Diet-sensitive prognostic markers for cardiovascular and renal disease

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Contents

Introduction and Aims of Thesis

General Introduction

Over the past decades, the prevalence of cardiovascular and renal disease has steadily risen all over the world, and these noncommunicable diseases are currently among the leading global and regional causes of death (1-3). These growing numbers are likely to reflect the aging of the population and rising prevalence of lifestyle-related diseases like obesity, type 2 diabetes, and hypertension; all known to be important risk factors for the development and progression of cardiovascular and renal disease (3,4). A wide range of pathophysiological pathways and risk factors are involved in the development and progression of cardiovascular and renal disease (5-10). Therefore, a comprehensive approach is needed to prevent and 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 will use serum markers to explore several pathophysiological pathways that are involved in the development and progression of cardiovascular and renal disease and accessible through dietary intervention.

Renal Risk Prediction

The incidence and prevalence of end-stage renal disease (ESRD) have steadily increased worldwide (11,12). For example, the number of patients with ESRD requiring renal replacement therapy (i.e., dialysis or transplantation) has doubled over the last 15 years in the Netherlands (13). This increase in prevalence is likely contributable to the rising rates of lifestyle-related diseases like type 2 diabetes and hypertension, which are major risk factors for the development of chronic kidney disease (CKD) and progression to ESRD (3,14). Although patients with type 2 diabetes and hypertension are at increased risk for CKD, not all patients in these high-risk groups will eventually develop CKD or progress to ESRD. Risk prediction may help to early identify patients at risk for renal disease and guide the initiation of appropriate treatment to further reduce the incidence and progression of CKD.

Over the past decades, several models have been developed to predict the risk of CKD in the general population (15-18). However, patients with diabetes and (micro)albuminuria are also at a particularly high risk of death prior to reaching ESRD (19,20). However, existing models predicting the risk of kidney disease fail to take this potential competing risk of death into account. Therefore, in **chapter 1**, we aimed to investigate the effects of accounting for the presence of competing risks in renal risk

prediction in type 2 diabetes. To this end, we compared the predictive performance of Cox regression and competing risk models using traditional risk factors for 10-year risk prediction of early- and late-stage renal complications (i.e., [micro]albuminuria and 50% increase in serum creatinine, respectively) in patients with type 2 diabetes.

Exploration of Novel Pathophysiological Pathways

Traditional risk factors, however, only partially explain the risk for future renal and cardiovascular events. Interventions targeting these traditional risk factors including glycemic control (21,22), blood pressure lowering (23,24), and lipid management (25-27) were found to delay cardiovascular and renal disease progression. In addition, the use of renin-angiotensin aldosterone system (RAAS) inhibitors has been shown to lower blood pressure and decrease albuminuria which leads to additional protective renal and cardiovascular effects (28-30). Despite these current treatment regimens, the residual risk for cardiovascular and renal disease remains extremely high (31). Thus, new targets for therapeutic intervention and monitoring of disease progression are required to further slow the progression of cardiovascular and renal disease.

Laboratory medicine may be a valuable tool to identify and explore pathophysiological pathways that are involved in the development and progression of cardiovascular and renal disease. In addition, the discovery of novel biomarkers may refine and complement risk prediction and monitoring of disease progression. In this thesis, we will focus on two pathophysiological pathways involved in cardiovascular and renal disease progression that may be accessible through dietary interventions (i.e., fluid balance and vascular calcification).

Effects of Sodium and Potassium Intake on Fluid Balance

Hypertension is one of the leading causes for the development and progression of cardiovascular and renal disease (32). High sodium intake has been found to be associated with increased blood pressure, whereas potassium intake was found to be inversely associated with blood pressure (33). Moreover, lifestyle measures including dietary sodium restriction and increased potassium intake are recognized to lower blood pressure and cardiovascular risk (34-36).

A key aspect in long-term regulation of blood pressure is fluid balance, which is regulated by means of osmoregulation and volume regulation. Normally, body fluid volume and electrolyte concentration are maintained within very narrow limits despite wide variations in dietary sodium and potassium intake. The mechanisms involved in counterbalancing the blood pressure raising effects of sodium have been investigated repeatedly. These include suppression of RAAS, resulting in a decreased tendency for

sodium reabsorption (37), and stimulation of release of natriuretic peptides, allowing for increased natriuresis (Figure 1) (38).

