Introduction
Kidney disease is an important problem worldwide. The prevalence of chronic kidney disease (CKD) is steadily increasing, with more than 10% of the population affected. Lifestyle-related conditions like obesity, hypertension and diabetes mellitus (DM) type 2 significantly contribute to the growing population of CKD patients. Additionally, the ageing population also plays a major role in the increased prevalence of CKD, with more than 20% of the people older than 60 years affected and 35% of the ones older than 70 years.

Kidney disease is defined as abnormalities in kidney structure or function, with implications for general health. When present for more than 3 months, the disease is qualified as chronic. The stage of CKD is based on glomerular filtration rate (GFR; <60 mL/min/1.73m²), category and extent of albuminuria (>30 mg/g creatinine). In 2012, the KDIGO clinical practice guideline for chronic kidney disease added “cause of the disease” (e.g. kidney disease in light of a systemic disease like diabetes-induced nephropathy, due to autosomal dominant polycystic kidney disease or as a result of focal segmental glomerulosclerosis) to this qualification, because it can affect outcomes and the choice of treatment. Acute kidney disease (an increase in serum creatinine by 50% within 7 days; or an increase in serum creatinine by 0.3 mg/dL within 2 days) was thought to be harmless for the kidney and reversible over time, provided that patients survived the condition. However, nowadays it is recognized that even small and transient changes in serum creatinine are associated with an increased risk of progression to CKD and even increased risk of mortality. The opposite also holds true; with CKD being a risk factor for the development of acute kidney injury (AKI). An integrated approach of the entire clinical syndrome of diminished GFR, with acute and chronic stages, is proposed, rather than subdividing and treating both disease entities separately.

As above stated, an important cause for the development of CKD includes hypertension, which has a prevalence of 20-40% in developed countries and is predicted to affect more than 1.5 billion people worldwide in 2025. Hypertension-induced kidney injury can lead to microvascular damage, peritubular capillary loss and the infiltration of inflammatory cells. This in turn leads to oxidative stress and intrarenal activation of the renin-angiotensin-aldosterone-system (RAAS), which finally has end-stage renal disease (ESRD) as a consequence. Other factors leading to CKD include obesity and DM, two conditions which have increased substantially in the past decade. The prevalence of DM in adults is estimated to be 6.4%, affecting 285 million people in 2010 and is expected to increase to 7.7% by 2030 with a total of 439 million cases. Especially individuals with the metabolic syndrome are prone to develop CKD. One explanation for the development of kidney disease in patients with metabolic syndrome is that they might share the same pathophysiological pathway: endothelial dysfunction in combination with oxidative stress. The combined presence of CKD and the metabolic syndrome in turn significantly increases the risk of cardiovascular disease. Another important cause of the increase in CKD is the ageing population. The older population continues to increase, with 420 million persons of 65 years and older
in 2000 and an expected increase of these individuals to 550-973 million in 2030.\textsuperscript{13,14} Structural and functional changes take place in the kidneys as a consequence of aging. Furthermore, the repair capacity in the older kidney is decreased, among others due to impaired adaptive and homeostatic mechanisms leading to cellular senescence. Older people are therefore more prone to develop kidney disease and because of increased co-morbidity, treating these patients is a challenge.

Patients with CKD are vulnerable for the development of systemic complications (\textit{Figure 1}), especially premature cardiovascular disease.\textsuperscript{15} Acute myocardial infarction\textsuperscript{16}, stroke\textsuperscript{17} and heart failure\textsuperscript{18} are more prevalent disorders in patients with CKD when compared to people with normal kidney function. In addition, a direct link between kidney function and damage of extrarenal tissues has been suggested. For example, the cardiorenal syndrome describes the interaction between deteriorated cardiac function and kidney disease.\textsuperscript{19} Kidney disease accelerates the initiation and progression of atherosclerosis and enhances the development of vascular calcifications.\textsuperscript{20} For these reasons early detection of CKD is crucial, so that intervention can take place in an early stage with the goal to dampen or prevent the progression of both renal and cardiovascular disease.\textsuperscript{21} However, the asymptomatic course of the early stages of

\textbf{Figure 1. Factors associated with increased risk of kidney disease (blue), stages of disease (green) and complications (purple)}

Horizontal arrows show transitions between stage, solid arrows pointing from left to right indicate progression of disease. Arrowheads pointing from right to left indicate remission. Grey triangles demonstrate changes in glomerular filtration and kidney damage. \textit{Figure adapted from Eckardt et al.}\textsuperscript{2}
CKD makes this a difficult task. Screening programmes targeted at known diabetics, hypertensives and those over 55 years have been described to detect 93% of all CKD in the community.22

