Diet-sensitive prognostic markers for cardiovascular and renal disease

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General Discussion and Future Perspectives

Along with the aging of the population and rising prevalence of lifestyle-related diseases like type 2 diabetes and hypertension, the prevalence of cardiovascular and renal disease has steadily risen all over the world, posing a serious health problem (1-3). Because a wide range of pathophysiological pathways are involved in the development and progression of cardiovascular and renal diseases (4-9), a comprehensive approach is needed to reduce the impact of these lifestyle-related diseases and their subsequent health consequences. Dietary and nutritional factors are relevant players in the development and progression of lifestyle-related diseases. Moreover, pathophysiological pathways that are accessible through dietary intervention are of particular interest for prevention and treatment of cardiovascular and renal disease. In this thesis, we first investigated the effects of accounting for the presence of competing risks in renal risk prediction in patients with type 2 diabetes using traditional risk factors. Second, because traditional risk factors only partially explain the risk for future cardiovascular and renal disease, we used several serum markers to further explore pathophysiological pathways that are involved in the development and progression of cardiovascular and renal disease and are accessible through dietary intervention.

Renal Risk Prediction in Type 2 Diabetes: a Work in Progress

The incidence and prevalence of type 2 diabetes are increasing rapidly, affecting ~347 million people worldwide in 2008, a number that is expected to almost double by 2030 (6,10). Because type 2 diabetes is an important risk factor for chronic kidney disease (CKD) and end-stage renal disease (ESRD) (11,12), the incidence and prevalence of CKD and ESRD are expected to rise along with the increasing incidence and prevalence of type 2 diabetes. Early identification of patients with type 2 diabetes at risk for renal disease may allow optimization of preventive measures to reduce the incidence and progression of renal disease. However, large prospective cohorts of patients with type 2 diabetes with a long follow-up period and available data on albuminuria and renal function are needed to develop renal risk prediction models. These data are extremely scarce, which hinders the development and subsequent validation of prediction models that can be used to early predict the development of diabetic renal disease. Furthermore, patients with diabetes and (micro)albuminuria are also at a particularly high risk of death prior to reaching ESRD (13,14). However, existing models predicting the risk of kidney disease fail to take this potential competing risk of death into account.

In **chapter 1**, we investigated the effects of accounting for the presence of competing risks in 10-year risk prediction of early- and late-stage renal complications in type 2 diabetes. As a consequence of the long time interval before development and progression of diabetic nephropathy, the number of patients that reached doubling of

serum creatinine (SCr) or ESRD was limited in this study population. To identify patients with the most progressive renal function loss within a period of 10 years, we used a surrogate end point of 50% increase of baseline SCr. In **chapter 1**, we demonstrated that in case of a substantial number of competing events as in prediction of late-stage renal complications it is important to account for the competing risk of death when performing survival analyses for renal risk prediction in patients with type 2 diabetes. Furthermore, we found that age was significantly associated with development of late-stage renal complications in standard survival analyses, but age lost significance in competing risk analyses. Evidently, age is a more important risk factor for mortality than it is for renal function decline.

Although the predicted risks for renal complications corresponded better with the observed risks after accounting for the competing risk of death, the ability of the models to distinguish between patients with and without the adverse renal outcomes remained moderate, especially for the models predicting early-stage renal complications. From a preventive medicine point of view, early identification of high risk patients is of particular interest, because it allows to allocate resources, efforts, and the burden of treatment to those patients in whom the possible health gains are highest and to alleviate the burden of treatment in those patients with a low risk. The use of novel biomarkers in renal risk prediction may refine and complement risk prediction. However, so far, results have been disappointing and no single biomarker has been discovered that really adds to established risk factors and albuminuria in particular, a strong predictor for renal disease progression, in renal risk prediction (15). Because multiple underlying pathophysiological pathways are involved in the development and progression of cardiovascular and renal disease (4-9), it may be more likely that a combination of novel biomarkers, reflecting the various pathophysiological processes, could improve renal risk prediction (15).

