UiT
THE ARCTIC
UNIVERSITY
OF NORWAY

Faculty of Health Sciences
Institute of Clinical Medicine

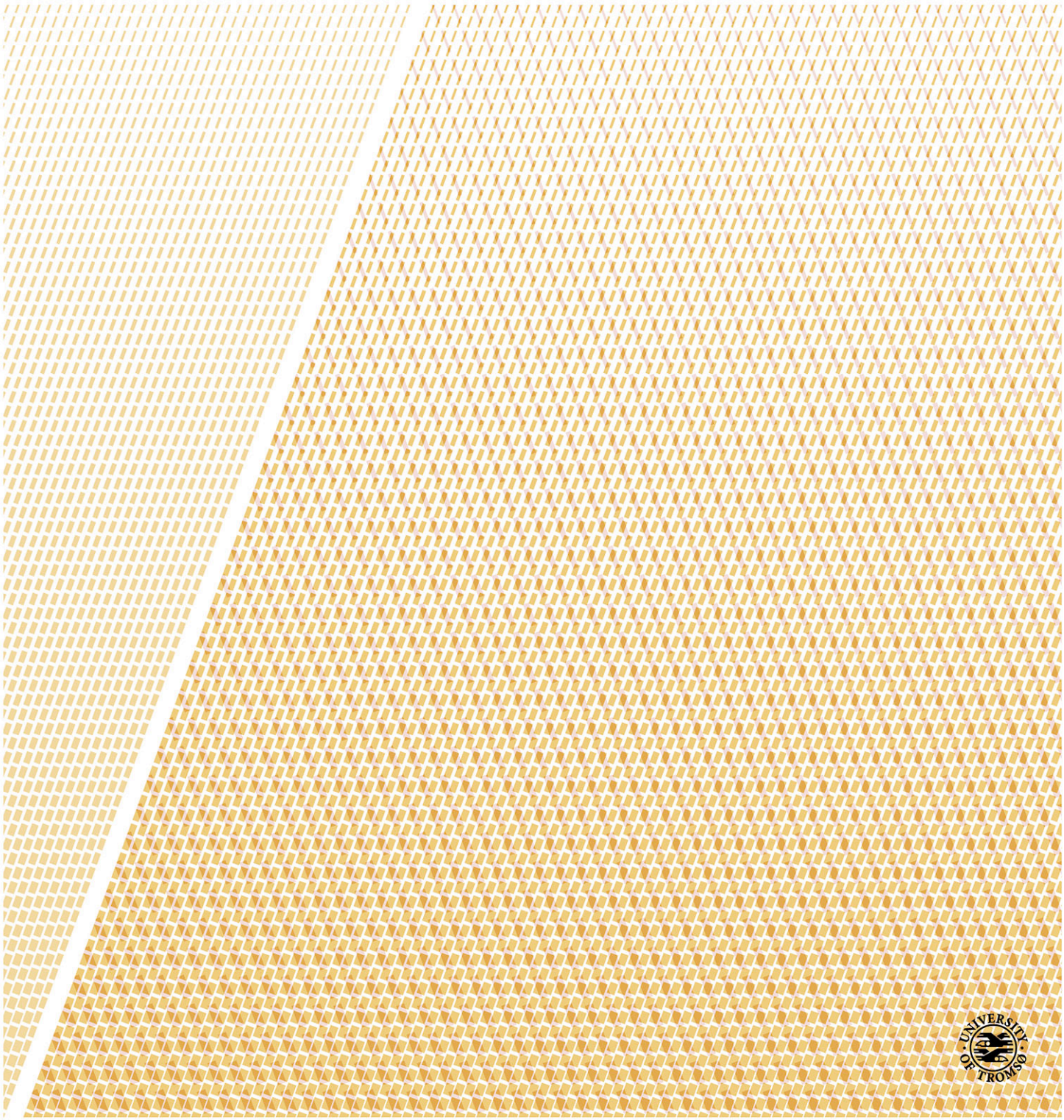
Oxidative stress and inflammation as risk factors for accelerated age-related GFR decline and albuminuria in the general population

The Renal Iohexol Clearance Survey Follow-Up Study

—

Jørgen Schei

A dissertation for the degree of Philosophiae Doctor – June 2018



CONTENTS

LIST OF FIGURES

ACKNOWLEDGMENTS

SUMMARY

The prevalence of chronic kidney disease (CKD) increases rapidly with age, affecting more than one-third of people over 70 years old. The age-related loss of kidney function, assessed by the glomerular filtration rate (GFR), is an important risk factor for CKD and kidney failure. However, there is a large variation in age-related GFR decline between individuals that cannot be fully explained by traditional CKD risk factors, such as diabetes, obesity, and hypertension. Identifying novel risk factors for early kidney disease, manifested as accelerated GFR decline or low-grade albuminuria, may suggest underlying pathologic mechanisms for the development of CKD and prompt the opportunity for early-targeted treatment.

Low-grade inflammation and oxidative stress are linked to aging and age-related chronic diseases and may represent key processes in CKD development. In particular, soluble TNF receptors and urinary markers of oxidatively damaged nucleic acids have predicted GFR decline and albuminuria in people with diabetes. However, it remains unclear whether these biomarkers predict an accelerated age-related GFR decline or albuminuria in the general population without CKD and diabetes. The few previous studies from the general population have limitations, most importantly, the use of estimated GFR (eGFR), which is inaccurate in the near-normal range of GFR and may be biased by non-GFR-related factors, such as inflammation, obesity and muscle wasting.

In the Renal Iohexol Clearance Survey in Tromsø 6 (RENIS-T6), we measured the GFR (mGFR) in 1627 middle-aged people without self-reported cardiovascular disease, diabetes, or kidney disease. After a median follow-up of 5.6 years, 1324 participants (81%) underwent the same GFR measurements in the RENIS Follow-Up (RENIS-FU) study. In cross-sectional analyses from RENIS-T6, we found that the eGFR values based on creatinine and cystatin C were associated with inflammatory biomarkers independent of mGFR, indicating a non-GFR-related influence of inflammation on eGFR. In longitudinal analyses, we found that a higher baseline serum level of high-sensitivity C-reactive protein, but not of TNF receptor 2, was associated with an accelerated age-related mGFR decline and an increased risk of incident CKD. Unexpectedly, higher baseline soluble TNF receptor type 2 was associated with a non-linear, slower age-related mGFR decline. Markers of oxidative stress, measured as the urinary excretion of oxidatively damaged DNA and RNA (8-oxodG and 8-oxoGuo), were not significantly associated with the age-related mGFR decline, but higher urinary excretion of 8-oxoGuo predicted low-grade albuminuria at follow-up. Studies with an even longer observation period, multiple biomarkers, and repeated GFR measurements are needed to fully evaluate the effects of low-grade inflammation and oxidative stress on age-related GFR decline in the general population.

LIST OF PRESENTED PAPERS

Paper 1

Schei J, Stefansson VTN, Mathisen UD, Eriksen BO, Solbu MD, Jenssen TG, Melsom T: Residual Associations of Inflammatory Markers with eGFR after Accounting for Measured GFR in a Community-Based Cohort without CKD. *Clinical journal of the American Society of Nephrology : CJASN.* 2016;11:280-286.

Paper 2

Schei J, Stefansson VTN, Eriksen BO, Jenssen TG, Solbu MD, Wilsgaard T, Melsom T: Association of TNF receptor 2 and CRP with GFR Decline in the General Nondiabetic Population. *Clinical journal of the American Society of Nephrology : CJASN.* 2017;12:624-634.

Paper 3

Schei J, Fuskevåg OM, Stefansson VTN, Solbu MD, Jenssen TG, Eriksen BO, Melsom T: Urinary Markers of Oxidative Stress Are Associated With Albuminuria but Not GFR decline. *Kidney International Reports* 2017

ABBREVIATIONS

ACEi	angiotensin-converting-enzyme inhibitor
ARB	angiotensin receptor blocker
BMI	body mass index
CKD	chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CV	coefficient of variation
CVD	cardiovascular disease
eGFR	estimated glomerular filtration rate
eGFRcre	estimated glomerular filtration rate based on creatinine
eGFRcys	estimated glomerular filtration rate based on cystatin C
eGFRcrecys	estimated glomerular filtration rate based on creatinine and cystatin C
ESRD	end-stage renal disease
GFR	glomerular filtration rate
HDL	high-density lipoprotein
hs-CRP	high-sensitive C-reactive protein
LDL	low-density lipoprotein
LQ-MS/MS	liquid chromatography-tandem mass spectrometry
mGFR	measured glomerular filtration rate
NF- κB	nuclear factor-κB
Nrf2	nuclear factor erythroid 2-related factor 2
NSAIDs	non-steroidal anti-inflammatory drugs
RENIS-T6	Renal Iohexol Clearance Survey in the Tromsø 6 study
RENIS-FU	Renal Iohexol Clearance Survey Follow-up study
TNFR1	soluble tumor necrosis factor receptor type 2
TNFR2	soluble tumor necrosis factor receptor type 2
UACR	urine albumin-creatinine ratio
8-oxodG	8-oxo-7,8-dihydro-2`-deoxyguanosine
8-oxoGuo	8-oxo-7,8-dihydroguanosine
8-oxodG^{UCR}	8-oxo-7,8-dihydro-2`-deoxyguanosine divided on urinary creatinine
8-oxoGuo^{UCR}	8-oxo-7,8-dihydroguanosine divided on urinary creatinine

1 INTRODUCTION

Loss of kidney function, assessed as the glomerular filtration rate (GFR), is a prominent feature of aging and an important cause of the high prevalence of chronic kidney disease (CKD) and end-stage renal disease (ESRD) in old age.[1, 2] CKD is an independent risk factor for ESRD, cardiovascular disease (CVD), and all-cause mortality.[3] Approximately 10% of the world's population have CKD, with an increasing prevalence partly due to an aging population.[1, 4] The mean rate of GFR decline has been reported to be 0.75-1.00 ml/min/1.73 m^2 per year in middle-aged persons.[5, 6]

The magnitude of the age-related GFR decline is based on a few studies using the estimated GFR (eGFR), which may have introduced uncertain estimates due to the limited accuracy of the eGFR equations. Although there is limited evidence, most previous studies found large inter-individual variations in the GFR decline rates. Some individuals have a low and steady GFR decline and maintain their kidney function in old age, while the GFR in others declines more rapidly and leads to CKD. Persons with a rapid GFR decline (>3 ml/min/year) have a higher risk of CVD and premature mortality, regardless of age and other cardiovascular risk factors.[7, 8] However, the underlying mechanisms for the variation in age-related GFR decline between individuals is unclear and only partly explained by traditional risk factors, such as diabetes, obesity and hypertension. Identifying novel risk factors for an accelerated GFR decline may suggest underlying pathological mechanisms and yield the possibility of an early and targeted treatment for the prevention of CKD.[9]

Inflammation and oxidative stress play important roles in aging and have been associated with several age-related chronic diseases, including CVD and CKD.[10-14] However, most previous studies on the association between inflammation, oxidative stress and kidney dysfunction have been cross-sectional and investigated in populations with established CKD or diabetes. In addition, these studies have been limited by the use of estimates of the GFR based on serum creatinine or cystatin C, which lack precision in the near-normal range of GFR. Importantly, eGFR may also be biased by factors that are not related to kidney function. Previous studies have found that traditional cardiovascular risk factors influence eGFR along non-GFR related pathways,[15, 16] but whether low-grade inflammation influence creatinine- or cystatin C-based eGFR needs to be established. A non-GFR-related association between inflammation and eGFR may create bias in longitudinal studies that investigate the relationship between eGFR and different outcomes, such as eGFR change rates, CVD and mortality.

The RENIS-FU study was designed to examine risk factors associated with age-related GFR decline using the measured GFR (mGFR) in a cohort representative of the general population. In the present

thesis, we examined the possible non-GFR-related influence of inflammatory markers on eGFR using mGFR as the gold standard for the true GFR. We also investigated the association of inflammatory and oxidative stress biomarkers with age-related GFR decline and with the development of increased urinary albumin excretion during 5.6 years of follow-up.

2 BACKGROUND

2.1 KIDNEY FUNCTION

The kidney is essential in several functions necessary for life, such as regulation of electrolytes, fluids and blood pressure; filtration and excretion of waste products from metabolism; and maintenance of acid-base homeostasis in the blood. The functional unit of the kidney is the nephron, where filtration, reabsorption, and secretion occur. The total number of nephrons in each kidney varies widely in healthy persons, from approximately 200,000 to 2.5 million.[17] The GFR is considered the best overall measure of kidney function.

2.1.1 Glomerular filtration rate

The GFR equals the total volume of fluid filtered through all of the functional nephrons per unit of time (ml/min) and is determined by the oncotic and hydrostatic pressure gradients between the capillaries and Bowman`s capsule, the surface area, and the hydraulic conductivity of the glomerular membranes. Because GFR varies with body size, it is usually corrected for body surface area and is expressed as ml/min/ 1.73 m^2. The normal GFR range varies, with a mean of approximately 130 ml/min/1.73 m^2 in young men and 120 ml/min/1.73 m^2 in young women (Figure 2).[6]

2.1.2 The measurement of GFR by exogenous filtration markers

The ideal filtration marker for measuring GFR is inert, freely filtered by the glomerulus and not secreted, metabolized, synthesized, or reabsorbed in the renal tubules or in any other organ. The urinary or plasma clearance of an ideal filtration marker is identical to the true GFR by definition. Inulin is an invert, uncharged polymer of fructose with a molecular weight of 5200 D and is the only known ideal filtration marker. Inulin clearance is considered the gold standard for measuring GFR.[18] However, the method is time consuming, complicated and expensive, making it unfeasible for clinical practice and epidemiological studies.[18] Thus, other exogenous markers, such as iothalamate, ^{51}Chrom-EDTA, and iohexol, are more often used. Iohexol is a non-radioactive contrast agent that can be measured as either urinary or plasma clearance. Urinary clearance is cumbersome and susceptible to measurement error due to the collection of urine. Thus, plasma clearance is more convenient and precise than urinary clearance.[18] Plasma clearance of iohexol can be measured with a multi-sample or single-sample method. The single-sample method has correlated well with the multi-sample method and with inulin and ^{51}Chrom-EDTA clearance.[18-20] Advantages of iohexol clearance include low costs and sensitive high-performance liquid chromatography (HPLC) assays for low doses, with rare adverse reactions. Limitations include possible tubular reabsorption or protein binding, contraindication in patients with allergies against iodine, and nephrotoxicity and risk for allergic

reactions at high doses.[18] A recent review has suggested plasma clearance of iohexol as a favorable marker for implementing a standardized GFR measurement protocol.[21, 22]

2.1.3 Estimated GFR by endogenous filtration markers

All methods to measure GFR are relatively time-consuming and expensive. Thus, endogenous filtration markers are widely used to estimate GFR in clinical practice and in epidemiologic research. The two most commonly used filtration markers are serum creatinine and cystatin C. Creatinine is a breakdown product from creatine phosphate, has a molecular mass of 113 D, is freely filtered by the glomerulus and, to a lesser extent, is secreted by the tubular cells. The rate of creatinine production depends primarily on muscle mass and diet. Therefore, estimating equations of GFR incorporate age and gender and correct for African-American ethnicity to minimize the non-GFR-related effects of muscle mass and thus improve GFR estimation. The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was developed in both CKD patients and healthy people.[23] Compared to the Modification of Diet in Renal Disease (MDRD) equation, which was developed in a CKD cohort, the CKD-EPI equation has performed better in the normal range of GFR.[24-26] International guidelines have recommended the use of the CKD-EPI equation to estimate GFR.[27] However, other estimating equations using serum creatinine have been developed[28-30] that may be more accurate than the CKD-EPI equation in the elderly[28] and across the full age-spectrum of the general population.[29]

Cystatin C is a non-glycosylated protein with a molecular mass of 13 kD that is freely filtered by the glomerulus and nearly completely absorbed and metabolized by the tubules. Since cystatin C is not affected by muscle mass and has improved the correlation with cardiovascular risk compared to creatinine, it was introduced as a promising filtration marker. Thus, several eGFR equations using serum cystatin C have been proposed.[31] However, the ability of cystatin C to estimate GFR among populations varies, and there have been considerable method-specific differences in cystatin C measurements.[32]

2.1.4 Non-GFR-related factors

Endogenous filtration markers may be influenced by factors other than GFR, referred to as non-GFR-determinants or non-GFR-related factors. By far, the most described example is muscle mass, which influences the serum creatinine level. Thus, an estimation of GFR by creatinine-based equations can be inaccurate in individuals with decreased or increased muscle mass, e.g., elderly individuals with chronic diseases, patients with paralysis or amputations, and body-builders.[18] Cystatin C is not affected by muscle mass but has been associated with other non-GFR-related factors, particularly cardiovascular risk factors, such as obesity and smoking.[15, 33, 34] Some studies have suggested that

cystatin C is also influenced by inflammation,[16, 34] while others have not.[35] Whether eGFR is influenced by inflammation through non-GFR-related pathways or not is important to resolve in order to interpret the results of studies assessing the relationship between inflammation and eGFR. Non-GFR-related factors may also confound the association between eGFR and cardiovascular risk found in epidemiologic studies, as outlined below.

