Nephrol Dial Transplant (2011) 26: 4054–4061
doi: 10.1093/ndt/gfr171
Advance Access publication 4 May 2011

Impact of vitamin A on clinical outcomes in haemodialysis patients

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Abstract

Background. Patients on maintenance haemodialysis treatment experience an excessive risk of cardiovascular disease and mortality. The vitamin A concentration is known to be higher in these patients compared to the general population where elevated vitamin A concentrations are associated with adverse outcome. The impact of vitamin A on morbidity and mortality in end-stage renal disease patients is controversial and is the topic of this study.

Methods. We analysed plasma retinol and retinol-binding protein 4 (RBP4) in 1177 diabetic haemodialysis patients, who participated in the German Diabetes and Dialysis Study (median follow-up 4 years). By Cox regression analyses hazard ratios (HRs) were determined for pre-specified, adjudicated end points according to baseline concentrations.

Results. Patients had a mean age of 66 ± 8 years, mean retinol and RBP4 concentrations of 3.28 (0.71–7.44) and 4.02 (1.28–10.1) μmol/L, respectively. Patients with retinol concentrations in the first quartile (<2.6 μmol/L) had an almost 2-fold increased risk of all-cause mortality compared to patients of the fourth quartile [>3.9 μmol/L; HR 1.81, 95% confidence interval (CI) 1.43–2.30]. There was a strong association between low retinol and the risk of sudden cardiac death (SCD, HR 2.22, 95% CI 1.41–3.50) and fatal infection (HR 2.19, 95% CI 1.26–3.82). Patients with RBP4 concentrations in the lowest quartile (<3.0 μmol/L) were more likely to die of any cause (HR 1.43, 95% CI 1.14–1.80), experience SCD (HR 1.97, 95% CI 1.28–3.03) and cardiovascular events (HR 1.43, 95% CI 1.10–1.85).

Conclusion. This large cohort study shows a strong association of low retinol and RBP4 concentrations with SCD and all-cause mortality in diabetic haemodialysis patients.

Keywords: haemodialysis; mortality; retinol; retinol-binding protein 4; sudden death

Introduction

Despite recent advances in renal replacement therapy, mortality of dialysis patients remains high. The ERA-EDTA reported a first-year mortality rate of 18% among European dialysis patients [1]. Particularly in diabetic patients, mortality is excessive with a 5-year survival rate of only 35% [2]. The development of atherosclerosis and cardiovascular disease (CVD) is one major cause for mortality. Sudden cardiac death (SCD) represents the most frequent event in these patients. Under diabetic conditions, the increased formation of reactive oxidative species (ROS) that result in endothelial dysfunction and chronic inflammation accelerates the progression of atherosclerosis and has been suggested as a potential risk factor for CVD [3].

Besides well-established factors such as chronic hyperglycaemia elevated free fatty acids and uraemic toxins, an impaired vitamin A metabolism might also have an impact on increased ROS formation, oxidative stress and oxidative tissue damage in diabetic patients with end-stage renal disease (ESRD) [4]. The concentrations of plasma retinol are increased in patients with kidney failure, which is mainly caused by a dysregulation of the plasma retinol homeostasis. Physiologically, retinol concentration is regulated by the hepatic synthesis of its specific carrier retinol-binding protein 4 (RBP4), a small visceral protein (21-kDa), which is catabolized by the kidney after delivering retinol to target tissues. Thus, the impaired renal catabolism of the retinol–RBP4 complex in consequence of kidney failure leads to an accumulation and abnormal supply of retinol to peripheral tissues in ESRD patients [5].

Surprisingly, one recent case–control study reported a significant association between low vitamin A plasma concentrations and carotid atherosclerosis in a small cohort of ESRD patients [6]. Furthermore, it could be shown that low retinol concentrations are associated with increased mortality in renal transplant recipients [7]. On the other hand, increased concentrations of plasma RBP4 were shown to be associated with the presence of microalbuminuria, chronic kidney disease and clinical atherosclerosis in type 2 diabetic patients [8-11].

Taken together, the current data on retinol and RBP4 metabolism in renal patients are inconclusive. Furthermore, the effects on specific clinical outcomes are unknown. The aim of this study, therefore, was to investigate components of the retinol transport complex and its association with specific adverse outcomes in haemodialysis patients. To that end, data of a large well-characterized cohort of diabetic haemodialysis patients were analysed [12].

