CHAPTER 13
Summary and discussion
Aims of this thesis
The general aim of this thesis was to explore the relevance of selected lifestyle factors in the preventive treatment of CKD, including the relation between lifestyle factors and pharmacological treatment, with focus on pathophysiological mechanisms. Body composition is an important factor influenced by lifestyle factors, reflecting the influence of nutritional habits and physical activity. In the first part of this thesis we investigated the association between body composition parameters (BMI, body fat distribution, and muscle mass) and outcome in renal patients. In the second part of the thesis we explored the potential of dietary sodium restriction, as a modifiable lifestyle factor, as a tool to modify outcome in renal patients, with focus on diabetic nephropathy, and finally, in the third part we investigated intermediate mechanisms by which sodium restriction may modify outcome in CKD.

Part I: Body composition parameters as risk factors in renal disease
In chapter 2 we investigated the relationship between creatinine excretion rate (CER), as a body composition parameter for muscle mass, with cardiovascular and all-cause mortality in patients with type 2 diabetes and renal disease. To do so, we used the combined database of two landmark studies on the impact of angiotensin receptor blockers on patient survival in type 2 diabetes and renal disease.1,2 We found that lower CER was independently associated with higher risk of both cardiovascular and all-cause mortality. These results are in line with data in renal transplant recipients and in the general population, but it is remarkable that the hazard ratio for mortality was quantitatively larger (roughly three-fold per halving of CER) in the current study than for transplant recipients and the general population where the hazard ratio increased about two-fold per halving of CER.3,4 This underlines the relevance of CER as a risk factor for mortality, which may be especially true for patients with diabetic nephropathy. As CER can be considered a proxy for muscle mass, thereby reflecting physical fitness, it may imply that physical fitness should be a target for intervention in these patients.

We subsequently focused on BMI and body fat distribution as body composition parameters. Previous epidemiological studies showed that weight excess and central body fat distribution are associated with an increased renal risk in the general population5-12 as well as in renal patients.13-16 This increased renal risk in association with weight excess is believed to be mediated, at least in part, by comorbid conditions, such as hypertension, dyslipidemia, insulin resistance, and diabetes. Interestingly, experimental data suggest a pathogenetic role for renal hemodynamics as well, either related to these comorbid conditions or independently.17-20 The hemodynamic changes include an increased glomerular filtration rate (GFR) relative to effective renal plasma flow (ERPF), reflecting an increased glomerular filtration pressure, as apparent from an elevated filtration fraction (FF). In the subsequent
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In chapter 3 we presented an overview on the available literature on the effect of weight excess and central body fat distribution on renal hemodynamics, and its possible role in progressive renal damage. We provided a summary of studies that link weight excess and body fat distribution to renal damage, showing that the effects of weight excess on the kidney are not limited to overt obesity, but extend to the much more prevalent overweight range, both for the short-term effects on renal hemodynamics and the long-term effects on renal outcome. These data strongly suggest that weight excess may become the main renal risk factor for the near future, especially in subjects with a central body fat distribution. In chapter 4 we described the impact of overall weight excess on renal hemodynamics and their (separate and combined) impact on long-term outcome in a large cohort of stable renal transplant recipients. We found an effect of BMI on GFR and FF, with a higher GFR and FF one year after transplantation in recipients with higher BMI. Of note, the effect of BMI was not explained by the presence of diabetes mellitus. This finding in transplanted kidneys demonstrates that the kidney somehow ‘senses’ the BMI of the recipients, and responds to the body dimensions of its new owner. It also demonstrates the robustness of the effect of BMI on renal hemodynamics, notwithstanding effects of multiple other influences on the transplanted kidney, such as ischemia-reperfusion injury, CNI toxicity and rejection episodes. A lower GFR and a higher FF were independent determinants of overall graft loss and graft loss by patient mortality. Higher BMI, and lower GFR were independent determinants of death-censored graft loss, with a contribution of higher FF as well. To our knowledge, this is the first study demonstrating the predictive effect of a higher FF for a worse long-term renal outcome in humans. It is therefore the first human evidence for the Brenner hypothesis, that dates back to the 1980’s, on the pathogenetic role of elevated glomerular pressure for progressive renal damage. This hypothesis, based on experimental studies in rat models where glomerular flow and filtration pressure can be measured directly, has been guiding renal research for quite a long time, but so far was not supported by direct evidence in man.21