2.2 CHRONIC KIDNEY DISEASE

CKD is defined as decreased kidney function (i.e., GFR < 60 ml/min/1.73 m^2) and/or the presence of kidney damage (i.e., albuminuria) for 3 months or more, irrespective of clinical diagnosis. The prevalence of CKD is estimated to be 8-14% in high-income countries, including Norway, Europe in general, and the USA.[4, 36, 37] In the elderly, CKD prevalence is particularly high, affecting more than one-third of people over 70 years.[1] CKD is classified according to the level of GFR and albuminuria (Figure 1). The CKD stages are progressively associated with increased risk for CVD, ESRD, and all-cause mortality.[27, 38, 39] CKD stage G5 is associated with reduced quality of life, poor outcomes, and high healthcare costs and necessitates dialysis or kidney transplantation in many patients. Whether mildly reduced GFR without albuminuria, i.e., GFR between 45 and 60 ml/min/1.73 m^2 (CKD stage G3aA1), is an independent risk factor for CVD and mortality has been debated, particularly due to the imprecise estimation of GFR and the difference in risk prediction by eGFR based on cystatin C and creatinine.[3]

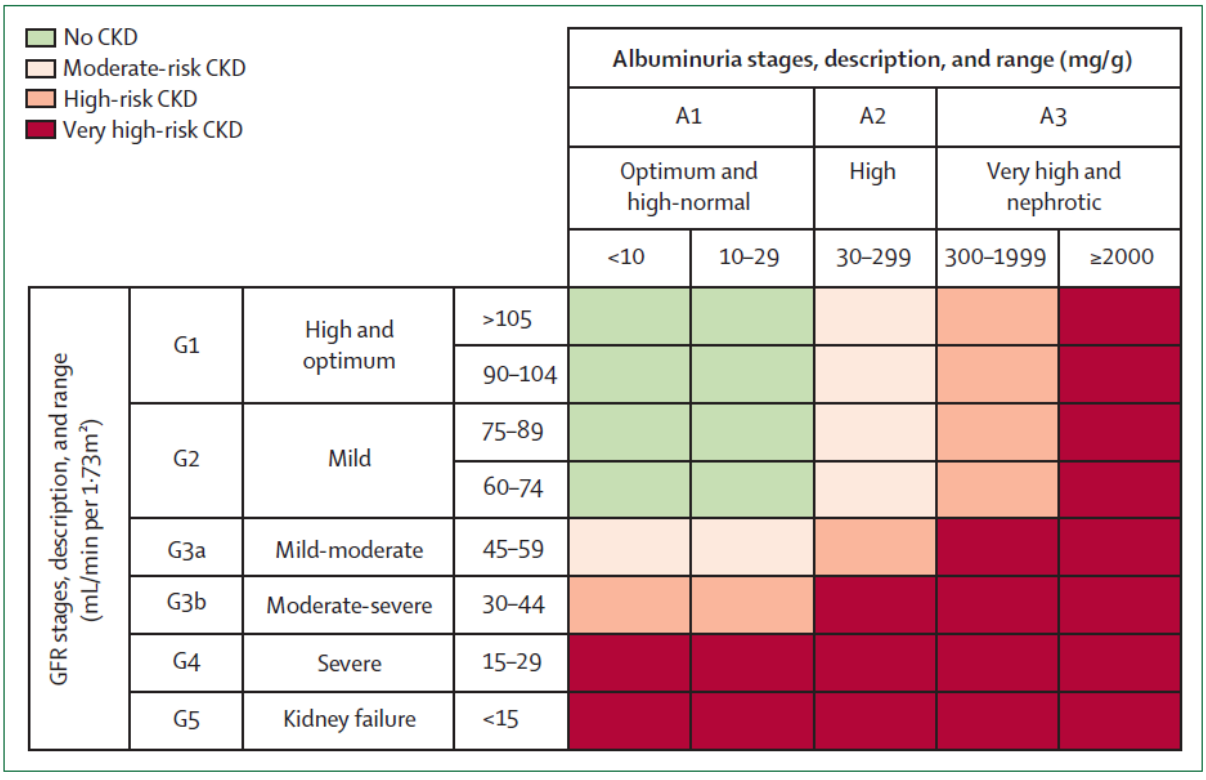


Figure 1. Classification of CKD according to KDIGO guidelines. Adapted from Levey A., Lancet 2012. [40]

2.2.1 The age-related GFR decline

Loss of kidney function is an indisputable feature of aging and a considerable contributor to the high prevalence of CKD in old age.[2] Cross-sectional studies using mGFR have demonstrated a strong correlation between age and GFR, even in healthy individuals (Figure 2). Kidney biopsies taken from healthy kidney donors at different ages have revealed fibrosis, tubular atrophy and loss of nephrons as hallmarks of renal aging.[41] Thus, a gradual decline in GFR may be a normal aging process rather than a disease state. However, the independent association between reduced GFR and mortality across all age groups is considered an argument for moderate CKD as a state of disease.[42] The risk factors for accelerated age-related GFR decline are not settled mainly because there are few longitudinal studies of age-related GFR decline from the general population. Moreover, all previous longitudinal studies were based on eGFR or creatinine clearance. Data from these studies indicate that the GFR declines with a mean annual rate of approximately 0.75-1.00 ml/min/1.73 m^2 in middle-aged persons but that the decline rate varies considerably between individuals .[5, 6]

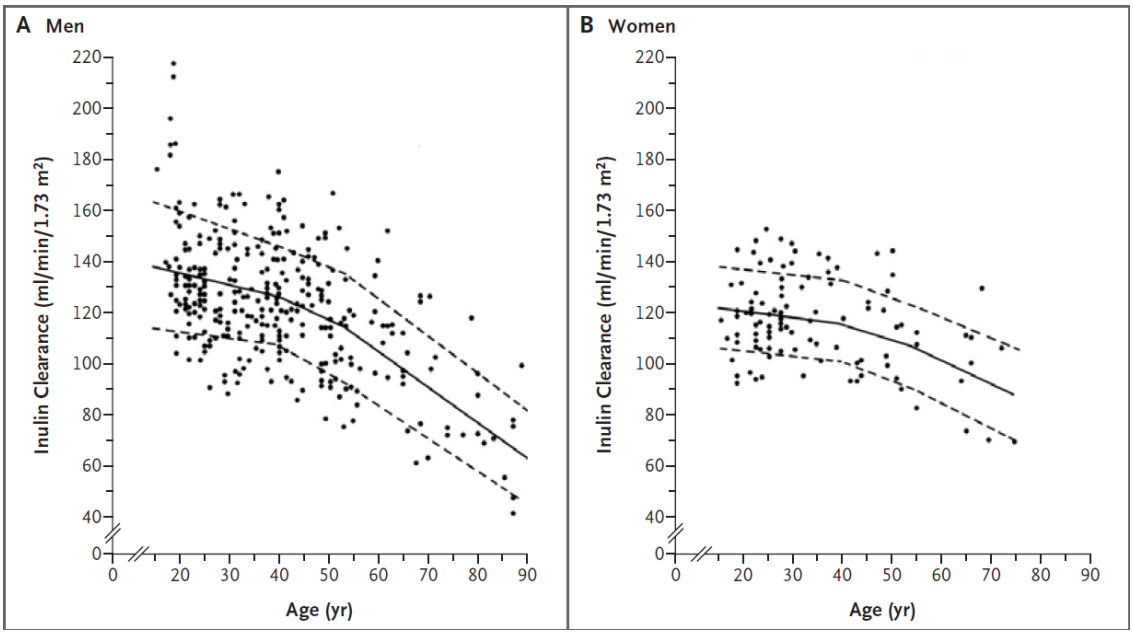


Figure 2. GFR measured by inulin clearance in men and women of different ages. Adapted from Wesson[43], reprinted in NEJM 354;23 2006[6]

2.2.2 Albuminuria

Albuminuria is a marker of kidney damage resulting from a dysfunctional glomerular filtration barrier and/or reduced proximal tubular reabsorption.[44] The gold standard for measuring urinary albumin excretion is 24-hour urine sampling. However, because this method is time consuming and prone to

sampling error, the spot urine albumin-creatinine ratio (UACR) is the recommended method to assess albuminuria.[27] To avoid variability, a first morning sample is preferred.[45] An increased UACR (> 3 mg/mmol) lasting for more than three months is an independent criterion for the definition of CKD.[27] A UACR ≥ 3 mg/mmol (30 mg/g) predicts CVD, mortality and progression of CKD, but even levels below this threshold, i.e., 1-3 mg/mmol (10-29 mg/g), have been associated with increased risks in general populations.[27, 46-48]

2.3 INFLAMMATION AND KIDNEY FUNCTION

2.3.1 The hypothesis of inflammation as a mechanism for CKD development

Low-grade inflammation has been linked to CKD and ESRD, playing a role in the underlying pathophysiologic process of the disease and being partly accountable for the increased risk of CVD and mortality.[49, 50] Indeed, low-grade inflammation play important roles in several age-related chronic diseases, including atherosclerosis and type 2 diabetes.[51, 52] In the kidneys, low-grade inflammation is proposed to be a key mechanism for the development of interstitial fibrosis and tubular atrophy, which characterizes CKD.[13] As noted above, these histologic changes have also been observed during aging,[41] suggesting inflammation as a contributing cause in renal aging.[53]
There is a vast literature describing the cross-sectional association of inflammatory biomarkers with reduced kidney function, suggesting inflammation as a cause of CKD development and as a cause of CVD in patients with CKD. In patients with diabetes, markers of inflammation have been found to predict eGFR decline, CKD and ESRD[54-56]. However, whether inflammation is a risk factor for age-related GFR decline in the general non-diabetic population is not settled; in the following paragraphs of the present thesis, this issue will be discussed in further detail.

2.3.2 Tumor necrosis factor receptors, C-reactive protein and kidney disease

Tumor necrosis factor (TNF) is a cytokine belonging to the TNF superfamily, which includes several cytokines with central roles in the immune and inflammatory systems.[57] TNF exerts its biological actions via two cell surface receptors, TNF receptor type 1 (TNFR1) and TNF receptor type 2 (TNFR2), which also circulate in soluble forms and can be measured with better sensitivity and reliability than TNF itself.[58] Activation of the transmembrane receptors by TNF leads to intracellular signaling and activation of several complexes and transcription factors, e.g., nuclear factor-κB (NF- κB), which eventually leads to apoptosis, proliferation, necroptosis, and the generation of pro-inflammatory mediators.[59]

Bertani and colleagues first described the involvement of TNF in kidney disease in 1989 in rabbits injected with human recombinant TNF. The damage induced by TNF included glomerular endothelial damage, polymorphonuclear cell accumulation, and fibrin deposition in the capillary lumen.[60] Since then, experimental studies have shown TNF involvement in various kidney diseases, including diabetic nephropathy, glomerulonephritis, and glomerulosclerosis.[61]

TNF exerts several effects on the kidneys, including reduction of glomerular blood flow and GFR, increment of albumin permeability and tubular sodium reabsorption, disruption of the glomerular permeability barrier, and increased production of several pro-inflammatory and hemodynamic mediators.[59, 61]

C-reactive protein (CRP) is an acute-phase protein produced by the liver after the stimulation of interleukin-6 and is a non-specific marker of inflammation.[62] CRP is widely used in clinical practice and in epidemiologic research and is regarded as an established risk factor for coronary heart disease.[10, 63] Recent guidelines have recommended the use of CRP as a cardiovascular risk-stratification tool.[64] Experimental studies indicate a role of CRP in the pathogenesis of acute kidney failure[65, 66] and diabetic nephropathy through inflammatory pathways similar to that of TNF, i.e., activation of NF-κB.[67, 68]

2.3.3 Epidemiological studies on inflammation and kidney function

Several cross-sectional studies have found an inverse correlation of CRP and soluble TNF receptors with kidney function, both in the general population, and in patients with type 2 diabetes, CKD, and ESRD.[69-77] Thus, low-grade inflammation has been suggested as a cause of CKD. However, this hypothesis has been debated because the level of inflammatory biomarkers may be increased by reduced renal clearance. Particularly, soluble TNF receptors have been found in animal studies to be eliminated mainly by renal clearance, indicating reverse causality.[78]

In longitudinal studies of the general population, some researchers have found an association between the baseline CRP level and risk of eGFR decline and CKD,[76, 79, 80] while others have not.[77, 81] There is growing evidence for an association between soluble TNF receptors and kidney function decline in type 2 diabetes, suggesting an important role of TNF activity in the development of diabetic nephropathy.[54-56, 82-85] However, there are few longitudinal investigations from the general population without diabetes.[77, 81, 86] Similar to the results from diabetic populations, these studies found an increased risk of rapid eGFR decline and CKD associated with elevated baseline soluble TNF receptors. However, they did not adjust for baseline eGFR in the multivariable adjusted models, and they did not exclude participants with established CVD or diabetes, which may have confounded the results. Finally, non-GFR related factors may have influenced the results when using cystatin C- or creatinine-based eGFR. Thus, whether higher CRP or soluble TNF receptors predict an accelerated age-related GFR decline and CKD in the general non-diabetic population remains unknown.

