Kidney oxygenation under pressure
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CHAPTER 1
Introduction and thesis outline
INTRODUCTION

Chronic kidney disease (CKD) is characterized by a progressive decrease of the kidney’s filtration capacity – i.e. the glomerular filtration rate (GFR) – with or without increased urinary excretion of albumin. It is an increasing public health issue, with an estimated worldwide prevalence of 8 to 16% in 2013. Hypertension together with diabetes mellitus are the leading causes of CKD and ultimately end-stage renal failure when renal replacement therapy via either dialysis and/or kidney transplantation are the only therapeutic options remaining. Cardiovascular disease is the most common cause of death among CKD patients. In fact hypertension is not only a causal factor in CKD, it is a consequence as well and blood pressure management is often difficult in these patients.

Thus, hypertension is a highly prevalent and relevant comorbidity in CKD. There is substantial evidence that nephrogenic hypertension can be attributed to increased sympathetic nerve activity (SNA) in these patients. However, the mechanisms underlying increased SNA in CKD are not completely understood. Several studies have reported an attenuation of SNA and blood pressure following bilateral nephrectomy. This has founded the concept that the trigger of the enhanced central sympathetic outflow in CKD patients resides in the affected kidneys themselves. Deterioration of renal oxygenation by altered renal perfusion and increased metabolic demand has been postulated as a common factor in the progression of CKD and nephrogenic sympathetic hyperactivity and hypertension. This has led to the conceptual framework depicted in Figure 1.

![Figure 1](image)

Figure 1 Conceptual framework of blood pressure regulation and kidney hypoxia in the progression of chronic kidney disease. The color-coded items and paths are investigated the different chapters of this thesis. Green is chapter 2, blue chapter 3, red chapter 4 and orange chapter 5.
Kidney function Each kidney – containing about 1 million nephrons (see figure) – receives about 600 mL of blood per minute. Almost all blood passes through the glomeruli, where it is filtered. This is a passive process, as the vessel wall acts as a sieve, the local pressure inside the glomerular capillaries is regulated and determines the amount of filtrate, or pre-urine, that is produced. This is the Glomerular Filtration Rate (GFR). The total amount of blood plasma that passes through the kidneys’ glomeruli is the Effective Renal Plasma Flow (ERPF) and the ratio of the filtrate to the total plasma flow is the Filtration Fraction (FF). Thus, with an average FF ~20%, about 175 liters of pre-urine is produced daily. Almost all pre-urine is resorbed in the tubuli (i.e. ~99%). Part of this process, sodium resorption in particular, requires oxygen. In most organs an increase in oxygen demand is simply resolved by increasing blood flow. However, kidney oxygenation is unique as an increase in blood supply leads to more urine production, requiring higher resorption rates and thus increases oxygen demand $^{25,26}$. 

Figure: Cancer Research UK / Wikimedia Commons (2015, 8 December).$^5$ “Diagram showing how the kidneys work.”

In summary this concept holds kidney metabolism at the center and shows how the sympathetic nervous system and renin angiotensin system (RAS) may affect kidney perfusion and metabolic load, leading to kidney hypoxia and disease progression (i.e. nephrosclerosis). At first, this concept may sound surprising considering the kidneys together receive about 20% of cardiac output $^{20}$, which is more than any other organ including the brain. Thus, sufficient oxygen should be available. However, in order for the kidneys to function properly a steep osmotic gradient needs to be applied from the outer to the inner medulla to aid resorption of solutes from the pre-urine. To maintain this gradient the blood perfusion in this area needs to be tightly regulated, while simultaneously the most metabolic demanding processes take place in the outer medulla. This results in a steep oxygen gradient in the medulla making the kidneys susceptible to hypoxia $^{21,22}$. According to the conceptual framework at hand, early oxygenation dysregulation then easily transients further into hypoxia due to negative feedback loops present in the system (Fig. 1 circular arrows). This framework also founded the rationale for catheter based renal denervation as potential antihypertensive treatment $^{23,24}$.

Described in Figure 1 is the conceptual framework as it was, at the on-set of the studies presented in this thesis. It shows the central role of the sympathetic nervous system and the renin angiotensin system in regards to blood pressure regulation and their effect on tubular oxygen consumption and medullar perfusion. However, at the outset of these studies several conceptual caveats remained, regarding:
1. the uniqueness of the sympathetic dysregulation in CKD patients (Figure 1 in green).
2. the effect of hyperoxia on blood pressure (Figure 1 blue path)
3. the link between RAS, SNA and kidney hypoxia in humans, (Figure 1 orange and red paths).

