Metabolic alterations in dialysis patients

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Contents

CHAPTER 1

Introduction and scope of the thesis

Background

Chronic kidney disease (CKD) is defined as a reduction of glomerular filtration rate and / or an abnormal urinary excretion of protein. It is frequent, and according to the most recent analysis of the National Health and Nutrition Examination Survey (NHANES) alarmingly increasing. Its prevalence in the United States currently is about 13% [1,2], and in Europe about 10%. The increasing trend observed in the recent decade translates into important problems, both from an individual and a public health perspective. First, more patients develop end-stage renal disease, which is present at an approximately 90% reduction of kidney function. The affected patients require renal replacement therapy, which is provided in the form of dialysis - one of the most expensive chronic therapies - or kidney transplantation. Second, chronic kidney disease and end-stage renal disease per se are strong risk factors for morbidity and mortality [3]. Patients with a moderately impaired kidney function already have a high risk to develop cardiovascular and non-cardiovascular complications[4]. Mortality risk further increases inversely proportionate to the decline in kidney function, and the majority of patients with chronic kidney disease die before reaching end-stage renal disease.

In the patients undergoing maintenance dialysis, the rate of death is excessive and similar to that of cancer. The ERA-EDTA registry reports a 18% first year mortality rate [5]. An even higher annual mortality rate of >20% is reported by the USRDS [6]. Compared to the general population, mortality ranges from a 500 fold increased risk in young patients aged 25-35 years to a 5-fold increased risk in individuals of a high age of 85 years or more [7,8]. Cardiovascular events represent with 43% a major cause of death among dialysis patients [6], and efforts have been undertaken to decrease risk. However, reducing mortality among patients undergoing dialysis has been proven difficult and remains a global challenge. The past 10 years have seen trials of many interventions designed to improve survival and CV outcomes in these patients. Unfortunately, few of these interventions have been shown to be effective, despite beneficial effects in surrogate markers [9-17] (Table 1).

Table 1. Randomized trials in hemodialysis (HD) patients

HD Patients: Randomised Trials with Endpoint Cardiovascular Disease or Death

Intervention	Year	N	Event	RR (95% CI)
EPO-Hematokrit [9]	1998	1233	366	1.3 (0.9-1.9)
Vitamin E [10]	2000	196	48	0.46 (0.27-0.78)
Acetylcysteine [11]	2003	134	51	0.60 (0.38-0.95)
Carvedilol [12]	2003	114	71	0.51 (0.32-0.82)
Dialyse Dosis x Flux [13]	2004	1846	871	0.92 (0.81-1.05)
Atorvastatin [14]	2005	1255	469	0.92 (0.77-1.10)
ACE-Inhibitor [15]	2006	397	130	0.93 (0.68-1.26)
Folic acid [16]	2007	2056	884	1.04 (0.91-1.18)
Rosuvastatin [17]	2009	2776	804	0.96 (0.84-1.11)

[9] Besarab et al, *N Engl J Med* 1998;339:584
[10] Boaz et al, *Lancet* 2000;356:1231
[11] Tepel et al, *Circ* 2003;107:992
[12] Cice et al, *JACC* 2003;41:1438
[13] Cheung et al, *Kidney Int* 2004;65:2380
[14] Wanner et al, *N Engl J Med* 2005;353:238
[15] Zannad et al, *Kidney Int* 2006;70:1318
[16] Jamison et al, *JAMA* 2007;298:1163
[17] Fellström et al, *NEJM* 2009;360:1395

For example, with very promising results from the general population, statins have been tested in dialysis patients in the 4D study [14] and the Aurora study [17]. As a surprise, both studies did not show a significant reduction in their primary endpoint of combined cardiovascular events and of mortality, raising the question of underlying reasons and future options.

Caution is required in translating findings from the general population to patients with chronic kidney disease, and general treatment recommendations can unfortunately not be applied to this patient group. Extrapolation of data from the general population may not meet the special disease pattern of kidney disease patients. One central question is - why?

Metabolic disturbances, which are common and strongly affecting morbidity and mortality in the general population, are also highly prevalent in dialysis patients. In this context, obesity is known to be a risk factor for the development of chronic

kidney disease and progression to end-stage renal disease. Furthermore, up to one half of the dialysis patients suffer from diabetes mellitus type 2, which in most cases is the cause for the failing kidneys and reaching end-stage renal disease (ESRD) [6, 18]. Similarly, Dyslipidemia in dialysis patients is more frequent than in the general population [19] and is characterized by hypertriglyceridemia and low levels of HDL. However, the effects of metabolic disturbances on clinical outcomes, including residual renal function in dialysis, cardiovascular events, and mortality, are not clear. Epidemiological studies in part even show paradoxical associations, which do not necessarily reflect causality however. With the combination of pathphysiological knowledge and epidemiologic reasoning, it is a challenge to reveal and approach true effects. True effects are essential to be identified, separated from clinical and methodological "distorsions", in order to develop new strategies and effectively treat dialysis patients for an improved survival.

Assessing metabolic risk in dialysis patients, three main aspects may be important: 1) the pathophysiologic effects of metabolic disturbances including obesity, diabetes mellitus, adipokine metabolism and lipid abnormalities are unlikely to completely reverse once patients reach dialysis. 2) Specific additional problems related to chronic kidney disease, in particular protein-energy wasting, may act as "competing risk", overshadow other effects and interfere in various hormonal regulations. 3) In advanced chronic kidney disease and end-stage renal disease, the pattern and composition of risk is changing. While myocardial infarction represents the most frequent cause of death in the general population, dialysis patients predominantly die of sudden cardiac death, accounting as a single cause for one quarter of all deaths [6, 14]. Certain disease states may predominantly affect macrovascular events such as myocardial infarction, while others may lead to microvascular complications, contributing differently to overall mortality.

Aim of this thesis

The aim of this thesis is to

1) Detect specific effects of metabolic alterations in dialysis patients
2) Provide explanations for conflicting results in the literature
3) Provide a rationale for novel interventions.

In this thesis, the metabolic status of dialysis patients is adressed and its consequences for the decline in residual kidney function, cardiovascular events and survival. The metabolic status includes alterations in nutritional and hormonal status. We will focus on:

1) lipid metabolism
2) diabetes mellitus type 2 and the importance of glycemic control
3) obesity and loss of residual kidney function
4) the role of adipokines and its longitudinal changes for clinical outcomes
5) specific effects of protein-energy wasting, including its impact in hormonal regulations of the bone-mineral axis
6) Vitamin D status and clinical consequences

Study Populations

The investigations are performed in two large cohorts of dialysis patients, the 4D and NECOSAD studies:

4D study

The 4D study was a prospective randomized controlled trial including 1255 patients with type 2 diabetes mellitus, age 18 – 80 years, and on hemodialysis for less than 2 years. Between March 1998 and October 2002, patients were recruited in 178 dialysis centres in Germany, and randomly assigned to double-blinded treatment with either 20mg atorvastatin (n=619) or placebo (n=636) once daily. Patients were followed up and visited in 6 monthly intervals until the date of death, censoring, or end of the study in March 2004 (median follow-up was 4 years). The primary endpoint of the 4D study was defined as a composite of death from cardiac causes, fatal or nonfatal stroke and nonfatal myocardial

infarction (MI), whichever occurred first (composite cardiovascular endpoint; CVE). Secondary endpoints included death from all causes, cardiac events, and cerebrovascular events.

Necosad study

The Netherlands Cooperative Study on the Adequacy of Dialysis (NECOSAD) is an observational, prospective cohort study, in which consecutive patients who were at least 18 years old, had ESRD and started chronic dialysis were included. A total of 2045 patients were enrolled in 38 participating dialysis centres between 1997 and 2006 in the Netherlands (19;21). They were followed up and visited in intervals of 6 months until the date of death, transplantation, withdrawal or end of the study at 01-01-2009. Necosad participants include both patients on hemodialysis and on peritoneal dialysis. Apart from demographic and clinical data, 24 hour urine collections were obtained at all visits. The latter allowed for the unique assessment of residual renal function over time, in addition to cardiovascular and non-cardiovascular mortality.

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CHAPTER 2

Evidence based management of lipid disorders

What are the consequences of renal insufficiency or the nephrotic syndrome for lipid levels?

Christiane Drechsler
Christoph Wanner
Ton Rabelink

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An introduction to chronic kidney disease

Chronic kidney disease is characterized by the failure of the kidneys to remove waste products and excess fluid from the body. It is defined by a sustained impairment of kidney function, as reflected by an abnormal excretion of urinary protein or a reduction of the glomerular filtration rate (GFR). When the glomerular filtration rate reaches levels below 15 ml/min (corresponding to a reduction in kidney function by approximately 90%), patients require renal replacement therapy, which is provided in the form of dialysis or transplantation. The etiology of chronic kidney disease (CKD) is heterogeneous, involving both primary kidney diseases, and a variety of non-renal diseases, which affect the kidneys. The main causes among primary kidney diseases are glomerulonephritis and renal vascular diseases, while diabetes mellitus, hypertension, and atherosclerosis are the most frequent non-renal causes potentially leading to a loss of kidney function.

The National Kidney Foundation - Kidney Disease Outcomes Quality Initiative (NKF-K/DOQI) workgroup has defined CKD by a glomerular filtration rate <60 ml/min/1.73m^2, or the presence of a marker of kidney damage.[1,2] Based upon these definitions, a classification of CKD by stages has been recommended and internationally accepted (Table 1).

Chronic kidney disease represents a large public health problem. Its prevalence in the United States currently is about 13% [3,4], with an increasing trend in the recent decade. Data from the United States Renal Data System (USRDS) furthermore show an increase in the incidence rate of end-stage renal disease, reaching 360 per million population in 2006. Accordingly, the number of patients entering the end-stage renal disease (ESRD) program rose from 106.912 in 2005 to 110.854 patients in 2006 [3,5]. The costs of treatment, which in the form of dialysis represents one of the most expensive chronic therapies, put an enormous burden on health care resources. Prevention of disease progression and associated complications therefore is highly important, requiring the knowledge of risk factors and appropriate treatment.

Table 1. Stages of chronic kidney disease

Stage		GFR (ml/min/1.73m^2)	Prevalence
1	Kidney damage with normal or increased GFR	≥ 90	3.3%
2	Kidney damage with mild decreased GFR	60 – 89	3.0%
3	Moderately decreased GFR	30 – 59	4.3%
4	Severely decreased GFR	15 – 29	0.2%
5	Kidney failure	< 15 (or dialysis)	0.1%

GFR = glomerular filtration rate.

Dyslipidemia in the patient with kidney disease

Proteinuria induced dyslipidemia

Abnormal lipid metabolism is common in patients with renal disease, and most prominent in the nephrotic syndrome. Studies have shown that about half of the patients with nephrotic syndrome (proteinuria > 3g/day) had total cholesterol concentrations above 300 mg/dL [6,7], and 80% of the patients had LDL cholesterol levels above 130 mg/dL [8].

The main abnormalities of lipid metabolism in the nephrotic syndrome include increases in total cholesterol, low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) cholesterol, apolipoproteins B (Apo B), C-III and triglycerides, while Apo A-I is reduced. Furthermore, the high-density lipoproteins are distributed abnormally (increased HDL_3 fraction and decreased HDL_2 fraction). Hyperlipidemia in the nephrotic syndrome results from increased hepatic synthesis and decreased catabolism of lipoproteins, whereby the contribution of each to establishing blood lipid levels has not been characterized in detail. Increased triglyceride rich lipoprotein concentration, VLDL and intermediate density lipoprotein (IDL) primarily results from decreased clearance [9], partly due to a reduced lipoprotein lipase (LPL) activity. Lipoprotein lipase is necessary for endothelial binding of VLDL and for normal lipolysis. The loss of the cofactor apoCII (a lipoprotein lipase activator), which is especially common in proteinuric renal disease, and a depletion of the endothelial bound lipoprotein lipase (LPL)

pool [10] may contribute to the reduced LPL-activity. In addition, both LDL and lipoprotein(a) [Lp(a)] synthesis are increased [9,11], whereby evidence exists that LDL synthesis may be augmented through a mechanism bypassing its normal precursor VLDL. Finally, due to decreased activity of lecithin:cholesterol acyltransferase (LCAT) in proteinuric renal disease [12], mature, the number of spherical HDL particles are decreased. These particles are important carriers for several cofactors, amongst which apoCII, affecting LPL activity and VLDL level.

The impact of CKD on serum lipids and lipoproteins

Dyslipidemia in patients with CKD and no nephrotic syndrome can be characterized by high triglyceride levels and low HDL concentrations, while total and LDL cholesterol are normal or even low [13,14]. Although the lipid abnormalities captured by routine laboratory measurements may not be impressive, more sophisticated analyses reveal profound disturbances in lipid metabolism. Mainly as a result of decreased catabolism, the concentration of triglyceride-rich lipoproteins (VLDL, IDL) is increased, in particular in the post prandial phase. Lipolysis of the highly atherogenic VLDL and chylomicron (CM) remnants is impaired partly due to the decreased lipoprotein lipase (LPL) on the vascular endothelium, and partly due to increased levels of the major LPL inhibitory apolipoprotein apo CIII. Furthermore, kidney failure is associated with a shift in the size distribution of LDL to increased content of small dense LDL. A reduction in LDL size, resulting in increased levels of small dense LDL, results from increased TG concentration, which via the action of cholesterol ester transfer protein (CETP) and hepatic lipase (HL) [15] result in the formation of small dense LDL. Due to the increased oxidative stress in patients with CKD and the reduced clearance, the fraction of highly atherogenic oxidized LDL is increased.

Disorders in HDL maturation and catabolism add to the dyslipidemic profile in CKD patients. Processes favoring HDL maturation are associated with a greater abundance of large mature HDL and greater HDL levels. The adenosine triphosphate binding cassette 1 (ABCA 1) is responsible for initial lipidation of apoAI and transfer of cholesterol and small (native) HDL particles. The formation of large, mature HDL particles is mediated by LCAT -resulting in the esterification

of cholesterol- as well as by LPL. As previously mentioned, functional deficiencies in LCAT and LPL activity may therefore affect HDL maturation.

In addition, elevated levels of Lp(a) have been found in CKD. [16-20], partly due to the diminished renal clearance [17]. Lp(a) is an LDL-like particle which has an additional protein, apolipoprotein(a), and constitutes an important risk factor for atherosclerosis. The mechanisms of lipid metabolism and alterations in renal failure are shown in Figure 1.

Lipid disorders in dialysis

The lipid abnormalities in CKD stages 2-4 as characterized by an increase in plasma triglycerides, VLDL and IDL, along with a reduction in HDL cholesterol, generally also apply for dialysis patients. Dyslipidemia becomes more pronounced as kidney failure advances to CKD stage 5 requiring dialysis.

Hemodialysis and peritoneal dialysis can both provide an adequate relief of uremic symptoms, but the two techniques appear to have different effects on uremic dyslipidemia [21,22]. Patients on peritoneal dialysis (PD) show higher cholesterol, triglyceride, LDL and Lp(a) levels than patients on maintenance hemodialysis (HD). Possible reasons may be a considerable loss of protein (7-14g/day) into the peritoneal dialysate, and the absorption of glucose (150-200g/day) from the dialysis fluid. Plasma concentrations of apoB100 were shown to be increased in peritoneal dialysis patients, whereas normal concentrations of apoB100 were found in hemodialysis patients [21]. The increase in apoB100 thereby was most markedly in the VLDL fraction, and only to a minor extent in IDL and LDL. An overproduction of VLDL-1 and VLDL-2 apoB100 has been suggested secondary to reduced insulin sensitivity and increased free fatty acid availability in PD patients. This leads to an increase in the poolsize of triglycerides and apoB100 in the VLDL fraction of PD patients. Although lipid profiles differ between PD and HD patients, abnormalities in lipid metabolism qualitatively have similarities regarding the pathogenesis of atherosclerosis and endothelial dysfunction in these groups.

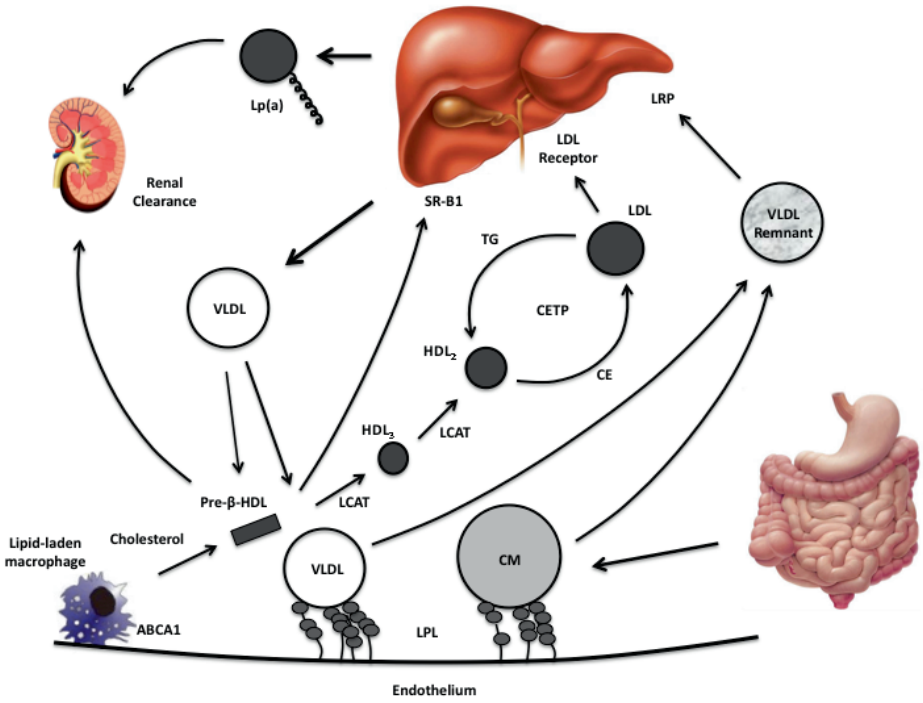


Figure 1. Triglyceride-rich lipoproteins are secreted by the gut (chylomicrons) or liver (VLDL) and then are processed on the vascular endothelium by lipoprotein lipase (LPL), yielding remnant particles. LPL is activated by apo CII and inhibited by apo CI and apo CIII. Apo CI and apo CIII are increased in chronic kidney disease (CKD). HDL formation is initiated by the combination of apo AI with cholesterol and phospholipids through interaction with the adenosine triphosphate binding cassette 1 (ABCA1). The nascent pre-β-HDL is then matured by cholesterol esterification through lecithin cholesterol ester transfer protein (LCAT), first to HDL_3 and then to HDL_2. HDL_2 is taken up by the liver by the scavenger receptor B1 (SR-B1). Alternatively, it transfers its cholesterol ester-rich core to VLDL, creating LDL (via CETP activity). LCAT protein mass and activity are both reduced in CKD. This results in accumulation of pre-β or discoidal HDL and HDL_3, which are subject to accelerated degradation in part by the kidney. While HDL_3 is usually rich in the antioxidant enzyme paraoxonase 1 (PON1), this is not the case in HDL_3 in patients having CKD. Lipoprotein(a) levels are increased in CKD as a result of decreased clearance.

Abbreviations: TG = triglyceride, VLDL = very low density lipoprotein, LPL = lipoprotein lipase, CKD = chronic kidney disease, HDL = high density lipoprotein, ABCA1 = adenosine triphosphate binding cassette 1, LCAT = lecithin cholesterol ester transfer protein, SR-B1 = scavenger receptor B1, CETP = cholesterol ester transfer protein, Lp(a) = lipoprotein (a), LRP = lipoprotein like receptor, CE = cholesteryl ester.

Lipid disorders after kidney transplantation

Lipid abnormalities in kidney transplant recipients are common, occurring in 60% to 70% of renal transplant recipients receiving immunosuppressive therapy [23,24]. Major characteristics are the increases in levels of LDL, VLDL and triglycerides, while HDL is usually normal.

Risk factors contributing to the development of dyslipidemia after transplantation are age, male gender, proteinuria, obesity, pretransplant hyperlipidemia and diabetes mellitus. Furthermore, dyslipidemia following kidney transplantation is associated with the use and dose of immunosuppressive agents such as corticosteroids, calcineurin-inhibitors, and inhibitors of the mammalian target of rapamycin (mTOR). The cumulative dose of corticosteroids thereby appears to be the most significant risk factor. Among calcineurin-inhibitors, a higher incidence of hyperlipidemia was shown with the use of ciclosporine as compared to tacrolimus. Sirolimus and everolimus however, both mTOR inhibitors, seem to even more impact on lipid metabolism with significant increases in triglyceride and cholesterol levels [25]. Mycophenolate mofetil is the only available immunosuppressive agent with no adverse effects on lipids.

Dyslipidemia and progression of kidney disease

Dyslipidemia, partly explained by its association with proteinuria, predicts progressive loss of kidney function [26]. This was seen particularly in early stages of diabetic nephropathy [27,28]. Elevated levels of triglycerides seem to contribute to the progression of albuminuria [29], diabetic nephropathy [30], and retinopathy.

They were furthermore associated with a higher risk of end-stage renal disease requiring renal replacement therapy [31]. In contrast, higher levels of HDL were found to be protective of albuminuria in type 1 diabetes [32].

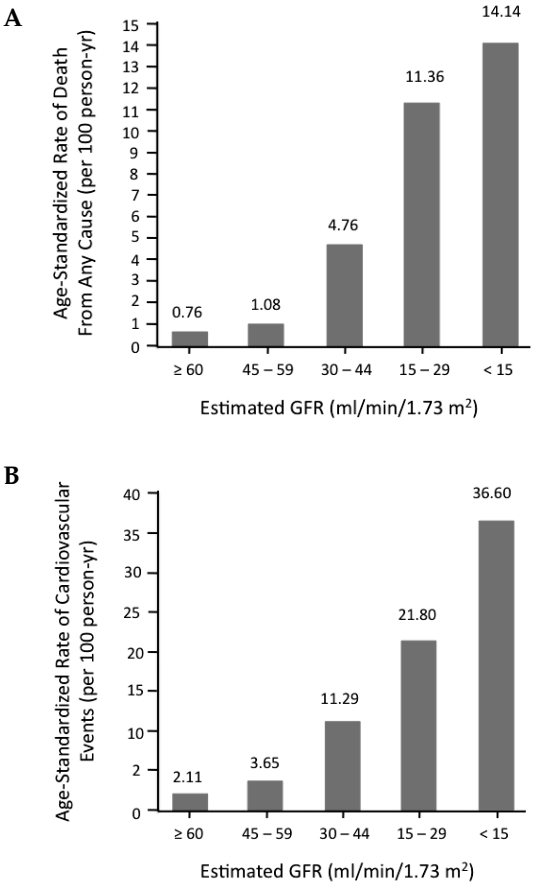
Dyslipidemia, cardiovascular risk and mortality

Chronic kidney disease per se has been shown to be a strong risk factor for cardiovascular morbidity and mortality [33]. Patients with a moderately impaired kidney function already have a high risk to develop cardiovascular complications [34]. Cardiovascular risk further increases inversely proportionate to the decline in kidney function (Figure 2), and the majority of patients with chronic kidney disease die of cardiac and vascular events before reaching end-stage renal disease.

In patients on long-term dialysis, cardiac and vascular disease is the leading cause of death, accounting for 43% of all-cause mortality [3]. Compared to the general population, mortality from cardiovascular disease is excessively high: it ranges from a 500 fold increased risk in young patients aged 25-35 years to a 5-fold increased risk in individuals of a high age of 85 years or more [35]. Possible reasons may involve the increased prevalence of traditional risk factors as known from the general population, and further uremia-related risk factors. Lipid abnormalities, being common in patients with kidney disease, have been suggested to play a major role. Therefore, it is tempting to propose a general need for treatment of lipid disorders in this patient group.

However, while a loglinear relation between blood cholesterol levels and cardiovascular risk is well established in the general population, this is not the case in renal patients. Many studies in patients with CKD, mainly stage 5, have failed to show a similar, clear pattern of high plasma total cholesterol, LDL cholesterol and triglycerides being associated with increased cardiovascular mortality. In fact, a number of studies have even found that low (not high) serum total cholesterol was associated with increased mortality [36-39]. U-shaped curves, and

recently, J-shaped curves have been described for the relationship between serum cholesterol and mortality. This probably reflects the influence of malnutrition and chronic inflammation, resulting in the phenomenon known as reverse causation. Concomitant illnesses accompanied by inflammation are associated with an increased risk of death; when they furthermore induce a decrease in cholesterol synthesis, the result may be artifactually negative associations between cholesterol and mortality. Supporting this hypothesis, hypercholesterolemia was shown to be an independent risk factor for cardiovascular and all-cause mortality in dialysis patients without, but not in those with evidence of malnutrition or inflammation [40].



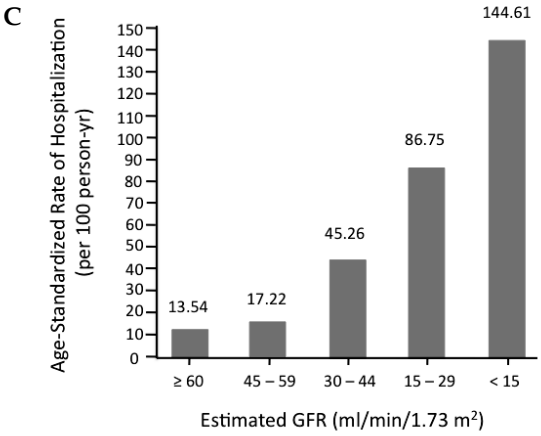


Figure 2. Age-standardized rates of death from any cause (A), cardiovascular events (B), and hospitalization (C), according to the estimated glomerular filtration rate (GFR).©

In general, caution is required on translating observational findings into possible therapeutic treatments. Furthermore, concern is also justified when applying recommendations for the treatment of lipid disorders from the general population –which are based upon many prospective randomized placebo-controlled trials- to patients with chronic kidney disease. Extrapolation of data from the general population may not meet the special disease pattern of kidney disease patients:

Despite cardiovascular deaths being a major cause of mortality in dialysis patients, the proportion of myocardial infarctions in cardiac deaths is much lower in patients with chronic kidney disease as compared to the general population. Only 25 per cent of the cardiac deaths in hemodialysis patients can be attributed to myocardial infarctions, while the majority of events constitutes of sudden cardiac deaths[3]. Although sudden cardiac death may to some extent also result from infarctions and arrhythmias, other reasons such as structural heart diseases presumably play an important role. Whether these may be modifiable with cholesterol lowering treatment, is unlikely.

Therefore, it is obvious that the classical guidelines, such as the national cholesterol education program adult panel III cannot generally be applied to renal patients. In particular, treatment indications may differ according to the severity of chronic kidney disease. In the subsequent chapter, we therefore present the available evidence for lipid lowering therapy according to the stages of chronic kidney disease as defined by K/DOQI (Kidney Disease Outcomes Quality Initiative) (Tab. 1)[2,41].

Lipid lowering therapy in patients with the nephrotic syndrome

Studies have shown that patients with persistent nephrotic syndrome and hyperlipidemia are at increased risk for atherosclerotic disease, particularly if other risk factors are present.[7,42,43]. Furthermore, it has been suggested from animal experiments and observations in humans that hyperlipidemia may also enhance the rate of progressive glomerular injury, possibly by promoting an intraglomerular equivalent of atherosclerosis. Thus, it appears reasonable that lowering lipid levels may both protect against systemic atherosclerosis and slow the progression of the underlying kidney disease. Studies have shown that statins can efficiently lower total and LDL cholesterol concentrations by 20 to 45%, and to a lesser extent triglyceride levels and Lp(a) levels [43-47]. Despite the lack of studies using "hard" endpoints, statins are suggested as the treatment of choice for persistent hyperlipidemia in the nephrotic syndrome **(IV/C)**. Due to side effects, other lipid lowering medication as nicotinic acid, fibric acid, probucol or bile acid sequestrants are not generally recommended. Instead, additional therapeutic options include dietary modification[48] and angiotensin inhibition **(IV/C)**, the latter being associated with a 10 to 20% decline in the plasma levels of total and LDL-cholesterol and Lp(a) [49].

Statins in patients with chronic kidney disease

Early renal disease: CKD stage 1-3 (GFR ≥30 ml/min/1.73m²)

The effects of a lipid lowering therapy on cardiovascular, cerebrovascular and renal endpoints were investigated in a subgroup analysis of the *Pravastatin Pooling Project*, including 12333 patients with mild CKD (stage 2) and 4491 patients with moderate CKD (stage 3). Pravastatin 40mg/day resulted in a significant 23% relative risk reduction in the combined endpoint of non-fatal myocardial infarction, cardiac death, percutaneous or surgical revascularizations in patients with moderate CKD. A similar effect was seen in patients with mild CKD, among whom even total mortality was reduced. The achieved relative risk reduction corresponds to the effect, which would have been expected in the general population without kidney disease. The corresponding absolute risk reduction was - due to the higher event rate – even more than twice as high compared to patients with normal kidney function (6.3 vs 2.9%) [50] **(IIb/B)**.

Furthermore, a prespecified subgroup analysis of 6.517 patients with kidney dysfunction was performed among the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT), including 19.000 hypertensive patients with at least 3 other risk factors for coronary artery disease, and non-fasting cholesterol levels ≤ 6.5mmol/l. It showed that patients receiving 10mg atorvastatin per day had a significantly lower risk of reaching the composite primary endpoint, consisting of nonfatal myocardial infarction and cardiac death, compared to patients receiving placebo [51] **(IIb/B)**.

Similar results were obtained from analyses of 1.329 patients with slightly elevated creatinine (110 to 200 μmol/L) participating in the Heart Protection Study (HPS). A total of 268 of the patients receiving placebo experienced a vascular event vs. 182 of the simvastatin treated patients, This corresponds to a 25% relative risk reduction in the simvastatin group [52] **(IIb/B)**. Recent findings from the Treatment to New Targets (TNT) study confirmed that treatment of patients having less severe CKD does reduce the cardiovascular risk [53] **(IIb/B)**.

Finally, there are data suggesting that statins may also slow the rate of decline in kidney function and lower urinary protein excretion. One subanalysis within the GRACE-study showed that in untreated patients with coronary heart disease, dyslipidemia and normal baseline creatinine, the glomerular filtration rate (GFR) decreased over a period of 3 years. Treatment with a statin could prevent this decline and lead to a significant improvement in kidney function [54]. In the TNT study, patients on 80mg atorvastatin per day had a significantly lower rate of decline of renal function than did patients receiving 10mg/day [55] **(IIb/B)**. Similarly, a post-hoc subgroup analysis of the CARE study had shown a lower GFR decline in pravastatin as compared to placebo treated patients[56] **(IIb/B)**. The reduction of GFR decline as achieved by statins (reduction of 0.1 ml/min/1.73m^2 per year in the latter study) however is relatively small (in comparison: reductions of 3 to 4 ml/min/1.73m^2 by the control of hypertension and ACE-inhibitor therapy in proteinuric patients [57,58]).

Although some data also exist for fibrates suggesting beneficial effects on the rate of progression of renal disease and cardiovascular risk [59,60] **(IIb/B)**, these drugs have been considered with caution in patients with CKD. This is mainly because most fibrates or their active metabolites accumulate in renal failure and occasionally cause rhabdomyolysis.

In conclusion, despite the absence of direct evidence from randomized controlled trials in patients with CKD, these data provide indirect evidence that patients with CKD stages 2 and 3 may benefit from a lipid lowering intervention. Based on the above post hoc analyses of past statin trials on subcohorts of patients with early CKD, data are sufficiently suggestive to justify the administration of statins in these patients **(IIb/B)**.

Advanced renal disease: CKD stage 4-5 and dialysis patients

Unfortunately, patients with more advanced CKD (stage 4) were either absent in the above described subgroup analyses, or their numbers were too small to be

analyzed. There is a complete lack of controlled studies addressing the effect of lipid lowering medication on outcome in patients at CKD stage 4. These patients represent a population with advanced kidney failure, where all-cause mortality markedly increases and the pattern of cardiovascular disease may change, compared to CKD stages 2 and 3. One open study has been performed in a small group of patients (n=143) over 20 months, and showed that atorvastatin decreased the primary cardiac endpoint in patients with pre-end stage renal disease, while this was not the case in dialysis patients [61].

More definite evidence has been provided by the prospective, randomized controlled 4D study (The German Diabetes and Dialysis Study). This study evaluated the effect of 20 mg atorvastatin / day vs placebo in 1255 hemodialysis patients with type 2 diabetes mellitus during 4 years of follow-up [62]. Although Atorvastatin effectively lowered LDL cholesterol by 42%, the composite primary cardiovascular endpoint, consisting of death from cardiac causes, non-fatal myocardial infarction and stroke, was only reduced by 8%, which was not statistically significant (RR 0.92, 95%CI 0.77-1.10, p=0.37) **(Ib/A)**. Similarly, all-cause mortality was not significantly reduced (RR 0.93, 95%CI 0.79-1.08, p=0.33). There was a positive result however for the secondary endpoint of all cardiac events combined, which were lowered by 18% in the atorvastatin group as compared to the placebo group (RR 0.82, 95%CI 0.86-0.99, p=0.03). Further evidence was contributed by AURORA: A study to evaluate the Use of Rosuvastatin in subjects On Regular Dialysis: an Assessment of survival and cardiovascular events[63]. In this international prospective randomized controlled trial, 2776 hemodialysis patients were assigned to receive rosuvastatin 10mg daily or placebo, and followed for a median of 3.8 years. Despite the mean reduction in LDL cholesterol of 43% in the intervention group, the combined primary endpoint of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke could not be reduced (HR 0.96, 95%CI 0.84-1.11, p=0.59) **(Ib/A)**. Rosuvastatin neither had an effect on individual components of the primary endpoint, nor on all-cause mortality (HR 0.96, 95%CI 0.86-1.07, p=0.51). It remains unclear whether these data can be generalized to peritoneal dialysis patients. This will be clarified by the ongoing

Study of Heart and Renal Protection (SHARP), which is a large-scale randomized controlled trial comparing the use of simvastatin and ezetimibe vs. placebo in 9489 patients in CKD stage 4 and on dialysis – the results are awaited in 2011 [64].

In conclusion, the 4D and AURORA studies do not provide a rationale to start statin treatment in hemodialysis patients **(Ib/A)**. Treatment aiming at primary prevention in the absence of signs and symptoms of coronary heart disease presumably comes too late once the patient has advanced to end-stage renal disease. It has been suggested however that patients who are already on statins when entering chronic dialysis should be left on the medication [14] .

Patients after kidney transplantation

Recipients of kidney transplants had been investigated in the ALERT study, which was a randomized controlled trial comparing fluvastatin (40-80 mg/day) vs. placebo in 2.102 patients with long-term stable graft function. Despite a mean reduction in LDL cholesterol of 1 mmol/l during 5.1 ± 1.1 years, there was no significant risk reduction for the combined primary endpoint, consisting of cardiac death, non-fatal myocardial infarction and coronary revascularization (RR 0.83 (95% CI 0.64-1.06), p=0.139) **(Ib/A)**. Furthermore, total mortality and graft loss did not differ significantly between the groups (In the fluvastatin and placebo groups there were 143 deaths compared with 138, and 146 graft losses compared with 137, respectively.) Rates in two of the three subcomponents of the primary endpoint - cardiac death and non-fatal myocardial infarction- were observed to be lower in the intervention group (RR 0.65 (0.48-0.88), p=0.005). The authors suggested that the trial may have been too small to detect a significant effect on the primary endpoint because the event rate was lower than expected. [65].

A post hoc analysis of the ALERT trial showed that the success of a lipid lowering therapy in kidney transplant recipients is dependent on when treatment is initiated. Indeed, a significant reduction in cardiac endpoints was observed in patients who started fluvastatin treatment within the first 4.5 years after renal transplantation (4.6% in fluvastatin group vs 9.2% in placebo group)[66] **(IIb/B)**.

Despite the lack of direct evidence it can therefore be suggested that renal transplant recipients with hyperlipidemia should be treated with a statin so that target LDL cholesterol levels can be achieved **(IIb/B)**. Importantly, for any statin therapy in renal transplant recipients, potential interactions with other medication and changes of immunosuppressive regimens should be taken into account. All statins, except Pravastatin, primarily undergo metabolism by the CYP 450 isoenzymes in the liver. The CYP 2C9 isoenzyme is responsible for the metabolism of fluvastatin and rosuvastatin, whereas atorvastatin, lovastatin, and simvastatin are metabolized by the CYP 3A4 isoenzyme. Concomitant intake of further drugs being metabolized by the CYP 3A4 system – such as the immunosuppressant Ciclosporine in renal transplant recipients, macrolide antibiotics or calcium channel blockers like verapamil - may dramatically increase the plasma concentration of the statin, placing patients at risk for adverse events like myopathy or rhabdomyolysis. Pravastatin and Rosuvasatin are less likely to induce drug-drug interactions and considered as more safe.

In summary, results from the 4D and ALERT trials do not necessarily doubt the validity of the subgroup analyses done for patients with CKD stages 2 and 3, in whom lipid lowering therapy appears to be just as effective as in patients with normal kidney function. It remains less clear whether lipid lowering therapy is still effective when started in patients with more advanced stages of chronic kidney disease.

Further therapeutic concepts for lipid-lowering in CKD

Nutritional interventions and physical activity

Nutritional interventions and physical activity play an important role as lipid lowering treatments in the general population. They presumably have similar importance in patients with chronic kidney disease in early stages 1-3 **(IV/C)**.

In stage 4, reduction in nutrient intake without sufficient physical activity may lead to a catabolic state with reduction of muscle mass. Therefore, nutritional interventions should not generally be applied to patients in advanced stages of CKD, but carefully considered individually.

Antiproteinuric therapy

Any intervention leading to a reduction of urinary protein excretion also leads to a reduction in LDL-cholesterol and Lp(a). ACE-inhibitors and AT1-receptor-blockers have antiproteinuric properties, and thus result in a reduction of the –microalbuminuria or proteinuria-induced- dyslipidemia.

Treatment guidelines

Guidelines including the European Best Practice guidelines (EBPG) [67] and the US National Kidney Foundation´s Kidney Disease Outcomes Quality Initiative (K/DOQI) [68,69] have been difficult to define due to the lack of randomized controlled trials addressing dyslipidemia in chronic kidney disease. Kidney transplant recipients are represented by the Kidney Disease Improving Global Outcomes (KDIGO)[70] guideline, which for the treatment of dyslipidemia refers to K/DOQI. Table 2 summarizes guideline treatment recommendations, and –as the randomized controlled studies were still ongoing once the guidelines were developed- reflects that they are mainly based on ***opinion***.

It is to be expected that further ongoing randomized controlled trials will add reliable evidence for the treatment of dyslipidemia especially in advanced chronic kidney disease (stages 4-5 and dialysis), so that new treatment guidelines with higher evidence levels may be established in the near future.

Table 2. Recommendations for the treatment of dyslipidemia according to the K/DOQI guidelines for patients with CKD and the KDIGO guidelines for kidney transplant recipients

Treatment of patients with CKD stage 1-4
In general, the K/DOQI working group recommended that the ***NCEP/ATP III guidelines*** [71] were applicable to patients with CKD stages 1-4, with some specific aspects deserving further consideration: 1) CKD should be classified as a CVD risk equivalent. 2) Complications of lipid-lowering therapies resulting from reduced kidney function should be anticipated. 3) It should be considered whether there may be indications for the treatment of dyslipidemia other than preventing CVD. 4) It should be determined whether the treatment of proteinuria may also be an effective treatment for dyslipidemias. Supporting the treatment of dyslipidemia, assessment and treatment of other modifiable traditional risk factors as hypertension, smoking, obesity and diabetes should be performed.
Treatment of patients with CKD stage 5
1. For adults with Stage 5 CKD and fasting triglycerides ≥500 mg/dL (≥5.65 mmol/L) that cannot be corrected by removing an underlying cause, treatment with therapeutic lifestyle changes (TLC) and a triglyceride-lowering agent should be considered. (C)
2. For adults with Stage 5 CKD and LDL ≥100 mg/dL (≥2.59 mmol/L), treatment should be considered to reduce LDL to <100 mg/dL (<2.59 mmol/L). (B)
3. For adults with Stage 5 CKD and LDL <100 mg/dL (<2.59 mmol/L), fasting triglycerides ≥200 mg/dL (≥2.26 mmol/L), and non-HDL cholesterol (total cholesterol minus HDL) ≥130 mg/dL (≥3.36 mmol/L), treatment should be considered to reduce non-HDL cholesterol to <130 mg/dL (<3.36 mmol/L). (C)
Treatment of patients after kidney transplantation
1. For patients with fasting triglycerides ≥ 500 mg/dL (≥ 5.65 mmol/L) that cannot be corrected by removing an underlying cause, we suggest therapeutic lifestyle changes and a triglyceride-lowering agent. [Based on KDOQI Recommendation 4.1 for patients with CKD stage 5, Evidence Level C]
2. For patients with elevated low-density lipoprotein (LDL) cholesterol, we suggest: If LDL ≥ 100 mg/dL (≥ 2.59 mmol/L), treat to reduce LDL to <100 mg/dL (< 2.59 mmol/L) [Based on KDOQI Guideline 4.2 for patients with CKD stage 5, Evidence Level B]
3. For patients with normal LDL cholesterol, elevated triglycerides and elevated non-HDL cholesterol, we suggest: If LDL < 100 mg/dL (< 2.59 mmol/L), fasting triglycerides ≥ 200 mg/dL (≥ 2.26 mmol/L), and non-HDL ≥ 130 mg/dL (≥ 3.36 mmol/L), treat to reduce non-HDL to < 130 mg/dL (< 3.36 mmol/L) [Based on KDOQI Guideline 4.3 for patients with CKD stage 5, Evidence Level C]

Conclusions

In conclusion, disturbances in lipid metabolism are common in patients with the nephrotic syndrome, patients with chronic kidney disease (CKD) and patients after kidney transplantation. The classical guidelines on lipid lowering therapy –such as the national cholesterol education program adult panel III- however cannot generally be applied to renal patients, as extrapolation of data from the general population may not meet the special disease pattern of patients with CKD. Although post-hoc analyses of statin trials support the administration of statins in patients with early stages of CKD (stages 1-3), patients with advanced chronic kidney disease do not benefit to the same extent from lipid-lowering therapy. In particular, there is no rationale to start statin treatment in patients, once they require maintenance hemodialysis.