2.4 OXIDATIVE STRESS AND KIDNEY FUNCTION

2.4.1 The hypothesis of oxidative stress as a mechanism for CKD development

Oxidative stress is a condition in which the generation of reactive oxygen species (ROS) exceeds the antioxidant repair and defense system. ROS (also called free radicals) contain unpaired electrons, making them highly reactive to other molecules, such as lipids, proteins and nucleic acids, which lead

to oxidation and damage of the attacked molecule.[87] Due to the unstable nature of ROS, direct measurement of oxidative stress in humans is difficult. Instead, an indirect approach to measure stable end products from oxidation in tissue, plasma or urine is used.[88]

There is a considerable evidence for an important role of oxidative stress in aging, mediated by damage to intracellular macromolecules.[14] Oxidative stress increases with age and is influenced by lifestyle-related factors such as smoking, hyperglycemia, lack of exercise, and diet.[89-92] In experimental studies, increased oxidative stress has been associated with the development of various kidney diseases. Epidemiologic studies have found an association between markers of oxidative stress and reduced GFR, but they are limited to cross-sectional design, and they have mainly been examined in people with established CKD.[93-100] Thus, it remains unclear whether markers of oxidative stress predict kidney function decline in the general population.

Several randomized controlled trials have investigated possible benefits of antioxidants in patients with CKD. According to a Cochrane report from 2012, there is no evidence of reduced risk of CVD and mortality in CKD patients on antioxidant therapy, but antioxidants may prevent the progression of CKD.[101] Previous clinical trials were small and revealed various results.[102-109] The most promising antioxidant so far is bardoxolone methyl, which has significantly improved mGFR and eGFR decline in patients with Alport syndrome, stage G3 CKD, and diabetic kidney disease (stage G4A2-3), although the follow-up times were too short to draw any conclusions.[103, 105, 110] The effect of antioxidants on the levels of inflammatory- and oxidative stress biomarkers in patients with CKD and ESRD has not been statistically significant,[109, 111, 112] but in a randomized controlled trial in healthy individuals, olive oil consumption reduced oxidative stress significantly.[113] Furthermore, in a study of twins, environmental factors, but not genetic factors, influenced the level of “whole-body” oxidative stress, measured as oxidatively damaged deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and lipids in urine, which suggests that oxidative stress is a modifiable condition.[114] We are not aware of any interventional study of an anti-inflammatory or antioxidant treatment with potential effects on GFR decline or albuminuria in the general population.

2.4.2 Urinary markers of oxidatively damaged DNA and RNA

DNA may be the most important target molecule for oxidative stress during aging.[12, 14] Oxidative damage to mitochondrial DNA has been proposed to induce age-related degenerative processes in the brain, heart, and kidneys.[115-117] Mitochondrial DNA may be more prone to oxidation than nuclear DNA.[118, 119] The proximal tubular cells in the kidneys contain a large number of mitochondria and may be particularly vulnerable to oxidative stress. Guanine in DNA and RNA is the nucleic acid most susceptible to oxidation. The oxidation rate of DNA and RNA can be estimated from the urinary

excretion of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) and 8-oxo-7,8-dihydroguanosine (8-oxoGuo), respectively, which represent the total systemic oxidative stress.[120]

2.4.3 Experimental studies on oxidative DNA and RNA damage and kidney function

A large body of evidence has shown that the accumulation of oxidatively damaged DNA and RNA in animal kidneys and their excretion in urine increase with aging.[12, 121] In experimental studies, markers of oxidative stress have been associated with inflammation, endothelial dysfunction, mitochondrial dysfunction, glomerulosclerosis, and kidney function decline, suggesting an important role of oxidative stress in the development of kidney disease.[13, 89, 116, 122] Intrarenal and urinary excretion of 8-oxodG has been reported in various CKD models.[99, 116, 118, 122] Diabetic rats exhibit increased renal expression and urinary excretion of 8-oxodG,[99, 118, 122], which are associated with the development of diabetic nephropathy, including proteinuria and renal failure.[99, 122] Accumulation of 8-oxodG has also been found in the mitochondria of the glomeruli and tubules in mice with induced toxic damage.[116] In the same study, the urinary excretion of 8-oxodG increased during the following days and was accompanied by the onset of albuminuria and glomerular and tubular injury,[116] which was also evident for diabetic mice.[122]

2.4.4 Epidemiological studies on oxidative DNA and RNA damage and kidney function

Similar to the findings discussed above, accumulation of mitochondrial DNA damage was found in kidney biopsies from patients with focal segmental glomerular sclerosis and diabetic kidney disease, but not in healthy controls, indicating a role of mitochondrial DNA oxidation in the development of these diseases.[116, 122] In epidemiologic studies, higher oxidative stress has been cross-sectionally associated with the severity of CKD.[93-96, 98, 100] The plasma level of F_2-isoprostane, a marker of lipid peroxidation, has been associated with CKD stages and has been found to be higher in CKD patients than in healthy controls,[94, 98, 100].

Few studies have investigated the relationship of oxidative DNA and RNA damage to kidney function. In a study of 51 CKD patients, the serum 8-oxodG level was inversely associated with the GFR, assessed as the creatinine clearance, while the urinary excretion of 8-oxodG was not.[93] The serum 8-oxodG level is mainly determined by the kidney function due to renal clearance. Thus, the serum level is not regarded as a valid measure of oxidative stress, whereas urinary excretion is.[120]

We are only aware of one longitudinal study that has examined oxidatively damaged DNA or RNA as a predictor of CKD. In a study of patients with type 2 diabetes, Hinokio et al. found that higher baseline urinary 8-oxodG excretion predicted the progression of albuminuria after 5 years of follow-up.[123] Interestingly, the urinary 8-oxodG level was the strongest predictor of diabetic nephropathy compared to blood pressure, HbA_{1c} and duration of diabetes. Unfortunately, the study did not

include GFR as an outcome. Studies on oxidative RNA damage and renal function are scarce in the literature presumably due to little interest in the oxidation of these nucleic acids over the years. Nevertheless, RNA may be more prone to oxidative damage due to its cytosolic location closer to the mitochondria, its single-stranded structure, and fewer protective proteins. To the best of our knowledge, no longitudinal study has investigated the association of oxidative DNA and RNA damage with GFR decline or albuminuria in the general population.

2.5 POSSIBLE INTERACTIONS BETWEEN OXIDATIVE STRESS AND INFLAMMATION IN AGE-RELATED GFR DECLINE

The mechanisms that lead to increased inflammation in CKD are not settled, but oxidative stress has been proposed as a contributor to inflammation when the GFR declines.[124] Oxidative stress promotes pro-inflammatory oxidized lipids and activate leukocytes and pro-inflammatory transcription factors, such as NF-kB.[13] Inflammation may also enhance oxidative stress, causing a destructive feedback loop that eventually leads to glomerular damage and renal fibrosis.[13] Thus, it is possible that the effect of oxidative stress and inflammation may interact to cause age-related GFR decline. The hypothesized interaction between inflammation and oxidative stress inducing kidney dysfunction is illustrated in Figure 3.

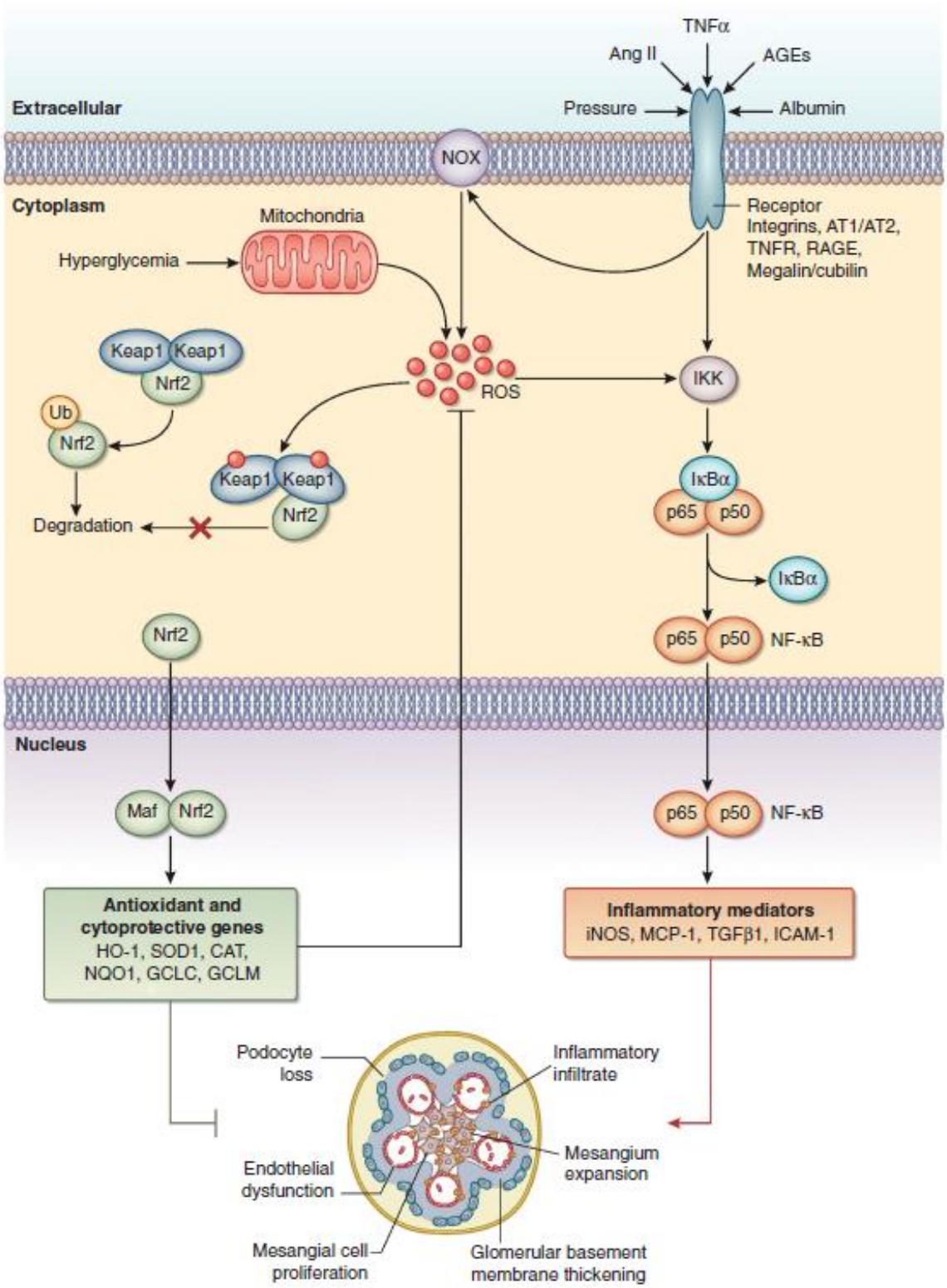


Figure 3. Intracellular signaling of oxidative stress (ROS) and inflammation that leads to kidney dysfunction. Adapted from Ruiz et al., Kidney International 2013. [13]

3 AIMS OF THE STUDY

In a general population without diabetes, kidney disease or CVD, our aims were as follows:

- To investigate whether serum markers of inflammation influence eGFR via non-GFR related mechanisms
- To investigate whether serum markers of inflammation predict an accelerated age-related mGFR decline
- To investigate whether urinary markers of oxidatively damaged DNA and RNA predict an accelerated age-related mGFR decline or low-grade albuminuria

4 METHODS

4.1 STUDY POPULATION

The renal iohexol clearance survey in Tromsø 6 (RENIS-T6) is a substudy of the Tromsø 6 study. The Tromsø 6 study is a population-based cohort that included an age-stratified representative sample of 12,984 inhabitants of the municipality of Tromsø in Northern Norway and was conducted from October of 2007 to December of 2008. [125] All inhabitants between the ages of 60 and 62 years and 40% of the inhabitants between the ages of 50 and 59 years were invited to participate, and 3564 (65%) individuals from these two age groups completed the main part of the Tromsø 6 study.

The RENIS-T6 study was performed from November of 2007 to June of 2009 and included 826 women and 801 men aged between 50 and 62 years. This age group was chosen in order to study a group of persons with sufficient risk of developing CVD and age-related GFR decline (which tend to accelerate after the age of 40), but without a high prevalence of comorbidity or medication. Of those individuals who completed the main Tromsø 6 study in the age group of interest, 739 participants were excluded due to self-reported previous myocardial infarction, angina pectoris, stroke, diabetes mellitus, or renal disease. The remaining 2,825 persons were invited to participate in the RENIS-T6 study, of which 2,107 (74%) agreed. In total, 77 persons were excluded due to allergy to contrast media, iodine, or latex or for other reasons, and 48 persons did not appear at their appointments. Thus, 1,632 participants of the remaining 1,982 were included according to a predetermined cohort target size based on the expected effect of GFR on the primary cardiovascular end point and stratified by sex and age groups. Five of these participants were excluded because of technical failures with the iohexol clearance measurements, leaving 1627 participants in the RENIS-T6 cohort (Figure 4).

The renal iohexol clearance survey follow-up (RENIS-FU) study was a follow-up study from the RENIS-T6 study, which was performed from September of 2013 to January of 2015. All participants in RENIS-T6 were invited to the RENIS-FU, except 23 participants who had died and seven who had a possible delayed allergic reaction to iohexol. Of the 1597 people invited, 1368 (86%) agreed. There were 39 participants who did not attend their appointments, and five participants were excluded because the antecubital vein could not be cannulated. Thus, 1324 persons (667 women and 657 men) participated in the RENIS-FU study (Figure 4).

To investigate the intra-individual variation in the GFR measurements, we measured GFR a third time in a random sample of 88 (5.5%) participants after two weeks and within 2 months after the RENIS-FU study. This approach allowed us to use mixed model regression with a random intercept, a

random slope and an unstructured covariance matrix to investigate risk factors associated with the GFR decline rates.

In papers 2 and 3, we excluded 33 participants who were diagnosed with diabetes mellitus at baseline according to their fasting plasma levels of glucose (≥ 7.0 mmol/l) and/or HbA1c (≥ 6.5%), four persons with missing values of TNFR2 and three with missing 8-oxodG/8-oxoGuo.