Effects of Potassium Intake on Fluid Balance

Hypertension is an important risk factor for development and progression of cardiovascular and renal disease (16,17). Lifestyle measures including dietary sodium restriction and increased potassium intake are recognized to lower blood pressure and cardiovascular risk (18-20). Previous studies have suggested that the effects of increased potassium intake on blood pressure are more pronounced at higher levels of sodium intake (20-22). During sodium restriction, increased potassium intake was found to have little or no effect on blood pressure (23). This suggests interaction between potassium intake and sodium and volume status, but this interaction has not been well characterized. In particular, the neurohumoral responses of osmoregulation

and volume regulation pathways to potassium supplementation have not been documented. Therefore, in **chapter 2**, our aim was to investigate the neurohumoral responses of potassium supplementation, as well as the effects on blood pressure, during a fully controlled sodium-restricted diet using a panel of markers that are involved in osmoregulation and volume regulation.

In **chapter 2**, we demonstrated that potassium supplementation has significant, albeit relatively small, blood pressure-lowering effects during sodium restriction in (pre) hypertensive subjects. Potassium is suggested to exert its blood pressure-lowering effects, at least in part, through stimulation of natriuresis (24,25), and may thus act as a diuretic agent. Furthermore, in **chapter 2**, we found that the blood pressure-lowering effects were mitigated by activation of several counter regulatory mechanisms (i.e., increased secretion of vasopressin, stimulation of the renin-angiotensin-aldosterone system [RAAS], and increased heart rate) during potassium supplementation: most likely this counterbalances the decrease in blood pressure. Our study provides new insights in and a biologically plausible explanation for the diminished blood pressure-lowering effects of potassium supplementation during sodium restriction. Although we cannot draw a definitive conclusion regarding the effects of potassium supplementation on extracellular volume and cardiac output, our findings strongly suggest that, at least in a large part of the included (pre)hypertensive subjects, increased potassium intake during sodium restriction decreases effective circulating volume and cardiac output to such an extent that several counter regulatory mechanisms are activated to maintain volume homeostasis and counterbalance the decrease in blood pressure. A study of Grobbee et al. that demonstrated that the fall in blood pressure during a low sodium and high potassium diet was accompanied by a significant decrease in cardiac index, suggesting that the combination of a low sodium and high potassium diet may indeed lower blood pressure by affecting cardiac output (26). Furthermore, these studies were performed in (pre)hypertensive subjects. In normotensive subjects, potassium supplementation was found to have no detectable effect on blood pressure (20), but the humoral effects and the effects on measured extracellular volume have not been well described. It would not only be of interest to more extensively investigate the neurohumoral effects of potassium supplementation, but also to investigate the effects of potassium supplementation on measured extracellular volume and cardiac output during a regular and sodium-restricted diet in both hypertensive and healthy subjects.

Cardiovascular and Renal Disease – a Role for Vasopressin?

Arginine vasopressin (AVP) is one of the key hormones involved in osmoregulation and volume regulation (27). Apart from its role in normal physiology, elevated AVP has

been hypothesized to have deleterious renal and cardiovascular effects. AVP levels are higher in patients with diabetes compared with healthy individuals (28,29). In addition, it has been shown that AVP infusion induces hypertension, glomerular hyperfiltration, and albuminuria in various experimental models, including rodent models of diabetes (30-32). However, epidemiological studies investigating the association between AVP levels and the rate of kidney function decline are lacking. To this end, we investigated cross-sectionally and longitudinally whether copeptin, a surrogate for vasopressin, is associated with renal function decline in patients with type 2 diabetes (**chapter 3**). In cross-sectional analyses, we found that copeptin was positively associated with urinary albumin-to-creatinine ratio (ACR) and inversely associated with estimated glomerular filtration rate (eGFR). In longitudinal analyses in patients without RAAS inhibition at baseline, we found that baseline copeptin is significantly associated with renal function decline. This association was independent of and stronger than the association of traditional risk factors with renal function decline. However, in additional analyses that are not included in this thesis, we found that copeptin had no significant added value in risk prediction of albuminuria or renal function decline on top of established risk factors for renal function decline or albuminuria.

Furthermore, copeptin was also found to be strongly associated with cardiovascular events and mortality in patients with type 2 diabetes and ESRD (33). However, patients with type 2 diabetes and ESRD represent a very small, highly selected group of patients with a strongly increased risk for cardiovascular diseases and mortality. It is not known whether copeptin is associated with cardiovascular and all-cause mortality in regular ambulatory patients with type 2 diabetes. Therefore, in **chapter 4**, we investigated whether copeptin is associated with cardiovascular and all-cause mortality in patients with type 2 diabetes treated in primary care. In this study, we found that copeptin is associated with an increased cardiovascular and all-cause mortality risk in patients with type 2 diabetes. After adjustment for established cardiovascular risk factors, we observed a trend between copeptin and cardiovascular mortality, while the association of baseline plasma copeptin with all-cause mortality remained significant. Although copeptin had additional predictive value on top of age and gender for risk prediction of cardiovascular mortality, copeptin did not substantially improve risk prediction for cardiovascular and all-cause mortality beyond currently used clinical markers.