Key points

Lipid abnormalities in the nephrotic syndrome and in renal insufficiency

Abnormal lipid metabolism is common in patients with the nephrotic syndrome and in patients with chronic kidney disease (CKD) or after kidney transplantation.

Main abnormalities of lipid metabolism in the nephrotic syndrome include increases in total cholesterol, low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) cholesterol, apolipoproteins B, C-III and triglycerides, while apo-A1 is reduced.

The profound disturbances in lipid metabolism in patients with chronic kidney disease include increased concentrations of triglyceride-rich lipoproteins, small dense and oxidized LDL, and impaired HDL maturation and catabolism. These alterations are not captured by routine laboratory measurements.

Immunosuppressive therapy importantly contributes to the development of dyslipidemia after kidney transplantation.

Chronic kidney disease per se is a strong risk factor for cardiovascular morbidity and mortality. Cardiovascular risk and mortality increase inversely proportionate to the decline in kidney function.

Treatment recommendations	*Evidence level*
The classical guidelines on lipid lowering therapy –such as the national cholsterol education program adult panel III- cannot generally be applied to renal patients, as extrapolation of data from the general population may not meet the special disease pattern of patients with chronic kidney disease.	
Patients with the nephrotic syndrome should be treated with a statin. Additional therapeutic options include dietary modification and inhibition of the renin-angiotensin system (angiotensin-converting enzyme inhibitors).	IV/C
Post-hoc analyses of statin trials support the administration of statins in patients with early stages of chronic kidney disease (CKD stage 1-3)	IIb/B
Patients with advanced chronic kidney disease do not benefit to the same extent from lipid-lowering therapy as do patients with early stages of CKD. In particular, there is no rationale to start statin treatment in patients, once they require maintenance hemodialysis.	Ib/A
Despite the lack of direct evidence, it is suggested that renal transplant recipients with hyperlipidemia should be treated with a statin. Importantly, potential interactions with immunosuppressants and other medication should be taken into account.	IIb/B
The present guidelines (EBPG and K/DOQI) on the treatment of lipid disorders in patients with chronic kidney disease do not reflect the current evidence.	

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CHAPTER 3

Association of body mass index with decline in residual kidney function after initiation of dialysis

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Abstract

Background: Obesity is a risk factor for loss of kidney function in the general population, but it is unknown whether it proceeds to affect residual kidney function once patients require dialysis. Our aim was to study the effects of BMI on the decline of kidney function and on the risk to develop anuria after initiation of dialysis.

Study Design: Prospective cohort study.

Setting & Participants: 1271 incident dialysis patients from 38 centers in The Netherlands, participating in the Netherlands Cooperative Study on the Adequacy of Dialysis (NECOSAD) between 1997 and 2006.

Predictor: Body mass index (BMI) assessed at 3 months after initiation of dialysis (baseline), and categorized into 4 groups: <20, ≥20-25, ≥25-30, ≥30 kg/m^2.

Outcomes & Measurements: The decline of measured glomerular filtration rate (mGFR) was determined with linear mixed models and adjusted for age, sex, primary kidney disease, dialysis modality, smoking, CVD, nPNA, and additionally for proteinuria, blood pressure and baseline mGFR. Cox regression analysis was used to calculate hazard ratios (HR) for the development of anuria.

Results: Patients had a mean age of 59±15 yr, BMI 24.8±4.1 kg/m^2, mGFR 4.7±3.3 ml/min. During 18 months follow-up, the decline of mGFR in patients with normal weight was 1.2 ml/min/yr (95%CI: 0.7;1.6). Compared to those, the adjusted loss of mGFR was 0.4 (0.02;0.8) ml/min/yr higher for overweight and 1.2 (0.5;1.8) ml/min/yr higher for obese patients. In contrast, the decline in underweight patients was 0.6 (-0.1;1.3) ml/min/yr lower. Anuria occurred in 297 patients; the risk being similar among BMI groups after adjustment for confounders and baseline diuresis.

Limitations: Patients with missing BMI or mGFR at baseline were excluded.

Conclusion: Obesity is a strong risk factor for the decline of kidney function after initiation of dialysis. Whether obese dialysis patients might benefit from a healthy weight reduction needs to be further studied.

Introduction

Residual kidney function is known to be beneficial for dialysis patients. Higher residual kidney function is associated with a lower mortality risk and a better quality of life (1-6). Therefore, its preservation remains important among the strategies to reduce mortality and improve quality of life of maintenance dialysis patients.

Obesity is a risk factor for the decline of kidney function in the general population. Several studies have found excess weight to be associated with a higher risk to develop chronic kidney disease (CKD) (7-12) and end-stage renal disease (ESRD) (13;14). The underlying pathophysiological mechanisms may be the development of glomerular hyperfiltration and glomerulomegaly (15-17), and structural changes in the kidney are also referred to as „obesity-related glomerulopathy" (18). These consequences of obesity negatively affecting kidney function may proceed even once patients already require dialysis treatment. Whether obesity is a risk factor for the decline of kidney function after initiation of dialysis, remains unclear.

In the dialysis population, a low rather than a high body mass index (BMI) increases the risk for mortality (19-22). A low BMI in these patients may be a consequence of the protein-energy-wasting syndrome, which represents a severe and complex process of muscle loss, poor food intake, inflammation and development of comorbidities (23-25). This severe condition may influence kidney function, as cross-sectional studies in patients with decreased kidney function have shown that low BMI and malnutrition correlated with low glomerular filtration rate (GFR) (26;27). It is unknown whether a low BMI is associated with a faster decline of kidney function.

Given the uncertainty whether obesity, underweight, or both, influence the decline of kidney function after initiation of dialysis, the primary aim of this study was to assess the effect of BMI on the decline of residual kidney function in dialysis

patients. The second aim was to determine the effect of BMI on the risk to develop anuria in these patients.
To that end, data from the Netherlands Cooperative Study on the Adequacy of Dialysis (NECOSAD), a prospective, multicentre cohort study of incident dialysis patients, were analyzed.

Methods

Study design

The Netherlands Cooperative Study on the Adequacy of Dialysis (NECOSAD) is an observational, prospective follow-up study, in which patients with ESRD who started with long-term dialysis treatment for the first time were enrolled in 38 participating dialysis centres between 1997 and 2006 in the Netherlands. Follow-up visits took place at 3 months after the start of dialysis, at 6 months and subsequently at intervals of 6 months. Baseline demographic and clinical data were obtained between 4 weeks prior to and 2 weeks after the start of maintenance dialysis treatment. Blood and 24-hour urine samples were obtained at all visits.
For the present analysis, the baseline was defined as 3 months after the start of dialysis treatment, when the patients' fluid and metabolic conditions had stabilized. Patients were censored at the date of loss to follow-up (death, kidney transplantation or transfer to a non-participating dialysis centre), the end of the follow-up at the 1st of January 2007, or at a set maximum of 18 months.

Patients

Patients with ESRD, who were at least 18 years old and started long-term dialysis for the first time, were invited to participate in NECOSAD.
In the present analysis, all patients with recorded height, weight and residual kidney function (measured GFR (mGFR) > 0 ml/min, diuresis ≥ 200 ml/day) at 3 months after initiation of dialysis treatment were included. The medical ethical committees of the participating centres approved the NECOSAD study and all patients gave their written informed consent before inclusion.

Data collection

Baseline demographic and clinical data

Information on age, gender and smoking status was obtained through patient interviews. Primary kidney disease and comorbidity were reported by the patients´ nephrologists. Primary kidney disease was classified according to the coding system of the European Renal Association – European Dialysis and Transplant Association (28). Patients were classified as being at low, medium or high risk for mortality according to the scores of Khan (29). Cholesterol and albumin levels were routinely determined in the dialysis centres. Protein nitrogen appearance (PNA) was calculated from urea concentrations in blood and urine as well as urine protein concentration, and was normalized to standard body weight. Blood pressure was measured in sitting position.

Body mass index

Height and weight were measured after dialysis sessions, and BMI was calculated as weight (kg) divided by height (m) squared. The study population was divided into four groups, based on the BMI at baseline. Following the World Health Organization guidelines, obesity was defined as a BMI of ≥30kg/m^2 and overweight as a BMI of ≥25.0 and <30.0 kg/m^2 (30). We defined normal weight (reference group) as a BMI of ≥20.0 and <25.0 kg/m^2 and underweight as a BMI of <20.0 kg/m^2.

Glomerular filtration rate, anuria

Plasma creatinine, plasma urea, urine creatinine and urine urea from 24h urine collections were measured at each study visit. In hemodialysis patients, blood was thereby taken before a dialysis session (D1), after D1 and before the next dialysis session (D2). Between D1 and D2, urine was collected. For calculations, the mean of D1 after and D2 before were being used.

Creatinine and urea clearances were assessed by the ratio of the respective urine levels (mmol/day) to plasma levels (mmol/l), multiplied by (1000/1440). Residual kidney function was calculated as the mean of creatinine and urea clearances, and expressed as measured glomerular filtration rate (mGFR). In our investigations of

the association of BMI with residual kidney function, we did not adjust mGFR for body surface area.
Anuria was defined as a urine volume of less than 200ml/day.

Statistical analyses
Continuous variables were expressed as mean with standard deviation, and categorical variables were expressed as percentages.
First, we first investigated the natural course of mGFR in each BMI group using repeated measurements analyses. We assessed mean mGFR values within BMI categories for each follow-up visit, taking into account between and within person correlations as well as missing values.
Second, since the course of mGFR mainly showed a linear pattern in the repeated measurements analyses, we chose a linear mixed effects model to analyze the effect of baseline BMI on the decline of mGFR. This model allows a random intercept, random slope and an unstructured covariance matrix. In the case of at least two consecutive follow-up mGFR being zero, the second and following mGFR measures were censored in the analyses. The model was repeated while adjusting for the potential confounders age, sex, primary kidney disease, dialysis modality, smoking, cardiovascular disease (CVD) and nPNA (=main analyses, model 2), and additionally for the potential intermediate variables proteinuria and mean arterial pressure (MAP) (model 3). Investigations to test the robustness of the analyses also included baseline mGFR (model 4). In further analyses, we stratified for diabetes mellitus (diabetic and non-diabetic patients) and dialysis treatment (hemodialysis and peritoneal dialysis).
Third, we calculated absolute (incidence) rates to develop anuria within each BMI category per 100 person years of follow-up. To that end, incident cases of anuria were assigned to their BMI category at baseline. Survival time until the development of anuria was defined as the number of days between the baseline of the present study and the date of anuria or the date of censoring.
Finally, Cox regression analysis was used to calculate hazard ratios (HR) and the corresponding 95% confidence intervals (95% CI) for the relative risks to develop anuria, using patients with a normal BMI as the reference group. The crude

associations were adjusted for the confounders mentioned above (model 2) and for baseline diuresis (=main analyses, model 3), and additionally for the potential intermediates proteinuria and MAP (model 4).
All analyses were performed using SPSS for Windows version 12.0.1 and SAS version 9.1.

Results

Patients

Between January 1997 and December 2006, a total of 1916 patients with ESRD, who started long-term dialysis and were included, still participated in NECOSAD at 3 months after initiation of dialysis (baseline). Of the 1724 patients presenting with recorded height and weight, information on mGFR was available in 1468 patients. Of those, all patients with a mGFR > 0 ml/min and a urine volume ≥ 200 ml/day were included in the present analysis (n=1271).
In the final study population (n=1271), the mean (SD) age was 59 (15) years, and BMI was 24.8 (4.1) kg/m^2. Sixty-two per cent of the patients were men, and 57% started with hemodialysis treatment. Of the participants, 8.8% were underweight, 48.8% had a normal weight, 32.8% were overweight and 9.6% were obese (Table 1). The mean mGFR measured right before the initiation of dialysis was 6.0 (3.2) ml/min; it was 6.7 ml/min in obese, 6.4 ml/min in overweight, 5.8 ml/min in normal weight and 5.0 ml/min in underweight patients. At baseline, i.e. at 3 months after initiation of dialysis, mean mGFR was overall 4.7 (3.3) ml/min, being highest (5.8 ml/min) in obese patients, followed by overweight (5.1 ml/min), normal weight (4.6 ml/min) and underweight patients (3.5 ml/min) (Table 2). Hemodialysis patients were treated 3 times per week with a mean duration of 3.64 hours per dialysis session and an ultrafiltration volume of 1.45 liter. Membranes were in 68% synthetic and in 32% cellulose derived; there was no use of cuprophane membranes. Peritoneal dialysis patients had a mean exchange volume of 8,3 liter per day. No significant differences in dialytic management were observed among the BMI groups.

Table 1: Baseline patient characteristics, presented per BMI category; study population n=1271

Characteristic	BMI (kg/m²)			
	< 20 (n=112)	≥ 20 < 25 (n=620)	≥ 25 < 30 (n=417)	≥ 30 (n=122)
Age *years*	53.6 (17.9)	58.3 (15.6)	60.6 (12.6)	58.8 (12.5)
Gender *% men*	45.5	65.5	66.2	49.2
Dialysis modality *% HD*	67.9	54.7	55.4	61.5
BMI *kg/m²*	18.8 (1.0)	22.8 (1.3)	27.1 (1.4)	33.3 (3.4)
Diuresis *ml/day*	812 (589)	1006 (664)	1140 (699)	1239 (893)
Primary kidney disease				
Diabetes mellitus *%*	16.1	10.5	17.0	41.0
Glomerulonephritis *%*	8.9	15.5	15.6	8.2
Renal vascular disease *%*	16.1	18.1	14.6	13.1
Other *%*	58.9	56.0	52.8	37.7
Ethnicity				
White *%*	87.3	92.7	94.8	88.6
Black *%*	3.6	1.7	1.6	2.6
Asian *%*	9.1	5.6	3.6	8.8
Comorbidity				
Diabetes mellitus* *%*	21.1	15.1	24.7	50.0
Cardiovascular Disease *%*	23.9	31.3	34.8	40.0
Comorbidity Khan score				
Low *%*	41.1	45.3	36.7	24.6
Intermediate *%*	28.6	31.0	37.6	44.3
High *%*	30.4	23.7	25.7	31.1
Current smoker *%*	33.6	26.8	16.5	15.8
Mean arterial pressure *mmHg*	101 (14)	103 (12)	105 (12)	102 (12)
Cholesterol *mg/dL*	195 (51)	198 (52)	199 (47)	201 (54)
Albumin *g/dL*	3.5 (0.6)	3.6 (0.5)	3.7 (0.5)	3.7 (0.5)
nPNA *g/kg/day*	1.1 (0.3)	1.0 (0.2)	1.0 (0.2)	1.0 (0.3)
Use of ACE-I or ARB *%*	33.0	38.2	36.0	39.3

Values are presented as means (SD) or *%*.
To convert serum cholesterol in mg/dL to mmol/L, multiply by 0.02586; albumin in g/dL to g/L, multiply by 10;
Abbreviations: BMI = body mass index; HD = hemodialysis; BP = blood pressure; nPNA = normalized protein nitrogen appearance, ACE-I = Angiotensin converting enzyme inhibitors; ARB = Angiotensin II receptor blockers.
* Numbers include patients who have diabetes mellitus as their primary kidney disease.

Table 2: Baseline mGFR and decline of mGFR until 18 months of follow-up in categories of BMI, determined by linear mixed model analysis; n=1271

Variables	Baseline mGFR at 3 months	Decline in mGFR unadjusted	Difference in decline of mGFR; unadjusted	Adjusted † Difference in decline of mGFR	Adjusted ‡ Difference in decline of mGFR	Adjusted # Difference in decline of mGFR
			(Model 1)	(Model 2)	(Model 3)	(Model 4)
BMI < 20 kg/m²	3.5 (2.1)	0.5 (-0.6;1.6)	-0.6 (-1.8;0.6)	-0.6 (-1.3;0.1)	-0.5 (-1.3;0.2)	-0.5 (-1.4;0.4)
BMI ≥ 20 < 25 kg/m²	4.6 (3.5)	1.2 (0.7;1.6)	0*	0*	0*	0*
BMI ≥ 25 < 30 kg/m²	5.1 (2.9)	1.6 (1.0;2.1)	0.4 (-0.3;1.1)	0.4 (0.02;0.8)	0.3 (-0.1;0.7)	0.2 (-0.3;0.8)
BMI ≥ 30 kg/m²	5.8 (3.8)	1.8 (0.8;2.8)	0.7 (-0.5;1.8)	1.2 (0.5;1.8)	1.0 (0.4;1.7)	1.1 (0.3;2.0)
Age (*years*)				-0.01 (-0.03;0.003)	-0.01 (-0.02;0.01)	-0.005 (-0.02;0.01)
Sex (*men*)				0.4 (0.05;0.8)	0.4 (-0.03;0.8)	0.3 (-0.2;0.8)
Dialysis modality (*HD*)				0.1 (-0.3;0.5)	-0.06 (-0.5;0.3)	-0.06 (-0.6;0.5)
Primary kidney disease						
Diabetes mellitus				0.4 (-0.3;1.1)	0.3 (-0.4;0.9)	0.4 (-0.4;1.3)
Glomerulonephritis				0	0	0
Renal vascular				0.5 (-0.2;1.1)	0.6 (-0.1;1.3)	0.7 (-0.2;1.6)
Other				0.3 (-0.2;0.8)	0.6 (0.03;1.1)	0.5 (-0.2;1.1)
Cardiovascular disease				-0.03 (-0.5;0.4)	0.1 (-0.4;0.5)	0.01 (-0.6;0.6)
Current smoker				0.1 (-0.3;0.6)	0.1 (-0.3;0.6)	0.2 (-0.4;0.8)
nPNA (*g/kg/day*)				2.2 (1.3;3.0)	2.0 (1.1;2.8)	1.8 (0.7;3.0)
Proteinuria (*g/day*)					0.2 (0.1;0.3)	0.2 (0.1;0.3)
Mean arterial pressure (*mmHg*)					0.03 (0.01;0.04)	0.03 (0.01;0.05)
P for trend		<0.001	0.045	<0.001	0.003	0.006

To convert mGFR in mL/min to mL/s, multiply by 0.01667.
Abbreviations: mGFR = measured glomerular filtration rate; BMI = body mass index; HD = hemodialysis; nPNA = normalized protein nitrogen appearance.
* Patients with a body mass index ≥ 20 and < 25 were used as the reference group.
Note: The linear mixed models include the variables as such, and their interactions with time. The coefficients provided in models 1 to 4 express the interaction terms of the variables with time, representing differences in slopes (= i.e. the amount by which the slope increases or decreases over categories (levels) of the variable.
†Model 2 (main model): Adjustments were made for age, gender, primary kidney disease, dialysis modality, smoking, cardiovascular disease and nPNA.
‡Model 3: Model 2 + additional adjustments for proteinuria and mean arterial pressure.
#Model 4: Model 3 + additional adjustments for baseline mGFR (inclusion of baseline mGFR; no interaction term with time).

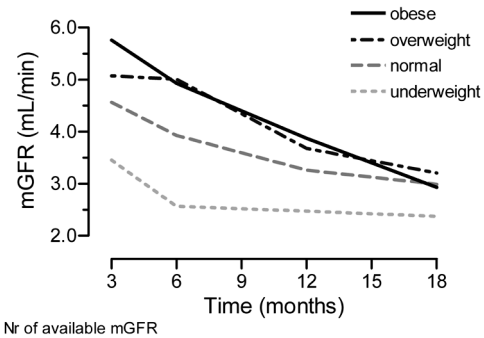
During follow-up, 297 patients became anuric. Reasons for censoring other than the inability to produce urine included transplantation (n=111), death (n=144), recovery of kidney function (n=17), patient refusal (n=45), transfer to a non-participating dialysis centre (n=12), or other (n=12). The proportion of censoring was thereby equally distributed over the BMI groups (30% censoring in underweight, 27% in normal weight, 28% in overweight and 26% in obese patients, respectively).

Decline of mGFR

The results of the repeated measurements analyses investigating the natural course of mGFR over time are shown in Figure 1a. Since the course of mGFR mainly showed a linear pattern, a linear mixed effects model was used in the subsequent analyses to analyze the effect of baseline BMI on the decline of mGFR. The mean (95% CI) decline of mGFR in the total study population was 1.3 (1.0;1.6) ml/min/year. Compared to patients with normal weight (reference group), who lost 1.2 (0.7;1.6) ml/min of their mGFR per year, the adjusted decline of mGFR was 0.4 (0.02;0.8) ml/min/year higher for overweight and 1.2 (0.5;1.8) ml/min/year higher for obese patients (model 2). Thus, obese patients had a 100% higher loss of mGFR per year than normal weight patients. In contrast, underweight patients lost 0.6 (-0.1;1.3) ml/min less mGFR per year (Figures 1b, 2 and Table 2). Additional adjustments for proteinuria and blood pressure (model 3) only slightly diminished the associations, supporting the existence of further pathways of BMI to impact the decline of mGFR. Results remained stable after further adjustment for baseline mGFR (model 4).

To test the robustness of our results, we performed additional analyses in strata of diabetes mellitus, in order to see whether our results were explained by the presence of the disease. In general, baseline mGFR and the decline of mGFR were higher in diabetic patients as compared to non-diabetic patients. In both patient groups, the effect of BMI on the decline of mGFR persisted (obese patients showing the highest and underweight patients the lowest decline of mGFR). Furthermore, stratified analyses according to dialysis modality showed that patients treated with peritoneal dialysis had higher levels of mGFR than

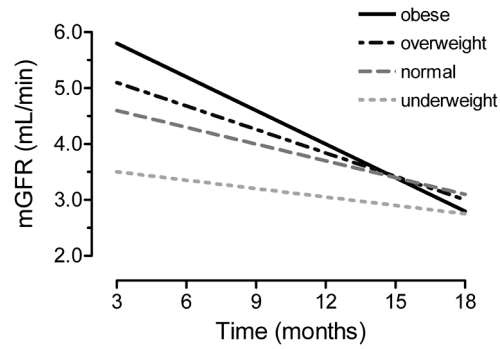
those treated with hemodialysis. In both treatment groups, obese patients had a significantly higher decline of mGFR as compared to patients with normal weight (data of the additional analyses not shown).



Nr of available mGFR

Obese	122	102	77	58
Overweight	417	343	283	221
Normal	620	519	419	312
Underweight	112	91	69	45

Figure 1a: Course of measured glomerular filtration rate (mGFR) over time per category of body mass index (BMI), determined by repeated measurements analysis.



Nr of available mGFR

Obese	122	102	77	58
Overweight	417	343	283	221
Normal	620	519	419	312
Underweight	112	91	69	45

Figure 1b: Decline of measured glomerular filtration rate (mGFR) over time per category of body mass index (BMI), determined by linear mixed model analysis.

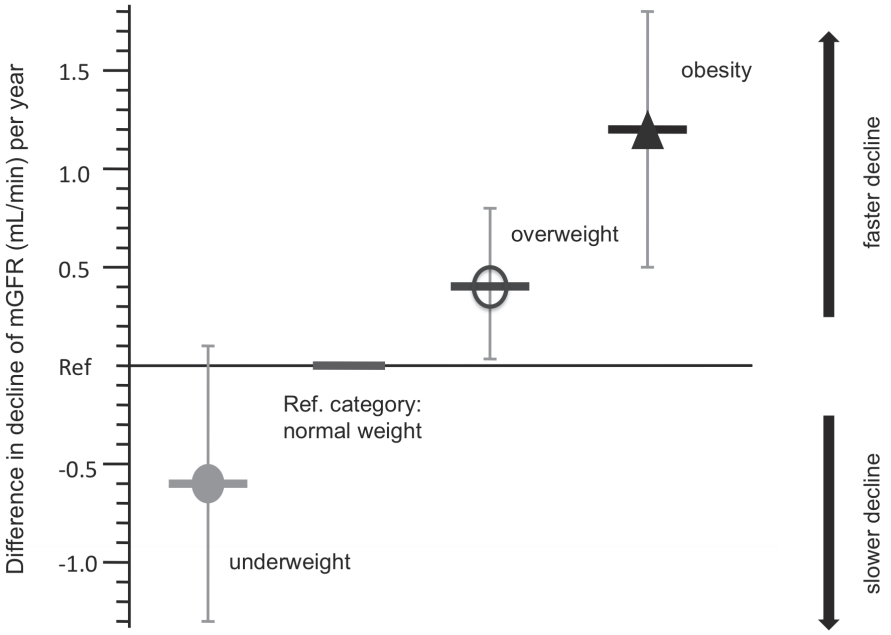


Figure 2: Adjusted differences in decline (β and 95% CI) of measured glomerular filtration rate (mGFR) over time, compared to the reference group of patients with normal weight.

Risk to develop anuria

Table 3 shows the absolute incidence rates and relative risks for the development of anuria. The absolute rate to develop anuria was 22 per 100 person years in patients with a normal weight. After adjustment for confounders and baseline diuresis, the relative risk to develop anuria was similar among the BMI groups (Table 3).

Table 3: Risks to develop anuria within 18 months of follow-up in categories of BMI; n=1271

Variables	Person years	Number of patients with anuria	Incidence rate of anuria per 100py	Crude Hazard ratio (95% CI)	Adjusted† Hazard ratio (95% CI)	Adjusted ‡ Hazard ratio (95% CI)	Adjusted # Hazard ratio (95% CI)
				(Model 1)	(Model 2)	(Model 3)	(Model 4)
BMI < 20 kg/m²	101	34	34	1.53 (1.05;2.24)	1.42 (0.96;2.10)	1.13 (0.77;1.68)	1.10 (0.74;1.63)
BMI ≥ 20 < 25 kg/m²	631	141	22	1*	1*	1*	1*
BMI ≥ 25 < 30 kg/m²	427	90	21	0.92 (0.71;1.21)	0.89 (0.67;1.17)	1.11 (0.84;1.47)	1.07 (0.81;1.42)
BMI ≥ 30 kg/m²	123	32	26	1.10 (0.74;1.64)	0.95 (0.63;1.44)	1.22 (0.81;1.85)	1.18 (0.77;1.79)
Age (*years)*					1.00 (0.99;1.01)	0.99(0.98;1.00)	0.99 (0.98;1.00)
Sex (*men*)					0.82 (0.64;1.05)	0.81 (0.63;1.04)	0.77 (0.60;0.99)
Dialysis modality (*HD*)					1.37 (1.05;1.79)	1.16 (0.89;1.51)	1.08 (0.82;1.42)
Primary kidney disease							
Diabetes mellitus					1.11 (0.73;1.68)	1.13 (0.74;1.71)	1.03 (0.67;1.57)
Glomerulonephritis					1	1	1
Renal vascular					1.10 (0.72;1.68)	1.18 (0.77;1.80)	1.19 (0.77;1.83)
Other					0.73 (0.51;1.04)	0.89 (0.62;1.26)	0.93 (0.65;1.34)
Cardiovascular disease					0.99 (0.75;1.31)	1.00 (0.76;1.32)	1.03 (0.78;1.36)
Current smoker					1.03(0.77;1.37)	1.06 (0.79;1.40)	1.06 (0.79;1.41)
nPNA (*g/kg/day*)					0.57(0.33;0.99)	0.96 (0.54;1.68)	0.91 (0.52;1.62)
diuresis *(ml/100/day)*						0.87(0.84;0.89)	0.86(0.83;0.89)
Proteinuria (*g/day)*							1.11 (1.05;1.71)
Mean arterial pressure (*mmHg*)							1.01 (0.99;1.02)
P for trend				0.29	0.64	0.40	0.57

Abbreviations: BMI = body mass index; HD = hemodialysis; nPNA = normalized protein nitrogen appearance.
* Patients with a body mass index ≥ 20 and < 25 were used as the reference group.
†Model 2: Model 1 + Adjustments were made for age, gender, primary kidney disease, dialysis modality, smoking, cardiovascular disease and nPNA.
‡Model 3 (main model): Model 2 + additional adjustments for baseline diuresis.
#Model 4: Model 3 + additional adjustments for proteinuria and mean arterial pressure.

Discussion

In this prospective cohort study of incident dialysis patients, overweight and obesity significantly increased the decline of kidney function during 18 months after initiation of dialysis, whereas underweight did not. In crude and in adjusted analyses, obese patients had the highest decline of mGFR, which consistently diminished with decreasing BMI and was lowest in underweight patients. The relative risk to develop anuria was similar among the BMI groups after adjustment for confounders and baseline diuresis.

Our study is the first, which prospectively assessed the impact of obesity and underweight on the decline of mGFR in dialysis patients. It revealed that the negative effects of obesity on kidney function are not limited to the general population, but remain important even in ESRD, when patients already have started dialysis treatment. These data extend results from prior studies, showing that excess weight is a risk factor for CKD and ESRD (7-14). Data from the Framingham Offspring Study for example showed that a higher BMI is a risk factor for the development of CKD in the general population (8;9), and two large observational studies reported a correlation between BMI and subsequent onset of ESRD in Japanese men and US Americans (13;14). Furthermore, in the dialysis population, several studies have aimed to identify predictors for the decline of kidney function (31-33). One study in 242 patients starting continuous peritoneal dialysis found that a high BMI predicted a faster decline of kidney function (33). The data from our study show that obesity is a risk factor for the decline of kidney function in peritoneal-, as well as in hemodialysis patients, with obese patients in both treatment groups having a significantly higher decline of mGFR as compared to those with normal weight.

We also showed that underweight was not a risk factor for the decline of kidney function in dialysis patients. Our findings add information to results found from cross-sectional studies, which showed that malnutrition and low BMI were correlated with low levels of GFR (26;27). Due to the cross-sectional design,

interpretation about causes and consequences is difficult. Several studies showed that underweight was associated with an increased risk for mortality in dialysis patients (19;20;22), whereby underweight may not be considered causal, but suspected as a consequence of underlying illnesses leading to increased short-term mortality (19). In the context of low BMI correlating with low levels of GFR, our current results therefore suggest that underweight may rather be a consequence than a risk factor of kidney failure.

Potential limitations of our study need to be acknowledged. First, the finally selected study cohort may not be completely representative of all dialysis patients at 3 months after treatment start, as the excluded patients due to missing BMI or mGFR measurement at baseline (448 out of the 1916) were older, mainly on hemodialysis, and more often presented with CVD (data not shown). This suggests that the missing baseline data, which lead to exclusion from the present analysis, probably were related to a poorer disease status. However, with the favourable design of including incident rather than prevalent patients, further selection that may occur in the course of maintenance dialysis could largely be reduced.

Furthermore, we did not measure mGFR by exogeneous markers of renal clearance such as iothalamate or iohexol which show excellent agreement with the inulin clearance (34-37), the gold standard for measuring mGFR (38). However, we measured mGFR with the best available alternative using creatinine and urea clearances as determined from 24h urine collections. Patients were clearly and personally instructed in educational programs to collect urine according to strict protocols and standard time periods. Study nurses regularly inquired collection procedures, so that the mGFR measurements are unlikely to be affected by collection errors.

Finally, missing values may have influenced the strength of our results, particularly in the Cox regression analyses on the risk to develop anuria. Since Cox regression models rely on the occurrence of events, missing information on urine production and possibly fewer recorded anuric events may have lead to a weaker precision

of the associations. However, the limitation of missing values could largely be reduced in our slope-based analyses by the use of linear mixed models, under the assumption of missing at random. These models estimate slopes of mGFR, using information from multiple longitudinal mGFR measurements in the study population.

Mechanisms by which BMI is related to kidney function are not yet fully understood. First, baseline mGFR levels in our study were higher in higher BMI categories. Considering the influence of dietary intake and muscle mass, which contribute to urea and creatinine levels, one may reflect potential confounding. However, the equality of blood creatinine and urea levels among the BMI groups, along with differences only being present in urine levels support evidence for real differences in baseline mGFR. Reasons for these differences may be sought in practical aspects regarding the initiation of long-term dialysis. According to the Kidney Disease Outcomes Quality Initiative (KDOQI) (39) and the European Best Practice Guidelines (EBPG) (40;41), considerations to initiate dialysis include GFR levels as well as comorbidities and the clinical condition of a patient. In line with the EBPG, diabetic patients in our study may have started dialysis treatment earlier (40;41), which in context of their high proportion among the obese patients, may partly explain the higher baseline mGFR of the obese group. However, in analyses stratified by the presence of diabetes mellitus, baseline mGFR levels were also higher in higher BMI categories of non-diabetic patients. This might be explained with regard to the clinical conditions of the patients, influencing the decision to start long-term dialysis. Obese patients may suffer more from uremic symptoms, and are expected to develop complications with their access, reason why in practice they may start earlier with dialysis treatment. In contrast, underweight patients often feel well despite low mGFR levels, and may therefore start dialysis at a later point in time.

Second, the decline of kidney function was higher in overweight and obese patients as compared to normal weight patients. One possible pathophysiological pathway is, that overweight and obese patients are more likely to have or to develop diabetes mellitus (42) and hypertension (43), which are established risk factors for the decline

of kidney function (32;44). However, further risk factors seem to play an important role, as obesity has been found to be associated with the development of glomerular lesions, independent of hypertension and hyperglycemia (17;45). These structural changes in the kidney include increased prevalences of focal segmental or global glomerulosclerosis, mesangial cell proliferation and mesangial matrix expansion, podocyte hypertrophy and interstitial fibrosis (45;46), and are partly referred to as "obesity-related glomerulopathy" (18;47). Underlying pathophysiological mechanisms may include the development of renal hyperperfusion, glomerular hyperfiltration and glomerulomegaly in overweight and obese patients, which in turn may cause proteinuria and contribute to the development of secondary focal segmental glomerulosclerosis (15;16;48-51). Furthermore, investigators have suggested that leptin, produced from adipose tissue, may directly promote the development of renal fibrosis (52). In addition, adipose tissue is recognized as a source of inflammatory cytokines such as tumor necrosis factor-α, interleukin-6 and CRP (53). Obesity therefore may be associated with a state of low-grade systemic inflammation (49;54), which may contribute to progressive kidney injury and thereby lead to an accelerated decline of mGFR.

In summary, patients with normal weight had a mean decline of mGFR of 1.2 ml/min per year. Obese patients lost 1.2 ml/min *more* of their mGFR per year, reflecting a 100% higher loss of residual kidney function per year as compared to patients with normal weight. We conclude that obesity is a strong risk factor for the decline of kidney function after initiation of dialysis. The relative risk to develop anuria was similar among the BMI groups after adjustment for confounders and baseline diuresis. Future interventional studies must show whether obese patients might benefit from a healthy weight reduction.

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CHAPTER 4

Glycemic control and cardiovascular events in diabetic hemodialysis patients

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Abstract

Background: Patients on maintenance dialysis treatment experience an excess mortality, predominantly of sudden cardiac death. Poor glycemic control is associated with cardiovascular comorbidities in the general population. This study investigated the impact of glycemic control on cardiac and vascular outcomes in diabetic hemodialysis patients.

Methods and Results: Glycated hemoglobin A1c (HbA1c) was measured in 1255 hemodialysis patients with type 2 diabetes mellitus, who participated in the German Diabetes and Dialysis Study (4D Study), and were followed for a median of 4 years. By Cox regression analyses, we determined hazard ratios to reach pre-specified, adjudicated endpoints according to HbA1c levels at baseline: sudden cardiac death (SD; n=160), myocardial infarction (MI, n=200), stroke (n=103), cardiovascular events (CVE; n=469), death due to heart failure (n=41) and all-cause mortality (n=617). Patients had a mean age of 66±8 years (54% male), and mean HbA1c of 6.7±1.3%. Patients with a HbA1c >8% had a more than 2fold higher risk of sudden death compared to those with a HbA1c ≤6% (hazard ratio 2.14; 95% confidence interval 1.33-3.44), persisting in multivariate models. Per 1% increase in HbA1c, the risk of sudden death significantly rose by 18%, and similarly, CVE and mortality increased by 8%, respectively. There was a trend for higher risks of stroke and deaths due to heart failure, while myocardial infarction was not affected. Both, the increased risks of CVE and mortality were mainly explained by the impact of HbA1c on sudden death.

Conclusions: Poor glycemic control was strongly associated with sudden cardiac death in diabetic hemodialysis patients, which accounted for increased CVE and mortality. In contrast, myocardial infarction was not affected. Whether interventions achieving tight glycemic control decrease sudden death, requires further evaluation.

Introduction

The rate of death of dialysis patients is abysmal. The ERA-EDTA registry reports a 18% first year mortality rate[1], whereas the USRDS reports an even higher annual mortality rate of >20% [2]. Cardiac disease represents with 41% the leading cause of death among dialysis patients[2]. The major occurring event is sudden cardiac death, which as a single cause accounts for 26-27% of all deaths in dialysis patients[2, 3]. It is important to identify risk factors for sudden cardiac death in relation to other cardiac and vascular events, in order to develop interventional strategies and reduce the excess mortality of these patients.

Diabetes mellitus is a growing health problem, and a risk factor for the development and progression of chronic kidney disease (CKD). Almost one half of the US dialysis patients developed end-stage renal disease (ESRD) due to type 2 diabetes mellitus (T2DM)[2]. These patients have a higher co-morbidity and poorer outcome as compared to non diabetic patients on dialysis[2], as reflected by a five year survival of only 35%[4].

Poor glycemic control is associated with the development of comorbidities including coronary artery disease and myocardial infarction in the general population[5, 6]. It has been shown that these are predisposing conditions for sudden cardiac death[7]. Glycemia is furthermore known to influence the electrolyte balance, the function of potassium and calcium-channels, and sympathetic activity - all relevant in the arrhythmogenesis of patients with kidney failure[8-10]. We therefore hypothesized that glycemic state is a risk factor for sudden cardiac death in dialysis patients.

To that end, we investigated the association of hemoglobin A1c (HbA1c) with the risk of sudden cardiac death, myocardial infarction, stroke, combined cardiovascular events, death due to heart failure, and all-cause mortality in hemodialysis patients. We analyzed data from the German Diabetes Dialysis Study (4D Study: Die Deutsche Diabetes Dialyse Studie), which evaluated atorvastatin in 1255 patients with T2DM on maintenance hemodialysis[3].

Materials and methods

Study Design and Participants

The 4D study methodology has previously been reported in detail[11]. Briefly, the 4D study was a prospective randomized controlled trial including 1255 patients with T2DM, age 18 – 80 years, and on hemodialysis for less than 2 years. Between March 1998 and October 2002, patients were recruited in 178 dialysis centres in Germany. After a period of 4 weeks, patients were randomly assigned to double-blinded treatment with either 20mg atorvastatin (n=619) or placebo (n=636) once daily. Study visits took place three times before randomization (visit 1-3), at randomization (visit 4), and at four weeks (visit 5) and every six months (visit 6 etc.) after randomization until the date of death, censoring, or end of the study in March 2004. The primary endpoint of the 4D study was defined as a composite of death from cardiac causes, fatal or nonfatal stroke and nonfatal myocardial infarction (MI), whichever occurred first (composite cardiovascular endpoint; CVE). Death from cardiac causes comprised sudden death, fatal MI, death due to congestive heart failure, death due to coronary heart disease during or within 28 days after an intervention, and all other deaths ascribed to coronary heart disease. Sudden death was considered as: death as verified by terminal rhythm disorders in an electrocardiogram; by witnesses observed death within one hour after the onset of cardiac symptoms; confirmed by autopsy; unexpected death, presumably or possibly of cardiac origin and in the absence of a potassium level greater or equal to 7.5 mmol per liter before the start of the three most recent sessions of hemodialysis. Myocardial infarction was diagnosed when two of the following three criteria were met: typical symptoms, elevated levels of cardiac enzymes (i.e. a level of creatine kinase MB above 5 percent of the total level of creatine kinase, a level of lactic dehydrogenase 1.5 times the upper limit of normal, or a level of troponin T greater than 2 ng per milliliter), or diagnostic changes on the electrocardiogram. When death occured within 28 days after a MI as diagnosed above, it was specified as death due to MI. The classifications were made exclusively with fatal MI only being classified as MI deaths, and not sudden cardiac deaths. 4D Study endpoints were centrally adjudicated by three

members of the endpoint committee blinded to study treatment and according to pre-defined criteria.