Materials and methods

Study design and participants

The 4D study design has previously been reported in detail [13]. Briefly, the 4D study was a prospective randomized controlled trial including 1255 patients with type 2 diabetes mellitus (T2DM), age 18–80 years and on haemodialysis for <2 years. Between March 1998 and October 2002, patients were recruited in 178 dialysis centres in Germany. After 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 at baseline and regularly after randomization until the date of death, censoring or end of the study in March 2004. At each follow-up visit, a blood sample was taken and an electrocardiogram and clinical information including adverse events was recorded. Plasma was prepared by centrifuging blood and kept frozen at −80°C.

Definition of end points

The primary end point of the 4D study was defined as a composite of death from cardiac causes, stroke and myocardial infarction (MI), whichever occurred first. Death from cardiac causes comprised fatal MI (death within 28 days after an MI), SCD, 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; death by witnesses observed within 1 h after onset of cardiac symptoms; death confirmed by autopsy; unexpected death, presumably or possibly of cardiac origin and in the absence of a potassium level ≥7.5 mmol/L before start of the three most recent haemodialysis sessions. Stroke was defined as a neurologic deficit lasting longer than 24 h. Computed tomographic or magnetic resonance imaging was available in all but 16 cases. MI was diagnosed when at least two of three criteria were fulfilled: typical symptoms; elevated levels of cardiac enzymes; diagnostic changes in the electrocardiogram. 4D Study end points were centrally adjudicated by three members of the end point committee blinded to study treatment and according to predefined criteria [12].

For the present analysis, the primary end point of cardiovascular events (CVE), SCD, stroke, MI, death due to infection and all-cause mortality were chosen to be separate outcome measures and based on the primary judgment of the end point 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. Co-morbidities 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 a history of MI, coronary artery bypass grafting surgery and percutaneous coronary intervention, and as documented by angiography. CHF was defined according to the classification system of the New York Heart Association. Blood pressure was measured in a sitting position. Body mass index (BMI) was calculated as weight (kilogram) divided by height (metre squared).

Analytical determination of retinol and RBP4

Retinol was determined using a modified gradient reversed-phase HPLC-system (Waters, Eschborn, Germany) after organic extraction as previously described [14]. The detection limit for retinol was 2.0 ng, the coefficient of variation (CV) between runs was 4%, and the recovery rate was >95%. Concentrations of RBP4 in plasma were quantified using enzyme-linked immunosorbent assay as previously described [10]. The intra-assay CV was 2.9% and the inter-assay CV was 4.2%.

Statistical analysis

Patient characteristics are presented according to quartiles of retinol and RBP4 concentrations. Continuous variables were expressed as mean with standard deviation or median with interquartile range as appropriate, and categorical variables were expressed as percentages. First, we performed correlation analyses for retinol and RBP4 with selected parameters representative of nutrition and inflammation. The effects of retinol and RBP4 on clinical outcomes were assessed by Kaplan–Meier estimates for incidences

of the pre-specified end points. Furthermore, relative risks were derived from Cox regression analyses, i.e. hazard ratios (HRs) and corresponding 95% confidence intervals (CI). The Cox regression analyses were adjusted for the following confounders: age, gender, atorvastatin treatment, time on dialysis, smoking status, systolic blood pressure, CAD and levels of phosphate and glycohaemoglobin A1c. Second, we performed additional Cox regression analyses with inclusion of parameters reflecting lipid metabolism, heart disturbances and anaemia, nutrition and inflammation, which may represent intermediate conditions lying in the causal pathway of the effect of retinol on adverse clinical events. The variables were included stepwise in order to see the magnitude, by which the effect estimate for the risk of retinol on adverse events changed, respectively. The analyses to investigate the effect of RBP4 on adverse outcomes were performed in the same way. 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. 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. Retinol and RBP4 were measured in 1177 patients, in whom plasma was available at baseline. Of those, 579 patients died during follow-up, of whom 154 patients died of SCD and 118 due to infection. A total of 447 patients reached the CVE with MI and stroke occurring in 193 and 94 patients, respectively.

