomerular filtration rate and left ventricular function in two independent community-based cohorts of elderly. (Manuscript)

V. Nerpin E, Ingelsson E, Risérus U, Sundström J, Larsson A, Jobs E, Jobs M, Hallan S, Zethelius B, Berglund L, Basu S, and Ärnlöv J. The combined contribution of albuminuria and glomerular filtration rate to the prediction of cardiovascular mortality in elderly men. *Nephrol Dial Transplant.* 2011 Sep;26(9):2820-7.

Contents

Abbreviations vii

Introduction 9
History of cardiovascular disease 9
The cardiovascular continuum 9
Cardio-renal syndrome 10
Kidney damage and dysfunction 11
Kidney damage (albuminuria) 11
Kidney dysfunction (reduced glomerular filtration rate) 11
Chronic kidney disease 12
Cardiovascular risk factors and the kidney 13
Insulin resistance (Study I) 13
Inflammation and oxidative stress (Study II) 14
Inflammation 14
Oxidative stress 15
Sub-clinical organ damage 15
Endothelial function (Study III) 15
Left ventricular dysfunction (Study IV) 18
Cardiovascular disease 19
Kidney damage and dysfunction and the risk of cardiovascular death (Study V) 19

Aims 21

Subjects and methods 22
The Uppsala Longitudinal Study of Adult Men (ULSAM) cohort 22
The Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) cohort 23
Study I (ULSAM) 23
Study II (ULSAM) 23
Study III (PIVUS) 24
Study IV (PIVUS and ULSAM) 24
Study V (ULSAM) 24
Clinical and metabolic investigations 25
Ethics 29
Statistical analyses 29
Study I 29

Study II 29
Study III 30
Study IV 30
Study V 31

Results 34
Insulin sensitivity and glomerular filtration rate (study I) 34
Inflammation, oxidative stress, glomerular filtration rate, and albuminuria (study II) 35
Glomerular filtration rate and endothelial function (study III) 36
Glomerular filtration rate and left ventricular function (study IV) 37
The combined contribution of albuminuria and glomerular filtration rate to the prediction of cardio-vascular mortality (study V) 38

Discussion 42
Comparison with the literature 42
Insulin sensitivity and glomerular filtration rate (study I) 42
Inflammation, oxidative stress, glomerular filtration rate, and albuminuria (study II) 43
Glomerular filtration rate and endothelial function (study III) 44
Glomerular filtration rate and left ventricular function (study IV) 44
The combined contribution of albuminuria and glomerular filtration rate to the prediction of cardiovascular mortality (study V) 45
General discussion 46
Modifiable risk factors 47
Changes in lifestyle 48
Pharmacological improvement of insulin sensitivity 48
Strengths and limitations 51

Conclusions 52

Summary in Swedish (Sammanfattning på svenska) 53

Acknowledgements 55

References 57

Abbreviations

ACE	Angiotensin converting enzyme
ACR	Albumin-creatinine ratio
ASA	Acetylsalicylic acid
BMI	Body mass index
CI	Confidence interval
CKD	Chronic kidney disease
CKD-EPI	Chronic kidney disease epidemiology collaboration
COX	Cyclooxygenase
CRP	C-reactive protein
CV	Variation coefficient
CVD	Cardiovascular disease
Cyst	Cystatin C
DAG	Directed acyclic graphs
EDV	Endothelial-dependent vasodilatation
eGFR	Estimated glomerular filtration rate (cystatin C-based)
EIDV	Endothelial-independent vasodilatation
ESRD	End-stage renal disease
FBF	Forearm blood flow
FMD	Flow-mediated dilatation
GFR	Glomerular filtration rate
HDL	High-density lipoprotein
HOMA	Homeostasis model assessment
HR	Hazard ratio
ICD	International classification of disease
IDI	Integrated discrimination improvement
IL-6	Interleukin 6
IVRT	Isovolumic relaxation time
LDL	Low-density lipoprotein
ln	Natural logarithm
LV	Left ventricular
LVEDV	Left ventricular diastolic volume
LVEF	Left ventricular ejection fraction
LVESV	Left ventricular systolic volume
M	Glucose disposal rate
MDRD	Modification of diet in renal disease
M/I ratio	Insulin sensitivity index

MPI	Myocardial performance index
NRI	Net reclassification improvement
NT-proBNP	N-terminal pro brain natriuretic peptide
OGTT	Oral glucose tolerance test
OR	Odds ratio
$PGF_{2\alpha}$	Prostaglandin F_2alpha
PIVUS	Prospective Investigation of the Vasculature in Uppsala Seniors
RAAS	Renin angiotensin aldosterone system
RERI	Relative excess risk due to interaction
SAA	Serum amyloid A
SD	Standard deviation
UAER	Urinary albumin excretion rate
ULSAM	The Uppsala Longitudinal Study of Adult Men
WHO	World health organization

Introduction

History of cardiovascular disease

At the beginning of the 1900s, cardiovascular mortality accounted for less than 10% of all mortality. During the last century, the social and economic factors changed, which contributed to an increased prevalence of cardiovascular disease (CVD). Between 1940 and 1967, the rate of CVD increased so strikingly that the World Health Organization (WHO) called it the world's most serious epidemic. Today, CVD is the main cause of death in the world and according to the WHO, 17.1 million die from CVD each year.

Through the years, many studies have identified major CVD-related risk factors such as high blood pressure, high blood cholesterol, smoking, obesity, diabetes, and physical inactivity.[1] However, other CVD-related risk factors such as left ventricular dysfunction, inflammation, oxidative stress, and kidney disease have also been proposed.

The causal mechanism behind CVD is not fully understood, but it appears to be multifactorial, with both genetic and environmental components; and a pathogenic process that spans over decades.[2]

The cardiovascular continuum

The concept of the cardiovascular continuum was first proposed by Dzau and Braunwald[3] in 1991 as a new paradigm for CVD (Fig. 1). CVD is linked by a chain of events that starts with a number of cardiovascular risk factors and continues as a progressive pathogenic process lasting for decades. Later in this process, cardiovascular events such as myocardial infarction (MI), stroke, or heart failure appear; which in turn can lead to further cardiovascular events and death. Atherosclerosis, myocardial necrosis, and heart failure cannot be reversed using current medical treatments, so it is important to prevent early components of the continuum such as hypertension, diabetes, hyperlipidaemia, and smoking, which offers a chance to delay the progression of CVD at an early stage.

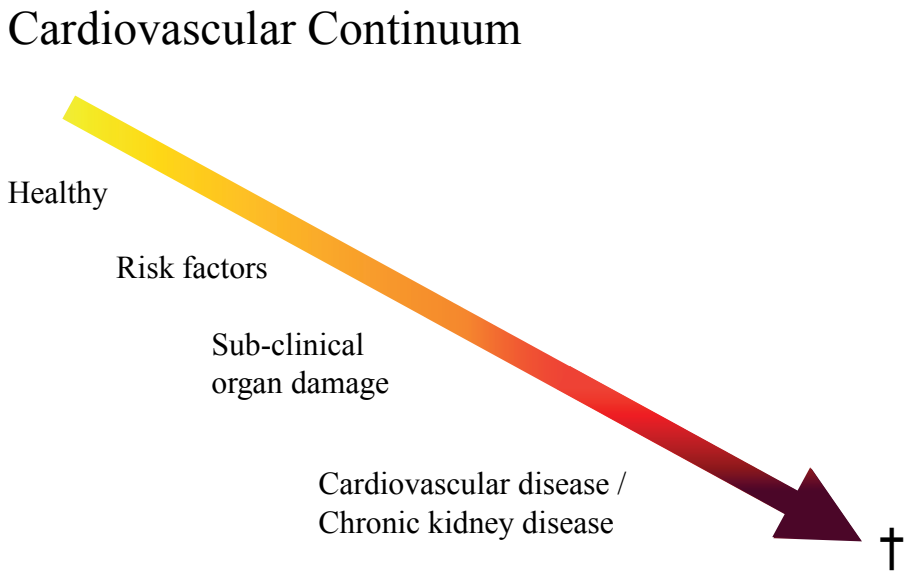

Figure 1. The cardiovascular continuum. Adapted from Dzau V. and Braunwald E. Am Heart J 1991;121:1244-63.

Cardio-renal syndrome

Numerous epidemiological studies have shown an association between cardiovascular morbidity and mortality and reduced kidney function, regardless of whether cardiac disease or kidney disease was the initial event.[4,5] The term "cardio-renal syndrome" has been defined as a pathophysiological disorder of the heart and kidneys by which acute or chronic dysfunction in one organ may induce acute or chronic dysfunction in the other organ.[6] Numerous studies have shown that it is a symbiotic relationship between CVD and the late stages of chronic kidney disease, but it has received less attention at early stages.

Cardiorenal syndrome has been sub-classified in five defined entities. Type 4 describes the complex interactions between the physiological and pathophysiological consequences of declining renal function which can lead to heart failure. These physiological responses may be due to underlying diseases such as hypertension or diabetes or can be a response to the functional decline in the kidney. The renal response to impaired GFR can lead to activation of multiple compensatory pathways including up-regulation of the renin-angiotensin- aldosterone system (RAAS) and sympathetic nervous system and also activation of the calcium-parathyroid system.[7]

Kidney damage and dysfunction

Kidney damage (albuminuria)

One way of assessing kidney damage is to measure the amount of albumin in the urine. Under physiological conditions, the glomerular filter forms a barrier to prevent macromolecules such as albumin from reaching the urinary space. Albuminuria has been suggested to be caused by glomerular basal membrane damage.[8] However, experimental studies have shown that quantities of albumin may reach the primary filtrate and that the proximal tubule is equipped with an effective albumin reabsorption system that subsequently metabolizes albumin to protein fragments and amino acids; indicating that albuminuria may also reflect tubular damage.[9]

Albuminuria is assessed either from a timed urine collection or, more commonly, from elevated concentrations in a spot sample, i.e. albumin-to-creatinine ratio (Table 1).[10] Increased microalbuminuria is common in hypertensive[11] and diabetic patients[12], but also in apparently healthy individuals.[13] Albuminuria is a predictor of systemic vascular damage[14], progression of kidney disease, and of the development of CVD.[15-17]

Table 1. Definition of micro- and macroalbuminuria

Urine collection method	***Normal***	***Micro-albuminuria***	***Macro-albuminuria***
Urinary albumin excretion rate	< 20 µg/min	> 20 µg/min	> 200 µg/min
Urine albumin-to-creatinine ratio	≤ 3mg/mmol	> 3mg/mmol	30 mg/mmol

Albuminuria has been associated with increased inflammation, coagulation defects, insulin resistance, hyperglycaemia, and hypertension that may explain the link with the development of CVD.[18] Interestingly, albuminuria has also been suggested to be a marker of systemic vascular damage.[14]

Kidney dysfunction (reduced glomerular filtration rate)

Glomerular filtration rate (GFR) describes the flow rate of filtered fluid through the kidney. GFR slowly decreases as a normal biological phenomenon linked to cellular and organ ageing. The most common causes of kidney dysfunction are atherogenic diseases such as hypertension, dyslipidaemia, and type-2 diabetes, diseases in which the underlying histological alteration is commonly represented by nephroangiosclerosis.

The effect of low estimated GFR on CVD may be mediated by loss of nephrons and parenchymal fibrosis, leading to CVD though accumulation of uremic toxins, impaired volume and blood pressure regulation, and multiple metabolic abnormities, including anaemia, disturbance in calcium phosphate homeostasis, increased sympathetic nervous activity, oxidative stress, and inflammation; all which are associated with accelerated atherosclerosis.[19]

There are a number of different equations to estimate GFR, which are based on serum creatinine, serum cystatin C, or both. In this thesis, we have mainly focused on cystatin C-based GFR (eGFR). Cystatin C is a protease inhibitor and it is produced by all nucleated cells at a constant rate. It has a stable production rate and is removed from the bloodstream by glomerular filtration, and it is completely reabsorbed and degraded in the tubules. Cystatin C has been suggested to be a better marker of GFR than creatinine-based GFR, since creatinine-based equations are influenced by age, gender, and muscle mass, which can misclassify individuals.[4]

Even so, recent studies have suggested that the incorporation of both creatinine and cystatin C in the same formula provides the most reliable estimate of GFR.20 In the present work, it was not possible to use this combined creatinine/cystatin formula, as it requires that both the creatinine and cystatin measurements should be calibrated against a new international reference standard.[21,22]

Chronic kidney disease

CKD is defined as either kidney damage (defined as pathological abnormalities or markers of damage, including abnormalities in blood, urine tests or imaging tests) or GFR < 60 mL/min/1.73 m^2 for ≥ 3 months.[23] The cut-off level for GFR of < 60 mL/min/1.73 m^2 is selected because it represents a reduction by more than half of the normal value of ~125 mL/min/1.73 m^2 in young people.[19] The severity of CKD can be divided into five stages based on kidney damage and/or level of glomerular filtration rates (Table 2). Even so, in clinical practice in Sweden the cut-off of < 50 mL/min/1.73 m^2 is also used to define CKD, particularly in the elderly.

Table 2. Stages of chronic kidney disease

Stage	Description	GFR (mL/min/1,73 m^2)
1	Kidney damage with normal or high GFR	≥ 90
2	Kidney damage with mildly depressed GFR	60–89
3a	Mildly to moderately decreased	45–59
3b	Moderately to severely decreased	30-44
4	Severely depressed GFR	15–29
5	Kidney failure	< 15 or dialysis

Cardiovascular risk factors and the kidney

Insulin resistance (Study I)

The underlying pathophysiology of insulin resistance is a gradual decrease in insulin sensitivity; when insulin sensitivity begins to fall, it results in an increased insulin production from pancreatic ß-cells in order to maintain glycaemic control. With time, the ß-cells will not be able to compensate for the degree of insulin resistance and the individual will pass from normal glucose tolerance to impaired glucose tolerance.

Impaired insulin sensitivity and compensatory hyperinsulinaemia have been suggested to contribute to development of renal injury through a number of different pathophysiological pathways:

1. Insulin *per se* stimulates the expression and activation of insulin-like growth factor 1, transforming growth factor-ß, endothelin-1, and components of the renin-angiotensin-aldosterone system. These factors have been shown to promote mitogenic and fibrotic processes in the kidney, such as proliferation of mesangial cells and extracellular matrix expansion.[24]
2. Insulin resistance and hyperinsulinaemia is also closely associated with oxidative stress[25], which could promote renal injury through decreased production and availability of nitric oxide[26], accelerated formation of glycol-oxidation, and lipid peroxidation products.[27-29]
3. Moreover, insulin resistance is linked to increased activity of pro-inflammatory cytokines and adipokines, factors that have been suggested to contribute to the progression of renal disease.[30]
4. There are also data suggesting that renal insufficiency suppresses renal clearance of insulin, which leads to higher circulating levels of insulin and thus further stimulates the deleterious effect of insulin on the kidney, i.e. leading to a vicious circle.[31]

Today, diabetes is the leading cause of end-stage renal disease[32] and reduced insulin sensitivity is a key component in the pathogenesis of diabetes.[33] Lower insulin sensitivity has also been suggested to be associated with impaired renal function in individuals without overt diabetes.[34] For instance, insulin resistance has been shown to predict end-stage renal disease in patients with mild renal impairment due to IgA nephritis.[35] Furthermore, the opposite chain of events has also been observed: patients with end-stage renal disease without diabetes have been shown to develop insulin resistance in the later stage of the disease.[35,36]

Based on previous data, we hypothesized that reduced insulin sensitivity may be involved in the development of renal dysfunction through pathways that are not primarily mediated by increased glucose levels.

Inflammation and oxidative stress (Study II)

Many of the traditional and untraditional cardiovascular risk factors that could affect endothelial function can be found in association with CKD. Systemic inflammation and oxidative stress has been proposed to be one of the untraditional mechanisms contributing to higher CVD burden in individuals with CKD.[37-39]

In this work, we measured 4 different inflammatory markers: one marker of COX-mediated inflammation (urinary prostaglandin $F_{2\alpha}$ [$PGF_{2\alpha}$]) and 3 markers of cytokine-mediated inflammation (serum C-reactive protein [CRP], interleukin-6 [IL-6], and serum amyloid A [SAA]). We also assessed one marker of oxidative stress (urinary F_2-isoprostanes). All of these were investigated for their independent associations with kidney damage and dysfunction with pre-specified subgroup analyses in individuals with albuminuria and with GFR in the normal range.

Inflammation

Inflammation *in vivo* can be measured with various indicators reflecting different segments of the inflammation reaction. Many studies have found an association between CKD and markers of inflammation, suggesting that CKD may be a low-grade inflammatory process.[40] Moreover, inflammatory markers have been shown to be predictors of decline in kidney function.[41] In addition, it has been shown that elevated CRP and IL-6 levels are independent predictors of cardiovascular outcomes in patients with CKD.[42-44] The mechanisms that contribute to the high prevalence of inflammation in CKD are unknown, but oxidative stress has been proposed as one possible mechanism.

Cyclooxygenase activity

$PGF_{2\alpha}$, a bioactive compound derived from arachidonic acid and catalysed by cyclooxygenase (COX), is an important mediator of inflammatory processes. $PGF_{2\alpha}$ can be quantified by measuring 15-keto-dihydro-$PGF_{2\alpha}$, which is a major metabolite of $PGF_{2\alpha}$. The latter has been shown to be a potent indicator of COX-mediated inflammatory processes *in vivo*.[45]

Cytokine-mediated inflammation

IL-6 is an interleukin that acts as both a pro-inflammatory and an anti-inflammatory cytokine. It is secreted by T cells and macrophages, and induces secretion of acute-phase proteins in hepatocytes (such as CRP and SAA). It stimulates the immune response to trauma, especially burns or other tissue damage that leads to inflammation.

CRP is an acute-phase protein that is synthesized in the liver in response to acute and chronic inflammation. Inflammation causes release of cytokines such as interleukin-6, which trigger the synthesis of CRP.