For the present analysis, sudden cardiac death, MI (fatal and nonfatal), stroke (fatal and nonfatal), the primary endpoint (CVE), death due to congestive heart failure and all-cause mortality were all chosen to be separate outcome measures and were based on the primary judgement of the endpoint committee during the 4D Study. The study was approved by the medical ethical committee, and all patients gave their written informed consent before inclusion.

Data collection

Information on age, gender and smoking status was obtained through patient interviews. Smoking status was classified as never, former or current. Comorbidities, including the presence of coronary artery disease (CAD) and congestive heart failure (CHF), as well as the duration of diabetes mellitus and dialysis treatment were reported by the patients´ nephrologists. Coronary artery disease was defined by the history of MI, coronary artery bypass grafting surgery; percutaneous coronary intervention; and the presence of coronary heart disease, as documented by coronary angiography. Blood pressure was measured in sitting position. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. All laboratory measurements of the 4D-Study were performed centrally at the Department of Clinical Chemistry, University of Freiburg, Germany. HbA1c was measured in blood samples taken at baseline at study visit 3 (1 week before randomization), using high performance liquid chromatography[12]. Inter-assay coefficients of variance were <5%. All blood samples were taken before the start of dialysis sessions and administration of drugs.

Statistical Analysis

Continuous variables were expressed as mean with standard deviation or median with interquartile range (IQR) as appropriate, and categorical variables were expressed as percentages.

The study population was divided into three groups, according to HbA1c levels at baseline: normal ≤6%, elevated >6% ≤8%, high >8%. First, we assessed the association of baseline HbA1c with sudden death both as continuous and as

categorical variable. For the latter, the patients with a HbA1c ≤6% were used as the reference group. Absolute (incidence) rates were calculated as the number of events occuring per 100 person years of follow-up. Kaplan-Meier curves were performed in each HbA1c group, and the log rank test was computed to compare curves. By Cox regression analyses, hazard ratios and corresponding 95% confidence intervals were calculated. The Cox regression analyses were adjusted for the confounders age, sex, atorvastatin treatment, systolic blood pressure, duration of T2DM, time on dialysis, smoking status, BMI, levels of LDL-cholesterol, triglycerides, albumin, haemoglobin, calcium, phosphate, C-reactive protein, and for the presence of CAD and CHF (main model). Second, we performed additional Cox regression analyses with inclusion of potassium and electrocardiographic variables, which may represent intermediate conditions lying in the causal pathway of the effect of glycemic control on sudden death. The variables were included one at a time in order to see the magnitude, by which the effect estimate for the risk of HbA1c on sudden death changed, respectively. Furthermore, In an analysis aiming for the impact of all intermediate variables together, they were simultaneously added to the main model. Third, we investigated HbA1c and the risk of other adverse cardiac and vascular outcomes including MI, stroke, the combined primary endpoint (CVE), and death due to heart failure, to distinguish whether potential effects of glycemic control are specific for sudden death, or generally influencing cardiac and vascular outcomes. In order to see whether a potential impact on the primary endpoint is mainly explained through the effect of HbA1c on sudden death, we also assessed the risks of HbA1c on CVE except for sudden death. Fourth, we similarly determined the association of HbA1c with all-cause mortality, and assessed the risks of HbA1c on all deaths except for sudden death. Finally, to test the robustness of our results, we divided the study population into quartiles of HbA1c at baseline, and repeated our analyses on the effect of glycemic control on all outcomes using this alternative categorization of HbA1c. We furthermore repeated all analyses in the placebo group only, in order to eliminate any potential influence by atorvastatin treatment. All p-values are reported two-sided. Analyses were performed using SPSS version 16.0. The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

Patient characteristics

Between March 1998 and October 2002, a total of 1255 patients were included into the 4D study, and had a HbA1c measurement at baseline. The mean follow-up period was 3.96 years (median 4.0 years) on atorvastatin and 3.91 years (median 4.08 years) on placebo. During follow-up, 469 patients reached the primary endpoint of CVE. A total of 617 patients died, of whom 160 patients died of sudden cardiac death. Furthermore, 41 patients died due to congestive heart failure, 200 patients experienced a MI (fatal or non-fatal), and 103 patients experienced a stroke (fatal or non-fatal).

In the study population (n=1255), the mean (SD) age was 65.7 (8.3) years, and 54% of the patients were male. The mean (SD) baseline HbA1c level was 6.7 (1.3) %; with no significant difference between the atorvastatin and placebo groups. The baseline patient characteristics are shown in Table 1.

Baseline HbA1c and risk of sudden death

The absolute rates of sudden cardiac death were high and increasing over the categories of HbA1c: 3.0 per 100 person years (py) in the group with HbA1c ≤6%, 5.0/100py in patients with a HbA1c between 6 and 8%, and 6.3/100py in patients with a HbA1c >8%. Figure 1A provides Kaplan-Meier curves for the time to sudden cardiac death per HbA1c category. Cox regression analyses pointed out the twice as high hazards of sudden death in patients with a HbA1c >8% as compared to those with normal HbA1c levels ≤6%, which persisted after the adjustment for confounders (Table 2). Evaluating potential intermediate conditions, we considered serum potassium, cardiac arrhythmia as documented by an electrocardiogram (ECG), left ventricular hypertrophy and differences in QRS axis (left axis type), signs of MI, repolarisation disorders, and corrected QT interval (Table 2). Adding any of the intermediate factors to the main model had little influence on the hazard ratios for HbA1c. The additional adjustment for all intermediates together also did not materially impact on the association of HbA1c with sudden death, suggesting mechanisms other than the investigated

ones to largely explain the higher risk of sudden death at higher levels of HbA1c. Investigating HbA1c as a continuous variable, the hazard to experience sudden cardiac death increased significantly by 18% per unit (i.e. 1%) increase in HbA1c (hazard ratio (HR) 1.18, 95% confidence interval (CI) 1.05-1.32, Table 3). The association was even stronger after adjustment for confounders, showing a 21% greater hazard of sudden death per unit increase in HbA1c (HR 1.21, 95% CI 1.06-1.38).

Additional analyses using quartiles of HbA1c (≤5.8%, >5.8 to ≤6.6%, >6.6 to ≤7.4%, >7.4%) showed similar results: Patients of the third and fourth HbA1c quartile had significantly increased risks of sudden cardiac death as compared to patients of the first quartile ($HR_{3rd\ quartile}$ 2.00, 95% CI 1.24-3.23; $HR_{4th\ quartile}$ 1.83, 95% CI 1.11-3.03, respectively).

Baseline HbA1c and risk of myocardial infarction, stroke, the primary endpoint of combined cardiovascular events and death due to heart failure

Investigating further cardiac and vascular outcomes, we found no association of HbA1c with the risk of MI. Both in continuous (adjusted HR 0.94, 95% CI 0.83-1.07) and in categorical analyses, the risk of MI did not increase (Tables 3 and 4). When non-fatal and fatal MI were analyzed separately, the results were similar showing no relation to HbA1c.

Higher levels of HbA1c by trend, but not significantly, affected the risk of stroke. Similarly, higher effect estimates for death due to heart failure were observed with higher levels of HbA1c, however the confidence intervals were wide (Tables 3 and 4).

The primary endpoint of combined CVE was markedly increased with higher levels of HbA1c (Table 3). Patients with a HbA1c >8% had an adjusted 37% higher risk of experiencing a CVE as compared to patients with a HbA1c ≤6%. This relation was mainly explained by the impact of HbA1c on sudden cardiac death, since no association was found for CVE except sudden death.

To strengthen our results, we eliminated any potential influence by atorvastatin treatment and repeated all analyses in the placebo group only. The results were similar, indicating no interaction and supporting the use of the complete data.

Table 1: Baseline patient characteristics, presented per HbA1c* category; study population n=1255

Characteristic	HbA1c (%)		
	≤ 6 (n=404)	> 6 ≤ 8 (n=664)	> 8 (n=187)
Age *years*	66 (8)	66 (8)	65 (9)
Gender *% men*	59.4	50.9	52.9
HbA1c *%*	5.4 (0.4)	6.9 (0.5)	8.9 (0.8)
Atorvastatin treatment *%*	48.3	51.5	52.9
Systolic BP* *mmHg*	145 (21)	147 (22)	144 (23)
Diastolic BP *mmHg*	76 (11)	76 (11)	76 (11)
BMI* *kg/m²*	26.9 (4.8)	27.7 (4.8)	28.2 (4.8)
Duration of diabetes *years*	15.5 (8.4)	18.9 (8.9)	20.9 (8.1)
Time on dialysis *months*	7.8 (6.9)	8.2 (6.9)	9.6 (6.8)
Smoker / Ex-smoker *%*	43.6	37.8	42.8
Arrhythmia *%*	18.1	19.0	19.8
History of			
CAD* *%*	29.2	29.4	29.9
CHF* *%*	31.9	36.7	38.0
Laboratory parameters			
LDL* cholesterol *mmol/L*	3.2 (0.7)	3.3 (0.8)	3.3 (0.8)
Triglycerides *mmol/L*	2.3 (1.5-3.5)	2.5 (1.7-3.6)	2.9 (1.9-4.5)
Hemoglobin *mmol/L*	6.6 (0.8)	6.8 (0.8)	6.9 (0.9)
Albumin *g/L*	38 (3)	38 (3)	38 (3)
C-reactive protein *mg/L*	4.4 (2.0-11.4)	5.2 (2.5-12.1)	6.1 (2.8-15.7)
Calcium *mmol/L*	2.3 (0.2)	2.3 (0.2)	2.3 (0.2)
Phosphate *mmol/L*	1.9 (0.5)	2.0 (0.5)	1.9 (0.5)
Potassium *mmol/L*	5.32 (0.9)	5.14 (0.9)	4.95 (0.7)
ECG* characteristics			
Sinus rhythm %	91	89	86
AV*– block %	7.4	7.7	5.3
QRS – left axis type %	60	63	71
Ventricular conduction Defects %	7	10	8
Repolarisation disorder %	62	61	70
LVH* %	12	12	14
QT interval corrected *ms*	423 (39)	427 (39)	426 (39)
Signs of MI* %	13	14	17
Atrial fibrillation / flutter %	8	9	12
Heart rate *bpm*	78 (15)	80 (16)	79 (15)

Values are presented as means (SD) or median (interquartile range) or *%*.
*Abbreviations: HbA1c = hemoglobin A1c; BP = blood pressure;
BMI = body mass index; CAD = coronary artery disease,
CHF = congestive heart failure; LDL = low density lipoprotein,
ECG = electrocardiogram (resting), AV = atrio – ventricular,
LVH = left ventricular hypertrophy, MI = myocardial infarction.

Baseline HbA1c and risk of all-cause mortality

In univariate analyses, all-cause mortality increased by 8% per unit increase in HbA1c (HR 1.08, 95% CI 1.02-1.15; Table 3). Multivariable adjustment resulted in a hazard ratio of 1.09 (95% CI 1.02-1.17). The results of categorical analyses are shown in Table 4 and Figure 1B. Patients with a HbA1c >6% were 34% more likely to die, as were those with a HbA1c >8%, compared to patients with normal HbA1c levels ≤6%.

Additional analyses revealed that the association of HbA1c with all-cause mortality was mainly explained by its effect on sudden cardiac death, since no association was seen for mortality except for sudden death (Tables 3 and 4).

Table 2: Baseline HbA1c* and the risk of sudden death; study population n=1255

	HbA1c (%)				
	≤ 6 (n=404)	> 6 ≤ 8 (n=664)		> 8 (n=187)	
Model		HR (95% CI)	p	HR (95% CI)	p
Crude	1	1.69 (1.14-2.49)	0.008	2.14 (1.33-3.44)	0.002
Adjusted†	1	1.82 (1.20-2.77)	0.005	2.25 (1.32-3.81)	0.003
Adjusted† + CAD*, CHF* (main model)	1	1.85 (1.22-2.81)	0.004	2.26 (1.33-3.85)	0.003
Main model plus					
Potassium	1	1.90 (1.25-2.89)	0.003	2.35 (1.38-4.01)	0.002
Rhythm disorders	1	1.84 (1.21-2.80)	0.004	2.23 (1.31-3.79)	0.003
LVH*, QRS left axis type	1	1.86 (1.23-2.83)	0.004	2.18 (1.28-3.72)	0.004
Signs of MI*	1	1.85 (1.22-2.80)	0.004	2.25 (1.32-3.84)	0.003
Repolarisation disorders	1	1.86 (1.22-2.83)	0.004	2.20 (1.29-3.76)	0.004
All intermediate factors	1	1.89 (1.24-2.89)	0.003	2.20 (1.29-3.78)	0.004

*Abbreviations: HbA1c = hemoglobin A1c; CAD = coronary artery disease, CHF = congestive heart failure; K= Potassium
LVH = left ventricular hypertrophy, MI = myocardial infarction.

†Adjusted: Adjustments were made for age, sex, atorvastatin treatment, systolic blood pressure, duration of diabetes, time on dialysis, smoking status, body mass index, levels of LDL-cholesterol, triglycerides, albumin, hemoglobin, calcium, phosphate and C-reactive protein.

Main model: further adjustments were made for the presence of coronary artery disease (as defined by history of myocardial infarction, coronary artery bypass grafting surgery, percutaneous coronary intervention, coronary heart disease documented by coronary angiography), and the presence of congestive heart failure.

QT interval and corrected QT interval were analyzed separately due to missing values and had no impact in the association of HbA1c with sudden death.

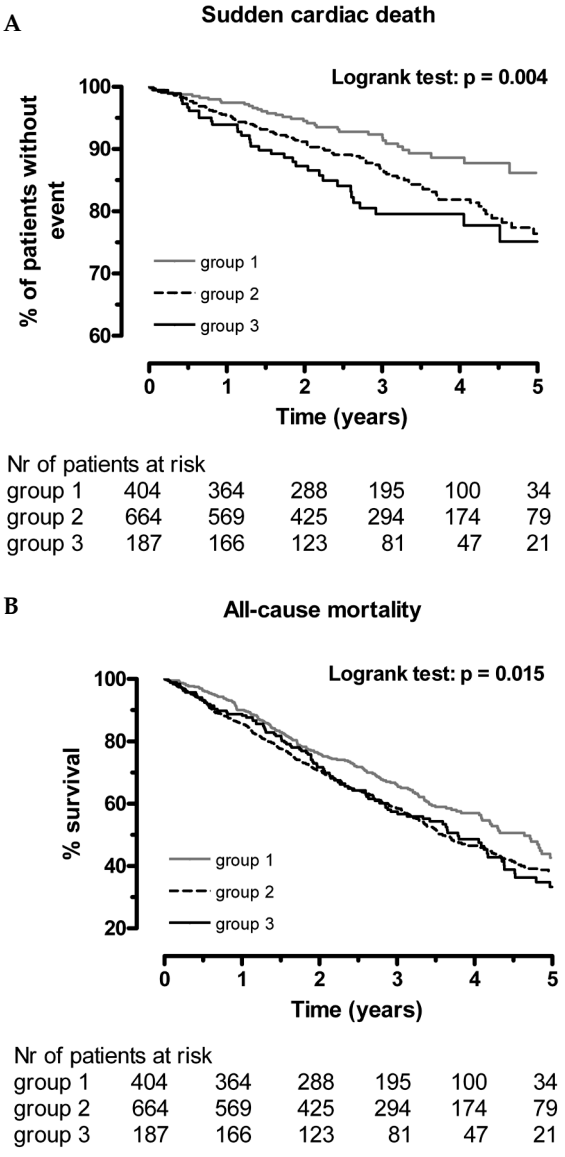

Figure 1A-B: Kaplan Meier curves for the time to A) sudden cardiac death, B) all-cause mortality in subgroups of patients according to baseline hemoglobin A1c (HbA1c) levels (group1: HbA1c ≤6% (=reference group), group 2: HbA1c >6 ≤8%, group 3: HbA1c >8%).

Table 3: Absolute rates of sudden death, myocardial infarction (MI), stroke, the primary endpoint, all-cause mortality, heart failure death and mortality except for sudden death, and hazard ratios with 95% confidence intervals (HR, 95% CI) per unit increase in HbA1c as continuous variable; n=1255

	Sudden death	MI	stroke	Primary endpoint*
events	160	200	103	469
Person-years (py)	3555	3368	3465	3287
Incidence rate / 100 py	4.5	5.9	3.0	14.3
HbA1c crude HR (95%CI)	1.18 (1.05-1.32)	0.98 (0.87-1.09)	1.13 (0.98-1.31)	1.08 (1.01-1.16)
HbA1c adj. HR[1] (95%CI)	1.21 (1.06-1.38)	0.94 (0.83-1.07)	1.11 (0.93-1.32)	1.09 (1.01-1.18)

	All-cause mortality	Heart failure death	Mortality except for sudden death
events	617	41	457
Person-years (py)	3555	3555	3555
Incidence rate / 100 py	17.4	1.2	12.9
HbA1c crude HR (95%CI)	1.08 (1.02-1.15)	1.14 (0.91-1.43)	1.05 (0.98-1.13)
HbA1c adj. HR[1] (95%CI)	1.09 (1.02-1.17)	1.30 (1.00-1.68)	1.04 (0.96-1.13)

[1]Adjusted: Adjustments were made for age, sex, atorvastatin treatment, systolic blood pressure, duration of diabetes, time on dialysis, smoking status, body mass index, levels of LDL-cholesterol, triglycerides, albumin, hemoglobin, calcium, phosphate, C-reactive protein, the presence of coronary artery disease (as defined by history of myocardial infarction, coronary artery bypass grafting surgery, percutaneous coronary intervention, coronary heart disease documented by coronary angiography), and the presence of congestive heart failure.

*The primary endpoint was a composite of death from cardiac causes, fatal stroke, non-fatal myocardial infarction, or non-fatal stroke, whichever occurred first.

Table 4: Hazard ratios and 95% confidence intervals (HR, 95% CI) for sudden cardiac death, myocardial infarction (MI), stroke, the primary endpoint, death due to heart failure, all-cause mortality, and mortality except for sudden cardiac death according to categories of HbA1c at baseline; n=1255

		Sudden death		MI		Stroke		Primary endpoint*	
model	HbA1c	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
crude	≤6	1		1		1		1	
	>6≤8	1.69 (1.14-2.49)	0.008	1.04 (0.77-1.41)	0.814	1.58 (0.98-2.54)	0.058	1.29 (1.04-1.59)	0.019
	>8	2.14 (1.33-3.44)	0.002	0.80 (0.50-1.28)	0.358	1.74 (0.96-3.18)	0.070	1.32 (0.99-1.75)	0.056
Adj[1]	≤6	1		1		1		1	
	>6≤8	1.85 (1.22-2.81)	0.004	0.94 (0.68-1.30)	0.707	1.56 (0.93-2.62)	0.093	1.31 (1.05-1.65)	0.018
	>8	2.26 (1.33-3.85)	0.003	0.77 (0.47-1.26)	0.299	1.67 (0.84-3.30)	0.142	1.37 (1.00-1.87)	0.050
		All-cause mortality		Heart failure death		Mortality except for sudden death			
crude	≤6	1		1		1			
	>6≤8	1.29 (1.07-1.55)	0.006	1.34 (0.65-2.75)	0.427	1.19 (0.97-1.47)	0.098		
	>8	1.31 (1.02-1.68)	0.033	1.44 (0.56-3.71)	0.452	1.10 (0.82-1.47)	0.543		
Adj[1]	≤6	1		1		1			
	>6≤8	1.34 (1.10-1.63)	0.004	1.53 (0.70-3.33)	0.288	1.19 (0.96-1.50)	0.117		
	>8	1.34 (1.02-1.76)	0.039	2.12 (0.75-5.98)	0.155	1.10 (0.80-1.52)	0.546		

Adjusted[1]: Adjustments were made for age, sex, atorvastatin treatment, systolic blood pressure, duration of diabetes, time on dialysis, smoking status, body mass index, levels of LDL-cholesterol, triglycerides, albumin, hemoglobin, calcium, phosphate, C-reactive protein, the presence of coronary artery disease (as defined by history of myocardial infarction, coronary artery bypass grafting surgery, percutaneous coronary intervention, coronary heart disease documented by coronary angiography), and the presence of congestive heart failure.

*The primary endpoint was a composite of death from cardiac causes, fatal stroke, non-fatal myocardial infarction, or non-fatal stroke, whichever occurred first.

Discussion

We analyzed data from 1255 hemodialysis patients with type 2 diabetes mellitus who took part in the 4D Study and experienced a high incidence of pre-specified and centrally adjudicated endpoints. In the present analysis, baseline HbA1c was a strong risk factor for sudden cardiac death during 4 years of follow-up. In patients with a HbA1c above 8 percent, the risk of dying suddenly was more than twice as high than in those with a HbA1c below 6 percent. There was a trend for higher risks of stroke and deaths due to heart failure, while myocardial infarction was not affected. Both, combined cardiovascular events and mortality were increased at higher levels of HbA1c, which was mainly explained by the impact of HbA1c on sudden death.

When enrolling into the 4D Study, patients had an average history of known diabetes for 18 years and had been on maintenance hemodialysis for an average of 8 months. They had a significant burden of microangiopathic complications (diabetic retinopathy: 71%, polyneuropathy: 60%) and macroangiopathic complications (about one third of these patients had coronary artery disease at baseline). Sudden death accounted with 26% for the highest proportion of deaths in the 4D Study, while only 11% of deaths were attributed to MI and adjudicated coronary heart disease[3]. Similar information was reported from the United States Renal Data System which showed that 27% of deaths in dialysis patients had been classified as cardiac arrest or cardiac arrhythmia, and only 8% as deaths due to acute MI and atherosclerotic heart disease[2].

A number of different causes may account for sudden death in dialysis patients, including micro- and macrovascular disease, sympathetic overactivity, structural heart disease, cardiac fibrosis, and electrolyte and volume shifts due to the hemodialysis procedure [9, 13, 14]. Our present study adds glycemic control to the list, which raises the question of potential mechanisms.

Hyperglycemia has been shown to play a significant role in the development of microangiopathy, endothelial dysfunction[15], and impaired myocardial vasodilator function[16], which contribute to cardiac microvessel disease and structural heart disease[17]. It has been reported that hyperglycemia induced excess generations of

highly reactive free radicals causing oxidative stress, and inflammatory cytokines[17, 18]. In this context, it is important to note that HbA1c is presumably an indicator of a higher load of the Amadori derived advanced glycation end products (AGEs), which are known to excert and amplify oxidative stress and vice versa can also be a consequence of oxidative stress. These AGE toxins are profibrotic and directly involved in the pathogenesis of the inflammatory reponse syndrome and vascular complications.

Myocardial fibrosis has mechanical and electrical sequelae which impact on cardiovascular prognosis[19]. It reduces the ventricular compliance and promotes arrhythmia by causing local delay in the spread of the action potential[20, 21]. In an animal model of mild diabetes, researchers observed an enhanced susceptibility to ventricular arrhythmias, with increased electrophysiological sensitivity to catecholamines and nonhomogenous collagen accumulation affecting local conduction[22]. Studies in diabetic patients found regional cardiac denervation and sympathetic overactivity, potentially resulting in life-threatening myocardial electrical instability[23, 24]. In line with these findings, HbA1c was reported to be a predictor of spontaneous ventricular arrhythmias in diabetic patients with an implantable-cardioverter-defibrillator[25].

We further found HbA1c to be a risk factor for all-cause mortality, which is in line with previous studies in diabetic patients from the general population[26, 27]. In chronic kidney disease patients the literature is not unequivocal. While several studies reported lower survival rates for diabetic patients with poor glycemic control [28-30], another study in 24,875 maintenance hemodialysis patients from Fresenius dialysis clinics in the United States did not indicate any association between HbA1c and 1 year survival[31]. Even though the lack of a survival association in this study could have been due to the short-term follow-up and further methodological differences, this study has led to some confusion about the role of glycemic control in dialysis patients[32]. Data from 23,618 patients in DaVita outpatient clinics over 3 years showed in unadjusted survival analyses paradoxically lower death hazard ratios with higher HbA1c values. However, after adjusting for a large number of potential confounders, higher HbA1c values were incrementally associated with higher death risks, and lower HbA1c levels

not related to malnutrition or anemia appeared to be associated with improved survival[33].

Surprisingly, in patients without renal failure, treatment that improved glycemic control did not achieve the predicted benefit in recent trials with regard to macrovascular complications. In fact, the question arose if tight glycemic control might even increase the risk of death querying the role of glycemic control to be a risk factor[34, 35]. In this context, it is of special interest to distinguish between micro- and macrovascular complications. Importantly, the risk of MI as a major macrovascular complication was not affected by glycemic control in our study. There was also no convincing effect on stroke, which is discussed to result from both macro- and microvascular components in diabetic patients [36]. These observations are in line with results from the ADVANCE study, which showed that the beneficial effect of intensive glycemic control was mainly achieved by the reduction of microvascular complications, while macrovascular events alone could not significantly be reduced[35]. However, macrovascular events with predominantly MI account for the majority of deaths in diabetic patients from the general population[37], possibly partly explaining why the intervention trials did not show a reduction in all-cause death. In contrast, patients with renal disease experience a strikingly different risk pattern with high proportions of sudden cardiac deaths, and atherosclerotic deaths playing a minor role only [2, 3]. Whereas the effect of tight glycemic control on macrovascular complications is still under debate, its effect on microvascular complications has repeatedly been shown[27, 34, 35, 38, 39]. Further studies will clarify whether better glucose control may decrease the risk of sudden cardiac death in dialysis patients.This study had certain **limitations**. It was a post-hoc analysis within a selected cohort of German patients with T2DM on hemodialysis. Therefore, the relationship between HbA1c and risk may not be generalisable to other patient populations. HbA1c measurement in patients on hemodialysis treatment might have been compromised by reduced red blood cell lifespan[40] and the widespread use of erythropoietin increasing the proportion of reticulocytes and younger red blood cells with less time for glycosylation to occur[41]. This may have lead to an underestimation of HbA1c levels, but due to its general nature it is unlikely to have influenced our results,

which were based on comparisons within the same study population. The specific outcomes, among which sudden cardiac death, and their association with HbA1c to be analyzed was the main **strength** of this study. In this context, the long-term follow-up, adequate sample size and high incidence of pre-specified and centrally adjudicated endpoints are further to be mentioned.
In conclusion, glycemic control as represented by the level of HbA1c was strongly associated with sudden cardiac death in hemodialysed type 2 diabetic patients. While myocardial infarction was not affected, the risks of the combined primary endpoint and mortality significantly increased at higher levels of HbA1c, and were mainly explained by the impact of glycemic control on sudden cardiac death. Whether tight glucose control decreases the risk of sudden death without causing side effects, should be examined in future trials.

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CHAPTER 5

Change in Adiponectin and the risk of sudden death, stroke, myocardial infarction and mortality in hemodialysis patients

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Abstract

Adiponectin levels increase and cardiovascular (CV) risk profile changes during progression of chronic kidney disease. This study examined the association of baseline and longitudinally changing adiponectin with the components of CV outcome and mortality in 1255 diabetic hemodialysis patients from the German Diabetes and Dialysis Study. Within 4 yrs follow-up, hazard ratios (95%CI) to reach pre-specified, adjudicated endpoints were determined: sudden death (SD; n=160), stroke (n=99), myocardial infarction (MI; n=200), combined CV events (CVE; n=469) and all-cause mortality (n=617). Baseline adiponectin was associated with an increased risk of CVE (HR 1.26; 1.05-1.51), mainly due to the high risks of SD (1.40; 1.02-1.90) and stroke (1.66; 1.12-2.45). Adiponectin was negatively correlated with CRP ($p<0.001$) and positively with NT-pro-BNP ($p<0.001$), the latter meaningfully attenuating the associations with adverse outcome. The risks for patients with longitudinally rising adiponectin markedly increased by 33% (CVE), 51% (SD), 66% (MI) and 29% (all-cause death), compared to patients with decreasing levels. Adjustments for the change in NT-pro-BNP weakened the associations. In conclusion, SD and stroke contribute largely to the CV risk being associated with high adiponectin in hemodialysis patients. Increasing adiponectin over time is associated with poor clinical outcome, likely in part as a consequence of rising NT-pro-BNP, and keeping its potential to counteract inflammation.

Introduction

Adiponectin is an adipocyte-specific cytokine, which is inversely correlated with body mass index [1;2] and has been suggested to play a protective role in the development of cardiovascular (CV) comorbidities. Adiponectin has been found to improve hepatic [3] and muscular insulin sensitivity [4], to ameliorate endothelial function [5], and to counteract inflammation [5-7]. In the general population, high levels of adiponectin were found to be associated with less CV complications, such as coronary artery disease [8;9] and myocardial infarction (MI) [10].

Serum levels of adiponectin are more than twofold increased in renal failure [11]. Similar observations of high adiponectin being linked with better CV outcome have been reported [11;12], but some studies showed contrary results: high adiponectin was found to be associated with high CV mortality [13], and progressive decline of renal function [14].

In advanced chronic kidney disease and end-stage renal disease, the pattern and composition of risk is changing. Cardiovascular risk is determined by various components such as sudden death (SD), stroke and MI, and may vary due to changing proportions of the components. By now, the association of baseline adiponectin with SD, stroke and MI is unclear in renal patients, and associations of longitudinal changes of adiponectin with these outcomes have not been investigated so far. Furthermore, uncertainty exists about pathways that underlie the associations of adiponectin with adverse outcomes in patients with renal failure.

The aim of this study was threefold; first to assess the association of baseline adiponectin with SD, stroke, MI, combined cardiovascular events (CVE) and all-cause mortality in dialysis patients, second to assess the association of longitudinal changes of adiponectin with these outcomes, and third to investigate the role of potential new factors in the association of adiponectin with outcome. To that end, data from the German Diabetes Dialysis Study (4D-Study (Die Deutsche

Diabetes Dialyse Studie)), which evaluated atorvastatin in 1255 patients with type 2 diabetes mellitus on maintenance hemodialysis treatment [15], were analyzed.

Results

Patient characteristics

Between March 1998 and October 2002, a total of 1255 patients were included into the 4D study. Of those, 1249 patients had a baseline and 1205 had a post baseline adiponectin measurement after a median of 182 days (interquartile range 177 – 185 days), with 1202 patients having both. The mean follow-up period was 3.96 years (median 4.0 years) on atorvastatin and 3.91 years (median 4.08 years) on placebo. During follow-up, 617 patients died (160 of SD). Furthermore, 469 patients reached the composite cardiovascular endpoint (CVE: cardiac death, stroke, MI) with stroke and MI occurring in 99 and 200 patients, respectively.

In the study population (n=1249), the mean age was 65.7 ± 8.3 years, and 54% of the patients were male. The median baseline adiponectin level was 13.8 (IQR 10-20) mg/L; the median post-baseline adiponectin level (after 6 months follow-up) was 13.7 (10.0-20.3) mg/L, with no significant differences being present between the atorvastatin and placebo group. The baseline patient characteristics are shown in Table 1.

Baseline adiponectin and outcome

Baseline adiponectin (continuous variable) was in crude analyses significantly associated with the risks of stroke, SD and CVE. Per unit increase in log transformed adiponectin, the risk of stroke increased by 66%, SD by 40%, and CVE by 26% (Table 2). When patients were divided into categories according to their adiponectin level at baseline, those in the highest adiponectin quartile had a >2-fold higher risk of stroke (HR (95%CI) 2.39 (1.28 – 4.48)), a 51% higher risk of SD (1.51 (0.99-2.31)) and a 33% higher risk of experiencing a CVE (1.33 (1.03-1.72)), as compared to patients in the lowest quartile (Table 3).

Table 1: Patient characteristics according to quartiles of adiponectin at baseline; study population n=1249

Characteristic	Adiponectin (mg/L)			
	≤9.97 (n=314)	>9.97 to ≤13.80 (n=311)	>13.80 to ≤19 (n=314)	>19.85 (n=310)
Age *years*	65 (9)	66 (8)	66 (8)	66 (8)
Gender *% male*	61	51	53	51
BMI kg/m^2	28.2 (4.7)	28.3 (4.9)	27.9 (4.9)	25.8 (4.3)
Atorvastatin treatment *%*	51	51	51	50
Smoker / Ex-smoker *%*	46	37	37	42
Systolic BP *mmHg*	144 (22)	145 (23)	147 (23)	147 (20)
Diastolic BP *mmHg*	74 (10)	75 (12)	77 (11)	77 (11)
HbA1c *%*	6.8 (1.3)	6.7 (1.2)	6.6 (1.3)	6.7 (1.3)
Duration of diabetes *years*	16.7 (9.0)	18.7 (8.8)	18.0 (8.9)	18.9 (8.3)
Time on dialysis *months*	8 (6)	8 (7)	9 (8)	8 (7)
History of				
CAD *%*	34	29	22	32
CHF *%*	41	35	31	35
Lipid values *mg/dl*				
Total cholesterol	221 (42)	221 (42)	219 (43)	216 (43)
LDL cholesterol	122 (30)	125 (28)	128 (31)	127 (30)
HDL cholesterol	31 (11)	33 (10)	37 (11)	44 (16)
Triglycerides	284 (189-414)	242 (171-360)	213 (143-293)	157 (116-245)
Adiponectin *mg/L*	7.9 (1.5)	11.8 (1.1)	16.3 (1.7)	29.2 (11.0)
C-reactive protein *mg/l*	7.7 (3.3-17.6)	5.5 (2.8-13.4)	4.7 (2.1-12.0)	3.3 (1.5-7.5)
Albumin *g/dl*	3.8 (0.3)	3.8 (0.3)	3.8 (0.3)	3.8 (0.3)
Hemoglobin *g/dl*	10.9 (1.4)	10.8 (1.3)	10.9 (1.4)	11.1 (1.4)
NT-pro-BNP *pg/ml*	2075 (948-5604)	3426 (1338-7346	3212 (1487-9262)	5689 (2351-16694)

Values are presented as means (SD) or median (interquartile range) or *%*.
Abbreviations: BMI, body mass index; BP, blood pressure; HbA1c, glycated hemoglobin A1c;
CAD, coronary artery disease; CHF, congestive heart failure; LDL, low density lipoprotein; HDL, high density lipoprotein; NT-pro-BNP, N-terminal-pro-B-type natriuretic peptide

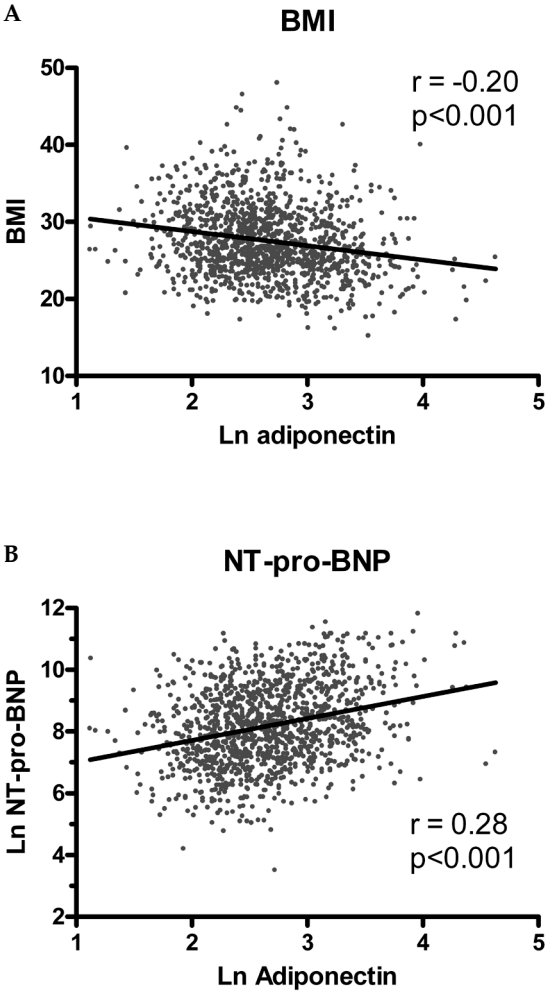
For the building of our multivariate models, we distinguished between confounding and intermediate conditions as known from the literature (the latter being part of a causal pathway). We especially evaluated further potentially important factors: body mass index (BMI), N-terminal-pro-B-type natriuretic peptide (NT-pro-BNP) and C-reactive protein (CRP). Adiponectin was inversely correlated to BMI ($r=-0.20$, $p<0.001$; Figure 1a), and positively to NT-pro-BNP ($r=0.28$, $p<0.001$; Figure 1b), both identified as confounders. As experimental data showed that adiponectin suppresses inflammation and CRP, (negative correlation $r=-0.27$, $p<0.001$; Figure 1c), the latter constitutes an intermediate condition, adjustments for which would not be warranted in epidemiological analyses. If performed, the resulting effect estimates would represent the association of adiponectin with outcome excluding the mechanism via its beneficial action on inflammation.

Subsequently, multivariate analyses were done with stepwise inclusion of confounders. Adjustments for age, gender, atorvastatin treatment, coronary heart disease, congestive heart failure, duration of type 2 diabetes mellitus, smoking status and blood pressure did not meaningfully change the association of adiponectin with adverse outcomes except for stroke (Table 2). While further inclusion of BMI hardly changed the effect estimates, interestingly NT-pro-BNP strongly impacted and attenuated the associations of adiponectin with outcomes (final models; Table 2). Results from the categorical analyses including all above mentioned confounders are shown in Table 3.

To strengthen the assumption of CRP being an intermediate condition, we performed explorative analyses. When the final multivariate models, described above, were additionally adjusted for CRP, i.e. when the suggested mechanism of adiponectin to improve inflammation was excluded, the risks expectedly became significantly higher: hazard ratios for log adiponectin to reach CVE increased from 1.03 to 1.11, sudden death from 1.12 to 1.22, stroke from 1.14 to 1.22, MI from 0.86 to 0.90 and all-cause mortality from 0.88 to 1.03, respectively. Further adjustments for triglycerides and high-density lipoprotein (HDL) cholesterol, which may also represent intermediate conditions, did not materially change the results.

Additional analyses on adiponectin and adverse outcomes in subgroups of patients free of cardiovascular disease at baseline yielded similar results, as did

stratified analyses by male and female gender. In order to study the potential impact of informative censoring by deaths, we did further investigations using combined endpoints of death and MI, and death and stroke. Similarly to our primary analyses, no association of adiponectin with MI was found, while an increase in stroke was noted in crude analyses (data not shown).



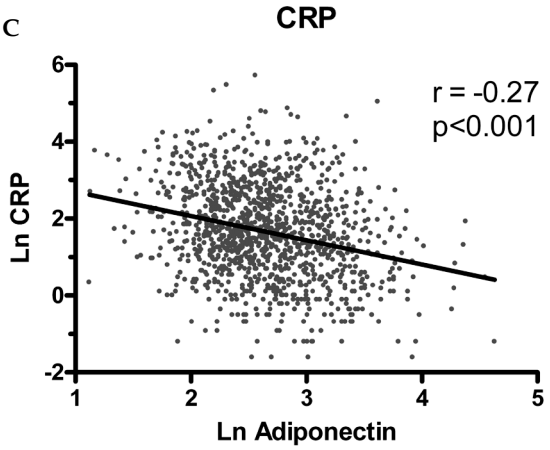


Figure 1a-c: Correlations of adiponectin (log transformed) with 1a) Body mass index, 1b) NT-pro-BNP (log transformed), 1c) C-reactive protein (log transformed)

Table 2: Baseline adiponectin (continuous variable, log transformed) and the risk of sudden death, stroke, myocardial infarction, combined cardiovascular events and all-cause mortality; study population n=1249

Outcome	Hazard ratio (HR) and 95% CI			
	crude	adj.*	adj.* + BMI	adj.* + BMI, NT-pro-BNP
Combined cardiovascular events	1.26 (1.05-1.51) p=0.013	1.27 (1.05-1.52) p=0.011	1.23 (1.02-1.48) p=0.028	1.03 (0.85-1.25) p=0.731
Sudden death	1.40 (1.02-1.90) p=0.036	1.39 (1.02-1.89) p=0.037	1.31 (0.96-1.79) p=0.093	1.12 (0.81-1.54) p=0.511
Stroke	1.66 (1.12-2.45) p=0.011	1.44 (0.97-2.14) p=0.074	1.45 (0.97-2.18) p=0.071	1.14 (0.75-1.73) p=0.555
Myocardial infarction	0.91 (0.68-1.20) p=0.487	0.94 (0.71-1.25) p=0.671	0.96 (0.72-1.28) p=0.789	0.86 (0.64-1.16) p=0.321
All-cause death	1.10 (0.94-1.30) p=0.239	1.10 (0.94-1.30) p=0.233	1.06 (0.90-1.25) p=0.474	0.88 (0.74-1.04) p=0.129

Abbreviations: BMI, body mass index; NT-pro-BNP, N-terminal-pro-B-type natriuretic peptide
* Analyses were adjusted for age, gender, atorvastatin treatment, coronary heart disease, congestive heart failure, duration of type 2 diabetes mellitus, smoking status, systolic blood pressure.