The median baseline concentrations of retinol were 3.18 μmol/L (range: 0.71-7.44 μmol/L) and of RBP4 3.84 μmol/L (range: 1.28–10.1 μmol/L). The baseline characteristics of all patients are shown in Table 1. Patients with higher retinol and RBP4 concentrations (fourth quartile) were predominantly male (67%), had a history of previous or current smoking, shorter duration of diabetes and lower percentage of CHF and arrhythmia compared to patients of the first quartile. With decreasing retinol and RBP4, parameters of nutritional status including total cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol were lower. Similarly, albumin and phosphate levels were lower with decreasing retinol and RBP4, while C-reactive protein (CRP) was elevated (Table 1).

Relation of retinol and RBP4 to BMI, albumin and CRP

In correlation analyses, retinol and RBP4 were highly related to each other (Pearson's correlation coefficient $r = 0.59$, $P < 0.001$). Retinol showed a strong correlation to albumin ($r = 0.38$, $P < 0.001$) and to CRP ($r = -0.25$, $P < 0.001$), but not to BMI ($r = 0.02$, $P = 0.483$). Similarly, RBP4 was strongly correlated to albumin ($r = 0.30$, $P < 0.001$), but considerably less to CRP ($r = -0.12$, $P < 0.001$). Again, no link between RBP4 and BMI was detected ($r = 0.007$, $P = 0.802$).

Furthermore, we investigated the levels of retinol and RBP4 in subgroups of patients with different CRP, albumin and BMI status. In patients with high CRP (highest CRP quartile >12.4 mg/L), mean retinol levels were lowest with 2.9 μmol/L as were the RBP4 levels with 3.8 μmol/L,

Table 1. Patient characteristics according to quartiles of baseline retinol and RBP4; study population $n = 1177$[a,b]

	Retinol (μmol/L)				RBP4 (μmol/L)			
Characteristic	≤ 2.6, ($n = 295$)	>2.6 ≤ 3.2, ($n = 295$)	>3.2 ≤ 3.9, ($n = 293$)	>3.9, ($n = 294$)	≤ 3.0, ($n = 296$)	>3.0 ≤ 3.8, ($n = 293$)	>3.8 ≤ 4.9, ($n = 294$)	>4.9, ($n = 294$)
Age, years	68 (7)	66 (8)	65 (8)	63 (8)	68 (8)	65 (8)	65 (8)	64 (9)
Gender, % male	39	48	61	67	44	52	59	60
BMI, kg/m^2	27.3 (5.0)	27.9 (5.0)	27.7 (4.6)	27.6 (4.7)	27.2 (4.9)	28.2 (5.0)	27.4 (4.6)	27.7 (4.8)
Atorvastatin treatment, %	51	48	49	49	50	52	52	49
Smoker/ex-smoker, %	33	40	44	44	36	42	39	45
Systolic BP, mmHg	143 (22)	148 (22)	149 (23)	145 (21)	145 (22)	146 (22)	147 (22)	146 (22)
Diastolic BP, mmHg	75 (11)	76 (11)	77 (12)	75 (11)	75 (10)	76 (11)	77 (12)	76 (11)
HbA1c, %	6.8 (1.3)	6.9 (1.2)	6.7 (1.2)	6.5 (1.2)	6.9 (1.3)	6.8 (1.3)	6.6 (1.2)	6.6 (1.2)
Duration of diabetes, years	19.4 (9.0)	19.0 (8.1)	17.6 (8.9)	16.7 (8.9)	19.2 (8.7)	19.2 (8.0)	17.9 (9.0)	16.3 (9.0)
Time on dialysis months	7 (6)	8 (7)	9 (7)	9 (8)	7 (7)	8 (7)	9 (7)	10 (7)
History of								
CAD, %	30	29	27	32	34	26	25	33
CHF, %	44	36	37	29	40	38	35	32
Arrhythmia, %	21	22	15	16	26	16	16	16
Lipid values, mg/dL								
Total cholesterol	208 (42)	218 (43)	223 (40)	228 (43)	206 (41)	219 (41)	221 (40)	232 (44)
LDL cholesterol	122 (30)	126 (31)	127 (30)	127 (29)	120 (29)	127 (29)	127 (30)	129 (31)
HDL cholesterol	37 (13)	36 (13)	36 (13)	36 (14)	37 (12)	36 (13)	37 (14)	36 (14)
Triglycerides	233 (145)	258 (167)	277 (178)	290 (72)	224 (136)	267 (171)	259 (157)	307 (189)
Phosphate, mg/L	5.5 (1.5)	6.0 (1.5)	6.2 (1.7)	6.4 (1.6)	5.5 (1.4)	5.9 (1.6)	6.2 (1.6)	6.5 (1.7)
CRP, mg/L	8.7 (3.8– 20.5)	5.4 (2.7–13.8)	4.2 (2.0– 9.4)	3.5 (1.5–7.7)	7.2 (3.4–20.6)	4.9 (2.6–11.7)	4.2 (1.7–9.6)	5.2 (2.0–11.1)
Albumin, g/dL	3.66 (0.30)	3.78 (0.30)	3.86 (0.28)	3.95 (0.27)	3.69 (0.31)	3.78 (0.29)	3.86 (0.27)	3.93 (0.29)

