Chapter 1

Introduction and Aims of Thesis
Introduction

*Chronic Kidney Disease – When Renal Function Sinks*

The prevalence of chronic kidney disease (CKD) varies among European countries between 3 and 17 per 100 persons (1). In the Netherlands, CKD prevalence has been estimated between 7.6 and 10.4 patients per 100 persons, according to the large observational cohort studies LifeLines (1) and PREVEND (2). A reduced renal function, commonly expressed as the estimated glomerular filtration rate (eGFR), is associated with a 3–19-fold increased mortality risk and an increased risk of progression to end stage renal disease (ESRD), depending on age and disease severity (3). Blockade of the renin-angiotensin-aldosterone system (RAAS) is the mainstay of CKD treatment. It aims at reduction of rate of renal function loss and of cardiovascular complications by reduction of blood pressure and proteinuria (4, 5). RAAS blockade interferes with the hemodynamic effects of increased RAAS-activity, i.e. systemic and renal vasoconstriction, as well as with its effects on volume status, and its pro-fibrotic effects. Despite proven efficacy however, risk reduction is at best modest. Treatment with angiotensin receptor blockers (ARB) delays progression to ESRD with a mere four to eight months (6, 7). RAAS blockade was introduced in the 1980’s as antihypertensive treatment. Its renoprotective properties were investigated in the 90’s, and large RCTs corroborated use of RAAS blockade for reno- and cardioprotection in CKD around 2000. Unfortunately, from that time until now in 2016 little progress has been made to further retard renal function loss or protect from cardiovascular complications. Our understanding of renal disease, and the factors driving progressive renal function loss, is far from complete, and other mechanisms than the RAAS are likely also involved. In this thesis, we address the interaction of RAAS-activity, sodium- and volume homeostasis, with another major hormonal system, that governs mineral-bone homeostasis.

In CKD the RAAS is aberrantly activated. In line, the prevalence of hypertension increases as renal function declines: for instance from 18.1 per cent when eGFR is 90–119 mL/min/1.73m² to 82.1 per cent when eGFR has dropped below 30 mL/min/1.73m² (8). RAAS blockade (e.g. angiotensin-converting enzyme [ACE] inhibitors or ARBs) reduces blood pressure and proteinuria, and these effects are assumed to be main factors for cardiovascular and renal protection. However, ACE inhibitors or ARBs reduce proteinuria only by some 40% on the average (9). The more ‘residual’ proteinuria remains, the higher the residual risk for subsequent cardiovascular events (10) and for progressive renal function loss (11). Combination of ACE inhibition with ARBs was initially thought to enhance RAAS blockade efficacy and improve outcomes. However, large RCTs pointed out that dual-blockade does not provide additional benefits, and even may cause more harm (12-14), as discussed more elaborately in (→ Chapter 2). Therefore, additional strategies to improve efficacy of RAAS blockade are urgently needed. The role of extracellular volume overload has long since been identified as an important mediator of RAAS blockade efficacy, based on the consistent observation that high salt intake blunts the effect of RAAS
blockade in patients with hypertension (15) and with CKD (16). Moreover, volume overload correlates with proteinuria, and identifies patients that have greater therapeutic benefit from sodium restriction and/or diuretics on blood pressure and proteinuria (17, 18). The other way round, proteinuria in itself may induce sodium retention and thus promote volume overload (19). All in all, there is compelling evidence for an interaction of volume overload with the effects of RAAS blockade, with blunting of efficacy during volume-overload. More recent data suggest that phosphate status may interact with efficacy of RAAS blockade as well, and moreover, that phosphate status may interact with volume status. Intriguingly, nephrotic—and hence probably volume overloaded—pediatric patients have higher serum phosphate concentrations despite preserved eGFR (20). Moreover, higher serum phosphate levels are associated with impaired cardiorenal protective effect of ACE-inhibitors (21). This suggests interaction between bone-mineral and volume-hemodynamic pathways in pathophysiology, and in the therapeutic response to RAAS blockade in CKD. This placed phosphate and mineral bone homeostasis firmly in our sights.

**Phosphate and Fibroblast Growth Factor 23 – Between Balancing and Capsizing**

The kidney controls serum phosphate concentrations by regulating its excretion. Only in the setting of ESRD, hyperphosphatemia occurs and is strongly associated with increased mortality (22). However, in earlier stages of CKD, serum phosphate is regulated to remain within the normal range (23). Even within this normal range, higher serum phosphate concentrations are associated with a higher risk of mortality in patients with patients with CKD (24, 25) and the general population (26), suggesting that mild disturbances in the regulatory system already have clinical impact. Of note, in CKD patients with higher serum phosphate concentrations, cardiorenal protection by RAAS blockade is less effective (21). Notwithstanding the unequivocal beneficial effect of reducing serum phosphate concentrations in haemodialysis patients (27), strategies to reduce serum phosphate in earlier stages of CKD lack currently evidence for long-term cardiovascular protection.