To summarize, we found copeptin to be significantly associated with renal function decline, cardiovascular and all-cause mortality, but copeptin did not substantially improve risk prediction for albuminuria, renal function decline, or mortality beyond currently used clinical markers. The lack of additional predictive value of copeptin beyond these established risk factors could be explained by the fact that the

plasma copeptin concentration is correlated with these established risk factors such as HbA_{1c}, renal function, and a history of cardiovascular disease. However, from a pathophysiological point of view, the associations of copeptin, as a surrogate for AVP, with renal function decline and mortality are of interest because the AVP system is a potentially modifiable system through pharmacological and non-pharmacological interventions and could provide a potential target for treatment and prevention of renal function decline, cardiovascular events, and mortality in type 2 diabetes. Unfortunately, it is impossible to draw a definite conclusion about the causality of the association of AVP with renal function decline and mortality given the observational design of our studies (**chapter 3** and **chapter 4**). It is important to distinguish whether the association of AVP with outcome is a causal one or whether AVP is merely a marker for an unfavorable risk profile (34): if copeptin is a correlated prognostic marker, it could serve as a prognostic marker for long-term renal and cardiovascular outcomes in patients with type 2 diabetes. However, if AVP is causally related with outcomes, it may serve as a potential target for treatment and prevention of renal and cardiovascular disease in type 2 diabetes.

Serum Sodium as a Prognostic Marker for Mortality

Elevated secretion of AVP may also result in hyponatremia, a disorder of water balance, with a relative excess of body water compared to total sodium content (35). For example, in patients with heart failure, the AVP concentration increases severely due to effective circulating volume depletion (35,36). The resulting water retention attenuates the effective circulating volume depletion, but ultimately at the expense of the occurrence of hyponatremia (36). Hyponatremia has been associated with an increased mortality risk in various study populations including patients with CKD (37), heart failure (38,39), and the general population (40-43). Diabetes is a condition predisposing for elevated levels of AVP and heart failure (28,44), both common causes of hyponatremia. Therefore, in **chapter 5**, we investigated whether serum sodium is associated with mortality in patients with type 2 diabetes, and whether a potential association of serum sodium with mortality could be explained by copeptin or NT-proBNP.

We found that low serum sodium, high copeptin, and high NT-proBNP are, independent of each other, associated with an increased cardiovascular and all-cause mortality risk in patients with type 2 diabetes (**chapter 5**). Thus, the association of serum sodium with mortality is not explained by either elevated secretion of AVP or heart failure. Whether low serum sodium itself leads to poor outcome remains to be elucidated. The serum sodium concentration may reflect the severity of comorbities

such as heart failure. If the serum sodium concentration reflects disease severity, combining of serum sodium concentration with copeptin and/or NT-proBNP may provide additional information on disease severity and prognosis.

Vitamin K and Vascular Calcification

Another pathophysiological pathway that may contribute to the development and progression of cardiovascular and renal disease, in particular in type 2 diabetes and CKD, is vascular calcification (45,46). Accumulating evidence indicates that vascular calcification is an active, tightly regulated, and complex process, with competition between factors promoting calcification and inhibitors of mineralization, but the exact underlying mechanisms are not completely understood (45,47). Matrix Gla protein (MGP) is a vitamin K-dependent inhibitor of soft tissue calcification (48). High plasma desphospho-uncarboxylated MGP (dp-ucMGP) concentrations, indicative of functional vitamin K insufficiency, have been associated with an increased cardiovascular and mortality risk (49-51). However, data regarding the prevalence and the clinical impact of functional vitamin K insufficiency are incomplete. To this end, in **chapter 6**, we assessed the prevalence of functional vitamin K insufficiency, as derived from plasma dp-ucMGP, and its health consequences in a large Dutch general population-based cohort.