4.2 DATA COLLECTION AND MEASUREMENTS

All measurements in both RENIS-T6 and RENIS-FU were done at the Clinical Research Unit at the University Hospital of North Norway, except fibrinogen and high-sensitivity CRP (hs-CRP) levels, which were measured a few months before RENIS-T6 as a part of Tromsø 6.[126] Participants met between 08:00 and 10:00 AM after an overnight fast. They were instructed to avoid large meals with meat and to avoid taking any non-steroid anti-inflammatory drugs the last 2 days prior to examination. In addition, they were instructed to refrain from tobacco smoking during the 12 hours prior the examination and to drink two glasses of water before arrival. Participants with an acute illness were rescheduled to another appointment. After arrival, all participants answered a written questionnaire regarding current alcohol, tobacco, and medication use. Body weight and height were measured, and the body mass index (BMI) was calculated (kg/m^2). Fasting plasma samples were drawn for biochemical analyses. Three samples of first-void morning spot urine were collected on consecutive days before the examination in RENIS-T6 and RENIS-FU. In addition, a second-void morning spot urine was collected in the baseline RENIS-T6 examination. The blood pressure was measured as both automated office blood pressure and as ambulatory in the RENIS-T6 study. The office blood pressure was measured three times at two-minute intervals using an automatic device (A&D Model UA-799; Tokyo, Japan) after two minutes of rest in a seated position. The average of the last two measurements was used in the analyses. Ambulatory blood pressure was measured with a Spacelab 90207 device (Spacelabs Healthcare, Redmond, USA) at 20-minutes intervals from 08:00 to 22:00. A more detailed description of these measurements have been published elsewhere.[127]

4.2.1 Iohexol clearance measurements

The GFR was measured using the single-sample plasma clearance of iohexol. The same procedure was used in the RENIS-T6 and RENIS-FU, and a more detailed description of the analyses is previously published.[128] A Teflon catheter was placed in an antecubital vein, and a null sample was drawn. Then, 5 ml of iohexol (Omnipaque, 300 mgI/ml; Amersham Health, London, UK) was injected, and the syringe was weighed before and after injection. The catheter was flushed with 30 ml isotonic saline and used to draw blood samples for iohexol measurements. The optimal time for measuring the

iohexol concentration after the injection was calculated using Jacobssons`s method based on the GFR estimated by creatinine measured in the Tromsø 6 study.[129] The exact time from injection to sampling was measured in minutes using a stopwatch for each participant. The serum concentration of iohexol was measured by high-performance liquid chromatography as described by Nilsson-Ehle.[130] The coefficient of variation (CV) for the analysis was 3.0% in RENIS-T6 and 3.1% in RENIS-FU. To adjust for a possible drift in the GFR measurements in the RENIS-FU, a 6% random sample of blood specimens collected in RENIS-T6 was reanalyzed during the RENIS-FU study. There was a mean difference in GFR of 2.28 ml/min/1.73 m^2 (95% confidence interval, 1.05-3.51) between the analyses, as described previously.[131] Accordingly, all of the baseline GFR measurements reported in papers 2 and 3 were adjusted by adding this difference to the baseline values. The mean CV for the intra-individual variation in GFR was 4.2% (95% confidence interval, 3.4-4.9%).

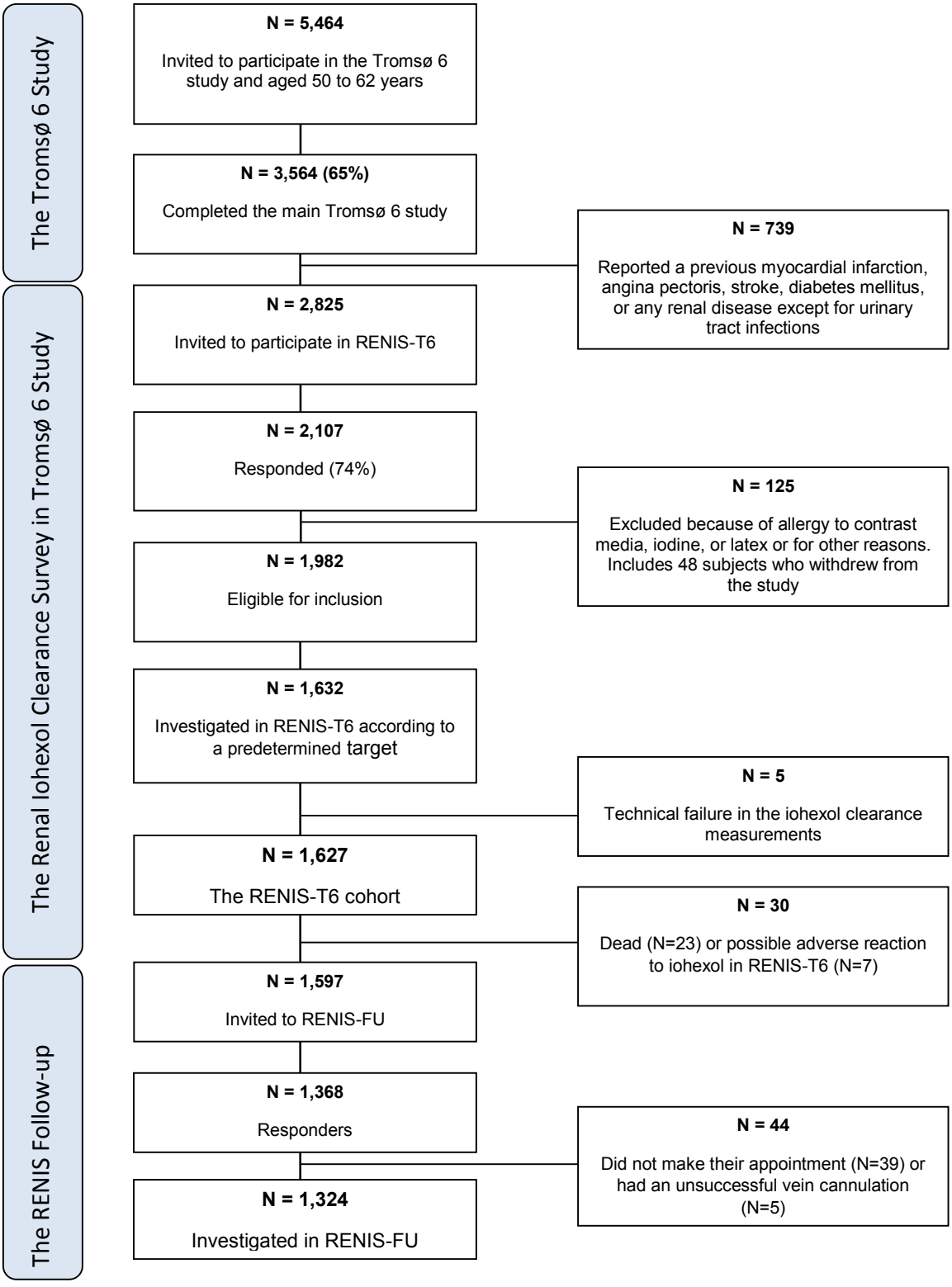


Figure 4. Flowchart of the Tromsø 6, RENIS-T6 and RENIS-FU studies

4.2.2 Measurements of inflammatory markers

The serum TNFR2 levels were measured by a quantitative sandwich enzyme-linked immunosorbent assay (ELISA) with a QuantiKine kit from R&D Systems, Inc. (Minneapolis, MN 55413, USA), at the Metabolic and Renal Research Lab, UiT The Arctic University of Norway. The serum samples were collected in RENIS-T6, stored at -80°C and thawed at the time of analysis. TNFR2 has been found to be stable through several freezing and thawing cycles.[132] The color intensity was measured on a Mikroplate Spectrophotometer (BioTek Instruments, Inc; Highland Park, P.O. Box 998, Vermont, USA). The interindividual and intraindividual CVs were 6.0% and 3.0%, respectively. The serum concentrations of hs-CRP were measured in the Tromsø 6 study 5.2 (95% confidence interval, 3.0-6.2) months earlier than the RENIS-T6 study. The hs-CRP level was analyzed by a particle-enhanced immunoturbidimetric assay on a Modal PPE autoanalyzer (Roche Diagnostics Norway AS; Norway).[126] The inter-individual and intra-individual CVs were 2.8% and 1.1%, respectively.

4.2.3 Measurements of oxidative stress markers

The urinary 8-oxodG and 8-oxoGuo levels were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) from second-void morning spot urine collected in the RENIS-T6 study. The samples were centrifuged, and the supernatant was immediately put on ice and frozen at -80°C within a few hours. The frozen urine samples were thawed and mixed before the analysis. The samples were prepared by adding 50 µl of a 50 nM aqueous internal standard (8-oxodG, $^{15}N_5$) to 60 µl of urine in a 1 ml collection plate (Waters, Milford, MA). To each of the wells, 500 µl of 0.1% formic acid was then added and mixed. The samples were analyzed by LC-MS/MS using the Waters Acquity^{TM} UPLC *I*-class system interfaced to the Waters Xevo TQ-S benchtop tandem quadrupole mass spectrometer (Waters, Manchester, UK). The urinary 8-oxodG and 8-oxoGuo concentrations were divided by urinary creatinine concentration in the analyses. The inter-assay and intra-assay CVs were <4.7% and <3.6% for 8-oxodG and <5.1% and <3.7% for 8-oxoGuo. A more detailed description of the analysis is given in the Supplemental Appendix of paper 3.

4.2.4 Other measurements

Serum creatinine was measured using an enzymatic assay standardized to the isotope dilution mass spectroscopy method (CREA Plus, Roche Diagnostics GmbH; Mannheim, Germany). Serum cystatin C was measured by a particle-enhanced turbidimetric immunoassay (Gentian, Moss, Norway) and calibrated to the international reference ERM-DA471/IFCC.[133] The urinary levels of albumin and creatinine were measured with commercial kits (ABX Diagnostics; Montpellier, France). Three samples of first-void morning urine were collected on separate days and analyzed unfrozen. The urinary albumin was corrected for urinary creatinine (urinary albumin-to-creatinine ratio (UACR), mg/mmol) to account for different degrees of dilution of the urine. The mean of the three UACR

values, calculated from the three consecutive urine samples, was used in the analyses. Fasting serum glucose, HbA_{1c}, triglycerides, LDL and HDL cholesterol levels were measured with standard methods and analyzed on the same day.

4.2.5 Definition of variables

In papers 1 and 2, we used the three CKD-EPI equations to estimate the GFR based on creatinine ($eGFR_{cre}$), cystatin C ($eGFR_{cys}$), or both ($eGFR_{crecys}$).[134] Incident CKD in paper 2 or low mGFR in paper 3 was defined as mGFR < 60 ml/min/1.73 m^2 at follow-up. In papers 2 and 3 we defined rapid GFR decline as an annual GFR decline of > 3.0 ml/min/1.73 m^2 as previously suggested.[7, 8] Annual GFR loss was calculated as the GFR at follow-up minus the GFR at baseline (in ml/min per 1.73 m^2) divided by the follow-up time in years. Low-grade albuminuria was defined as an UACR > 1.13 mg/mmol (corresponds to UACR >10 mg/g; Figure 2) according to the "high-normal albuminuria" cut off proposed by the CKD Prognosis Consortium.[27, 47]

4.3 STATISTICAL ANALYSES

The characteristics of the study population are presented as the mean (standard deviation [SD]), median (interquartile range), and number (percent). The characteristics were grouped by gender in paper 1. In paper 2, we presented the study characteristics according to the quartiles of soluble TNF receptor 2. In paper 3, we presented the study characteristics according to the quartiles of 8-oxodG and 8-oxoGuo, respectively, normalized for urinary creatinine and adjusted for sex. A linear trend over increasing quartiles in papers 2 and 3 was tested with linear and median regression for continuous variables and logistic regression for dichotomous variables. We tested the difference between the baseline and follow-up variables with paired t-tests for continuous and normally distributed variables, Wilcoxon signed-rank sum test for skewed variables, and McNemar's test for paired dichotomous variables. Differences between subjects included in RENIS-FU and those lost to follow-up were tested with the two independent samples t-test, Wilcoxon-Mann-Whitney test, and chi-square or Fisher's exact test, as appropriate.

In paper 1, we used multiple linear regression models to examine the associations between the mGFR and eGFR as dependent variables and fibrinogen, log hs-CRP, and sTNFR2 as independent variables. To assess the residual associations between eGFR and the inflammatory markers after accounting for mGFR, we used generalized estimating equations as described by Rule et al.[16] In the analyses, mGFR, $eGFR_{cre}$, $eGFR_{cys}$, and $eGFR_{crecys}$ were regressed simultaneously on fibrinogen, TNFR2, or log hs-CRP, with multivariable adjustment. To detect a significant deviation of the risk factor's association with eGFR compared with mGFR, the interaction between the risk factor and an indicator

variable for eGFR method was tested. A statistically significant interaction was interpreted as a non-GFR-related association with the eGFR.[16] We tested for interactions of age and gender with the associations between eGFR and the inflammatory markers after accounting for mGFR.

In papers 2 and 3, we used linear mixed regression models with a random intercept and slope to investigate the associations of the inflammatory and oxidative stress markers with the annual mGFR change rate. mGFR standardized to the body surface area (ml/min/1.73 m^2) was used as the dependent variable. In paper 2, chronological age was used as the independent time variable, while in paper 3, observation time from baseline to follow-up was used as the independent time variable. The associations of the risk factors with the GFR decline rate were modeled as interactions between the risk factors and the time variable. All study participants at baseline were included in the model regardless of the number (0, 1 or 2) of follow-up GFR measurements because a linear mixed regression model allows for missing observations at one or more points in time.[135]

The odds ratios for rapid GFR decline, incident CKD, and incident low-grade albuminuria were analyzed using multiple logistic regression models in papers 2 and 3.

To test for non-linear associations between the mGFR decline and inflammatory or oxidative stress markers in papers 2 and 3, we used a second-degree fractional polynomial transformation of the inflammatory or oxidative stress markers in the interaction with time in the linear mixed model.[136]

To evaluate the classification power of urinary 8-oxoGuo (divided by urinary creatinine) for predicting low-grade albuminuria at follow-up in paper 3, we compared the area under the receiver operating characteristic (ROC) curve (AUC) for the logistic regression models with and without 8-oxoGuo using the likelihood test.

5 MAIN RESULTS

5.1 PAPER 1. Residual associations of inflammatory markers with eGFR after accounting for measured GFR in a community-based cohort without CKD

There were 1627 participants in the RENIS-T6 cohort with a valid GFR measurement. The mean mGFR was 87.8 ml/min/1.73 m^2 in women and 95.7 ml/min/1.73 m^2 in men. In multiple linear regression models with mGFR and eGFR as dependent variables, a higher concentration of fibrinogen was associated with lower mGFR and $eGFR_{cys}$ but not $eGFR_{cre}$ and $eGFR_{crecys}$. A higher concentration of sTNFR2 was associated with a lower mGFR and eGFR in all of the estimating formulas. A higher concentration of hs-CRP was associated with a lower mGFR, $eGFR_{cys}$ and $eGFR_{crecys}$ but not $eGFR_{cre}$.

In generalized estimated equations, the residual associations between the eGFR equations and the inflammatory markers were examined after accounting for mGFR. In the model adjusted for age, sex, and the use of angiotensin-converting-enzyme inhibitors (ACEis) or angiotensin II receptor blockers (ARBs), higher concentrations of fibrinogen, sTNFR2 and hs-CRP were associated with higher $eGFR_{cre}$ and lower $eGFR_{cys}$ after accounting for mGFR. In the fully adjusted model, $eGFR_{cre}$ remained associated with fibrinogen, sTNFR2 and hs-CRP, whereas sTNFR2 and hs-CRP were associated with $eGFR_{cys}$, again in the opposite direction. The combined $eGFR_{crecys}$ equation was not associated with any inflammatory markers after accounting for mGFR.

5.2 PAPER 2. Association of soluble TNF receptor 2 and CRP with GFR decline in the general nondiabetic population

There were 1590 study participants at baseline after excluding those with diagnostic criteria for diabetes or missing values of TNFR2 in the current study. Among these, 1296 participants had follow-up data after a median of 5.6 years. All characteristics changed between baseline and follow-up ($p<0.05$), except for the body weight, BMI, and fasting triglycerides. Participants with higher TNFR2 levels had a lower mGFR and eGFR, were older, were more likely to be men, had a worse metabolic profile, had a higher hs-CRP and were more likely to use ARBs, smoke and consume less alcohol ($p<0.05$).