[a]Values are presented as means (SD) or median (interquartile range) or percentage.
[b]BP, blood pressure; HbA1c, glycated haemoglobin A1c; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

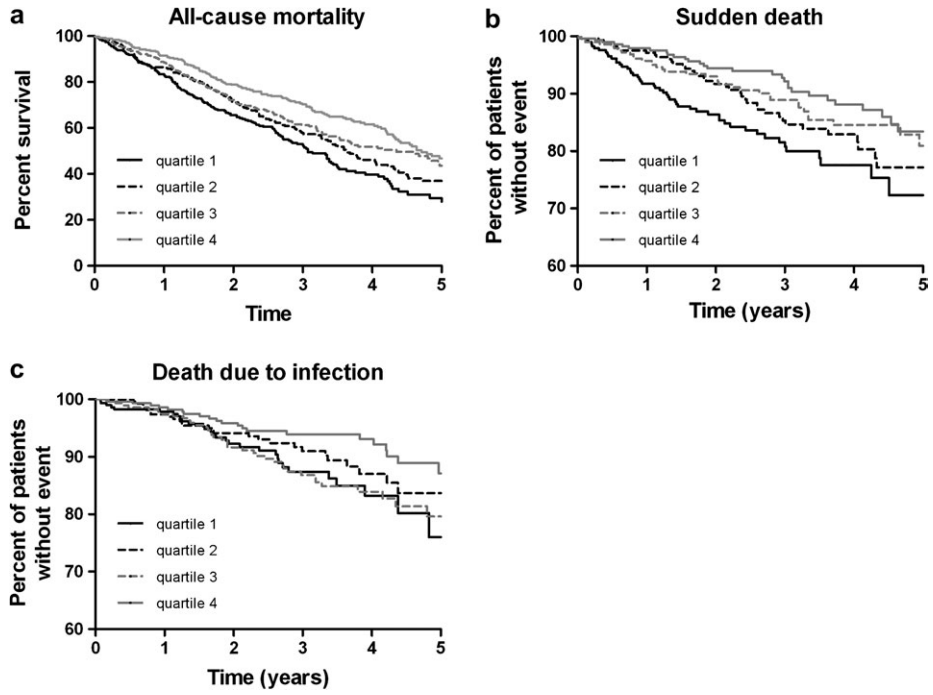

Fig. 1. Kaplan–Meier curves for the time to all-cause mortality (**a**) SCD (**b**) and death due to infection (**c**) in subgroups of patients according to baseline retinol plasma concentration (retinol Quartile 1: ≤2.6 μmol/L; Quartile 2: >2.6 ≤ 3.2 μmol/L; Quartile 3: >3.2 ≤ 3.9 μmol/L; Quartile 4: >3.9 μmol/L).