SAA has been linked to functions related to inflammation, pathogen defence, HDL metabolism, and cholesterol transport. It has been shown that SAA levels are elevated in CKD patients, and the protein is known to bind to HDL.[46,47] When pro-inflammatory SAA accumulates, HDL loses its anti-inflammatory capacity, and due to this finding it has been implicated in pathological conditions such as atherosclerosis.[48]

Oxidative stress

Oxidative stress takes place when oxidant production exceeds anti-oxidant capacity. It is caused by free radicals, which are extremely reactive and react instantly with important macromolecules such as proteins, lipids, carbohydrates and damaged DNA of structures.

In this thesis, oxidative stress was measured as non-enzymatically produced F_2-isoprostanes (8-Iso-$PGF_{2\alpha}$) in urine. The isoprostanes belong to a family of PG-like compounds mainly generated by the non-enzymatic peroxidation of arachidonic acid in membrane phospholipids (without the action of COX enzyme).[49] Today, F_2-isoprostanes have become the gold standard for measurement of lipid peroxidation.[50]

Sub-clinical organ damage

Endothelial function (Study III)

As mentioned earlier, CKD is associated with increased morbidity and mortality in CVD. The increased risk of CVD in patients with CKD has been

attributed to a cluster of traditional and untraditional cardiovascular risk factors (e.g. hypertension, dyslipidaemia, diabetes, smoking, oxidative stress, and chronic inflammation) which all can cause endothelial dysfunction and subclinical cardiovascular damage.

The potential underlying mechanisms in the interplay between renal dysfunction and endothelial dysfunction in arteries are incompletely understood. Animal experiments have shown that systemic administration of nitric oxide synthase inhibitor induces renal vasoconstriction and injury that is characterized by glomerulosclerosis and interstitial fibrosis.[51,52] But the opposite chain of events is also possible; clinical studies have shown that renal dysfunction can increase oxidative stress and inflammation[53,54], which may in turn cause endothelial dysfunction and atherosclerosis in the systemic vasculature.[55]

The majority of studies of endothelial function in renal disease have focused on CKD of stages 3–5; however, little is known about endothelial function in the general population. In this study, endothelial function was measured with 3 different aspects of endothelial function, flow-mediated dilatation, endothelium-dependent vasodilation, and endothelium-independent vasodilatation.

Flow-mediated dilatation

The assessment of brachial artery flow-mediated dilation (FMD) from ultrasound imaging was developed and widely used because of its non-invasive nature and its feasibility.[56] The most popular method is reactive hyperaemia test. The test employs a temporary occlusion of, for example, the forearm in order to create an ischaemia-induced reactive hyperaemia and a corresponding increase in shear stress in the conduit artery (Fig. 2). The technique provokes release of nitric oxide, resulting in vasodilation that can be quantitated as an index of vasomotor function.[57]

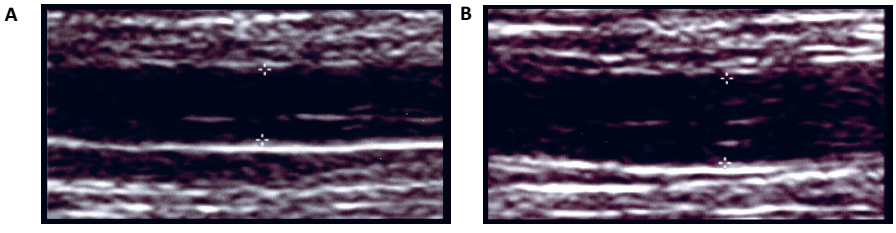

Figure 2. Flow-mediated dilation at rest (A) and during hyperaemia (B)

Endothelium-dependent vasodilation (EDV)

EDV is an invasive forearm technique that involves infusion of acetylcholine in the brachial artery. Acetylcholine is used to stimulate L-arginine, which in turn affects the enzyme endothelial NO synthase. The latter then diffuses into the vessel wall and provides vasodilation through activation of cyclic guanosine monophosphate (cGMP) (Fig. 3). This technique mainly evaluates endothelium-dependent vasodilation in forearm resistance arteries and was described by different groups in 1990.[58] Reduced EDV has been found in patients with coronary heart disease[59], hypertension[58], hypercholesterol-aemia[60], diabetes[61], smoking[62], and chronic kidney disease.[63]

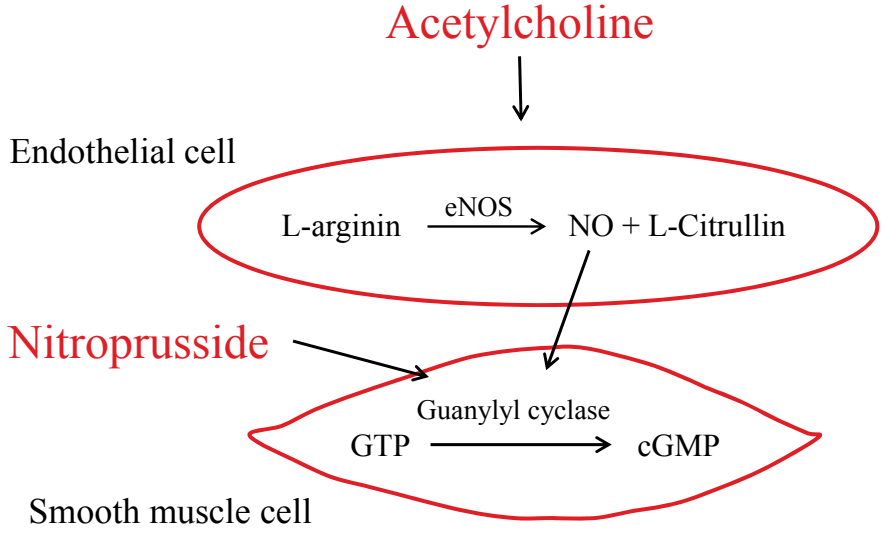

Figure 3. Regulation of the contractility of arterial smooth muscle by NO and cGMP.

Endothelium-independent vasodilatation (EIDV)

Endothelium-independent vasodilation is also an invasive forearm technique, this time involving infusion of sodium nitroprusside in the brachial artery. This technique mainly evaluates endothelium-independent vasodilation in forearm resistance arteries. Nitric oxide synthesized in endothelial cells diffuses locally through tissue and activates guanylate cyclase in nearby smooth muscle cells. The resulting rise in cyclic guanosine monophosphate (cGMP) leads to relaxation of the muscle and vasodilation.

Left ventricular dysfunction (Study IV)

Cardio-renal syndrome type 4, is a condition in which primary CKD can contribute to a reduction in cardiac function, such as cardiac remodelling, left ventricular dysfunction, or hypertrophy. Anomalies of left ventricular structure and function are very frequent in patients with advanced renal dysfunction (eGFR < 60 ml/min/1.73 m^2), and have a negative effect on cardiovascular prognosis.[7,64]

One possible mechanism could be sodium retention and increased extracellular fluid volume in the setting of mild kidney dysfunction leading to chronic activation of the renin-angiotensin system (RAAS). Persistent activation of RAAS has damaging effects on cardiac function and contributes to the progression of heart failure through promotion of cardiac remodelling and myocardial fibrosis.[65] An experimental study by Martin *et al.*[66] demonstrated that mild renal insufficiency in rats resulted in early cardiac fibrosis and impaired diastolic function, which progressed to more global LV remodelling and dysfunction; and then on to heart failure.

Another possible mechanism could be that CKD often co-exists with cardiovascular risk factors such as dyslipidaemia, hypertension, smoking, and diabetes.[67] Elevated cardiovascular risk factors contribute to accelerated atherosclerosis in these patients through increased production of reactive oxygen species, which could then lead to increased incidence of heart failure in the general population.[4,68]

Whether eGFR may be associated with left ventricular function in the community has been less well studied. In the study, 3 different aspects of left ventricular function were measured: left ventricular systolic ejection fraction (LVEF), diastolic isovolumic relaxation time (IVRT), and myocardial performance index (MPI) reflecting global ventricular function.

LVEF is one of the most commonly reported measures of left ventricular systolic function and can be determined using several invasive and non-invasive methods. It is defined as the stroke volume (the difference between ventricular end-diastolic volume and end-systolic volume), and is expressed as a percentage of left ventricular end-diastolic volume. Reduced LVEF indicates deteriorated left ventricular systolic function.

Isovolumic relaxation time (IVRT) is the interval in the cardiac cycle from aortic valve closure to mitral valve opening. Prolonged IVRT indicates poor myocardial relaxations (Fig. 4).

MPI, also known as the Tei index, is defined as the sum of isovolumic contraction time and isovolumic relaxation time divided by the ejection time, and it reflects both systolic and diastolic time.[69,70] Higher values are attributable to prolonged isovolumic intervals and a shortening of ejection time, which are both associated with pathological states involving overall cardiac dysfunction (Fig. 4).

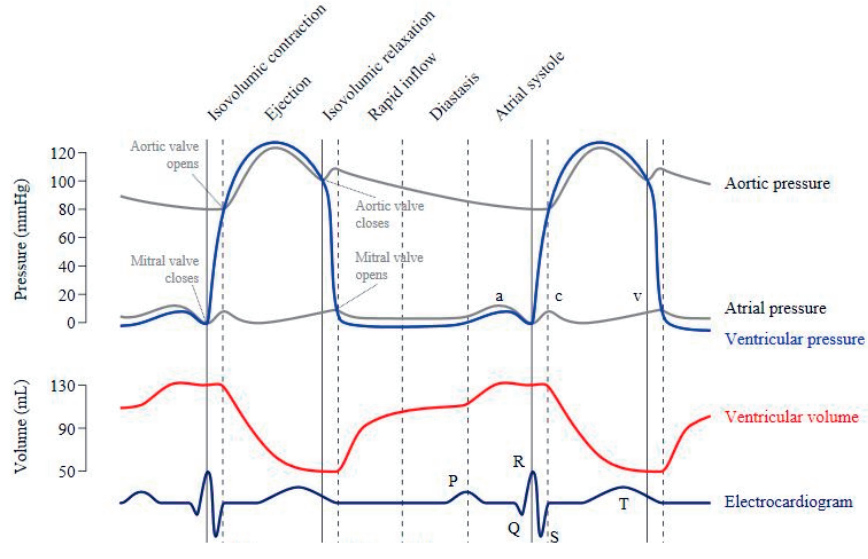

Figure 4. The heart cycle.

Cardiovascular disease

Kidney damage and dysfunction and the risk of cardiovascular death (Study V)

International guidelines have recommended screening for albuminuria and GFR in selected patient groups, such as patients with hypertension or diabetes, in order to identify individuals with increased risk of CVD.[10,71] It is less well studied, however, whether screening for the kidney biomarkers albuminuria and eGFR substantially improves prediction of cardiovascular risk in the general population.

Before new biomarkers are introduced into clinical practice, they must be properly evaluated. They must be able to improve risk prediction for an individual. One way to obtain a prognosis is to use mathematical equations describing the relationship between one or more prognostic biomarkers and a given outcome. There are three commonly used methods to assess the accuracy of biomarkers in predicting clinical outcomes: discrimination, calibration, and reclassification.[72,73]

In study V, we wanted to evaluate albuminuria and eGFR as risk markers for CVD by using global model fit, model discrimination, calibration, and reclassification to look for improvement in terms of cardiovascular risk prediction. As the clinical relevance of an improved cardiovascular risk prediction is highest in the primary preventive setting, we performed pre-specified analyses in participants without any evidence of CVD at baseline.

Aims

The overall aim of this thesis was to investigate the influence of mild kidney damage and dysfunction on the different stages of the cardiovascular continuum; from risk factors such as insulin resistance (study I), inflammation and oxidative stress (study II), via sub-clinical cardiovascular damage such as endothelial dysfunction (study III) and left ventricular dysfunction (study IV), to overt CVD and death (study V).

Specific aims:

Paper I: To determine whether impaired kidney function (cystatin C-based glomerular filtration rate) is associated with insulin resistance.

Paper II: To determine whether albuminuria and impaired kidney function are associated with inflammation and oxidative stress.

Paper III: To determine whether impaired kidney function is associated with deteriorated endothelial function.

Paper IV: To determine whether impaired kidney function is associated with deteriorated left ventricular function.

Paper V: To investigate whether albuminuria and cystatin C-based GFR improve cardiovascular risk prediction.

Subjects and methods

The Uppsala Longitudinal Study of Adult Men (ULSAM) cohort

ULSAM is an ongoing, longitudinal, epidemiologic study based on all available men who were born between 1920 and 1924 and who resided in Uppsala County, Sweden, in September 1970. Of the 2,841 men invited, 2,322 (82%) chose to participate. The men were re-investigated at the ages of 60, 70, and 77 (Fig. 5).

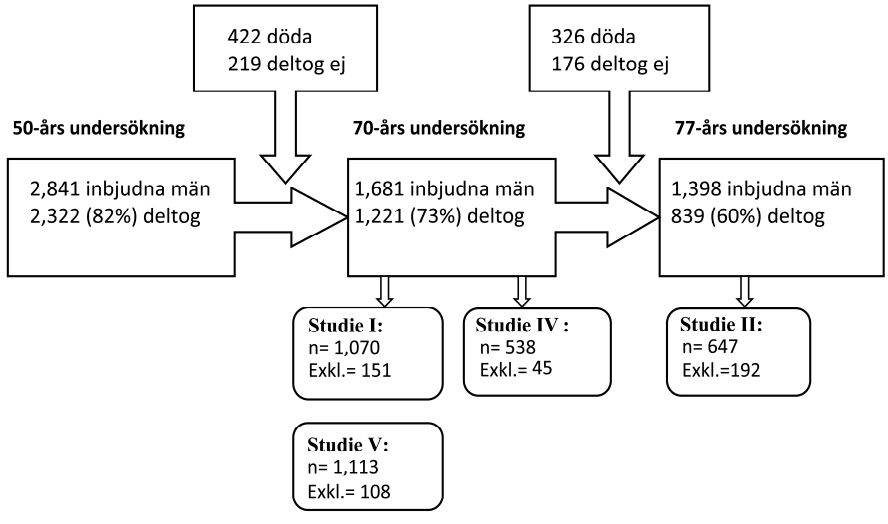

Figure 5. Uppsala Longitudinal Study of Adult Men: study populations for studies I, II, IV, and V.

Investigation at 70 years of age

Studies I, IV, and V were based on the third cycle of examination (1991–1995). During the intervening 20 years, 422 had died and 219 had moved out of the Uppsala region. Of the 1,681 men invited, 460 did not participate in this follow-up, leaving 1,221 men (73%) with an average age of 71 years.

Investigation at age 77 years of age

Study II was based on the fourth examination cycle, when the subjects were approximately 77 years old (1997–2001). At that time, 748 of the 2,322 participants who were alive at age 50 had died and another 176 men were not eligible for other reasons. In total, 1,398 men were invited to participate in this investigation; and of those invited, 839 men (60%) participated.

The Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) cohort

Men and women living in Uppsala, Sweden, were chosen from the community register and were invited (by letter) to participate within two months after their 70th birthday. Of 2,025 people invited, 1,016 (50%) participated (51% of them women).[74]

Study I (ULSAM)

We excluded 151 men because of unavailable baseline data at the third examination cycle. Thus, the study sample comprised 1,070 individuals. We also performed analyses in participants with normal fasting glucose and glucose tolerance (n = 517) and participants with normal fasting glucose and glucose tolerance, and normal GFR (> 50 ml/min/1.73 m^2, n = 433).

Follow-up data were available for 694 participants. We excluded 108 participants with impaired GFR at baseline (<50ml/min/1.73 m^2) which left 586 participants. Renal impairment during follow-up was defined as having a GFR of < 50ml/min/1.73 m^2 at the fourth examination cycle (after ~7 year), or being hospitalized for renal failure during follow-up. Subjects who were hospitalized for renal failure were identified from the Swedish Hospital Discharge Register using the following international classification of disease (ICD) codes: renal failure; 584–588 (ICD-9), N17–N19 (ICD-10).

Study II (ULSAM)

The analyses were based on the fourth examination cycle of the ULSAM cohort (n = 839). Of these, 647 (77%) had valid measurements of serum cystatin C, urinary albumin-creatinine ratio (ACR), IL-6, CRP, SAA, and urinary $PGF_{2\alpha}$, F_2-isoprostanes, and covariates. We also performed analyses in participants with normal eGFR (n = 514, eGFR > 60ml/min/1.73 m^2) and normal ACR (n = 522, ACR < 3 mg/mmol).

Study III (PIVUS)

For this study, we excluded 64 participants because of missing data on eGFR or covariates. After these exclusions, 952 individuals aged 70 (49.3% women) were eligible and constituted the study sample. Measurements of FMD, EDV, and EIDV were available for 952, 835, and 852 of these participants, respectively. We also performed the above analyses in a subgroup with eGFR > 60 ml/min/1.73 m^2. FMD, EDV, and EIDV were available on 888, 778, and 796 participants, respectively.

Study IV (PIVUS and ULSAM)

In the fourth study, in PIVUS, we excluded 49 participants who had not undergone the echocardiography examination, 8 participants with LVEF < 40%, 33 participants with a previous diagnosis of heart failure, 14 participants with missing data on cystatin C, and 1 participant with eGFR > 270 ml/min/1.73 m^2. After these exclusions, 911 individuals aged 70 (50.6% of them women) were eligible. Of these individuals, 785 had valid measurements of LVEF, 850 of isovolumic relaxation time (IVRT), and 732 of myocardial performance index (MPI).

In ULSAM, at the third re-investigation, an echocardiographic Doppler examination was performed consecutively on the first 583 participants. We excluded 15 participants where it was not possible to obtain reliable data from the echocardiographic examination, 14 participants with LVEF < 40%, 4 participants who had previously been hospitalized for heart failure, and 12 participants with missing data on cystatin C. After these exclusions, 538 individuals aged 70 were eligible. Of these individuals, 407 had valid measurements of LVEF, 494 had valid measurements of IVRT, and 424 had valid measurements of MPI.