In **chapter 6**, we demonstrated that functional vitamin K insufficiency is a common phenomenon especially among elderly and subjects with type 2 diabetes, hypertension, CKD, and cardiovascular disease. In addition, we found J-shaped associations of plasma dp-ucMGP concentrations with all-cause and cardiovascular mortality. Furthermore, we found a significant negative interaction of plasma dp-ucMGP levels with systolic blood pressure on both cardiovascular and cardiac events, and we found that plasma dp-ucMGP levels were inversely associated with cardiac events in subjects with hypertension. These apparent conflicting results may be explained by the fact that MGP is predominantly implicated in arterial medial calcification, which is highly prevalent in CKD and type 2 diabetes (52-55), whereas growth arrest-specific 6 (gas6), a proinflammatory and prothrombotic vitamin K-dependent protein, may enhance plaque inflammation in atherosclerotic calcification (56). However, further research is needed to test this hypothesis and clarify the mechanisms involved.

Thus, functional vitamin K insufficiency is a common phenomenon that may have serious health consequences. A number of factors may contribute to functional vitamin K insufficiency, for example inadequate dietary intake or impaired vitamin K recycling. Direct quantification of vitamin K in plasma was reported to be the best indicator of recent dietary intake (57-59). In **chapter 7**, we describe a simple and rapid LC-MS/MS

method for determination of vitamin K_1 and vitamin K_2 (i.e., menaquinone-4 and -7) in human plasma. Measurement of status markers like plasma levels of vitamin K may provide relevant additional information on top of functional vitamin K status.

Conclusion and Future Perspectives

The global burden of cardiovascular and renal disease is increasing, therefore it is important to more extensively explore the underlying pathophysiological pathways and identify targets for non-pharmacological and pharmacological treatment of cardiovascular and renal disease. Accordingly, pathways that are accessible through dietary intervention are of particular interest. In this thesis, we used serum markers to explore pathophysiological pathways involved in the development and progression of cardiovascular and renal disease and demonstrated that several diet-sensitive prognostic markers are associated with the development and progression of cardiovascular and renal disease.

One diet-sensitive pathophysiological pathway that is involved in cardiovascular and renal disease that we explored is fluid balance. We investigated the effects of potassium supplementation, on top of a fully controlled sodium-restricted diet, on markers of osmoregulation and volume regulation in (pre)hypertensive subjects. The results of our study suggested that potassium supplementation during sodium restriction decreases effective circulating volume to such an extent that several counter regulatory mechanisms (i.e., increased secretion of vasopressin, stimulation of the RAAS, and increased heart rate) are activated to maintain volume homeostasis and counterbalance the decrease in blood pressure. It is important to further investigate the neurohumoral effects and dose-effect relationship of potassium and sodium supplementation in future randomized, double-blind, placebo-controlled crossover trials. If our findings are confirmed by future intervention studies, this is important knowledge for the recommendations on sodium restriction and increasing potassium intake, and on dietary counseling that takes not only the separate nutrients into account, but the balance in their combination as well.

Another diet-sensitive pathophysiological pathway that we explored is vascular calcification. MGP is a strong vitamin K-dependent endogenous inhibitor of soft tissue calcification. We demonstrated that functional vitamin K insufficiency is common, especially among elderly and subjects with type 2 diabetes, hypertension, CKD, and cardiovascular disease, which may have important health consequences. We found that functional vitamin K insufficiency, as derived from plasma dp-ucMGP concentrations, was associated with an increased all-cause and cardiovascular mortality risk. This may have relevance for intervention strategies, as vitamin K supplementation is cheap

and easy, and likely to be safe. Therefore, future, preferably long-term, intervention studies are needed to investigate whether vitamin K supplementation could prevent the development and progression of cardiovascular and renal disease.

Furthermore, since vascular calcification is a complex process with competition between inhibitors and promoters of calcification, it may be important to not only focus on a single marker such as MGP, but also to investigate potential interactions and joint effects of factors that are known to affect vascular calcification. Vitamin D, for example, was suggested to have a biphasic dose-response curve association with vascular calcification: i.e., vitamin D deficiency as well as an excess of vitamin D were found to be associated with an increased risk for vascular calcification through inflammation and hyperphosphatemia/hypocalcemia, respectively (60,61). It would be of interest to investigate a potential interaction between vitamin D levels and functional vitamin K status in respect to vascular calcification.

In conclusion, if the findings in this thesis are confirmed by future preferably long-term intervention studies, this could have several clinical implications. Awareness should be raised on diet composition (i.e., the separate nutrients as well as the balance in their combinations) and its role in cardiovascular and renal health. Furthermore, individual titration of non-pharmacological as well as pharmacological treatment strategies based on serum markers could be new approaches in personalized medicine aiming to reduce the risk for cardiovascular and renal disease.

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