Table 2. HRs and 95% CI for all-cause mortality according to retinol and RBP4 (continuous variables) at baseline

	Retinol	P	RBP4	P
Crude model	0.79 (0.72–0.87)	<0.001	0.91 (0.86–0.97)	0.004
Model 1[a]	0.82 (0.75–0.91)	<0.001	0.93 (0.87–0.99)	0.030
Model 2[b]	0.85 (0.77–0.93)	0.001	0.95 (0.89–1.01)	0.083
Model 3[c]	0.85 (0.77–0.94)	0.002	0.96 (0.90–1.02)	0.184
Model 4[d]	0.98 (0.88–1.09)	0.687	1.01 (0.95–1.08)	0.693

[a]Main model: adjusted for confounders age, gender, atorvastatin treatment, time on dialysis, glycated haemoglobin A1c, phosphate, smoking status, CAD and systolic blood pressure.
[b]Model 1 plus additional adjustments for CHF, arrhythmia, haemoglobin.
[c]Model 2 plus additional adjustments for total, HDL and LDL cholesterol, triglycerides and BMI.
[d]Model 3 plus additional adjustments for albumin and CRP.

respectively. Retinol and RBP4 were in contrast highest with 3.6 and 4.3 μmol/L in patients of the first CRP quartile (≤2.3 mL/L).

Similarly, retinol and RBP4 levels were lowest (2.8 and 3.4 μmol/L, respectively) in patients with low albumin (first quartile ≤3.6 g/dL), while no meaningful difference in retinol and RBP4 levels was found over the categories of BMI. Combined analyses showed that patients with both a high CRP (Quartile 4) and low albumin (Quartile 1) had even lower retinol levels of 2.5 μmol/L and RBP4 levels of 3.2 μmol/L, with similar results being obtained from the subgroup of patients with all three conditions (high CRP, low albumin and low BMI) being present: retinol levels were 2.4 μmol/L and RBP4 3.0 μmol/L, respectively.

Retinol status and total mortality

Retinol status at baseline was significantly associated with all-cause mortality (see Figure 1). In unadjusted Cox regression analyses, the risk of death decreased significantly by 21% per unit increase (1 μmol/L) in retinol (HR 0.79, 95% CI 0.72–0.87; Table 2). The association was not meaningfully changed after adjustment for confounders, showing an 18% decrease in mortality per unit increase in retinol (HR 0.82, 95% CI 0.75–0.91). We especially evaluated further factors that potentially lie in the causal pathway and may mediate the effect of retinol on mortality (Table 2). With the stepwise inclusion of CHF, arrhythmia, haemoglobin, total and LDL and HDL cholesterol as well as BMI, the effect estimate did not meaningfully change (Table 2). With additional inclusion of CRP and albumin, however, and therewith 'taking out' these potential pathways by the adjustments, the risk of death was attenuated (Table 2), suggesting inflammation may play a major role in the mechanisms of retinol to affect mortality.

In categorical analyses, the unadjusted hazard to die was almost 2-fold higher in patients with low retinol concentrations (first quartile) as compared to those with high concentrations (fourth quartile) (HR 1.81, 95% CI 1.43–2.30), (Table 3). This association remained stable after controlling for confounders. Adjustments for the intermediate variables as mentioned above slightly reduced the effect estimate (HR 1.54, 95% CI 1.19–2.00), with additional adjustments for albumin and CRP attenuating the association (HR 1.08, 95% CI 0.82–1.43).

Table 3. Risk HR and 95% CI of combined CVE, sudden death, death due to MI, death due to stroke, death due to infection and all-cause mortality by quartiles of baseline retinol; study population $n = 1177$

	HR (95% CI) according to baseline retinol			
Outcome	≤2.6, ($n = 295$)	>2.6 ≤ 3.2, ($n = 295$)	>3.2 ≤ 3.9, ($n = 293$)	>3.9, ($n = 294$)
CVE				
Crude	1.33 (1.03–1.72)	1.20 (0.93–1.54)	0.75 (0.57–0.99)	1
Adjusted[a]	1.27 (0.96–1.66)	1.13 (0.87–1.47)	0.74 (0.56–0.98)	1
SCD				
Crude	2.22 (1.41–3.50)	1.50 (0.93–2.42)	1.27 (0.78–2.07)	1
Adjusted[a]	1.92 (1.18–3.11)	1.32 (0.81–2.15)	1.22 (0.74–2.00)	1
MI				
Crude	1.01 (0.68–1.50)	1.02 (0.70–1.48)	0.74 (0.49–1.10)	1
Adjusted[a]	1.07 (0.70–1.62)	1.06 (0.72–1.56)	0.78 (0.52–1.17)	1
Stroke				
Crude	1.51 (0.85–2.61)	1.51 (0.87–2.62)	0.62 (0.32–1.23)	1
Adjusted[a]	1.15 (0.62–2.12)	1.17 (0.66–2.06)	0.54 (0.27–1.07)	1
Death due to infection				
Crude	2.19 (1.26–3.82)	1.61 (0.91–2.84)	1.97 (1.15–3.37)	1
Adjusted[a]	2.28 (1.28–4.07)	1.61 (0.90–2.87)	1.91 (1.11–3.29)	1
All-cause death				
Crude	1.81 (1.43–2.30)	1.43 (1.12–1.81)	1.25 (0.98–1.60)	1
Adjusted[a]	1.67 (1.30–2.14)	1.32 (1.03–1.68)	1.19 (0.93–1.52)	1