In both PIVUS and ULSAM, missing data on covariates were handled by multiple imputation techniques to deal with the loss of information on covariates in the dataset.

Study V (ULSAM)

Based on the third examination cycle, we excluded 108 patients because of lack of valid measurements of serum cystatin C, urinary albumin excretion rate (UAER), and/or covariates needed for the present study. We also examined a subgroup of 649 men who did not have CVD at baseline. For this subgroup, the following exclusion criteria were used: previous MI or angina pectoris, as noted in the medical history; Q or QS waves or left bundle-branch block (Minnesota codes 1.1 to 1.3 and 7.1, respectively) on the base-

line electrocardiogram; a history of any CVD, as noted in the Swedish Hospital Discharge Register (International Classification of Diseases, 10th revision [ICD-10] codes I00 to I99); or current treatment with nitroglycerin or cardiac glycosides. Cardiovascular mortality was defined using the Swedish Cause of Death Register (ICD-10 codes I00 to I99).

Clinical and metabolic investigations

The investigations in PIVUS and ULSAM were performed using the same standardized methods, which included anthropometrical measurements, blood pressure, fasting blood, and a questionnaire regarding their medical history, smoking habits, and regular medication.

All participants were investigated in the morning after an overnight fast, with no medication or smoking allowed after midnight. Venous blood samples were drawn in the morning after an overnight fast and stored at –70°C. Body mass index (BMI) was calculated as the ratio of the weight to the height squared (kg/m^2). Blood pressure was measured by a calibrated mercury sphygmomanometer to nearest even mmHg after at least 10 min of rest and the average of three (PIVUS) or two (ULSAM) recordings was used. Lipid variables and fasting blood glucose were measured by standard laboratory techniques. Use of diabetes medication was ascertained through self-report questionnaires. Diabetes was defined as fasting plasma glucose >7.0 mmol/l or 2-h postload glucose level >11.1 mmol/l or by the use of oral hypoglycaemic agents or insulin. Impaired glucose tolerance was defined as a 2-h postload glucose value of 7.8 –11 mmol/l. Impaired fasting glucose was defined as fasting plasma glucose of 5.6 – 6.9 mmol/l. Hypertension was defined as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or receiving treatment for hypertension.

Serum cystatin C, serum creatinine, and albuminuria

In ULSAM, serum cystatin C was measured on a BN ProSpec analyser (Siemens) using a Siemens assay.[75] In PIVUS, serum cystatin C was measured using a Gentian assay (Moss, Norway) on an Architect Ci8200 (Abbott Laboratories, Abbott Park, IL, USA).[76] Based on these measurements of cystatin C, estimated GFR was calculated by assay-specific formulae, both of which have been shown to be closely correlated with iohexol clearance (Table 3).[75,76]

Serum/plasma creatinine in ULSAM subjects was measured by spectrophotometry using Jaffe's reaction and reagents from Boehringer Mannheim. The instrument used was the Hitachi 717 or 911 (Hitachi, Japan). For PIVUS subjects, compensated Jaffe method was used (reagent 14.3600.01; Synermed International, Westfield, IN, USA) and measurements were performed on an Architect Ci8200 analyzer (Abbott).

GFR was calculated from creatinine by using Modification of Diet in Renal Disease ($eGFR_{MDRD}$)[22] and Chronic Kidney Disease Epidemiology Collaboration ($eGFR_{CKD\text{-}EPI}$) equation (Table 3).[77]

Table 3. Different GFR equations used in this thesis

GFR equation		
MDRD (IDMS)		$GFR = 175 \times (S_{cr})^{-1.154} \times (Age)^{-0.203}$
Cystatin C (Siemens assay)		$GFR = 77.24 \times CystC^{-1.2623}$
Cystatin C (Gentian assay)		$GFR = 79.901 \times CystC^{-1.4389}$
CKD-EPI (IDMS)	$Scr \leq 80$	$GFR = 141 \times Scr^{-0.411} \times (0.993)^{Age}$
CKD-EPI (IDMS)	$Scr > 80$	$GFR = 141 \times Scr^{-1.209} \times (0.993)^{Age}$

CKD-EPI = Chronic Kidney Disease Epidemiology Collaboration; S-Creatinine (Scr) = µmol/L; MDRD = Modification of Diet in Renal Disease; GFR = glomerular filtration rate; IDMS = isotope-dilution mass spectrometry.

UAER was calculated from the amount of albumin in urine collected during the night. The subjects were instructed to void immediately before going to bed and to record the time. All urine samples during the night and the first sample of urine after rising were collected and used for the analyses (Albumin RIA 100; Pharmacia, Uppsala, Sweden).

Urine albumin-to-creatinine ratio was measured by analysing urine albumin (Dade Behring, Deerfield, IL, USA) using a Behring BN ProSpec® analyzer (Dade Behring) and urine creatinine using a modified kinetic Jaffe reaction on an Architect Ci8200® analyzer (Abbott), and is reported in S.I. units (mmol/L). Creatinine-related urine albumin was then calculated from the Prospec® results.

Euglycaemic hyperinsulinaemic clamp technique

The euglycaemic hyperinsulinaemic clamp technique was used according to DeFronzo[78], with a slight modification to suppress hepatic glucose production[79], for estimation of *in vivo* sensitivity to insulin. Insulin (Actrapid Human®; Novo, Copenhagen, Denmark) was infused in a primary dose for the first 10 min and then as a continuous infusion (56 mU/min per body surface area [m^2], whereas DeFronzo[78] used 40 mU/min per body surface area [m^2]) for two hours to maintain steady-state hyperinsulinaemia. The target plasma glucose level was 5.1 mmol/L and was maintained by measuring plasma glucose every five minutes.

The glucose infusion rate during the last hour was used as a measure of glucose disposal rate (*M* value). The insulin sensitivity index (*M/I* ratio) was calculated by dividing *M* by the mean insulin concentration during the same

period of the clamp. *M/I* therefore represent the amount of glucose metabolized per unit of plasma insulin (Fig. 6).

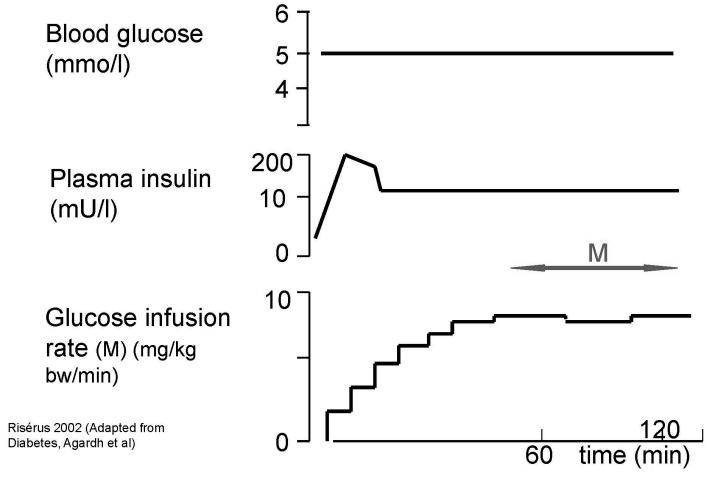

Figure 6. Euglycaemic hyperinsulinaemic clamp.

Oxidative stress

Urinary F_2-isoprostanes were analysed by radioimmunoassay without any previous extraction or purification.[49]

Inflammation

High-sensitivity serum CRP and SAA measurements were performed with latex-enhanced reagent (Dade Behring) using a Behring BN ProSpec analyzer (Dade Behring). IL-6 measurements on serum were performed with an ELISA kit (IL-6 HS; R&D Systems, Minneapolis, MN, USA). Urinary 15-keto-dihydro-$PGF_{2\alpha}$ was analysed by radioimmuno-assay.[45]

The brachial artery ultrasound technique

The brachial artery was assessed by external B-mode ultrasound imaging 2–3 cm above the elbow (AcusonXP128 with a 10-MHz linear transducer; Acuson, Mountain View, CA, USA) according to the International Brachial Artery Reactivity Task Force.[80]

A cuff was placed below the elbow and inflated to a pressure of at least 50 mmHg above systolic blood pressure for 5 min. FMD was defined as the maximal brachial artery diameter recorded between 30 and 90 s following cuff release minus the diameter at rest, all divided by the diameter at rest, using electronic calipers for measurements. FMD was successfully evaluated

in 97% of the participants. The reproducibility (CV) was 3% for baseline brachial artery diameter and 29% for FMD.[81]

The invasive forearm technique

Forearm blood flow (FBF) was measured by venous occlusion plethysmography (Elektromedicin, Kullavik, Sweden). Venous occlusion was achieved by a blood pressure cuff applied proximal to the elbow and inflated to 50 mmHg by a rapid cuff inflator. Evaluations of FBF were made by calculation of the mean of at least five consecutive recordings.

An arterial cannula was placed in the brachial artery. Resting FBF was measured 30 min after cannula insertion. After evaluation of resting FBF, local intra-arterial drug infusions were given over 5 min for each dose, with a 20-min wash-out period between the drugs. The infused dosages were 25 and 50 mg/min for acetylcholine (Clin-Alpha, Läufelfingen, Switzerland) to evaluate EDV and 5 and 10 mg/min for sodium nitroprusside (SNP) (Nitropress; Abbott Pharmaceutical, Abbott Park, IL, USA) to evaluate EIDV.

EDV was defined as FBF during infusion of 50 mg/min of acetylcholine minus resting FBF, all divided by resting FBF. EIDV was defined as FBF during infusion of 10 mg/ min of SNP minus resting FBF, all divided by resting FBF. The CV of the ultrasound assessments when repeating the measurements was 8% for EDV and 10% for EIDV.[82]

Ventricular function

A 2- to 5-MHz transducer was used for two-dimensional and Doppler echocardiography, which was performed with an Acuson XP124 cardiac unit (Acuson, CA, USA) in PIVUS subjects and with a Hewlett-Packard Sonos 1500 cardiac ultrasound unit (Hewlett-Packard, Andover, MA, USA) in ULSAM subjects. Examinations and readings of the images were performed by two experienced physicians (Dr Lind, PIVUS, and Dr Andrén, ULSAM) who were unaware of any other data on the subjects.

Left ventricular dimensions were measured with M-mode. Left ventricular volumes (left ventricular diastolic volume [LVEDV] and left ventricular systolic volume [LVESV]) were calculated according to the Teichholz M-mode formula: volume = $7D^3/(2.4 + D)$, where D is the diameter.[83,84]

LVEF, reflecting left ventricular systolic function and was calculated as (LVEDV – LVESV)/LVEDV. Impaired LVEF was defined as LVEF < 40%.[85] Ventricular diastolic function was measured with isovolumic relaxation time (IVRT) as the interval between aortic valve closure and the onset of mitral flow, using the Doppler signal from the area between the LV outflow tract and mitral flow. MPI, reflecting global left ventricular function, was calculated as (isovolumic contraction time + isovolumic relaxation time)/left ventricular ejection time.

Ethics

The ULSAM and PIVUS studies were approved by the Ethics Committee of the University of Uppsala. The participants gave informed written consent before entering the study.

Statistical analyses

Data are given as mean ± standard deviation (SD) for continuous variables and as number and percentage for categorical variables. Two-tailed 95% confidence intervals and p-values are given, with p-values of < 0.05 being regarded as significant. Statistical software packages STATA 10, 11, or 12 (Stata Corporation, College Station, TX, USA) and SAS 9.1 for Windows (SAS Institute, Cary, NC, USA) were used.

The distributions of continuous variables were tested using the Shapiro-Wilk test. Logarithmic transformation was performed to obtain a normal distribution. To rule out the possibility that an outcome; either in total or in part; had been affected by factors other than the exposure itself, we adjusted for different known confounders. In studies II and IV, we used a directed acyclic graphs (DAGs) approach to establish a parsimonious model with minimised confounding of effect estimates in model B.

Study I

Linear regression analyses were used to assess the cross-sectional associations between insulin sensitivity index (M/I; independent variable) and cystatin C-based GFR (dependent variable). We adjusted for age, glucometabolic variables, cardiovascular risk factors, lifestyle factors and a combined model of all factors in different models.

We also performed the above analyses in 2 subgroups: (1) normal fasting glucose and normal glucose tolerance (n = 517); and (2) normal fasting glucose and normal glucose tolerance, and normal GFR (> 50 ml/min/1.73 m^2, n = 433). Logistic regression was used to relate insulin sensitivity to renal dysfunction during follow-up.

Study II

Linear regression analyses were used to assess the cross-sectional associations between CRP, $PGF_{2\alpha}$, IL-6, SAA, and F_2-isoprostanes (independent variable), and cystatin C-based GFR or ACR (dependent variables in separate models). The following models were used:

- Model A: age-adjusted;
- Model B: adjusted according to directed acyclic graphs (DAGs): age, BMI, smoking, systolic and diastolic blood pressure, LDL cholesterol, HDL cholesterol, and triglycerides, statin treatment, ACE-inhibitor, ASA, anti-inflammatory and cortisone medication;
- Model C: adjusted as in model B, but also for diabetes and CVD.

We also performed the above analyses in one subgroup: participants with normal eGFR (> 60 ml/min/1.73 m^2) and normal ACR (< 3 mg/mmol).

Study III

Linear regression analyses were used to assess the cross-sectional associations of cystatin C-based GFR (eGFR) (independent variable) with FMD, EDV, or EIDV (dependent variables in separate models). We adjusted for age and sex, and for established CVD risk factors in separate models.

We also performed the above analyses in a subgroup with eGFR > 60 ml/min/1.73 m^2.

In order to evaluate the individual effects of different CVD risk factors on the association between eGFR and endothelial function, we also performed separate exploratory models adjusted for variables reflecting blood pressure, dyslipidaemia, impaired glucose metabolism, adiposity, inflammation, or smoking.

Study IV

Linear regression analyses were used to assess the cross-sectional associations of eGFR (independent variable) with LVEF, IVRT, and MPI (dependent variables in separate models). Missing data on covariates were handled via multiple imputation techniques to deal with the loss of information on covariates in the dataset.

The following models were used:

- Model A: adjusted for age and sex (PIVUS);
- Model B: DAG-adjusted; adjusted for age, sex (PIVUS), systolic and diastolic blood pressure, BMI, LDL cholesterol, HDL cholesterol, smoking, and diabetes.

We also performed the above analyses in a pre-specified subgroup with normal eGFR (> 60 ml/min/1.73 m^2). PIVUS: n = 743/802/688; ULSAM: n = 224/268/243 for LVEF/IVRT/MPI analyses, respectively). Moreover, we investigated the association between creatinine-based eGFR (Chronic Kidney Disease Epidemiology Collaboration formula, [CKD-EPI])[22] and LVEF. In secondary analyses, we used a model adjusted for age, sex (PIVUS) and NT-proBNP.

Study V

Different statistical tests were performed to investigate whether combined addition of albuminuria and cystatin e GFR with established cardiovascular risk factors would improve the risk prediction for cardiovascular death (Fig. 7).[72] All analyses were also performed for the participants who did not have CVD at baseline.

Cox-regression models

Multivariable Cox-regression models adjusted for established cardiovascular risk factors were used to calculate hazard ratios (HRs) for cardiovascular mortality. Proportional hazards assumptions were confirmed by Schoenfeld tests.

Global model fit

We performed likelihood-ratio tests to investigate whether the global model fit improved after the addition of kidney markers.

C statistic

Estimates of the C statistic for the Cox-regression models were calculated according to the method of Pencina *et al*.[86] Differences in C statistics (with 95% confidence intervals [CI]) after the addition of eGFR and UAER to the model with established risk factors were estimated using the method described by Antolini *et al*.[87] The C statistic measures how well a prognostic model distinguishes (discriminates between) individuals with and without the outcome of interest. The C-index has values ranging from 0.5 (no discrimination) to 1.0 (good discrimination).

Calibration

Calibration is another key measure of model performance. Calibration quantifies how closely the predicted probabilities of an event match the actual experience. When evaluating the performance of a model after addition of a new marker, it is essential to check for improvement in calibration (or at least for no adverse effect if other measures improve). We used the Grønnesby and Borgan calibration test,[88] which compares the number of events that are observed with those that are expected on the basis of estima-

tion from the models, within five risk score groups. A non-significant p-value indicates adequate calibration.

Net reclassification improvement (NRI)

The increased discriminative value of the biomarkers was further examined with NRI as described by Pencina *et al*.[89] NRI compares an "old" model (i.e. traditional risk factors) with a "new" model (i.e. traditional risk factors + new risk factors) by classifying the predicted risks into different risk categories (for example < 5%, 5–20%, > 20% 10-year CVD risk). The improvement in reclassification can be quantified as a sum of differences in the proportion of individuals moving up minus the proportion moving down for people who develop events, and the proportion of individuals moving down minus the proportion moving up for people who do not develop events.

