Chapter 1

General introduction and aims of the thesis
Introduction

For long no clear definition of impaired renal function and increased urinary protein loss existed. Researchers and clinicians used arbitrary terminology and cut-offs to indicate renal disease. In 2002 the Kidney Disease Outcomes Quality Initiative (KDOQI) organization introduced for the first time clear guidelines for the definition and classification of chronic kidney disease (CKD). Chronic kidney disease was defined based on the presence of kidney damage (such as manifest for example by elevated albuminuria), or glomerular filtration rate (GFR) less than 60 mL/min/1.73m² for at least 3 months and was classified into five stages on the level of GFR, as shown in Figure 1.(1)

<table>
<thead>
<tr>
<th>GFR (mL/min/1.73m²)</th>
<th>Normoalbuminuria</th>
<th>Micro/macroalbuminuria</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;90</td>
<td>No CKD</td>
<td>Stage 1</td>
</tr>
<tr>
<td>60-75</td>
<td></td>
<td>Stage 2</td>
</tr>
<tr>
<td>30-60</td>
<td></td>
<td>Stage 3</td>
</tr>
<tr>
<td>15-30</td>
<td></td>
<td>Stage 4</td>
</tr>
<tr>
<td>&lt;15</td>
<td></td>
<td>Stage 5</td>
</tr>
</tbody>
</table>

Figure 1. Staging of chronic kidney disease by glomerular filtration rate according to the 2002 KDOQI guidelines. (1)

In less than a decade, the definition and classification for chronic kidney disease increased attention for chronic kidney disease enormously in clinical practice, research and public health, but also generated debate.(2,3) In 2009 it was the position of KDIGO (Kidney Disease: Improving Global Outcomes) and KDOQI organizations that a new definition and classification of chronic kidney disease might be necessary, and that these should reflect patient prognosis. In their opinion an analysis of epidemiological data should answer key questions underlying the debate. Therefore a collaborative meta-analysis of general population cohorts was started by the CKD Prognosis Consortium, that showed that an eGFR less than 60 mL/min/1.73m² and an ACR of more than 1.1 mg/mmol (10 mg/g) are independent predictors of overall and cardiovascular mortality risk in the general population.(4) In another report of this consortium it was shown that a lower eGFR and higher albuminuria are also risk factors for end-stage renal disease (ESRD), acute kidney injury and progressive CKD in both general and high-risk populations, independent of each other and of cardiovascular risk factors.(5) So, it has been shown that not only GFR, but also higher albuminuria is associated with a worse
cardiovascular and renal prognosis. These findings had major impact. Based upon these data KDIGO published new guidelines for the definition and classification of chronic kidney disease, in which they took into account this prominent role of albuminuria (Figure 2).

\[\text{Prognosis of CKD by GFR and Albuminuria Categories: KDIGO 2012}\]

\[\text{Persistent albuminuria categories Description and range}\]

\begin{align*}
\text{A1} & : \text{Normal to mildly increased} \\
& <30 \text{ mg/g} <3 \text{ mg/mmol} \\
\text{A2} & : \text{Moderately increased} \\
& 30-300 \text{ mg/g} 3-30 \text{ mg/mmol} \\
\text{A3} & : \text{Severely increased} \\
& >300 \text{ mg/g} >30 \text{ mg/mmol}
\end{align*}

\begin{align*}
\text{G1} & : \text{Normal or high} \geq 90 \\
\text{G2} & : \text{Mildly decreased} 60-89 \\
\text{G3a} & : \text{Mildly to moderately decreased} 45-59 \\
\text{G3b} & : \text{Moderately to severely decreased} 30-44 \\
\text{G4} & : \text{Severely decreased} 15-29 \\
\text{G5} & : \text{Kidney failure} <15
\end{align*}

\[\text{Figure 2. Staging of chronic kidney disease by glomerular filtration rate and albuminuria according to the 2012 KDIGO guidelines (3)}\]

**Aim of the thesis**

Although valuable new information on the impact of albuminuria has been obtained in the last decade and albuminuria has achieved a place in defining and staging of chronic kidney disease, still many questions are unresolved yet. The studies described in this thesis cluster around a number of these questions. First, what are the mechanisms underlying albumin leakage? Second, what causes a rise in albuminuria, and third, why is albuminuria predictive for not only renal but also vascular damage?

