CHAPTER 1

GENERAL INTRODUCTION
Men are from Mars, Women are from Venus. In recent years a lot of debate has been going on about psychological and social differences between men and women. Currently, this discussion about gender differences and equality also takes place in the biomedical field. Far more than originally thought, increasing evidence reveals that men and women differ in their basic physiology, the susceptibility to and progression of diseases, and the response to therapy.

A recent survey of scientific literature in several disciplines identified that there is still a gender bias in research, especially in animal studies. Studies in males predominate in the biological literature, which suggests that basic biology from medical textbooks might better reflect the biology of males than females. Furthermore, there is the preferential use of male animals in drug development studies and an underrepresentation of females in clinical trials in which drug safety, dosing and efficacy were tested. Recently, it has been shown that this underrepresentation of women in clinical trials might be related to greater adverse effects in women than men.

A main reason for this neglect of females in research is that investigating physiology in females is more complicated due to the effects of menstrual cycle, resulting in reduced homogeneity of the study population. Focus on men only allows smaller studies, which are better affordable, however, at the expense of lack of data on female (patho-)physiology. Elucidation of the mechanism behind gender disparities, however, has important implications for the practice of both medicine and research. This will not only help explain why women or men are more prone to certain diseases, but also may translate into gender specific treatment and subsequently better outcomes in men and women.

Gender differences in cardiovascular and renal risk
In the field of cardiology and nephrology, awareness of gender differences is increasing as well; differences in overall risk, differences in risk factor profile and the impact of various risk factors are described. As to overall risk, it is known that women generally have a lower risk for developing chronic kidney disease (CKD), and progress slower to end stage renal disease (ESRD) after a renal insult compared with men. Furthermore, cardiovascular disease (CVD) develops later in life in women compared with men. These gender disparities suggest striking gender differences in the physiological and pathophysiological mechanisms regulating renal and vascular function. These are presumably mediated via sexual hormones, since this would also explain why protection against progression of renal disease and CVD is lost in postmenopausal women.
Men and women face gender specific risk profiles, as it has been shown that the impact of well-established renal and cardiovascular risk factors differs between men and women. It has been reported that premenopausal women have a lower blood pressure than men at similar age, and men have a higher prevalence of microalbuminuria. In addition, there are risk factors that occur in women only, namely those related to pregnancy and its complications.

**Adverse pregnancy outcome: a risk factor for cardiovascular and renal disease**

The normal physiological response to pregnancy represents several metabolic changes and up regulation of the inflammatory cascade. In addition, pregnancy also demands major changes in the cardiovascular and renal hemodynamic system. Pregnant women can double their blood volume and cardiac output, while systemic vascular resistance falls, leading to a decrease in arterial blood pressure. In parallel, renal hemodynamic function undergoes physiological adaptations, i.e. glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) increases by 40-60% and 60-80% respectively. Such metabolic and renal/cardiovascular responses to pregnancy could be considered as “stress” tests of maternal vascular function. In some women during pregnancy, the adaptations to metabolic changes and important alterations in hemodynamic function are insufficient, resulting in adverse pregnancy outcome. In this way, adverse pregnancy outcome may be an indicator of increased risk of vascular and renal diseases in later life.

Adverse pregnancy pathophysiology relevant to the (renO)-vascular bed are pregnancy induced hypertension and preeclampsia. Preeclampsia is characterized by de-novo development of hypertension and proteinuria in the second half of pregnancy and is associated with characteristic glomerular lesions. Complicating up to 5% of pregnancies, preeclampsia is the most common glomerular disease worldwide and remains a leading cause of infant and maternal morbidity and mortality. In the Netherlands, annually approximately 2500 women suffer from preeclampsia. Although it is a pregnancy-specific disease, evidence has mounted that preeclampsia has important long-term implications for maternal health, in particular cardiovascular and renal health. It is, however, uncertain whether the increased renal and cardiovascular risk in formerly preeclamptic women is explained by preeclampsia itself, or by underlying common risk factors and co-morbidity, such as hypertension, overweight or insulin resistance.

**The renin-angiotensin aldosterone system**

The mechanism behind the gender differences in the susceptibility for cardiovascular and renal disease and the increased cardiorenal risk in formerly preeclamptic women is not completely understood. The renin-angiotensin aldosterone system (RAAS), one of the main regulators of blood pressure, renal hemodynamics and volume homeostasis, might be involved, as suggested by several lines of evidence and discussed in part one of this thesis.