Table 3: Risk (Hazard Ratio (HR) and 95% confidence interval (95% CI)) of sudden death, stroke, myocardial infarction, combined cardiovascular events and all-cause mortality by quartiles of adiponectin at baseline; study population n=1249

Outcome	Adiponectin levels at baseline (mg/L)			
	≤9.97 (n=314)	>9.97 to ≤13.80 (n=311)	>13.80 to ≤19.85 (n=314)	>19.85 (n=310)
Cardiovascular events				
Crude HR (95% CI)	1	1.23 (0.94-1.60)	1.01 (0.77-1.31)	1.33 (1.03-1.72)
Adj.† HR (95% CI)	1	1.17 (0.88-1.55)	1.06 (0.79-1.40)	1.06 (0.80-1.40)
Sudden death				
Crude HR (95% CI)	1	1.18 (0.75-1.84)	0.77 (0.47-1.25)	1.51 (0.99-2.31)
Adj.† HR (95% CI)	1	1.05 (0.66-1.68)	0.72 (0.43-1.21)	0.99 (0.62-1.57)
Stroke				
Crude HR (95% CI)	1	1.87 (0.97-3.60)	1.92 (1.01-3.64)	2.39 (1.28-4.48)
Adj.† HR (95% CI)	1	1.78 (0.86-3.65)	1.65 (0.80-3.39)	1.72 (0.84-3.52)
Myocardial infarction				
Crude HR (95% CI)	1	1.01 (0.69-1.48)	0.91 (0.62-1.33)	0.81 (0.54-1.20)
Adj.† HR (95% CI)	1	0.99 (0.65-1.49)	1.08 (0.72-1.61)	0.80 (0.52-1.23)
All-cause death				
Crude HR (95% CI)	1	1.13 (0.90-1.41)	0.91 (0.73-1.15)	1.14 (0.91-1.42)
Adj.† HR (95% CI)	1	1.03 (0.81-1.31)	0.90 (0.70-1.15)	0.80 (0.62-1.02)

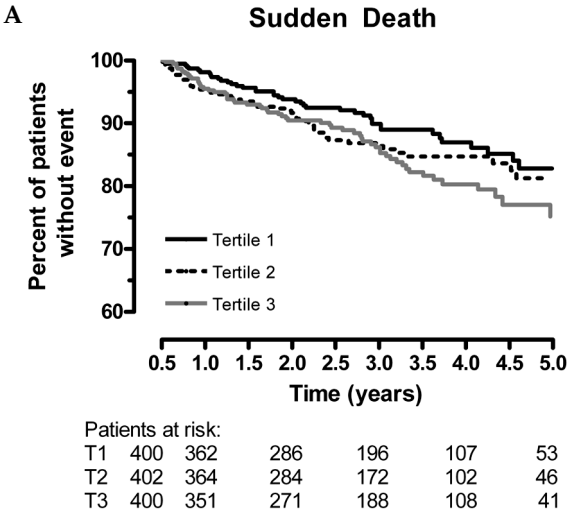
† Analyses were adjusted for age, gender, atorvastatin treatment, coronary heart disease, congestive heart failure, duration of type 2 diabetes mellitus, smoking status, systolic blood pressure, body mass index, N-terminal-pro-B-type natriuretic peptide‡

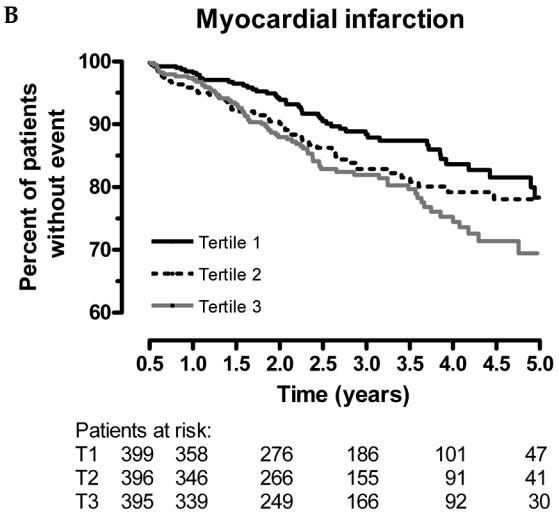
Change from baseline adiponectin and outcome

For our longitudinal analyses, we assessed the percentage change of adiponectin from baseline to the next available follow-up measurement (change in adiponectin = ratio $adiponectin_{f\text{-}up}$ / $adiponectin_{baseline}$). Correlation analyses showed that the change in adiponectin was strongly correlated to the change in NT-pro-BNP ($r=0.23$, $p<0.001$) and inversely to the change in BMI ($r= -0.12$, $p<0.001$). Furthermore, a rise in adiponectin was strongly correlated to a decrease in CRP ($r= -0.16$, $p<0.001$).

In Cox regression analyses on the association of changes in adiponectin with outcome, the crude relative risk of SD, MI, CVE and all-cause mortality increased considerably per unit increase in the log transformed adiponectin change (continuous variable). When patients were divided into tertiles according to the

percent change in adiponectin (patient characteristics are shown in Table 4), those with greater than 12.3% increasing adiponectin levels (3rd tertile) had in crude analyses a significant 51% higher risk of SD, a 66% higher risk of MI, a 33% higher risk of CVE and a 29% higher risk of all-cause death, compared to patients with decreasing adiponectin levels in the 1st tertile (reference group) (Table 5). After multivariate adjustments for baseline variables and the longitudinal changes in BMI and NT-pro-BNP, the association of increasing adiponectin with adverse outcomes attenuated largely. Again, NT-pro-BNP had a significant impact, with adjustments for its longitudinal change meaningfully contributing to attenuate the risks. The hazard ratios and 95% CIs from both crude and adjusted analyses of adiponectin on all outcomes are shown in Table 5. Additional adjustments for triglycerides and HDL cholesterol levels did not materially change the results.
Taking informative censoring by death into account, analyses of MI and stroke showed that rising adiponectin over 6 months (log transformed ratio $adiponectin_{f\text{-}up}$ / $adiponectin_{baseline}$), increased the crude risks of the combined endpoints MI and death and stroke and death significantly ($HR_{MI+death}$ 1.45 (1.17-1.78), $HR_{stroke+death}$ 1.39 (1.09-1.64)), and were attenuated after multivariate adjustments.





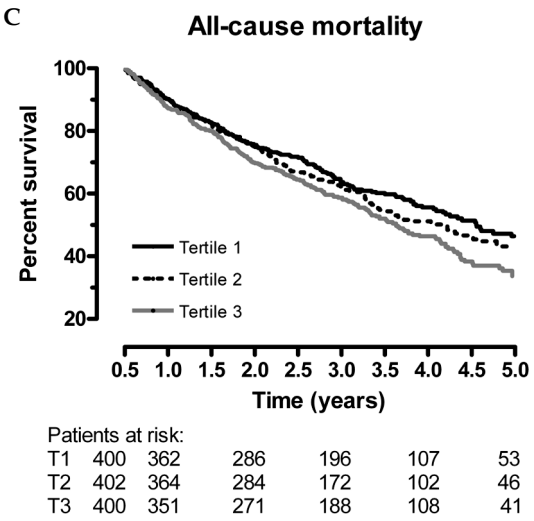


Figure 2a-c: Kaplan-Meier curves for the time to a) sudden death, b) myocardial infarction, and c) all-cause mortality by tertiles of the change in adiponectin from baseline. Tertile 1: patients with decreasing adiponectin greater than or equal to 12.8% (n=400), Tertile 2: patients with stable adiponectin levels (change in adiponectin between -12.8% and +12.3%; n=402), Tertile 3: patients with increasing adiponectin greater than 12.3% (n=400)

Table 4: Patient characteristics according to tertiles of the percent change in adiponectin from baseline; study population n=1202

Characteristic	Percent change in Adiponectin from baseline		
	Decrease ≤ - 12.8% (n=400)	Stable – 12.8% to +12.3% (n=402)	Increase > 12.3% (n=400)
Age *years*	65 (8)	66 (8)	66 (8)
Gender *% male*	56	53	52
BMI kg/m^2	28.0 (5.1)	28.0 (4.9)	26.8 (4.5)
Atorvastatin treatment *%*	52	48	49
Smoker / Ex-smoker *%*	39	39	42
Systolic BP *mmHg*	145 (22)	146 (22)	146 (22)
Diastolic BP *mmHg*	75 (11)	77 (11)	75 (11)
HbA1c *%*	6.8 (1.4)	6.8 (1.2)	6.6 (1.1)
Duration of diabetes *years*	18.4 (8.5)	18.6 (8.9)	17.3 (8.8)
Time on dialysis *months*	8 (7)	9 (7)	8 (7)
History of			
CAD *%*	31	29	28
CHF *%*	31	35	39
Lipid values *mg/dl*			
Total cholesterol	223 (42)	217 (41)	218 (43)
LDL cholesterol	128 (30)	124 (30)	126 (29)
HDL cholesterol	38 (14)	35 (14)	36 (12)
Triglycerides	226 (150;313)	224 (150;353)	216 (149;311)
Adiponectin change *mg/l*	- 4.4 (-7.8;-2.6)	-0.1 (-0.9;0.7)	4.6 (2.4;8.8)
C-reactive protein *mg/l*	4.3 (1.9;9.7)	4.8 (2.3;11.7)	5.8 (2.8;14.9)
Albumin *g/dl*	3.8 (0.3)	3.8 (0.3)	3.8 (0.3)
Hemoglobin *g/dl*	11.0 (1.3)	11.0 (1.3)	10.8 (1.4)
NT-pro-BNP *pg/ml*	3123 (1506;8585)	3544 (1460;8810)	3510 (1322;9659)

Values are presented as means (SD) or median (interquartile range) or *%*.
Abbreviations: BMI, body mass index; BP, blood pressure; HbA1c, glycated hemoglobin A1c; CAD, coronary artery disease; CHF, congestive heart failure; LDL, low density lipoprotein; HDL, high density lipoprotein; NT-pro-BNP, N-terminal-pro-B-type natriuretic peptide

Table 5: Change in adiponectin from baseline (tertiles) and the risk (Hazard Ratio (HR) and 95% confidence interval (95% CI)) of sudden death, stroke, myocardial infarction, combined cardiovascular events and all-cause mortality; study population n=1202

Outcome	Percent change in Adiponectin from baseline		
	Decrease ≤ - 12.8% (n=400)	Stable – 12.8% to +12.3% (n=402)	Increase > 12.3% (n=400)
Cardiovascular events			
Crude HR (95% CI)	1	1.23 (0.97-1.56)	1.33(1.05-1.69)
Adj.‡ HR (95% CI)	1	1.14 (0.87-1.50)	1.13 (0.84-1.51)
Adiponectin change as cont. variable#: HR_{crude} 1.32 (1.02 – 1.71); p=0.03			
		HR_{adj} 1.04 (0.75 – 1.44); p=0.81	
Sudden death			
Crude HR (95% CI)	1	1.33 (0.88-2.00)	1.51 (1.02-2.25)
Adj.‡ HR (95% CI)	1	0.94 (0.57-1.57)	1.27 (0.77-2.11)
Adiponectin change as cont. variable#: HR_{crude} 1.47 (0.95 – 2.27); p=0.08			
		HR_{adj}: 1.18 (0.66 – 2.11); p=0.58	
Stroke			
Crude HR (95% CI)	1	1.20 (0.72-2.00)	1.07 (0.63-1.80)
Adj.‡ HR (95% CI)	1	1.12 (0.63-1.98)	0.87 (0.46-1.66)
Adiponectin change as cont. variable#: HR_{crude} 1.14 (0.65 – 1.99); p=0.66			
		HR_{adj}: 0.94 (0.48 – 1.83); p=0.85	
Myocardial infarction			
Crude HR (95% CI)	1	1.40 (0.96-2.04)	1.66 (1.15-2.39)
Adj.‡ HR (95% CI)	1	1.31 (0.87-1.98)	1.45 (0.94-2.23)
Adiponectin change as cont. variable#: HR_{crude} 1.56 (1.05 – 2.31); p=0.03			
		HR_{adj} 1.31 (0.81 – 2.14); p=0.27	
All-cause mortality			
Crude HR (95% CI)	1	1.13 (0.92-1.39)	1.29 (1.06-1.57)
Adj.‡ HR (95% CI)	1	0.93 (0.73-1.19)	0.92 (0.71-1.18)
Adiponectin change as cont. variable#: HR_{crude} 1.33 (1.07 – 1.66); p=0.01			
		HR_{adj} 0.85 (0.64 – 1.13); p=0.27	

‡Analyses were adjusted for age, gender, atorvastatin treatment, coronary heart disease, congestive heart failure, duration of type 2 diabetes mellitus, baseline adiponectin levels, smoking status, systolic blood pressure, body mass index (BMI), N-terminal-pro-B-type natriuretic peptide (NT-pro-BNP) and C-reactive protein at baseline, longitudinal changes in BMI and longitudinal changes in NT-pro-BNP

#The adiponectin change (ratio $adiponectin_{f\text{-}up}/adiponectin_{baseline}$) as cont.variable was log transformed.

Discussion

This study investigated the association of two consecutive adiponectin measurements with sudden death, stroke, myocardial infarction as – pathophysiologically different – components of cardiovascular events, and all-cause mortality. The main findings within a large cohort of diabetic hemodialysis patients were that high adiponectin at baseline was associated with an increased risk of CVE, mainly due to the high risks of sudden death and stroke, but not of MI. Increasing adiponectin during follow-up was associated with increased risks of adverse outcomes. NT-pro-BNP and its longitudinal change turned out to be a strong confounder and meaningfully attenuated the associations of adiponectin with adverse outcomes. In addition, high levels of adiponectin were correlated with low levels of CRP, the latter potentially being an effect of adiponectin.

Mean adiponectin serum concentration in the general population is approximately 6mg/l [16]. Low levels of adiponectin were associated with coronary artery disease in men [9] and increased the risk of MI [10]. Similarly, investigations in hemodialysis patients and in patients with chronic renal disease found low adiponectin levels to be associated with poor CV outcome [11;12;17]. In contrast, recent studies suggested a high rather than a low adiponectin to be associated with increased CV and all-cause mortality, and a higher decline of renal function [13;14;18]. One possible reason for the discrepant results may lie in varying proportions of the –pathophysiologically different- components of CV outcome. The pattern of risk is changing in chronic kidney disease and end-stage renal disease, compared to healthy subjects. Whereas people from the general population mainly die from MI, sudden death represents the most prominent event in dialysis patients, accounting for 25% of all-cause mortality [19]. Therefore, our study adds important new knowledge, investigating the individual components of CV outcome, and showing that the association of a high baseline adiponectin with CVE was indeed mainly explained by the risks of sudden death and stroke, and not of MI.

One further reason for the differing results in literature may be sought in the complex mechanisms, by which adiponectin is regulated. The differentiation of causes from consequences, actions from reactions, and true effects from confounding, is essential. In this context, our study provides important information, distinguishing confounding from intermediate conditions. It also especially investigates potential new factors with their impact in the association of adiponectin with outcome by applying a stepwise approach: Adjusting the crude models for a variety of confounders as being known from the literature did not meaningfully change the effect estimates derived for the association of adiponectin with adverse outcomes. Thereafter we attempted to investigate poor nutritional status and wasting, as partly being reflected by a low BMI. Wasting is known to affect adiponectin levels [13;20] and is associated with poor outcome [21;22]. Experimental data suggest that adiponectin may be associated with weight loss secondary to increased energy expenditure [23;24]. Conversely, one study showed that weight loss increased plasma adiponectin levels [25]. In fact, additional adjustment of the analyses for BMI did either not or only slightly diminish the risks. This suggests that apart from the complexity of poor nutritional status, which may hardly be possible to adequately adjust for, other pathologic processes may also contribute and underlie the observed associations of high adiponectin with poor outcome.

In this context it is of particular interest that NT-pro-BNP appears to play a crucial role in adiponectin metabolism. Recent data showed a positive relation between adiponectin and brain natriuretic peptides [22;26-28], as well as with left ventricular dysfunction [26]. Levels of adiponectin, in elderly heart failure patients, were found particularly increased in those with underlying non-ischemic origin [29]. Importantly, one study showed that carperitide infusions (atrial natriuretic peptide) increased plasma levels of adiponectin in patients with heart failure [30]. As NT-pro-BNP also is a risk factor for adverse outcome [31], it represents an important confounder in the association of adiponectin with outcome. After further adjustments for NT-pro-BNP, we were surprised by the magnitude by which the effect estimates were lowered. In fact, NT-pro-BNP was the only variable, which largely influenced and attenuated the association. Thus,

our study suggests that the relation of a high adiponectin with adverse outcome in hemodialysis patients is essentially explained by NT-pro-BNP, potentially representative of cardiac function and possibly fluid overload.

In line with this, the data on follow-up measurements of adiponectin show that longitudinal increases in adiponectin levels were related to higher risks of sudden death, MI, combined CVE and all-cause mortality. Again, the increase in adiponectin was correlated to an increase in NT-pro-BNP, and adjustments for NT-pro-BNP and its changes resulted in meaningfully lower effect estimates. These data extend findings of a previous study suggesting an increased mortality with increasing adiponectin [32], not taking NT-pro-BNP into account. The proposed link of adiponectin with impaired cardiac function may further be supported by studies in men with angina pectoris [33], patients with coronary artery disease [28;34] and congestive heart failure [22;29], where elevated circulating adiponectin levels were independent markers of future MI, CVE and mortality. Compared to the general population, those patients presented with a poorer health condition, eventually suggesting that impaired cardiac function may have accounted for a modification of adiponectin levels and associated risk. In future,more research is needed to elucidate the impact of ventricular dysfunction, hemodynamic factors, cardiac ischemia and volume status [35;36], as well as the interesting hypothesis of an indirect stimulation of adiponectin by natriuretic peptides through increased lipid mobilization [22].

Adressing potential causal effects of adiponectin, experimental data suggest the hormone to have several anti-inflammatory properties. First, the production and action of TNFα, a key proinflammatory cytokine, is inhibited in various cell types including cardiac and vascular cells [37]. Second, nuclear factor-κ B activation in endothelial cells and the ability to activate AMP-kinase is inhibited, which resulted in downregulation of CRP, interleukin-8, and adhesion molecule expression [5;38-43]. Third, adiponectin promoted the clearance of apoptotic cells by macrophages [44], thus counteracting an exacerbation of inflammation and immune dysfunction [45]. Applying this information to the clinical situation,

adiponectin may be assumed to lower CRP in hemodialysis patients, which is a strong risk factor for adverse outcome [46;47]. In this case, adjustments for CRP in the analyses of adiponectin with outcome, i.e. excluding the potential pathway of lowering inflammation, are expected to result in a worse association (=higher risk estimates for adiponectin). Indeed, consistently higher effect estimates were seen, suggesting that the experimentally observed anti-inflammatory effects of adiponectin also hold true in the clinical setting of maintenance hemodialysis.

The strengths of this prospective cohort study include the precise and comprehensive recording of pre-specified, centrally adjudicated outcomes, the distinction of different components forming cardiovascular events, the longitudinal measures of adiponectin, the large number of patients and the long-term follow-up. A potential limitation may be that the adiponectin measurements did not account for the differentiation of isoforms, with the high molecular weight isoform being suggested as the most biologically active form [48]. However, the roles of the isoforms varying in different physiopathological conditions are not yet fully established [49;50]. In heart failure populations, total adiponectin has been recommended as preferred measure for mortality assessments [51].

In conclusion, the increased risk of cardiovascular events seen with a high adiponectin at baseline was mainly explained by high risks of sudden death and stroke, but not of myocardial infarction. Increasing adiponectin during follow-up was associated with higher risks of adverse cardiovascular outcomes and death, whereby the associations were mainly explained by a confounding effect of NT-pro-BNP. Furthermore, adiponectin appears to hold anti-inflammatory properties in hemodialysis patients. We therefore suggest that high and increasing adiponectin in the dialysis population largely reflects a consequence of disease circumstances, characterized by increasing NT-pro-BNP. Most likely it is a counterregulatory response to worsening health, while keeping its potential to counteract inflammation.

Materials and methods

Study Design and Participants

The 4D study methodology has previously been reported in detail [52]. Briefly, the 4D study was a prospective randomized controlled trial including 1255 patients with type 2 diabetes mellitus, 18 – 80 years, and previous duration of hemodialysis of less than 2 years. Between March 1998 and October 2002, patients were recruited in 178 participating dialysis centres in Germany. After a period of 4 weeks, patients were randomly assigned to double-blind treatment with either 20mg Atorvastatin (n=619) or placebo (n=636) once daily. Study visits took place three times before randomization (visit 1-3), at randomization (visit 4), and at four weeks (visit 5) and every six months (visit 6 etc.) after randomization until the date of death, censoring, or end of the study in February 2004. The primary endpoint of the 4D study was defined as a composite of death from cardiac causes, stroke and MI, whichever occurred first. Death from any cause, sudden death, stroke and MI were secondary study endpoints. 4D Study endpoints were centrally adjudicated by three members of the endpoint committee blinded to study treatment and according to pre-defined criteria [15].

For the present analysis, sudden death, stroke, MI, the combined cardiovascular endpoint (CVE: cardiac death, stroke, MI) and all-cause death were all chosen to be separate outcome measures and were based on the primary judgement of the endpoint committee during the 4D Study.

Data collection

Information on age, gender and smoking status was obtained through patient interviews. Smoking status was classified as never, former or current. Comorbidities, including the presence of coronary artery disease and congestive heart failure, as well as the duration of diabetes mellitus and dialysis treatment were reported by the patients´ nephrologists. Blood pressure was measured in sitting position. Body mass index was calculated as weight (kg) divided by height (m) squared. All laboratory measurements of the 4D-Study were performed centrally at the Department of Clinical Chemistry, University of Freiburg,

Germany. Adiponectin was measured in blood samples taken at study visit 3 (1 week before randomization) and visit 6 (6 months after randomization). If there was no sample available at visit 6 (n=20), a sample taken at the following study visit was chosen (visit 7: n=19; visit 11: n=1). Measurements of adiponectin were performed by enzyme linked immunosorbent assay (Human Adiponectin ELISA, BioVendor GmbH, Heidelberg, Germany). Inter-assay coefficients of variance were <5%. All blood samples were taken before the start of dialysis sessions and administration of drugs.

Statistical Analysis

The study population was divided into four groups, according to quartiles of the adiponectin levels at baseline. Continuous variables were expressed as mean with standard deviation or median with interquartile range (IQR) as appropriate, and categorical variables were expressed as percentages.

In general, associations of adiponectin with outcome were assessed by absolute (incidence) rates to reach the pre-specified endpoints, and by relative risks derived from Cox regression analyses, i.e. hazard ratios (HRs) and corresponding 95% confidence intervals. After identifying confounders according to epidemiologic criteria [53;54], multivariate Cox regression analyses were performed adjusting for age, gender, atorvastatin treatment, coronary artery disease, congestive heart failure, duration of type 2 diabetes mellitus, smoking status and systolic blood pressure.

In detail, the following analyses were performed. First, the association of baseline adiponectin with the pre-defined outcome measures of sudden death, stroke, MI, CVE and all-cause mortality was analyzed, both as continuous variable (logarithmically transformed because values were not normally distributed) and as categorical variable (baseline adiponectin quartiles).

Second, we investigated the role of BMI, NT-pro-BNP and CRP in the association of adiponectin with outcome. We therefore determined the correlation of adiponectin with BMI, NT-pro-BNP and CRP, and adjusted our analyses on adiponectin and outcome additionally and stepwise for these variables.

Third, to investigate the longitudinal variations in adiponectin, we calculated the relative change of adiponectin from baseline to the next available follow-up measurement, predominantly taken at 6 months (change in adiponectin = ratio $adiponectin_{f\text{-}up}$ / $adiponectin_{baseline}$). Patients were divided into tertiles according to the relative change in adiponectin. The association between the relative change of adiponectin and outcome was then assessed as continuous variable (log transformed), and as categorical variable using the tertiles. The observation period contributing for the Cox regression analyses thereby started from the day of the respective follow-up measurement, used to assess the change in adiponectin. Adjustments were made for baseline variables and additionally for longitudinal changes in BMI and NT-pro-BNP, its relations with adiponectin before being evaluated in correlation analyses.
To account for potential interaction by gender, we performed further analyses in strata of male and female patients.
In general, to compare adiponectin between the atorvastatin and placebo groups, the Wilcoxon rank-sum test was used, whereas the Wilcoxon signed-rank test was applied to compare baseline with post-baseline adiponectin levels. All p-values are reported two-sided. Analyses were performed using SPSS version 16.0.

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CHAPTER 6

Wasting is strongly associated with sudden cardiac death in hemodialysis patients

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Abstract

Background: Wasting is common in patients with end-stage renal disease (ESRD), and often accompanied by cardiovascular disease and inflammation. The cardiovascular risk profile meaningfully changes in ESRD, and little is known about the impact of wasting on specific clinical outcomes.

Objective: This study examined the effects of wasting on the various components of cardiovascular outcome, and on deaths due to infection in hemodialysis patients.

Design: Patients participating in the German Diabetes and Dialysis Study (4D Study) were categorized according to the presence or absence of wasting, defined by both a BMI ≤23 kg/m^2 and albumin level ≤3.8g/dl. By Cox regression analyses, the associations of wasting with sudden cardiac death (SCD; n=160), myocardial infarction (MI; n=200), combined cardiovascular events (CVE; n=469), and deaths due to infection (n=128) were investigated during 4 years of follow-up.

Results: Compared to the patients without wasting (n=1147), patients with wasting (n=108) had a 3 fold increased risk of SCD (HR 3.0, 95% CI 2.0-4.5), which hardly attenuated after multivariable adjustment (HR 2.5, 95% CI 1.6-3.8). There were trends for increased risks of stroke and deaths due to infection, while MI was not affected. The risk of CVE was significantly increased by 60% (HR_{adj} 1.6 (1.2-2.1)) and mainly explained by the effect of wasting on SCD.

Conclusions: Wasting was strongly associated with SCD, but not with MI in diabetic hemodialysis patients. Non-atherosclerotic cardiac disease potentially plays a major role to account for the increased CVE in patients with wasting, suggesting the need for novel treatment strategies.

Introduction

In advanced stages of chronic kidney disease (CKD), patients increasingly develop wasting. Wasting is a severe syndrome characterized by poor food intake, low muscle mass, inflammation and the development of comorbidities. It has also been referred to as the malnutrition-inflammation-atherosclerosis (MIA) or malnutrition-inflammation-complex syndrome (MICS)(1;2). In general, patients suffering from wasting experience an excessive risk of cardiovascular disease and death(3-6). However, little is known about the impact of wasting on specific clinical events, accounting for the poor overall outcome.

The pattern and composition of mortality risk is changing in advanced stages of CKD. While myocardial infarction represents the most frequent cause of death in the general population, sudden cardiac death (SCD) is the major occuring event in dialysis patients(7), which as a single cause accounts for one quarter of all deaths(8;9). Risk factors playing an important role in the pathogenesis of SCD include increased levels of cytokines, endothelial dysfunction and oxidative stress. Cardiac and vascular damage are predisposing conditions for SCD, with heart failure and left ventricular hypertrophy playing a major role(10;11). Furthermore, endocrine disorders, including insulin resistance and glycemic state, meaningfully increase the incidence of SCD, but are not associated with myocardial infarction(12). Similarly, all these conditions have been found important implications in the wasting syndrome(2;6). It may therefore be hypothesized that wasting is specifically associated with SCD rather than myocardial infarction in hemodialysis patients.

The concurrent presence of inflammation in the wasting syndrome has often been demonstrated. Furthermore, the immune system in patients with wasting has been shown compromised(13-15). Whether this translates into higher rates of inflammatory and infectious deaths, remains largely unknown however.

To that end, we investigated the association of wasting as represented by low body mass index (BMI) and hypoalbuminemia with the risk of SCD, myocardial infarction, stroke, combined cardiovascular events (CVE) and death due to infection in hemodialysis patients. We analyzed data from the German Diabetes

Dialysis Study (4D Study: Die Deutsche Diabetes Dialyse Studie), which evaluated atorvastatin in 1255 patients with type 2 diabetes mellitus on maintenance hemodialysis(9).

Subjects and methods

Study Design and Participants

As described previously(16), the 4D study was a prospective randomized controlled trial including 1255 patients with type 2 diabetes mellitus, age 18 – 80 years, and on hemodialysis for less than 2 years. Between March 1998 and October 2002, patients were recruited in 178 dialysis centres in Germany. After a run-in period of 4 weeks, patients were randomly assigned to double-blinded treatment with either 20mg atorvastatin (n=619) or placebo (n=636) once daily. Study visits took place three times before randomization (visit 1-3), at randomization (visit 4), and at four weeks (visit 5) and every six months (visit 6 etc.) after randomization until the date of death, censoring, or end of the study in March 2004. The primary endpoint of the 4D study was defined as a composite of death from cardiac causes, stroke and myocardial infarction, whichever occurred first. Death from cardiac causes comprised fatal myocardial infarction (death within 28 days after a myocardial infarction), SCD, death due to congestive heart failure, death due to coronary heart disease during or within 28 days after an intervention, and all other deaths ascribed to coronary heart disease. Patients who died unexpectedly and did not present with a potassium level greater than 7.5 mmol per liter before the start of the three most recent sessions of hemodialysis were considered to have had sudden death from cardiac causes. 4D Study endpoints were centrally adjudicated by three members of the endpoint committee blinded to study treatment and according to pre-defined criteria.

For the present analysis, SCD, myocardial infarction (including fatal and non-fatal events), stroke (fatal and non-fatal), the primary endpoint of combined CVE and death due to infection were chosen to be separate outcome measures. An additional endpoint comprised all combined CVE except SCD. The study was

conducted in accordance with the ethical standards, approved by the medical ethical committee, and all patients gave their written informed consent before inclusion.

Data collection

Information on age, gender and smoking status was obtained through patient interviews. Smoking status was classified as never, former or current. Comorbidities, including the presence of coronary artery disease and congestive heart failure, as well as the duration of diabetes mellitus and dialysis treatment were reported by the patients´ nephrologist. Blood pressure was measured in sitting position. Body mass index was calculated as weight (kg) divided by height (m) squared. All laboratory measurements of the 4D-Study were performed centrally at the Department of Clinical Chemistry, University of Freiburg, Germany. Concentrations of serum albumin, C-reactive protein, hemoglobin, calcium, phosphate, LDL, HDL and total cholesterol were measured in blood samples taken at baseline at study visit 3 (1 week before randomization). Albumin was measured photometrically using the anionic dye bromcresol green on a Roche Modular clinical chemistry analyser (Roche Diagnostics, Mannheim, Germany). Calibrators and quality control materials were also obtained by Roche Diagnostics. Inter-assay coefficients of variance were <5%. All blood samples were taken before the start of dialysis sessions and administration of drugs.

Statistical Analysis

The study population was categorized according to the presence or absence of wasting. Since wasting represents an unspecific condition with a number of contributing factors, and due to the absence of guidelines for classification(17), we used BMI and serum albumin concentration as commonly available markers in line with suggestions recently been given by an expert panel. Wasting was defined 1) by a BMI ≤23kg/m^2, 2) by albumin levels ≤3.8g/dl, and 3) the combination of both a BMI ≤23kg/m^2 and albumin ≤3.8g/dl. The latter represented the main categorization used for the present study. Continuous variables were expressed as mean with standard deviation or median with interquartile range (IQR) as appropriate, and categorical variables were expressed as percentages.

First, we assessed the association of wasting as defined by the combination of both a low BMI and low albumin with SCD. Absolute (incidence) rates were calculated as the number of events occuring per 100 person years of follow-up. Kaplan-Meier curves were performed in each group, and the log rank test was computed to compare the curves. By Cox regression analyses, hazard ratios and corresponding 95% confidence intervals were calculated, and adjusted for the confounders age, sex, atorvastatin treatment, duration of dialysis, smoking, coronary artery disease, congestive heart failure, systolic blood pressure, levels of LDL-cholesterol, haemoglobin, C-reactive protein, HbA1c, calcium and phosphate. Second, we performed additional Cox regression analyses with inclusion of potential intermediate variables, including left ventricular hypertrophy, levels of NT-pro-BNP. Third, we determined the relation of wasting with further cardiac and vascular outcomes, i.e. myocardial infarction, stroke, and cardiovascular events combined (CVE). Fourth, wasting was investigated regarding the risk of death due to infections. For the robustness of our results, wasting was furthermore analysed using the separate definitions by low BMI, or by low albumin. Finally, we investigated potential interaction of wasting with atorvastatin in the effect on the specified endpoints, and repeated all analyses stratified by treatment. All p-values are reported two-sided. Analyses were performed using SPSS version 16.0.

Results

Patient characteristics

Between March 1998 and October 2002, a total of 1255 patients were included into the 4D study, and had their BMI and albumin levels assessed at baseline. The mean follow-up period was 3.96 years (median 4.0 years) on atorvastatin and 3.91 years (median 4.08 years) on placebo. During follow-up, 469 patients reached the primary endpoint of combined CVE. A total of 617 patients died, of whom 160 patients died of SCD, and 128 patients died of infection. Furthermore, 200 patients experienced a fatal or non-fatal myocardial infarction, and 103 patients experienced a stroke (fatal or non-fatal).

In the study population (n=1255), the mean (SD) age was 65.7 (8.3) years, and 54% of the patients were male. A total of 189 patients had a BMI ≤ 23kg/m^2, and 1066 had a BMI > 23kg/m^2. Albumin levels were ≤3.8g/dl in 668 patients, and >3.8g/dl in 587 patients. According to our main classification of wasting based on the combination of both parameters, a total of 108 patients were categorized as suffering from wasting (i.e. having a BMI ≤ 23kg/m^2 and albumin ≤3.8g/dl). The baseline patient characteristics are shown in Table 1. Patients with wasting had a higher burden of coronary artery disease, congestive heart failure, left ventricular hypertrophy (LVH), and markedly higher levels of N-terminal-pro-B-type natriuretic peptide (NT-pro-BNP) compared to patients without wasting.

Wasting and the risk of sudden cardiac death

In the whole study group, the incidence rate of SCD was 4.5 events/100 person years (py). Compared to patients without wasting, who had an incidence rate of 4.0/100py, patients with wasting had a highly increased incidence rate of 11.8/100py (Table 2 and Figure 1A).

By Cox regression analyses, the unadjusted hazard to experience SCD was 3 fold higher in patients with wasting as compared to those without wasting (hazard ratio (HR) 3.0, 95% confidence interval (CI) 2.0-4.5). This association remained strong after adjustment for confounders (HR (95% CI) 2.5 (1.6-3.8)). To evaluate potential intermediate variables, we additionally included LVH, by which the adjusted hazard ratio for SCD was further reduced to 2.4 (1.5-3.7). When log NT-pro-BNP was added to the model, the association was attenuated to a hazard ratio of 2.1 (1.4-3.3). Addition of both potential intermediate variables to the same model resulted in a hazard ratio of 2.1 (1.3-3.3).

Findings were similar, when wasting was analysed using separate definitions by a low BMI, or by a low albumin in additional analyses. The hazard of SCD was more than 2 fold increased in patients with a BMI ≤ 23kg/m^2, as compared to those with a BMI >23kg/m^2. Similarly, patients with an albumin ≤ 3.8g/dl had a 80% higher hazard of SCD compared to patients with an albumin >3.8g/dl. The associations persisted after adjustment for confounders (Table 2).

Table 1: Patient characteristics according to the presence or absence of wasting at baseline; study population n=1255

Characteristic	wasting		BMI (kg/m²)		Albumin (g/dl)	
	BMI ≤ 23 & albumin ≤3.8 (n=108)	BMI > 23, albumin > 3.8 (n=1147)	≤23 (n=189)	>23 (n=1066)	≤3.8 (n=668)	>3.8 (n=587)
Age *years*	68 (8)	65 (8)	67 (8)	65 (8)	67 (8)	65 (8)
Gender *% male*	52	54	58	53	48	61
BMI *kg/m²*	21.1 (1.5)	28.2 (4.6)	21.1 (1.5)	28.7 (4.3)	27.7 (5.1)	27.4 (4.5)
Atorvastatin treatment *%*	53	49	47	50	50	49
Systolic BP *mmHg*	147 (22)	145 (22)	149 (23)	145 (22)	145 (23)	146 (21)
Diastolic BP *mmHg*	74 (11)	76 (11)	75 (12)	76 (11)	75 (11)	76 (11)
Smoker / Ex-smoker *%*	48	40	51	39	36	45
Duration of diabetes *years*	16.9 (7.8)	18.2 (8.9)	17.3 (8.2)	18.3 (8.9)	18.2 (8.8)	18.0 (8.8)
Time on dialysis *months*	6.5 (5.5)	8.4 (7.0)	7.8 (6.5)	8.4 (7.0)	7.3 (6.3)	9.4 (7.3)
History of						
CAD *%*	42	28	37	28	30	28
CHF *%*	53	34	44	34	39	31
arrhythmia *%*	21	19	21	18	19	19
PVD *%*	57	44	53	43	47	42
LVH *%*	28	11	29	10	13	12
Laboratory parameters						
Total cholesterol *mg/dl*	203 (37)	221 (43)	211 (38)	221 (43)	216 (43)	223 (42)
LDL cholesterol *mg/dl*	116 (28)	126 (30)	123 (30)	126 (30)	124 (31)	128 (29)
HDL cholesterol *mg/dl*	39 (15)	36 (13)	40 (16)	35 (12)	36 (13)	37 (14)
Albumin *g/dl*	3.55 (0.25)	3.84 (0.29)	3.77 (0.33)	3.83 (0.30)	3.59 (0.20)	4.07 (0.17)
Hemoglobin *g/dl*	10.7 (1.3)	10.9 (1.4)	10.9 (1.4)	10.9 (1.4)	10.7 (1.4)	11.1 (1.3)
Calcium *mmol/l*	2.3 (0.2)	2.3 (0.2)	2.3 (0.2)	2.3 (0.3)	2.3 (0.2)	2.3 (0.2)
Phosphate *mmol/l*	5.79 (1.83)	6.05 (1.59)	5.96 (1.77)	6.04 (1.58)	5.93 (1.66)	6.14 (1.54)
HbA1c *%*	6.5 (1.2)	6.7 (1.3)	6.5 (1.2)	6.8 (1.3)	6.8 (1.3)	6.6 (1.2)
Adiponectin *mg/l*	20.3 (15.5)	15.9 (9.0)	20.4 (13.7)	15.5 (8.7)	16.5 (10.8)	16.0 (8.6)
NT-pro-BNP *pg/ml*	6941 (2043-22302)	3143 (1373-8375)	6403 (2145-21800)	2977 (1336-7711)	3690 (1570-10220)	2905 (1290-8049)
C-reactive protein *mg/l*	5.5 (1.7-16.2)	4.9 (2.4-12.0)	4.1 (1.7-12.0)	5.1 (2.5-12.5)	6.4 (2.8-15.8)	3.9 (2.0-8.4)

Values are presented as means (SD) or median (interquartile range) or %.
Abbreviations: BP, blood pressure; HbA1c, glycated hemoglobin A1c;
CAD, coronary artery disease; CHF, congestive heart failure; PVD, peripheral vascular disease, LVH, left ventricular hypertrophy
LDL, low density lipoprotein; HDL, high density lipoprotein; NT-pro-BNP, N-terminal-pro-B-type-natriuretic peptide

Table 2: Absolute incidence rates, and hazard ratios with 95% confidence intervals (HR, 95% CI) for sudden cardiac death, myocardial infarction, the primary endpoint of combined cardiovascular events, and the primary endpoint except for sudden cardiac death according to the presence or absence of wasting at baseline; n=1255

Outcome	wasting		BMI		albumin	
	BMI≤23 & albumin ≤3.8 (n=108)	BMI>23 and/or albumin >3.8 (n=1147)	≤23 (n=189)	>23 (n=1066)	≤3.8 (n=668)	>3.8 (n=587)
Cardiovascular events						
Incidence rate / 100py	23.8	13.6	19.6	13.4	16.3	12.5
Crude HR (95% CI)	1.8 (1.3-2.4)	1	1.5 (1.2-1.9)	1	1.4 (1.1-1.6)	1
Adj.[1] HR (95% CI)	1.6 (1.2-2.1)	1	1.4 (1.1-1.8)	1	1.2 (1.01-1.5)	1
Sudden death						
Incidence rate / 100py	11.8	4.0	8.2	3.9	5.8	3.3
Crude HR (95% CI)	3.0 (2.0-4.5)	1	2.1 (1.5-3.0)	1	1.8 (1.3-2.5)	1
Adj.[1] HR (95% CI)	2.5 (1.6-3.8)	1	1.9 (1.3-2.8)	1	1.6 (1.1-2.2)	1
Stroke						
Incidence rate / 100py	4.3	2.9	3.2	2.9	4.1	2.0
Crude HR (95% CI)	1.5 (0.8-2.9)	1	1.1 (0.6-1.9)	1	2.2 (1.5-3.4)	1
Adj.[1] HR (95% CI)	1.6 (0.8-3.2)	1	1.2 (0.7-2.2)	1	2.0 (1.3-3.1)	1
Myocardial infarction						
Incidence rate / 100py	6.2	5.9	4.8	6.1	5.8	6.1
Crude HR (95% CI)	1.1 (0.6-1.9)	1	0.8 (0.5-1.2)	1	1.0 (0.7-1.3)	1
Adj.[1] HR (95% CI)	1.0 (0.5-1.7)	1	0.8 (0.5-1.2)	1	0.9 (0.7-1.3)	1
Cardiovascular events except sudden death						
Incidence rate / 100py	11.2	9.3	10.9	9.1	10	8.9
Crude HR (95% CI)	1.2 (0.8-1.9)	1	1.2 (0.9-1.6)	1	1.2 (0.95-1.5)	1
Adj.[1] HR (95% CI)	1.2 (0.8-1.8)	1	1.2 (0.9-1.7)	1	1.1 (0.9-1.4)	1
Death due to infection						
Incidence rate / 100py	6.3	3.4	4.8	3.4	4.5	2.8
Crude HR (95% CI)	2.0 (1.2-3.5)	1	1.5 (0.9-2.3)	1	1.8 (1.3-2.6)	1
Adj.[1] HR (95% CI)	1.5 (0.8-2.6)	1	1.2 (0.8-2.0)	1	1.7 (1.1-2.5)	1

[1] model 1: adjusted for age, sex, atorvastatin treatment, systolic blood pressure, coronary artery disease, congestive heart failure, smoking, duration of dialysis, levels of LDL cholesterol, hemoglobin, C-reactive protein, HbA1c, calcium and phosphate

Wasting and risk of myocardial infarction, stroke and combined cardiovascular events

In contrast, no association of wasting with myocardial infarction was found. The incidence rate of myocardial infarction was 5.9/100 py in the whole study group, and not markedly different in the presence (6.2/100py) or absence of wasting (5.9/100py) (Figure 1B). The crude hazard ratio for myocardial infarction associated with wasting was 1.1 (0.6-1.9). Similarly, the multivariable adjusted hazard ratio was 1.0 (0.5-1.7). When non-fatal and fatal MI were analyzed separately, the results were confirmed showing no association between wasting and myocardial infarction. The results were furthermore consistent throughout all definitions of wasting used.