With respect to the first question, *the mechanisms of albuminuria*, this is addressed in Chapters 3, 5 and 7. They deal on the evidence that albuminuria without the presence of diabetes and hypertension may have a comparable impact as microalbuminuria in subjects
with diabetes and/or hypertension (Chapter 3). These findings are contradictory to what was previously thought: microalbuminuria was seen as just the consequence of diabetes and hypertension and reflecting the severity of these underlying diseases. In Chapter 5 we tried to tackle the question whether albuminuria should be interpreted as a glomerular or tubular disease. In Chapter 7 we studied the renal handling of urate, which is a marker of proximal tubular reabsorption, in relation to albuminuria.

With respect to the second question, what causes progressive albuminuria, we evaluated the factors associated with a rise in albuminuria over time (Chapter 4). In addition, we evaluated in detail the impact of a high salt intake on albuminuria (and of serum uric acid) and studied the impact of salt intake on the development of de novo hypertension (Chapter 6).

In response to the third question why is albuminuria predictive for not only renal but also vascular damage we evaluated in Chapter 8 whether cardiac markers Troponin T and N-terminal pro-B-type natriuretic peptide (NT-pro-BNP) levels are cross-sectionally associated with estimated glomerular filtration rate (eGFR) and albuminuria, and whether these markers are associated with cardiovascular outcome, independent of eGFR, albuminuria and conventional cardiovascular risk factors. Chapter 3 also touches this question.

The PREVEND cohort

All analyses described in this thesis are performed using data of the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study that is carried out in Groningen, the Netherlands. The PREVEND study is a prospective cohort study with sequential follow-up designed to investigate the natural course of albuminuria and its relation to renal and cardiovascular outcome. In summary, in 1997-1998 all inhabitants of the city of Groningen aged 28–75 years were sent a questionnaire on demographics, disease history, smoking habits, use of medication and a vial to collect a first-morning-void urine sample. These were sent by mail to a central laboratory for assessment. Of these subjects, 40,856 responded (47.8%). From these subjects, the PREVEND cohort was selected with the aim to create a cohort enriched for the presence of high albuminuria. After exclusion of patients with type 1 diabetes mellitus (defined as requiring the use of insulin) and pregnant females (defined by self report), all subjects with a urinary albumin concentration of >10 mg/L (7,768) were invited, of which 6,000 participated. Furthermore, a randomly selected control group with a urinary albumin concentration of <10 mg/L (3,394) was invited, of which 2,592 participated. These 8,592 subjects constitute the actual PREVEND cohort and were studied in more detail.
These 8,592 subjects visited an outpatient clinic at regular intervals for detailed assessment on their health status. The first screening round took place in 1997-1998. In 2001-2003 6,894 subjects completed the second screening round. The third screening round was held after a median follow-up of 2.2 years in 2003-2006, which was attended by 5,862 subjects. The fourth screening round was completed by 5,086 participants after a median follow-up of 2.9 years (Figure 3).

Figure 3. Flow chart of the PREVEND cohort from 1997 to 2009.
At the baseline and follow-up screening rounds, all participants completed a questionnaire on demographics, disease history, smoking habits and use of medication. Information on drug use was completed with data from community pharmacies. For each screening round participants visited an outpatient unit. During this visit, height, weight and blood pressure was measured, and a fasting blood sample was drawn. In addition, subjects collected urine for two consecutive periods of 24 hours. Vital statistics were retrieved by record linkage.

