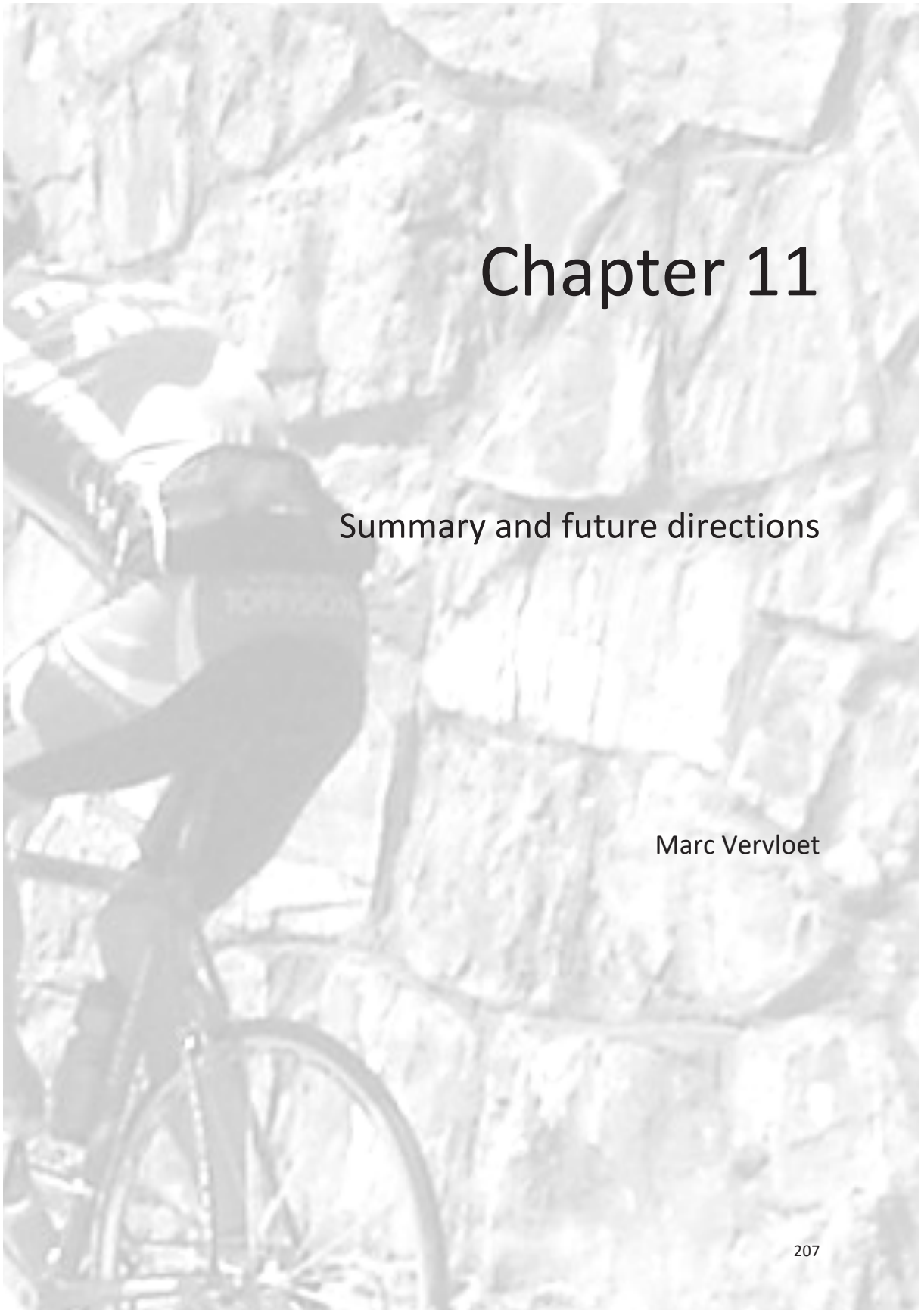

Chapter 11

Summary and future directions

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Chronic kidney disease (CKD) is characterised by a linear increase in cardiovascular risk, along with progression of renal failure. Several features of CKD have been identified to possibly encompass this increased risk. One of the most prominent features that may be responsible for this added risk is the syndrome called CKD-mineral and bone disease (CKD-MBD). CKD-MBD has been acknowledged as an entity, because of the strong relations that exist between changes in homeostasis of calcium, phosphate, PTH and vitamin D, histological changes of uremic bone disease, vascular calcification and clinical outcome. These intertwined relationships suggest that intervening in one aspect of CKD-MBD may have beneficial impact on outcome. Unfortunately, the complexity of this system frustrates conducting clinical trials that could provide clear-cut proof of clinical importance. For that reason most treatment guidelines have low levels of evidence. On top of that, the spectrum of CKD-MBD is expanding as novel regulating pathways are identified. Mediators of these new pathways, like FGF23 and klotho, subsequently emerged as possible independent markers or even inducers of morbidity and mortality, mainly related to cardiovascular disease.