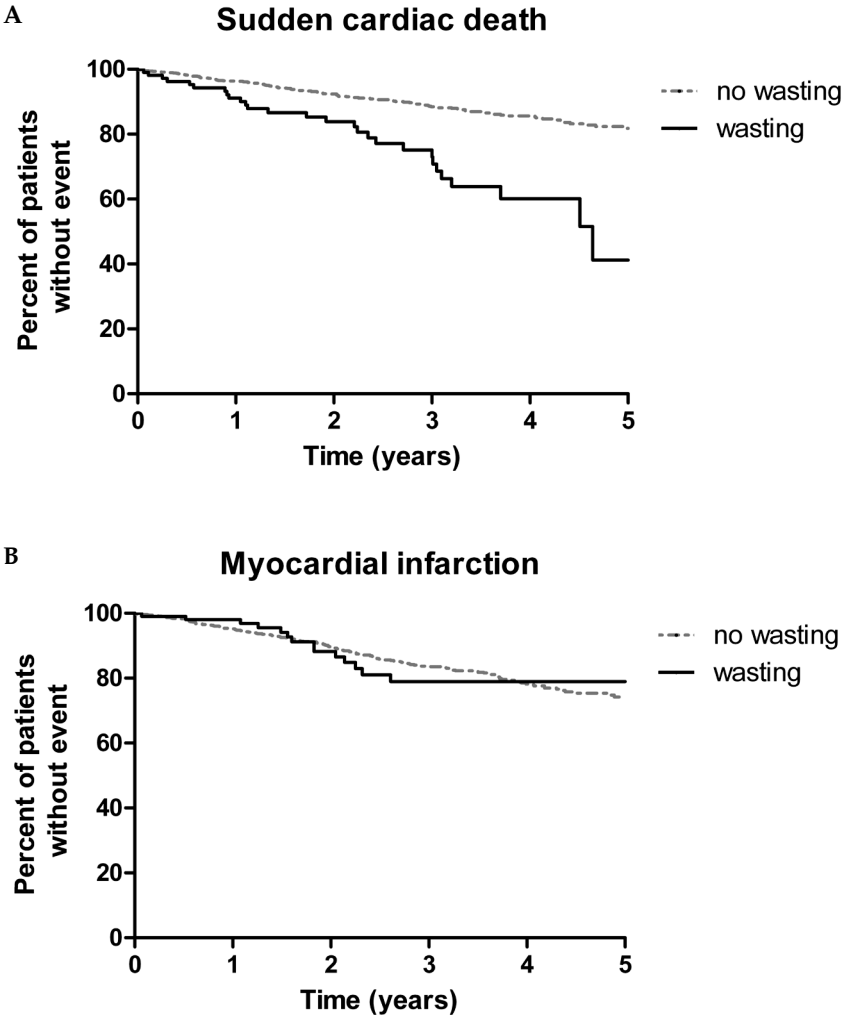
The presence of wasting affected the risk of stroke by trend, but not significantly (Table 2). In the additional analyses, low levels of albumin (below 3.8 g/dl) were associated with a twofold increased risk of stroke, while a low BMI (<23 kg/m^2) irrespective of the inflammatory status had no influence.

The endpoint of combined CVE was markedly increased in patients suffering from wasting (Table 2 and Figure 1C). Affected patients had an adjusted 60% higher risk of combined CVE as compared to patients without wasting. Additional analyses revealed that this relation was mainly explained by the impact of wasting on SCD, since no association was found for combined CVE except SCD. The findings were consistent in the additional analyses using BMI or albumin separately for the definition of wasting.

Wasting and risk of death due to infection

Deaths due to infection were increased in patients suffering from the wasting syndrome. They had a twofold increased risk compared to non-affected patients, but this association was significantly attenuated after multivariable adjustment. Similarly to what was seen for strokes, the inflammatory component of the wasting syndrome as represented by a low albumin appeared to strongly increase the risk, while a low BMI separately had no influence.

To strengthen our results, we eliminated any potential influence by atorvastatin treatment and repeated all analyses in the placebo group only. The results were similar, indicating no effect modification and supporting the use of the complete data.



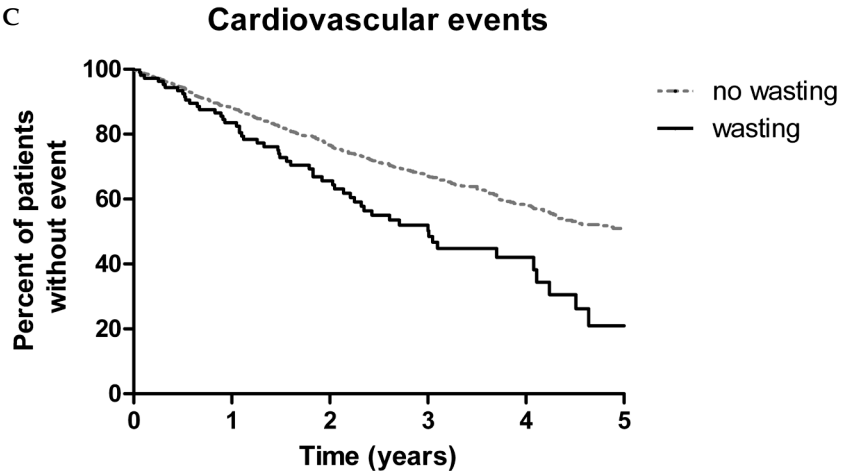


Figure 1 A-C: Kaplan-Meier curves for the time to A) sudden cardiac death, B) myocardial infarction, C) the primary endpoint of combined cardiovascular events in subgroups of patients according to the presence or absence of wasting.

Discussion

We investigated the effect of wasting on particular components of cardiovascular outcome, and death due to infection in a large prospective cohort of hemodialysis patients with type 2 diabetes mellitus. Within 4 years of follow-up, the presence of wasting was significantly associated with a three-fold increased risk of sudden cardiac death, and by trend with stroke and deaths due to infection. In contrast, the presence of wasting did not affect the risk of myocardial infarction. Furthermore, the strong impact of wasting on sudden cardiac death mainly explained the increased hazard of combined cardiovascular events in the wasting syndrome, being 60% higher in patients with wasting as compared to patients without wasting.

Wasting is common in patients with CKD and becoming most prevalent in end-stage renal disease. Estimated 18-75% of dialysis patients are suggested to show evidence of the wasting syndrome, depending on the measured parameters,

age and comorbidities(18). Wasting is characterized by a low body weight, low protein-energy stores, muscle loss and low concentrations of albumin and other proteins(19). In dialysis patients, many factors may affect nutritional status and the development of wasting, such as uremia induced low appetite, hormonal disturbances, metabolic acidosis, oxidative stress and comorbidities(17;20). In the context of its multifactorial character, wasting has also been referred to as „malnutrition-inflammation-complex (MICS)" syndrome, „malnutrition-inflammation-atherosclerosis (MIA)" syndrome, or „malnutrition-inflammation-cachexia" syndrome (1;2). The single contributions of the wasting components are difficult to assess, considering the variety of factors and their complex interactions. Pathways and specific outcome effects are therefore crucial to get further insights into which wasting factors and wasting mechanisms may -relatively to others- be most important to serve as promising targets for novel intervention strategies. Little is known so far on specific outcomes associated with wasting, and our study is the first to address this question in a large cohort of dialysis patients who experienced a high incidence of pre-specified and centrally adjudicated endpoints. Our finding that wasting was strongly associated with SCD is supported by previous research outlined in the literature. One study investigating risk factors for SCD in dialysis patients found markers of inflammation including albumin, hsCRP and IL-6 of major importance (21). Inflammation is commonly involved in the wasting syndrome and may increase the risk of sudden death via the development of premature atherosclerosis and cytokine-induced plaque instability(22), or by direct effects on the myocardium and electrical conduction system(23). Cytokines are also involved in the modulation of ion channel function and the generation of arrhythmias (24;25), as well as in the aggravation of sympathetic tone, leading to tachycardia and cardiac electrical instability. In our study, patients with wasting exhibited to a considerable extent left ventricular hypertrophy and increased levels of adiponectin and NT-pro-BNP. These factors are known to be strong predictors of SCD and may, at least in part, represent wasting related to structural changes in the heart(11;26-28). In this context, our study may offer novel treatment strategies for patients with wasting. Given that current treatments are limited, new therapeutic options are urgently needed. Our

finding that patients with the wasting syndrome are at increased risk of SCD suggests that the patients may be considered for particular treatments to prevent SCD, including ß-blocker or implantable cardioverter defibrillator therapy (29;30). Given that wasting is considered to contribute to atherosclerosis (6;31), our finding of no relation between wasting and the risk of myocardial infarction came rather as a surprise. Despite a significant burden of coronary artery disease in patients with wasting (about 40% at baseline), wasting did not translate into a higher risk of myocardial infarction as a major ischemic complication. Stroke risk, which is less related to atherosclerosis compared to myocardial infarction showed a remarkable trend for an association with wasting. It is of major interest, that the increased cardiovascular events seen in patients with wasting are mainly explained by the effect of wasting on SCD. Therefore, although a lot of attention is paid to atherosclerosis in the wasting syndrome, other factors like structural heart disease may be even more important, resulting in a relative excess of SCD. In addition, our findings may be particularly important concerning the paradoxical associations often seen for metabolic or nutritional status and outcome in dialysis patients. By now, explanations for the survival benefits largely seen for dialysis patients with obesity included time effects (short follow-up), age, differences among populations and reverse causation (32;33). While obesity is known to be a risk factor for myocardial infarction (34), our study shows that the latter may potentially be overruled by excessive incidences of SCD in patients with beginning or obvious wasting. This in turn may lead to a relative underrepresentation of (e.g. obesity induced) myocardial infarctions.

Finally, our results showing increased deaths due to infection in patients with wasting strengthen the notion that inflammation meaningfully impacts and contributes to the patients´ mortality. In line with findings on compromised immune status and higher rates of infections (13-15), wasting continues to translate into poor infection-specific outcome.

Potential limitations of the study need to be acknowledged. It was a post-hoc analysis within a selected cohort of German patients with type 2 diabetes mellitus on hemodialysis. Therefore, the relationship between the presence of wasting and risk may not be generalisable to other patient populations. Yet, it may be assumed

that the results also hold true for non-diabetic hemodialysis patients, similarly suffering from wasting. The group of patients with wasting, defined by using a BMI ≤ 23kg/m^2 was relatively small, and results may become stronger with larger numbers of patients in future studies. The specific outcomes, among which SCD, and their association with wasting to be analyzed was the main strength of this study. In this context, the long-term follow-up, adequate sample size and high incidence of pre-specified and centrally adjudicated endpoints are further to be mentioned.

In conclusion, the presence of wasting was strongly associated with sudden cardiac death, but not with myocardial infarction in diabetic hemodialysis patients. Non-atherosclerotic cardiovascular disease potentially plays a major role to account for the increased cardiovascular events in patients with wasting. In addition to current treatment, patients with the wasting syndrome should be targeted in the prevention of sudden cardiac death. Apart from regular examinations, patients with wasting may be considered for further treatments including ß-blocker or implantable cardioverter defibrillator therapy(29;30).

Acknowledgements

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CHAPTER 7

The association between parathyroid hormone and mortality in dialysis patients is modified by wasting

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Abstract

Background: The association between parathyroid hormone (PTH) level and mortality in dialysis patients is controversial. We hypothesized that wasting, a common condition potentially related to adynamic bone disease, modifies the association of PTH with mortality and cardiovascular events (CVE), respectively.

Methods: We analyzed data from 1255 diabetic hemodialysis patients, participating in the German Diabetes and Dialysis Study between 1998 and 2004. Patients were stratified by the presence or absence of wasting (albumin ≤3.8 vs albumin >3.8 g/dl; BMI ≤23 vs BMI >23 kg/m^2). Using Cox regression analyses, we calculated the risks of 1) all-cause mortality and 2) CVE according to baseline PTH levels. All analyses were adjusted for age, sex, atorvastatin treatment, duration of dialysis, comorbidity, HbA1c, phosphate, calcium, blood pressure, hemoglobin and C-reactive protein.

Results: Patients had a mean age of 66±8 years and 54% were male. Among patients without wasting (albumin >3.8g/dl, n=586), the risks of death and CVE during 4 years follow-up significantly increased by 23% and 20% per unit increase in logPTH. Patients in the highest PTH tertile had a 74% higher risk of death (HR_{adj} 1.74, 95%CI 1.27-2.40) and a 49% higher risk of CVE (HR_{adj} 1.49, 95%CI 1.05-2.11) compared to patients in the lowest PTH tertile. In contrast, no effect was found in patients with wasting. Accordingly, additional analyses in strata of BMI showed that PTH significantly impacted on death and CVE ($HR(logPTH)_{adj}$ 1.15 and 1.14, respectively) only in patients without, but not in patients with wasting.

Conclusions: Wasting modifies the association of PTH with adverse outcomes in diabetic dialysis patients. High PTH levels are of concern in the patients without wasting, while the effect of PTH on mortality is nullified in the patients with wasting.

Introduction

Parathyroid hormone (PTH) modulates calcium and phosphate homeostasis (1-3). Disorders with excess PTH secretion such as primary and secondary hyperparathyroidism, can lead to bone disease and vascular calcification. Accordingly, a high PTH was found to be associated with high risks of cardiovascular events and mortality in the general population (4;5). In patients with chronic kidney disease (CKD), however, the association is less clear: similar results were reported for patients with moderate to severe CKD (6), but studies in dialysis patients are controversial. While some studies indicated higher risks of death with increased PTH levels (7-10), other investigations either found no association (11), or a low PTH being related to a greater risk of adverse outcomes (12-14).

In CKD and end-stage renal disease (ESRD), wasting is a common problem, representing a severe and complex process of muscle loss, poor food intake, inflammation and the development of comorbidities (15;16). Patients with diabetes mellitus are especially affected by the syndrome, which was shown to be associated with a high mortality (17). Wasting was furthermore found to be related to adynamic bone disease, which is a severe state of renal osteodystrophy, and characterized by low levels of PTH, lack of bone cell activity, and a low bone turnover (18;19). This, in turn, was reported to be linked to increased fracture risks and higher cardiovascular complications such as aortic stiffening and arterial calcification (20;21).

Given the interrelations of wasting with parathyroid hormone metabolism, we hypothesized that wasting modifies the association of PTH with adverse outcomes in long-term dialysis patients with diabetes mellitus. We therefore assessed the effect of PTH on all-cause mortality and cardiovascular events in 1255 diabetic hemodialysis patients participating in the 4D study (the German Diabetes and Dialysis Study), stratified by the presence of wasting.

Subjects and methods

Study Design and Participants

The 4D study methodology has previously been reported in detail (22). Briefly, the 4D study was a prospective randomized controlled trial including 1255 patients with type 2 diabetes mellitus, 18 – 80 years, and previous duration of hemodialysis of less than 2 years. Between March 1998 and October 2002, patients were recruited in 178 participating dialysis centres in Germany. After a run-in period of 4 weeks, patients were randomly assigned to double-blind treatment with either 20mg Atorvastatin (n=619) or placebo (n=636) once daily. Study visits took place three times before randomization (visit 1-3), at randomization (visit 4), and at four weeks (visit 5) and every six months (visit 6 etc.) after randomization until the date of death, censoring, or end of the study in March 2004. The primary endpoint of the 4D study was defined as a composite of death from cardiac causes, nonfatal myocardial infarction, and stroke, whichever occurred first. Secondary endpoints included death from all causes, sudden death, all myocardial infarctions and stroke. 4D Study endpoints were centrally adjudicated by three members of the endpoint committee blinded to study treatment and according to pre-defined criteria (23).

For the present analysis, all-cause mortality and combined cardiovascular events (CVE) including cardiac death, myocardial infarction and stroke, were chosen to be separate outcome measures and were based on the primary judgement of the endpoint committee during the 4D Study. The study was approved by the medical ethical committee, and all patients gave their written informed consent before inclusion.

Data collection

Information on age, gender and smoking status was obtained through patient interviews. Smoking status was classified as never, former or current. Comorbidities, including the presence of coronary artery disease and congestive heart failure, as well as the duration of diabetes mellitus and dialysis treatment were reported by the patients´ nephrologists. Blood pressure was measured in

sitting position. Body mass index was calculated as weight (kg) divided by height (m) squared. All laboratory measurements of the 4D-Study were performed centrally at the Department of Clinical Chemistry, University of Freiburg, Germany. Concentrations of serum albumin, C-reactive protein, hemoglobin, calcium, phosphate, LDL-cholesterol, glycated hemoglobin A1c and intact parathyroid hormone were measured in blood samples taken at baseline at study visit 3 (1 week before randomization). Measurements of intact PTH were performed by the PTH STAT test on an Elecsys 2010 analyzer (Roche Diagnostics Mannheim, Germany). Albumin was measured photometrically using the anionic dye bromcresol green on a Roche Modular clinical chemistry analyzer (Roche Diagnostics Mannheim, FRG). Calibrators and quality control materials were also obtained by Roche Diagnostics. Inter-assay coefficients of variance were <5%. All blood samples were taken before the start of dialysis sessions and administration of drugs.

Statistical Analysis

The study population was divided into two groups, according to the presence or absence of wasting. Since wasting represents an unspecific condition with a number of contributing factors, and due to the absence of guidelines for classification, we used albumin and furthermore BMI as commonly available markers in line with suggestions recently being given by an expert panel (15). Therefore, wasting was defined by albumin levels ≤3.8g/dl, and in additional analyses by a BMI ≤ 23 kg/m^2. Continuous variables were expressed as mean with standard deviation or median with interquartile range (IQR) as appropriate, and categorical variables were expressed as percentages.

Associations of parathyroid hormone with all-cause mortality and CVE were assessed by absolute (incidence) rates, and by relative risks derived from Cox regression analyses, i.e. hazard ratios (HRs) and corresponding 95% confidence intervals. The Cox regression analyses were adjusted for the confounders age, sex, atorvastatin treatment, duration of hemodialysis, coronary artery disease, congestive heart failure, peripheral vascular disease, systolic blood pressure, glycemic control as represented by HbA1c, and levels of calcium, phosphate, hemoglobin and C-reactive protein.

In detail, the following analyses were performed. First, the study population was divided into tertiles according to the PTH levels at baseline. Associations of the PTH tertiles with mortality and CVE were assessed within the categories of the presence or absence of wasting (albumin ≤3.8 vs albumin >3.8 g/dl). Second, baseline PTH was analyzed as a continuous variable (log transformed) with regard to the adverse outcomes. Third, in order to test the robustness of our results, additional analyses were performed in strata of BMI as a further marker to classify the presence (BMI ≤23 kg/m^2) of absence (BMI >23 kg/m^2) of the wasting syndrome. Within the BMI groups, analyses on the association of PTH with mortality and CVE were performed according to the description given above. Similarly, PTH was investigated both as continuous and as categorical variable (tertiles).
All p-values are reported two-sided. Analyses were performed using SPSS version 16.0.

Results

Patient characteristics

Between March 1998 and October 2002, a total of 1255 patients entered the 4D study. Of those, 1248 patients had a baseline PTH measurement. The mean follow-up period was 3.96 years (median 4.0 years) on atorvastatin and 3.91 years (median 4.08 years) on placebo. During follow-up, 617 patients died. Furthermore, 469 patients reached the endpoint of combined cardiovascular events.
In the study population (n=1255), the mean age was 65.7 ± 8.3 years, and 54% of the patients were male. The mean baseline PTH level was 102 ± 119 pg/mL (median PTH 70 pg/mL; interquartile range 36 - 127 pg/mL). No significant differences were noted between the atorvastatin and placebo groups. The baseline patient characteristics are shown in Table 1.

Table 1: Baseline patient characteristics, presented according to the presence / absence of wasting, defined by albumin ≤ / > 3.8 g/dl; study population n=1255

Characteristic	Albumin (g/dl) ≤ 3.8 (wasting, n=668)	Albumin (g/dl) > 3.8 (no wasting, n=587)
Age *years*	67 (8)	65 (8)
Gender *% men*	47.5	61.3
Atorvastatin treatment *%*	50.3	51.1
Systolic BP *mmHg*	145 (23)	146 (21)
Smoker / Ex-smoker *%*	36.1	45.3
BMI kg/m^2	27.7 (5.1)	27.4 (4.5)
Duration of diabetes *years*	18.2 (8.8)	18.0 (8.8)
Time on dialysis *months*	7.3 (6.3)	9.4 (7.3)
History of		
CAD *%*	30.2	28.4
CHF *%*	38.6	31.7
PVD *%*	46.9	42.1
Laboratory parameters		
PTH *pg/mL*	90 (98)	116 (138)
LDL cholesterol *mg/dL*	124 (31)	128 (29)
Hemoglobin *g/dL*	10.7 (1.4)	11.1 (1.3)
HbA1c *%*	6.8 (1.3)	6.7 (1.2)
C-reactive protein *mg/L*	13.9 (24.0)	7.6 (10.3)
Calcium *mmol/L*	2.3 (0.2)	2.3 (0.2)
Phosphate *mmol/L*	5.9 (1.7)	6.1 (1.5)

Values are presented as means (SD) or *%*.
To convert serum albumin in g/dL to g/L, multiply by 10; PTH in pg/mL to ng/L, multiply by 1; LDL cholesterol in mg/dl to mmol/L, multiply by 0.02586; hemoglobin in g/dL to mmol/L, multiply by 0.62;
Abbreviations: PTH = Parathyroid hormone; BP = blood pressure;
BMI = body mass index; CAD = coronary artery disease,
CHF = congestive heart failure; PVD = peripheral vascular disease;
LDL = low density lipoprotein; HbA1c = hemoglobin A1c;

PTH, wasting and all-cause mortality

Within the whole study population, the mortality risks for patients in the second or third PTH tertile were not materially different compared to the reference of patients in the lowest PTH tertile (adjusted $HR_{Tertile2}$ 1.17, 95% CI 0.95-1.44; adjusted $HR_{Tertile3}$ 1.19, 95% CI 0.96-1.47). Investigating the hypothesis of effect modification by wasting, the analyses on PTH and mortality were stratified by wasting as

defined by baseline albumin levels. In patients without wasting (albumin >3.8g/dl, n=586), the absolute mortality rate during 4 years follow-up was 15 per 100 person years (py). All-cause mortality increased stepwise with higher PTH levels: it was 11/100 py for patients in the lowest tertile with a PTH ≤46.3pg/mL, 15/100py in patients with a PTH between 46.3 and 106 pg/mL (2nd tertile), and 17/100py in patients with a PTH >106 pg/mL (3rd tertile) (Figure 1B and Table 2). In unadjusted Cox regression analyses using PTH as a continuous variable, the relative risk of death increased significantly by 17% per unit increase in log transformed PTH (HR 1.17, 95% CI 1.04-1.31). The association was even stronger in multivariate analyses with a 23% increase in mortality per unit increase in log transformed PTH (HR 1.23, 95% CI 1.09-1.39). With multivariate Cox regression models using PTH as a categorical variable, patients in the second PTH tertile had a 37% higher risk of death compared to those in the lowest tertile. Patients with highest PTH levels (3rd tertile) had the highest mortality, being significantly increased by 74% as compared to patients of the lowest PTH tertile (Table 2). Similar results were found in additional analyses using a BMI > 23kg/m^2 to define the absence of wasting. All-cause mortality significantly rose by 15% per unit increase in log transformed PTH in patients without wasting. In contrast, in patients with the disease state expectedly showing a high incidence of death (absolute mortality rate of 21 per 100 py), no association of PTH with mortality was found: Death rates were similar across the tertiles of PTH, both in the analyses using albumin and BMI for the definition of wasting.

PTH, wasting and the risk of cardiovascular events

The absolute rate for cardiovascular events was 21 per 100 person years in patients with wasting, and 15/100py in patients without wasting as defined by baseline albumin levels.

When relative risks were investigated, crude Cox regression analyses in patients without the wasting syndrome (albumin > 3.8 g/dl) revealed a significant 19% increase in CVE (HR 1.19, 95% CI 1.04-1.36) per unit increase in log transformed PTH. The strong association persisted after multivariable adjustments (HR 1.20, 95% CI 1.04-1.38).

Table 2: Parathyroid hormone and risk of all cause mortality and CVE in strata of wasting, as defined by albumin levels ≤ / >3.8g/dl; n=1248

Variables	Wasting Albumin ≤3.8g/dl N=662			No Wasting Albumin >3.8g/dl N=586		
	PTH tertiles			PTH tertiles		
	Tertile 1 PTH ≤ 46.3 pg/mL n=239	Tertile 2 PTH > 46.3 ≤ 106 pg/mL n=231	Tertile 3 PTH > 106 pg/mL n=192	Tertile 1 PTH ≤ 46.3 pg/mL n=177	Tertile 2 PTH > 46.3 ≤ 106 pg/mL n=185	Tertile 3 PTH > 106 pg/mL n=224
All-cause mortality						
Incidence rate per 100py	21	22	18	11	15	17
Crude Hazard ratio (95% CI)	1*	1.07 (0.84-1.38) p=0.58	0.87 (0.67-1.15) p=0.33	1*	1.32 (0.96-1.81) p=0.08	1.48 (1.10-1.99) p=0.01
Adjusted† Hazard ratio (95% CI)	1*	1.07 (0.82-1.39) p=0.64	0.86 (0.63-1.16) p=0.32	1*	1.37 (0.98-1.92) p=0.07	1.74 (1.27-2.40) p=0.001
Adjusted† Hazard ratio (95% CI) for log PTH as cont. variable		1.03 (0.90-1.17) p=0.67			1.23 (1.09-1.39) p=0.001	
Cardiovascular events						
Incidence rate per 100py	20	22	19	13	13	18
Crude Hazard ratio (95% CI)	1*	1.10 (0.82-1.48) p=0.52	0.97 (0.71-1.32) p=0.85	1*	1.05 (0.73-1.50) p=0.81	1.44 (1.04-1.99) p=0.03
Adjusted† Hazard ratio (95% CI)	1*	1.20 (0.88-1.65) p=0.25	1.05 (0.74-1.48) p=0.80	1*	1.01 (0.69-1.48) p=0.94	1.49 (1.05-2.11) p=0.03
Adjusted† Hazard ratio (95% CI) for log PTH as cont. variable		1.09 (0.94-1.26) p=0.24			1.20 (1.04-1.38) p=0.012	

To convert serum albumin in g/dL to g/L, multiply by 10; PTH in pg/mL to ng/L, multiply by 1;
Abbreviations: PTH = parathyroid hormone
* Patients with a PTH ≤ 46.3 pg/mL were used as the reference group.
†Multivariate analyses: Adjustments were made for age, sex, atorvastatin treatment, duration of hemodialysis, coronary artery disease, congestive heart failure, peripheral vascular disease, systolic blood pressure, glycemic control as represented by HbA1c, and levels of calcium, phosphate, hemoglobin and C-reactive protein.

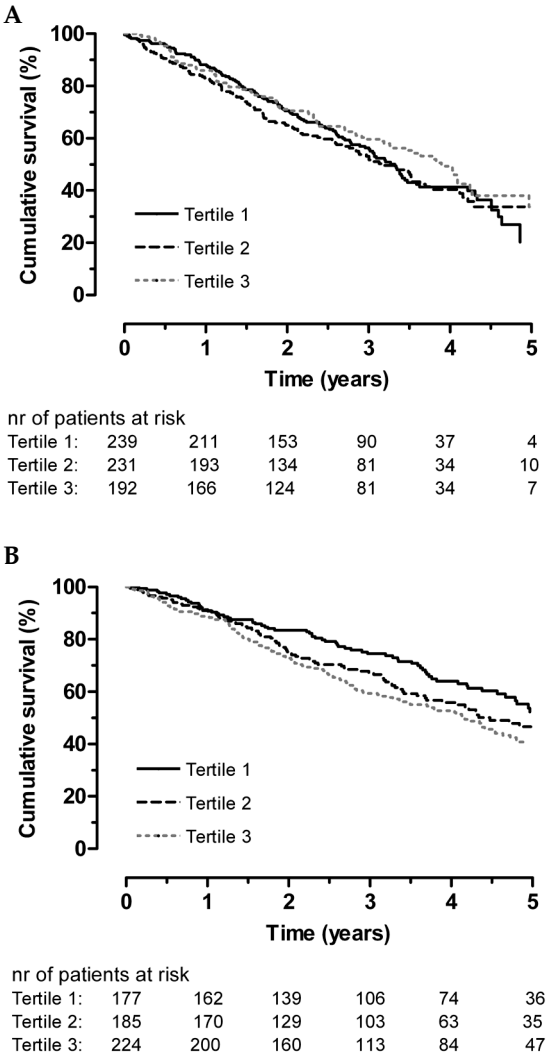

Figure 1A-B: Kaplan Meier curves for the time to all-cause mortality in patients with wasting (albumin ≤3.8g/dl, Figure 1A) and patients without wasting (albumin >3.8g/dl, Figure 1B); patients were grouped into tertiles of parathyroid hormone levels with the lowest tertile (Tertile 1) serving as the reference group.

Table 3: Table 2: Parathyroid hormone and risk of all cause mortality and CVE in strata of wasting, as defined by BMI ≤ / >23kg/m^2; n=1248

Variables	Wasting BMI ≤23kg/m^2 N=189			No Wasting BMI >23kg/m^2 N=1059		
	PTH tertiles			PTH tertiles		
	Tertile 1 PTH ≤ 46.3 pg/mL n=76	Tertile 2 PTH > 46.3 ≤ 106 pg/mL n=59	Tertile 3 PTH > 106 pg/mL n=54	Tertile 1 PTH ≤ 46.3 pg/mL n=340	Tertile 2 PTH > 46.3 ≤ 106 pg/mL n=357	Tertile 3 PTH > 106 pg/mL n=362
All-cause mortality						
Incidence rate per 100py	25	25	28	14	17	16
Crude Hazard ratio (95% CI)	1*	0.99 (0.64-1.51) p=0.95	1.13 (0.74-1.74) p=0.56	1*	1.23 (0.99-1.54) p=0.07	1.12 (0.89-1.39) p=0.34
Adjusted† Hazard ratio (95% CI)	1*	0.94 (0.58-1.54) p=0.81	0.94 (0.58-1.53) p=0.80	1*	1.27 (1.00-1.60) p=0.05	1.28 (1.00-1.63) p=0.05
Adjusted† Hazard ratio (95% CI) for log PTH as cont. variable		1.04 (0.84-1.28) p=0.71			1.15 (1.04-1.27) p=0.005	
Cardiovascular events						
Incidence rate per 100py	23	21	30	15	17	17
Crude Hazard ratio (95% CI)	1*	0.93 (0.55-1.57) p=0.78	1.32 (0.81-2.17) p=0.26	1*	1.11 (0.86-1.43) p=0.43	1.14 (0.89-1.46) p=0.30
Adjusted† Hazard ratio (95% CI)	1*	0.80 (0.44-1.46) p=0.46	1.01 (0.58-1.79) p=0.96	1*	1.19 (0.91-1.56) p=0.20	1.31 (1.00-1.72) p=0.05
Adjusted† Hazard ratio (95% CI) for log PTH as cont. variable		1.12 (0.88-1.42) p=0.35			1.14 (1.02-1.28) p=0.02	

To convert serum albumin in g/dL to g/L, multiply by 10; PTH in pg/mL to ng/L, multiply by 1; Abbreviations: PTH = parathyroid hormone

* Patients with a PTH ≤ 46.3 pg/mL were used as the reference group.

†Multivariate analyses: Adjustments were made for age, sex, atorvastatin treatment, duration of hemodialysis, coronary artery disease, congestive heart failure, peripheral vascular disease, systolic blood pressure, glycemic control as represented by HbA1c, and levels of calcium, phosphate, hemoglobin and C-reactive protein.

In analyses using PTH as a categorical variable, patients of the highest PTH tertile had a 49% higher risk of CVE (HR 1.49, 95% CI 1.05-2.11) as compared to patients of the lowest PTH tertile. When the absence of wasting was defined by a BMI > 23 kg/m^2 in additional analyses, similar results were found. The risk of CVE increased by 14% (HR 1.14, 95%CI 1.02-1.28) per unit increase in log transformed PTH. Furthermore, patients of the second and third PTH tertile were 19% and 31% more likely to exhibit cardiovascular events as compared to the lowest tertile, respectively. (Table 3).

Discussion

This study investigated the role of wasting in the association of parathyroid hormone levels with adverse outcomes in long-term dialysis patients with type 2 diabetes mellitus. We found that wasting modified the relation of PTH with all-cause mortality and cardiovascular events in 1255 diabetic hemodialysis patients participating in the 4D study, a prospective cohort with a high incidence of pre-specified and centrally adjudicated endpoints. High levels of PTH were associated with increased mortality and CVE in patients without wasting, but not in patients with the disease state. Among patients without wasting, those of the highest PTH tertile had 74% and 49% higher risks of death and CVE, respectively, as compared to patients of the lowest PTH tertile. In contrast, no effect of PTH on adverse outcomes was seen in patients with the wasting syndrome.

This study is the first, which investigated the association of PTH with adverse outcomes in dialysis patients, taking into account potential effect modification by wasting. It revealed that the negative effects of a high PTH are only observed in relatively healthy patients without the wasting syndrome, but do not appear in those suffering from the disease state. Our data contribute to explain divergent results found for the relation of PTH with adverse outcomes in previous studies of dialysis patients. Data from the Dialysis Outcomes and Practice patterns study for example showed that **mortality** was higher among patients with

high PTH levels above 600 pg/mL (9), and further large observational studies among dialysis patients reported higher risks of death with increased PTH levels (7;8;10). However, other studies found no association (11), or that a low PTH was associated with an increased mortality (12-14). Apart from partly methodological explanations including different follow-up times, our study adds important new knowledge showing that the impact of PTH on adverse outcomes depends on the disease state of the population studied. Furthermore, it provides a link to the results and the understanding of PTH metabolism in the general population and CKD patients of earlier stages. These patients in general are healthier and suffer to a much lesser extent from wasting. It is therefore not surprising that in these populations, many studies indicated a clear association of hyperparathyroidism with increased mortality (4-6;24), which was even reported to be independent of 25-hydroxy-vitamin D status, bone mass and renal function (24).

Mechanisms, by which a high PTH may affect mortality, include impaired insulin sensitivity (25), glucose intolerance (26) and abnormal lipid metabolism (27). Other mechanisms reported in literature refer to bone marrow fibrosis (28) with ineffective erythropoiesis (29), and abnormal immune function (30). Furthermore, PTH has been shown to directly affect vascular smooth muscle cells and ventricular myocytes (31), with the potential to impair cardiac energy production, and the accumulation of calcium in the myocardium (32). Excess PTH was suggested to play a role in the pathogenesis of myocardial hypertrophy and fibrosis, vascular calcification, impaired endothelial vasodilation and left ventricular diastolic filling dynamics (33-37). In this context, our finding of a high PTH being associated with **cardiovascular events** in patients without wasting is not surprising. In line with prior studies showing that high PTH was related to the development of cardiovascular disease (38), including coronary heart disease (39), our data support the evidence that part of the adverse effects of excess PTH result from its actions on the cardiovascular system.

In patients with the wasting syndrome, no association of PTH with mortality or cardiovascular events was found. Wasting is common in patients with chronic

kidney disease, and particularly present in those with diabetes mellitus (40;41). It represents a severe and complex process of muscle loss, poor food intake, inflammation and the development of comorbidities (15), and is associated with a high mortality (17;42). Malnutrition and hypoalbuminemia – characteristics of the wasting syndrome – have been found to be associated with adynamic bone disease, which is as a severe state of renal osteodystrophy characterized by low levels of PTH and low bone turnover (18;19;43;44). It may therefore be that low levels of PTH in patients with wasting represent a surrogate of an underlying disease process, with a low PTH possibly reflecting an impaired secretion due to the "illness" (i.e.wasting). The potential impact of wasting on bone metabolism may be supported by further experimental evidence: In vitro, inflammation has been shown to suppress PTH (45;46). Furthermore, leptin as a key anorexigenic hormone was shown to have antiosteogenic properties and to decrease bone mass (47). Additionally, weight loss which is frequently observed in wasting, was reported to lower PTH levels in humans (48). Therefore, the suggested impact of wasting on PTH metabolism may be a key reason to alter associations of PTH with cardiovascular events and mortality in dialysis patients. Finally, it could however also be argued that the impact of wasting on mortality is so strong that it overshadows the association of a high PTH with adverse outcomes. Further research is clearly warranted, with the results of our study indicating the need for stratification according to the presence or absence of wasting in clinical evaluations of bone metabolism.

Our study has potential **limitations**. It was a post-hoc analysis within a selected cohort of German patients with type 2 diabetes mellitus on hemodialysis. Therefore, the results cannot necessarily be extrapolated to other patient populations. Yet it may be assumed that the results also hold true for non-diabetic hemodialysis patients, suffering from wasting and PTH disorders, too. The group of patients with wasting as defined by a BMI $\leq 23 kg/m^2$ in our additional analyses was relatively small, and results may become stronger with larger numbers of patients in future studies. Furthermore, measurements of PTH might have been compromised by the high susceptibility of the hormone to preanalytic processes.