In this thesis we put the conceptual framework under pressure to test these paths and features and developed the methods to do so, i.e. to minimally invasive assess renal perfusion and oxygenation in humans.

**Sympatho-vagal balance and cardio-metabolic impairment**
Sympathetic hyperactivity is not merely present in CKD, it is common in many cardio-metabolic diseases. Therefore, before we study its role in kidney disease we need to explore its epidemiology. Sympathetic hyperactivity is a form of autonomic dysregulation that is characterized by a decreased arterial baroreflex function, amongst other characteristics. As changes in the arterial blood pressure are detected at baroreceptors in the aortic arch and carotid sinuses – i.e. sensory input to the (cardiovascular) autonomic nervous system – the autonomic nervous system acts to adjust the arterial pressure by altering the heart rate and/or the myocardial contractility, as well as the systemic vascular resistance. Alterations in the sensitivity of this mechanism to blood pressure changes can be quantified by measurement of the baroreflex sensitivity. This has already been established as an important determinant of the sympatho-vagal balance of the cardiovascular system 27. Baroreflex dysfunction is common in cardio-metabolic disease and BRS has been shown to be a clinically relevant and independent prognostic factor in cardiovascular disease, hypertension, metabolic syndrome and obesity, amongst others 28-35.

This wide range in applicability of BRS indicates a potential as an integrative risk factor for cardiovascular disease, that may be especially relevant to kidney disease patients. In Chapter 2 we therefore investigated the BRS in a large population study to evaluate and explore its associations between with cardiovascular risk factors and cardio-metabolic impairment.

**The effect of hyperoxia on blood pressure in CKD patients**
All visceral organs contain peripheral chemoreceptors, which communicate their signal to the autonomic nervous system upon activation, whereby they can directly influence cardiac and respiratory function (Figure 1, blue pathway). Among these receptors there are those sensitive to hypoxia that induce sympathetic activity, once activated. Various groups have found altered renal chemoreceptor activation in CKD 36-38. The proof of concept was provided by a study by Hering et al. who exposed CKD patients to 100% oxygen over a non-rebreathing mask for 15 min. This resulted in a 30% reduction in SNA accompanied by a lower pulse pressure 37. As this response was absent in healthy controls and non-CKD patient populations 39,40,
the observed effects on sympathetic nerve activity and blood pressure were attributed to CKD-specific hypoxia mediated renal chemo-reflex deactivation. Additional support for the existence of a kidney-derived chemo-reflex, were the observations in non-CKD sympathetically hyperactive patient groups not showing such a response.41,42

Thus, the hemodynamic response to oxygen supplementation appears to be uniquely different in CKD patients. However, these studies did not show the mechanism by which oxygen supplementation reduced SNA activity in CKD patients. Considering the conceptual framework described above (Fig. 1), sympathetic deactivation must lead to vasodilatation to lower blood pressure. Therefore we repeated the experiments by Hering et al.37, while including steps to measure the dose effect of the oxygen supplementation and include measurements of systemic vascular resistance and baroreflex function. For this study we literally put CKD patients under pressure in the hyperbaric chamber facility. The results are described in Chapter 3.

**MRI in a nutshell** MRI scanners use the magnetic properties of hydrogen atoms in the body. The nucleus of each hydrogen atom consists of one spinning proton, that thereby generates a tiny magnetic field. In the powerful MRI magnet these protons align and can be excited to change their orientation by sending resonating radio frequent (RF) signals into the body. When returning to their original position these protons act as tiny magnets 'echoing' the radio frequent signal, that is picked up by receiver coils. During this process the protons are affected by their surroundings, changing the magnitude of their 'echo'. This is unique to every substance (e.g. water, fat, bone etc.), this is what creates the contrast in MRI images. However, some of the disturbances are unwanted, such as motion (e.g. moving blood) or magnetically active substances (e.g. metal). While this can negatively affect standard MRI imaging, these disturbances can also be exploited to generate other contrasts in the eventual image.