One of the most striking observations connecting CKD-MBD to overall mortality was the association between the prescription of active compounds of vitamin D to hemodialysis patients and mortality. This association remained significant after correcting for potential confounders. In addition, its validity was confirmed using complex statistical methods, and was consistently found in different patient cohorts. In **chapter 2** we critically appraise the way these studies applied the often hard to comprehend statistical methods. The difference between historical cohort analysis and retrospective studies is clarified, and the validity of data arising from either study type is discussed. The meaning of sensitivity analysis and propensity scores, repeatedly performed to underline the reliability of the observation, is explained. The roles of confounding and mediation, as well as effect modification in relation to studies that link active vitamin D use to improved mortality are outlined and finally, the possible perception of clinicians when confronted with studies that use complex statistical analyses, that form the basis of rather bold conclusions, is discussed. We concluded that, within the limitations inherent of all observational studies, methods used were sound, and

that, in the absence of evidence from prospective clinical trials, it may be considered inconsiderate to dismiss these observations entirely.

In **chapter 3** we evaluated the efficiency of cinacalcet in real-world practice, prescribed when judged necessary by the treating physician, to improve levels of PTH and attainment of current treatment target in 13 Dutch dialysis units. This was studied in the Dutch subgroup of the pan-European ECHO cohort. At baseline 144 hemodialysis and peritoneal dialysis patients were followed for 12 month after initiation of cinacalcet. Also retrospectively data were collected from the 6 month period preceding the start of cinacalcet. Biochemical, pharmacological and clinical data were captured and analysed. Key objective was to determine the percentage of patients that attain the treatment targets as set by KDOQI-guidelines that were prevailing during the observation period. Also, the absolute change of key laboratory parameters was assessed. The percentage of patients within target increased from 8% to 17%, and from 14% to 41% for PTH and calcium respectively, from baseline to month 12. The absolute change in PTH was a 58% decline. When patients were subdivided into three categories, based on baseline level of PTH, we noticed that percentage change of PTH was similar for these three subgroups. This indicates that important improvements in more severe SHPT remain obtainable. We also noticed substantial improvements in calcium levels, while the use of active vitamin D compounds and calcium-containing binders was unchanged, suggesting that calcium release from bone contributed to baseline levels of this mineral.

In **chapter 4** we compared results for individual European countries that participated in the ECHO study. A total of 1865 patients were analysed from 12 countries. Remarkable differences appeared at baseline when cinacalcet was initiated for levels of PTH, calcium and phosphate. Highest baseline median levels for PTH were seen in The Netherlands and the UK (91 and 105 pmol/l respectively), lowest levels in Austria (67pmol/l). Percentage change of PTH ranged from 38% (UK) to 58% in The Netherlands, while achieving KDOQI targets for PTH appeared to be primarily dependent on baseline level of PTH. Covariate analysis revealed that baseline PTH, use of vitamin D and country significantly affected probability of attaining PTH targets. Reductions in levels for calcium were consistent throughout all countries, as was a decline in phosphate levels. The absolute changes in phosphate however,

differed between countries. Differences between countries could be accounted for by different reimbursement at time of observation, differences in local practice or differences in dietary habits between countries.

As epidemiological studies suggest that increases in phosphate levels most strongly predict mortality, when compared with other markers of CKD-MBD, and we noticed a decline in phosphate level for patients that initiated cinacalcet in the ECHO study, we analysed the possibility that this phosphate decrement could be attributed to the use cinacalcet in **chapter 5**. Rationale for this was that in patients with uncontrolled SHPT, an important source of phosphate could be bone instead of dietary intake. Phosphate changes can be induced by changes in phosphate binder therapy and vitamin D use. However, since these data were available in our data set as well, we were able to correct for these factors, using regression analysis with changes in phosphate binder therapy and active vitamin D as categorical parameters (decreased, unchanged, increased dose). For 45% of patients with a decrease in phosphate levels from baseline to end of observation period, this decrease could not be attributed for by a change in vitamin D or phosphate binder therapy, and thus possibly was induced by the initiation of cinacalcet. We established a significant ($p=0.03$) decline in phosphate levels between 2-5% for every 10% decline in level for PTH, depending on concomitant change in phosphate binder use. We subsequently used univariate and multivariate generalized linear models and multivariate regression logistic regression analysis to identify factors that predicted change in phosphate level. We identified dialysis vintage, hours of hemodialysis per week at cinacalcet start, history of parathyroidectomy, baseline PTH, absolute and percentage change in PTH from baseline to month 12 as predicting factors for a change in phosphate level. However, the change in PTH level was the key parameter predicting change in phosphate level. Changes in phosphate binder dosing did not predict change in phosphate level. We concluded that in patients with moderate and severe SHPT, controlling phosphate, aiming at controlling PTH is an effective strategy.