Levels of PTH, which are known to be generally lower in diabetic as compared to non-diabetic patients, therefore might have been additionally lowered. However, as all samples were treated in the same manner, preanalytic processes are unlikely to have affected our investigations, which focused on relative comparisons based on PTH tertiles. Finally, we did not have information on Vitamin D levels, which potentially may provide further insight into the mechanisms underlying the associations seen.

The main **strengths** of this study include the long-term follow-up, the high number of study participants and the high incidence of pre-specified and centrally adjudicated endpoints.

In **conclusion**, wasting modifies the association of PTH with adverse outcomes in dialysis patients with type 2 diabetes mellitus. High levels of PTH were associated with increased mortality and cardiovascular events in patients without wasting, but not in patients with the wasting syndrome. Our data provide evidence that the effect of PTH on adverse outcomes is markedly dependent on the patients´ disease states. We therefore suggest that risk assessments of PTH should be performed in strata of wasting. This may help clinicians in the decision and evaluation of treatment options, with our data suggesting that high PTH is of concern in relatively healthy dialysis patients without wasting, while the effect of PTH on mortality is nullified in patients with wasting.

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CHAPTER 8

Changes in parathyroid hormone, body mass index, and the association with mortality in dialysis patients

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Abstract

Background: Obesity is associated with secondary hyperparathyroidism in the general population. It is unknown whether BMI affects PTH level and its association with mortality in dialysis patients.

Methods: From a prospective cohort study of incident dialysis patients in the Netherlands (NECOSAD), we selected all patients with recorded BMI and PTH at 3 months (baseline) after the start of dialysis (n=1628, age 59±15yr, BMI 24.7±4.1 kg/m^2, median PTH 13.0 (IQR 5.3-29.0) pmol/l). We assessed associations between BMI and PTH at baseline and between their changes over 3 months by linear regression analyses. The effect of the changes in PTH on all-cause mortality during a subsequent mean follow-up of 3.2 ± 2 years was assessed by Cox regression analyses.

Results: Median PTH levels at baseline were lowest in underweight patients (10.2 pmol/l), followed by normal weight (12.1 pmol/l), overweight (14.0 pmol/l) and obese patients (17.5 pmol/l). The associations were similar in diabetic and non-diabetic patients. A ≥5% decrease in BMI (n=101) over 3 months was accompanied by a 26% decrease in PTH (PTH_{ratio} 0.74; p=0.039), whereas a ≥5% increase in BMI (n=143) was associated with an 11% increase in PTH (PTH_{ratio} 1.11; p=0.026). Compared to patients with stable PTH levels, patients with decreasing PTH in the presence of weight loss showed a 2-fold higher mortality (HR 2.02, 95% CI 1.45-2.83; p<0.001), in contrast to those with decreasing PTH in the absence of weight loss.

Conclusions: PTH is associated with BMI and its longitudinal changes in dialysis patients, both in patients with and without diabetes mellitus. A decrease in PTH in the presence of weight loss was associated with a high mortality. Low and decreasing PTH levels may be symptoms of wasting, and should be considered with caution in dialysis patients.

Introduction

Disturbances of mineral metabolism are common in patients undergoing maintenance dialysis. Secondary hyperparathyroidism develops early in the progression of chronic kidney disease (CKD) and worsens with advanced stages of the disease[1-5]. A variety of complications may develop, including bone demineralization, soft tissue and vascular calcification, anemia, cognitive dysfunction, and muscle and skin complaints[6]. Furthermore, high levels of parathyroid hormone (PTH) have been found associated with an increased mortality in patients with chronic kidney disease[7-10].
On the other hand, mineral disorders may also occur with low levels of PTH. In this context, adynamic bone disease has been reported as an important and serious complication[11, 12]. It has been shown that also low levels of PTH are associated with an increased mortality in dialysis patients[13-15]. The pathophysiology thereby is not clear, pointing out the need to identify risk factors that lead to alterations in PTH metabolism and associated mortality risk.

Nutritional status may impact on PTH metabolism, since obesity has been found related to increased levels of PTH in the general population[16-18] and in pre-dialysis patients[19]. It is unknown whether obesity is a risk factor for high PTH levels in dialysis patients. In contrast, dialysis patients suffer to a considerable extent from wasting, which is a complex process of muscle loss, poor food intake, inflammation and the development of comorbidities[20]. Underweight, frequently observed in patients with the wasting syndrome, has been shown to be associated with a high mortality in dialysis patients[21]. Considering the interrelations between body mass index and mineral metabolism in the general population, it is speculated that underweight and weight loss may result in low levels of PTH. This, in turn, may have a different impact on mortality as compared to low levels of PTH that are unrelated to wasting and either physiologic or intentionally achieved by medication.

The aim of this study was threefold. First, to investigate the association of BMI with levels of PTH in end-stage renal disease patients starting dialysis; second to assess the relation of longitudinal changes in BMI with changes in PTH; and third to assess the potential impact of changing PTH on mortality in dialysis patients, analyzing data of a prospective multicenter cohort study of incident dialysis patients in the Netherlands.

Subjects and methods

Study Design

NECOSAD is an observational prospective follow-up study in which incident dialysis patients have been enrolled in 38 participating dialysis centers since 1997 in The Netherlands. Study visits took place at the start of dialysis, at 3 months, 6 months, and subsequently at 6 months intervals until the date of loss to follow-up (death, kidney transplantation, or transfer to a non-participating dialysis center) or the end of the follow-up at January 1, 2007. Baseline demographic and clinical data were obtained between four weeks prior to and two weeks after the start of long-term dialysis treatment. Blood and 24-hour urine samples were obtained at all visits.

For the present analysis, baseline is defined as 3 months after the start of dialysis treatment, when the patients' fluid and metabolic conditions had stabilized.

Patients

Patients with ESRD who were at least 18 years old and started long-term dialysis therapy for the first time were invited to participate in NECOSAD.

In the present analysis, all patients with recorded height, weight, and measurements of PTH at 3 months after initiation of dialysis were included. The medical ethical committees of the participating centers approved the study, and all patients gave their written informed consent before inclusion.

Data collection

Demographic and clinical data included age, sex, ethnicity, smoking habits, primary kidney disease and comorbidity. Primary kidney diseases and causes of death were classified according to the coding system of the European Renal Association – European Dialysis and Transplant Association (ERA-EDTA). Diagnoses of comorbid conditions were reported by the patients nephrologists and used to calculate the comorbidity score according to Khan. Plasma calcium, phosphorus, intact PTH and albumin were measured at 3 and at 6 months after the initiation of dialysis by standard laboratory techniques in the different centers. Plasma calcium concentrations were corrected for the albumin concentrations. Height and weight were measured after dialysis sessions, and BMI was calculated as weight (kilograms) divided by height (meters) squared. Blood pressure was measured in sitting position.

Statistical analyses

Mean values with standard deviations (SD) were calculated for continuous variables, and median values with interquartile range (IQR) as appropriate. Categorical variables were expressed as proportions. Body mass index at baseline was categorized into four groups, with obesity being defined as a BMI ≥30 kg/m^2 and overweight as a BMI between 25 and 30 kg/m^2. Because of the lack of appropriate standards for dialysis patients[20], normal weight was defined as a BMI between 20 and 25 kg/m^2 (reference group), and underweight as less than 20 kg/m^2.

First, we assessed the association of BMI with PTH at baseline using linear regression analyses. Levels of PTH were logarithmically transformed and used as the dependent variable. The analyses were adjusted for age, sex, primary kidney disease, dialysis modality, diabetes mellitus, cardiovascular disease, and levels of calcium and phosphate. Since BMI is generally higher, and PTH lower in diabetic patients as compared to non-diabetic patients, we investigated potential effect modification by the disease and repeated the analyses in strata of diabetes mellitus. Second, we performed longitudinal analyses of the relation between BMI and PTH. We calculated the relative change in BMI from 3 months (baseline)

until the next available follow-up visit at 6 months ($BMI_{6m}/BMI_{baseline}$). This time period was examined in line with the suggestions by an expert panel to assess clinically meaningful changes in weight[20]. Accordingly, we divided the patients into 3 categories: patients with a decreasing BMI ≥5%, patients with a stable BMI ±5%, and patients with an increasing BMI ≥5%. Similarly, we assessed the relative change in PTH from baseline ($PTH_{6m}/PTH_{baseline}$). Linear regression analyses were then used to assess the association of the change in BMI with the change in PTH, and adjusted for the confounders mentioned above. Finally, we investigated the potential impact of this association on mortality in dialysis patients. Death rates according to the change in PTH from baseline (tertiles) were determined by Cox regression analyses, under consideration of changes in weight. In particular, we evaluated patients with decreasing PTH according to the presence or absence of weight loss. Hazard ratios and 95% confidence intervals were calculated for analyses, which were performed both univariately, and adjusted for confounders. Statistical analyses were performed with SPSS version 16.0.

Results

Patients

A total of 1916 patients with ESRD who started long-term dialysis and were included, still participated in NECOSAD at 3 months after the initiation of dialysis therapy (baseline). Of 1724 patients presenting with recorded height and weight, 1628 patients had measurements of PTH available at baseline and were included in the present analysis. Mean age of the patients was 59 ± 15 years, mean BMI was 24.7 ± 4.1 kg/m^2, and median PTH was 13.0 (IQR 5.3 – 29.0 pmol/l).

With higher BMI at baseline, more patients had diabetes and cardiovascular disease, and fewer patients had renal vascular diseases as primary kidney disease (Table 1). The percentage of male patients was smaller in underweight and in obese patients compared to patients with normal weight.

Table 1: Baseline patient characteristics, presented per BMI category; study population n=1628

Characteristic	BMI (kg/m²)			
	< 20 (n=153)	20 – 25 (n=819)	25 – 30 (n=499)	≥ 30 (n=157)
Age *years*	54.4 (18.1)	59.5 (15.8)	61.0 (13.0)	59.9 (12.9)
Gender *% men*	47.1	64.8	64.9	41.4
Dialysis modality *% HD*	71.2	62.8	60.1	66.2
BMI *kg/m²*	18.7 (1.0)	22.7 (1.4)	27.1 (1.4)	33.4 (3.3)
Primary kidney disease				
Diabetes mellitus %	11.8	10.6	18.6	40.1
Glomerulonephritis %	11.1	14.3	15.2	9.6
Renal vascular disease %	15.7	20.6	15.4	12.1
Comorbidity				
Diabetes mellitus %	16.7	15.9	26.4	50.3
Cardiovascular Disease %	27.3	35.0	36.9	38.1
Comorbidity Khan score				
Low %	40.5	41.3	35.1	24.8
Intermediate %	31.4	31.3	38.1	42.7
High %	28.1	27.5	26.9	32.5
Systolic blood pressure *mmHg*	146 (25)	149 (24)	150 (23)	151 (24)
Diastolic blood pressure *mmHg*	83 (15)	83 (14)	83 (13)	83 (13)
Albumin *g/dL*	3.5 (0.6)	3.6 (0.5)	3.7 (0.5)	3.6 (0.5)
Calcium *mmol/L*	2.4 (0.3)	2.4 (0.3)	2.4 (0.2)	2.4 (0.3)
Phosphate *mmol/L*	1.8 (0.6)	1.8 (0.6)	1.8 (0.5)	1.8 (0.5)

Values are presented as means (SD) or %.
To convert serum cholesterol in mg/dL to mmol/L, multiply by 0.02586; albumin in g/dL to g/L, multiply by 10;
Abbreviations: BMI = body mass index; HD = hemodialysis; BP = blood pressure;
* Numbers include patients who have diabetes mellitus as their primary kidney disease.

Association of BMI with PTH

Median PTH levels at baseline were lowest in underweight patients (10.2 pmol/l), followed by normal weight (12.1 pmol/l), overweight (14.0 pmol/l) and obese patients (17.5 pmol/l). The PTH levels as derived from adjusted linear regression analyses were significantly higher by 51% in obese patients and by 18% in overweight patients as compared to patients with normal weight. In line with this, PTH levels were 18% lower in underweight as compared to normal weight patients. The associations were similar in the analyses stratified for diabetes

mellitus, whereby overweight and obese patients with the disease generally had lower levels of PTH than patients without the disease. The levels of PTH and differences over the categories of BMI are shown in Table 2.

Table 2: Baseline PTH and adjusted differences in PTH over categories of BMI; n=1628

	BMI (kg/m²)			
	< 20 (n=153)	20 – 25 (n=819)	25 – 30 (n=499)	≥ 30 (n=157)
Whole study population				
Baseline PTH (IQR) *pmol/L*	10.2 (4.8-23.4)	12.1 (4.9-27.0)	14.0 (5.4-30.8)	17.5 (8.9-38.6)
adjusted† difference in PTH	-18%	0*	+18%	+51%
P-value	0.04		0.01	<0.001
With Diabetes mellitus				
Baseline PTH (IQR) *pmol/L*	10.7 (4.2-19.5)	12.5 (5.4-24.7)	11.0 (5.0-28.8)	15.6 (8.2-34.1)
adjusted‡ difference in PTH %	-15%	0*	+21%	+35%
P-value	0.48		0.15	0.04
Without Diabetes mellitus				
Baseline PTH (IQR) *pmol/L*	10.0 (5.0-24.0)	12.1 (4.7-28.0)	16.0 (5.8-31.0)	18.6 (9.8-43.0)
adjusted‡ difference in PTH %	-19%	0*	+17%	+66%
P-value	0.06		0.03	<0.001

* Patients with a body mass index ≥ 20 and < 25 were used as the reference group.
† Adjustments were made for age, sex, primary kidney disease, dialysis modality, diabetes mellitus, cardiovascular disease, calcium and phosphate levels.
‡ Adjustments were made for age, sex, primary kidney disease, dialysis modality, cardiovascular disease, calcium and phosphate levels.

Longitudinal changes of BMI and PTH

Of all 1628 patients with available BMI and PTH at baseline, a total of 1437 had both measurements also available at the subsequent follow-up visit, taking place at 6 months after the initiation of dialysis. These were included in the longitudinal analyses. Median PTH at follow-up was 11.7pmol/l. Patients with a stable BMI (ratio $BMI_{6m/baseline} \geq 0.95 \leq 1.05$, n=1197) showed stable PTH levels ($PTH_{6m}/_{baseline}$ 0.91). A ≥5% decrease in BMI (n=101) was accompanied by a 26% decrease in PTH ($PTH_{6m}/_{baseline}$ 0.74; p=0.039), whereas a ≥5% increase in BMI (n=143) was associated with an 11% increase in PTH ($PTH_{6m}/_{baseline}$ 1.11; p=0.026), (Figure 1).

In stratified analyses, patients with diabetes mellitus showed a decrease in

median PTH levels from 12.7 pmol/L at baseline to 9.5 pmol/L at follow-up. In contrast, the median PTH levels in patients without diabetes mellitus did not meaningfully alter, being 13.0 pmol/L at baseline and 12.0 pmol/L at follow-up. The longitudinal associations between changes in BMI and PTH applied to both groups (diabetic and non-diabetic patients), and were more pronounced in diabetic patients (Figure 1).

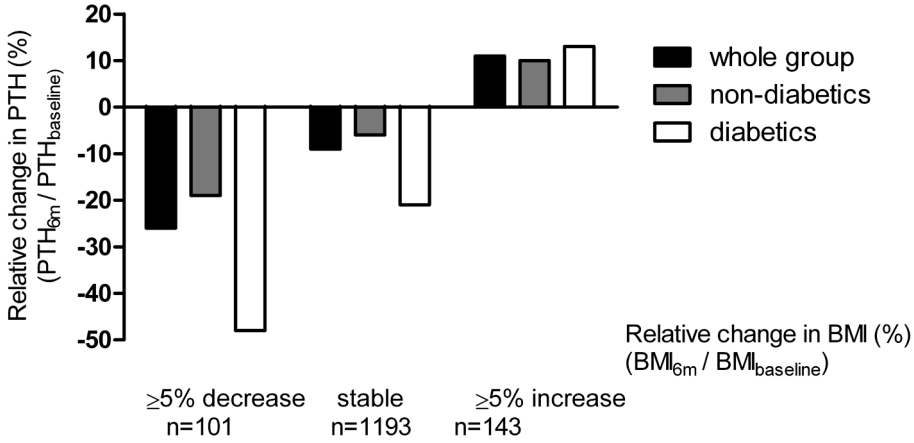

Figure 1: Relative change in PTH levels ($PTH_{6m/baseline}$) in groups of BMI change (decreasing / stable / increasing BMI)

Mortality risks of changing BMI and PTH

Patients were divided into tertiles according to the relative change in PTH from baseline ($PTH_{6m}/_{baseline}$). The tertiles corresponded to a PTH ratio of ≤0.64 (tertile 1, n=479), PTH ratio of >0.64 ≤ 1.20 (tertile 2, n=479), and a PTH ratio >1.20 (tertile 3, n=479). The majority of the patients with significant weight loss (58 out of the 101 patients with a decrease in BMI ≥5%) were among the lowest tertile of PTH change. These patients were subgrouped separately. Compared to patients with stable PTH (tertile 2), those with decreasing PTH in the presence of weight loss (n=58) had a 2 fold increased mortality (HR 2.02, 95% CI 1.45-2.83), while patients with decreasing PTH in the absence of weight loss (n=479-58=421) were not at

increased risk of death (HR 0.94, 95% CI 0.76-1.15). The results persisted after multivariable adjustments. Additional analyses revealed that the risks of death for patients with decreasing PTH due to weight loss was highest in the short term, being considered as the subsequent half year following the observed changes in PTH and BMI (data not shown). The results of all survival analyses are shown in Table 3 and Figure 2.

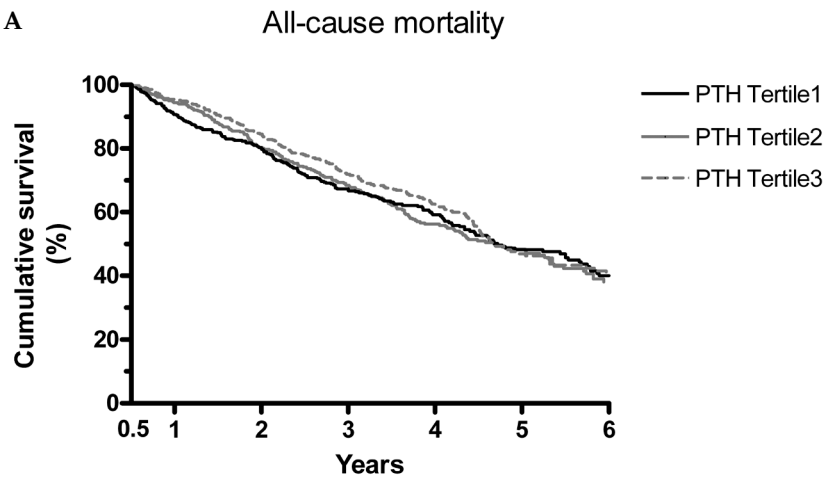

Figure 2 A: Kaplan-Meier curves for all-cause mortality in tertiles of the relative change in PTH from baseline ($PTH_{6m/baseline}$).

Tertile 1 = decreasing PTH (PTHratio ≤0.64, n=479);

Tertile 2 = stable PTH (PTHratio >0.64 ≤1.20; n=479);

Tertile 3 = increasing PTH (PTHratio >1.20, n=479);

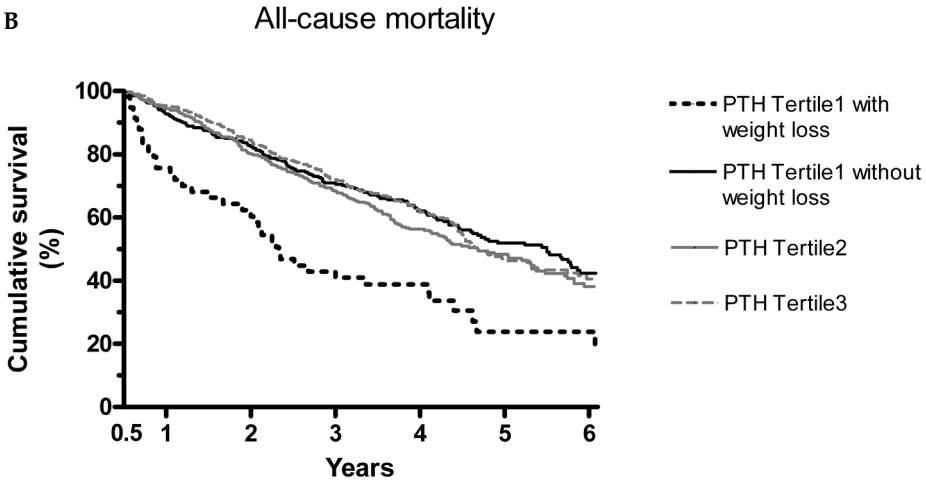

Figure 2 B: Kaplan-Meier curves for all-cause mortality in tertiles of the relative change in PTH from baseline ($PTH_{6m/baseline}$), differentiating the tertile 1 with decreasing PTH levels according to the presence or absence of weight loss.

Tertile 1 = decreasing PTH, and presence of weight loss (n=58)

Tertile 1 = decreasing PTH, and absence of weight loss (n=421)

Tertile 2 = stable PTH (n=479)

Tertile 3 = increasing PTH (n=479)

Table 3: Mortality (Hazard ratio and 95% CI) according to the change in PTH from baseline; n=1437

Model	Tertiles of PTH change (ratio PTH $_{6m/baseline}$)			
	Tertile 1 PTH ratio ≤0.64 n=479		Tertile 2 PTH ratio >0.64 ≤1.20 n=479	Tertile 3 PTH ratio >1.20 n=479
Crude	1.04 (0.86-1.27) p=0.66		1	0.92 (0.76-1.13) p=0.44
Adjusted†	1.02 (0.84-1.24) p=0.82		1	1.09 (0.88-1.36) p=0.42
	With weight loss n=58	Without weight loss n=421		
Crude	2.02 (1.45-2.83) p<0.001	0.94 (0.76-1.15) p=0.53	1	0.92 (0.76-1.13) p=0.44
Adjusted†	1.54 (1.09-2.18) p=0.015	0.95 (0.77-1.17) p=0.61	1	1.08 (0.87-1.34) p=0.47

*Patients with a PTH ratio >0.64 ≤1.20 were used as the reference group.
† Adjustments were made for age, sex, primary kidney disease, dialysis modality, diabetes mellitus, cardiovascular disease, calcium and phosphate levels.

DISCUSSION

In this prospective cohort study of incident dialysis patients, BMI was associated with PTH at baseline. Underweight patients had the lowest levels of PTH, followed by normal weight, overweight and obese patients. Furthermore, longitudinal changes in BMI over 3 months of follow-up were paralleled by longitudinal changes in PTH. Weight loss during follow-up was accompanied by decreases in PTH, while weight gain resulted in increases of PTH. Considering decreases in PTH, the relation with BMI strongly affected outcome: Compared to patients with stable PTH levels, patients with decreasing PTH due to weight loss had a >2fold higher risk of death, while those with decreasing PTH in the absence of weight loss did not.

Obesity has been shown to be a risk factor for secondary hyperparathyroidism in the general population[16]. Similarly, a higher BMI was found to be associated with higher PTH levels in men with chronic kidney disease not yet on dialysis[19]. In line with this, our study extends the previous findings, showing that the association of high BMI with PTH also holds true for the dialysis population, and in particular applies to both diabetic and non-diabetic patients. This is important, as diabetic patients usually present with higher weight, but lower PTH levels than non-diabetic patients[22]. Our findings therefore show that weight similarly relates to PTH in both patient groups and does not play a major role in explaining the differences in PTH levels between diabetic and non-diabetic patients.

The mechanisms underlying the association between BMI and PTH are not yet known in detail. One possible link may be 25 (OH) vitamin D levels, which were shown to be lower in persons with higher BMI in the general population[16, 18, 23-25]. Patients with a high BMI possibly experience less sun exposure due to lower mobility and clothing habits, leading to a lower bioavailability of vitamin D[25, 26]. In addition, obesity potentially results in a higher storage of vitamin D in adipose tissue, furthermore contributing to lower circulating levels of vitamin D. In this context, one study measuring body composition by dual-energy x-ray absorptiometry showed total body fat even better than BMI related to hyperparathyroidism, suggesting fat cells to play a main role for vitamin D availability[18]. Finally, a higher body weight may impose a greater strain on the skeleton, potentially resulting in decreased skeletal responses to the actions of PTH[16]. This in turn, may lead to compensatory higher PTH levels.

Furthermore, we showed that underweight was related to low PTH levels and that weight loss was paralleled by a decrease in PTH. These data extend results from prior studies in the general population, where weight loss achieved by intestinal bypass surgery in obese people was shown to lower their PTH levels[27]. Importantly, this present observation in renal patients may provide a link to explain why low levels of PTH were related to an increased mortality in some previous studies of dialysis patients[13-15]. In contrast to intentional weight loss in

the studies of the general population[27], partly achieved by dietary or surgical interventions, weight loss in patients with chronic kidney disease most likely occurs unintentionally in the context of wasting. Wasting is common in patients with CKD or ESRD, and represents a severe and complex process of muscle loss, poor food intake, inflammation and the development of comorbidities. The decrease in PTH, accompanying the weight loss, may be interpreted as a symptom of an underlying "illness" (wasting), which is associated with a high mortality[28]. Thus, decreasing PTH resulting in a high rate of death may -in the presence of weight loss- represent a surrogate of the wasting process[29].

This proposed link between wasting and low and decreasing PTH levels can be supported by further evidence. Wasting was found to be associated with adynamic bone disease[11, 12, 30, 31]. Furthermore, inflammation, which often accompanies the wasting syndrome, has in vitro been shown to suppress PTH[32, 33]. Finally, leptin as a key anorexigenic hormone potentially involved in wasting, was shown to have antiosteogenic properties and to decrease bone mass[34].

While decreases in PTH may be warranted in the context of intentional interventions, e.g. by medication, decreasing PTH cannot generally be considered favorable. Our study shows that for the evaluation and treatment of bone metabolism in dialysis patients, it is therefore especially important to consider nutritional status among the reasons for decreases in PTH. These may completely different relate to outcome, depending on the circumstances, such as the concurrent presence or absence of weight loss.

Potential limitations of our study need to be acknowledged. We did not have information on Vitamin D levels, which potentially may provide further insight into the mechanisms underlying the associations seen. The measurements of PTH were not centrally performed, but by various first-generation immunometric PTH assays depending on the different participating centres. With central measurements, the associations could even have been stronger. Despite the measurements in several laboratories however, we still were able to detect the

presented effects. Finally, due to the observational design, causality cannot be inferred.
The strengths of this study include the favorable design of incident dialysis patients reducing selection, the high number of participants, and the longitudinal measures of BMI and PTH.

In conclusion, PTH is associated with BMI and its longitudinal changes in dialysis patients, both in patients with and without diabetes mellitus. PTH decreased with weight loss, being associated with a high mortality. Low and decreasing PTH levels may be symptoms of wasting, and should be considered with caution in dialysis patients.

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CHAPTER 9

Vitamin D deficiency is associated with sudden cardiac death, combined cardiovascular events and mortality in haemodialysis patients

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Abstract

Aims: Dialysis patients experience an excess mortality, predominantly of sudden cardiac death (SCD). Accumulating evidence suggests a role of vitamin D for myocardial and overall health. This study investigated the impact of vitamin D status on cardiovascular outcomes and fatal infections in haemodialysis patients.

Methods and Results: 25-hydroxyvitamin D (25[OH]D) was measured in 1109 diabetic haemodialysis patients who participated in the German Diabetes and Dialysis Study (4D Study) and were followed-up for a median of 4 years. By Cox regression analyses, we determined hazard ratios (HR) for pre-specified, adjudicated endpoints according to baseline 25(OH)D levels: SCD (n=146), myocardial infarction (MI, n=174), stroke (n=89), cardiovascular events (CVE; n=414), death due to heart failure (n=37), fatal infection (n=111) and all-cause mortality (n=545). Patients had a mean age of 66±8 years (54% male), and mean 25(OH)D of 18.0±9.8 ng/ml. Patients with severe vitamin D deficiency (25[OH]D ≤ 10ng/ml) had a 3fold higher risk of SCD compared to those with sufficient 25(OH)D levels >30ng/ml (HR 3.0; 95% confidence interval 1.4-6.4). Furthermore, CVE and all-cause mortality were strongly increased (HR 1.8, 95% CI 1.2-2.7, and HR 1.7, 95% CI 1.2-2.5, respectively), all persisting in multivariate models. There was a trend for higher risks of stroke and fatal infection, while MI and deaths due to heart failure were not significantly affected.

Conclusions: Severe vitamin D deficiency was strongly associated with SCD, CVE and mortality, and by trend with stroke and fatal infection in diabetic haemodialysis patients. Whether vitamin D supplementation decreases adverse outcomes, requires further evaluation.

Introduction

Vitamin D deficiency is observed in the vast majority of haemodialysis patients and there is accumulating evidence that vitamin D, beyond its effects on bone and mineral metabolism, is also crucial for cardiovascular health and protection against infectious diseases.[1-9] In general, vitamin D from either ultraviolet-B induced synthesis in the skin or from nutritional intake is hydroxylated to 25-hydroxyvitamin D (25[OH]D) in the liver. 25(OH)D circulates in up to 1000 fold higher concentrations than the most potent vitamin D metabolite 1,25-dihydroxyvitamin D (1,25[OH]2D) (calcitriol).[8,9] The renal production of 1,25(OH)2D is tightly controlled by homeostatic mechanisms but becomes significantly dependent on substrate availability when circulating 25(OH)D are low.[8] In addition, various extrarenal tissues including the myocardium and vasculature have been shown to express 1α-hydroxylase and are thus capable of producing large amounts of 1,25(OH)2D.[8,9] Of note, locally produced 1,25(OH)2D, the synthesis of which is dependent on circulating 25(OH)D levels, exerts its effects predominantly in an autocrine and paracrine manner thereby regulating approximately three percent of the human genome.[8,9,11]

In patients with chronic kidney disease (CKD) limited sunlight exposure and reduced capacity of the skin to synthesize vitamin D as well as loss of vitamin D binding protein in the urine are mainly responsible for the high prevalence of depressed 25(OH)D levels which are used to assess vitamin D status.[1-10] Given that the kidney is the main source for circulating 1,25(OH)2D, which is crucial for calcium and phosphorus as well as parathyroid hormone (PTH) homeostasis, 1,25(OH)2D and its analogues are routinely supplemented in many end-stage CKD patients and this therapy is associated with improved survival.[11] Beside the use of this therapy with active vitamin D, little attention has been paid in the past to 25(OH)D levels which can be raised by supplementation with the precursor vitamin D. Recent studies have, however, shown that in CKD low 25(OH)D levels are a significant risk factor for cardiovascular diseases and mortality.[1-7] In a nested case control study among 1000 incident haemodialysis patients there was a significantly increased 90 day mortality in the group with the lowest 25(OH)D

levels.[4] However, data on Long-term mortality and specific cardiovascular events such as SCD or stroke, being significantly associated with vitamin D deficiency in patients undergoing coronary angiography,[13,14] are lacking for haemodialysis patients. In view of the particularly high incidence of SCD in dialysis patients, accounting for one quarter of all deaths, such data are needed to gain a better understanding of the diagnostic and probably therapeutic implications of vitamin D in these patients. Hence we investigated the effect of Vitamin D levels on SCD in relation to other cardiac, vascular, and infection-related outcomes in a large well-characterized cohort of haemodialysis patients.[15]

Materials and methods

Study Design and Participants

The 4D study methodology has previously been reported in detail.[15] Briefly, the 4D study was a prospective randomized controlled trial (RCT) including 1255 patients with type 2 diabetes mellitus, aged 18 – 80 years and on haemodialysis for less than 2 years. Between March 1998 and October 2002, patients were recruited in 178 dialysis centres in Germany. After a period of 4 weeks, patients were randomly assigned to double-blinded treatment with either 20 mg atorvastatin (n=619) or placebo (n=636) once daily. Study visits took place three times before randomization (visit 1-3), at randomization (visit 4), and at four weeks (visit 5) and every six months (visit 6 etc.) after randomization until the date of death, censoring, or end of the study in March 2004. The primary endpoint of the 4D study was defined as a composite of death from cardiac causes, fatal or nonfatal stroke and nonfatal myocardial infarction (MI), whichever occurred first (composite cardiovascular endpoint; CVE). Death from cardiac causes comprised SCD, fatal MI, death due to congestive heart failure (CHF), death due to coronary heart disease during or within 28 days after an intervention, and all other deaths ascribed to coronary heart disease. SCD was considered as: death verified by terminal rhythm disorders in an electrocardiogram; by witnesses observed death within one hour after the onset of cardiac symptoms; confirmed by autopsy;

unexpected death, presumably or possibly of cardiac origin and in the absence of a potassium level greater or equal to 7.5 mmol per liter before the start of the three most recent sessions of haemodialysis. MI was diagnosed when two of the following three criteria were met: typical symptoms, elevated levels of cardiac enzymes (i.e. a level of creatine kinase MB above 5 percent of the total level of creatine kinase, a level of lactic dehydrogenase 1.5 times the upper limit of normal, or a level of troponin T greater than 2 ng per milliliter), or diagnostic changes on the electrocardiogram. 4D Study endpoints were centrally adjudicated by three members of the endpoint committee blinded to study treatment and according to pre-defined criteria.

For the present analysis, SCD, MI (fatal and nonfatal), stroke (fatal and nonfatal), the primary endpoint (CVE), death due to CHF, death due to infection and all-cause mortality were all chosen to be separate outcome measures. The study complies with the Declaration of Helsinki, was approved by the medical ethical committee, and all patients gave their written informed consent before inclusion.

Data collection

Information on age, gender and smoking status was obtained through patient interviews. Smoking status was classified as never, former or current. Comorbidities, including the presence of coronary artery disease (CAD) and CHF, as well as the duration of diabetes mellitus and dialysis treatment were reported by the patients´ nephrologists. CAD was defined by the history of MI, coronary artery bypass grafting surgery; percutaneous coronary intervention; and the presence of coronary heart disease, as documented by coronary angiography. Blood pressure was measured in sitting position. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Levels of 25(OH)D were measured in blood samples taken at baseline at study visit 3 (1 week before randomization) and stored at -80°C. Determinations in serum were performed by means of a chemiluminescence assay (IDS, iSYS 25-hydroxyvitamin D; Immunodiagnostic systems Ltd, Boldon, England) on an IDS-iSYS multi-discipline automated analyser. Within-day coefficients of variation (CV) were 5.5 to 12.1 %, and inter-day CV were 8.9 to 16.9 %, respectively. All blood samples were taken before the start of dialysis sessions and administration of drugs.

Statistical Analysis

Continuous variables were expressed as mean with standard deviation or median with interquartile range (IQR) as appropriate, and categorical variables were expressed as percentages.

The study population was divided into three groups according to their 25(OH)D status at baseline. In line with widely used cut-off values, patients were grouped into severely vitamin D deficient (≤10.0 ng/ml), moderately vitamin D deficient (>10 ≤30 ng/ml), and vitamin D sufficient (>30 ng/ml).[4,8,13] First, we assessed the association of baseline 25(OH)D with SCD, both as continuous and as categorical variable. For the latter, the patients with sufficient 25(OH)D levels were used as the reference group. Absolute (incidence) rates were calculated, and relative risks derived from Cox regression analyses, i.e. hazard ratios (HRs) and corresponding 95% confidence intervals. The Cox regression analyses were adjusted for the confounders age, sex, atorvastatin treatment, CAD, CHF, systolic blood pressure, smoking status, duration of dialysis, ultrafiltration volume, BMI, levels of LDL-cholesterol, HDL-cholesterol, C-reactive protein and glycohaemoglobin A1c. To account for the seasonal variation of vitamin D, we furthermore adjusted our analyses for the season of blood draw. We therefore used a binary variable reflecting the months October to March and April to September, respectively. Second, in order to explore possible pathways, we performed additional analyses with inclusion of potential intermediate conditions including levels of calcium, PTH and phosphate. The use of active vitamin D treatment was furthermore considered in the additional multivariate analyses. Third, we investigated vitamin D and the risk of other adverse cardiac and vascular outcomes including MI, stroke, the combined primary endpoint and death due to CHF. Furthermore, we evaluated the association of 25(OH)D levels with all-cause mortality and fatal infections. In addition, we used an alternative approach to account for the seasonal fluctuation of vitamin D and calculated z-values of logarithmically transformed 25(OH)D levels based on their means and standard deviation values within each month of blood sampling (formula for z-values: X-mean/standard deviation). Finally, to exclude potential interaction by atorvastatin treatment, we repeated our analyses stratified by medication.

All p-values are reported two-sided. Analyses were performed using SPSS version 16.0.

Results

Patient characteristics

Between March 1998 and October 2002, a total of 1255 patients were included into the 4D study, of whom 1109 had a measurement of 25(OH)D at baseline. The mean follow-up period was 3.96 years (median 4.0 years) on atorvastatin and 3.91 years (median 4.08 years) on placebo. During follow-up, 414 out of the 1109 patients reached the primary endpoint of CVE. A total of 545 patients died, of whom 146 patients died of SCD. Furthermore, 37 patients died due to CHF and 111 patients died due to infection. A total of 174 patients experienced a MI (fatal or non-fatal), and 89 patients experienced a stroke (fatal or non-fatal).

In the study population (n=1109), the mean (standard deviation) age was 66 (8) years and 54% of the patients were male. In general, the mean (standard deviation) level of 25(OH)D at baseline was 18.0 (9.8) ng/ml. As expected, we observed a seasonal variation of 25(OH)D in our patients, with the lowest concentrations in February (13.8 (10.8) ng/ml) and the highest concentrations in August (23.0 (13.0) ng/ml). The patient characteristics are shown in Table 1. Patients with severe vitamin D deficiency were more likely to be female, and had higher levels of glycohaemoglobin $A1_c$. Furthermore, the burden of left ventricular hypertrophy was higher, as were the levels of LDL-cholesterol and NT-pro-BNP in patients with severe vitamin D deficiency compared to patients with sufficient vitamin D levels.

Table 1: Baseline patient characteristics, presented per Vitamin D category; study population n=1109

Characteristic	Vitamin D ≤ 10 (n=177)	> 10 ≤ 30 (n=818)	> 30 (n=114)
Age *years*	66 (8)	66 (8)	65 (8)
Gender *% men*	49.2	53.6	67.5
25-hydroxyitamin D *ng/mL*	8.0 (1.5)	17.1 (5.2)	39.8 (10.0)
Atorvastatin treatment *%*	52.0	49.6	49.1
Use of active Vitamin D *%*	22.6	17.9	14.9
Systolic BP* *mmHg*	146 (22)	146 (22)	147 (22)
Diastolic BP *mmHg*	76 (11)	76 (11)	77 (10)
BMI* kg/m^2	27.4 (5.3)	27.6 (4.7)	27.0 (4.1)
Duration of diabetes *years*	19.9 (8.4)	17.7 (8.8)	17.4 (9.0)
Time on dialysis *months*	8.3 (7.0)	8.2 (6.8)	9.4 (7.4)
Smoker / Ex-smoker *%*	42.9	39.2	44.7
History of			
CAD* *%*	28.8	28.7	36.8
CHF* *%*	37.9	34.6	40.4
Presence of LVH* *%*	14.1	11.6	12.4
Ultrafiltration volume *kg*	2.16 (1.17)	2.27 (1.19)	2.22 (1.25)
Laboratory parameters			
LDL* cholesterol *mg/dL*	131 (29)	125 (30)	122 (28)
HDL* cholesterol *mg/dL*	37 (14)	36 (13)	38 (12)
Triglycerides *mg/dL*	244 (165-322)	224 (153-324)	185 (135-304)
Hemoglobin *g/dL*	11.0 (1.4)	10.9 (1.4)	10.9 (1.2)
Albumin *g/dL*	3.8 (0.3)	3.8 (0.3)	3.9 (0.3)
C-reactive protein *mg/L*	4.9 (2.5-10.9)	5.2 (2.5-13.6)	4.5 (2.1-10.3)
HbA1c *%*	6.8 (1.3)	6.7 (1.3)	6.5 (1.2)
Calcium *mmol/L*	2.3 (0.2)	2.3 (0.2)	2.3 (0.2)
Phosphate *mmol/L*	6.2 (1.5)	6.0 (1.6)	5.7 (1.6)
NT-pro-BNP *pg/mL*	3690 (1479-8080)	3411 (1447-9568)	2608 (1087-7026)

Values are presented as means (SD) or median (interquartile range) or %.
*Abbreviations: HbA1c = haemoglobin A1c; BP = blood pressure;
BMI = body mass index; CAD = coronary artery disease,
CHF = congestive heart failure; LVH = left ventricular hypertrophy
LDL = low density lipoprotein, HDL = high density lipoprotein,
NT-pro-BNP = N-terminal-pro-B-type natriuretic peptide

Vitamin D status and risk of sudden cardiac death

Vitamin D status at baseline was strongly associated with the risk of SCD (see Figure 1A). By Cox regression analyses, the unadjusted hazard to experience SCD was 3 fold higher in patients with severe vitamin D deficiency as compared to those with sufficient vitamin D levels (HR 3.0, 95% CI 1.4-6.4), Table 2). This association was virtually unchanged after controlling for potential confounders and seasonal variation of vitamin D (HR 2.9, 95% CI 1.3-6.3). Additional adjustment for markers of mineral metabolism including PTH, calcium and phosphate also did not materially change the results (HR 2.9, 95% CI 1.3-6.4), which persisted even after further adjustment for the use of active Vitamin D treatment (HR 3.1, 95% CI 1.4-6,7). When vitamin D was analysed as a continuous variable, the hazard to die suddenly increased by 60% per unit decrease in 25(OH)D levels (Table 3). This association persisted in multivariate analyses. To strengthen our results, we performed additional analyses using z-values as an alternative approach to account for the seasonal variation of vitamin D. The results were similar, confirming a strong association of 25(OH)D levels with the risk of SCD (data not shown).