Integrated discrimination improvement (IDI)

IDI also compares an "old" model (i.e. traditional risk factors) with a "new" model (i.e. traditional risk factors + new risk factors). The difference is that it considers the change in the estimated prediction probabilities as a continuous variable as described by Pencina *et al*.[89] The IDI was also used to identify cut-off points of eGFR and UAER to achieve optimal discrimination as previously described.[90,91]

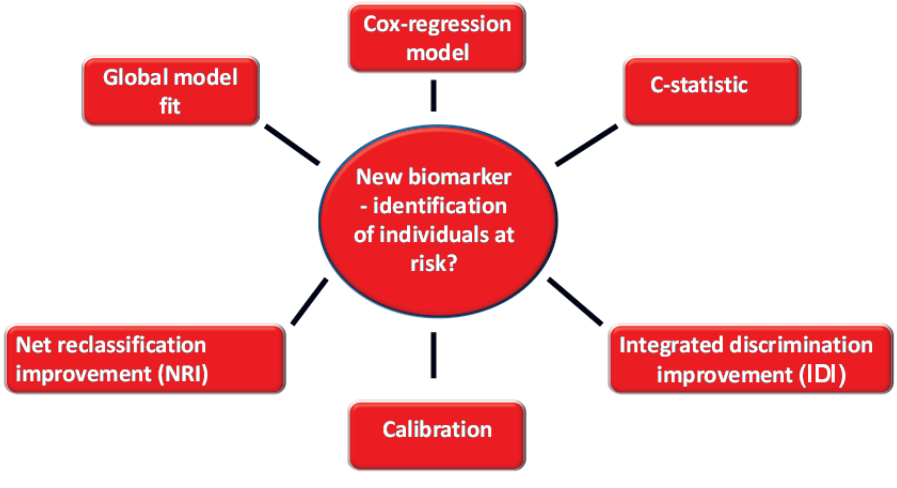

Figure 7. Different statistical methods to evaluate a new biomarker.

Effect modification

In studies III and IV, we performed a test for effect modification by gender, by including a multiplicative interaction term in multivariable model B.

Results

Insulin sensitivity and glomerular filtration rate (study I)

In the whole cohort, 1 unit higher of M/I (5.2 ± 2.5) was significantly associated with 0.85–1.19 ml/min/1.73 m^2 higher eGFR in all models (models A–E) (Table 4). In participants with normal fasting glucose and normal glucose tolerance, the positive association between insulin sensitivity and eGFR was essentially the same in all models. After further exclusion of participants with impaired eGFR (< 50 ml/min/1.73 m^2), the association between insulin sensitivity and eGFR remained statistically significant in all models, but with lower regression coefficients (Table 4).

Table 4. The association of insulin sensitivity index (M/I) and cystatin C-based glomerular filtration rate (eGFR): multivariable linear regression

Model	Total cohort (n = 1,070)		Normal fasting glucose and normal glucose tolerance (n = 517)		Normal fasting glucose, normal glucose tolerance, and eGFR > 50 ml/min/1.73 m^2 (n = 433)	
	β-coefficient (95% CI)	p-value	β-coefficient (95% CI)	p-value	β-coefficient (95% CI)	p-value
A	0.86 (0.53–1.19)	< 0.001	1.03 (0.57–1.50)	< 0.001	0.52 (0.11–0.93)	0.01
B	1.10 (0.67–1.53)	< 0.001	0.79 (0.25–1.33)	0.004	0.54 (0.07–1.00)	0.02
C	0.85 (0.52–1.19)	< 0.001	1.03 (0.56–1.56)	< 0.001	0.55 (0.14–0.97)	0.01
D	0.88 (0.45–1.31)	< 0.001	1.09 (0.51–1.67)	< 0.001	0.61 (0.11–1.10)	0.02
E	1.19 (0.69–1.68)	< 0.001	0.86 (0.23–1.49)	0.007	0.66 (0.12–1.19)	0.02

Data are regression coefficients for a 1-unit higher M/I. Model A was adjusted for age; model B was adjusted for age and glucometabolic factors (fasting plasma glucose, fasting plasma insulin, and 2-hour plasma glucose from an oral glucose tolerance test); model C was adjusted for age and cardiovascular risk factors (hypertension, dyslipidaemia, and smoking), model D was adjusted for age and lifestyle factors (BMI, physical activity, and consumption of tea, coffee, and alcohol), and model E was adjusted for all covariates in models A–D.

Of the participants with normal eGFR (> 50 ml/min/1.73 m^2) at baseline, 32 developed renal dysfunction during follow-up. In these participants, higher insulin sensitivity was borderline significantly associated with lower risk of developing renal dysfunction in the age- and glucometabolic-adjusted model (models A and B, Table 5). Interestingly, the association between insulin sensitivity and renal dysfunction appeared stronger in the sub-sample with normal fasting glucose and normal glucose tolerance (Table 5).

Table 5. The association of insulin sensitivity index (M/I) and the incidence of renal dysfunction in participants with eGFR > 50 ml/min/1.73 m^2 at baseline: multivariable logistic regression

Model	Total cohort (no. of events/no. at risk (32/586)		Normal fasting glucose and normal glucose tolerance (no. of events/no. at risk (16/295)	
	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value
Model A	0.85 (0.72–1.00)	0.055	0.67 (0.51–0.89)	0.006
Model B	0.82 (0.65–1.02)	0.071	0.58 (0.40–0.84)	0.004

Data are odds ratios for a 1-unit higher M/I. Model A was adjusted for age; model B was adjusted for age, fasting plasma glucose, fasting plasma insulin, and 2-hour glucose tolerance test.

Inflammation, oxidative stress, glomerular filtration rate, and albuminuria (study II)

In the whole cohort, higher eGFR was inversely associated with lower lnCRP, lower lnIL-6, lower lnSAA, and higher lnF_2-isoprostanes; higher ACR was positively associated with higher lnCRP, higher lnIL-6, higher lnSAA, and lower lnF_2-isoprostanes when adjusting for age, BMI, smoking, systolic and diastolic blood pressure, treatment for hypertension, LDL-cholesterol, HDL-cholesterol, triglycerides, and treatment with statin, ACE inhibitors, ASA, anti-inflammatory drugs, and cortisone (models A and B, Table 6).

After further exclusion of participants with impaired eGFR (< 60 ml/min/1.73 m^2) the association between eGFR and lnCRP, lnIL-6, remained statistically significant in all models but with lower regression coefficients. No significant association was seen between eGFR and urinary $lnPGF_{2\alpha}$ in the whole cohort or in participants with eGFR > 60 ml/min/1.73m^2. After exclusion of participants with ACR > 3 mg/mmol, ACR was found to be positively associated with $lnPGF_{2\alpha}$ and lnSAA adjusted for age (data for the subgroup analyse not shown in thesis, only in paper).

Table 6. Cross-sectional associations between high sensitive CRP, interleukin-6, prostaglandin F_2 alpha, SAA, F_2-isoprostane and cystatin C-based GFR (eGFR), and ACR at age 77: multivariable regression

	Cystatin C-estimated glomerular filtration rate (eGFR) n = 647		**lnAlbumin-creatinine ratio (ACR) n = 647**	
	β-coefficient (95% CI)	p-value	β-coefficient (95% CI)	p-value
Model A				
lnhsCRP	-0.22 (-0.30 to -0.15)	< 0.001	0.11 (0.03 to 0.18)	0.004
lnPGF_2 alpha	0.005 (-0.07 to 0.08)	0.89	-0.05 (-0.12 to 0.03)	0.23
lnIL-6	-0.28 (-0.35 to -0.20)	< 0.001	0.15 (0.08 to -0.23)	< 0.001
lnSAA	-0.15 (-0.22 to -0.07)	< 0.001	0.11 (0.03 to -0.19)	0.005
lnF_2-Isoprostane	0.08 (0.006 to 0.16)	0.04	-0.11 (-0.19 to -0.04)	0.004
Model B				
lnhsCRP	-0.19 (-0.26 to -0.11)	< 0.001	0.10 (0.02 to 0.17)	0.01
lnPGF_2 alpha	0.008 (-0.07 to 0.08)	0.83	-0.03 (-0.11 to 0.04)	0.38
lnIL-6	-0.23 (-0.30 to -0.15)	< 0.001	0.14 (0.06 to 0.22)	< 0.001
lnSAA	-0.13 (-0.21 to -0.06)	0.001	0.12 (0.04 to 0.20)	0.004
lnF_2-Isoprostane	0.09 (0.02 to 0.17)	0.01	-0.10 (-0.18 to -0.02)	0.01

Data are regression coefficients for a 1-SD higher lnC-reactive protein (CRP), lnInterleukin 6 (IL-6), lnProstaglandin F_2 alpha (PGF_2alpha), lnSerum amyloid protein (SAA), lnF_2-Isoprostane and eGFR and albumin-creatinine ratio (ACR). Model A was adjusted for age; model B was adjusted according to directed acyclic graphs (DAGs): age, smoking, BMI, systolic and diastolic blood pressure, LDL, HDL and triglycerides, statin treatment, and ACE-inhibitory, ASA-, anti-inflammatory, and cortisone medication.

Glomerular filtration rate and endothelial function (study III)

eGFR and FMD was not significantly associated in the whole cohort or in individuals with eGFR >60 ml/min/1.73m^2 (n = 888) in either age- and sex-, or multivariable- adjusted models (Table 7).

In the whole cohort, a 10 ml/min/1.73 m^2 higher eGFR was found to be associated with 3% higher lnEDV, after adjusting for age and sex (model A, Table 7). The association was attenuated after adjusting for established cardiovascular risk factors (model B, Table 7). In a sub-sample with eGFR > 60 ml/min/1.73 m^2 (n = 778), the association between eGFR and lnEDV was similar but with a wider confidence interval (model A, Table 7). No significant association was observed after further adjustment for cardiovascular risk factors (model B, Table 7).

A positive association between eGFR and lnEIDV was seen in the whole cohort. A 10 ml/min/1.73 m^2 higher eGFR was significantly associated with 2% higher lnEIDV in the age- and sex-adjusted model (model A, Table 7). No association was found after adjusting for cardiovascular risk factors. Furthermore, no association between eGFR and lnEIDV was observed in the sample with eGFR > 60 ml/min/ 1.73 m^2 (n = 796) (model B, Table 7).

In the study, there was no evidence of effect modification by gender on the association between eGFR and any vascular function.

Table 7. Cross-sectional associations between eGFR and FMD, EDV, or EIDV at age 70: multivariable regression

	Estimated glomerular filtration rate (eGFR)			
	Whole sample		eGFR > 60 ml/min	
	Regression coefficient (95% CI)	p-value	Regression coefficient (95% CI)	p-value
Model A; Sex and age				
FMD	0.02 (-0.09 to 0.12)	0.76	0.02 (-0.10 to 0.14)	0.79
lnEDV	0.03 (0.01 to 0.05)	0.001	0.02 (0.001 to 0.04)	0.04
lnEIDV	0.02 (0.007 to 0.04)	0.007	0.01 (-0.007 to 0.03)	0.21
Model B; Cardiovascular risk factors				
FMD	0.01 (-0.10 to 0.12)	0.85	0.008 (-0.12 to 0.14)	0.90
lnEDV	0.01 (-0.008 to 0.03)	0.26	0.009 (-0.02 to 0.02)	0.93
lnEIDV	0.003 (-0.02 to 0.02)	0.73	-0.007 (-0.03 to 0.01)	0.52

Abbreviations: FMD, flow-mediated dilatation; lnEDV, $\log_e$ endothelium-dependent vasodilatation; lnEIDV, log.endothelium-independent vasodilatation. Data are regression coefficients for 10 ml/min/1.73 m^2 higher eGFR. Model A was adjusted age and sex (PIVUS). Model B = model A + systolic and diastolic blood pressure, anti-hypertensive medication, BMI, fasting glucose, anti-diabetic medication, LDL-cholesterol, HDL-cholesterol and triglycerides, CRP, lipid-lowering medication, and smoking. Whole sample: FMD, n = 952, EDV, n = 835, EIDV, n = 852; eGFR > 60 ml/min: FMD, n = 888, EDV, n = 778, EIDV, n = 796.

Glomerular filtration rate and left ventricular function (study IV)

In both PIVUS and ULSAM, higher eGFR was significantly associated with higher LVEF, adjusted for age and sex (model A, Table 8). In addition, higher eGFR was significantly associated with lower IVRT and MPI (reflecting better ventricular function) in PIVUS. After further adjustment for systolic and diastolic blood pressure, BMI, diabetes, LDL- and HDL-cholesterol, and smoking, a significant association was found between eGFR and LVEF (model B, Table 8) in both cohorts.

Furthermore, in subgroup analyses of participants with eGFR > 60 ml/min/1.73 m^2, a significant association between eGFR and LVEF, IVRT, and MPI was seen in PIVUS but not in ULSAM, after adjustment for age and sex (data not shown in thesis, only in paper).

The association between creatinine-based GFR with LVEF in PIVUS and ULSAM was similar to that for eGFR, adjusted for age and sex, but was of borderline significance (the multivariable regression coefficient for a 1-SD

increase in LVEF was 0.07 [95% CI -0.07 to 0.14, p = 0.08] in PIVUS and 0.09 [95% CI -0.01 to 0.19, p = 0.08] in ULSAM).

There was no evidence of effect modification by gender on the association between eGFR, LVEF, IVRT or MPI in PIVUS.

Table 8. Cross-sectional associations between cystatin C-based glomerular filtration rate (eGFR) and LVEF, IVRT, or MPI at age 70 in PIVUS and ULSAM: multivariable regression; whole cohort with LVEF > 40%

Estimated glomerular filtration rate (eGFR) Whole cohort					
	β-coefficient (95% CI)	p-value		β-coefficient (95% CI)	p-value
PIVUS			***ULSAM***		
Model A; Sex and age			**Model A; Sex and age**		
LVEF	0.11 (0.03 to 0.18)	0.004	LVEF	0.14 (0.04 to 0.23)	0.005
IVRT	-0.12 (-0.18 to -0.05)	0.001	IVRT	-0.05 (-0.14 to 0.04)	0.24
MPI	-0.10 (-0.17 to -0.03)	0.006	MPI	-0.09 (-0.18 to 0.01)	0.08
Model B; DAG			**Model B; DAG**		
LVEF	0.10 (0.03 to 0.17)	0.008	LVEF	0.11 (0.02 to 0.21)	0.02
IVRT	-0.07 (-0.14 to -0.01)	0.02	IVRT	-0.03 (-0.12 to 0.06)	0.50
MPI	-0.07 (-0.14 to 0.0001)	0.051	MPI	-0.06 (-0.15 to 0.04)	0.25

Data are regression coefficients for a 1-SD higher eGFR; Abbreviations: LVEF, left ventricular ejection fraction; IVRT, isovolumic relaxation time; MPI, myocardial performance index. Model A was adjusted for age and sex. Model B, DAG-adjusted: age, sex, systolic and diastolic blood pressure, BMI, diabetes, LDL-cholesterol, and smoking; whole cohort PIVUS: LVEF, n = 785, IVRT, n = 850, MPI, n = 732; ULSAM: LVEF, n = 407, IVRT, n = 494, MPI, n = 424.

The combined contribution of albuminuria and glomerular filtration rate to the prediction of cardiovascular mortality (study V)

During follow-up (median 12.9 years; range 0.7–15.4 years), 208 participants died from CVD (mortality rate = 1.6 per 100 person-years at risk). In participants without CVD at baseline, 86 died from CVD (mortality rate = 1.1 per 100 person-years at risk).

Cox regression (continuous analysis)

In the sub-sample without CVD at baseline, higher UAER was significantly associated with higher risk of cardiovascular death, after adjustment for established risk factors and eGFR; and eGFR was significantly associated with cardiovascular mortality, after adjustment for established cardiovascular risk factors and UAER (Table 9). Models that included UAER and eGFR showed

better global fit than models with only the established risk factors ($p < 0.001$).

Table 9. The association between UAER, eGFR, and cardiovascular mortality: multivariable Cox regression (continuous analysis)

Variable	Participants without CVD at baseline (n = 649)	
	Hazard ratio for a 1-SD increase 95% CI	p-value
Urinary albumin excretion rate (UAER)		
Adjusted for cardiovascular risk factors	1.29 (1.07 to1.56)	0.006
Adjusted for cardiovascular risk factors + eGFR	1.26 (1.05 to 1.51)	0.01
eGFR		
Adjusted for cardiovascular risk factors	0.72 (0.58 to 0.90)	0.004
Adjusted for cardiovascular risk factors + UAER	0.74 (0.59 to 0.92)	0.007

Data are hazard ratios for 1-SD higher ln urinary albumin excretion rate (UAER) and estimated glomerular filtration rate (eGFR – cystatin C). All models were adjusted for cardiovascular risk factors (age, systolic blood pressure, anti-hypertensive treatment, total cholesterol, HDL-cholesterol, lipid-lowering treatment, diabetes, smoking, and BMI.

C statistics

In the whole cohort, the C statistic increased significantly for the prediction of cardiovascular mortality when UAER and eGFR were incorporated into a model with the established risk factors. In participants without CVD at baseline, the increment in the C statistic was of similar magnitude but with wider CIs, making the association non-significant ($p = 0.15$).

Calibration

The p-values for the Grønnesby and Borgan statistics indicate adequate calibration for the model with UAER and eGFR ($p = 0.88$).

Net reclassification

Reclassification after the addition of UAER and eGFR to the model with the established risk factors in participants without CVD at baseline is presented in Table 10. In 12 participants who died from cardiovascular causes, reclassification was more accurate when the model with both kidney markers was used, and for 7 participants it became less accurate. Of those subjects who did not die, 62 were reclassified in a lower risk category and 33 were reclassified in a higher risk category. The NRI was estimated to be 0.11 ($p = 0.04$).

Table 10. Reclassification of participants without CVD at baseline who died from cardiovascular causes or who did not die, when adding UAER and eGFR to a model with established risk factors

Model with established risk factors	**Model with established risk factors and UAER and eGFR**			
Participants who died from CVD	< 5% risk	5–20% risk	> 20% risk	Total no.
	Number (per cent)			
< 5% risk	1 (100)	0 (0)	0 (0)	1
5–20% risk	3 (5.0)	45 (75.0)	12 (20.0)	60
> 20% risk	0 (0)	4 (16.0)	21(84.0)	25
Total no.	4	49	33	86
Participants who did not die				
< 5% risk	27 (81.8)	6 (18.2)	0 (0)	33
5–20% risk	33(7.2)	399(86.9)	27 (5.9)	459
> 20% risk	0 (0)	29(40.8)	42(59.1)	71
Total no.	60	434	69	563

Established risk factors included age, systolic blood pressure, anti-hypertensive treatment, total cholesterol, HDL-cholesterol, lipid-lowering treatment, diabetes, smoking, and BMI. UAER and eGFR were modelled as continuous variables.