**Treatment of renal disease**

Therapies to slow down the rate of progression of CKD, independent of treatment of the underlying disease, are centered around blood pressure control and, if present, reduction of proteinuria. Angiotensin Converting Enzyme (ACE) inhibitors or Angiotensin Receptor Blockers (ARBs) are recommended as first-line therapy. In addition to RAAS inhibitors however, multimodal approaches including lifestyle changes and multidrug therapy will be required in most cases to optimize control of the several risk factors for CKD and related cardiovascular morbidity. Unfortunately, many patients with CKD finally progress to ESRD demanding dialysis of renal transplantation. In the Netherlands, the number of patients on renal replacement therapy i.e. dialysis and renal transplantation, increased with 18% over the last five years.23 Renal transplantation, however, is associated with better outcomes in regard to quality of life and reduced mortality when compared with maintenance dialysis therapy and therefore the preferred renal replacement treatment option. There is a marked increase in patients with a functioning renal transplant (Figure 2). As a consequence, costs due to the treatment of kidney disease enormously pressure the health care system of many countries, including the Netherlands.24,25

![Figure 2. Number of patients on renal replacement therapy in the Netherlands](image)

*Figure adapted from ‘Renine, stichting registratie nierfunctievervanging Nederland’.*23
Over the last decades, the treatment of ESRD, including renal replacement therapy, has improved significantly. Especially renal transplantation improved the quality of life and life expectancy of patients on dialysis.\textsuperscript{26} However, even after successful transplantation, morbidity and mortality rates are higher compared to those in the general population.\textsuperscript{27} Of those patients who survive the first year after a successful renal transplantation, 50\% of the grafts originating from deceased or living donors are lost within 12-15 years after transplantation. One of the main causes for graft loss is patient mortality with a functioning graft.

Cardiovascular disease, including hypertension, endothelial dysfunction and atherosclerosis, is the leading cause of death after transplantation.\textsuperscript{28} The prevalence is about five times higher when compared to the general population. In addition to that, infections, metabolic abnormalities and adverse effects of immunosuppressive medications significantly impact on the long-term morbidity and quality of life of renal transplant recipients (RTR).\textsuperscript{29} Therefore, much attention should be paid to find new treatment strategies to prevent or at least dampen the high morbidity and mortality burden in CKD patients and RTR.

**Gasotransmitters**

The biological and also the medical importance of the endogenously-generated gaseous transmitter nitric oxide (NO) was recognized by the 1998 Nobel Prize for Medicine/Physiology. NO together with hydrogen sulfide (H$_2$S) and carbon monoxide (CO) make up the family of labile biological mediators called “gasotransmitters”.\textsuperscript{30} Recently, it has been suggested that ammonia (NH$_3$) is the fourth member of the gasotransmitter family.\textsuperscript{31} Historically, these gases were considered to be highly toxic and hazardous to the environment. However, it was found that under normal physiological conditions in mammals these molecules are enzymatically regulated and endogenously produced. Due to this discovery, the biological and physiological functions of these gases have been re-evaluated and over the last decades, it has become apparent that each gasotransmitter possesses a number of physiological actions.\textsuperscript{32-34} Furthermore, recent studies have revealed that most likely these three gaseous molecules have redundant or overlapping pathophysiological functions, often involving similar molecular targets (Figure 3).\textsuperscript{35,36}

Concurrent with unravelling the biological functions, the translational research on gasotransmitters has exploded. Pharmaceutical interventions that alter the production or levels of gaseous mediators (either endogenous or exogenous) themselves or modulate the signalling pathways they act by, are already in use in clinical practice, and still, more are being evaluated in preclinical models and clinical trials. Furthermore, given the widespread (patho)physiological functions of these gaseous molecules, elucidating the therapeutic potential of gasotransmitters might lead to new treatment modalities in the battle against renal disease.
Introduction

Aim of this thesis

The aim of this thesis is to investigate the role of gasotransmitters as predictor of graft failure and mortality in human renal transplantation and whether these hypothesized associations relate to cardiovascular and renal risk factors. To further substantiate the human findings we therapeutically intervened in animal models of renal and cardiac disease with the goal to elucidate the therapeutic potential of gasotransmitters in the experimental setting.