[a]Analyses were adjusted for age, gender, atorvastatin treatment, time on dialysis, glycated haemoglobin A1c, phosphate, smoking status, CAD and systolic blood pressure.

Retinol status and risk of SCD, stroke, MI and combined CVE

Retinol concentration at baseline was strongly associated with risk of SCD (Table 3 and Figure 1). Patients of the lowest retinol quartile had a >2-fold increased risk of SCD compared to those of the highest quartile (unadjusted HR 2.22, 95% CI 1.41–3.50). Even after adjustment for confounders patients in the first retinol quartile showed a 92% greater hazard of SCD than patients of the fourth retinol quartile. There were moderate trends of low retinol to affect stroke and combined CVE, while no association with MI was found (Table 3).

Retinol and risk of death due to infection

Patients with plasma retinol concentrations in the first quartile had a 2.2-fold higher hazard for fatal infections than patients in the fourth retinol quartile (crude HR 2.19, 95% CI 1.26–3.82). The association became even stronger after adjustment for confounders (adjusted HR 2.28, 95% CI 1.28–4.07).

RBP4 and total mortality

Results of RBP4 were similar to that of retinol. RBP4 plasma concentrations at baseline were significantly associated with subsequent all-cause mortality (See Figure 2). Per unit increase in RBP4 (1 μmol/L), the risk of death decreased by 9% (HR 0.91, 95% CI 0.86–0.97; Table 2). The results remained stable after adjustment for confounders. Further adjustments for intermediate variables attenuated the effect, with inflammation as reflected by CRP and albumin hereby representing the major explanatory pathway (HR 1.01, 95% CI 0.95–1.08).

The results of the categorical analyses are shown in Table 4. Patients with RBP4 plasma concentrations of the first quartile had a 43% increased risk of death compared to those with high concentrations (fourth quartile) (HR 1.43, 95% CI 1.14–1.80), Table 4). After adjustment for confounders, this association was virtually unchanged.

RBP4 status and risk of SCD, stroke, MI, death due to infection and combined CVE

Similarly to retinol, RBP4 concentrations were strongly associated with the risk of SCD (Table 4 and Figure 2). Patients of the lowest RBP4 quartile had an almost 2-fold higher risk of SCD compared to those of the highest quartile (crude HR 1.97, 95% CI 1.28–3.03). Adjustment for confounders only slightly decreased this association (HR 1.74, 95% CI 1.10–2.74). There was a trend for an increased risk of stroke, while MI and deaths due to infection were not affected. Considering combined CVE, there was a 43% increased risk for patients in the lowest RBP4 quartile, which remained virtually unchanged after adjustments for confounders. Again, similar to the observations for retinol, intermediate variables including lipid status and particularly inflammation largely explained the effect of RBP4 on CVE by attenuating the association (HR 1.15, 95% CI 0.86–1.53).

Discussion

In this study, we investigated the retinol metabolism in 1177 haemodialysis patients with T2DM, who took part in the 4D Study and experienced a high incidence of prespecified and adjudicated end points. Our main findings were that plasma concentrations of retinol and RBP4 were elevated as compared to the general population. In outcome analyses, patients with higher retinol and RBP4 concentrations had a survival benefit. In this context, low concentrations of retinol were associated with increased