In the second part of this thesis we introduced fibroblast growth factor 23 (FGF23) and klotho in **chapter 6**. Current knowledge covering structure, mode of action, regulation and clinical significance of these two recently discovered compounds, involved in regulation other key components of CKD-MBD, are discussed and summarized. We concluded that the

stage is set for prospectively conducted clinical trials, targeting FGF23, and possibly klotho, aiming at improved clinical endpoints. However, mandatory for any additional study in which the level of FGF23 is a key determinant, solid knowledge of the characteristics of the assay that are being used, is required. Therefore, we performed formal validation testing of commercially available assays for both full-length FGF23 (intact FGF23, iFGF23) and an assay that detects both iFGF23 and its c-terminal fragment, the results of which are described in **chapter 7**. We assessed intra- and interassay variability, matrix interferences, linearity in both healthy volunteers, patients with moderate CKD and on dialysis, and in patients with hypophosphatemic osteomalacia. We found that the assay testing iFGF23 provided by Immutopics^{TM} has unacceptable high interassay variation. The cFGF23 by Immutopics^{TM} and the iFGF23 assay by Kainos^{TM} have acceptable characteristics, providing proper washing procedures is performed for the Kainos assay. For the cFGF23 assay EDTA plasma is required, while for iFGF23 both ADTE plasma and serum is suitable.

In **chapter 8** we conducted a detailed cross-over study in 10 healthy volunteers to detect the changes in levels of both iFGF23 and cFGF23 induced by a diet either low or high in calcium and phosphate content for 36 hours each. We also measured changes in serum phosphate, calcium, vitamin D metabolites, and PTH. For all study periods (regular diet, low-phosphate and high-phosphate diet) a so far not described circadian rhythm was observed, that differed for iFGF23 and cFGF23 suggesting the accumulation of FGF23 fragments during the day. On top of that a modest decline in cFGF23 on phosphate restriction was observed, while the phosphate-enriched diet induced an increase in levels of both cFGF23 and iFGF23. This increase in FGF23 was associated with a significant decline in levels of 1,25vitD, that could not be accounted for by changes in PTH, phosphate or calcium. Our findings are in line with the assumption that FGF23 is a better indicator for phosphate load, compared to plasma phosphate concentration, and that FGF23 levels may explain the increased cardiovascular risk that is associated with “high-normal” phosphate levels in the general population. On top of that, these changes in diet and subsequent changes in FGF23 levels inhibited the activation of vitamin D.

To further delineate the potential role of FGF23 as a cardiovascular risk marker, we examined its relation with a wide range of demographic, clinical and biochemical

characteristics of subjects included in the MASTERPLAN cohort in **chapter 9.** In this well-described cohort of 604 patients, with a mean GFR of 37 ml/min, we could confirm the previously described association with eGFR, PTH, and phosphate, factors involved in regulatory feedback loops for FGF23. On top of that, an association with the presence of diabetes and a history of cardiovascular disease was demonstrated. The most striking findings in this study was the strong association between FGF23, and both smoking and proteinuria. For the former the most likely explanation is a negative effect of smoking on FGF23 sensitivity and thus requirement of higher levels of FGF23 to induce 213hosphaturia. The relationship between FGF23 and proteinuria could be two-sided: FGF23 inducing more pronounced proteinuria, or proteinuria inducing more FGF23. In case of the latter, the most likely explanation could be proteinuria induced tubular damage, leading to FGF23 resistance. These hypotheses however require additional studies.