As an increasing body of evidence suggests that the body fat distribution rather than the weight excess per se, is important for identifying subjects at risk,10-12 we investigated the association between body fat distribution and renal hemodynamics in healthy subjects (chapter 5). We found that a higher waist-to-hip ratio (WHR) - reflecting central body fat distribution - was independently associated with lower GFR and ERPF, and with higher FF. As these healthy subjects had normal blood pressure and normal fasting plasma glucose levels, the association of renal hemodynamics with WHR could not be attributed to concomitant hypertension or impaired glucose tolerance. The previously reported association between BMI and renal hemodynamics was also present in this population, but the association
between WHR and renal hemodynamics remained significant after controlling for BMI. In this population body fat distribution was a stronger determinant of renal hemodynamics than weight excess, but the effect was particularly prominent in those with weight excess AND a central body fat distribution. In line with this unfavorable renal hemodynamic profile, in a follow-up study not included in this thesis, we found that high WHR, was a strong and independent predictor of death-censored graft failure, cardiovascular and all-cause mortality. Adjustment for BMI did not annihilate this association (unpublished data).

Thus, weight excess and central body fat distribution contribute to progressive renal damage at least partly, by their association with glomerular pressure. What would be the consequences for renoprotective intervention? First, it should be emphasized that weight loss is a main target in overweight subjects, that can reduce blood pressure and improve glucose tolerance. Also, weight loss reverses glomerular hypertension and associated proteinuria, as shown in studies after bariatric surgery. 22 Moreover, sodium restriction attenuated the elevated GFR and FF in overweight, but, otherwise healthy subjects. 23 Furthermore, pharmacological blockade of the renin-angiotensin-aldosterone system (RAAS) reversed the renal hemodynamic changes induced by obesity. 24 In chapter 6, we performed a randomized clinical trial to assess whether direct renin-inhibition (DRI) – a new potent inhibitor of the RAAS – can decrease glomerular pressure in subjects with weight excess and arterial hypertension. We found that DRI provides a reduction in both renal and systemic hemodynamics. This trial is thereby the first to demonstrate an effect of DRI on renal hemodynamics in subjects with weight excess and hypertension. In addition, it adds to the current understanding of the RAAS in response to DRI as we extensively measured plasma and urine RAAS constituents, showing a reduction in systemic and intrarenal RAAS activity. DRI may therefore play a role in the attenuating the susceptibility and progression of renal damage in subjects with increased renal risk.

Part II: Effects of sodium intake in diabetic renal patients: clinical

In the second part of this thesis we focused on a major complication of weight excess, namely type 2 diabetes mellitus, and specifically long-standing diabetes that is complicated by microvascular disease as manifested by renal disease. Therapy of choice in diabetes and renal disease is blockade of the RAAS with either ACEi or ARB which substantially improves renal and cardiovascular protection. 1,2,25 Unfortunately, progression of renal disease in diabetes occurs in many patients despite treatment with ACEi or ARB. Previous studies in non-diabetic renal disease from our group showed that treatment response to RAAS blockade can be potentiated by addition of dietary sodium restriction. 26,27 Whether this holds true for diabetes and renal disease is unknown as differences in pathophysiology between renal disease of diabetic and non-diabetic origin render straight forward extrapolation of these data to the diabetic population unwarranted. 28 Moreover, no randomized controlled
studies on the potentiation of RAAS blockade by sodium restriction in type 2 diabetes and renal disease are available (as reviewed in 29). Reluctance to prescribe sodium restriction in diabetes patient may relate to the so-called ‘salt paradox’. This phenomenon, that has been observed in experimental animals and uncomplicated type 1 diabetes, refers to the induction of a hyperfiltration pattern by sodium restriction. It is due to an increased proximal tubular reabsorption of anions and cations secondary to persistent hyperglycemia and proximal tubular hypertrophy, resulting in a decreased distal tubular delivery, aggravated by dietary sodium restriction and consequently activation of the tubuloglomerular feedback, with afferent vasodilation and a rise in GFR that serves to ensure stable delivery of anions and cations to the distal tubuli. Hence, sodium restriction in diabetes may induce hyperfiltration and thereby promote renal disease progression. Whether this paradox is present and clinical manifest in human type 2 diabetes, and, moreover, in association with renal disease, is unknown. We therefore performed, as described in chapter 7, a multicenter randomized controlled clinical trial to investigate the potentiating effects of sodium restriction, in respect to hydrochlorothiazide, on RAAS blockade efficacy in type 2 diabetes mellitus and renal disease. First, we found that in this population sodium intake was very high, namely 224 mmol/d which is the equivalent of a daily sodium chloride intake of 13.2 g. This high salt intake was representative for the outpatient population used for study recruitment, supporting the generalizability of our data. Sodium restriction decreased sodium intake to 150 mmol/d (daily sodium chloride intake of 8.8 g), thus leaving sodium intake still well-above the recommended target intake of current WHO guideline (sodium intake of 80 mmol/d or a daily sodium chloride intake of 5 g). Second, sodium restriction and HCT, added to RAAS blockade, reduced blood pressure and proteinuria, without affecting creatinine clearance. The largest decrease in proteinuria and blood pressure was obtained by combining RAAS blockade with the combination of sodium restriction and HCT, however, at the expense of a (reversible) decrease in creatinine clearance. We conclude that intervention in sodium status in an effective non-pharmacological intervention to increase RAAS blockade efficacy in type 2 diabetes and renal disease. Moreover, the effect on creatinine clearance provides no support for relevance of the salt-paradox in this clinical condition.