*Photograph: 3 Tesla MRI at the AMC-UvA*

**Angiotensin-II and kidney oxygenation using functional MRI of the kidney**
Kidney oxygenation can reliably be assessed by blood oxygen level dependent (BOLD) MRI.43,44 As BOLD MRI is sensitive to the blood deoxyhemoglobin level, the acquired signal is the composite result of oxygen extraction from the blood (i.e. metabolic demand) and the rate of oxygen delivery (i.e. perfusion)45. The technique was originally validated in a porcine model46. Also, in subsequent human studies the technique was shown to provide excellent intra-individual tracking of minor changes in kidney oxygenation.
Blood Oxygen Level Dependent MRI exploits the magnetic properties of deoxy-hemoglobin. By measuring signal decay over time, the amount of deoxyhemoglobin can be estimated. Whereby, fast decay means a lot of deoxyhemoglobin is present and the tissue oxygenation is low and vice versa. Thus, BOLD MRI indicates the local hemoglobin oxygen saturation and therefore, oxygenation (reflected by BOLD MRI) is a balance or competition between oxygen supply (blood perfusion) and oxygen extraction from the blood (metabolic demand).

Although BOLD MRI can track intra-individual changes in oxygenation, researchers have found only limited associations between kidney oxygenation measured by blood oxygen level dependent (BOLD) MRI and kidney function measured by estimated glomerular filtration rate (eGFR) \(^ {47,48}\). The GFR denotes the amount of fluid that is filtered through the glomerular filtration membrane in the kidney and is a measure of the filter performance and capacity of the kidneys. In CKD the GFR slowly declines due to gradual loss of nephrons. Therefore the GFR is used as a primary measure of disease progression in CKD. Also, GFR indirectly represents the kidney’s metabolic demand. The more blood is filtered (high GFR) the more water and solutes need to be resorbed, which is energy demanding. Thus, the kidney is unique compared to other organs in such a way that an increase in perfusion increases the GFR and thereby increases in metabolic demand as well. These unique attributes need to be taken into account in the interpretation of kidney MRI.

Therefore we propose that kidney oxygenation status is reflected by the perfusion (i.e. oxygen supply) to GFR (i.e. oxygen demand) ratio, which is the filtration fraction. The filtration fraction denotes the amount of pre-urine formed per amount of blood that passes through the kidneys. Thus, to relate kidney BOLD MRI to kidney function measure by GFR, the BOLD MRI measurement needs to be corrected for the kidney’s perfusion. This can be done using phase contrast MRI, which is a sequence sensitized to quantify motion by measuring the phase shift in the MRI signal. Using this technique we can measure the velocity of blood flowing through the renal artery and calculate the renal artery blood flow.

To investigate the added value of phase contrast MRI renal blood flow measurements to kidney BOLD MRI we performed an experiment using angiotensin II infusions in healthy subjects. The renin-angiotensin-aldosterone system (RAAS) is responsible for regulation of the arterial blood pressure in the long-term. This system is regulated by the kidney to compensate for loss in effective circulating volume by activating angiotensin II, a potent arteriolar vasoconstrictor. Activation of angiotensin II is of major consequents to kidney perfusion and oxygenation itself \(^ {49,50}\). Angiotensin-II predominantly provides vasoconstriction of the efferent glomerular arteriole. Kidney perfusion is thereby decreased while simultaneously the filtration pressure in the glomeruli is increased, thereby raising the filtration fraction.
(Figure 2, red pathway). A previous study by Schachinger et al showed an acute decrease in cortical oxygenation during bolus injections of angiotensin II (Ang-II) in healthy humans. Additionally, other studies showed that blocking the RAAS in CKD patients increases renal oxygenation.

Thus angiotensin-II infusion should put kidney oxygenation under pressure. The result of these studies are described in Chapter 4. There we also use gold standard radio isotope kidney function tests to assess the associations between kidney function, perfusion and oxygenation.

**Direct effect of sympathetic activity on kidney oxygenation not shown in humans**

The link between SNA and renal hypoxia has primarily been investigated in animal models using invasive measurement techniques that cannot be applied in humans. These studies found that kidney sympathetic activation decreases renal blood flow. Simultaneously, sympathetic nerves directly innervate the renal tubules inducing sodium reabsorption and thereby increasing metabolic demand. The net effect of which is a decreased renal blood flow and increased tubular demand is therefore a decrease in oxygenation (Figure 1, orange pathway). However, in humans direct observations of the effect of sympathetic activation on kidney oxygenation are lacking.

Lower Body Negative Pressure (LBNP) can be used to experimentally increase systemic SNA in humans. LBNP is therefore ideally suited to investigate the sympatho-renal effects on kidney oxygenation. In Chapter 5 we describe the use of LBNP in an MRI scanner during
multimodal functional MRI to evaluate kidney perfusion and oxygenation during increasing sympathetic activity by in healthy humans.