Integrated discrimination improvement

In the sub-sample without CVD at baseline, the separate and combined addition of UAER and eGFR to the model with established risk factors improved IDI beyond the established risk factors (Table 11).

Table 11. The integrated discrimination improvement when adding UAER and cystatin C-based eGFR to a model with established risk factors for the prediction of cardiovascular mortality in participants without CVD at baseline

Variable	**Participants without CVD at baseline (n = 649)**	
	IDI	**p-value**
A. Established risk factors	reference	
+ UAER	0.020	0.02
+ eGFR	0.015	0.02
+ UAER + eGFR	0.032	0.002
B. Established risk factors + UAER	reference	
+ eGFR	0.012	0.03
C. Established risk factors + eGFR	reference	
+ UAER	0.018	0.03

Data are integrated discrimination improvement (IDI) for difference with model with established cardiovascular risk factors (age, systolic blood pressure, anti-hypertensive treatment, total cholesterol, HDL-cholesterol, lipid-lowering treatment, diabetes, smoking, and BMI). UAER and eGFR, were modelled as continuous variables.

Identification of optimal cut-offs

Based on maximal improvement in IDI, we identified the following optimal cut-offs: eGFR 45 ml/min/1.73 m^2 and UAER > 6 μg/min. Interestingly, the established cut-offs for eGFR and UAER used to diagnose chronic kidney disease stage 3 and microalbuminuria (eGFR < 60 ml/min/1.73 m^2 and UAER > 20 μg/min, respectively) did not significantly improve IDI in participants who were free from CVD at baseline.

Discussion

Numerous studies have demonstrated a close link between chronic kidney disease and CVD, but few have investigated the association at the early stages of the kidney disease. Using an epidemiological approach, the overall aim of this thesis was to explore the influence of kidney damage and dysfunction on the different stages of the cardiovascular continuum.

Comparison with the literature

Insulin sensitivity and glomerular filtration rate (study I)

In study I, we investigated the association between insulin sensitivity and eGFR in the ULSAM cohort. The data indicate that impaired insulin sensitivity may be involved in the development of renal dysfunction, before the onset of diabetes or before diabetic glucose elevations.

Our findings are in accordance with previous community-based studies that have investigated the cross-sectional association between insulin sensitivity and GFR.[34,92,93] In these studies, reduced insulin sensitivity (assessed from serum insulin levels or HOMA-IR) was associated with impaired renal function. However, both fasting insulin and HOMA-IR are limited as indicators of insulin sensitivity because they are also highly influenced by the individual's beta cell function, i.e. insulin secretion. The most quantitatively important insulin-sensitive tissue, skeletal muscle, is also better reflected by the clamp method. The association between insulin sensitivity as evaluated by the gold standard euglycaemic clamp technique and GFR has not been reported previously.

Furthermore, no previous studies have analysed this association in individuals with normal glucose levels and normal GFR. However, we are aware of one previous study that has evaluated the longitudinal association between insulin sensitivity and incidence of renal dysfunction. In contrast to study I, Fox and co-workers reported that HOMA insulin resistance did not significantly predict renal dysfunction in participants with normal glucose levels.[94] The discrepant results could perhaps be explained by differences between the studies in the assessment of insulin sensitivity or that our study sample consisted exclusively of elderly men.

Inflammation, oxidative stress, glomerular filtration rate, and albuminuria (study II)

In study II, we investigated the association between inflammation, oxidative stress, eGFR, and albuminuria in the ULSAM cohort. We found that cytokine-mediated inflammation was involved at an early stage of impaired kidney function. Unexpectedly, we found an inverse relationship between higher urinary F_2-isoprostane (oxidative stress) concentrations and higher eGFR/lower albuminuria.

Our findings are in accordance with most, but not all[95], previous community-based studies that have found independent associations between biomarkers of cytokine-mediated inflammation (C-reactive protein, tumour necrosis factor alpha, interleukin-6, and fibrinogen) and eGFR, measured through serum creatinine[42,43,96,97], cystatin C[98,99], and albuminuria.[97,100,101] We are aware of one previous study that has found these associations in elderly individuals without any apparent signs of kidney damage or dysfunction.[99] Systemic inflammation has been considered to be a risk factor for CKD, but may also represent a common pathway by which cardiovascular risk factors interact to amplify renal injury.[54,102]

To our knowledge, this is the first study to have investigated the association between markers of kidney damage and dysfunction and *in vivo* $PGF_{2\alpha}$ concentrations. However, no independent associations were seen between these markers of kidney pathology and urinary $PGF_{2\alpha}$ metabolite in this study, indicating that cyclooxygenase-mediated inflammation is not involved in the early stages of chronic kidney disease.

Surprisingly, increased eGFR and reduced ACR were associated with higher levels of urinary F_2-isoprostanes in the whole cohort. Since oxidative stress has been suggested to play an important role in the development of kidney disease, based on experimental studies[103,104], this finding was contradictory to what we originally hypothesized. Yet, there was a similar finding in a recent study from the Framingham Offspring Study, where individuals with CKD had lower urinary isoprostanes than individuals without CKD.[97] In contrast, a study on obese children[105] failed to show any linear correlation between plasma cystatin C, albuminuria, and urinary F_2-isoprostanes.

The unexpected associations found between kidney biomarkers and intact urinary F_2-isoprostanes in study II may possibly be related to the fact that F_2-isoprostanes were quantified in urine but not in plasma.[106] Studies have shown that patients with proven moderate-to-severe chronic kidney disease[54] and dialysis patients[102,107-109] have higher plasma concentrations of F_2-isoprostane than healthy subjects.

Glomerular filtration rate and endothelial function (study III)

In study III, we investigated the relationship between eGFR and endothelial function in PIVUS cohort. Lower eGFR was associated with impaired endothelial function, but this association was largely explained by confounding of established cardiovascular risk factors.

Clinical studies have indicated that impaired endothelial function is observed in individuals with moderate-to-severe kidney function relative to healthy controls, using biomarkers of endothelial function[53,110] and brachial measures of endothelial function.[111-113] However, previous community-based data are both scarce and inconsistent. A report from the Hoorn study showed an inverse association between eGFR and different indirect biomarkers of endothelial function (von Willenbrand factor, vascular cell adhesion molecule-1, and urinary albumin excretion).[114] One community-based study (Study of Health in Pomerania [SHIP]) indicated that endothelial dysfunction measured with FMD was associated with mild reduction in renal function in females.[115] In contrast, the Framingham Heart Study[116] and the Multi-Ethnic Study of Atherosclerosis (MESA)[117] indicated that endothelial dysfunction measured with FMD was not a major correlate of moderate CKD.

Our data are in line with Framingham heart study and MESA study suggesting that there is no association between eGFR and endothelial function assessed by FMD. It is possible that some of the inconsistencies between previous studies have been due to the different methods used to estimate endothelial function, and also to age and gender differences between the study samples. To my knowledge, we are the first group to report the association between eGFR and endothelial function as assessed by the invasive forearm technique.

Glomerular filtration rate and left ventricular function (study IV)

In study IV, we investigated the relationship between eGFR and left ventricular function in both ULSAM and the PIVUS cohort. We found a positive association between eGFR and echocardiographic indices of left ventricular systolic, diastolic, and global function. However, after adjustment for cardiovascular risk factors, only the association between eGFR and LVEF was statistically significant in the 2 cohorts. Interestingly, an association between eGFR and systolic function was also seen in participants with eGFR > 60 ml/min/m^2 in the PIVUS cohort.

Our findings agree with the results of one previous community-based study that measured LV function with echocardiography in 60- to 70-year-old participants with coronary heart disease, but free from heart failure (n =

818).[118] Serum cystatin C was found to be associated with both diastolic and systolic dysfunction in both crude and multivariable models.

In contrast, the MESA, involving a cohort of 4,970 participants aged 44–80 years, found no significant association between mild-to-moderate reduction in kidney function measured with cystatin C and LVEF using cardiac magnetic resonance imaging.[119] Similarly, in the Dallas Heart Study[120], with a cohort of 2,548 individuals aged 30–65 years, no significant associations were found between cystatin C and LVEF.

Perhaps differences in clinical characteristics such as age and prevalence of CVD and CKD could explain these discrepant results between the studies. The participants in the previous negative studies were younger, healthier, and had less CVD and CKD co-morbidities. We are not aware of any previous study that has found an association between glomerular filtration rate and left ventricular function in individuals free from clinical heart failure and with GFR > 60 ml/min/1.73 m^2.

The combined contribution of albuminuria and glomerular filtration rate to the prediction of cardiovascular mortality (study V)

In study V, we added both albuminuria and eGFR to a model with established cardiovascular risk factors in an attempt to improve risk stratification for cardiovascular death, particularly in individuals free from CVD. Our results indicated that the combined addition of eGFR and albuminuria significantly improved model discrimination, calibration, and reclassification beyond established cardiovascular risk factors for the prediction of cardiovascular mortality.

The findings are in agreement with the results of previous community-based studies.[11,121-128] In a report from the HUNT-II study, a combination of eGFR and albuminuria improved the individual risk stratification, particularly in subjects over 70 years of age, as evaluated by measures of discrimination and reclassification.[124] In a recent report from the ARIC study, mildly reduced eGFR and albuminuria independently contributed to the risk of all-cause mortality, cardiovascular disease, and heart failure, evaluated by measures of discrimination and reclassification.[129] However, in the HUNT-II study, creatinine-based eGFR was used and the study sample included participants with CVD.

We are aware of only one previous paper that has reported associations between albuminuria, impaired GFR, and high risk of cardiovascular mortality in individuals free from CVD.[122] In that study, only relative risks were reported, and no analyses of model discrimination, calibration, or reclassification were presented. These statistical measures have been suggested to be

highly relevant in order to properly evaluate the clinical usefulness of a potential risk factor.[130]

General discussion

The overall aim of this thesis work was to investigate the influence of kidney damage and dysfunction on the different stages of the cardiovascular continuum. Our data showed that the interplay between the kidney and the cardiovascular system starts already at the risk factor stage of the cardiovascular continuum. We also showed that impaired kidney function is associated with sub-clinical dysfunction of the vasculature and the myocardium, and that the accuracy of cardiovascular risk prediction may be improved if different aspects of kidney damage and dysfunction are taken into account.

The interplay between the kidney and the cardiovascular system is multifaceted and the present thesis clarifies only a few aspects of this complex chain of events. Our first study suggested that lower insulin sensitivity was associated with lower renal function, even in individuals with normal glucose levels and normal eGFR. This indicates that impaired insulin sensitivity may be involved in the development of renal dysfunction at an early stage, before the onset of diabetes or pre-diabetes glucose elevations. These findings are in line with previous studies suggesting that insulin resistance and compensatory hyperinsulinemia could be partly responsible for harming the normal kidney[94,131]; and challenge the notion that diabetic nephropathy is solely due to long-term, poorly controlled glucose levels. It should also be noted that insulin resistance may also be an important factor in severe kidney disease.[36,132]

Some of the mechanisms linking insulin resistance to kidney dysfunction are oxidative stress, inflammation, and endothelial dysfunction.[44,133,134] In healthy individuals, the degree of insulin resistance was found to be closely associated with markers of oxidative stress and inversely associated with levels of anti-oxidant substances well before the development of glucose intolerance and type-2 diabetes. Insulin resistance was also associated with the production of free radicals, which in turn was responsible for the deterioration of insulin action.[135] However, an experimental study on rats showed that oxidative stress induces insulin resistance, which can lead to a vicious circle.[136] Also, other studies have suggested that chronic inflammation may be involved in the pathogenesis of insulin resistance and type-2 diabetes.[137] One possible pathway that generates cytokine-mediated inflammation is through increased oxidative stress.[138,139] This hypothesis is supported by *in vivo* studies showing that free fatty acids and glucose induce inflammation through oxidative stress, have a cumulative and independent effect, and that anti-oxidants reverse the phenomenon.[140-142]

Increased inflammation and oxidative stress are hallmarks of severe kidney disease.[37,54] The work for this thesis suggests that these pathways may be involved already early in the cardio-renal continuum.

Studies have also shown that insulin resistance, oxidative stress, and inflammatory cytokines affect vascular walls and promote endothelial dysfunction.[134,143,144] Chronic kidney disease is associated with endothelial dysfunction, although the precise associations with GFR and the level at which endothelial dysfunction begins are contentious. However, it has long been known that abnormal endothelial function is present in late-stage CKD[145] but this thesis work indicates that it is already evident at the early stages of impaired kidney function; albeit confounded by cardiovascular risk factors.

Insulin resistance has also been shown to directly affect the myocardium via metabolic, structural, and functional changes, leading to diabetic cardiomyopathy, myocardial ischaemia, and ultimately heart failure and death.[146]

RAAS is an important regulator of blood pressure, renal haemodynamic, and volume homeostasis in normal kidney physiology. A decline in renal function can lead to sodium retention and increased extracellular fluid volume, which leads to a chronic activation of the RAAS. Persistent activation of RAAS could have damaging effects on cardiac function and contribute to the progression of heart failure through promotion of cardiac remodelling and myocardial fibrosis.[65] Increased levels of angiotensin II and aldosterone have been shown to alter the insulin-signalling pathway and to promote the formation of reactive oxygen species, which induce endothelial dysfunction.[147,148] Studies have also demonstrated that RAAS contributes to impaired insulin secretion and insulin resistance, which increases the risk of type-2 diabetes.[149-151] Furthermore, persistent activation of RAAS could have damaging effects on cardiac function and contribute to the progression of heart failure by promotion of cardiac remodelling and myocardial fibrosis.[65]

In this thesis, the risk factors studied (insulin resistance, oxidative stress, inflammation, endothelial dysfunction, and ventricular dysfunction) describe a complex relationship with renal impairment, which in extension can lead to CVD. One can speculate that insulin resistance may be an important deleterious first step in the chain reaction of inflammation and oxidative stress that leads to kidney and heart dysfunction, and ultimately to overt CKD, CVD, and death.

Modifiable risk factors

In this thesis we have proposed a close interplay between insulin resistance, inflammation, oxidative stress, impaired kidney and cardiovascular function, and cardiovascular mortality. Thus, an important question arises: by preventing insulin resistance, inflammation, or oxidative stress, would it be possible to prevent the development of impaired kidney function and its severe consequences? Since no firm conclusions regarding causality can be drawn from

our data, more intervention trials or Mendelian randomization studies are needed to properly investigate these issues. Some recent data on this issue are discussed below.

Changes in lifestyle

In western cultures, the most common factor causing insulin resistance is obesity though low-grade inflammation.[152] The fundamental cause of obesity and overweight is high calorie intake relative to physical activity, resulting in a positive energy balance. It has been shown that in patients with CKD, lifestyle changes such as weight loss and exercise can increase insulin sensitivity, reduce proteinuria, and protect from oxidative stress and inflammation, which all seem to prevent further decline in renal function and reduce related CVD and mortality.[153-156]

Primary prevention is a vital element of health services, yet we know that it is difficult to increase the physical activity and improve the nutritional habits of patients in the long term. Further work in this area is clearly needed.

Pharmacological improvement of insulin sensitivity

The most common insulin sensitizer is probably biguanides (metformin). This is particularly effective in overweight subjects with type-2 diabetes, a condition usually characterized by insulin resistance. Animal studies have shown that metformin may protect against tubular cell injury in diabetic nephropathy by reduction of oxidative stress and inflammation.[157,158] However, metformin is eliminated through the kidneys unchanged and because of the risk of lactic acidosis due to accumulation of metformin[159], it may be necessary to adjust the dose in patients with impaired kidney function.[160]

Another type of medication that increases insulin sensitivity is thiazolidinedione (glitazones). This is a relatively new class of oral anti-diabetic drugs that improve metabolic control in patients with type-2 diabetes. Thiazolidinedione acts by binding to peroxisome proliferator-activated receptor gamma (PPARγ) in peripheral adipocytes to promote adipogenesis and uptake of free fatty acid, which leads to reductions in fat stored in the muscle and liver. It is also believed to increase the secretion of adiponectin and to decrease the production of resistin and tumour necrosis factor α (TNF-α). Human and animal studies have shown that thiazolidinediones have a protective effect on the kidney[161] by down-regulating the renin-angiotensin system[162], reducing oxidative stress and inflammation[163] and improving renal endothelial function.[164]

Another suggested link between insulin resistance and impaired kidney function is via advanced glycosulation end-products (AGEs) and increased expression of the receptor for AGEs (RAGE). The effects of AGEs are nor-

mally inhibited by AGE-degrading systems and anti-AGE receptors. When there is an imbalance between the two systems, it can cause oxidative stress and induce inflammation that progress to vascular complications in different organs.[165] Large AGE proteins are unable to enter the Bowman's capsule; instead, they binds to AGE receptors on endothelial and mesangial cells and to the mesangial matrix, leading to glomerular sclerosis and impaired kidney function.[166] AGEs can be generated under hyperglycaemic conditions, but are also derived exogenously from AGE-rich meals.[167] In other words, diet is a major environmental source of pro-inflammatory AGEs. One possible way to inhibit the risk of oxidative stress and inflammation could be to restrict food-derived AGEs. Studies have also shown that different drugs such as metformin[158], pravastatin[168], azelnidipine[169], sevelamer[170], and irbesartan[171] could inhibit the AGE-induced apoptosis, inflammatory and fibrotic reactions in the kidney. These studies are promising, but we need larger, randomized clinical trials before this can lead to clinical therapy for the prevention of kidney failure.

Anti-inflammatory drugs

Chronic inflammation appears to be a key player in the development of CKD, and many attempts have been made to find an effective anti-inflammatory drug.