In spite of these favorable short-term effects of sodium restriction during RAAS blockade, there is currently substantial debate on the role of sodium status and long term outcome in diabetes. Several observational studies in diabetes addressed this question by investigating the association of sodium intake, assessed by urinary sodium excretion in 24 hour urine collection, on renal prognosis and mortality. In chapter 8, we provided a brief overview of these studies along with our critical appraisal. In short, several studies found either a positive association, a J-curve phenomenon (Thomas et al.), or paradoxically, an inverse association (Ekinci et al.) with renal outcome and mortality rate. We believe that these observational studies could be subject to unmeasured and residual confounding which may
have played a dominant role in eliciting the paradoxical results. We state that although observational studies can be useful to generate interesting hypotheses, they never provide the true answer. Randomized controlled trials are necessary to truly assess the impact of salt reduction on mortality. Yet, an extremely low sodium intake may elicit adverse effects, either because it reflects a poor nutritional intake, or because it elicits excess reactive activation of the RAAS and/or renal hypoxia. We will discuss this in more detail in the final chapter of this paragraph.

Part III: Effects of sodium intake in renal patients: mechanisms

Sodium restriction exerts its cardiovascular and renal effects by blood pressure-dependent as well as blood pressure-independent pathways: several of these pathways were investigated in the last part of this thesis. First, we investigated whether the reduction in proteinuria by sodium restriction is exclusively due to the reduction in blood pressure in patients with diabetic and non-diabetic renal disease. Second, in humans, we tested the newly discovered experimental concept of a vascular endothelial growth factor C (VEGF-C) mediated extrarenal mechanism of sodium and blood pressure regulation. Third, we investigated two separate blood pressure-independent mechanisms by which sodium restriction can exert its effects.

In chapter 9, we investigated whether the antiproteinuric response to sodium restriction can be explained by the improved blood pressure control in patients with diabetic and non-diabetic nephropathy. We found that proteinuria reduction by sodium restriction, added to RAAS blockade, was not explained by the concomitant decrease in blood pressure. Of note, during addition of hydrochlorothiazide (HCT) to RAAS blockade the association between the reduction of blood pressure and proteinuria was stronger, suggesting that sodium restriction and diuretic may have a different mode of antiproteinuric action. We hypothesized that sodium restriction has additional specific blood pressure-independent renoprotective effects. In chapter 10, we focused on a recently discovered pathway of sodium and blood pressure homeostasis, that altered the traditional paradigm on sodium balance of the body. Traditionally, sodium balance is known to be linked to the regulation of the extracellular volume, by the interaction with osmoregulation that strives to keep serum sodium with the homeostatic margins. In this classical concept the kidney plays the main role in sodium and volume regulation. Recently, however, an additional, extrarenal pathway of sodium storage was discovered, with subcutaneous non-osmotic storage of sodium, by a pathway that includes subcutaneous lymphangiogenesis mediated by VEGF-C. In experimental animals VEGF-C was shown to respond to high sodium diet, but whether this is also the case in human, was unknown. We tested this in healthy volunteers and CKD patients, and found that a rise in sodium intake was associated with a rise in circulating levels of VEGF-C, along with activation of the conventional osmotic mechanism of sodium homeostasis, in both CKD.
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Patients with non-diabetic nephropathy and healthy subjects. Moreover, circulating levels of VEGF-C were higher in the CKD patients than in the healthy subjects on either sodium intake. Considering the sodium-sensitivity of blood pressure (and proteinuria) in the CKD patients as opposed to the sodium resistance in the healthy subjects, this suggests that the alleged VEGF-C mediated pathway was insufficiently effective to buffer the effect of increased sodium intake on blood pressure and the kidney in CKD patients. It would be of great interest to further explore the contribution of (deficient) non-osmotic sodium storage to sodium excess, hypertension and proteinuria in CKD patients.