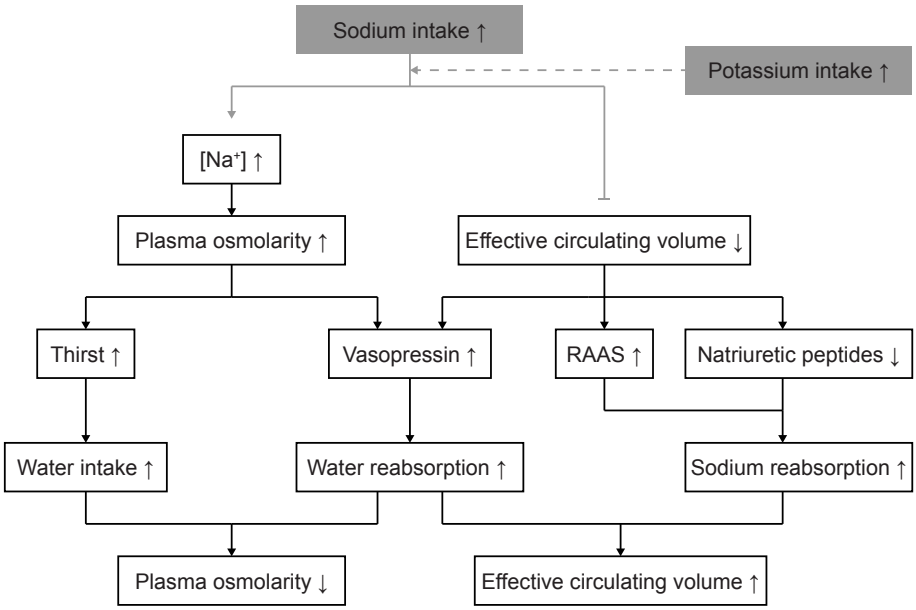

Figure 1. Simplified schematic overview of the potential effects of sodium and potassium intake on markers of osmoregulation and volume regulation.

Previous studies, including meta-analyses of randomized controlled trials, have suggested that the blood pressure lowering effects of potassium are more pronounced at higher levels of sodium intake (36,39,40). During sodium restriction, potassium intake was found to have little or no effect on blood pressure (41). 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**, we investigated the humoral effects of potassium supplementation during a fully controlled sodium-restricted diet using a panel of markers that are involved in osmoregulation and volume regulation (Figure 1). We additionally investigated the effects of sodium supplementation, with surmised opposite changes in markers of osmoregulation and volume regulation.

Cardiovascular and Renal Disease - A Role for Vasopressin?

Arginine vasopressin (AVP), also known as antidiuretic hormone (ADH), is one of the key hormones involved in osmoregulation and volume regulation (42). The primary stimuli for physiologic release of AVP are an increase in plasma osmolarity, hypotension, hypovolemia, and stress (42). AVP acts through three different vasopressin receptors, namely the V_{1a}, V_2, and V_3 (or V_{1b}) receptors, which mediate vasoconstriction, stimulate water retention, and facilitate secretion of adrenocorticotropic hormone (ACTH), respectively (43).

Apart from the role of AVP in normal physiology, elevated levels of AVP have been hypothesized to have deleterious renal and cardiovascular effects. It has been shown that AVP levels are higher in patients with diabetes compared with healthy individuals (44,45), especially in patients with diabetes and (micro)albuminuria (46). Furthermore, it has been shown that AVP infusion induces hypertension, glomerular hyperfiltration, albuminuria, and glomerulosclerosis in various experimental models, including rodent models of diabetes (47-49). In contrast, lowering AVP concentration by water loading resulted in less kidney damage (50). However, epidemiological studies investigating the association between AVP levels and the rate of kidney function decline are lacking. Therefore, in **chapter 3**, we investigated the association of copeptin, a sensitive surrogate marker for AVP (51), with renal function decline, both cross-sectionally and longitudinally, in patients with type 2 diabetes.

Several studies have reported that copeptin is also associated with cardiovascular events and mortality in patients with cardiovascular diseases (i.e., acute myocardial infarction, heart failure, and stroke) (42). Moreover, copeptin levels were found to be strongly associated with cardiovascular events and mortality in patients with type 2 diabetes and ESRD (52). 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. In **chapter 4**, we prospectively investigated whether plasma copeptin levels were associated with cardiovascular and all-cause mortality in patients with type 2 diabetes treated in primary care.

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 (53). For example, in patients with heart failure, the AVP concentration dramatically increases due to severe effective circulating volume depletion (53,54). The resulting sodium and water retention attenuates the effective circulating volume depletion, but

ultimately at the expense of the occurrence of hyponatremia (54). Hyponatremia has been associated with an increased mortality risk in various study populations including patients with CKD (55), heart failure (56,57), and the general population (58-61).

Diabetes is a condition predisposing for elevated levels of AVP and heart failure (44,62), both common causes of hyponatremia. Elevated levels of AVP and heart failure, however, are also associated with an increased mortality risk (63-65). Therefore, in **chapter 5**, we investigated whether serum sodium concentration is associated with cardiovascular and all-cause mortality in patients with type 2 diabetes, and whether a potential association of serum sodium with mortality could be explained by copeptin, a surrogate marker for AVP, or NT-proBNP, a marker of heart failure.