The mean (SD) change rate for the mGFR during the observation period was -0.84 (2.00) ml/min/1.73 m^2 per year. There was an inverse bivariate correlation between the TNFR2 level and mGFR at baseline ($r= -0.26$, $p<0.001$). The hs-CRP level was not correlated with the baseline mGFR. In linear mixed models, a higher TNFR2 level was not associated with the mean mGFR decline in unadjusted analyses, but it was associated with a slower mGFR decline in the fully adjusted models (mean annual

change in mGFR per SD increase in TNFR2: 0.11 ml/min/1.73 m^2 per year, 95% CI: 0.02-0.19). A higher TNFR2 level was also associated with a slower decline in $eGFR_{crecys}$ but not $eGFR_{cre}$ or $eGFR_{cys}$. Higher hs-CRP level was associated with a more rapid decline in mGFR and $eGFR_{cre}$ in all models, but not with changes in $eGFR_{cys}$ and $eGFR_{crecys}$. One mg/L higher hs-CRP was associated with a more rapid mGFR change of -0.03 ml/min/1.73 m^2 per year (95% CI: -0.05 to -0.01).

In a second-degree fractional polynomial transformation of the interaction between TNFR2 and time, a higher TNFR2 level was non-linearly associated with the mGFR decline in the multivariable adjusted model. There was an increasingly positive association between higher TNFR2 levels and the mGFR change rate, i.e., a slower GFR decline.

A higher hs-CRP level was associated with a higher odds ratio for rapid GFR decline (>3.0 ml/min/1.73 m^2 per year) in the unadjusted model and adjusted for sex, age, weight and height (OR: 1.03; 95% CI: 1.01-1.06 per 1 mg/L increase in hs-CRP). In addition, a higher hs-CRP level was associated with a higher odds ratio for incident CKD (mGFR<60 ml/min/1.73 m^2) at follow-up in the fully adjusted model (OR: 1.05; 95% CI: 1.01-1.09; per 1 mg/L increase in hs-CRP). These associations were no longer significant when we excluded persons with CRP above 10 mg/L.

5.3 PAPER 3. Urinary markers of oxidative stress are associated with albuminuria but not GFR decline

There were 1591 baseline participants in the current study after excluding those who fulfilled the diagnostic criteria for diabetes (n=33) and those with missing values of 8-oxodG/8-oxoGuo (n=4). After a median of 5.6 years, 1298 persons were included in the follow-up study. The baseline concentrations of 8-oxodG and 8-oxoGuo divided by the urinary creatinine concentration were named 8-oxodG^{UCR} and 8-oxoGuo^{UCR}, respectively. The median (IQR) ratios were 1.36 (1.04-1.74) nmol/mmol for 8-oxodG^{UCR} and 3.45 (2.68-4.44) nmol/mmol for 8-oxoGuo^{UCR}. Urinary 8-oxodG and 8-oxodG concentrations were higher in men than in women when the levels were not divided by urinary creatinine, but the ratios were lower in men. The baseline characteristics of the study population were presented according to the quartiles of 8-oxodG^{UCR} and 8-oxoGuo^{UCR}, and a linear trend over higher quartiles was adjusted for sex. Higher quartiles of both markers were associated with higher age and with current smoking but were not associated with mGFR or UACR. Study participants with higher quartiles of 8-oxoGuo^{UCR} had a higher weight, BMI, fasting glucose, and hs-CRP and a lower HDL. There was a significant positive linear correlation between log 8-oxodG and log 8-oxoGuo (R=0.88, P<0.001).

Neither 8-oxodG^{UCR} nor 8-oxoGuo^{UCR} was associated with the mGFR change rate in linear mixed regression models or with rapid GFR decline or incident CKD in logistic regression models (data not shown). Fifty-two study participants developed low-grade albuminuria at follow-up, defined as UACR > 1.13 mg/mmol.

In the logistic regression analyses, there was a higher odds ratio for incident low-grade albuminuria when 8-oxodG^{UCR} was used as a continuous variable in unadjusted analyses, but after adjustment for age, sex, weight and height, this association was no longer statistically significant. When using 8-oxoGuo^{UCR} as a continuous variable, there was a higher odds ratio for incident low-grade albuminuria in the unadjusted model and in the model adjusted for age, sex, weight and height but not in the fully adjusted model (Model 3, Table 4). However, there was a statistically significant trend for a higher odds ratio with increasing quartiles of 8-oxoGuo^{UCR} in all models (P<0.02). Persons with 8-oxoGuo^{UCR} in the highest quartile had an odds ratio of 2.64 (95% CI; 1.50-4.65) for incident low-grade albuminuria compared to persons with 8-oxoGuo^{UCR} in the three lowest quartiles. The odds ratio remained unchanged after an additional adjustment for the baseline mGFR (P<0.001). The AUC for predicting incident low-grade albuminuria increased from 0.67 (95% CI; 0.60-0.75) to 0.71 (95% CI; 0.63-0.78) by including 8-oxoGuo^{UCR} in the fully adjusted model, resulting in a statistically improved model (P=0.002).

5.4 ADDITIONAL ANALYSES

There were no interactions between markers of low-grade inflammation and markers of oxidatively damaged DNA and RNA in the association with the age-related mGFR decline.

6. GENERAL DISCUSSION

6.1 METHODOLOGICAL DISCUSSION

6.1.1 Design

The RENIS-T6 study was primarily designed to investigate the association between mGFR and cardiovascular risk in the general population. The collection of cardiovascular end points is currently on-going. The RENIS-FU Study was designed to assess the mGFR change rate during 5.6 years of follow-up and to investigate risk factors associated with age-related mGFR decline. This thesis is based on both baseline and longitudinal data from the RENIS-T6 study and the RENIS-FU study. Given the observational nature of the study, no conclusions regarding causality may be drawn.

6.1.2 Bias

Selection bias may occur when the exposure or outcome of interest in a study sample differ in a systematic way from the population of interest. Participants who were invited to the RENIS-T6 study were initially invited to the Tromsø 6 study. The attendance rate in the Tromsø 6 study was 66%, which is high compared to that in other population studies. Still, with one-third of the eligible population not attending, selection bias is likely. For example, responders may be more engaged in their own health, which in turn affect lifestyle-related risk factors, such as weight, smoking and alcohol consumption. To reveal the magnitude of selection bias, one should compare the characteristics between those who attended the study and those who were eligible and did not attend. Unfortunately, we lack information from those who did not attend the Tromsø 6 study, and therefore, we are not able to assess the degree of selection bias. Thus, some degree of selection bias in the current study cannot be excluded. The attendance rate in RENIS-T6 study was 77%, and there were only small differences in age and BMI between those included in the study (n=1627) and all eligible persons (n=2825).[128] The attendance rate for the RENIS-FU study was 83%. Differences in HbA_{1c} and BMI between those included in the follow-up study and those lost to follow-up were small, except for smoking and the UACR. In paper 3, there were higher urinary levels of 8-oxoGuo among those lost to follow-up than among those included in the follow-up study. If we assume a steeper GFR decline in non-respondents, our results may have been biased toward zero. However, the effect estimates were small, and the 95% CI was narrow for both 8-oxoGuo and 8-oxodG. Similarly, the difference in exposure may also have diluted the association between 8-oxoGuo and low-grade albuminuria.

6.1.3 Information bias

Information bias involves the misclassification of exposure or outcome and can be due to recall bias, reporting bias, observer bias, or measurement error. Some of the exposure variables in our study were obtained from questionnaires that may have introduced information bias. In particular, people have tend to underestimate their smoking and alcohol habits.

In the RENIS-FU study, we reanalyzed the iohexol concentration in a random sample (6%) of blood samples to correct for a possible drift in the measurements between baseline and follow-up. The blood samples from the baseline study were randomly selected among the participants of the follow-up study and were analyzed simultaneously during the follow-up study. The mean difference in mGFR between baseline and the random samples was 2.28 ml/min/1.73 m^2 (95% confidence interval 1.05-3.51).[131] The sequential steps in the process of measuring GFR may have changed over the 5.6 years, although we used the same measurement protocol and procedures. Thus, we adjusted all the baseline GFR measurements according to the drift. However, we cannot exclude the possibility that the drift may have introduced some random error in our study, leading to diluted estimates and reduced statistical power. To calculate the day-to-day variability of the GFR measurement, we performed a third GFR measurement in a random sample. The intra-individual CV (day-to-day variability of the GFR measurement) that consisted of both biological variation and measurement error was 4.2%, which is lower than most previous studies that measured GFR.

6.1.4 Confounding

We adjusted for known risk factors for GFR decline that are also associated with the inflammatory markers. However, we cannot exclude the influence of other confounders, e.g., unmeasured factors that are associated with the inflammatory markers and that also affect the age-related GFR decline, leading to spurious associations. Smoking was clearly associated with higher levels of soluble TNFR2 and urinary levels of oxidatively damaged DNA and RNA in papers 2 and 3. In addition, smoking was associated with a borderline steeper GFR decline (not presented). The most likely consequence of an underestimated smoking status would therefore be an attenuation of the effect estimates between these variables and the GFR decline rates.

6.1.5 External validity

The external validity or the generalizability of a study indicates how well its results apply to other populations. The participants in the RENIS-T6 study were Caucasians. Therefore, the results may not apply to other ethnic groups. For example, the non-GFR-related associations between inflammatory markers and the eGFR found in our study may be different in other ethnicities.[16] However, a recent study of elderly subjects found CRP to be associated with both eGFRcre and eGFRcys after accounting

for mGFR in a cohort where 45% were of black ethnicity.[137] The results are similar compared to ours, which suggest that inflammation, as a non-GFR-related factor, is consistent over ethnicities.

Regarding paper 2, a higher level of CRP was associated with a decline in eGFR in the Multi-Ethnic Study of Atherosclerosis (MESA), which included white, African-American, Hispanic, and Chinese participants.[76] Similar associations of soluble TNF receptors with the risk of ESRD have been observed in Caucasians and Pima Indians with diabetes mellitus, suggesting generalizability between ethnicities.[55, 83] We are not aware of previous population-based studies on the association between oxidative RNA damage and albuminuria. However, oxidative DNA damage predicted diabetic nephropathy, defined as albuminuria, in a Japanese cohort. Because the above-discussed studies include diabetic populations, they are not comparable to our study since we excluded participants with diabetes.

Participants in the RENIS-T6 were aged 50 to 62 years. Thus, our results may not be generalizable to other age groups. In paper 1, the non-GFR related association of fibrinogen and eGFRcre was attenuated with higher age. Although this interaction seems paradox, the non-GFR-related effect of fibrinogen may not be significant at higher ages.

The participants in the RENIS study were quite healthy. We excluded participants with self-reported kidney disease, CVD and diabetes mellitus at the entry of the study with the purpose to study factors associated with age-related mGFR decline in a general non-diabetic population. Thus, our results may not mirror associations of inflammation and oxidative stress with the risk of rapid GFR decline and CKD in other populations with other risk factors (e.g., diabetes and preexisting CVD).

6.2 DISCUSSION OF RESULTS

6.2.1 Inflammation and non-GFR-related factors

We found that the inflammatory markers TNFR2, hs-CRP, and fibrinogen associated differently with eGFR than with mGFR, indicating that eGFR based on cystatin C and creatinine are influenced by inflammation through non-GFR-related pathways. $eGFR_{cre}$ was positively associated with TNFR2, hs-CRP, and fibrinogen after multivariable adjustment and relative to mGFR. $eGFR_{cys}$ was associated with TNFR2 and hsCRP but in an opposite direction compared to $eGFR_{cre}$.

In a previous RENIS-T6 publication, Mathisen et al. found that several traditional cardiovascular risk factors, including BMI, smoking, and lipids, were associated with eGFR, particularly eGFR based on cystatin C, after a multivariable adjustment including mGFR.[15] In a study from the Genetic

Epidemiology Network of Arteriopathy (GENOA) cohort and the Epidemiology of Coronary Artery Calcification (ECAC) cohort, Rule et al. found CRP, BMI, and hypertension to be associated with $eGFR_{cys}$, whereas only urinary creatinine was associated with $eGFR_{cre}$ after accounting for mGFR.[16] Accordingly, the authors argued that $eGFR_{cre}$ is superior in reflecting the true GFR in association studies of eGFR and outcome. However, Melsom et al. found that eGFR based on both cystatin C and creatinine was influenced by insulin resistance and non-traditional cardiovascular risk factors (asymmetric dimethylarginine and symmetric dimethylarginine) relative to mGFR. Similarly, we observed non-GFR related associations for both $eGFR_{cre}$ and $eGFR_{cys}$. Taken together, these studies suggest that $eGFR_{cre}$ does not necessarily reflect the associations with true GFR better than $eGFR_{cys}$. eGFR based on creatinine is extensively used in epidemiologic studies of kidney function and risk of CVD and mortality. The non-GFR-related factors described by us and others imply that associations between the eGFR level in the near-normal range and the risk of CVD and mortality attributable to kidney function should be interpreted with caution. Non-GFR-related factors may also confound the association between eGFR and kidney outcomes such as GFR decline rates, incident albuminuria, and ESRD.

6.2.2 Non-GFR-related properties by $eGFR_{cre}$ and $eGFR_{cys}$

Our findings also suggest that the non-GFR-related associations of TNFR2 and hs-CRP operate in opposite directions. Similar opposite non-GFR-related associations were reported in two previous studies of the RENIS-T6 cohort[15, 138] and, for CRP, in a recent study of two community-based cohorts of the elderly.[137] Indeed, most identified non-GFR-related factors that are related to increased cardiovascular risk are associated with a lower $eGFR_{cys}$ but a higher $eGFR_{cre}$.[15, 137, 138] These results may partly explain the different risks with $eGFR_{cre}$ and $eGFR_{cys}$ in epidemiologic studies of the general population.[3] When using the combined equation with creatinine and cystatin C ($eGRF_{crecys}$), the residual associations with the inflammatory markers were not statistically significant relative to mGFR. Thus, using $eGFR_{crecys}$ may reduce confounding from non-GFR-related factors in epidemiologic studies, which has also been suggested by others.[137, 138]

6.2.3 Soluble TNF receptors and age-related GFR decline

In paper 2, we found that a higher baseline hs-CRP level was associated with an accelerated decline in mGFR and incident CKD during 5.6 years of follow-up. In contrast, we found that higher baseline TNFR2 level was associated with a slower mGFR decline. TNFR2 has been proposed as an important risk factor for GFR decline in diabetes, but only a few studies have investigated the relationship between soluble TNF receptors and eGFR decline in the general population.[77, 81, 86, 139] Shankar et al. found TNFR2 to be associated with an increased risk for incident CKD, defined as $eGFR_{cre}$ < 60 ml/min/1.73 m^2, during 15 years of follow-up.[77] Medenwald et al. found an association between

higher TNFR1 levels and accelerated eGFR decline in men, as well as a higher risk of incident CKD in both genders during 4 years of follow-up.[81] In two other cohort studies, one of middle-aged and elderly Chinese individuals and one of elderly persons from Sweden, TNFR1 and TNFR2, respectively, were associated with incident CKD during 5-6 years of follow-up.[86, 139]

There are several possible explanations for the opposite results reported by us compared to other studies from the general population.[77, 81, 86, 139] They used eGFR based on either creatinine or cystatin C, which may be problematic because of non-GFR-related confounding, e.g., by inflammation as demonstrated in paper 1.[16, 34] We included eGFR in our analyses and found that a higher TNFR2 level was associated with a slower decline rate of mGFR and $eGFR_{crecys}$ but not with $eGFR_{cre}$ or $eGFR_{cys}$. A higher hs-CRP level was associated with an accelerated decline in mGFR and $eGFR_{cre}$ but not with $eGFR_{cys}$ or $eGFR_{crecys}$. The different results observed with different GFR equations may also be explained by lower precision of eGFR in the normal range of GFR.