Phosphate regulation is under control of three different hormones (28). Active, 1,25-dihydroxy-vitamin D₃ (1,25D) increases serum calcium and phosphate levels, predominantly by increasing gastrointestinal and renal tubular reabsorption. Parathyroid hormone (PTH) decreases renal phosphate reabsorption—and stimulates the activation of vitamin D. In 2001, fibroblast growth factor 23 (FGF-23) was discovered as the culprit of several bone disorders that are characterized by excessive renal phosphate loss (29, 30). FGF-23 inhibits tubular phosphate reabsorption; inhibits 1-alpha-hydroxylase, the enzyme that converts inactive 25-hydroxy vitamin D₃ into 1,25D (31); and inhibits PTH secretion (32, 33). FGF-23 is able to bind to the FGF-receptor 1c only when the coreceptor α-klotho is present (34). Indeed, mice lacking αklotho or FGF-23 share the same phenotype of premature ageing and ectopic calcification, partly due to high calcium and phosphate concentrations (28, 35, 36). FGF-23 thus counterbalances the effects of vitamin
D on phosphate homeostasis. An additional layer of complexity is added by the interaction of FGF-23 and vitamin D with the RAAS (37). FGF-23 lowers 1,25D, and in this fashion attenuates the inhibiting effect of vitamin D on the RAAS (38). Moreover, FGF-23 may induce left ventricular hypertrophy (39), possibly by aberrant stimulation of the FGF-receptor 4 in the cardiac myocytes (40). The actions of FGF-23 are summarized in Figure 1.

**Figure 1.** Schematic representation of FGF-23 physiology. FGF-23 signals via the FGF recepotor 1c (FGFR1c) and its obligate coreceptor, α-klotho (Kl) the parathyroid gland to inhibit production of parathyroid hormone (PTH); inhibits expression of sodium-phosphate (NaPi) cotransporters in the kidney, thus stimulate phosphate excretion in the urine; inhibits conversion of inactive vitamin D to active, 1,25-dihydroxy-vitamin D₃ (1,25D). Dashed red arrow signifies pathophysiologic association of FGF-23 and left ventricular hypertrophy, possibly mediated by FGFR-4 signaling or FGF-23 induced volume overload.

The principal site of FGF-23 production is the osteocyte. FGF-23 production increases in response to 1,25D (31), PTH (41) and phosphate (42), effectively closing negative feedback loops. Also higher calcium concentrations may stimulate FGF-23 production (43), as may inflammatory cytokines or iron deficiency (44). The strongest determinant of FGF-23 concentrations, however, is renal function.

In persons with preserved renal function, the normal value of FGF-23 is typically <100 RU/mL (45). FGF-23 concentrations increase early in the course of CKD (46), long before disturbances in PTH and phosphate values are apparent (23). Allegedly, this increase serves to stimulate phosphate excretion in the remaining functioning nephrons. With the progression of CKD, FGF-23 rises exponentially from values in the hundreds in CKD stage 3–4 to reach extreme values in haemodialysis patients: 10 000 to even 100 000 RU/mL, as illustrated in Figure 2.
Figure 2. Summary of FGF-23 concentrations reported in this thesis. FGF-23 rises exponentially as renal function declines. This occurs earlier and to a more extreme extent than parathyroid hormone (PTH, left Y-axis) and serum phosphate concentrations (right Y-axis). Data points reflect patients with CKD (chapter 4, 7), haemodialysis patients (chapter 6) and renal transplant recipients (chapter 5).

Even the presence of minimal residual renal function in haemodialysis patients is associated with lower FGF-23 levels (47, 48). After renal transplantation and thus restoration of renal function, FGF-23 levels generally return to normal values within 3 months (49). Higher FGF-23 concentrations are associated with an increased mortality risk in haemodialysis patients (50, 51), predominantly cardiovascular mortality in predialysis CKD (52, 53), and even with cardiovascular and all-cause mortality in the general population (54, 55). Because the adverse consequences of high FGF-23 concentrations have repeatedly been demonstrated, FGF-23 has been hypothesized to be a target for intervention. Accordingly, known determinants of FGF-23 have been targeted relentlessly in efforts to lower FGF-23 levels.