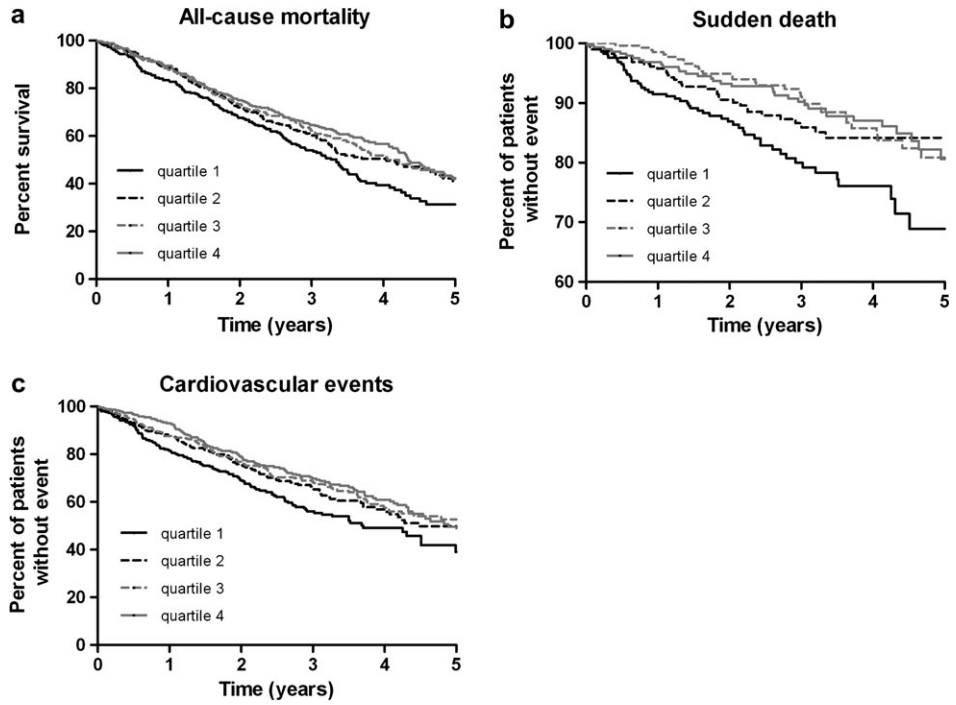

Fig. 2. Kaplan–Meier curves for the time to all-cause mortality (**a**) SCD (**b**) and CVE (**c**) in subgroups of patients according to baseline RBP4 plasma concentration (RBP4 Quartile 1: ≤3.0 μmol/L; Quartile 2: >3.0 ≤ 3.8 μmol/L; Quartile 3: >3.8 ≤ 4.9 μmol/L; Quartile 4: >4.9 μmol/L).

Table 4. Risk HR and 95% CI of combined CVE, sudden death, death due to MI, death due to stroke, death due to infection and all-cause mortality by quartiles of baseline RBP4; study population $n = 1177$

	HR (95% CI) according to baseline RBP4			
Outcome	≤3.0, ($n = 296$)	>3.0 ≤ 3.8, ($n = 293$)	>3.8 ≤ 4.9, ($n = 294$)	>4.9, ($n = 294$)
CVE				
Crude	1.43 (1.10–1.85)	1.07 (0.82–1.40)	1.00 (0.77–1.30)	1
Adjusted[a]	1.41 (1.07–1.84)	1.12 (0.86–1.47)	1.01 (0.78–1.33)	1
SCD				
Crude	1.97 (1.28–3.03)	1.15 (0.72–1.84)	0.94 (0.58–1.53)	1
Adjusted[a]	1.74 (1.10–2.74)	1.15 (0.71–1.86)	0.97 (0.60–1.58)	1
MI				
Crude	1.14 (0.76–1.70)	1.11 (0.75–1.63)	0.88 (0.59–1.31)	1
Adjusted[a]	1.25 (0.83–1.90)	1.23 (0.83–1.83)	0.90 (0.60–1.35)	1
Death due to stroke				
Crude	1.70 (0.96–3.03)	1.04 (0.56–1.93)	1.20 (0.66–2.17)	1
Adjusted[a]	1.45 (0.79–2.65)	0.96 (0.51–1.82)	1.15 (0.63–2.08)	1
Death due to infection				
Crude	1.04 (0.61–1.77)	1.00 (0.60–1.68)	1.19 (0.73–1.92)	1
Adjusted[a]	0.98 (0.56–1.71)	0.97 (0.57–1.63)	1.15 (0.71–1.86)	1
All-cause death				
Crude	1.43 (1.14–1.80)	1.09 (0.86–1.38)	1.06 (0.84–1.33)	1
Adjusted[a]	1.31 (1.03–1.67)	1.09 (0.86–1.39)	1.05 (0.83–1.33)	1

[a]Analyses were adjusted for age, gender, atorvastatin treatment, time on dialysis, glycated haemoglobin A1c, phosphate, smoking status, CAD and systolic blood pressure.

mortality, in particular, SCD and death due to infection. Similarly, low concentrations of RBP4 were related to higher risks of sudden and all-cause death and combined CVE.