Shankar et al. and Medenwald et al. used traditional regression analyses to assess the eGFR decline and risk of CKD, and they did not adjust for baseline eGFR.[77, 81] Similar to other studies, we found an inverse baseline association between TNFR2 and mGFR, which is likely due to the renal excretion of soluble TNF receptors.[78] The baseline association between soluble TNF receptors and eGFR could have confounded the longitudinal analyses, particularly when the outcome was defined as incident CKD (eGFR < 60 ml/min/1.73 m^2). Indeed, in a community-based cohort of the elderly, the odds ratios for the 5-year incidence of CKD associated with a higher TNFR1 were attenuated and not statistically significant after additional adjustment for baseline eGFRcys.[86] There were only 14 participants in our study population who developed incident CKD defined as $eGFR_{cys}$ < 60 ml/min/1.73 m^2. Still, a higher TNFR2 at baseline was associated with a higher odds ratio for incident CKD (based on cystatin C) in a logistic model without adjusting for the baseline $eGFR_{cys}$. However, this association lost its statistical significance after adjusting for the baseline $eGFR_{cys}$, suggesting an impact of the baseline association between TNFR2 and eGFR on the longitudinal analysis. In contrast, there were no baseline associations between hs-CRP and mGFR or eGFR. Thus, the odds ratio for incident CKD based on mGFR associated with higher hs-CRP was not influenced by an additional adjustment for baseline mGFR. The issue of adjusting for baseline level or not when using ordinary regression to analyze change (i.e., in GFR), has been widely debated.[140] Both methods may lead to biased results.[140] Accordingly, we used mixed model regression to analyze the change in GFR, which is considered the gold standard method to analyze the GFR trajectory in longitudinal studies.[135, 141, 142]

Our finding of a positive relationship between TNFR2 and mGFR decline may seem contradictory compared to the results of other studies. A higher TNFR2 was independently associated with a slower

GFR decline in linear and non-linear models. Although these results should be interpreted carefully, we speculate that they represent an association between inflammation and renal hyperfiltration, which is similar to what has been found in animal studies and in patients with diabetes.[143-145] In a cohort from general Japanese and American populations, higher IL-6 and CRP levels were associated with a higher eGFR in younger adults but with a gradually lower eGFR at higher ages, indicating a possible phase of renal hyperfiltration.[69] On the other hand, it has also been suggested that higher concentrations of soluble TNF receptors may abrogate the inflammatory effects of TNFα.[146] Thus, it is possible that higher levels of TNF receptors may be a beneficial physiologic response in healthy conditions and harmful with increasing severity of disease. However, we did not find any interactions between TNFR2 and fasting glucose, hypertension, or oxidative stress markers that would support a notion of a multi-hit mechanism, or different risk in different persons within this relatively homogenous cohort.

6.2.4 CRP and age-related GFR decline

Previous studies of the relationship between CRP and GFR decline have shown inconsistent results.[76, 77, 79, 81, 147] In the Multi-Ethnic Study of Atherosclerosis, Hiramoto et al. found that higher CRP levels were associated with a decline in $eGFR_{cre}$ but not $eGFR_{cys}$, which is in accordance with our findings.[76] Similar, CRP was not associated with eGFR decline based on cystatin C in the Cardiovascular Health Study.[147] The different results obtained by eGFR based on creatinine or cystatin C may partly be explained by different non-GFR-related effects of CRP.[16] We are not aware of other studies of the relationship between CRP and GFR decline that have used mGFR. We found an accelerated GFR decline with both mGFR and $eGFR_{cre}$ but not with $eGFR_{cys}$. However, the risk of incident CKD with higher hs-CRP was only statistically significant using mGFR. Our finding of an accelerated GFR decline disappeared after excluding 48 participants with an acute inflammatory state, defined as hs-CRP > 10 mg/L.[148] Thus, it is possible that these participants had a disease (e.g., cancer or rheumatic disease) that results in steeper GFR decline, which is not related to the chronic inflammation process per se. It may also indicate that overt inflammation and not low-grade inflammation in itself increases the risk of GFR decline. Alternatively, low-grade inflammation may lead to GFR decline in persons with additional risks, such as diabetes, according to the multi-hit hypothesis.

We excluded participants with diabetes, kidney disease, and CVD at the entry of the study. Most of the previous studies did not exclude participants with diabetes or CVD, which may have influenced the association between low-grade inflammation and CKD.[76, 77, 81, 86, 149] It is possible that the underlying pathophysiology that leads to GFR decline differ in early stages compared to more advanced stages of CKD. It should also be noted that there is not necessarily any contradiction

between the effect of TNFR2 on the mean GFR decline in our study and a possibility of soluble TNF receptors as risk factors for CKD in populations with different risk (e.g., diabetes).

6.2.5 Oxidative stress and GFR

In paper 3, we investigated the association of urinary markers of oxidative DNA and RNA damage with the age-related GFR decline. None of the markers was associated with the GFR decline, but urinary 8-oxoGuo excretion was associated with an increased risk of low-grade albuminuria. We are not aware of previous studies that have described the association between markers of oxidative stress and renal function decline using mGFR in the general population. Most of the prior studies have been cross-sectional and mainly conducted in patients with established CKD.[93-96, 98, 100] Only two of these studies found that the plasma level of oxidative stress markers correlated with the eGFR or creatinine clearance.[93, 94] Akagi et al. found a strong inverse correlation between serum 8-oxodG levels and creatinine clearance in CKD patients.[93] However, the urinary 8-oxodG level was not correlated with creatinine clearance, suggesting that the plasma 8-oxodG level reflects the GFR and that the increased plasma 8-oxodG level in CKD patients is mainly due to reduced urinary clearance of the marker. Similar, the plasma 8-isoprostane level, a marker of lipid peroxidation, was correlated with CKD stages.[94] The urinary concentrations of 8-oxodG and 8-oxoGuo in our study did not correlate with the baseline mGFR or the UACR but were positively associated with several cardiovascular and CKD risk factors. This was particularly evident for urinary 8-oxoGuo.

6.2.6 Oxidative stress and albuminuria

Hinokio et al. reported that higher urinary 8-oxodG excretion was associated with an increased risk of the progression of albuminuria in patients with diabetic nephropathy.[123] We found an association between urinary 8-oxodG excretion and increased risk of low-grade albuminuria in unadjusted analyses, but this association disappeared when adjusting for sex, baseline age, weight, and height. In contrast, the association of urinary 8-oxoGuo excretion with the risk of low-grade albuminuria remained statistically significant even in the fully adjusted model. This finding is noteworthy because low-grade albuminuria, defined as a UACR between 1.13 and 3.40 mg/mmol, associates with increased risk of GFR decline and CKD, but most of all CVD and mortality in the general population.[27, 46-48] Some have suggested that low-grade albuminuria may represent generalized endothelial dysfunction, and a study of patients on dialysis found an inverse relationship between endothelial function and oxidative stress, measured as plasma 8-oxodG.[97] Furthermore, in a previous study of patients with type 2 diabetes, higher urinary 8-oxoGuo excretion was associated with increased risks of all-cause and diabetes-related mortality.[150] Our finding of an increased risk of low-grade albuminuria associated with higher 8-oxoGuo may therefore represent endothelial dysfunction

rather than kidney disease per se. However, we recently reported that even a minimal increase in albuminuria is a risk factor for an accelerated age-related GFR decline in our cohort.[48]

6.2.7 Antioxidants and CKD

Intervention with an antioxidant treatment or diet may reduce oxidative stress. A previous Cochrane report has suggested that antioxidant therapy may prevent the progression of GFR decline in CKD patients.[101] Particularly, bardoxolone methyl, a potent activator of nuclear factor-erythroid-2-related factor 2 (Nrf2), has been investigated as treatment for maintaining GFR in CKD patients. Nrf2 regulates several genes with antioxidant and anti-inflammatory properties that are important in maintaining kidney function.[151] In the Bardoxolone Methyl Treatment: Renal Function in CKD/Type 2 Diabetes (BEAM) trial, patients who received bardoxolone methyl with type 2 diabetes and stage G3-4 CKD had significant improvement in the eGFR at 24 and 52 weeks compared with placebo.[106] The later Bardoxolone Methyl Evaluation in Patients with Chronic Kidney Disease and Type 2 Diabetes Mellitus: the Occurrence of Renal Events (BEACON) trial was terminated due to unforeseen adverse effects, and the authors concluded that bardoxolone methyl did not reduce the risks of ESRD and cardiovascular mortality among patients with severe CKD and type 2 diabetes.[152] However, a recent post hoc analysis of the BEACON trial reported that patients who received bardoxolone methyl had an increased eGFR that was sustained after 48 weeks and a 52% reduced risk of progression to ESRD.[110] Preliminary results from the TSUBAKI trial showed that Japanese patients with type 2 diabetes and stage G3 CKD treated with bardoxolone methyl had a significant improvement in mGFR, measured by inulin clearance, compared to placebo at 16 weeks, and that patients with both G3 and G4 CKD tolerated bardoxolone methyl treatment well.[105] However, these short term changes in GFR may have been caused by hemodynamic effects of bardoxolone methyl without any beneficial long-term effects on kidney function.[153] Whether antioxidant treatment can prevent an accelerated GFR decline or CKD in the general population remains unresolved.

6.3 STRENGTHS AND LIMITATIONS

The primary strength of the current thesis was the repeated measurements of GFR in a large population-based cohort. Single-sample iohexol clearance has been validated against gold standard methods, and the intra-individual CV in the GFR measurements in our study was lower than reported in previous studies.[18, 20-22] To the best of our knowledge, this is the first longitudinal study of a general population to include GFR measurements. Furthermore, the measurements of creatinine and cystatin C were both calibrated to international standards, and we measured soluble TNF receptors and the urinary excretion of 8-oxodG and 8-oxoGuo with recommended methods. We used state-of-the-art

statistical methods in order to investigate risk factors associated with the age-related GFR trajectory.[135]

However, the study also has limitations that need to be addressed. The generalizability of our findings is restricted to relatively healthy middle-aged Caucasians. Thus, comparing our results to other race and age groups with different risks of CKD and CVD should be done cautiously. An observation time of 5.6 years may not be sufficient to detect associations of low-grade inflammation and oxidative stress with age-related GFR decline or CKD.

In paper 1, we were not able to adjust for dietary factors or muscle mass, which probably are the main non-GFR-related factors of creatinine-based eGFR. However, we obtained largely the same results after adjusting for waist-to-hip ratio and physical exercise, which are both correlated with muscle mass. The observed associations between inflammation and the age-related GFR decline were small and therefore may not be clinically relevant. We only measured the baseline level of soluble TNFR2 as a marker of TNF activity. However, both TNF receptors are highly correlated, and the same results would be expected for soluble TNFR1.[55, 75] Moreover, TNFR2 levels have been reported to be stable during a prolonged period with repeated measurements.[54] In paper 3, we used morning spot-urine for the analysis of 8-oxodG and 8-oxoGuo instead of 24-hour urine sampling, which is considered the gold standard. To reduce the intra-individual variation, we adjusted the levels of 8-oxodG and 8-oxoGuo for urinary creatinine, which has shown good correlations with 24-hour sampling.[154]

7 CONCLUSIONS AND PERSPECTIVES

7.1 CONCLUSIONS

We found that inflammatory markers associated differently with eGFR based on creatinine and cystatin C compared to mGFR, which suggests that inflammation may bias eGFR equations and the association between eGFR and risk of CKD and CVD. In the longitudinal study, we found that the age-related mGFR decline over 5.6 years was 0.84 ml/min/1.73 m^2 per year. A higher baseline level of hs-CRP was associated with an accelerated age-related mGFR decline as well as a higher risk of incident CKD, while a higher level of TNFR2 was associated with a slower age-related mGFR decline. Oxidatively damaged DNA and RNA were not statistically significantly associated with the age-related mGFR decline, but higher urinary excretion of oxidatively damaged RNA was associated with an increased risk of low-grade albuminuria.

7.2 PERSPECTIVES

Our finding of several cardiovascular risk factors as non-GFR-related factors in the RENIS-T6 study should prompt longitudinal studies to investigate the relationship between mGFR, rather than eGFR, and cardiovascular risk and all-cause mortality. To do so, we are currently collecting cardiovascular endpoints from the RENIS study.

Multiple biomarkers and longer follow-up with several mGFR measurements are needed to assess the long-term effect of inflammation and oxidative stress on the age-related GFR decline and risk of CKD in the general non-diabetic population. We are planning a new follow-up study (RENIS 3) at the end of 2018 and will invite all baseline participants from the RENIS-T6 study to a new GFR measurement with iohexol clearance. The follow-up time will then be approximately 10-11 years. Factors related to the age-related GFR decline will be investigated using several baseline biomarkers, such as proteomics, and the associations with low-grade inflammation and oxidative stress will be examined further. Additionally, the associations of low-grade inflammation and oxidative stress with the risk of CVD and mortality will be investigated.

8 REFERENCES

1. Coresh J, Selvin E, Stevens LA*, et al.* Prevalence of chronic kidney disease in the United States. *JAMA : the journal of the American Medical Association* 2007; **298:** 2038-2047.

2. Stevens LA, Coresh J, Levey AS. CKD in the elderly--old questions and new challenges: World Kidney Day 2008. *American journal of kidney diseases : the official journal of the National Kidney Foundation* 2008; **51:** 353-357.

3. Shlipak MG, Matsushita K, Ärnlöv J*, et al.* Cystatin C versus Creatinine in Determining Risk Based on Kidney Function. *New England Journal of Medicine* 2013; **369:** 932-943.

4. Webster AC, Nagler EV, Morton RL*, et al.* Chronic Kidney Disease. *Lancet* 2016.

5. Lindeman RD, Tobin J, Shock NW. Longitudinal studies on the rate of decline in renal function with age. *Journal of the American Geriatrics Society* 1985; **33:** 278-285.

6. Stevens LA, Coresh J, Greene T*, et al.* Assessing kidney function--measured and estimated glomerular filtration rate. *The New England journal of medicine* 2006; **354:** 2473-2483.

7. Shlipak MG, Katz R, Kestenbaum B*, et al.* Rapid decline of kidney function increases cardiovascular risk in the elderly. *Journal of the American Society of Nephrology : JASN* 2009; **20:** 2625-2630.

8. Rifkin DE, Shlipak MG, Katz R*, et al.* Rapid kidney function decline and mortality risk in older adults. *Archives of internal medicine* 2008; **168:** 2212-2218.

9. Obrador GT, Mahdavi-Mazdeh M, Collins AJ. Establishing the Global Kidney Disease Prevention Network (KDPN): a position statement from the National Kidney Foundation. *American journal of kidney diseases : the official journal of the National Kidney Foundation* 2011; **57:** 361-370.

10. Danesh J, Wheeler JG, Hirschfield GM*, et al.* C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. *The New England journal of medicine* 2004; **350:** 1387-1397.

11. Kroese LJ, Scheffer PG. 8-hydroxy-2'-deoxyguanosine and cardiovascular disease: a systematic review. *Current atherosclerosis reports* 2014; **16:** 452.