A Barrage of Interventions Targeting Fibroblast Growth Factor-23

Efforts to reduce FGF-23 concentrations consist of strategies aimed at various elements, including dietary, immunological and hemodynamic interventions summarized in table 1.

Table 1. Strategies to reduce FGF-23 concentrations or limit its consequences. Strategies in italic are based on unpublished data.

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dietary strategies</strong></td>
<td></td>
</tr>
<tr>
<td>Phosphate restriction</td>
<td>Reduce intake of a phosphate.</td>
</tr>
<tr>
<td>Phosphate binders</td>
<td>Reduce intestinal absorption of phosphate</td>
</tr>
<tr>
<td>Sodium restriction</td>
<td>May attenuate effects of FGF-23 on volume status</td>
</tr>
<tr>
<td>Potassium supplementation</td>
<td>May lower FGF-23 and increase serum phosphate</td>
</tr>
<tr>
<td>Antibodies to FGF-23</td>
<td>Inhibit FGF-23 action in body</td>
</tr>
<tr>
<td>Intensified haemodialysis schedule</td>
<td>Reduce phosphate and fluid accumulation, both determinants of FGF-23</td>
</tr>
</tbody>
</table>
Because FGF-23 regulates phosphate homeostasis, dietary phosphate was considered first. Net phosphate reabsorption can be reduced by limiting intake by a phosphate-restricted diet, and deployment of phosphate binders, which are commonly used in ESRD. High dietary phosphate intake results in higher FGF-23 concentrations in healthy volunteers (42, 56, 57). However, in patients with CKD, dietary phosphate restriction did not achieve a reduction of FGF-23 (58). The efficacy of phosphate binders on FGF-23 varies, with absent effects on FGF-23 by calcium-based phosphate binders (59); the effect of lanthanum carbonate that varied from none (60) to strong if combined with dietary phosphate restriction (58); and marked reductions by sevelamer treatment (59). Third, antibodies to FGF-23 have been tested, however full-blockade of FGF-23 function resulted in extreme calcifications and premature death of animals (61), underscoring the physiological necessity of FGF-23 for keeping calcium-phosphate balance in check. Fourth, in haemodialysis, better phosphate control can be achieved by more frequent, or longer dialysis sessions (62); or longer hemodiafiltration (63): both strategies also achieve lower FGF-23 concentrations in haemodialysis (63, 64), however both are obviously not applicable to the predialysis CKD population. A fifth strategy follows from the finding that FGF-23 is a particular strong predictor of heart failure rather than ischemic cardiovascular events (53, 65). These observational data have led to the hypothesis that FGF-23 may induce volume overload. Experimental studies found that FGF-23 induces expression of the sodium-chloride cotransporter (NCC) to stimulate sodium retention and induce hypertension (66). This effect could be overcome by the diuretic hydrochlorothiazide. This emphasizes that FGF-23 and the renal regulation of the elements phosphorus (P) and sodium (Na) are possibly intertwined, and interventions that target sodium (diuretics, a low sodium diet) may thus influence phosphate regulation. Consequently, both elements may be identified as twin-targets for dietary restriction. A sixth strategy involves potassium supplementation. Potassium supplementation increases serum phosphate levels (67). This suggest that potassium is another dietary component capable of influencing phosphate regulation, possibly potassium could lower FGF-23 concentrations. If so, potassium would be another target for dietary intervention. However, achievement of dietary intake targets is difficult: notwithstanding dietary recommendations (4), few patients with CKD achieve adherence to dietary sodium restriction (68). Therefore, better strategies for sustained changes in dietary habits are direly needed.

Need for a Multidisciplinary Approach for Enhancing Elements of a Healthy Lifestyle