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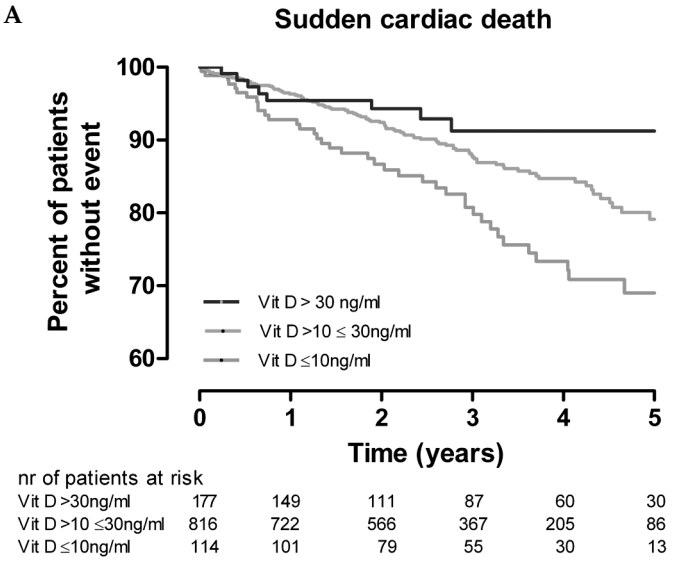

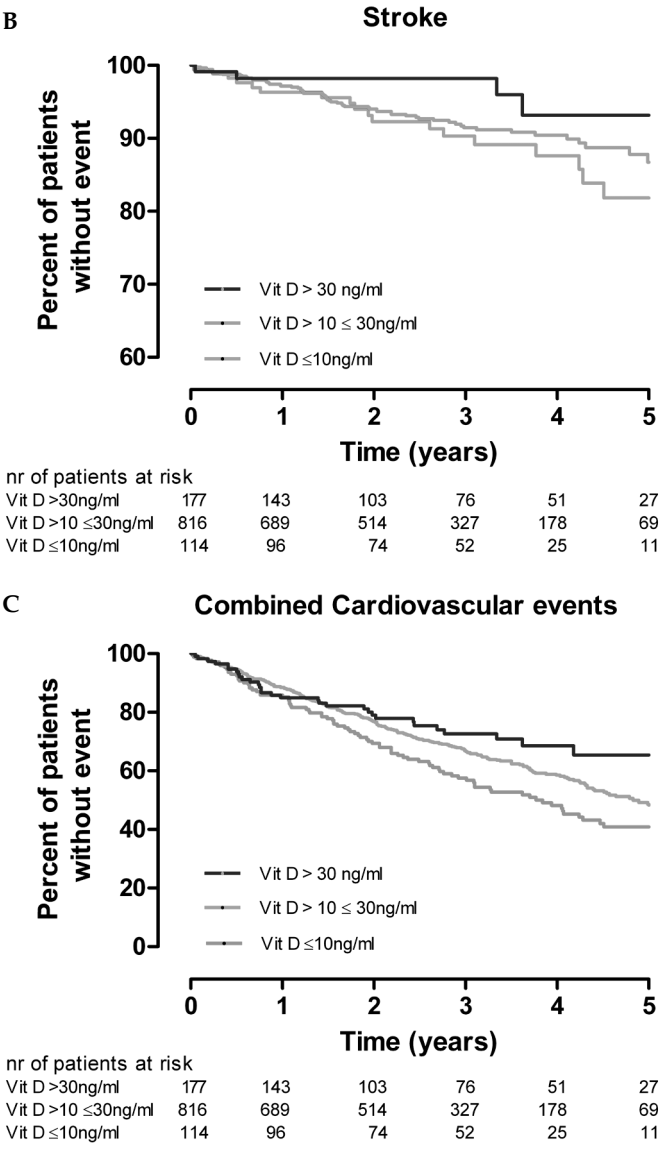

Figure 1: Kaplan-Meier curves for the time to A) sudden cardiac death, B) stroke, C) combined cardiovascular events in subgroups of patients according to 25(OH)Vitamin D levels at baseline (severely vitamin D deficient (≤10.0 ng/ml), moderately vitamin D deficient (>10 ≤30 ng/ml), and vitamin D sufficient (>30 ng/ml))

Vitamin D status and risk of myocardial infarction, stroke, death due to heart failure, and combined cardiovascular events

There was a trend for higher risks of stroke with lower vitamin D levels (see Figure 1B). Per unit decrease in vitamin D, the risk of stroke increased by 30% after adjustment for confounders. In categorical analyses, patients with severe vitamin D deficiency had an almost 3 fold increased risk of stroke compared to those with normal levels (adjusted HR 2.9, 95% CI 0.9-10.0). In contrast, no association of vitamin D status with MI was found. Both in continuous (adjusted HR 1.1, 95% CI 0.8-1.5) and in categorical analyses, the risk of MI did not increase at lower levels of vitamin D (Tables 2 and 3). When non-fatal and fatal MI were analyzed separately, the results were similar showing no relation to vitamin D status. The number of deaths due to CHF was small in the present study (n=37). These deaths similarly were not meaningfully affected by vitamin D status.

Finally, the primary endpoint of combined CVE was markedly increased with lower levels of 25(OH)D (Table 2 and Figure 1C). Patients with severe vitamin D deficiency had an adjusted 80% higher risk of experiencing a CVE as compared to patients with sufficient vitamin D status (Table 3).

Vitamin D status and risk of death due to infection and all-cause mortality

Deaths due to infection were almost 2 fold increased in patients suffering from severe vitamin D deficiency (adjusted HR 1.9, 95% CI 0.8-4.6). Per unit decrease in log transformed vitamin D, the rate of fatal infections rose by 50% (adjusted HR 1.5, 95% CI 1.0-2.3). Furthermore, deaths due to all causes increased significantly by 40% per unit decrease in log vitamin D. Patients with levels below 10 ng/ml had an adjusted 90% higher risk of death as compared to patients with sufficient 25(OH)D levels (Tables 2 and 3).

To strengthen our results, we repeated all analyses in the placebo group only. The results were similar, supporting the use of the complete data.

Table 2: Absolute incidence rates, and hazard ratios with 95% confidence intervals (HR, 95% CI) for sudden cardiac death, stroke, myocardial infarction and death due to heart failure according to levels of 25-hydroxyitamin D at baseline; n=1109

Outcome	Vitamin D *ng/mL*		
	≤10 (n=177)	>10 ≤30 (n=818)	>30 (n=114)
Sudden cardiac death			
Incidence rate / 100py	7.4	4.3	2.5
Crude HR (95% CI)	3.0 (1.4-6.4)	1.8 (0.9-3.6)	1
Adj.[1] HR (95% CI)	2.9 (1.3-6.3)	1.7 (0.8-3.5)	1
Stroke			
Incidence rate / 100py	3.8	2.9	1.2
Crude HR (95% CI)	3.0 (1.0-8.8)	2.3 (0.9-6.4)	1
Adj.[1] HR (95% CI)	2.9 (0.9-10.0)	2.3 (0.7-7.5)	1
Myocardial infarction			
Incidence rate / 100py	6.7	5.7	5.2
Crude HR (95% CI)	1.3 (0.7-2.4)	1.1 (0.7-1.8)	1
Adj.[1] HR (95% CI)	1.4 (0.8-2.7)	1.2 (0.7-2.0)	1
Death due to heart failure			
Incidence rate / 100py	1.6	1.1	1.2
Crude HR (95% CI)	1.3 (0.4-4.2)	0.9 (0.3-2.5)	1
Adj.[1] HR (95% CI)	1.5 (0.4-5.0)	0.8 (0.3-2.4)	1
Cardiovascular events			
Incidence rate / 100py	18.2	13.8	10.2
Crude HR (95% CI)	1.8 (1.2-2.7)	1.4 (0.9-2.0)	1
Adj.[1] HR (95% CI)	1.8 (1.2-2.8)	1.4 (0.9-2.0)	1
Death due to infection			
Incidence rate / 100py	4.3	3.5	2.2
Crude HR (95% CI)	1.9 (0.8-4.5)	1.7 (0.8-3.7)	1
Adj.[1] HR (95% CI)	1.9 (0.8-4.6)	1.6 (0.7-3.4)	1
All-cause mortality			
Incidence rate / 100py	22.9	16.6	13.0
Crude HR (95% CI)	1.7 (1.2-2.5)	1.3 (0.9-1.8)	1
Adj.[1] HR (95% CI)	1.9 (1.3-2.7)	1.3 (0.9-1.8)	1

[1] model 1: adjusted for age, sex, atorvastatin treatment, season, coronary artery disease, congestive heart failure, systolic blood pressure, smoking, duration of dialysis, ultrafiltration volume, body mass index, levels of LDL, HDL cholesterol, C-reactive protein, HbA1c

Table 3: Risk of cardiovascular events, sudden cardiac death, stroke, myocardial infarction, death due to heart failure, death due to infection and all-cause mortality per unit decrease in 25-hydroxyvitamin D (continuous variable, log transformed); study population n=1109

Outcome	Hazard ratio (HR) and 95% CI		
	crude	adjusted[1]	adjusted[2]
Sudden cardiac death	1.6 (1.1-2.2) p=0.007	1.5 (1.1-2.2) p=0.019	1.6 (1.1-2.2) p=0.016
Stroke	1.4 (0.9-2.2) p=0.09	1.3 (0.8-2.1) p=0.23	1.3 (0.8-2.1) p=0.26
Myocardial infarction	1.0 (0.7-1.3) p=0.96	1.1 (0.8-1.5) p=0.71	1.0 (0.8-1.4) p=0.83
Death due to heart failure	1.0 (0.5-2.0) p=0.90	1.1 (0.5-1.9) p=0.83	1.0 (0.5-2.0) p=0.94
Cardiovascular events	1.2 (1.0-1.4) p=0.11	1.2 (1.0-1.5) p=0.12	1.2 (0.9-1.4) p=0.20
Death due to infection	1.4 (1.0-2.1) p=0.06	1.5 (1.0-2.3) p=0.05	1.4 (1.0-2.2) p=0.08
All-cause mortality	1.4 (1.2-1.6) p<0.001	1.4 (1.2-1.7) p<0.001	1.4 (1.2-1.7) p<0.001

[1] model 1: adjusted for age, sex, atorvastatin treatment, season, coronary artery disease, congestive heart failure, systolic blood pressure, smoking, duration of dialysis, ultrafiltration volume, body mass index, levels of LDL, HDL cholesterol, C-reactive protein, HbA1c
[2] model 2: additional adjustments for PTH, Calcium and Phosphate

Discussion

We have shown that vitamin D deficiency is an independent risk factor for SCD, CVE and all-cause mortality in diabetic haemodialysis patients. These associations were independent of common known risk factors. In patients with severe vitamin D deficiency there was also a remarkable trend towards increased risks of stroke and fatal infections.

Our study is the first to highlight the role of vitamin D deficiency as a risk factor of adverse long-term outcomes in diabetic haemodialysis patients. These data are in line with observations in other cohorts of CKD patients,[2-7] in patients referred for coronary angiography,[13,14] as well as in population based cohorts.[16-19] These latter studies showed an increased risk of mortality and/or CVE in individuals with low 25(OH)D levels.[2-7,13,14,16-19] Given that most haemodialysis patients are vitamin D deficient, we believe that our findings might have significant clinical implications when considering that natural vitamin D supplementation is considered a relatively safe, easy and cheap therapy.[20] We are aware of a history of promising data on risk factors of mortality in haemodialysis patients leading to the initiation of RCTs which failed to show significant effects of targeted treatments.[21] Without claiming causality for our findings we want to stress that vitamin D exerts various effects which might, in a causal manner, underlie harmful consequences of vitamin D deficiency.[11]

First, we want to point out that classic effects of vitamin D related to calcium and phosphorus homeostasis as well as PTH regulation might of course play an important role for cardiovascular risk in CKD.[22] Reduced vitamin D metabolites lead to hypocalcaemia and secondary hyperparathyroidism, which is associated with increased mortality risk.[12,23] In this context, previous studies indicate that natural vitamin D or 25(OH)D supplementation might have beneficial effects on mineral metabolism including reductions in PTH levels. [12,24] Results on this latter topic are, however, inconsistent and further studies are required.[12,24] Interestingly, our prospective results did not materially change even after adjustments for various parameters of mineral metabolism suggesting that other mechanisms might have mainly driven the association of low 25(OH)D and adverse outcomes in the 4D study. Associations of vitamin D deficiency with cardiovascular risk factors including type 2 diabetes mellitus,[25] arterial hypertension,[26] malnutrition and inflammation may hypothetically explain the increased mortality risk in patients with low 25(OH)D levels. Adjustments for these latter risk factors had, however, only little impact on our prospective analyses. Hence other mechanisms may be relevant. Data from the Multi-Ethnic Study of Atherosclerosis suggest that vitamin D deficiency is prospectively associated with increased risk of

coronary artery calcification.[27] This relationship seemed to be stronger for patients with lower estimated GFR and there was no significant association of 1,25(OH)2D and coronary artery calcification.[27] Vitamin D might exert direct anti-atherosclerotic effects on endothelial and vascular smooth muscle cells as well as on macrophages whose foam cell formation was inhibited by 1,25(OH)2D.[28,29] The strong association of vitamin D deficiency with SCD but not with MI might, however, suggest that atherosclerosis related to vitamin D deficiency might not be the main pathophysiological link for our findings. Direct vitamin D effects on the myocardium, which expresses the VDR as well as 1α-hydroxylase,[30,31] may therefore be of importance. Experimental animal studies revealed myocardial hypertrophy and dysfunction with a hypercontractile state in both conditions of vitamin D deficiency as well as in VDR knockout models,[11,32,33] even if VDR knockout was exclusively performed in cardiomyocytes.[34] Clinical studies confirmed associations of vitamin D deficiency with CHF and in particular with diastolic dysfunction but our results regarding heart failure deaths, which are limited by relatively low numbers of events, do not support an important role of vitamin D in this context.[30,35,36] Furthermore, altered myocardial calcium flux and increased risk of SCD related to a poor vitamin D status suggest a link to cardiac arrhythmias.[13,30] This notion is in line with observations in haemodialysis patients showing that calcitriol reduced a prolonged QT interval, which is a risk factor for SCD, the single largest cause of death in dialysis patients.[37,38] Apart from this, detrimental consequences of vitamin D deficiency might also be mediated by an increased risk of infections, which is supported by our data showing an important trend for fatal infections in patients with severe vitamin D deficiency.[39]

Our data should be viewed in light of a meta-analysis, which showed significantly improved survival of natural vitamin D supplementation in individuals without end-stage renal failure.[40] We are aware that findings from RCTs among patients free of advanced CKD cannot be uncritically extrapolated to haemodialysis patients.[12,21] We also want to underline that physicians, impressed by previous data in favour of multiple health benefits of natural vitamin D and by the magnitude of the present observed associations should not abstain from future RCTs, which are urgently needed. Unless these trial results are published our vitamin D

prescription among haemodialysis patients should be guided by considerations that weight the probable benefit versus the probable risks and costs of this therapy. Supplementation of natural vitamin D to reach proposed optimal 25(OH)D levels of 30 to 60 ng/mL is considered safe and it should be kept in mind that sunbathing can produce up to 20,000 IU vitamin D per day.[8,9,20] This latter dose is much higher than required to reach 25(OH)D target levels by using the rule of thumb that 1,000 IU vitamin D can increase 25(OH)D levels by approximately 10 ng/mL in patients without severe CKD.[8,9,20]

Our data are limited due to the observational nature of our study which precludes any conclusion regarding cause and effect relationships of our observed results. Despite extensive adjustments we cannot exclude residual confounding. Another drawback of our work is that due to lack of data we were not able to study interactions and confounding by levels of 1,25(OH)2D or fibroblast growth factor-23 (FGF-23) which suppresses 1,25(OH)2D synthesis.[41] Associations of 25(OH)D and outcome measures were, however, independent of parameters of mineral metabolism and the use of active vitamin D treatment.

In conclusion, we observed that low 25(OH)D levels are associated with increased risks of SCD, CVE and mortality, and there was furthermore a trend for higher risks of stroke and fatal infections. The magnitude of the observed associations, as well as previous data in favour of multiple health benefits of natural vitamin D, point to the urgent need for an RCT. Such study can clarify whether the relatively easy, safe and cheap supplementation therapy with vitamin D can decrease adverse outcomes, in particular SCD, in haemodialysis patients.

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CHAPTER

10

Vitamin D status and clinical outcomes in incident dialysis patients: results from the NECOSAD study

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Abstract

Objective: The majority of dialysis patients suffer from vitamin D deficiency, which might contribute to an adverse health outcome. We aimed to elucidate whether European dialysis patients with low 25-hydroxyvitamin D (25[OH]D) levels are at increased risk of mortality and specific fatal events.
Design: Prospective cohort study of incident dialysis patients in the Netherlands (NECOSAD).

Methods: We selected all patients with measured 25(OH)D at 12 months after the start of dialysis, the baseline for our study. By Cox regression analyses, we assessed the impact of 25(OH)D levels on short-term (6 months follow-up) as well as long-term mortality (3 years follow-up). Associations of 25(OH)D levels with cardiovascular and non-cardiovascular mortality were also determined.

Results: Data from 762 patients (39% females, age 59±15yr, 25(OH)D=18±11 ng/ml) were available. Fifty-one and 213 patients died during a follow-up of 6 months and 3 years, respectively. After adjustments for possible confounders the hazard ratio (HR) (with 95% CI) for mortality was 1.9 (1.0-3.6) for short-term and 1.4 (1.0-1.9) for long-term mortality when comparing patients with 25(OH)D levels ≤ 10 ng/ml with those presenting with 25(OH)D levels > 10 ng/ml. Adjusted HRs for cardiovascular mortality were 2.8 (1.1-6.7) and 1.6 (1.0-2.5) for early and long-term mortality, respectively. For non-cardiovascular mortality we observed no relevant association.

Conclusions: Vitamin D deficiency in dialysis patients is associated with an adverse health outcome, in particular with short-term cardiovascular mortality. Intervention studies are urgently needed to evaluate whether vitamin D supplementation improves health outcomes of dialysis patients.

Introduction

Accumulating evidence supports the hypothesis that vitamin D deficiency might contribute to the extraordinary high mortality risk among dialysis patients [1-8]. Most patients on maintenance dialysis suffer from vitamin D deficiency because ultraviolet-B (UV-B) induced vitamin D production in the skin, the main source for vitamin D, is usually limited due to reduced sun exposure and impaired dermal vitamin D synthesis [1-8]. This high prevalence of vitamin D deficiency is increasingly recognised as an important health issue because it has been shown that (i) approximately 3% of the human genome is regulated by the vitamin D endocrine system and (ii) that the vitamin D receptor (VDR) is almost ubiquitously expressed [2, 9]. Data from patients with and without chronic kidney disease (CKD) suggest that beyond its classic effects on bone and mineral metabolism, vitamin D may also protect against cardiovascular diseases, immune disorders or cancer [2, 9, 10].

The traditional view of vitamin D metabolism is that vitamin D is hydroxylated to 25-hydroxyvitamin D (25[OH]D) in the liver. Then, 25(OH)D is further converted to the most active vitamin D metabolite 1,25-dihydroxyvitamin D (1,25[OH]2D) by the enzyme 1α-hydroxylase in the kidney. Serum levels of 1,25(OH)2D, which are mainly determined by renal production, are tightly regulated by parameters of mineral metabolism (i.e. parathyroid hormone [PTH], fibroblast growth factor 23 [FGF-23]), decline with lower glomerular filtration rate (GFR), and are usually not closely associated with 25(OH)D levels [2, 9, 10]. The discovery that apart from the kidney many other organs are also able to produce 1,25(OH)2D on a local level revolutionized our understanding of vitamin D physiology [9, 10]. Given that this extrarenal production of 1,25(OH)2D seems to be significantly dependent on substrate availability of 25(OH)D, the vitamin D status is classified according to circulating 25(OH)D levels [2, 10].

Previous studies among patients with CKD largely indicate that low 25(OH)D levels are associated with increased mortality and in particular with cardiovascular events [1, 4-8], but data among dialysis patients are sparse [5, 7, 8]. Wolf et al performed the largest study in this field and found that among 1000 incident hemodialysis

patients, low 25(OH)D levels were significantly associated with 90-day mortality in a nested case-control analysis [8]. Data on the association of 25(OH)D with long-term mortality in hemodialysis patients are unknown. The Vitamin D status and association with outcome is furthermore of interest in other patient populations, which meaningfully differ in duration or modality of dialysis, primary kidney disease prevalences and ethnic composition. There is also no previous study in CKD patients which addressed associations of vitamin D deficiency with both long-term as well as short-term outcome data for all-cause, cardiovascular, and non-cardiovascular mortality. Hence we aimed to assess the effect of 25(OH)D on morbidity and mortality in dialysis patients, analyzing data of a prospective multicenter cohort study of incident dialysis patients in the Netherlands.

Subjects and methods

Study Design

NECOSAD is an observational prospective follow-up study in which incident dialysis patients have been enrolled in 38 participating dialysis centers since 1997 in The Netherlands [11, 12]. Study visits took place at the start of dialysis, at 3 months, 6 months, and subsequently at 6 months intervals until the date of loss to follow-up (death, kidney transplantation, or transfer to a non-participating dialysis center) or the end of the follow-up at January 1, 2009. Baseline demographic and clinical data were obtained between four weeks prior to and two weeks after the start of long-term dialysis treatment. Blood and 24-hour urine samples were obtained at all visits. For the present analysis, baseline is defined as 12 months after the start of dialysis treatment, when the patients' fluid and metabolic conditions had stabilized and when adequate amounts of plasma material for laboratory measurements were available.

Patients

Patients with ESRD who were at least 18 years old and started long-term dialysis therapy for the first time were invited to participate in NECOSAD. In the present analysis, all patients with available blood samples to perform measurements of 25(OH)D at 12 months after initiation of dialysis were included. The medical ethical committees of the participating centers approved the study, and all patients gave their written informed consent before inclusion.

Data collection

Demographic and clinical data included age, sex, ethnicity, smoking habits, primary kidney disease and comorbidity. Primary kidney diseases and causes of death were classified according to the coding system of the European Renal Association – European Dialysis and Transplant Association (ERA-EDTA). Diagnoses of comorbid conditions were reported by the patients nephrologists and used to calculate the comorbidity score according to Khan. Plasma 25(OH) D levels were measured in samples taken at 12 months after the start of dialysis using a chemiluminescence-immunoassay on the Liaison autoanalyzer (DiaSorin, Saluggia/ Italy). This timepoint was chosen because of the adequate availability of plasma material and the measurements were performed centrally at the laboratory of the Department of Nephrology, University of Aachen, Germany. Plasma calcium, phosphorus, intact PTH, total alkaline phosphatase and albumin were measured by standard laboratory techniques in the different centers. Height and weight were measured after dialysis sessions, and BMI was calculated as weight (kilograms) divided by height (meters) squared. Blood pressure was measured in the sitting position.

Definition of endpoints

Cardiovascular mortality was defined as death due to the following causes: myocardial ischemia and infarction; hyperkalemia; hypokalemia; cardiac arrest; (hypertensive) cardiac failure; fluid overload; cerebrovascular accident; haemorrhage from ruptured vascular aneurysm; mesenteric infarction; cause of death uncertain/unknown. All other causes of death were designated as non-cardiovascular mortality.

Statistical analyses

Mean values with standard deviations (SD) were calculated for continuous variables, and median values with interquartile range (IQR) as appropriate. Categorical variables were expressed as proportions.

In line with widely used cut-off values, patients were categorized into severely vitamin D deficient (≤10.0 ng/ml), moderately vitamin D deficient (>10 ≤30 ng/ml), and vitamin D sufficient (>30 ng/ml). Due to the relatively low numbers of fatal events and due to results from previous studies suggesting that 10 ng/ml is an appropriate threshold to identify patients at high mortality risk, we mainly performed a categorization into only two groups: patients with 25(OH)D levels ≤ 10 ng/ml and patients with 25(OH)D levels > 10 ng/ml. These two groups were mainly used to assess the associations of 25(OH)D levels with all-cause mortality and death from cardiovascular and non-cardiovascular causes.

We calculated Cox proportional hazard ratios (HR) with 95% confidence intervals (95% CI) for subsequent short-term (6 months) and long-term (3 years) periods, according to 25(OH)D levels at baseline. In addition, we investigated conditional risks, i.e. the risks to die within 3 years, conditional on having survived the first half year. HRs were calculated for patients with 25(OH)D levels ≤ 10 ng/ml compared to those with higher 25(OH)D levels as well as for comparisons between the groups with severe vitamin D deficiency, moderate vitamin D deficiency and vitamin D sufficiency. The highest category of 25(OH)D was used as the reference group. We calculated a crude model and a model adjusted for potential confounders including age, sex, ethnicity, dialysis modality, primary kidney disease, diabetes mellitus, cardiovascular disease, blood pressure, body mass index, use of vitamin supplements, levels of serum albumin, creatinine and hemoglobin. To account for the seasonal variation of vitamin D, we furthermore adjusted our analyses for the season of blood draw. We therefore used a binary variable reflecting the months October to March and April to September, respectively. In order to explore possible pathways, we performed further analyses with additional inclusion of parameters of bone mineral metabolism including levels of calcium, PTH, phosphate and alkaline phosphatase. Finally, we tested potential interactions of Vitamin D with the use of vitamin supplements and with levels of alkaline phosphatase.

Cumulative mortality curves for CV mortality and non-CV mortality were made by competing risk analysis [13]. All p-values are reported two-sided, and considered significant at a level smaller than 0.05. Analyses were performed using SPSS version 16.0.

Results

Patients

A total of 1753 patients with ESRD who started long-term dialysis and were included, still participated in NECOSAD at 12 months after the initiation of dialysis therapy (baseline). Of those, vitamin D was measured in 762 patients, in whom the amount of collected blood was sufficient for the measurement of Vitamin D. These patients were included in the present analyses. Of note, the included patients were not different from the remainder (excluded patients 1753-762=991). Both patient groups were very similar with regard to demographic and clinical characteristics including co-morbidity and levels of routine laboratory markers.

In the study population (n=762), the mean (standard deviation) age was 59 (15) years and 61% of the patients were male. In general, the mean (standard deviation) level of 25(OH)D at baseline was 18.2 (11.0) ng/ml. As expected, we observed a seasonal variation of 25(OH)D in our patients, with the lowest concentrations in March (14.1 (6.9) ng/ml) and the highest concentrations in August (25.0 (13.0) ng/ml).

The patient characteristics are shown in Table 1. Significant findings at baseline were that with lower 25(OH)D levels, more patients had diabetes mellitus either as the primary kidney disease or as comorbidity. Patients on hemodialysis and males were less common in groups with lower 25(OH)D levels. Levels of alkaline phosphatase were higher in the patients with low 25(OH)D levels (for further baseline data see Table 1).

During the 3 years follow-up period, 213 patients died, of whom 118 patients died of cardiovascular causes, and 95 patients died of non-cardiovascular causes. Of

all deaths, 51 occurred in the short-term, i.e. within 6 months after baseline. These included 29 cardiovascular and 22 non-cardiovascular deaths.

Table 1. Baseline characteristics of the study population, and according to levels of 25(OH)Vitamin D

	whole group	**Vit D categories**		
		1	**2**	**3**
		VitD <=10	10-30	>30
Numbers	n=762	n=193	n=469	n=100
Age (yrs)	59 ± 15	57 ± 15	60 ± 15	57 ± 15
Male (%)	61	50	63	78
Dialysis modality (%HD)	64	55	68	76
Primary kidney disease				
Diabetes mellitus (%)	15	23	14	6
Glomerulonephritis (%)	15	12	15	21
Renal vascular disease (%)	17	16	17	15
Other (%)	53	49	54	58
Body mass index (kg/m²)	24.9 ± 4.1	25.0 ± 4.6	25.0 ± 3.8	24.6 ± 4.2
Blood pressure (mmHG)				
Systolic	149 ± 23	149 ± 24	150 ± 23	146 ± 22
Diastolic	83 ± 13	82 ± 13	83 ± 13	83 ± 13
Active smokers (%)	22	27	20	18
Vitamin supplementation (%)	94	92	94	94
Comorbidity				
Diabetes mellitus (%)	20	31	18	8
Cardiovascular disease (%)	32	27	35	25
Khan score				
low (%)	38	35	37	53
intermediate (%)	34	42	34	20
high (%)	28	24	29	28
GFR (mL/min per 1.73m²)	2.0 (0.7-3.9)	1.9 (0.7-4.2)	2.1 (0.7-4.0)	1.7 (0.5-3.3)
Laboratory values				
Hemoglobin (g/dL)	11.4 ± 1.4	11.2 ± 1.4	11.5 ± 1.4	11.4 ± 1.2
Albumin (g/L)	36 ± 6	35 ± 6	36 ± 6	36 ± 5
25-hydroxyvitamin D (ng/mL)	18 ± 11	8 ± 2	18 ± 5	40 ± 10
Alkaline phosphatase	78 ± 53	90 ± 72	75 ± 46	72 ± 38
Serum calcium (mmol/L)	2.4 ± 0.2	2.4 ± 0.3	2.4 ± 0.2	2.4 ± 0.2
Serum phosphate (mmol/l)	1.8 ± 0.5	1.8 ± 0.6	1.8 ± 0.5	1.9 ± 0.5
PTH (pmol/l)	13.0 (5.0-27.0)	14.0 (4.8-26.8)	12.2 (4.8-29.3)	13.1 (5.6-25.5)
Cholesterol (mmol/l)	4.85 ± 1.31	5.0 ± 1.5	4.9 ± 1.3	4.6 ± 1.2

Continuous data are expressed as means ± standard deviation and as medians with interquartile range, and categorical data are shown as percentages.
GFR = glomerular filtration rate

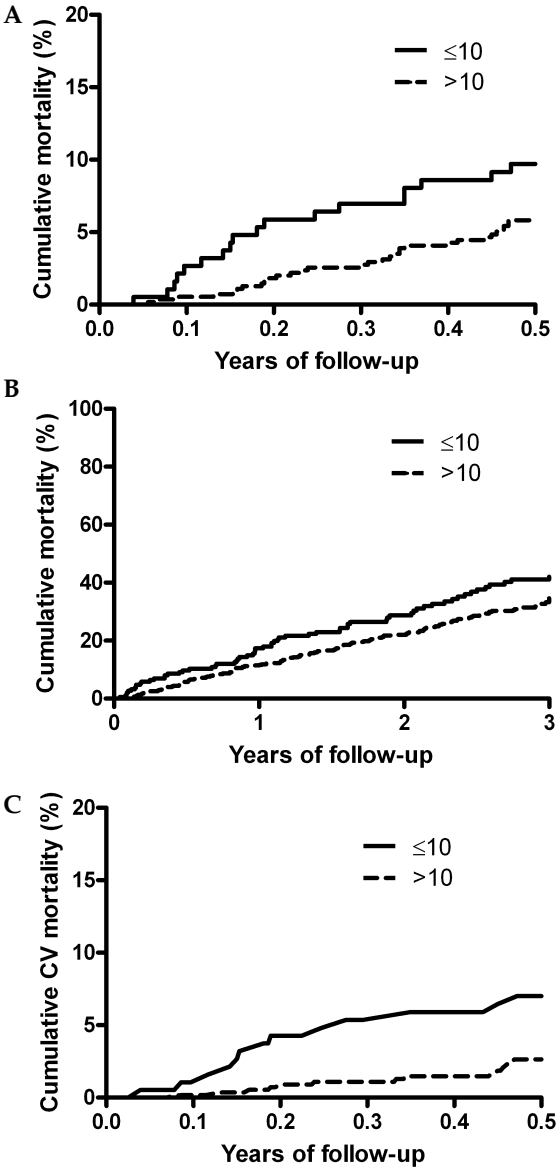
Vitamin D status and total mortality

We investigated short term and long term mortality according to vitamin D status. The respective follow-up intervals were 6 months and 3 years following the measurements of 25(OH)D. We observed marginally significant associations when comparing patients with severe vitamin D deficiency with all other study participants (Table 2 and Figures 1A, 1B). In detail, the adjusted HRs for the 6 months and 3 years follow-up periods were 1.89 (0.99-3.62, p=0.06) and 1.35 (0.95-1.91, p=0.09), respectively. Additional adjustments for parameters of bone mineral metabolism decreased these HRs to 1.53 (0.78-2.99) for the 6 months follow-up and to 1.34 (0.95-1.90) for the 3 years follow-up analyses. Conditional on having survived the first 6 months after assessment of the Vitamin D status, there was no meaningful increase in deaths for the severely deficient patients any more (conditional analyses, Table 2). Of note, there was no interaction observed between Vitamin D status and the use of vitamin supplements, nor with levels of alkaline phosphatase in the association with mortality. Using the three category approach for vitamin D status we observed a trend for an increased mortality in patients with severe vitamin D deficiency when compared to patients with vitamin D sufficiency (Table 3). Adjusted HRs for the group with severe vitamin D deficiency were 1.32 (95% CI: 0.48-3.61) for the 6 months follow-up and 1.16 (0.66-2.02) for the 3 years follow-up. These HRs remained materially unchanged after additional adjustments for parameters of bone mineral metabolism (data not shown).

Vitamin D status and cardiovascular mortality

Cardiovascular mortality was significantly increased in patients with severe vitamin D deficiency (Table 2 and Figures 1C, 1D). Compared to patients with 25(OH)D levels above 10 ng/ml the adjusted HRs were 2.75 (1.13-6.69, p=0.03) for the 6 months follow-up and 1.59 (1.00-2.54, p=0.05) for 3 the years follow-up (Table 2). After adjustments for parameters of bone mineral metabolism these HRs were 2.38 (0.93-6.13) and 1.60 (1.00-2.56) for the 6 months and the 3 years follow-up, respectively. Comparing patients with severe vitamin D deficiency with the vitamin D sufficient group the adjusted HRs were 2.50 (0.51-12.35) and 1.85

(0.79-4.33) for the 6 months and 3 years follow-up analyses, respectively. Further adjustments for parameters of bone mineral metabolism did not materially alter the results (data not shown).



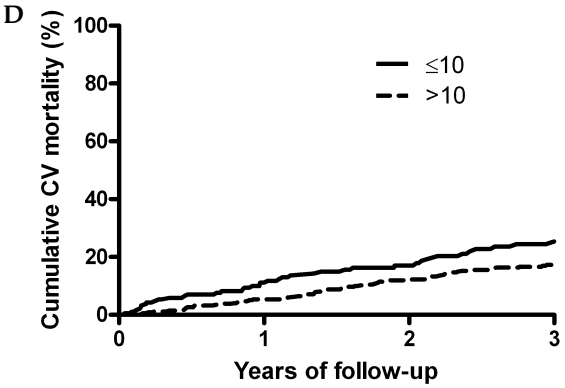


Figure 1 A-D: Cumulative mortality curves for A) all-cause mortality within 6 months, B) all-cause mortality within 3 years, C) cardiovascular mortality within 6 months and D) and cardiovascular mortality within 3 years according to Vitamin D status at baseline

Vitamin D status and non-cardiovascular mortality

There was no meaningful association of vitamin D status with non-cardiovascular mortality in any of our Cox regression analyses (Table 2 and Table 3). Compared to patients with 25(OH)D levels above 10 ng/ml, the risk of non-cardiovascular mortality was tentatively higher in patients with severe vitamin D deficiency with adjusted HRs of 1.23 (0.44-3.40, p=0.73) for the 6 months follow-up and 1.12 (0.67-1.87, p=0.67) for the 3 years follow-up (Table 2). Furthermore, no association was seen in the analyses comparing three categories of vitamin D status (Table 3). Additional adjustments for parameters of bone mineral metabolism did not materially change these results for the associations of 25(OH)D levels and non-cardiovascular mortality (data not shown).

Table 2. Hazard ratios with 95% confidence intervals for all-cause mortality, cardiovascular and non-cardiovascular mortality according to Vitamin D status: severely defieient vs non-severely deficient or sufficient

All-cause mortality				
		6 months	**3 years**	**conditional: between 6 months and 3 years**
crude	≤ 10	1.74 (0.98-3.11)	1.34 (1.00-1.80)	1.23 (0.88-1.73)
	>10	1	1	1
adjusted	≤ 10	1.89 (0.99-3.62)	1.35 (0.95-1.91)	1.29 (0.86-1.93)
	>10	1	1	1
Cardiovascular mortality				
		6 months	**3 years**	**conditional: between 6 months and 3 years**
crude	≤ 10	2.79 (1.31-5.93)	1.55 (1.06-2.28)	1.27 (0.81-2.01)
	>10	1	1	1
adjusted	≤ 10	2.75 (1.13-6.69)	1.59 (1.00-2.54)	1.42 (0.81-2.47)
	>10	1	1	1
Non-cardiovascular mortality				
		6 months	**3 years**	**conditional: between 6 months and 3 years**
crude	≤ 10	0.88 (0.33-2.39)	1.11 (0.70-1.75)	1.18 (0.71-1.98)
	>10	1	1	1
adjusted	≤ 10	1.23 (0.44-3.40)	1.12 (0.67-1.87)	1.17 (0.65-2.10)
	>10	1	1	1

* adjusted for age, sex, dialysis modality, ethnicity, primary kidney disease, diabetes mellitus, cardiovascular disease, body mass index, systolic blood pressure, use of vitamin supplements, levels of albumin, hemoglobin and creatinine, and for the seasonal variation of vitamin D

Table 3. Hazard ratios with 95% confidence intervals for all-cause mortality, cardiovascular and non-cardiovascular mortality according to levels of 25-hydroxyitamin D at baseline (severely vitamin D deficient, vitamin D deficient, vitamin D sufficient)

All-cause mortality				
		6 months	**3 years**	**conditional: between 6 months and 3 years**
crude	≤ 10	1.50 (0.60-3.78)	1.38 (0.86-2.23)	1.34 (0.77-2.34)
	10-30	0.83 (0.34-2.03)	1.04 (0.67-1.62)	1.11 (0.66-1.85)
	>30	1	1	1
adjusted	≤ 10	1.32 (0.48-3.61)	1.16 (0.66-2.02)	1.21 (0.62-2.34)
	10-30	0.66 (0.26-1.66)	0.85 (0.52-1.38)	0.93 (0.53-1.65)
	>30	1	1	1
Cardiovascular mortality				
		6 months	**3 years**	**conditional: between 6 months and 3 years**
crude	≤ 10	3.25 (0.73-14.40)	1.89 (0.87-4.12)	2.15 (0.88-5.21)
	10-30	1.20 (0.27-5.36)	1.53 (0.73-3.19)	1.82 (0.78-4.21)
	>30	1	1	1
adjusted	≤10	2.50 (0.51-12.35)	1.85 (0.79-4.33)	1.84 (0.68-5.02)
	10-30	0.97 (0.21-4.45)	1.18 (0.55-2.52)	1.32 (0.55-3.17)
	>30	1	1	1
Non-cardiovascular mortality				
		6 months	**3 years**	**conditional: between 6 months and 3 years**
crude	≤ 10	0.63 (0.17-2.33)	0.83 (0.44-1.57)	0.90 (0.43-1.89)
	10-30	0.65 (0.21-2.00)	0.70 (0.40-1.24)	0.72 (0.37-1.40)
	>30	1	1	1
adjusted	≤ 10	0.72 (0.18-2.87)	0.75 (0.35-1.60)	0.82 (0.34-2.00)
	10-30	0.53 (0.16-1.70)	0.64 (0.34-1.21)	0.67 (0.31-1.44)
	>30	1	1	1

* adjusted for age, sex, dialysis modality, ethnicity, primary kidney disease, diabetes mellitus, cardiovascular disease, body mass index, systolic blood pressure, use of vitamin supplements, levels of albumin, hemoglobin and creatinine, and for the seasonal variation of vitamin D

Discussion

Our data from incident dialysis patients show an increased mortality in patients with severe vitamin D deficiency compared to those without severe vitamin D deficiency. In analyses of specific fatal events we found a strong association of severe vitamin D deficiency with cardiovascular mortality, in particular for short term follow-up analyses. For non-cardiovascular mortality we observed no significant association with vitamin D status.