A schematic and simplified representation of the RAAS is given in figure 1. The RAAS is activated by sympathetic activation and volume depletion, such as low...
perfusion pressure in the kidney or low sodium/chloride supply to the macula densa, and starts with the release of renin from the juxtaglomerular apparatus in the kidneys. Renin activates the peptide angiotensinogen, converting it into angiotensin I (ang I). Ang I is further, by actions of the angiotensin-converting enzyme (ACE), converted to angiotensin II (ang II), one of the main effector hormones of the system. Ang II is an active peptide that induces vasoconstriction in the systemic and renal vascular bed. Furthermore, it stimulates aldosterone release from the adrenal cortex, leading to distal tubular sodium reabsorption. Together, these effects of ang II play a main role in the homeostatic response to volume depletion. Moreover, ang II stimulates cell proliferation and hypertrophy, and triggers pro-inflammatory and pro-fibrotic processes, which may contribute to progressive renal damage.  

**Figure 1:** Pathway of the renin-angiotensin aldosterone system.

**Figure 2:** Overview of the angiotensin related receptors. Adapted from Conti S et al. Hypertension 2012;60:878-883.
Ang II mediates its effect mainly through binding to the ang II type 1 (AT1) receptor, but it can also bind to the ang II type 2 (AT2) receptor (figure 2). Next to the long existing classical ACE/angII/AT1-receptor axis, a new depressor arm has been identified within the RAAS, including angiotensin converting enzyme 2 (ACE2), ang(1-7) and its receptors (Mas receptor and AT2-receptor). It seems that this new pathway counter-regulates the classical ACE/ang II/AT1-receptor pathway and exerts protective effects against an overstimulation of the AT1-receptor. Intervention in the RAAS with ACE-inhibitors and AT1-receptor blockers has allowed great advances in the prevention of progressive renal function and cardiovascular disease.23-24

In the last few years, gender differences in the components and regulation of the RAAS have been recognized to be of functional importance. Several studies have shown the influence of sex hormones on different components of the RAAS. Moreover, evidence suggests that men and women respond differently to RAAS inhibition and it has been shown that women respond less sensitive to exogenous stimulation of the RAAS than men.25-27 Moreover, during normal pregnancy women have marked changes in the RAAS including a sharp decrease in sensitivity to ang II. However, during preeclampsia this decrease in ang II sensitivity is incomplete or absent.28,29 Differential angiotensin receptor expression and activation (possibly via auto-antibodies), or differences in ang II metabolism might be involved. Therefore, the RAAS might be a possible mediator of the differences in risk profile between men and women and women, and in the long term adverse effects of preeclampsia on cardiorenal risk profile.

Renal hemodynamics
Selective filtration of the blood by the kidney is possible because of the unique characteristics of the glomerulus and renal circulation. Glomerular capillaries are interposed between an afferent and efferent arteriole, which permits modulation of the perfusion and pressure in the glomerular capillaries, despite systemic changes in blood pressure. This function is important, because it does not only control renal blood flow, but also glomerular filtration, tubular absorption, and medullary perfusion, and it protects the glomerular capillaries from the harmful impact of arterial blood pressure and thereby from a major contributor to CKD.

The pathogenic potential of altered renal hemodynamics to induce CKD has been extensively studied in remnant kidney models. In this animal model, removal of one kidney and infarction/removal of two-thirds of the remaining kidney results in progressive glomerular hyperfiltration, which is an important factor in progressive renal function loss. The adaptive response in the remnant nephrons, characterized by glomerular capillary hypertension, serves to preserve glomerular filtration in the short term, but leads to glomerular capillary damage, glomerular protein leakage and consequent nephron loss on the long term, thus eliciting a vicious circle of progressive renal damage. The concept that renal hyperfiltration attributes independently to the mechanism of CKD has been shown in our research group by investigating renal hemodynamics in obesity.30,31
Both renal hemodynamics and RAAS are modified by sodium status
RAAS activity and renal hemodynamic function is extremely modifiable by sodium homeostasis. The RAAS is activated during low sodium intake; renin is increased, down-stream aldosterone is increased and ang II sensitivity is decreased compared to high sodium. Sodium intake modulates renal hemodynamics in healthy subjects as well as in subjects with hypertension. In risk populations like sodium-sensitive hypertensive and overweight subjects, high dietary sodium intake elicits an unfavorable renal hemodynamic profile, which is absent during low sodium diet. Our research group have shown in overweight subjects that this unfavourable renal hemodynamic profile was associated with an exaggerated rise in extra cellular volume (ECV) in response to high sodium intake.