Vitamin K and Vascular Calcification

Another pathophysiological pathway that may contribute to the development and progression of renal and cardiovascular disease, in particular in CKD and type 2 diabetes, is vascular calcification (66,67). Until recently, vascular calcification was thought to result from passive precipitation of calcium and phosphate that was closely linked to ageing (66-68). However, 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 remain not completely understood (66,68).

Matrix Gla protein (MGP) is a strong endogenous inhibitor of soft tissue calcification (69). Activation of MGP by carboxylation is vitamin K-dependent and essential for MGP's activity as a calcification inhibitor (Figure 2) (70,71). Plasma desphospho-uncarboxylated (dp-ucMGP) was found to be a sensitive marker for vascular vitamin K status (72). High plasma dp-ucMGP concentrations, indicative of functional vitamin K insufficiency, are common in patients with CKD and have been associated with an increased cardiovascular risk (73,74). In addition, a recent study showed that high plasma dp-ucMGP concentrations are associated with adverse health outcomes in a general population cohort (75). However, data regarding the prevalence of functional vitamin K insufficiency, and thus its clinical impact, are incomplete. In **chapter 6**, we assessed the prevalence of functional vitamin K insufficiency, as derived from plasma dp-ucMGP, in a large Dutch general population-based cohort. Furthermore, we investigated whether plasma dp-ucMGP concentration is associated with cardiovascular events and mortality and whether these associations are modified by comorbidities such as diabetes, hypertension, and CKD.

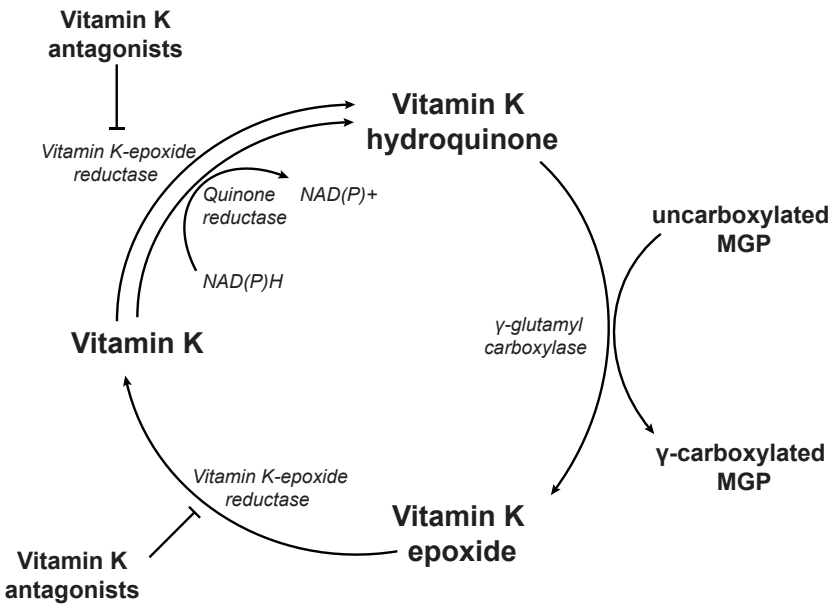

Figure 2. Schematic illustration of the vitamin K cycle. Vitamin K serves as a crucial cofactor in activation of matrix Gla protein (MGP). The vitamin K cycle can be inhibited by vitamin K antagonists.

As mentioned, functional vitamin K insufficiency may be a serious health problem. Inadequate dietary intake of vitamin K may contribute to functional vitamin K insufficiency. However, vitamin K recycling may also be impaired. For example, in a rat model of CKD, it was shown that the activity of γ-glutamyl carboxylase, the enzyme that catalyzes the posttranslational γ-carboxylation of vitamin K-dependent proteins such as MGP (Figure 2), was reduced (76). Status markers like plasma levels of vitamin K_1 (phylloquinone [PK]) and vitamin K_2 (menaquinones [MK-n]) are likely to provide relevant additional information in this rapidly emerging field. Given the growing interest in vitamin K status and intake, the development of new LC-MS/MS methods can be useful for speeding-up the sample preparation process and for obtaining a higher sample throughput. In **chapter 7**, we describe a simple and rapid LC-MS/MS method for determination of PK, MK-4, and MK-7 in human plasma. Furthermore, we investigated the association of plasma vitamin K concentration with vitamin K intake and functional vitamin K status, as derived from plasma dp-ucMGP, in renal transplant recipients.

Aims of this Thesis

In this thesis, we will explore several pathophysiological pathways involved in the development and progression of cardiovascular and renal disease that are accessible through dietary interventions (Figure 3).