Giving the well-established connection between the renin angiotensin system (RAS) with proteinuria and clinical outcomes, the impact of active vitamin D on proteinuria and its epidemiological association with clinical outcome, and finally the recent findings of FGF23, and to some extent klotho, as an independent cardiovascular risk factor, we explored the possibility of cross-talk between these systems in **chapter 10.** Using data from the literature we concluded that calcitriol has a direct effect on renin-expression and that some favourable effects attributed to vitamin D may be mediated by attenuated downstream effects of the RAS. The clinical relevance of this is that currently most pharmacological interventions on the RAS intervene more downstream than renin, frequently inducing a compensatory hyperreninism, and the other way around: vitamin D deficiency can induce an activated RAS. On top of that, evidence is emerging that angiotensin II downregulates the expression of klotho, an indispensible factor for FGF23 signaling. Vitamin D deficiency can also induce klotho deficiency, while inducing FGF23 production. Besides an obligate factor for FGF23 signal transduction, klotho has a role in calcium homeostasis and distant cardiovascular protection. Taken together, we conclude that these systems are intertwined, and that future tailored therapy for intervention in one system may require monitoring in another system for optimizing therapy.

This thesis provide several data indicating that current accepted treatments can be further optimized and that new insights into the regulation of key aspects of CKD-MBD hold promise for improved future avenues for improving care for patients suffering kidney disease.

Future direction

As mentioned in the introduction section, even those aspects of management of CKD-MBD that are generally considered to be key treatment targets, like controlling hyperphosphataemia, improving or preventing SHPT, and correcting vitamin D deficiency, lack convincing evidence from clinical trials. This is acknowledged in the recent KDOQI guidelines on diagnosis and treatment of CKD-MBD published in 2009, Although a prospective controlled trial comparing attempts to control hyperphosphaetemia versus no control could still be justified, this is unlikely feasible anymore. However, useful information could come from a trial aiming at different levels of phosphate control, with clinically important endpoints. Differences between currently available phosphate binders in terms of clinical outcome, and even biochemical profile are still poorly understood. In collaboration with the MCA hospital Alkmaar and MUMC Maastricht we will explore differences in vitamin K binding capacity between phosphate binders, and their impact on MGP metabolism. Considering control of hyperphosphatemia, current treatment options are still insufficient, given the pill burden, and insufficient efficacy of monotherapy in more advanced CKD. More options are needed like blockers of sodium-phosphate transporters in the gastrointestinal tract, but will require in-depth study, before application in the clinical situation.

Despite decades of research, the exact role of PTH as a potential culprit in the excess morbidity and mortality is far from clarified. Epidemiological studies in patients suffering from CKD are equivocal, and basic research points to both beneficial and maladaptive effects of increasing levels of PTH in CKD. The identification of PTH as an inducer of hyperphosphatemia in specific circumstances, and both FGF23 production and secretion, may help to determine optimal PTH levels for individual patients, balancing these presumed maladaptive effects to its phosphaturic effects, and its stimulating effect on the production of calcitriol. Clearly, the assumption that an optimal, individualized PTH level can outperform generally applicable treatment targets, requires clinical research. The expanding knowledge on the development of secondary hyperparathyroidism (SHPT), and increased therapeutic options, make tailored treatment of SHPT feasible.

Reducing phosphate uptake by either dietary intervention or pharmacological intervention is an effective means of lowering FGF23, especially in more early stages of CKD-MBD. In the

current “FGF23-era” it is imperative that studies aiming to reduce FGF23 by any means should not make the same mistake as has been done for phosphate-lowering therapies, but should aim for clinically meaningful endpoints. From a mechanistic point of view considering the role of FGF23, a big piece is the puzzle is missing, namely its presumed biological effects in uremia. Published animal studies and our own preliminary data point to direct effects in the heart and possible effects in the arterial wall, but this research is at its dawn only. Exploring beneficial or maladaptive effects of FGF23 at different tissues hold promise of unravelling new mechanism of disease or defence, that ultimately may lead to the development of entire new treatment strategies. This also holds true for klotho. For this substance data are even more preliminary, but possibly even more promising, because effects on endothelial function, the process of vascular calcifications, and premature aging, preceded data, that indicate an overall deficiency of klotho in CKD. Biological effects, changes in tissue and circulating levels of klotho in CKD, and possibilities to modulate its expression and secretion, all require further exploring. The ever-increasing understanding of intense cross-talk between biological systems mandates an open-mind looking for effects of interventions in unexpected systems, like effects of RAS inhibition on phosphate handling, and vitamin D treatment for upregulating klotho levels.

Several key aspects of the abovementioned future directions are currently being addressed by our institution in close collaboration with the UMCG Groningen and UMC St Radboud Nijmegen, and supported by the Dutch Kidney Foundation.