Overall aim of thesis
The aim of this thesis is to get more knowledge about the mechanisms behind the gender differences in susceptibility to cardiovascular and renal disease and the increased cardiorenal risk after preeclampsia. In part one of this thesis (chapter 2-4) we discuss the involvement of an important mediator of renal and vascular (patho-) physiology: the RAAS. In addition, in the second part (chapter 5-7) we focus on renal hemodynamic function and volume status as possible mechanism for an increased risk for renal and cardiovascular disease.

Outline of thesis
Chapter two describes the results of a translational study, in which we evaluated gender differences in the function of the RAAS, including the responsiveness to ang II, which might provide a possible explanation for the gender difference in progression to renal disease. We evaluated the response to acute administration of exogenous ang II in healthy female and male subjects. However, to substantiate relevance for chronic renal damage, we studied in healthy female and male rats the effect of chronic ang II administration on the kidney. Furthermore, we investigated the direct vascular responsiveness to ang II in isolated aortic rings of healthy female and male rats and the role of angiotensin receptors.

Chapter three provides a review, in which we describe the characteristics of preeclampsia with a focus on the mechanisms of angiogenesis and the RAAS and its role in the pathogenesis of preeclampsia. The imbalance of the RAAS and the imbalance between angiogenic and anti-angiogenic factors, which may be both common to preeclampsia and CKD, might explain why a history of preeclampsia predisposes women to develop CKD. In this chapter we focus on the association between preeclampsia and the subsequent increased risk of developing CKD and the potential mechanisms by which the risk of CKD is elevated in women with a history of preeclampsia.

In chapter four we evaluate the role of the RAAS in the increased risk for CVD and renal disease in formerly preeclamptic women. Women with a hypertensive pregnancy show an increased sensitivity to ang II during pregnancy as compared with healthy pregnant women. As persistent ang II sensitivity might play a role in the
increased risk for cardiovascular and renal disease, we investigated ang II sensitivity in women after early-onset preeclampsia. To explore whether preeclampsia itself could attribute to this abnormality in ang II sensitivity, we carefully selected healthy women, without co-morbidity. To further test the hypothesis that preeclampsia itself may induce persistent increased ang II sensitivity, we studied in rats with experimental preeclampsia, the blood pressure and renal response to chronic ang II infusion after pregnancy as well as ex vivo ang II sensitivity in the aorta.

In the following chapters we focus on renal hemodynamics and the link with sodium and volume homeostasis and the RAAS in human. Chapter five provides a general overview on renal hemodynamics and the possible role of renal hemodynamics in progressive renal damage.

In chapter six, we evaluate gender differences in renal hemodynamics as possible mechanism for differences in risk profile for renal and cardiovascular disease. In this chapter, we mainly focus on differences in volume status between men and women. Since both volume status and the RAAS are strongly modulated by dietary sodium intake, we investigated blood pressure and extra cellular volume (ECV) in steady state during low and high sodium intake in both healthy male and female subjects without hypertension or obesity. Furthermore, we studied the circulating RAAS in more detail and specifically the adrenal response to ang II infusion. We previously described differences in systemic and renal vascular sensitivity to ang II between normotensive men and women (chapter three). In this chapter, we present data of RAAS-activity and adrenal responsiveness to ang II.

In chapter seven, we investigate the renal hemodynamic profile in women with a history of early-onset preeclampsia compared with healthy controls. In steady state during low and high sodium intake, glomerular filtration rate (GFR), effective renal plasma flow (ERPF) and filtration fraction (FF) were measured at baseline and during graded ang II infusion. To explore whether preeclampsia itself could attribute to renal disease, we carefully selected healthy women, without co-morbidity, with a body mass index (BMI) < 30 kg/m² and excluded hypertensive formerly preeclamptic women. We tested the hypothesis that an unfavorable renal hemodynamic profile might be present, be it or not dependent on sodium intake, as candidate mechanism for the increased risk for long-term renal damage in formerly preeclamptic women.

Finally in chapter eight the results described in chapter 2-7 are summarized and discussed.
REFERENCES