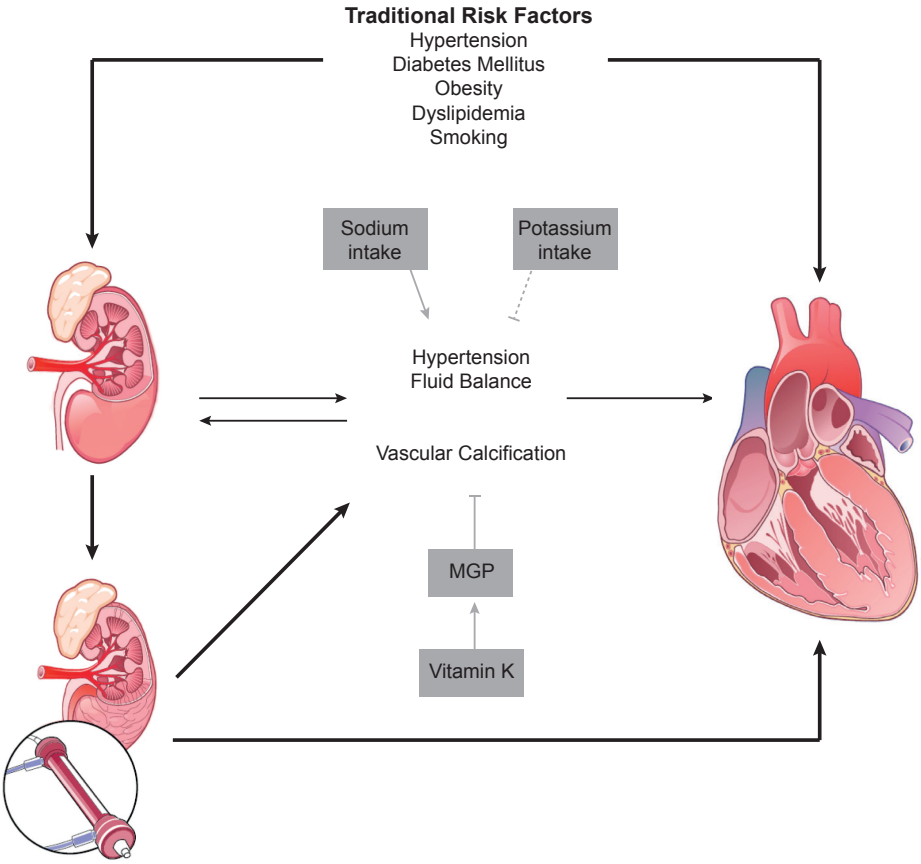

Figure 3. Simplified schematic overview of several diet-sensitive pathophysiological pathways involved in the development and progression of cardiovascular and renal disease. Adapted from references 77 and 78 with permission from Elsevier.

Risk prediction may help to early identify patients at risk for CKD and guide the initiation of appropriate treatment to further reduce the incidence and progression of CKD. In **chapter 1**, we will investigate the effects of accounting for the presence of competing risks in renal risk prediction in type 2 diabetes using traditional risk factors.

Traditional risk factors, however, only partially explain the risk for future renal and cardiovascular events and the residual risk for cardiovascular and renal disease remains extremely high despite the current treatment regimens targeting traditional risk factors. Novel insights in the pathophysiological mechanisms underlying the development and progression of cardiovascular and renal disease as well as novel targets for (dietary) intervention are required to further slow the progression of cardiovascular and renal disease.

In **chapter 2**, we will focus on osmoregulation and volume regulation and we will assess the effects of sodium and potassium supplementation, on top of a fully controlled sodium-restricted diet, on markers of osmoregulation and volume regulation in (pre) hypertensive subjects. In **chapters 3 and 4**, we will zoom in on vasopressin, one of the key hormones involved in osmoregulation and volume regulation and investigate whether copeptin is associated with renal function decline and cardiovascular and all-cause mortality in type 2 diabetes. In **chapter 5**, we will investigate whether low serum sodium concentration, a potential consequence of an elevated AVP concentration, is associated with mortality in type 2 diabetes, and whether this association could be explained by copeptin or NT-proBNP.

Another pathophysiological pathway that may contribute to the development and progression of cardiovascular disease is vascular calcification, which is common in high-risk populations such as type 2 diabetes, hypertension, and CKD. MGP is a strong vitamin K-dependent inhibitor of soft tissue calcification. In **chapter 6**, we will investigate the prevalence of functional vitamin K insufficiency and its health consequences in a large Dutch general population-based cohort. In **chapter 7**, we will describe a simple and rapid LC-MS/MS method for determination of vitamin K_1 and K_2 (MK-4 and -7) in human plasma.

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