12. Moller P, Lohr M, Folkmann JK*, et al.* Aging and oxidatively damaged nuclear DNA in animal organs. *Free radical biology & medicine* 2010; **48:** 1275-1285.

13. Ruiz S, Pergola PE, Zager RA*, et al.* Targeting the transcription factor Nrf2 to ameliorate oxidative stress and inflammation in chronic kidney disease. *Kidney international* 2013; **83:** 1029-1041.

14. Vina J, Borras C, Miquel J. Theories of ageing. *IUBMB life* 2007; **59:** 249-254.

15. Mathisen UD, Melsom T, Ingebretsen OC*, et al.* Estimated GFR associates with cardiovascular risk factors independently of measured GFR. *Journal of the American Society of Nephrology : JASN* 2011; **22:** 927-937.

16. Rule AD, Bailey KR, Lieske JC*, et al.* Estimating the glomerular filtration rate from serum creatinine is better than from cystatin C for evaluating risk factors associated with chronic kidney disease. *Kidney international* 2013; **83:** 1169-1176.

17. Bertram JF, Douglas-Denton RN, Diouf B*, et al.* Human nephron number: implications for health and disease. *Pediatric nephrology (Berlin, Germany)* 2011; **26:** 1529-1533.

18. Stevens LA, Levey AS. Measured GFR as a confirmatory test for estimated GFR. *Journal of the American Society of Nephrology : JASN* 2009; **20:** 2305-2313.

19. Brandstrom E, Grzegorczyk A, Jacobsson L*, et al.* GFR measurement with iohexol and 51Cr-EDTA. A comparison of the two favoured GFR markers in Europe. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* 1998; **13:** 1176-1182.

20. Bird NJ, Peters C, Michell AR*, et al.* Comparison of GFR measurements assessed from single versus multiple samples. *American journal of kidney diseases : the official journal of the National Kidney Foundation* 2009; **54:** 278-288.

21. Delanaye P, Ebert N, Melsom T*, et al.* Iohexol plasma clearance for measuring glomerular filtration rate in clinical practice and research: a review. Part 1: How to measure glomerular filtration rate with iohexol? *Clinical kidney journal* 2016; **9:** 682-699.

22. Delanaye P, Melsom T, Ebert N*, et al.* Iohexol plasma clearance for measuring glomerular filtration rate in clinical practice and research: a review. Part 2: Why to measure glomerular filtration rate with iohexol? *Clinical kidney journal* 2016; **9:** 700-704.

23. Levey AS, Stevens LA, Schmid CH*, et al.* A new equation to estimate glomerular filtration rate. *Annals of internal medicine* 2009; **150:** 604-612.

24. Stevens LA, Coresh J, Feldman HI*, et al.* Evaluation of the modification of diet in renal disease study equation in a large diverse population. *Journal of the American Society of Nephrology : JASN* 2007; **18:** 2749-2757.

25. Stevens LA, Schmid CH, Greene T*, et al.* Comparative performance of the CKD Epidemiology Collaboration (CKD-EPI) and the Modification of Diet in Renal Disease (MDRD) Study equations for estimating GFR levels above 60 mL/min/1.73 m2. *American journal of kidney diseases : the official journal of the National Kidney Foundation* 2010; **56:** 486-495.

26. McFadden EC, Hirst JA, Verbakel JY*, et al.* Systematic Review and Metaanalysis Comparing the Bias and Accuracy of the Modification of Diet in Renal Disease and Chronic Kidney Disease Epidemiology Collaboration Equations in Community-Based Populations. *Clinical chemistry* 2017.

27. Levey AS, de Jong PE, Coresh J*, et al.* The definition, classification, and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. *Kidney international* 2011; **80:** 17-28.

28. Schaeffner ES, Ebert N, Delanaye P*, et al.* Two novel equations to estimate kidney function in persons aged 70 years or older. *Annals of internal medicine* 2012; **157:** 471-481.

29. Pottel H, Hoste L, Dubourg L*, et al.* An estimated glomerular filtration rate equation for the full age spectrum. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* 2016; **31:** 798-806.

30. Bjork J, Back SE, Sterner G*, et al.* Prediction of relative glomerular filtration rate in adults: new improved equations based on Swedish Caucasians and standardized plasma-creatinine assays. *Scandinavian journal of clinical and laboratory investigation* 2007; **67:** 678-695.

31. Tidman M, Sjostrom P, Jones I. A Comparison of GFR estimating formulae based upon s-cystatin C and s-creatinine and a combination of the two. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* 2008; **23:** 154-160.

32. Eckfeldt JH, Karger AB, Miller WG*, et al.* Performance in Measurement of Serum Cystatin C by Laboratories Participating in the College of American Pathologists 2014 CYS Survey. *Archives of pathology & laboratory medicine* 2015; **139:** 888-893.

33. Knight EL, Verhave JC, Spiegelman D*, et al.* Factors influencing serum cystatin C levels other than renal function and the impact on renal function measurement. *Kidney international* 2004; **65:** 1416-1421.

34. Stevens LA, Schmid CH, Greene T*, et al.* Factors other than glomerular filtration rate affect serum cystatin C levels. *Kidney international* 2009; **75:** 652-660.

35. Grubb A, Bjork J, Nyman U*, et al.* Cystatin C, a marker for successful aging and glomerular filtration rate, is not influenced by inflammation. *Scandinavian journal of clinical and laboratory investigation* 2011; **71:** 145-149.

36. Hallan SI, Coresh J, Astor BC*, et al.* International comparison of the relationship of chronic kidney disease prevalence and ESRD risk. *Journal of the American Society of Nephrology : JASN* 2006; **17:** 2275-2284.

37. Hallan SI, Ovrehus MA, Romundstad S*, et al.* Long-term trends in the prevalence of chronic kidney disease and the influence of cardiovascular risk factors in Norway. *Kidney international* 2016; **90:** 665-673.

38. Nitsch D, Grams M, Sang Y*, et al.* Associations of estimated glomerular filtration rate and albuminuria with mortality and renal failure by sex: a meta-analysis. *BMJ (Clinical research ed)* 2013; **346:** f324.

39. Peralta CA, Shlipak MG, Judd S*, et al.* Detection of chronic kidney disease with creatinine, cystatin C, and urine albumin-to-creatinine ratio and association with progression to end-stage renal disease and mortality. *JAMA : the journal of the American Medical Association* 2011; **305:** 1545-1552.

40. Levey AS, Coresh J. Chronic kidney disease. *Lancet* 2012; **379:** 165-180.

41. Denic A, Lieske JC, Chakkera HA*, et al.* The Substantial Loss of Nephrons in Healthy Human Kidneys with Aging. *Journal of the American Society of Nephrology : JASN* 2017; **28:** 313-320.

42. Hallan SI, Matsushita K, Sang Y*, et al.* Age and association of kidney measures with mortality and end-stage renal disease. *JAMA : the journal of the American Medical Association* 2012; **308:** 2349-2360.

43. L W. *Physiology of the human kidney.* Grune & Stratton: New York, 1969.

44. Zhang A, Huang S. Progress in pathogenesis of proteinuria. *International journal of nephrology* 2012; **2012:** 314251.

45. Stephen R, Jolly SE, Nally JV, Jr*., et al.* Albuminuria: when urine predicts kidney and cardiovascular disease. *Cleveland Clinic journal of medicine* 2014; **81:** 41-50.

46. 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* 2005; **112:** 969-975.

47. 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* 2010; **375:** 2073-2081.

48. Melsom T, Solbu M, Schei J*, et al.* Mild albuminuria is a risk factor for faster GFR decline in the non-diabetic population. *Kidney International Reports (2018), doi: 101016/jekir201801015* 2018.

49. Yilmaz MI, Solak Y, Saglam M*, et al.* The relationship between IL-10 levels and cardiovascular events in patients with CKD. *Clinical journal of the American Society of Nephrology : CJASN* 2014; **9:** 1207-1216.

50. Yeun JY, Levine RA, Mantadilok V*, et al.* C-Reactive protein predicts all-cause and cardiovascular mortality in hemodialysis patients. *American journal of kidney diseases : the official journal of the National Kidney Foundation* 2000; **35:** 469-476.

51. Garcia-Garcia PM, Getino-Melian MA, Dominguez-Pimentel V*, et al.* Inflammation in diabetic kidney disease. *World journal of diabetes* 2014; **5:** 431-443.

52. Libby P. Inflammation in atherosclerosis. *Arteriosclerosis, thrombosis, and vascular biology* 2012; **32:** 2045-2051.

53. O'Sullivan ED, Hughes J, Ferenbach DA. Renal Aging: Causes and Consequences. *Journal of the American Society of Nephrology : JASN* 2017; **28:** 407-420.

54. Gohda T, Niewczas MA, Ficociello LH*, et al.* Circulating TNF receptors 1 and 2 predict stage 3 CKD in type 1 diabetes. *Journal of the American Society of Nephrology : JASN* 2012; **23:** 516-524.

55. Niewczas MA, Gohda T, Skupien J*, et al.* Circulating TNF receptors 1 and 2 predict ESRD in type 2 diabetes. *Journal of the American Society of Nephrology : JASN* 2012; **23:** 507-515.

56. Skupien J, Warram JH, Niewczas MA*, et al.* Synergism between circulating tumor necrosis factor receptor 2 and HbA(1c) in determining renal decline during 5-18 years of follow-up in patients with type 1 diabetes and proteinuria. *Diabetes care* 2014; **37:** 2601-2608.

57. Aggarwal BB. Signalling pathways of the TNF superfamily: a double-edged sword. *Nature reviews Immunology* 2003; **3:** 745-756.

58. Diez-Ruiz A, Tilz GP, Zangerle R*, et al.* Soluble receptors for tumour necrosis factor in clinical laboratory diagnosis. *European journal of haematology* 1995; **54:** 1-8.

59. Al-Lamki RS, Mayadas TN. TNF receptors: signaling pathways and contribution to renal dysfunction. *Kidney international* 2015; **87:** 281-296.

60. Bertani T, Abbate M, Zoja C*, et al.* Tumor necrosis factor induces glomerular damage in the rabbit. *The American journal of pathology* 1989; **134:** 419-430.

61. Navarro JF, Mora-Fernandez C. The role of TNF-alpha in diabetic nephropathy: pathogenic and therapeutic implications. *Cytokine & growth factor reviews* 2006; **17:** 441-450.

62. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. *The Journal of clinical investigation* 2003; **111:** 1805-1812.

63. Wang A, Liu J, Li C*, et al.* Cumulative Exposure to High-Sensitivity C-Reactive Protein Predicts the Risk of Cardiovascular Disease. *Journal of the American Heart Association* 2017; **6**.

64. Pearson TA, Mensah GA, Alexander RW*, et al.* Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation* 2003; **107:** 499-511.

65. Lai W, Tang Y, Huang XR*, et al.* C-reactive protein promotes acute kidney injury via Smad3-dependent inhibition of CDK2/cyclin E. *Kidney international* 2016; **90:** 610-626.

66. Tang Y, Huang XR, Lv J*, et al.* C-reactive protein promotes acute kidney injury by impairing G1/S-dependent tubular epithelium cell regeneration. *Clinical science (London, England : 1979)* 2014; **126:** 645-659.

67. Li ZI, Chung AC, Zhou L*, et al.* C-reactive protein promotes acute renal inflammation and fibrosis in unilateral ureteral obstructive nephropathy in mice. *Laboratory investigation; a journal of technical methods and pathology* 2011; **91:** 837-851.

68. Liu F, Chen HY, Huang XR*, et al.* C-reactive protein promotes diabetic kidney disease in a mouse model of type 1 diabetes. *Diabetologia* 2011; **54:** 2713-2723.

69. Costello-White R, Ryff CD, Coe CL. Aging and low-grade inflammation reduce renal function in middle-aged and older adults in Japan and the USA. *Age (Dordrecht, Netherlands)* 2015; **37:** 9808.

70. Gupta J, Mitra N, Kanetsky PA*, et al.* Association between albuminuria, kidney function, and inflammatory biomarker profile in CKD in CRIC. *Clinical journal of the American Society of Nephrology : CJASN* 2012; **7:** 1938-1946.

71. Keller C, Katz R, Cushman M*, et al.* 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 nephrology* 2008; **9:** 9.

72. Kurashina T, Nagasaka S, Watanabe N*, et al.* Circulating TNF receptor 2 is closely associated with the kidney function in non-diabetic Japanese subjects. *Journal of atherosclerosis and thrombosis* 2014; **21:** 730-738.

73. Lin J, Hu FB, Rimm EB*, et al.* The association of serum lipids and inflammatory biomarkers with renal function in men with type II diabetes mellitus. *Kidney international* 2006; **69:** 336-342.

74. Niewczas MA, Ficociello LH, Johnson AC*, et al.* Serum concentrations of markers of TNFalpha and Fas-mediated pathways and renal function in nonproteinuric patients with type 1 diabetes. *Clinical journal of the American Society of Nephrology : CJASN* 2009; **4:** 62-70.

75. Carlsson AC, Larsson TE, Helmersson-Karlqvist J*, et al.* Soluble TNF Receptors and Kidney Dysfunction in the Elderly. *Journal of the American Society of Nephrology : JASN* 2014.

76. Hiramoto JS, Katz R, Peralta CA*, et al.* Inflammation and coagulation markers and kidney function decline: the Multi-Ethnic Study of Atherosclerosis (MESA). *American journal of kidney diseases : the official journal of the National Kidney Foundation* 2012; **60:** 225-232.

77. Shankar A, Sun L, Klein BE*, et al.* Markers of inflammation predict the long-term risk of developing chronic kidney disease: a population-based cohort study. *Kidney international* 2011; **80:** 1231-1238.

78. Bemelmans MH, Gouma DJ, Buurman WA. Tissue distribution and clearance of soluble murine TNF receptors in mice. *Cytokine* 1994; **6:** 608-615.

79. Kugler E, Cohen E, Goldberg E*, et al.* C reactive protein and long-term risk for chronic kidney disease: a historical prospective study. *Journal of nephrology* 2015; **28:** 321-327.

80. Stuveling EM, Hillege HL, Bakker SJ*, et al.* C-reactive protein is associated with renal function abnormalities in a non-diabetic population. *Kidney international* 2003; **63:** 654-661.

81. Medenwald D, Girndt M, Loppnow H*, et al.* Inflammation and renal function after a four-year follow-up in subjects with unimpaired glomerular filtration rate: results from the observational, population-based CARLA cohort. *PloS one* 2014; **9:** e108427.

82. Lin J, Hu FB, Mantzoros C*, et al.* Lipid and inflammatory biomarkers and kidney function decline in type 2 diabetes. *Diabetologia* 2010; **53:** 263-267.

83. Pavkov ME, Nelson RG, Knowler WC*, et al.* Elevation of circulating TNF receptors 1 and 2 increases the risk of end-stage renal disease in American Indians with type 2 diabetes. *Kidney international* 2015; **87:** 812-819.

84. Pavkov ME, Weil EJ, Fufaa GD*, et al.* Tumor necrosis factor receptors 1 and 2 are associated with early glomerular lesions in type 2 diabetes. *Kidney international* 2015.

85. Coca SG, Nadkarni GN, Huang Y*, et al.* Plasma Biomarkers and Kidney Function Decline in Early and Established Diabetic Kidney Disease. *Journal of the American Society of Nephrology : JASN* 2017.