Our findings are of particular interest because the majority of dialysis patients is vitamin D deficient and it has been shown that the vitamin D endocrine system is involved in various pathophysiological processes beyond the classic vitamin D effects on bone health and mineral metabolism [2, 9, 10]. Vitamin D deficiency has been associated with cardiovascular diseases [14, 15], cancer [16], infections [17] and autoimmune diseases [18] but these data were largely derived from patients without end-stage renal disease. There is also increasing evidence that a sufficient vitamin D status might be renoprotective by inhibition of the renin-angiotensin-aldosterone system, decreasing proteinuria or anti-inflammatory properties [19, 20]. In dialysis patients, 1,25(OH)D or its analogues are frequently used and this therapy is associated with improved survival although it should be mentioned that further studies are still needed to establish the benefit of this active vitamin D treatment [21, 22]. Natural vitamin D supplementation, which increases 25(OH) D levels, is currently not an integral part of the treatment of dialysis patients although most of them are vitamin D deficient [1, 3-8]. However, even patients receiving active vitamin D treatment might benefit from natural vitamin D intake because in organs expressing 1α-hydroxylase, tissue levels of 1,25(OH)2D are mainly determined by local conversion of 25(OH)D to 1,25(OH)2D and not by circulating 1,25(OH)2D levels [10].

Whether natural vitamin D supplementation in dialysis patients reduces mortality and cardiovascular events is now the burning question. Current knowledge on this topic is based on only a few observational studies [5, 7, 8]. The NECOSAD study provides novel data, in particular because it is the first study among both chronic hemodialysis as well as peritoneal dialysis patients that addresses the association

of vitamin D status with short-term as well as long-term mortality. Our data showed that 25(OH)D were associated with overall survival, the effect sizes however being smaller compared to two previous studies investigating vitamin D deficiency in hemodialysis patients [5, 8]. Apart from a possible publication bias, we believe that underlying differences in the study populations (i.e. duration and mode of dialysis, primary kidney disease or follow-up time) might be a reasonable explanation for these partly differing results. Among 1000 hemodialysis patients Wolf et al reported about an association of low 25(OH)D levels with increased mortality within 90 days after initiating hemodialysis therapy, whereas the baseline examination for our present analysis was performed after 12 months of dialysis therapy [8]. In another obviously heterogeneous cohort of 102 hemodialysis patients, low 25(OH)D levels were also significantly associated with early mortality [5]. Consistent with our results, there was no significant association of 25(OH)D and mortality in a 3 year follow-up study of 230 Chinese patients with a median peritoneal dialysis duration of 26 months [7]. Taken together, the currently available literature suggests that vitamin D deficiency is a better predictor of short-term mortality than long-term mortality. This is supported by our results of the conditional analyses, showing no meaningful increase in deaths for the severely Vitamin D deficient patients any more, once they had survived the first 6 months after Vitamin D assessment. Considering that Vitamin D levels were determined only once in single measurements, potential changes in Vitamin D status over time may contribute to explain the time-differentiating effects, which remains to be investigated in future studies.

Interestingly, there was a significant association of vitamin D deficiency with increased risk of cardiovascular events in peritoneal dialysis patients [7], which is in line with our data and the findings by Wolf et al [8]. Hence, these data suggest a possible relationship between vitamin D deficiency and cardiovascular events. It is difficult to draw conclusions regarding causality from the observational NECOSAD study but accumulating evidence suggests that vitamin D supplementation might decrease cardiovascular risk [2, 9, 10, 23]. Beneficial vitamin D effects on CKD-mineral and bone disorders (CKD-MBD) (i.e. secondary hyperparathyroidism) might protect the cardiovascular system when considering

the observed associations of CKD-MBD and increased cardiovascular risk [24-26]. Concerning classic cardiovascular risk factors there exists evidence that vitamin D has anti-diabetic [27], anti-hypertensive [28] and anti-inflammatory properties [2, 9, 10, 19]. In addition, vitamin D effects seem to be important for the maintenance of normal myocardial structure and function [29]. In this context vitamin D deficiency has been associated with sudden cardiac death and heart failure [29, 30], in particular with diastolic dysfunction [31, 32]. However, when discussing a possible role of vitamin D in cardiovascular diseases it should also be underlined that despite careful adjustments of our analyses for various cardiovascular risk factors and parameters of bone mineral metabolism, 25(OH)D levels may simply be an indicator of a poor health status which is associated with malnutrition and reduced outdoor exposure leading to vitamin D deficiency.

Randomized controlled trials (RCTs) are therefore urgently needed to elucidate whether vitamin D supplementation in dialysis patients reduces cardiovascular risk or mortality. Waiting for the results of these RCTs we remain with the unanswered question whether we should prescribe natural vitamin D to our dialysis patients. Without raising a general recommendation, we want to stress that natural vitamin D doses to reach proposed target levels of 25(OH)D of 30 to 60 ng/mL (75 to 150 nmol/L) are considered absolutely safe when using the rule of thumb that 1,000 IU vitamin D increase 25(OH)D levels by 10 ng/mL (25 nmol/L) [33]. It should also be considered that vitamin D has been shown to exert multiple health benefits including a significant reduction in total mortality in a meta-analysis of RCTs, although we have to acknowledge that most of these data were derived from study cohorts without significant CKD [2, 9, 10, 16-18, 27-29, 34].

Our results are limited because it is difficult to draw conclusions regarding causality from an observational study. Furthermore, we have relatively wide confidence intervals for the results of our Cox regression analyses. Existence of residual confounding cannot be excluded despite careful adjustments of our statistical analyses. On the other hand it can be hypothesized that several covariates of our Cox regression models may partially lie in the causal pathway of adverse effects of vitamin D deficiency. The data on vitamin supplementation also include further vitamins apart from vitamin D, so that the exact percentage of

Vitamin D supplementation is expected to be lower. Other limitations of our study are missing data on 1,25(OH)2D and fibroblast growth factor-23 (FGF-23), which suppresses 1α-hydroxylase activity [35].

In conclusion we found an increased mortality risk in severe vitamin D deficient incident dialysis patients. Risk of cardiovascular mortality was strongly increased in patients with severe vitamin D deficiency, whereas there was no significant association with non-cardiovascular mortality. Associations of vitamin D status with adverse outcomes were more pronounced in short-term when compared to long-term follow-up analyses. RCTs are urgently needed to elucidate whether vitamin D supplementation reduces mortality and cardiovascular events in dialysis patients.

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CHAPTER 11

Summary and Discussion

In this thesis, metabolic alterations in dialysis patients have been adressed, and their consequences for the decline in residual kidney function, cardiovascular events and survival. Particular attention was thereby given to detect specific effects on –pathophysiologically different- events in dialysis patients, to provide explanations for conflicting results in the literature, and to provide a rationale for novel interventions.

With an annual mortality of about 20%, dialysis patients urgently require novel and effective treatment strategies. Results from the general population, indicating beneficial effects of interventions to control standard and novel risk factors, have been motivating. Although similarly expected to improve survival in the high risk population of dialysis patients, many trials however have been negative, not offering substantial improvements for cardiovascular and overall health by the tested treatments in dialysis patients.

This raises the question of why interventions failed to improve survival in this patient group. Understanding potential reasons is highly important as it may offer the possibility for designing new strategies.

With this aim, the thesis investigated the risk of metabolic alterations on adverse outcomes considering specific pathophysiologies in dialysis patients, and methodological challenges to reveal „true“ effects. One particular aspect thereby was the changing risk pattern in dialysis patients to be taken into account. While myocardial infarction represents the most frequent cause of death in the general population, dialysis patients mainly experience non-ischemic adverse events. With risk factors potentially differently affecting the various outcomes, the thesis investigated specific endpoints to get insight into relevant pathways and effects of metabolic alterations.

The need for such strategy is introduced in **chapter 2**, which outlines lipid disorders in patients with renal disease, clinical effects and current treatments. This review illustrates an abnormal lipid metabolism being common in patients

with the nephrotic syndrome and in patients with chronic kidney disease (CKD) or after kidney transplantation. Main abnormalities of lipid metabolism in the nephrotic syndrome include increases in total cholesterol, low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) cholesterol, apolipoproteins B, C-III and triglycerides, while apo-A1 is reduced. The profound disturbances in lipid metabolism in patients with chronic kidney disease include increased concentrations of triglyceride-rich lipoproteins, small dense and oxidized LDL, and impaired HDL maturation and catabolism. These alterations however are not captured by routine laboratory measurements. The classical guidelines on lipid lowering therapy –such as the national cholsterol education program adult panel III- cannot generally be applied to renal patients, as extrapolation of data from the general population may not meet the special disease pattern of patients with chronic kidney disease. Although post-hoc analyses of statin trials support the administration of statins in patients with early stages of chronic kidney disease (CKD stage 1-3), patients with advanced chronic kidney disease do not benefit to the same extent from lipid-lowering therapy. In particular, there is no rationale to start statin treatment in patients, once they require maintenance hemodialysis. The present guidelines (EBPG and K/DOQI) on the treatment of lipid disorders in patients with chronic kidney disease do not reflect the current evidence.

In **chapter 3**, Obesity was identified as a strong risk factor for the decline in residual renal function (RRF) after patients start dialysis. This finding is important because prior studies have shown that preservation of residual renal function in dialysis patients is associated with better survival. In summary, patients with normal weight had a mean decline of mGFR of 1.2 ml/min per year. Obese patients lost 1.2 ml/min *more* of their mGFR per year, reflecting a 100% higher loss of residual kidney function per year as compared to patients with normal weight. The relative risk to develop anuria was similar among the BMI groups after adjustment for confounders and baseline diuresis. This study provides a rationale for future interventional trials, which must show whether obese patients might benefit from a healthy weight reduction.

Chapter 4 focusses on poor glycemic control and its effects on cardiac and vascular outcomes. Cardiac disease represents the leading cause of death, particularly sudden cardiac death. Evidence suggests that poor glycemic control may impact in the arrhythmogenesis of patients with kidney failure, since it affects the development of comorbidities, the electrolyte balance, the function of potassium and calcium channels, and sympathetic activity. In the present study of 1255 hemodialysis patients with type 2 diabetes mellitus, poor glycemic control as represented by elevated levels of glycated hemoglobin A1c was associated with an increased risk of sudden cardiac death. Patients with a HbA1c above 8% had a twofold higher risk of sudden death compared to those with a HBA1c ≤6%, independently of other known risk factors. While myocardial infarction was not affected, the risks of the combined primary endpoint and mortality significantly increased at higher levels of HbA1c, and were mainly explained by the impact of glycemic control on sudden cardiac death. By pointing out the importance of distinguishing the –pathophysiologically different- causes of death, the present study offers new perspectives for future research on glucose control in populations with high incidences of sudden death. Importantly, these results may suggest novel therapeutic strategies in diabetic hemodialysis patients, in whom sudden cardiac death accounts for a quarter of all deaths. Whether tight glucose control decreases the risk of sudden death without causing side effects, remains to be studied.

In the context of body weight regulation, and related processes of insulin sensitivity, inflammation, and endothelial function, adiponectin represents a hormone of major interest. It is an adipocyte-specific cytokine with a protective role in the development of cardiovascular morbidities in the general population. As chronic kidney disease progresses, adiponectin levels increase and cardiovascular risk profiles change. In **chapter 5** we determined the association of baseline and longitudinal changes in adiponectin with different cardiovascular outcomes in 1255 type 2 diabetic hemodialysis patients in the German Diabetes and Dialysis Study. Within 4 years of follow-up, the hazard ratios to reach pre-specified, adjudicated end points were determined. The increased risk of cardiovascular events observed

with high adiponectin levels at baseline was associated with high risks of sudden death and stroke but not of myocardial infarction. Adiponectin was negatively correlated with C-reactive protein and positively correlated with NT-pro-BNP, the latter significantly attenuating the associations with adverse outcome. Increased longitudinal levels of adiponectin during follow-up were associated with higher risks of adverse cardiovascular outcomes and death; associations weakened by a confounding effect of increased NT-pro-BNP. The study importantly suggests that high basal and increasing adiponectin levels in the dialysis population largely reflect a consequence of disease circumstances. Most likely, this rise is a counter-regulatory response to worsening health in keeping with adiponectin's potential to counteract inflammation.

One very important aspect of „worsening health" in dialysis patients is the wasting syndrome. Wasting increasingly develops, as kidney function declines, and is a complex process of muscle loss, poor food intake, hypercatabolism and inflammation. The wasting syndrome is adressed in **chapter 6**. Known to be associated with a high mortality and cardiovascular events, the processes however are poorly understood and treatments are lacking. In chapter 6, we therefore studied the impact of wasting on specific clinical outcomes, including sudden cardiac death, myocardial infarction, and deaths due to infection in dialysis patients of the 4D study. Compared to the patients without wasting, patients with the wasting syndrome had a 3 fold increased risk of sudden cardiac death, which hardly attenuated after multivariable adjustment. There was a trend for increased risks of stroke and death due to infection, while myocardial infarction was not associated with wasting. The increased risk of combined cardiovascular events by 60 percent was mainly explained by the effect of wasting on sudden cardiac death, since no association was seen for combined cardiovascular events except sudden death. This finding of wasting being strongly associated with sudden cardiac death, but not with myocardial infarction in diabetic hemodialysis patients is important, as it suggests non-atherosclerotic cardiac disease to play a major role for the increased cardiovascular events in patients with wasting. This offers the potential of new treatment strategies, in which patients with the wasting

syndrome should be targeted in the prevention of sudden cardiac death. In this context, they may be considered for further treatments including ß-blocker or implantable cardioverter defibrillator therapy in addition to regular examinations.

Previous studies had identified wasting as a condition strongly affecting metabolic systems and altering conditions associated with outcomes (e.g. the impact of wasting on cholesterol metabolism and the associated mortality). Hints in the literature of a potential influence of wasting in parathyroid hormone metabolism lead to a clinical study presented in **chapter 7**. This study investigated the impact of wasting in the association of parathyroid hormone with cardiovascular events and mortality in dialysis patients. It revealed that the negative effects of a high PTH are observed only in relatively healthy patients without the wasting syndrome, but are nullified in patients suffering from the disease state. The study contributes to explain conflicting results in the literature, showing that the association of parathyroid hormone with adverse outcomes depends on the presence or absence of the wasting syndrome, and more generally speaking on the disease state of the population studied. This finding that wasting was an effect modifier to play a role in the associations of PTH with adverse outcomes is supported by further research presented in **chapter 8**. Here, we performed a study investigating the associations of parathyroid hormone with body mass index, the longitudinal changes, and their effect on mortality in dialysis patients. The results show that PTH importantly varies with BMI, being lowest in underweight and highest in obese patients, and that this association applies to both diabetic and non-diabetic patients. The study points out the impact of nutritional status on PTH levels and associated outcome, as it shows that a decreasing PTH was associated with a high mortality only in the presence, but not in the absence of weight loss. With weight loss being an important part of the wasting syndrome, low and decreasing PTH levels may be symptoms of wasting, and should be considered with caution in dialysis patients.

Vitamin D status is essentially important in the regulation of PTH metabolism, but moreover suggested to play a meaningful role for myocardial and overall health. In **chapter 9**, we investigated the impact of vitamin D status on specific cardiovascular outcomes and fatal infections in haemodialysis patients, who participated in the German Diabetes and Dialysis Study (4D Study). Patients with severe vitamin D deficiency (25[OH]D ≤ 10ng/ml) had a 3fold higher risk of sudden cardiac death compared to those with sufficient 25(OH)D levels >30ng/ml. Furthermore, combined cardiovascular events and all-cause mortality were strongly increased by 80 and 70 percent, respectively. There was a trend for higher risks of stroke and fatal infection, while myocardial infarction and deaths due to heart failure were not considerably affected. The study provides a rationale for future interventions on nutritional Vitamin D supplementation, strongly supported by our further analyses outlined in **chapter 10**. We analyzed the impact of Vitamin D status on adverse outcome including all-cause, cardiovascular, and non-cardiovascular mortality in incident dialysis patients from the NECOSAD study, who included both hemo- and peritoneal dialysis patients, and patients with and without diabetes mellitus. Particular attention was given to potential time-differentiating risks in the short-term (6 months follow-up) and longer term (3 years follow-up). We found an increased risk of mortality in patients with severe vitamin D deficiency. In analyses of specific fatal events we found a strong association of severe vitamin D deficiency with cardiovascular mortality, in particular for short term follow-up analyses. For non-cardiovascular mortality we observed no meaningful association with vitamin D status.
Given that most haemodialysis patients are vitamin D deficient, these findings might have significant clinical implications when considering that natural vitamin D supplementation is considered a relatively safe, easy and cheap therapy. Randomized controlled trials (RCTs) are therefore urgently needed to elucidate whether vitamin D supplementation in dialysis patients reduces cardiovascular risk or mortality.

In summary, the clinical research performed in this thesis detected specific effects of metabolic alterations in dialysis patients. Since mortality comprises various causes of death, the composition of which is meaningfully different as

compared to the general population, this implies that particular interventions may not generally decrease deaths but only the specific outcomes being mediated by the targeted risk factors. One consequence should be that in interventional trials in dialysis patients, the endpoints ought to be chosen very critically and carefully. Another consequence is that dialysis patients potentially require a combination of treatments rather than single interventions, in order to decrease the –pathophysiologically different- problems resulting in overall deaths.

Secondly, the thesis contributes to explain conflicting results in the literature, as thorough methodological concepts were applied to dissect true effects from confounding, and considering effect modification as appropriate. As mentioned within the various chapters of this thesis, many observational studies in dialysis patients did not yield unequivocal results. With our major methodological challenge to provide risk estimates as accurate as possible based on current knowledge, we used an etiologic approach as recommended by clinical epidemiologists, and thereby considered the recommendations to distinguish between confounding and intermediate variables. Furthermore, the analyses were not based on forward or backward selection procedures, by which important confounders may be missed, or spurious factors (e.g. by chance large hazard ratio in the data) included. In line with the scientific epidemiological suggestions, we primarily approached pathophysiological effect sizes, using (apart from a crude model) multivariate adjustments with carefully selected variables based on clinical suspicion and literature knowledge to ascertain independent effects.

Third, the present work outlined in this thesis provides a strong rationale for novel interventions in dialysis patients. Considering the excess mortality and paucity of data for effective treatments in dialysis patients, randomized controlled trials are urgently needed. With new information on specific endpoints, methodological advances to approach true effects, and furthermore emerging new risk factors being addressed in this thesis, challenges to design and conduct promising interventional studies are ready to meet. By proving the hypotheses being generated in this work, dialysis patients may face novel treatments to substantially improve cardiovascular and overall health.

CHAPTER

12

Samenvatting

In dit proefschrift werden metabole veranderingen in dialysepatiënten behandeld en de gevolgen daarvan op achteruitgang in nierfunctie, cardiovasculaire aandoeningen en overleving. Hierbij werd extra aandacht gegeven aan op het opsporen van specifieke effecten op -pathofysiologisch verschillende- uitkomsten in dialysepatiënten, zodat verklaringen gegeven kunnen worden voor tegenstrijdige resultaten in de literatuur en om beweegredenen te geven voor nieuwe interventies.

Met een jaarlijkse sterfte van ongeveer 20% hebben dialysepatiënten dringend nieuwe en effectieve behandelstrategieën nodig. Resultaten vanuit de algemene bevolking, die gunstige effecten van interventies op standaard en nieuwe risicofactoren aantonen, zijn erg motiverend. Hoewel verwacht werd dat op eenzelfde manier de overleving van de hoogrisico populatie van dialysepatiënten verbetert zou worden, zijn echter veel experimentele onderzoeken tot nu toe negatief gebleven, zodat geen substantiële verbeteringen in de cardiovasculaire en de algehele gezondheid door middel van de onderzochte behandelingen in dialysepatiënten kon worden bereikt.

Dit roept de vraag op waarom interventies niet in staat bleken te zijn om de overleving in deze patiëntengroep te verbeteren. Het begrijpen van mogelijke redenen hiervoor is erg belangrijk, omdat dit de mogelijkheid biedt voor de ontwikkeling van nieuwe strategieën.

Met dit als doel, is in dit proefschrift het risico van metabole veranderingen op ongunstige uitkomsten onderzocht, rekening houdend met de specifieke pathofysiologische processen in dialysepatiënten en methodologische uitdagingen om 'echte' effecten te onthullen. Een belangrijk aspect daarbij was dat rekening gehouden moest worden met het veranderende risicopatroon in dialysepatiënten. Terwijl myocardinfarct de meest voorkomende doodsoorzaak is in de algemene populatie, lijden dialysepatiënten voornamelijk aan niet-ischemische uitkomsten. Rekening houdend met het feit dat risicofactoren mogelijk verschillende effecten kunnen hebben op de diverse uitkomsten, zijn in dit proefschrift specifieke

eindpunten onderzocht om inzicht te krijgen in relevante mechanismen en effecten op metabole veranderingen.

De noodzaak voor een dergelijke strategie is geïntroduceerd in **hoofdstuk 2**, dat lipiden-afwijkingen in patiënten met een nierziekte, klinische effecten en huidige behandelingen schetst. Dit review-artikel illustreert dat een abnormaal lipiden-metabolisme veel voorkomt in patiënten met het nefrotisch syndroom en in patiënten met een chronische nierziekte (CKD) of na een niertransplantatie. De belangrijkste afwijkingen in het lipiden-metabolisme in het nefrotisch syndroom zijn een toename in het totale cholesterol, low-density lipoprotein (LDL) en very low-density lipoprotein (VLDL) cholesterol, apolipoproteïne B, C-III en triglyceriden, terwijl apo-A1 is afgenomen. De ernstige verstoringen in het lipiden-metabolisme in patiënten met een chronische nierziekte zijn toegenomen concentraties van triglyceriden-rijke lipoproteïnen, lage dichtheid en geoxideerd LDL en verslechterde HDL vorming en afbraak. Deze veranderingen worden echter niet waargenomen bij routine laboratorium bepalingen. De klassieke richtlijnen voor lipiden-verlagende therapie, zoals de 'National cholesterol education program adult panel III', kunnen niet in het algemeen worden toegepast op nierpatiënten, omdat extrapolatie van gegevens van de algemene populatie mogelijk niet overeenkomt met het specifieke ziektepatroon van patiënten met een chronische nierziekte. Hoewel post-hoc analyses van experimentele onderzoeken naar statines ondersteunen dat statines gegeven moeten worden aan patiënten in de begin stadia van chronische nierziekte (CKD stadium 1-3), hebben patiënten met een vergevorderde chronische nierziekte geen baat bij dezelfde mate van lipiden-verlagende behandeling. Voornamelijk is er geen grondreden om te starten met statines in patiënten wanneer zij eenmaal chronische hemodialyse nodig hebben. De huidige richtlijnen (EBPG en K/DOQI) voor de behandeling van lipiden-afwijkingen in patiënten met een chronische nierziekte laten het huidige bewijs nog niet zien.

In **hoofdstuk 3** werd obesitas geïdentificeerd als sterke risicofactor voor de achteruitgang in restnierfunctie (RRF) nadat patiënten met dialyseren zijn gestart. Deze bevinding is belangrijk omdat eerdere studies hebben aangetoond

dat handhaving van restnierfunctie in dialysepatiënten is geassocieerd met een betere overleving. Samengevat, patiënten met een normaal gewicht hadden een gemiddelde achteruitgang in mGFR van 1.2 ml/min per jaar. Obese patiënten verloren 1.2 ml/min meer van hun mGFR per jaar, wat betekent dat zij een 100% groter verlies van restnierfunctie per jaar hebben vergeleken met patiënten met een normaal gewicht. Het relatieve risico voor het ontwikkelen van anurie was gelijk tussen de BMI groepen na correctie voor confounders en diurese op baseline. Deze studie geeft een grondreden voor toekomstige interventie trials, die moeten aantonen of obese patiënten baat zouden kunnen hebben bij een gezonde gewichtsafname.

Hoofdstuk 4 focust op slechte handhaving van bloedsuiker levels en de effecten daarvan op cardio- en vasculaire uitkomsten. Hartziekten, voornamelijk plotselinge hartdood, zijn de belangrijkste doodsoorzaak. Er is bewijs dat suggereert dat een slechte handhaving van bloedsuiker levels invloed kan hebben op het ontstaan van aritmiën in patiënten met nierfalen, omdat het de ontwikkeling van comorbiditeiten, de elektrolyten balans, de functie van kalium en calcium kanalen en sympathische activiteit beïnvloed. In deze studie onder 1255 hemodialysepatiënten met type 2 diabetes mellitus, was een slechte handhaving van bloedsuiker levels, gerepresenteerd door verhoogde levels van geglyceerd hemoglobine A1c, geassocieerd met een verhoogd risico op plotselinge hartdood. Patiënten met een HbA1c boven 8% hadden een tweevoudig hoger risico op plotselinge hartdood vergeleken met patiënten met een HbA1c ≤6%, onafhankelijk van andere bekende risicofactoren. Hoewel myocardinfarct niet werd beïnvloed, was het risico van het gecombineerde primaire eindpunt en mortaliteit significant verhoogt bij hogere levels van HbA1c, dit werd voornamelijk verklaard door de invloed van handhaving van bloedsuiker levels op plotselinge hartdood. Deze studie biedt, door uit te leggen wat het belang is van het onderscheid maken tussen -pathofysiologisch verschillende- doodsoorzaken, nieuwe perspectieven voor toekomstig onderzoek naar glucose handhaving in populaties met hoge incidenties van plotselinge dood. Belangrijk is dat deze resultaten suggesties bieden voor nieuwe therapeutische strategieën in diabetische hemodialysepatiënten,

in wie een kwart van alle doodsoorzaken plotselinge hartdood is. Of een strikte handhaving van glucose levels het risico van plotselinge hartdood vermindert zonder enige bijwerking te veroorzaken dient verder onderzocht te worden.

Binnen de context van gewichtsregulatie en gerelateerde processen van insuline gevoeligheid, inflammatie en endotheel functie, is adiponectine een hormoon dat van groot belang is. Het is een adipocyte-specifiek cytokine met een beschermende rol in de ontwikkeling van cardiovasculaire morbiditeit in de algemene bevolking. Bij progressie van een chronische nierziekte nemen adiponectine levels toe en verandert het cardiovasculaire risico profiel. In **hoofdstuk 5** bepaalden we de associatie van baseline en longitudinale veranderingen in adiponectine met verschillende cardiovasculaire uitkomsten in 1255 type 2 diabetische hemodialysepatiënten in de Duitse Diabetes en Dialyse Study. Binnen 4 jaar follow-up werden de hazard ratio's om van tevoren gespecificeerde, beoordeelde eindpunten te behalen bepaald. Het verhoogde risico op cardiovasculaire eindpunten met hoge adiponectine levels op baseline was geassocieerd met hoge risico's op plotselinge hartdood en hersenbloeding, maar niet op myocardinfarct. Adiponectine was negatief gecorreleerd met C-reactive protein en positief gecorreleerd met NT-pro-BNP, de laatste bleek significant de associatie met ongunstige uitkomst te veranderen. Verhoogde longitudinale levels van adiponectine tijdens follow-up werden geassocieerd met hogere risico's op ongunstige cardiovasculaire uitkomsten en dood; associaties werden verzwakt door een confounding effect van toegenomen NT-pro-BNP. De belangrijke suggestie van deze studie is dat hoge basale en toegenomen adiponectine levels in de dialyse populatie grotendeels een gevolg zijn van de ziekte omstandigheden. Hoogstwaarschijnlijk is deze toename een counter-regulatoire respons op een verslechterde gezondheid om zo de potentie van adiponectine om inflammatie tegen te gaan te behouden.

Een zeer belangrijk aspect van "verslechtering van de gezondheid" in dialysepatiënten is het "wasting syndroom". Wasting ontwikkeld steeds vaker, als de nierfunctie afneemt en is een complex proces van spier verlies, slechte

voedsel inname, hyperkatabolisme en inflammatie. Het wasting syndroom wordt behandeld in **hoofdstuk 6**. Het is bekend dat het geassocieerd is met hoge mortaliteit en cardiovasculaire eindpunten, echter, de onderliggende processen zijn niet bekend en ook ontbreekt het aan behandelingen. In hoofdstuk 6 hebben we daarom de impact van wasting op specifieke klinische eindpunten bestudeerd, waaronder plotselinge hartdood, myocardinfarct en overlijden door infectie in dialysepatiënten van de 4D studie. Vergeleken met patiënten zonder wasting, hadden patiënten met het wasting syndroom een drievoudig verhoogd risico op plotselinge hartdood, wat bijna niet veranderde na multivariate adjustering. Er was een trend voor een verhoogd risico op hersenbloeding en sterfte door infectie, terwijl myocardinfarct niet was geassocieerd met wasting. Het 60% verhoogde risico op gecombineerde cardiovasculaire eindpunten werd voornamelijk verklaard door het effect van wasting op plotselinge hartdood, omdat geen associatie was gevonden voor gecombineerde cardiovasculaire eindpunten behalve plotselinge dood. Deze bevinding dat wasting sterk geassocieerd is met plotselinge hartdood, maar niet met myocardinfarct in diabetische hemodialysepatiënten is belangrijk, omdat het suggereert dat niet-atherosclerotische hartziekten een belangrijke rol spelen in de verhoogde kans op cardiovasculaire eindpunten in patiënten met wasting. Dit beidt mogelijkheden voor nieuwe behandelstrategieën, waarin patiënten met het wasting syndroom een belangrijke doelgroep zijn in de preventie van plotselinge hartdood. In deze context zouden bij hen verdere behandelingen met β-blokkers of geïmplanteerde defibrillators als toevoeging op de reguliere onderzoeken overwogen kunnen worden.

Eerdere studies hebben wasting geïdentificeerd als een conditie die metabole systemen sterk beïnvloed en condities die met uitkomsten geassocieerd zijn sterk verandert (bijv. de impact van wasting op cholesterol metabolisme en de daarmee geassocieerde mortaliteit). Suggesties in de literatuur van een mogelijke invloed van wasting op het parathyroid hormoon metabolisme hebben geleid tot een klinische studie die is gepresenteerd in **hoofdstuk 7**. Deze studie onderzocht de impact van wasting in de associatie van parathyroid hormoon met cardiovasculaire uitkomsten en mortaliteit in dialysepatiënten. Er werd onthuld dat de negatieve

effecten van een hoog PTH alleen geobserveerd worden in relatief gezonde patiënten zonder het wasting syndroom, maar worden opgeheven in patiënten die lijden aan de ziekte status. De studie draagt bij aan de verklaring van conflicterende resultaten in de literatuur, door aan te tonen dat de associatie van parathyroid hormoon en ongunstige uitkomsten afhangt van de aanwezigheid of afwezigheid van het wasting syndroom en meer in het algemeen van de ziekte status van de populatie die bestudeerd wordt. De bevinding dat wasting een effect modifier was door een rol te spelen in de associaties van PTH met ongunstige uitkomsten wordt ondersteund door nader onderzoek dat gepresenteerd is in **hoofdstuk 8**. Hierin voerden wij een studie uit naar de associatie van parathyroid hormoon met body mass index, de longitudinale veranderingen en hun effect op mortaliteit in dialysepatiënten. De resultaten tonen aan dat PTH in belangrijke mate varieert met BMI, het is het laagst is in patiënten met ondergewicht en het hoogst in obese patiënten en dat deze associatie geldt voor zowel diabetische als niet-diabetische patiënten. De studie laat de impact zien van voedingsstatus op PTH levels en de daarmee geassocieerde uitkomst, omdat het laat zien dat een verlaagd PTH was geassocieerd meer een hoge mortaliteit alleen in de aanwezigheid, maar niet in de afwezigheid van gewichtsverlies. Omdat gewichtsverlies een belangrijk onderdeel is van het wasting syndroom zijn lage en verlaagde PTH levels mogelijke symptomen van wasting en zullen deze zorgvuldig moeten worden bekeken in dialysepatiënten.

Vitamine D status is van essentieel belang in de regulatie van PTH metabolisme, maar lijkt bovendien een belangrijke rol te spelen voor wat betreft myocard en algehele gezondheid. In **hoofdstuk 9** onderzochten we de impact van vitamine D status op specifieke cardiovasculaire eindpunten en fatale infecties in hemodialysepatiënten die deelnamen aan de Duitse Diabetes en Dialyse Studie (4D Studie). Patiënten met ernstige vitamine D deficiëntie (25[OH]D ≤ 10 ng/ml) hadden een drievoudig hoger risico op plotselinge hartdood vergeleken met degenen met voldoende 25(OH)D levels >30 ng/ml. Bovendien waren gecombineerde cardiovasculaire eindpunten en all-cause mortaliteit sterk verhoogd met respectievelijk 80 en 70 procent. Er was een trend voor hogere

risico's voor hersenbloeding en fatale infectie, terwijl myocardinfarct en dood door hartfalen niet aanmerkelijk werden beïnvloed. De studie verschaft een gegronde reden voor toekomstige interventies naar vitamine D suppletie via voeding, wat extra wordt ondersteund door onze verdere analyses die beschreven zijn in **hoofdstuk 10**. We analyseerden de impact van vitamine D status op ongunstige uitkomsten waaronder all-cause, cardiovasculaire en niet-cardiovasculaire mortaliteit in incidente dialysepatiënten van de NECOSAD studie, waarin zowel hemo- als peritoneaal dialysepatiënten en patiënten met en zonder diabetes mellitus zijn geïncludeerd. Bijzondere aandacht werd geschonken aan mogelijk tijdsafhankelijke risico's op de korte termijn (6 maanden follow-up) en langere termijn (3 jaar follow-up). We vonden een verhoogd risico op mortaliteit in patiënten met ernstige vitamine D deficiëntie. In analyses naar specifieke fatale uitkomsten vonden we een sterke associatie van ernstige vitamine D deficiëntie met cardiovasculaire mortaliteit, vooral voor de korte termijn follow-up analyses. Voor niet-cardiovasculaire mortaliteit observeerden we geen betekenisvolle associatie met vitamine D status. Gegeven dat de meeste hemodialysepatiënten vitamine D deficiënt zijn, kunnen deze bevindingen mogelijk significante klinische betekenis hebben, overwegende dat natuurlijke vitamine D suppletie beschouwd wordt als een relatief veilige, gemakkelijke en goedkope therapie. Randomized controlled trials (RCTs) zijn daarom in het bijzonder nodig om op te helderen of vitamine D suppletie in dialysepatiënten het cardiovasculaire risico of mortaliteit verlaagt.

Samengevat, het klinische onderzoek dat in dit proefschrift is uitgevoerd heeft specifieke effecten van metabole veranderingen in dialysepatiënten aan het licht gebracht. Omdat mortaliteit bestaat uit verschillende doodsoorzaken, waarvan de samenstelling in belangrijke mate verschilt van die van de algemene populatie, betekent dit dat bepaalde interventies mogelijk niet alleen in het algemeen het aantal overlijdens verlaagt, maar alleen de specifieke uitkomsten die worden beïnvloed door de risicofactoren waarop de behandeling is gericht. Een consequentie zal zijn dat in interventie trials in dialysepatiënten de eindpunten erg kritisch en zorgvuldig gekozen zullen moeten worden. Een andere consequentie is

dat dialysepatiënten mogelijk een combinatie van behandelingen nodig hebben in plaats van enkele interventies om de -pathofysiologisch verschillende- problemen die leiden tot sterfte in het algemeen te verminderen.
Ten tweede, dit proefschrift draagt bij aan de verklaring van conflicterende resultaten in de literatuur, omdat grondige methodologische concepten
toegepast zijn om echte effecten van confounding te onderscheiden en om effect modificatie als mogelijkheid te kunnen beschouwen. Zoals is genoemd in de verschillende hoofdstukken van dit proefschrift, hebben veel observationele studies in dialysepatiënten niet geleidt tot gelijkluidende resultaten. Met onze grote methodologische uitdaging om zo accuraat mogelijke risico schattingen, gebaseerd op huidige kennis, te kunnen geven, gebruikten we een etiologische benadering zoals aanbevolen door klinische epidemiologen en hebben daarbij de aanbevelingen in overweging genomen om onderscheid te maken tussen confounding en intermediaire variabelen. Bovendien zijn de analyses niet gebaseerd op forward of backward selectie procedures, waarbij belangrijke confounders gemist zouden kunnen worden, of op onechte risicofactoren (bijv. door toeval een hoge hazard ratio in de data) die waren geïncludeerd. In lijn met de wetenschappelijk epidemiologische suggesties, hebben we voornamelijk pathofysiologische effect groottes benaderd door gebruik te maken van (naast ruwe modellen) multivariate adjustering met zorgvuldig geselecteerde variabelen, gebaseerd op klinische verdenking en kennis van de literatuur om onafhankelijke effecten te kunnen bepalen.
Ten derde, het onderzoek dat is beschreven in dit proefschrift biedt een sterke gegronde reden voor nieuwe interventies in dialysepatiënten. Overwegende de overmatige mortaliteit en schaarsheid van data over effectieve behandelingen in dialysepatiënten, zijn randomized controlled trials bijzonder nodig. Met nieuwe informatie over specifieke eindpunten, methodologische voordelen om echte effecten te benaderen en daarnaast het ontdekken van nieuwe risicofactoren die zijn behandeld in dit proefschrift, is het een grote uitdaging om veelbelovende interventie studies te ontwikkelen en uit te voeren. Door de hypothesen te bewijzen die aan dit proefschrift zijn ontleend, kunnen dialysepatiënten nieuwe behandelingen tegemoet zien die de cardiovasculaire en algehele gezondheid substantieel verbeteren.

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List of publications

(1) Pilz S, Tomaschitz A, **Drechsler C**, Ritz E, Boehm B, Grammer T, März W. Parathyroid hormone level is associated with mortality and cardiovascular events in patients undergoing coronary angiography. *European Heart Journal*, in press.

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Curriculum Vitae

Christiane Drechsler was born on February 18, 1978 in Plauen, Germany. After she passed secondary school (Abitur) in 1997 at the Gymnasium Lohr am Main, she attended Würzburg University to study medicine. During her medical studies, she was introduced to the field of clinical research and was involved in the German Diabetes and Dialysis Study at the Nephrology Unit of the University Hospital in Würzburg. Being attracted by clinical research, she performed her medical thesis under supervision of Prof. C. Wanner, working on family recruitment and phenotyping to study the heredity of diabetes mellitus and diabetic nephropathy. After her internships at the Universities of Oxford, Lausanne and Bern, she graduated with her final medical examination in November 2004.

As of December 2004, she worked as medical doctor and researcher at the Division of Nephrology at the University Hospital in Würzburg. Within the multicenter "Study of Heart and Renal Protection (SHARP)", she worked as coordinator, investigator and clinical monitor, recruiting study sites and patients, educating study nurses and supporting investigators. She entered her residency in internal medicine, taking care of patients of the University Hospital. Continuing her education in clinical research, she joined the Master of Science Programme in Clincal Epidemiology at the Netherlands Institute of Health Sciences (NIHES) at the Universities of Rotterdam and Leiden, graduating in 2007. Her Master thesis entitled "Obesity is a risk factor for decline of renal function after the start of dialysis" won the NIHES award for the best research paper of the academic year. A research fellowship from the European Renal Association-European Dialysis and Transplant Association enabled her to continue scientific work at the Department of Clinical Epidemiology of the Leiden University Medical Center, under the supervision of Prof. Friedo W. Dekker, Prof. Christoph Wanner, Prof. Frits Rosendaal and Dr. Diana Grootendorst. The findings of this research are described in this thesis. During her PhD work she collaborated with research groups in the Netherlands, Germany and Austria, and followed courses in epidemiology and biostatistics. In 2008, she was admitted to join the young investigators academy by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) and awarded a research grant to work on a clinical prediction study in dialysis patients. The research of her PhD thesis was presented at international congresses, where it received various awards. At present, Christiane is working at the Nephrology Unit of the University Hospital in Würzburg, continuing her residency and scientific work.