Today, non-steroidal anti-inflammatory (NSAID) drugs are the most commonly used pain medicines in adults. Unfortunately, NSAIDs including cyclooxygenase-2 (COX-2) inhibitors have been shown to have an adverse effect on renal function, especially in patients with CKD.[172-174] The adverse effects of NSAIDs are mediated through inhibition of prostaglandin synthesis, leading to vasoconstriction, acute kidney injury, sodium retention, oedema, hypertension, and hyperkalaemia.[175]

It has also become apparent that certain cyclooxygenase-2 inhibitors increase the risk of serious cardiovascular events, including hypertension, MI, heart failure, and stroke. The mechanism is believed to be caused by inhibition of prostaglandin I_2 by thromboxane A_2, which promotes thrombosis formation.[176,177]

Anti-oxidant supplementation

Oxidative stress is believed to be caused by an imbalance between free radical production and anti-oxidant defence. Oxidative stress has been detected in people with CKD, and because oxidation products may mediate inflammation in CKD patients, nutrients or anti-oxidants (such as vitamin C and E), or both, may be of particular interest in this patient group. It has been shown that high-dose vitamin E supplementation reduces markers of inflammation in patients with diabetes[178], coronary artery disease[179], ESRD[180], and haemodialysis[181], which could indicate an anti-inflammatory action. Moreover, vitamin E supplementations might also reduce sensitivity to LDL

oxidation[179] and improves endothelial function.[182,183] However, several studies have not been able to demonstrate any beneficial effect of vitamin E supplementation in reducing cardiovascular risk.[184-186] It is clear that further studies are needed in this area, particularly regarding the effects on kidney disease.

Statins (HMG-CoA reductase inhibitors)

Another class of drugs that is believed to have anti-inflammatory properties is the statins. These have been proven to reduce cardiovascular morbidity and mortality through a lipid-lowering effect. Interestingly, there is experimental and clinical evidence that statins can also improve endothelial dysfunction[187], oxidative stress[188] and inflammation.[189] Several studies have found that kidney disease is associated with low-grade inflammation as measured by elevated CRP levels, even in patients with mild-to-moderate renal impairment.[190,191] Statins may protect the kidney by reducing lipid levels and modulating glomerular mesangial cells and to improve inflammatory process and increase GFR.[192-194] Today, it is recommended that CKD patients be treated with lipid-lowering drugs, which is supported by guidelines[195], meta-analyses, and randomized, placebo-controlled trial studies.[196-198]

Renin-angiotensin-aldosterone

Several RAAS inhibitors, such as direct renin inhibition, angiotensin converting enzyme inhibitors, angiotensin II receptor blockers, and mineralcorticoid receptors are all drugs that have been shown to reduce the risk of CKD and CVD. The mechanism is possibly mediated in part by increased insulin sensitivity, attenuated oxidative stress, and improved endothelial function.[148,199] However, it still remains to be seen whether reduced insulin resistance by these drugs can prevent the development of CKD in insulin-resistant individuals.

In summary, although several different treatment options are available that could be beneficial in theory, firm evidence of a favourable effect in slowing down or reversing the progression of chronic kidney disease and thus preventing CVD is limited for most treatment alternatives. Given the current global increase in CKD, an improved treatment would have major clinical implications. A better understanding of the cardio-renal interplay at the early stages of the disease may improve the patient's outcome in the long term, delaying not only early renal disease but also the progression to CVD and death. Further research is needed to fully clarify all the potential mechanisms that connect insulin resistance, inflammation, and oxidative stress with renal damage, and to provide solid evidence; based on which specific treatment recommendations could be made.

Strengths and limitations

The strengths of the thesis include the use of two large, homogenous, community-based studies with detailed characterizations of participants with regards to glucometabolic variables, cardiovascular risk factors, and lifestyle factors. To my knowledge, the ULSAM cohort is the largest cohort in the world to be examined with the gold standard for measurement of insulin resistance, the euglycaemic clamp technique. PIVUS also has unique comprehensive measures of non-invasive and invasive arterial measurements of endothelial function.

Some limitations are worth mentioning, the most obvious ones being the unknown generalizability to women (ULSAM), and to other age and ethnic groups. Furthermore, the PIVUS cohort had a moderate participation rate. However, an analysis of non-participants showed the present sample to be representative of the total population in terms of most cardiovascular disorders and medications.[63]

In addition, in the present study we used a cystatin C-based GFR formula, which is an indirect approximation of GFR, because direct measurement of GFR by the gold standard method (exogenous clearance measurements) was not available. Even so, exogenous clearance measurements are seldom used in epidemiological research, as this is a time-consuming and costly procedure. Importantly, cystatin C-based GFR have been shown to be closely correlated with GFR as assessed by exogenous clearance measurements, also in the normal range of GFR.[75,76]

In 2012, Inker *et al.* introduced a new GFR equation (the CKD-EPI formula) involving serum cystatin C in combination with serum creatinine level, and also including age, sex, and race, which is considered to provide a more accurate formula to estimate GFR.[20] We did not use this formula because it is based on standardized cystatin C and creatinine measurements and these were not available at the start (baseline) of the present studies.

Furthermore, four of the studies were based on cross-sectional design; thus, we could not assess causality. Another limitation worth mentioning is the "healthy cohort effect", which means that those subjects who tend to participate in cohort studies are generally healthier than those who choose not to participate. This may lead to limited generalizability regarding the whole general population.

Conclusions

I. Lower insulin sensitivity was associated with lower renal function, even in individuals with normal glucose levels and normal eGFR. Impaired insulin sensitivity may be involved in the development of renal dysfunction at an early stage, prior to the onset of diabetes or pre-diabetic glucose elevations.

II. Cytokine-mediated inflammation, but not COX-mediated inflammation, was inversely associated with eGFR and positively associated with ACR. Conversely, higher levels of F_2-isoprostanes, reflecting increased oxidative stress, were associated with higher eGFR and lower ACR.

III. Lower eGFR was associated with impaired endothelial function, even in individuals with normal kidney function, but this association was largely explained by confounding by established cardiovascular risk factors.

IV. Lower levels of eGFR were independently associated with reduced left ventricular function. The data suggested that the detrimental interplay between the kidney and the heart begins in the early stages of left ventricular dysfunction, before the development of symptomatic heart failure and CKD.

V. The combined addition of eGFR and UAER significantly improved model discrimination, calibration, and reclassification beyond established cardiovascular risk factors for the prediction of cardiovascular mortality in a community-based sample of elderly men who were free from CVD.

The current established cut-offs for microalbuminuria (20 μg/min) and chronic kidney disease stage 3 (< 60 ml/min/1.73 m^2) used in clinical practice appeared less suitable for prediction of cardiovascular risk in elderly men.

Summary in Swedish (Sammanfattning på svenska)

Hjärt-kärlsjukdom är en av de vanligaste dödsorsakerna i världen. En annan sjukdom på frammarsch är kronisk njursjukdom, vilket har visats vara vanligare än vi tidigare trott och drabbar idag var tionde svensk. Nedsatt njurfunktion är en lika stark riskfaktor för hjärt-kärlsjukdom som diabetes. Vad som orsakar detta samband mellan njursjukdom och hjärt-kärlsjukdom är inte helt klarlagt men nedsatt känslighet för insulin, sk insulinresistens, inflammation samt oxidativ stress, har föreslagits spela en nyckelroll.

Syftet med avhandlingen var att undersöka om det fanns något samband mellan lätt nedsatt njurfunktion och olika stadier i utvecklingen av hjärtkärlsjukdom, från riskfaktorer såsom insulinresistens (studie I), inflammation och oxidativ stress (studie II), till förstadiet i utvecklandet av hjärtkärlskador såsom skador på kärlväggen (studie III) och nedsatt pumpförmåga av vänster kammare (studie IV), till förbättrad hjärt-kärlriskprediktion (studie V).

Avhandlingen baseras sig på två pågående populationsbaserade kohorter, Uppsala Longitudinal Study of Adult Men (ULSAM) och Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS). ULSAM startade 1970 och inkluderade 2322, 50-åriga män. Deltagarna har vidare undersökts vid 60, 70, 77, 82 och 88 års ålder. PIVUS startade 2001 och inkluderade 70-åriga män och kvinnor boende i Uppsala, Sverige. Av 2025 inbjudna personer, deltog 1016 personer (51% kvinnor).

Studie I visade att det fanns ett samband mellan insulinresistentes och lätt nedsatt njurfunktion, även hos personer med normala glukosnivåer och normal njurfunktion. Vi kunde även visa på att riskfaktorer som insulinresistens kan vara involverad i utvecklingen av nedsatt njurfunktion, även hos personer utan diabetes. Studie II, visade på samband mellan cytokin medierad inflammation (CRP, IL-6) och lätt nedsatt njurfunktion. Tvärtemot vår initiala hypotes, sågs även ett samband mellan högre nivåer av oxidativ stress och förbättrad njurfunktion och lägre nivåer av njurskada. Studie III och IV, visade på samband mellan lätt försämrad njurfunktion och nedsatt kärl-, och hjärtfunktion, men sambandet mellan njurfunktion och kärlfunktion var till stor del förklarade av etablerade kardiovaskulära riskfaktorer. Studie V visade, att genom att lägga till två njurmarkörer till en modell med etablerade hjärt-kärlriskfaktorer förbättrades riskprediktionen för kardiovaskulär död.

Vi visade samtidigt att nuvarande etablerade gränsvärde som används i klinisk praxis, mikroalbuminuri (20 mikrogram/min) och kronisk njursjuk-

dom stadium 3 (<60 ml/min/1.73 m^2), var mindre lämplig för att bedöma risken för hjärtkärl död hos äldre män.

Sammanfattningsvis, avhandlingen visar att samspelet mellan njuren och hjärt-kärlträdet spelar en viktig roll för utvecklingen för hjärt-kärlsjukdomar och att detta samspel börjar redan på ett tidigt stadium i sjukdomsprocessen, långt innan man får symtom.

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References

1. Mendis S. The contribution of the Framingham Heart Study to the prevention of cardiovascular disease: a global perspective. *Prog Cardiovasc Dis.* Jul-Aug 2010;53(1):10-14.

2. WHO. World Health Organization. Neglected Global Epidemics: Three Growing Threats. 2003.

3. Dzau V, Braunwald E. Resolved and unresolved issues in the prevention and treatment of coronary artery disease: a workshop consensus statement. *Am Heart J.* Apr 1991;121(4 Pt 1):1244-1263.

4. Shlipak MG, Sarnak MJ, Katz R, et al. Cystatin C and the risk of death and cardiovascular events among elderly persons. *N Engl J Med.* May 19 2005;352(20):2049-2060.

5. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med.* Sep 23 2004;351(13):1296-1305.

6. Ronco C. Cardiorenal syndromes: definition and classification. *Contrib Nephrol.* 2010;164:33-38.

7. House AA. Cardio-renal syndrome type 4: epidemiology, pathophysiology and treatment. *Semin Nephrol.* Jan 2012;32(1):40-48.

8. Remuzzi G, Bertani T. Pathophysiology of progressive nephropathies. *N Engl J Med.* Nov 12 1998;339(20):1448-1456.

9. Comper WD, Russo LM. The glomerular filter: an imperfect barrier is required for perfect renal function. *Curr Opin Nephrol Hypertens.* Jul 2009;18(4):336-342.

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

11. Brantsma AH, Bakker SJ, de Zeeuw D, de Jong PE, Gansevoort RT. Urinary albumin excretion as a predictor of the development of hypertension in the general population. *J Am Soc Nephrol.* Feb 2006;17(2):331-335.

12. de Boer IH, Katz R, Cao JJ, et al. Cystatin C, albuminuria, and mortality among older adults with diabetes. *Diabetes Care.* Oct 2009;32(10):1833-1838.

13. Arnlov J, Evans JC, Meigs JB, et al. Low-grade albuminuria and incidence of cardiovascular disease events in nonhypertensive and nondiabetic individuals: the Framingham Heart Study. *Circulation.* Aug 16 2005;112(7):969-975.

14. Hermans MM, Henry RM, Dekker JM, Nijpels G, Heine RJ, Stehouwer CD. Albuminuria, but not estimated glomerular filtration rate, is associated with maladaptive arterial remodeling: the Hoorn Study. *J Hypertens.* Apr 2008;26(4):791-797.

15. Brantsma AH, Bakker SJ, Hillege HL, et al. Cardiovascular and renal outcome in subjects with K/DOQI stage 1-3 chronic kidney disease: the importance of urinary albumin excretion. *Nephrol Dial Transplant.* Dec 2008;23(12):3851-3858.

16. Cirillo M, Senigalliesi L, Laurenzi M, et al. Microalbuminuria in nondiabetic adults: relation of blood pressure, body mass index, plasma cholesterol levels, and smoking: The Gubbio Population Study. *Arch Intern Med.* Sep 28 1998;158(17):1933-1939.

17. Ljungman S, Wikstrand J, Hartford M, Berglund G. Urinary albumin excretion--a predictor of risk of cardiovascular disease. A prospective 10-year follow-up of middle-aged nondiabetic normal and hypertensive men. *Am J Hypertens.* Aug 1996;9(8):770-778.

18. Russo LM, Comper WD, Osicka TM. Mechanism of albuminuria associated with cardiovascular disease and kidney disease. *Kidney Int Suppl.* Nov 2004(92):S67-68.

19. Sarnak MJ, Levey AS, Schoolwerth AC, et al. Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. *Hypertension.* Nov 2003;42(5):1050-1065.

20. Inker LA, Schmid CH, Tighiouart H, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med.* Jul 5 2012;367(1):20-29.

21. Grubb A, Blirup-Jensen S, Lindstrom V, Schmidt C, Althaus H, Zegers I. First certified reference material for cystatin C in human serum ERM-DA471/IFCC. *Clin Chem Lab Med.* Nov 2010;48(11):1619-1621.

22. Levey AS, Coresh J, Greene T, et al. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Intern Med.* Aug 15 2006;145(4):247-254.

23. Stevens PE, Levin A. Evaluation and management of chronic kidney disease: synopsis of the kidney disease: improving global outcomes 2012 clinical practice guideline. *Ann Intern Med.* Jun 4 2013;158(11):825-830.

24. Sarafidis PA, Ruilope LM. Insulin resistance, hyperinsulinemia, and renal injury: mechanisms and implications. *Am J Nephrol.* 2006;26(3):232-244.

25. Riserus U, Basu S, Jovinge S, Fredrikson GN, Arnlov J, Vessby B. Supplementation with conjugated linoleic acid causes isomer-dependent oxidative stress and elevated C-reactive protein: a

potential link to fatty acid-induced insulin resistance. *Circulation.* Oct 8 2002;106(15):1925-1929.

26. Prabhakar SS. Role of nitric oxide in diabetic nephropathy. *Semin Nephrol.* Jul 2004;24(4):333-344.

27. Horie K, Miyata T, Maeda K, et al. Immunohistochemical colocalization of glycoxidation products and lipid peroxidation products in diabetic renal glomerular lesions. Implication for glycoxidative stress in the pathogenesis of diabetic nephropathy. *J Clin Invest.* Dec 15 1997;100(12):2995-3004.

28. Suzuki D, Miyata T, Saotome N, et al. Immunohistochemical evidence for an increased oxidative stress and carbonyl modification of proteins in diabetic glomerular lesions. *J Am Soc Nephrol.* Apr 1999;10(4):822-832.

29. Miyata T, Sugiyama S, Suzuki D, Inagi R, Kurokawa K. Increased carbonyl modification by lipids and carbohydrates in diabetic nephropathy. *Kidney Int Suppl.* Jul 1999;71:S54-56.

30. Knight SF, Imig JD. Obesity, insulin resistance, and renal function. *Microcirculation.* Jun-Jul 2007;14(4-5):349-362.

31. Rabkin R, Ryan MP, Duckworth WC. The renal metabolism of insulin. *Diabetologia.* Sep 1984;27(3):351-357.

32. Brancati FL, Whelton PK, Randall BL, Neaton JD, Stamler J, Klag MJ. Risk of end-stage renal disease in diabetes mellitus: a prospective cohort study of men screened for MRFIT. Multiple Risk Factor Intervention Trial. *JAMA.* Dec 17 1997;278(23):2069-2074.

33. Boden G. Pathogenesis of type 2 diabetes. Insulin resistance. *Endocrinol Metab Clin North Am.* Dec 2001;30(4):801-815, v.

34. Chen J, Muntner P, Hamm LL, et al. Insulin resistance and risk of chronic kidney disease in nondiabetic US adults. *J Am Soc Nephrol.* Feb 2003;14(2):469-477.

35. Kaartinen K, Syrjanen J, Porsti I, et al. Insulin resistance and the progression of IgA glomerulonephritis. *Nephrol Dial Transplant.* Mar 2007;22(3):778-783.

36. DeFronzo RA, Alvestrand A, Smith D, Hendler R, Hendler E, Wahren J. Insulin resistance in uremia. *J Clin Invest.* Feb 1981;67(2):563-568.

37. Cottone S, Lorito MC, Riccobene R, et al. Oxidative stress, inflammation and cardiovascular disease in chronic renal failure. *J Nephrol.* Mar-Apr 2008;21(2):175-179.

38. Stenvinkel P, Ketteler M, Johnson RJ, et al. IL-10, IL-6, and TNF-alpha: central factors in the altered cytokine network of uremia--the good, the bad, and the ugly. *Kidney Int.* Apr 2005;67(4):1216-1233.

39. Upadhyay A, Larson MG, Guo CY, et al. Inflammation, kidney function and albuminuria in the Framingham Offspring cohort. *Nephrol Dial Transplant.* Aug 3 2010.

40. Goicoechea M, de Vinuesa SG, Lahera V, et al. Effects of atorvastatin on inflammatory and fibrinolytic parameters in patients

with chronic kidney disease. *J Am Soc Nephrol.* Dec 2006;17(12 Suppl 3):S231-235.

41. Fried L, Solomon C, Shlipak M, et al. Inflammatory and prothrombotic markers and the progression of renal disease in elderly individuals. *J Am Soc Nephrol.* Dec 2004;15(12):3184-3191.