Restriction of phosphate uptake can be achieved by pharmacological and dietary interventions. Pharmacological phosphate binder therapy is costly, not fully effective and comes with a substantial pill-burden for patients (69). Compliance to a phosphate-restricted diet is difficult and challenging, as phosphate is ubiquitously present in our food products (70), although avoidance of phosphate-rich additives is feasible and facilitates control of hyperphosphatemia.
Several dietary components affect outcome in CKD and the general population. A healthy lifestyle must thus address such dietary factors including, but not restricted to, phosphate. A higher intake of sodium puts patients with CKD at higher risk for progression to ESRD (71) and increased risk for cardiovascular complications despite treatment (72). Even in the general population, high sodium intake is associated with higher blood pressure (73) and increased cardiovascular mortality especially in overweight subjects (74), whereas higher potassium intake may be protective against development of hypertension (75) and mortality (76). Phosphate intake is not routinely analyzed, however the results are mixed between detrimental (77), absent (25), or protective effects (78) in the general, CKD and mixed population respectively. Conversely, the concentration of phosphate in the serum phosphate is a strong predictor of renal disease progression (21) and mortality (24). Sodium restriction has been assessed in several trials in our center (79-82), and elsewhere (83, 84). These studies typically consist of short-term (6 weeks) interventions, and many patients relapse in old habits after participation (68). Of course, a sustained change resulting from dietary interventions is needed to elicit true impact in clinical practice. A sustained change in health behavior however is notoriously difficult to achieve. To achieve and maintain a successful change in health behavior, behavioral interventions can be deployed, for example based on the self-regulation therapy (85). Guidance in the attainment of goals throughout the phases of behavioral change in self-regulation theory however can be time-extensive—and hence costly. E-health technology has emerged rapidly over the last few years, and may have potential to support the process of behavioral change and self-management skills of patients with CKD, but experience so far is limited, and not up to the level of evidence-based medicine. All in all, there is an urgent need for novel approaches that can achieve long-term beneficial changes in dietary elements of a healthy lifestyle.

Outline of Thesis

RAAS blockade is the ship-of-the-line in prevention of progressive renal function loss. However, RAAS blockade only delays end stage renal disease by 4–8 months and indiscriminate combination of several RAAS blocking agents together may be harmful, so alternative approaches to enhance the protective effects of RAAS blockade are needed. These approaches could be pharmacological or dietary intervention, or their combination. This thesis addresses interactions between RAAS/volume status, and regulators of phosphate-, bone- and mineral homeostasis, to identify better treatment strategies based on combined modulation of RAAS/volume status, and of factors involved in phosphate–bone-mineral homeostasis. We expand this introduction with a review of vitamin D as adjunct to improve antiproteinuric efficacy of RAAS blockade (→ Chapter 2). As became clear, sodium intake remains a treatment target in CKD. This deserves a more elaborate explanation, as we review in (→ Chapter 3). Because FGF-23 may interact with the renin-angiotensin-aldosterone system (37) and higher serum phosphate concentrations
reduce the protective effects of RAAS blockade (21), we set out to investigate whether higher FGF-23 concentrations may impair the efficacy of intensification of RAAS blockade by a low sodium diet (Chapter 4). FGF-23 emerged as a robust risk factor for cardiovascular mortality in haemodialysis patients (50, 51), but after renal transplantation FGF-23 concentrations return to near-normal values (49). We hypothesized that relatively higher FGF-23 concentrations in stable renal transplant recipients still herald a high risk of cardiovascular mortality (Chapter 5). Because FGF-23 is correlated more strongly with volume overload rather than ischemic cardiovascular events (65), we suggested that volume overload itself may be a determinant of FGF-23 levels. Therefore, we assessed in haemodialysis patients the relation between ultrafiltration volume – the amount of volume that needs to be withdrawn during a haemodialysis session and FGF-23 concentrations (Chapter 6). We also investigated whether the steep drop in serum phosphate during haemodialysis may lower FGF-23 values (Chapter 6). To expand the hypothesis that FGF-23 may directly induce volume overload by effects on sodium handling (66), we assessed, the other way round, whether changes in volume status may influence FGF-23 concentrations in patients with preserved renal function (Chapter 7). The elements sodium and phosphorus are literally connected at the molecular level in many food products as sodium-phosphate salts (86), and a diet rich of these additives increases FGF-23 levels (87). In a broader perspective, we investigated if sodium intake and phosphate intake are correlated in different patient populations (Chapter 8). Another viewpoint arose from the observation that potassium supplementation increases serum phosphate levels (67). We suggested that potassium exerts this effect by reducing FGF-23 concentrations (Chapter 9).

This thesis addresses several dietary elements that affect renal and cardiovascular outcomes. However, to realize the therapeutic potential of dietary improvements in clinical practice, a sustained change in dietary behavior must be established. Individual behavioral counseling of patients is time-extensive and costly. E-health technology could facilitate counseling and support behavioral approaches based on self-regulation theory. Therefore, we investigated the feasibility and cost-efficacy of a multidisciplinary e-health approach aimed at reduction of dietary sodium intake in renal patients in the randomized, multicenter SUBLIME trial (Chapter 10). A general discussion of all these chapters in the perspective of current literature will be provided in (Chapter 11).
References


Part I
Minerals, Volume and Fibroblast Growth Factor 23