Kidney specific intravoxel incoherent motion analysis
A major drawback of any MRI based perfusion measurements – including phase contrast MRI – is their inability to discriminate between blood and urine perfusion. To overcome this problem we included a diffusion weighted imaging (DWI) sequence optimized for kidney specific intravoxel incoherent motion (IVIM) analysis. Using this method we show its ability to detect and follow changes in kidney perfusion. Not only blood perfusion, but urine perfusion as well.

The DWI sequence is not only sensitive Brownian motion, but to any form of motion including that caused by perfusion. However, the higher the magnitude of the gradient pulse the less sensitive the signal is to fast perfusion motion. By introducing additional gradient pulses at low b-values, the proportion of fast bulk motion to slow Brownian motion can be quantified. This is the basis of IVIM analysis. IVIM analysis of employs a bi-exponential decay model to distinguish between capillary perfusion and tissue diffusion fractions \(^{55,66}\). Since its introduction, IVIM modelling has been applied to human kidneys, e.g. to identify altered perfusion in native kidney lesions and hypoperfused regions in transplanted kidneys \(^{67-71}\). However, as Muller et al \(^{67}\) already noticed, the kidneys are rheologically more complex than other organs and a bi-exponential model may not be sufficient to model renal physiology.

Incorporating a third exponent in the IVIM model could solve this problem. Such a model could enable discrimination between blood and pre-urine flow. Recently, it was shown by Van Baalen et al \(^{72}\) that such a tri-exponential IVIM model may be preferable in the kidney. In their implementation, the tri-exponential model produced three distinct signal fractions: a diffusion fraction, an intermediate bulk motion fraction \((f_i)\) and a fraction of fast bulk motion \((f_f)\). These fractions were shown to be consistent with the distinct functional regions within the kidney \(^{72}\). However, it remains unclear how this model relates to changes in kidney perfusion.

**Diffusion Weighted Imaging** MRI can be sensitized to diffusion by introducing a linear variance in the magnetic field using a pulsed field gradient. As the precession frequency of protons is proportional to the magnetic field strength, the orientation of the proton spins will change at different rates. This results in dispersion of the spin phase and loss of signal. If then a gradient pulse is applied of the same magnitude but opposite direction, the proton spins will start to refocus or rephase. However, the result of this refocusing will not be perfect for protons that have moved during the pulse interval and the measured signal is reduced. This reduction is proportional to speed and distance of proton movement. Thus, dense tissues will only allow little diffusion (Brownian motion) and thus show a high signal after phase refocusing and vice versa.
In **Chapter 6** we describe our findings using the tri-exponential approach to IVIM analysis in relation to perfusion changes caused by Ang-II infusion and relate the shifts in perfusion fractions to changes in gold standard measured GFR and renal plasma flow. This approach may provide a MRI based method that can simultaneously quantify and map shifts in kidney perfusion and filter function, to identify hyper filtration.

**OUTLINE OF THIS THESIS**

Blood pressure regulation is disturbed not only in kidney disease patients, but in most cardio-metabolic disease. It may even be an early integrative marker for cardiovascular disease. Therefore, in **Chapter 2**, BRS is evaluated in the HELIUS cohort to explore BRS in a large relatively healthy population.

In **Chapter 3** we investigate whether oxygen supplementation can reduce blood pressure in CKD patients. A session of hyperbaric oxygen was part of this study. In **Chapter 3B** we describe the conversion of a Portapres® device for use under hyperbaric conditions.

Evaluation of kidney hypoxia in humans is not possible using traditional kidney function measurements. **Chapter 4** describes the use of MRI techniques to evaluate kidney perfusion and oxygenation while depressed by Angiotensin II infusion to induce kidney hypoxia. There we compare these results to gold standard radioisotope kidney function tests.

In **Chapter 5** we apply multimodal functional MRI to evaluate kidney perfusion and oxygenation during increased sympathetic activity by lower body negative pressure in healthy humans.

A different approach to measure tissue perfusion is the application of Intravoxel Incoherent Motion (IVIM) analysis. The study described in **Chapter 4** included DWI sequences optimized to investigate whether a kidney specific tri-exponential approach to IVIM is able to detect and follow changes in kidney perfusion. Not only blood perfusion, but urine perfusion as well. Our findings using this technique are described in **Chapter 6**.

In **Chapter 7** the results of the studies are discussed in respect to one another and other recent research in the field to assess how the conceptual framework holds up and how this information may be of consequence to science and clinic in the future. Specific recommendations are made on how to improve and apply multi modal MRI of the kidneys for clinical research and possibly implementation.
REFERENCES