42. Stuveling EM, Hillege HL, Bakker SJ, Gans RO, De Jong PE, De Zeeuw D. C-reactive protein is associated with renal function abnormalities in a non-diabetic population. *Kidney Int.* Feb 2003;63(2):654-661.

43. Shlipak MG, Fried LF, Crump C, et al. Elevations of inflammatory and procoagulant biomarkers in elderly persons with renal insufficiency. *Circulation.* Jan 7 2003;107(1):87-92.

44. Dogra G, Irish A, Chan D, Watts G. Insulin resistance, inflammation, and blood pressure determine vascular dysfunction in CKD. *Am J Kidney Dis.* Dec 2006;48(6):926-934.

45. Basu S. Radioimmunoassay of 15-keto-13,14-dihydro-prostaglandin F2alpha: an index for inflammation via cyclooxygenase catalysed lipid peroxidation. *Prostaglandins Leukot Essent Fatty Acids.* May 1998;58(5):347-352.

46. Tolle M, Huang T, Schuchardt M, et al. High-density lipoprotein loses its anti-inflammatory capacity by accumulation of pro-inflammatory-serum amyloid A. *Cardiovasc Res.* Apr 1 2012;94(1):154-162.

47. Weichhart T, Kopecky C, Kubicek M, et al. Serum amyloid A in uremic HDL promotes inflammation. *J Am Soc Nephrol.* May 2012;23(5):934-947.

48. Urieli-Shoval S, Linke RP, Matzner Y. Expression and function of serum amyloid A, a major acute-phase protein, in normal and disease states. *Curr Opin Hematol.* Jan 2000;7(1):64-69.

49. Basu S. Radioimmunoassay of 8-iso-prostaglandin F2alpha: an index for oxidative injury via free radical catalysed lipid peroxidation. *Prostaglandins Leukot Essent Fatty Acids.* Apr 1998;58(4):319-325.

50. Yin H. New techniques to detect oxidative stress markers: mass spectrometry-based methods to detect isoprostanes as the gold standard for oxidative stress in vivo. *Biofactors.* 2008;34(2):109-124.

51. Ochodnicky P, Henning RH, Buikema H, et al. Renal endothelial function and blood flow predict the individual susceptibility to adriamycin-induced renal damage. *Nephrol Dial Transplant.* Feb 2009;24(2):413-420.

52. Baylis C, Mitruka B, Deng A. Chronic blockade of nitric oxide synthesis in the rat produces systemic hypertension and glomerular damage. *J Clin Invest.* Jul 1992;90(1):278-281.

53. Cottone S, Palermo A, Vaccaro F, et al. Inflammation and endothelial activation are linked to renal function in long-term kidney transplantation. *Transpl Int.* Jan 2007;20(1):82-87.

54. Oberg BP, McMenamin E, Lucas FL, et al. Increased prevalence of oxidant stress and inflammation in patients with moderate to severe chronic kidney disease. *Kidney Int.* Mar 2004;65(3):1009-1016.
55. Kals J, Kampus P, Kals M, et al. Inflammation and oxidative stress are associated differently with endothelial function and arterial stiffness in healthy subjects and in patients with atherosclerosis. *Scand J Clin Lab Invest.* 2008;68(7):594-601.
56. Pyke KE, Tschakovsky ME. The relationship between shear stress and flow-mediated dilatation: implications for the assessment of endothelial function. *J Physiol.* Oct 15 2005;568(Pt 2):357-369.
57. Sinoway LI, Hendrickson C, Davidson WR, Jr., Prophet S, Zelis R. Characteristics of flow-mediated brachial artery vasodilation in human subjects. *Circ Res.* Jan 1989;64(1):32-42.
58. Panza JA, Quyyumi AA, Brush JE, Jr., Epstein SE. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N Engl J Med.* Jul 5 1990;323(1):22-27.
59. Schachinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation.* Apr 25 2000;101(16):1899-1906.
60. Creager MA, Cooke JP, Mendelsohn ME, et al. Impaired vasodilation of forearm resistance vessels in hypercholesterolemic humans. *J Clin Invest.* Jul 1990;86(1):228-234.
61. Johnstone MT, Creager SJ, Scales KM, Cusco JA, Lee BK, Creager MA. Impaired endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *Circulation.* Dec 1993;88(6):2510-2516.
62. Celermajer DS, Sorensen KE, Georgakopoulos D, et al. Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. *Circulation.* Nov 1993;88(5 Pt 1):2149-2155.
63. Annuk M, Soveri I, Zilmer M, Lind L, Hulthe J, Fellstrom B. Endothelial function, CRP and oxidative stress in chronic kidney disease. *J Nephrol.* Nov-Dec 2005;18(6):721-726.
64. Foley RN, Parfrey PS, Harnett JD, et al. Clinical and echocardiographic disease in patients starting end-stage renal disease therapy. *Kidney Int.* Jan 1995;47(1):186-192.
65. Sun Y. The renin-angiotensin-aldosterone system and vascular remodeling. *Congest Heart Fail.* Jan-Feb 2002;8(1):11-16.
66. Martin FL, McKie PM, Cataliotti A, et al. Experimental mild renal insufficiency mediates early cardiac apoptosis, fibrosis, and diastolic dysfunction: a kidney-heart connection. *Am J Physiol Regul Integr Comp Physiol.* Jan 15 2012;302(2):R292-299.
67. Kasiske BL. The kidney in cardiovascular disease. *Ann Intern Med.* Apr 17 2001;134(8):707-709.
68. Chen YT, Vaccarino V, Williams CS, Butler J, Berkman LF, Krumholz HM. Risk factors for heart failure in the elderly: a

prospective community-based study. *Am J Med.* Jun 1999;106(6):605-612.

69. Tei C, Dujardin KS, Hodge DO, et al. Doppler echocardiographic index for assessment of global right ventricular function. *J Am Soc Echocardiogr.* Nov-Dec 1996;9(6):838-847.

70. Tei C, Ling LH, Hodge DO, et al. New index of combined systolic and diastolic myocardial performance: a simple and reproducible measure of cardiac function--a study in normals and dilated cardiomyopathy. *J Cardiol.* Dec 1995;26(6):357-366.

71. Perk J, De Backer G, Gohlke H, et al. [European Guidelines on Cardiovascular Disease Prevention in Clinical Practice (version 2012). The Fifth Joint Task Force of the European Society of Cardiology and other societies on cardiovascular disease prevention in clinical practice (constituted by representatives of nine societies and by invited experts)]. *G Ital Cardiol (Rome).* May 2013;14(5):328-392.

72. Tripepi G, Jager KJ, Dekker FW, Zoccali C. Statistical methods for the assessment of prognostic biomarkers (Part I): discrimination. *Nephrol Dial Transplant.* May 2010;25(5):1399-1401.

73. Tripepi G, Jager KJ, Dekker FW, Zoccali C. Statistical methods for the assessment of prognostic biomarkers(part II): calibration and re-classification. *Nephrol Dial Transplant.* May 2010;25(5):1402-1405.

74. Lind L, Fors N, Hall J, Marttala K, Stenborg A. A comparison of three different methods to evaluate endothelium-dependent vasodilation in the elderly: the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study. *Arterioscler Thromb Vasc Biol.* Nov 2005;25(11):2368-2375.

75. Larsson A, Malm J, Grubb A, Hansson LO. Calculation of glomerular filtration rate expressed in mL/min from plasma cystatin C values in mg/L. *Scand J Clin Lab Invest.* 2004;64(1):25-30.

76. Flodin M, Jonsson AS, Hansson LO, Danielsson LA, Larsson A. Evaluation of Gentian cystatin C reagent on Abbott Ci8200 and calculation of glomerular filtration rate expressed in mL/min/1.73 m(2) from the cystatin C values in mg/L. *Scand J Clin Lab Invest.* 2007;67(5):560-567.

77. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med.* May 5 2009;150(9):604-612.

78. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol.* Sep 1979;237(3):E214-223.

79. Pollare T, Vessby B, Lithell H. Lipoprotein lipase activity in skeletal muscle is related to insulin sensitivity. *Arterioscler Thromb.* Sep-Oct 1991;11(5):1192-1203.

80. Corretti MC, Anderson TJ, Benjamin EJ, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International

Brachial Artery Reactivity Task Force. *J Am Coll Cardiol.* Jan 16 2002;39(2):257-265.

81. Lind L, Hall J, Larsson A, Annuk M, Fellstrom B, Lithell H. Evaluation of endothelium-dependent vasodilation in the human peripheral circulation. *Clin Physiol.* Nov 2000;20(6):440-448.

82. Lind L, Sarabi M, Millgard J. Methodological aspects of the evaluation of endothelium-dependent vasodilatation in the human forearm. *Clin Physiol.* Mar 1998;18(2):81-87.

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

84. Wallerson DC, Ganau A, Roman MJ, Devereux RB. Measurement of cardiac output by M-mode and two-dimensional echocardiography: application to patients with hypertension. *Eur Heart J.* Dec 1990;11 Suppl I:67-78.

85. Paulus WJ, van Ballegoij JJ. Treatment of heart failure with normal ejection fraction: an inconvenient truth! *J Am Coll Cardiol.* Feb 9 2010;55(6):526-537.

86. Pencina MJ, D'Agostino RB. Overall C as a measure of discrimination in survival analysis: model specific population value and confidence interval estimation. *Stat Med.* Jul 15 2004;23(13):2109-2123.

87. Antolini L, Nam B-H, D'Agostino RB. Inference on correlated discrimination mesures in survival analysis: a non parametric apprach. *Statist Theory Methods.* 2004;33:2117-2135.

88. Gronnesby JK, Borgan O. A method for checking regression models in survival analysis based on the risk score. *Lifetime Data Anal.* 1996;2(4):315-328.

89. Pencina MJ, D'Agostino RB, Sr., D'Agostino RB, Jr., Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. *Stat Med.* Jan 30 2008;27(2):157-172; discussion 207-112.

90. Zethelius B, Berglund L, Sundstrom J, et al. Use of multiple biomarkers to improve the prediction of death from cardiovascular causes. *N Engl J Med.* May 15 2008;358(20):2107-2116.

91. Soveri I, Arnlov J, Berglund L, Lind L, Fellstrom B, Sundstrom J. Kidney function and discrimination of cardiovascular risk in middle-aged men. *J Intern Med.* Oct 2009;266(4):406-413.

92. Kubo M, Kiyohara Y, Kato I, et al. Effect of hyperinsulinemia on renal function in a general Japanese population: the Hisayama study. *Kidney Int.* Jun 1999;55(6):2450-2456.

93. Onat A, Hergenc G, Uyarel H, et al. Association between mild renal dysfunction and insulin resistance or metabolic syndrome in a random nondiabetic population sample. *Kidney Blood Press Res.* 2007;30(2):88-96.

94. Fox CS, Larson MG, Leip EP, Meigs JB, Wilson PW, Levy D. Glycemic status and development of kidney disease: the Framingham Heart Study. *Diabetes Care.* Oct 2005;28(10):2436-2440.

95. Pruijm M, Ponte B, Vollenweider P, et al. Not all inflammatory markers are linked to kidney function: results from a population-based study. *Am J Nephrol.* 2012;35(3):288-294.

96. Muntner P, Hamm LL, Kusek JW, Chen J, Whelton PK, He J. The prevalence of nontraditional risk factors for coronary heart disease in patients with chronic kidney disease. *Ann Intern Med.* Jan 6 2004;140(1):9-17.

97. Upadhyay A, Larson MG, Guo CY, et al. Inflammation, kidney function and albuminuria in the Framingham Offspring cohort. *Nephrol Dial Transplant.* Mar 2011;26(3):920-926.

98. Keller C, Katz R, Cushman M, Fried LF, Shlipak M. Association of kidney function with inflammatory and procoagulant markers in a diverse cohort: a cross-sectional analysis from the Multi-Ethnic Study of Atherosclerosis (MESA). *BMC Nephrol.* 2008;9:9.

99. Keller CR, Odden MC, Fried LF, et al. Kidney function and markers of inflammation in elderly persons without chronic kidney disease: the health, aging, and body composition study. *Kidney Int.* Feb 2007;71(3):239-244.

100. Kshirsagar AV, Bomback AS, Bang H, et al. Association of C-reactive protein and microalbuminuria (from the National Health and Nutrition Examination Surveys, 1999 to 2004). *Am J Cardiol.* Feb 1 2008;101(3):401-406.

101. Sabanayagam C, Lee J, Shankar A, Lim SC, Wong TY, Tai ES. C-reactive protein and microalbuminuria in a multi-ethnic Asian population. *Nephrol Dial Transplant.* Apr 2010;25(4):1167-1172.

102. Cottone S, Mule G, Guarneri M, et al. Endothelin-1 and F2-isoprostane relate to and predict renal dysfunction in hypertensive patients. *Nephrol Dial Transplant.* Feb 2009;24(2):497-503.

103. Chade AR, Best PJ, Rodriguez-Porcel M, et al. Endothelin-1 receptor blockade prevents renal injury in experimental hypercholesterolemia. *Kidney Int.* Sep 2003;64(3):962-969.

104. Cheng ZJ, Vaskonen T, Tikkanen I, et al. Endothelial dysfunction and salt-sensitive hypertension in spontaneously diabetic Goto-Kakizaki rats. *Hypertension.* Feb 2001;37(2 Part 2):433-439.

105. Savino A, Pelliccia P, Giannini C, et al. Implications for kidney disease in obese children and adolescents. *Pediatr Nephrol.* May 2011;26(5):749-758.

106. Halliwell B, Lee CY. Using isoprostanes as biomarkers of oxidative stress: some rarely considered issues. *Antioxid Redox Signal.* Jul 15 2010;13(2):145-156.

107. Handelman GJ, Walter MF, Adhikarla R, et al. Elevated plasma F2-isoprostanes in patients on long-term hemodialysis. *Kidney Int.* May 2001;59(5):1960-1966.

108. Himmelfarb J, Stenvinkel P, Ikizler TA, Hakim RM. The elephant in uremia: oxidant stress as a unifying concept of cardiovascular disease in uremia. *Kidney Int.* Nov 2002;62(5):1524-1538.
109. Ikizler TA, Morrow JD, Roberts LJ, et al. Plasma F2-isoprostane levels are elevated in chronic hemodialysis patients. *Clin Nephrol.* Sep 2002;58(3):190-197.
110. Landray MJ, Wheeler DC, Lip GY, et al. Inflammation, endothelial dysfunction, and platelet activation in patients with chronic kidney disease: the chronic renal impairment in Birmingham (CRIB) study. *Am J Kidney Dis.* Feb 2004;43(2):244-253.
111. Khaira A, Mahajan S, Kumar A, et al. Endothelial function and oxidative stress in chronic kidney disease of varying severity and the effect of acute hemodialysis. *Ren Fail.* 2011;33(4):411-417.
112. Passauer J, Pistrosch F, Bussemaker E, Lassig G, Herbrig K, Gross P. Reduced agonist-induced endothelium-dependent vasodilation in uremia is attributable to an impairment of vascular nitric oxide. *J Am Soc Nephrol.* Apr 2005;16(4):959-965.
113. Thambyrajah J, Landray MJ, McGlynn FJ, Jones HJ, Wheeler DC, Townend JN. Abnormalities of endothelial function in patients with predialysis renal failure. *Heart.* Feb 2000;83(2):205-209.
114. Stam F, van Guldener C, Becker A, et al. Endothelial dysfunction contributes to renal function-associated cardiovascular mortality in a population with mild renal insufficiency: the Hoorn study. *J Am Soc Nephrol.* Feb 2006;17(2):537-545.
115. Reffelmann T, Krebs A, Ittermann T, et al. Mild renal dysfunction as a non-traditional cardiovascular risk factor?-Association of cystatin C-based glomerular filtration rate with flow-mediated vasodilation. *Atherosclerosis.* Aug 2010;211(2):660-666.
116. Foster MC, Keyes MJ, Larson MG, et al. Relations of measures of endothelial function and kidney disease: the Framingham Heart Study. *Am J Kidney Dis.* Nov 2008;52(5):859-867.
117. Peralta CA, Jacobs DR, Jr., Katz R, et al. Association of Pulse Pressure, Arterial Elasticity, and Endothelial Function With Kidney Function Decline Among Adults With Estimated GFR >60 mL/min/1.73 m(2): The Multi-Ethnic Study of Atherosclerosis (MESA). *Am J Kidney Dis.* 2012;59(1):41-49.
118. Ix JH, Shlipak MG, Chertow GM, Ali S, Schiller NB, Whooley MA. Cystatin C, left ventricular hypertrophy, and diastolic dysfunction: data from the Heart and Soul Study. *J Card Fail.* Oct 2006;12(8):601-607.
119. Agarwal S, Thohan V, Shlipak MG, et al. Association between Cystatin C and MRI Measures of Left Ventricular Structure and Function: Multi-Ethnic Study of Atherosclerosis. *Int J Nephrol.* 2011;2011:153868.
120. Patel PC, Ayers CR, Murphy SA, et al. Association of cystatin C with left ventricular structure and function: the Dallas Heart Study. *Circ Heart Fail.* Mar 2009;2(2):98-104.

121. Astor BC, Hallan SI, Miller ER, 3rd, Yeung E, Coresh J. Glomerular Filtration Rate, Albuminuria, and Risk of Cardiovascular and All-Cause Mortality in the US Population. *Am J Epidemiol.* Apr 2 2008;2:2.

122. Cirillo M, Lanti MP, Menotti A, et al. Definition of kidney dysfunction as a cardiovascular risk factor: use of urinary albumin excretion and estimated glomerular filtration rate. *Arch Intern Med.* Mar 24 2008;168(6):617-624.

123. Foster MC, Hwang SJ, Larson MG, et al. Cross-classification of microalbuminuria and reduced glomerular filtration rate: associations between cardiovascular disease risk factors and clinical outcomes. *Arch Intern Med.* Jul 9 2007;167(13):1386-1392.