86. Carlsson AC, Nordquist L, Larsson TE*, et al.* Soluble Tumor Necrosis Factor Receptor 1 Is Associated with Glomerular Filtration Rate Progression and Incidence of Chronic Kidney Disease in Two Community-Based Cohorts of Elderly Individuals. *Cardiorenal medicine* 2015; **5:** 278-288.

87. Cross CE, Halliwell B, Borish ET*, et al.* Oxygen radicals and human disease. *Annals of internal medicine* 1987; **107:** 526-545.

88. Sies H, Berndt C, Jones DP. Oxidative Stress. *Annual Review of Biochemistry* 2017; **86:** 715-748.

89. Harrison D, Griendling KK, Landmesser U*, et al.* Role of oxidative stress in atherosclerosis. *The American journal of cardiology* 2003; **91:** 7a-11a.

90. Pierce GL, Donato AJ, LaRocca TJ*, et al.* Habitually exercising older men do not demonstrate age-associated vascular endothelial oxidative stress. *Aging cell* 2011; **10:** 1032-1037.

91. Vlassara H, Torreggiani M, Post JB*, et al.* Role of oxidants/inflammation in declining renal function in chronic kidney disease and normal aging. *Kidney international Supplement* 2009**:** S3-11.

92. Mesaros C, Arora JS, Wholer A*, et al.* 8-Oxo-2'-deoxyguanosine as a biomarker of tobacco-smoking-induced oxidative stress. *Free radical biology & medicine* 2012; **53:** 610-617.

93. Akagi S, Nagake Y, Kasahara J*, et al.* Significance of 8-hydroxy-2'-deoxyguanosine levels in patients with chronic renal failure. *Nephrology (Carlton, Vic)* 2003; **8:** 192-195.

94. Dounousi E, Papavasiliou E, Makedou A*, et al.* Oxidative stress is progressively enhanced with advancing stages of CKD. *American journal of kidney diseases : the official journal of the National Kidney Foundation* 2006; **48:** 752-760.

95. Himmelfarb J, McMonagle E, McMenamin E. Plasma protein thiol oxidation and carbonyl formation in chronic renal failure. *Kidney international* 2000; **58:** 2571-2578.

96. Karamouzis I, Sarafidis PA, Karamouzis M*, et al.* Increase in oxidative stress but not in antioxidant capacity with advancing stages of chronic kidney disease. *American journal of nephrology* 2008; **28:** 397-404.

97. Kaya Y, Ari E, Demir H*, et al.* Accelerated atherosclerosis in haemodialysis patients; correlation of endothelial function with oxidative DNA damage. *Nephrology, dialysis,*

transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 2012; **27:** 1164-1169.

98. 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 international* 2004; **65:** 1009-1016.

99. Prabhakar S, Starnes J, Shi S*, et al.* Diabetic nephropathy is associated with oxidative stress and decreased renal nitric oxide production. *Journal of the American Society of Nephrology : JASN* 2007; **18:** 2945-2952.

100. Ramos LF, Shintani A, Ikizler TA*, et al.* Oxidative stress and inflammation are associated with adiposity in moderate to severe CKD. *Journal of the American Society of Nephrology : JASN* 2008; **19:** 593-599.

101. Jun M, Venkataraman V, Razavian M*, et al.* Antioxidants for chronic kidney disease. *The Cochrane database of systematic reviews* 2012; **10:** Cd008176.

102. Alborzi P, Patel NA, Peterson C*, et al.* Paricalcitol reduces albuminuria and inflammation in chronic kidney disease: a randomized double-blind pilot trial. *Hypertension* 2008; **52:** 249-255.

103. Block GA, Pergola PE, Inker L*, et al.*: Initial Data Report from "CARDINAL": A Phase 2/3 Study of Bardoxolone Methyl in Patients with Alport Syndrome. In *Abstract form, American Society of Nephrology ASN Kidney Week*, New Orleans LA, USA, 2017

104. Hadjiyannakos D, Filiopoulos V, Trompouki S*, et al.* Treatment with oral paricalcitol in daily clinical practice for patients with chronic kidney disease stage 3–4: a preliminary study. *Clinical kidney journal* 2013; **6:** 164-168.

105. Nangaku M, Shimazaki R, Akizawa T: Bardoxolone Methyl Improved GFR Measured by Standard Inulin Clearance: The TSUBAKI Study. In *Abstract form, American Society of Nephrology ASN Kidney Week*, New Orleans, LA, USA, 2017

106. Pergola PE, Raskin P, Toto RD*, et al.* Bardoxolone Methyl and Kidney Function in CKD with Type 2 Diabetes. *New England Journal of Medicine* 2011; **365:** 327-336.

107. Saldanha JF, Leal Vde O, Stenvinkel P*, et al.* Resveratrol: why is it a promising therapy for chronic kidney disease patients? *Oxidative medicine and cellular longevity* 2013; **2013:** 963217.

108. Semba RD, Ferrucci L, Bartali B*, et al.* Resveratrol Levels and All-Cause Mortality in Older Community-Dwelling Adults. *JAMA internal medicine* 2014.

109. Thethi TK, Bajwa MA, Ghanim H*, et al.* Effect of paricalcitol on endothelial function and inflammation in type 2 diabetes and chronic kidney disease. *Journal of diabetes and its complications* 2015; **29:** 433-437.

110. Chin MP, Bakris GL, Block GA*, et al.* Bardoxolone Methyl Improves Kidney Function in Patients with Chronic Kidney Disease Stage 4 and Type 2 Diabetes: Post-Hoc Analyses from Bardoxolone Methyl Evaluation in Patients with Chronic Kidney Disease and Type 2 Diabetes Study. *American journal of nephrology* 2018; **47:** 40-47.

111. Himmelfarb J, Ikizler TA, Ellis C*, et al.* Provision of antioxidant therapy in hemodialysis (PATH): a randomized clinical trial. *Journal of the American Society of Nephrology : JASN* 2014; **25:** 623-633.

112. Saldanha JF, Leal VO, Rizzetto F*, et al.* Effects of Resveratrol Supplementation in Nrf2 and NF-kappaB Expressions in Nondialyzed Chronic Kidney Disease Patients: A Randomized, Double-Blind, Placebo-Controlled, Crossover Clinical Trial. *Journal of renal nutrition : the official journal of the Council on Renal Nutrition of the National Kidney Foundation* 2016; **26:** 401-406.

113. Machowetz A, Poulsen HE, Gruendel S*, et al.* Effect of olive oils on biomarkers of oxidative DNA stress in Northern and Southern Europeans. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 2007; **21:** 45-52.

114. Broedbaek K, Ribel-Madsen R, Henriksen T*, et al.* Genetic and environmental influences on oxidative damage assessed in elderly Danish twins. *Free radical biology & medicine* 2011; **50:** 1488-1491.

115. Conti S, Cassis P, Benigni A. Aging and the renin-angiotensin system. *Hypertension* 2012; **60:** 878-883.

116. Daehn I, Casalena G, Zhang T*, et al.* Endothelial mitochondrial oxidative stress determines podocyte depletion in segmental glomerulosclerosis. *The Journal of clinical investigation* 2014; **124:** 1608-1621.

117. Sastre J, Pallardo FV, Vina J. The role of mitochondrial oxidative stress in aging. *Free radical biology & medicine* 2003; **35:** 1-8.

118. Kakimoto M, Inoguchi T, Sonta T*, et al.* Accumulation of 8-hydroxy-2'-deoxyguanosine and mitochondrial DNA deletion in kidney of diabetic rats. *Diabetes* 2002; **51:** 1588-1595.

119. Richter C, Park JW, Ames BN. Normal oxidative damage to mitochondrial and nuclear DNA is extensive. *Proceedings of the National Academy of Sciences of the United States of America* 1988; **85:** 6465-6467.

120. Poulsen HE, Nadal LL, Broedbaek K*, et al.* Detection and interpretation of 8-oxodG and 8-oxoGua in urine, plasma and cerebrospinal fluid. *Biochimica et biophysica acta* 2014; **1840:** 801-808.

121. Gan W, Nie B, Shi F*, et al.* Age-dependent increases in the oxidative damage of DNA, RNA, and their metabolites in normal and senescence-accelerated mice analyzed by LC-MS/MS: urinary 8-oxoguanosine as a novel biomarker of aging. *Free radical biology & medicine* 2012; **52:** 1700-1707.

122. Qi H, Casalena G, Shi S*, et al.* Glomerular Endothelial Mitochondrial Dysfunction Is Essential and Characteristic of Diabetic Kidney Disease Susceptibility. *Diabetes* 2017; **66:** 763-778.

123. Hinokio Y, Suzuki S, Hirai M*, et al.* Urinary excretion of 8-oxo-7, 8-dihydro-2'-deoxyguanosine as a predictor of the development of diabetic nephropathy. *Diabetologia* 2002; **45:** 877-882.

124. Tucker PS, Scanlan AT, Dalbo VJ. Chronic kidney disease influences multiple systems: describing the relationship between oxidative stress, inflammation, kidney damage, and concomitant disease. *Oxidative medicine and cellular longevity* 2015; **2015:** 806358.

125. Jacobsen BK, Eggen AE, Mathiesen EB*, et al.* Cohort profile: the Tromso Study. *International journal of epidemiology* 2012; **41:** 961-967.

126. Eggen AE, Mathiesen EB, Wilsgaard T*, et al.* The sixth survey of the Tromso Study (Tromso 6) in 2007-08: collaborative research in the interface between clinical medicine and epidemiology: study objectives, design, data collection procedures, and attendance in a multipurpose population-based health survey. *Scandinavian journal of public health* 2013; **41:** 65-80.

127. Mathisen UD, Melsom T, Ingebretsen OC*, et al.* Ambulatory blood pressure is associated with measured glomerular filtration rate in the general middle-aged population. *Journal of hypertension* 2012; **30:** 497-504.

128. Eriksen BO, Mathisen UD, Melsom T*, et al.* Cystatin C is not a better estimator of GFR than plasma creatinine in the general population. *Kidney international* 2010; **78:** 1305-1311.

129. Jacobsson L. A method for the calculation of renal clearance based on a single plasma sample. *Clinical physiology (Oxford, England)* 1983; **3:** 297-305.

130. Nilsson-Ehle P. Iohexol clearance for the determination of glomerular filtration rate: 15 years´ experience in clinical practice. . *eJIFCC* 2006; **13(2)**.

131. Eriksen BO, Stefansson VT, Jenssen TG*, et al.* Elevated blood pressure is not associated with accelerated glomerular filtration rate decline in the general non-diabetic middle-aged population. *Kidney international* 2016; **90:** 404-410.

132. Aziz N, Nishanian P, Mitsuyasu R*, et al.* Variables that affect assays for plasma cytokines and soluble activation markers. *Clinical and diagnostic laboratory immunology* 1999; **6:** 89-95.

133. Eriksen BO, Lochen ML, Arntzen KA*, et al.* Estimated and Measured GFR Associate Differently with Retinal Vasculopathy in the General Population. *Nephron* 2015; **131:** 175-184.

134. Inker LA, Schmid CH, Tighiouart H*, et al.* Estimating glomerular filtration rate from serum creatinine and cystatin C. *The New England journal of medicine* 2012; **367:** 20-29.

135. Leffondre K, Boucquemont J, Tripepi G*, et al.* Analysis of risk factors associated with renal function trajectory over time: a comparison of different statistical approaches. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* 2015; **30:** 1237-1243.

136. Royston P, Sauerbrei W. *Multivariable Model-building*. Chichester: John Wiley & Sons Ltd, 2008.

137. Foster MC, Levey AS, Inker LA*, et al.* Non-GFR Determinants of Low-Molecular-Weight Serum Protein Filtration Markers in the Elderly: AGES-Kidney and MESA-Kidney. *American journal of kidney diseases : the official journal of the National Kidney Foundation* 2017.

138. Melsom T, Fuskevag OM, Mathisen UD*, et al.* Estimated GFR Is Biased by Non-Traditional Cardiovascular Risk Factors. *American journal of nephrology* 2015; **41:** 7-15.

139. Liu G, Deng Y, Sun L*, et al.* Elevated plasma tumor necrosis factor-alpha receptor 2 and resistin are associated with increased incidence of kidney function decline in Chinese adults. *Endocrine* 2016; **52:** 541-549.

140. Glymour MM, Weuve J, Berkman LF*, et al.* When is baseline adjustment useful in analyses of change? An example with education and cognitive change. *American journal of epidemiology* 2005; **162:** 267-278.

141. Boucquemont J, Heinze G, Jager KJ*, et al.* Regression methods for investigating risk factors of chronic kidney disease outcomes: the state of the art. *BMC nephrology* 2014; **15:** 45.

142. Montez-Rath ME, Winkelmayer WC, Desai M. Addressing missing data in clinical studies of kidney diseases. *Clinical journal of the American Society of Nephrology : CJASN* 2014; **9:** 1328-1335.

143. Har R, Scholey JW, Daneman D*, et al.* The effect of renal hyperfiltration on urinary inflammatory cytokines/chemokines in patients with uncomplicated type 1 diabetes mellitus. *Diabetologia* 2013; **56:** 1166-1173.

144. Har RL, Reich HN, Scholey JW*, et al.* The urinary cytokine/chemokine signature of renal hyperfiltration in adolescents with type 1 diabetes. *PloS one* 2014; **9:** e111131.

145. Kodera R, Shikata K, Kataoka HU*, et al.* Glucagon-like peptide-1 receptor agonist ameliorates renal injury through its anti-inflammatory action without lowering blood glucose level in a rat model of type 1 diabetes. *Diabetologia* 2011; **54:** 965-978.

146. Aderka D, Engelmann H, Maor Y*, et al.* Stabilization of the bioactivity of tumor necrosis factor by its soluble receptors. *The Journal of experimental medicine* 1992; **175:** 323-329.

147. Keller C, Katz R, Sarnak MJ*, et al.* Inflammatory biomarkers and decline in kidney function in the elderly: the Cardiovascular Health Study. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* 2010; **25:** 119-124.

148. Kushner I, Rzewnicki D, Samols D. What does minor elevation of C-reactive protein signify? *The American journal of medicine* 2006; **119:** 166.e117-128.

149. 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 international* 2007; **71:** 239-244.

150. Broedbaek K, Siersma V, Henriksen T*, et al.* Urinary markers of nucleic acid oxidation and long-term mortality of newly diagnosed type 2 diabetic patients. *Diabetes care* 2011; **34:** 2594-2596.

151. Li W, Khor TO, Xu C*, et al.* Activation of Nrf2-antioxidant signaling attenuates NFkappaB-inflammatory response and elicits apoptosis. *Biochemical pharmacology* 2008; **76:** 1485-1489.

152. de Zeeuw D, Akizawa T, Audhya P*, et al.* Bardoxolone methyl in type 2 diabetes and stage 4 chronic kidney disease. *The New England journal of medicine* 2013; **369:** 2492-2503.

153. Baigent C, Lennon R. Should We Increase GFR with Bardoxolone in Alport Syndrome? *Journal of the American Society of Nephrology : JASN* 2018; **29:** 357-359.

154. Barregard L, Moller P, Henriksen T*, et al.* Human and methodological sources of variability in the measurement of urinary 8-oxo-7,8-dihydro-2'-deoxyguanosine. *Antioxidants & redox signaling* 2013; **18:** 2377-2391.