In chapter 2 we review the role of the gaseous mediator H$_2$S in renal physiology, disease and renal transplantation. In part 1, we investigate the role of two gasotransmitters, i.e. H$_2$S and NO, in human renal transplantation. Since H$_2$S metabolites such as sulfate and thiosulfate are associated with a beneficial cardiovascular risk profile and reduced all-cause mortality in RTR, in chapter 3 we examine whether these associations also

Figure 3. NO, CO and H$_2$S share unique and overlapping pathways by which they exert their protective effects

All three gaseous molecules play a role in cellular apoptosis, inflammation and proliferation, by influencing mitogen-activated protein (MAP) kinases. They can also induce vascular endothelial growth factor (VEGF), which is an important pro-survival factor that is important in angiogenesis. By downregulation of nuclear factor kappa B (NF-κB) inflammation is reduced. In addition, they regulate oxidant/antioxidant balance through nuclear factor-like-2 (Nrf-2). NO and H$_2$S together regulate vascular smooth muscle relaxation via ATP-sensitive potassium (K$_{ATP}$) channels. Both NO and CO regulate vasodilatation and neutrophil adhesion through the guanylate cyclase (GC) pathway where guanosine triphosphate (GTP) is converted to cyclic guanosine monophosphate (cGMP) by GC. H$_2$S inhibits conversion from cGMP to guanosine monophosphate (GMP) by inhibiting phosphodiesterase-5 (PDE-5) thereby stabilizing the present cGMP. **Figure adapted from Snijder et al.**

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hold true for graft survival in RTR. In RTR, oxidative stress contributes significantly to graft failure, morbidity and mortality. In humans, serum free sulfhydryl groups (reduced thiols, R-SH) are the transducers of redox-regulated events, since free thiols rapidly react with free (oxidative) radicals to form disulfides. In chapter 4 we test our hypothesis that high levels of free thiols as a reflection of a favorable redox status are positively associated with cardiovascular risk parameters, patient and graft survival in RTR.

Nitric oxide (NO) is considered to be a crucial signalling molecule in various physiological processes and both protective and detrimental properties have also been described for NO in renal transplantation. Findings from experimental studies in renal tissue of RTR suggest a role for NO in acute and chronic transplant rejection. Chapter 5 investigates whether nitrite and nitrate, the metabolites of NO, and nitroso species are associated with graft and patient survival in stable RTR. Additionally, we examine the involvement of possible inducers and inhibitors of NO metabolism in survival after renal transplantation. In chapter 6 we therefore investigate the associations of the NO substrate and cardiovascular risk parameter homoarginine with graft and patient survival in RTR, whereas in chapter 7 we determined the levels of the endogenous NO inhibitor asymmetric dimethylarginine (ADMA) and checked whether higher levels predict for graft failure and all-cause mortality in the renal transplantation setting.

Part 2 of this thesis focuses on the involvement and therapeutic potential of H2S and its metabolites in hypertension-induced renal and cardiac disease. Chapter 8 describes the influence of hypertension on renal expression of H2S enzymes and examines to what extent renal functional and structural changes induced by angiotensin II infusion are reversible over time. Since evidence is accumulating that H2S has an important role in renal (patho)physiology, H2S treatment is evolving as a very promising strategy in patients with kidney disease. Major challenges are H2S' rapid volatility, short half-life, and the legitimate fear for toxicity. However, the translation of H2S to the clinical setting becomes more within reach since thiosulfate, one of the major H2S metabolites, which is already safely administered to humans, is demonstrated to be protective in various conditions. These beneficial effects of thiosulfate are described to be, at least in part, due to its conversion to H2S. In chapter 9 we studied the protective properties of sodium thiosulfate (STS) and the sulfide salt sodium hydrosulfide (NaHS) in experimental angiotensin II-induced hypertensive renal disease. To confirm these potential protective effects of STS and NaHS, in chapter 10 we tested these compounds in experimental hypertensive cardiac disease. Since effects of increasing and inhibiting endogenous H2S levels are not consistent and might be concentration and cell type or organ dependent, in chapter 11 we studied the effect of H2S inhibition by DL-propargylglycine (PAG, a CSE inhibitor) on renal morphology and function in healthy rats and in a hypertensive renal injury model based on angiotensin II infusion.

In chapter 12 the content of this thesis is summarized and discussed. Based on our findings we discuss the possibilities for gasotransmitters to be part of novel treatment modalities for patients that suffer from renal disease.
References