In chapters 11 and 12, we focused on important blood pressure-independent effects of sodium restriction on fibrosis/inflammation and lipoprotein regulation, respectively. First, in chapter 11, we determined the effect of sodium restriction on circulating levels of the anti-inflammatory and anti-fibrotic peptide n-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP). As AcSDKP is specifically degraded by the ACE and its plasma levels rise substantially by inhibition of ACEi, AcSDKP is thought to be involved in the therapeutic effects of RAAS blockade, in particular its anti-inflammatory and antifibrotic properties. We hypothesized that sodium restriction on top of RAAS blockade further increases AcSDKP, as a possible explanation for the enhanced effects of RAAS blockade during sodium restriction. Indeed, we demonstrated that in CKD patients, sodium restriction, on top of single and dual RAAS blockade, increased circulating levels of AcSDKP independent of blood pressure. This increase in AcSDKP is well in line with data demonstrating anti-inflammatory and antifibrotic effects of sodium restriction. AcSDKP may therefore contribute to the increased cardiovascular and renal protection of RAAS blockade during sodium restriction. Finally, in chapter 12, we described the effects of maximal antiproteinuric treatment by addition of sodium restriction and ARB to ACEi - on lipoprotein levels in patients with proteinuria of non-diabetic origin. Proteinuria is characterized by distinct dyslipidemia. The pro-protein convertase subtilisin kexin type 9 (PCSK9) pathway plays a key role in lipoprotein metabolism by promoting hepatic LDL-receptor degradation, which may provide a mechanism which contributes to atherogenic lipoprotein abnormalities in experimental nephrosis and in humans with glomerular proteinuria. We tested whether plasma PCSK9 is elevated in proteinuric states, and determined relationships of PCSK9 with lipoprotein responses to proteinuria reduction. We found that plasma PCSK9 was elevated in proteinuria, predicted lipoprotein responses to proteinuria reduction but remained unchanged after proteinuria reduction. In view of these findings which favor the hypothesis that PCSK9 may contribute to atherogenic lipoprotein abnormalities in proteinuric subjects, proteinuric subjects may be a relevant patient category for PCSK9 inhibition in the future. We focused in our paper mainly on the relation between PCSK9 and decreased hepatic lipoprotein clearance, however, new data suggests that PCSK9 may be involved in increased VLDL production as well. Furthermore, others found that PCSK9 exerts its effect on lipoprotein metabolism also
independently from hepatic production or clearance by binding of PCSK9 to apolipoprotein B lipoproteins, thereby diminishing the effects on LDL receptor deficiency.\textsuperscript{47,48}

**GENERAL CONCLUSIONS AND LOOK TOWARDS THE FUTURE**

Based on the findings of this thesis, we state that body composition parameters that reflect lifestyle (muscle mass, fat mass, and body fat distribution) are pathophysiological involved in renal disease. To test whether intervention in lifestyle can intervene in renal disease progression, we studied the effect of sodium restriction. We found that sodium reduction had a pronounced effect on blood pressure and albuminuria, as well as on inflammation/fibrosis and lipoprotein metabolism in renal patients. Taken together, we conclude that lifestyle intervention is a feasible and elegant measure to treat disease progression in renal patients.

Taken into account the effect of our intervention in sodium intake on renal parameters, we are currently carrying this concept further. First, we are conducting a large multicenter randomized clinical trial to assess the extent to which sodium restriction lowers proteinuria and blood pressure over a prolonged period of time – i.e. 6-months. By inclusion of no less than 150 renal patients, this trial exceeds by far the patient numbers of any other intervention trial on the impact of sodium in the field of Nephrology. Furthermore, this trial assesses also the effect of motivational counseling and self-management strategies (for instance home measurement of sodium excretion using lab-on-a-chip technology) on long-term compliance to sodium restriction. Of note, due to the less demanding inclusion and exclusion criteria in respect to other randomized clinical trials - thereby facilitating the inclusion of patients in the full spectrum of renal disease - this trial is of high clinical importance as results can easily be extrapolated to the overall outpatient nephrology population. Second, we are investigating to which extent sodium restriction potentiates relatively new pharmacological interventions – i.e. stimulation of the vitamin D and blockade of the mineralocorticoid receptor, respectively.
REFERENCES


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