124. Hallan S, Astor B, Romundstad S, Aasarod K, Kvenild K, Coresh J. Association of kidney function and albuminuria with cardiovascular mortality in older vs younger individuals: The HUNT II Study. *Arch Intern Med.* Dec 10 2007;167(22):2490-2496.

125. Hermans MM, Henry R, Dekker JM, et al. Estimated glomerular filtration rate and urinary albumin excretion are independently associated with greater arterial stiffness: the Hoorn Study. *J Am Soc Nephrol.* Jun 2007;18(6):1942-1952.

126. de Boer IH, Katz R, Cao JJ, et al. Cystatin C, albuminuria, and mortality among older adults with diabetes mellitus. *Diabetes Care.* Jul 8 2009.

127. van der Velde M, Matsushita K, Coresh J, et al. Lower estimated glomerular filtration rate and higher albuminuria are associated with all-cause and cardiovascular mortality. A collaborative meta-analysis of high-risk population cohorts. *Kidney Int.* Jun 2011;79(12):1341-1352.

128. Matsushita K, van der Velde M, Astor BC, et al. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. *Lancet.* Jun 12 2010;375(9731):2073-2081.

129. Waheed S, Matsushita K, Sang Y, et al. Combined association of albuminuria and cystatin C-based estimated GFR with mortality, coronary heart disease, and heart failure outcomes: the Atherosclerosis Risk in Communities (ARIC) Study. *Am J Kidney Dis.* Aug 2012;60(2):207-216.

130. Hlatky MA, Greenland P, Arnett DK, et al. Criteria for evaluation of novel markers of cardiovascular risk: a scientific statement from the American Heart Association. *Circulation.* May 5 2009;119(17):2408-2416.

131. Gu DF, Shi YL, Chen YM, et al. Prevalence of chronic kidney disease and prediabetes and associated risk factors: a community-based screening in Zhuhai, Southern China. *Chin Med J (Engl).* Apr 2013;126(7):1213-1219.

132. Shinohara K, Shoji T, Emoto M, et al. Insulin resistance as an independent predictor of cardiovascular mortality in patients with end-stage renal disease. *J Am Soc Nephrol.* Jul 2002;13(7):1894-1900.

133. Wei Y, Chen K, Whaley-Connell AT, Stump CS, Ibdah JA, Sowers JR. Skeletal muscle insulin resistance: role of inflammatory cytokines and reactive oxygen species. *Am J Physiol Regul Integr Comp Physiol.* Mar 2008;294(3):R673-680.

134. Wang G, Liu Y, Wang A, Tong W, Zhang Y. Biomarkers of inflammation, endothelial dysfunction and insulin resistance in adults of Inner Mongolia, China. *Diabetes Metab Res Rev.* Sep 2010;26(6):490-495.

135. Ceriello A. Oxidative stress and glycemic regulation. *Metabolism.* Feb 2000;49(2 Suppl 1):27-29.

136. Ogihara T, Asano T, Katagiri H, et al. Oxidative stress induces insulin resistance by activating the nuclear factor-kappa B pathway and disrupting normal subcellular distribution of phosphatidylinositol 3-kinase. *Diabetologia.* May 2004;47(5):794-805.

137. Pradhan AD, Cook NR, Buring JE, Manson JE, Ridker PM. C-reactive protein is independently associated with fasting insulin in nondiabetic women. *Arterioscler Thromb Vasc Biol.* Apr 1 2003;23(4):650-655.

138. Chen SJ, Yen CH, Huang YC, Lee BJ, Hsia S, Lin PT. Relationships between inflammation, adiponectin, and oxidative stress in metabolic syndrome. *PLoS One.* 2012;7(9):e45693.

139. Roebuck KA. Oxidant stress regulation of IL-8 and ICAM-1 gene expression: differential activation and binding of the transcription factors AP-1 and NF-kappaB (Review). *Int J Mol Med.* Sep 1999;4(3):223-230.

140. Ceriello A, Motz E. Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. *Arterioscler Thromb Vasc Biol.* May 2004;24(5):816-823.

141. Esposito K, Nappo F, Marfella R, et al. Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: role of oxidative stress. *Circulation.* Oct 15 2002;106(16):2067-2072.

142. Tripathy D, Mohanty P, Dhindsa S, et al. Elevation of free fatty acids induces inflammation and impairs vascular reactivity in healthy subjects. *Diabetes.* Dec 2003;52(12):2882-2887.

143. Banerjee D, Recio-Mayoral A, Chitalia N, Kaski JC. Insulin resistance, inflammation, and vascular disease in nondiabetic predialysis chronic kidney disease patients. *Clin Cardiol.* Jun 2011;34(6):360-365.

144. Costa-Hong V, Bortolotto LA, Jorgetti V, Consolim-Colombo F, Krieger EM, Lima JJ. Oxidative stress and endothelial dysfunction

in chronic kidney disease. *Arq Bras Cardiol.* May 2009;92(5):381-386, 398-403, 413-388.

145. Locatelli F, Canaud B, Eckardt KU, Stenvinkel P, Wanner C, Zoccali C. Oxidative stress in end-stage renal disease: an emerging threat to patient outcome. *Nephrol Dial Transplant.* Jul 2003;18(7):1272-1280.

146. Gray S, Kim JK. New insights into insulin resistance in the diabetic heart. *Trends Endocrinol Metab.* Oct 2011;22(10):394-403.

147. Lastra G, Habibi J, Whaley-Connell AT, et al. Direct renin inhibition improves systemic insulin resistance and skeletal muscle glucose transport in a transgenic rodent model of tissue renin overexpression. *Endocrinology.* Jun 2009;150(6):2561-2568.

148. Whaley-Connell AT, Chowdhury NA, Hayden MR, et al. Oxidative stress and glomerular filtration barrier injury: role of the renin-angiotensin system in the Ren2 transgenic rat. *Am J Physiol Renal Physiol.* Dec 2006;291(6):F1308-1314.

149. Suzuki K, Nakagawa O, Aizawa Y. Improved early-phase insulin response after candesartan treatment in hypertensive patients with impaired glucose tolerance. *Clin Exp Hypertens.* Jul 2008;30(5):309-314.

150. Townsend RR, Zhao H. Plasma renin activity and insulin sensitivity in normotensive subjects. *Am J Hypertens.* Oct 1994;7(10 Pt 1):894-898.

151. Henriksen EJ, Jacob S, Kinnick TR, Teachey MK, Krekler M. Selective angiotensin II receptor antagonism reduces insulin resistance in obese Zucker rats. *Hypertension.* Oct 2001;38(4):884-890.

152. Reaven GM. The insulin resistance syndrome: definition and dietary approaches to treatment. *Annu Rev Nutr.* 2005;25:391-406.

153. Navaneethan SD, Yehnert H, Moustarah F, Schreiber MJ, Schauer PR, Beddhu S. Weight loss interventions in chronic kidney disease: a systematic review and meta-analysis. *Clin J Am Soc Nephrol.* Oct 2009;4(10):1565-1574.

154. Wang YL, Shu KH, Yang MF, et al. The Impact of Body Weight Management in Chronic Kidney Disease Patients With Obesity. *J Ren Nutr.* Jun 18 2013.

155. Corpeleijn E, Bakker SJ, Stolk RP. Obesity and impaired renal function: potential for lifestyle intervention? *Eur J Epidemiol.* 2009;24(6):275-280.

156. Straznicky NE, Grima MT, Lambert EA, et al. Exercise augments weight loss induced improvement in renal function in obese metabolic syndrome individuals. *J Hypertens.* Mar 2011;29(3):553-564.

157. Sayed AA, Khalifa M, Abd el-Latif FF. Fenugreek attenuation of diabetic nephropathy in alloxan-diabetic rats: attenuation of diabetic nephropathy in rats. *J Physiol Biochem.* Jun 2012;68(2):263-269.

158. Ishibashi Y, Matsui T, Takeuchi M, Yamagishi S. Metformin inhibits advanced glycation end products (AGEs)-induced renal tubular cell injury by suppressing reactive oxygen species generation via reducing receptor for AGEs (RAGE) expression. *Horm Metab Res.* Nov 2012;44(12):891-895.

159. Scheen AJ. Clinical pharmacokinetics of metformin. *Clin Pharmacokinet.* May 1996;30(5):359-371.

160. Lalau JD, Vermersch A, Hary L, Andrejak M, Isnard F, Quichaud J. Type 2 diabetes in the elderly: an assessment of metformin (metformin in the elderly). *Int J Clin Pharmacol Ther Toxicol.* Aug 1990;28(8):329-332.

161. Dagenais GR, Gerstein HC, Holman R, et al. Effects of ramipril and rosiglitazone on cardiovascular and renal outcomes in people with impaired glucose tolerance or impaired fasting glucose: results of the Diabetes REduction Assessment with ramipril and rosiglitazone Medication (DREAM) trial. *Diabetes Care.* May 2008;31(5):1007-1014.

162. Sarafidis PA, Bakris GL. Protection of the kidney by thiazolidinediones: an assessment from bench to bedside. *Kidney Int.* Oct 2006;70(7):1223-1233.

163. Taguchi K, Okada A, Yasui T, et al. Pioglitazone, a peroxisome proliferator activated receptor gamma agonist, decreases renal crystal deposition, oxidative stress and inflammation in hyperoxaluric rats. *J Urol.* Sep 2012;188(3):1002-1011.

164. Yamagishi T, Saito Y, Nakamura T, et al. Troglitazone improves endothelial function and augments renal klotho mRNA expression in Otsuka Long-Evans Tokushima Fatty (OLETF) rats with multiple atherogenic risk factors. *Hypertens Res.* Nov 2001;24(6):705-709.

165. Ramasamy R, Vannucci SJ, Yan SS, Herold K, Yan SF, Schmidt AM. Advanced glycation end products and RAGE: a common thread in aging, diabetes, neurodegeneration, and inflammation. *Glycobiology.* Jul 2005;15(7):16R-28R.

166. Yan HD, Li XZ, Xie JM, Li M. Effects of advanced glycation end products on renal fibrosis and oxidative stress in cultured NRK-49F cells. *Chin Med J (Engl).* May 5 2007;120(9):787-793.

167. Cai W, Ramdas M, Zhu L, Chen X, Striker GE, Vlassara H. Oral advanced glycation endproducts (AGEs) promote insulin resistance and diabetes by depleting the antioxidant defenses AGE receptor-1 and sirtuin 1. *Proc Natl Acad Sci U S A.* Sep 25 2012;109(39):15888-15893.

168. Ishibashi Y, Yamagishi S, Matsui T, et al. Pravastatin inhibits advanced glycation end products (AGEs)-induced proximal tubular cell apoptosis and injury by reducing receptor for AGEs (RAGE) level. *Metabolism.* Aug 2012;61(8):1067-1072.

169. Nakamura T, Sato E, Fujiwara N, et al. Calcium channel blocker inhibition of AGE and RAGE axis limits renal injury in nondiabetic

patients with stage I or II chronic kidney disease. *Clin Cardiol.* Jun 2011;34(6):372-377.

170. Vlassara H, Uribarri J, Cai W, et al. Effects of sevelamer on HbA1c, inflammation, and advanced glycation end products in diabetic kidney disease. *Clin J Am Soc Nephrol.* Jun 2012;7(6):934-942.

171. Matsui T, Nishino Y, Maeda S, Takeuchi M, Yamagishi S. Irbesartan inhibits advanced glycation end product (AGE)-induced up-regulation of vascular cell adhesion molecule-1 (VCAM-1) mRNA levels in glomerular endothelial cells. *Microvasc Res.* May 2011;81(3):269-273.

172. Patel K, Diamantidis C, Zhan M, et al. Influence of creatinine versus glomerular filtration rate on non-steroidal anti-inflammatory drug prescriptions in chronic kidney disease. *Am J Nephrol.* 2012;36(1):19-26.

173. Sturmer T, Erb A, Keller F, Gunther KP, Brenner H. Determinants of impaired renal function with use of nonsteroidal anti-inflammatory drugs: the importance of half-life and other medications. *Am J Med.* Nov 2001;111(7):521-527.

174. Winkelmayer WC, Waikar SS, Mogun H, Solomon DH. Nonselective and cyclooxygenase-2-selective NSAIDs and acute kidney injury. *Am J Med.* Dec 2008;121(12):1092-1098.

175. Clive DM, Stoff JS. Renal syndromes associated with nonsteroidal antiinflammatory drugs. *N Engl J Med.* Mar 1 1984;310(9):563-572.

176. Strand V, Hochberg MC. The risk of cardiovascular thrombotic events with selective cyclooxygenase-2 inhibitors. *Arthritis Rheum.* Aug 2002;47(4):349-355.

177. Fitzgerald GA. Coxibs and cardiovascular disease. *N Engl J Med.* Oct 21 2004;351(17):1709-1711.

178. Devaraj S, Jialal I. Alpha tocopherol supplementation decreases serum C-reactive protein and monocyte interleukin-6 levels in normal volunteers and type 2 diabetic patients. *Free Radic Biol Med.* Oct 15 2000;29(8):790-792.

179. Devaraj S, Tang R, Adams-Huet B, et al. Effect of high-dose alpha-tocopherol supplementation on biomarkers of oxidative stress and inflammation and carotid atherosclerosis in patients with coronary artery disease. *Am J Clin Nutr.* Nov 2007;86(5):1392-1398.

180. Boaz M, Smetana S, Weinstein T, et al. Secondary prevention with antioxidants of cardiovascular disease in endstage renal disease (SPACE): randomised placebo-controlled trial. *Lancet.* Oct 7 2000;356(9237):1213-1218.

181. Galli F, Varga Z, Balla J, et al. Vitamin E, lipid profile, and peroxidation in hemodialysis patients. *Kidney Int Suppl.* Feb 2001;78:S148-154.

182. Skyrme-Jones RA, O'Brien RC, Berry KL, Meredith IT. Vitamin E supplementation improves endothelial function in type I diabetes mellitus: a randomized, placebo-controlled study. *J Am Coll Cardiol.* Jul 2000;36(1):94-102.

183. Plantinga Y, Ghiadoni L, Magagna A, et al. Supplementation with vitamins C and E improves arterial stiffness and endothelial function in essential hypertensive patients. *Am J Hypertens.* Apr 2007;20(4):392-397.
184. McKechnie R, Rubenfire M, Mosca L. Antioxidant nutrient supplementation and brachial reactivity in patients with coronary artery disease. *J Lab Clin Med.* Mar 2002;139(3):133-139.
185. Kinlay S, Behrendt D, Fang JC, et al. Long-term effect of combined vitamins E and C on coronary and peripheral endothelial function. *J Am Coll Cardiol.* Feb 18 2004;43(4):629-634.
186. Mann JF, Lonn EM, Yi Q, et al. Effects of vitamin E on cardiovascular outcomes in people with mild-to-moderate renal insufficiency: results of the HOPE study. *Kidney Int.* Apr 2004;65(4):1375-1380.
187. Mason JC, Ahmed Z, Mankoff R, et al. Statin-induced expression of decay-accelerating factor protects vascular endothelium against complement-mediated injury. *Circ Res.* Oct 18 2002;91(8):696-703.
188. Kakuda H, Kanasaki K, Koya D, Takekoshi N. The administration of pitavastatin augments creatinine clearance associated with reduction in oxidative stress parameters: acute and early effects. *Clin Exp Nephrol.* Apr 2013;17(2):240-247.
189. Pruefer D, Scalia R, Lefer AM. Simvastatin inhibits leukocyte-endothelial cell interactions and protects against inflammatory processes in normocholesterolemic rats. *Arterioscler Thromb Vasc Biol.* Dec 1999;19(12):2894-2900.
190. Tonelli M, Sacks F, Pfeffer M, Jhangri GS, Curhan G. Biomarkers of inflammation and progression of chronic kidney disease. *Kidney Int.* Jul 2005;68(1):237-245.
191. Sarnak MJ, Poindexter A, Wang SR, et al. Serum C-reactive protein and leptin as predictors of kidney disease progression in the Modification of Diet in Renal Disease Study. *Kidney Int.* Dec 2002;62(6):2208-2215.
192. Di Lullo L, Addesse R, Comegna C, et al. Effects of fluvastatin treatment on lipid profile, C-reactive protein trend, and renal function in dyslipidemic patients with chronic renal failure. *Adv Ther.* Nov-Dec 2005;22(6):601-612.
193. Sawara Y, Takei T, Uchida K, et al. Effects of lipid-lowering therapy with rosuvastatin on atherosclerotic burden in patients with chronic kidney disease. *Intern Med.* 2008;47(17):1505-1510.
194. Gyebi L, Soltani Z, Reisin E. Lipid nephrotoxicity: new concept for an old disease. *Curr Hypertens Rep.* Apr 2012;14(2):177-181.
195. K/DOQI clinical practice guidelines for management of dyslipidemias in patients with kidney disease. *Am J Kidney Dis.* Apr 2003;41(4 Suppl 3):I-IV, S1-91.
196. Fried LF, Orchard TJ, Kasiske BL. Effect of lipid reduction on the progression of renal disease: a meta-analysis. *Kidney Int.* Jan 2001;59(1):260-269.

197. Sharp Collaborative G. Study of Heart and Renal Protection (SHARP): randomized trial to assess the effects of lowering low-density lipoprotein cholesterol among 9,438 patients with chronic kidney disease. *Am Heart J.* Nov 2010;160(5):785-794 e710.

198. Barylski M, Nikfar S, Mikhailidis DP, et al. Statins decrease all-cause mortality only in CKD patients not requiring dialysis therapy--a meta-analysis of 11 randomized controlled trials involving 21,295 participants. *Pharmacol Res.* Jun 2013;72:35-44.

199. Ceconi C, Fox KM, Remme WJ, et al. ACE inhibition with perindopril and endothelial function. Results of a substudy of the EUROPA study: PERTINENT. *Cardiovasc Res.* Jan 1 2007;73(1):237-246.

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