**Outline of studies presented in this thesis**

For many years 24h-urinary albumin excretion (UAE) used to be the gold standard for measuring albuminuria. In the Netherlands in general people are that interested in optimal health care that they are cooperative enough to collect 24 hour urine samples. However, in many other countries urine collections over 24 hour are not feasible and spot urine samples are used to measure albumin over creatinine ratio. Therefore, new international guidelines advocate the use of albumin to creatinine ratio (ACR) instead of 24h-urinary albumin excretion for staging albuminuria. Concern has been expressed that the use of ACR instead of 24h-UAE may result in misclassification because of interindividual differences in the urinary creatinine excretion. In **Chapter 2** we studied whether such misclassification occurs frequently, and if so, what the consequences of misclassification might be. As such a research question more reliable could be answered in a group of subjects with a wide range in albuminuria, and as PREVEND itself includes only a few subjects with albumin excretions over 300 mg/24h, we planned this study as a cooperation between the PREVEND and the RENAAL investigators. The RENAAL study is a multinational double-blind randomised placebo-controlled study and evaluated the renal protective effects of losartan in patients with type 2 diabetes and nephropathy.

Many studies the last years evaluated the association between albuminuria and cardiovascular events.(6-11) Albuminuria, or more specific microalbuminuria, is often regarded as the consequence of end-organ damage due to diabetes mellitus and/or hypertension, and as such associated with an increased risk for cardiovascular events. It has not been studied thus far whether isolated microalbuminuria, which is microalbuminuria in absence of diabetes, hypertension and a cardiovascular disease history, has clinical relevance. This is investigated in **Chapter 3**.

Various studies evaluated the factors that are associated with a progressive fall in GFR.(12-15) As an impaired GFR is a late consequence of kidney damage while albuminuria is considered an early consequence of kidney damage, it might well be that the factors associated with a fall in GFR differ from the factors that lead to a rise in albuminuria. To that purpose, we studied
in **Chapter 4** factors associated with progressive albuminuria. When these factors can be specified, they can be of help to delineate measures to prevent progressive albuminuria, and thus progressive kidney damage.

In **Chapter 5** we tried to more specifically study the mechanisms that underlie progressive albuminuria. Is it related to glomerular and/or proximal or distal tubular damage? To that purpose we measured urinary markers representing damage to different parts of the nephron in subjects with progressive as compared to subjects with stable albuminuria. IgG and IgG-4 were measured as glomerular markers; KIM-1, NAG, β2-microglobulin, Cystatin C as proximal tubular markers; H-FABP as marker of distal tubular damage marker; and finally NGAL and MCP-1 as inflammatory markers.

It has been shown earlier in the PREVEND database that a high sodium intake cross-sectionally was associated with a higher albuminuria. As albuminuria is considered to be the consequence of vascular endothelial damage, we next studied whether an increased salt intake is also related to a rise in albuminuria over time and to a rise in serum uric acid (which can also be regarded as the consequence of endothelial damage). In that study, that was performed as a collaboration between the Harvard University in Boston and the University Medical Center of Groningen, we moreover, hypothesized that a chronically high sodium intake would be associated with incident hypertension among those with higher serum uric acid and urinary albumin excretion (Chapter 6).

A high serum uric acid is associated with future cardiovascular events. In several studies serum uric acid concentration has also been found to be associated with albuminuria. This association has been explained as serum uric acid causing vascular damage, resulting in an increase in albuminuria. In **Chapter 7** we studied whether the opposite relation may also be true, i.e. whether albuminuria can be a cause for an increase in serum uric acid, and -if so- what the underlying mechanism would be.

It has been suggested that markers of cardiovascular damage as troponins and natriuretic peptides can be falsely elevated in subjects with impaired kidney function because of decreased renal clearance. The value of these biomarkers in subjects with impaired kidney function and/or albuminuria has therefore been debated. We analyzed in **Chapter 8**, first, whether high sensitive troponin T (hsTnT) and N-terminal pro-B-type natriuretic peptide (NT-pro-BNP) levels are cross-sectionally associated with eGFR and albuminuria, and second, whether these markers are associated with cardiovascular outcome, independent of eGFR, albuminuria and conventional cardiovascular risk factors.
References