Our finding that plasma concentrations of retinol and RBP4 were highly elevated in our patients as compared to the general population, are in line with results from previous studies addressing retinol status in patients with chronic kidney disease [5, 6, 8–11]. The mechanism by which physiological homeostatic regulated retinol is increased in plasma of haemodialysis patients is unknown so far. Apo-RBP4 (RBP4 unbound to retinol) is considered as a positive feedback signal from the periphery indicating the demand of target cells for retinol. Thus, an increased utilization of retinol by tissues results in increased apo-RBP4 levels stimulating the hepatic release of retinol. A loss of kidney function leads to a decreased filtration and degradation of RBP4 and a subsequent increase of apo-

RBP4 serum concentration which possibly triggers an enhanced retinol release from the liver [5, 15].

This study demonstrates a survival advantage in type 2 diabetic haemodialysis patients with high retinol concentrations compared to patients with lower retinol concentrations. It is important to note that low retinol plasma concentrations in haemodialysis patients are still above the recommended retinol plasma concentrations in healthy individuals. The significant association between low plasma retinol concentration and all-cause mortality persisted after adjustment for potential confounders. Investigations in other study cohorts of haemodialysis patients and renal transplant recipients have shown similar results demonstrating a higher risk for mortality, coronary heart disease and/or CVE with lower retinol concentrations [6, 7, 16]. Our study adds important new information, addressing specific end points. In this context, our study identified SCD and death due to infection as major targets of low retinol concentrations. This observation of a reverse association of vitamin A with adverse outcome is similar to that seen for BMI or adiponectin in haemodialysis patients [17, 18]. Several studies demonstrated a better survival of overweight or obese haemodialysis patients compared to those with normal weight or are underweight. In contrast to the general population, being underweight is considered as a major risk factor for mortality. Thus, highly elevated retinol concentrations could possibly facilitate survival advantages in haemodialysis patients. But what are the mechanisms behind that?

Vitamin A is important for normal morphology and function of epithelial cells in many organs of animals and humans. It regulates monocytic differentiation and function and influences the secretion of key cytokines by macrophages [19]. Therefore in vitamin A deficiency, the epithelial cells as first barrier to infection and the specific immune response are affected. Vice versa, inflammation results in a physiologic transient decline of vitamin A concentration during the acute phase response which returns to pre-infection levels within a few days [19–21]. Patients with ESRD are at a greater risk for inflammation due to uraemia and the haemodialysis treatment *per se* [22]. In line with these observations, a strong association between retinol and CRP as well as albumin concentrations was seen in this study. Interestingly, there was no association of retinol with BMI. Regarding the increased mortality of patients with low retinol levels, inflammation potentially plays a major role, as the association of retinol with adverse outcomes was attenuated after adjustment for albumin and CRP. In this context, the literature is not unequivocal. A study by Connolly *et al.* [7] (2007) demonstrated a significant survival advantage at higher retinol concentrations even after stratification for CRP in renal transplant patients and explained this by anti-inflammatory or anti-infective mechanisms. Apart from medication used in kidney transplantation, differences in design, size and study population need to be acknowledged, however, and may contribute to explaining the different results when comparing data from various studies.

With regard to molecular mechanisms, it is important to mention that retinol is a precursor for the synthesis of retinoic acid isomers that influence the transcription of several proteins by interacting with retinoic acid receptor and retinoid X receptor (RXR) nuclear receptors. One partner of RXR is the peroxisome proliferator-activated receptor-alpha (PPARα), which is expressed in heart and vascular cells. PPARα plays a role in controlling the myocardial fatty acid oxidation and prevents cardiac hypertrophy in animal studies [23–25]. Of note, cardiac hypertrophy is a major risk factor for SCD, the latter being strongly reduced at high concentrations of retinol in our study. In this context, the beneficial effect of high retinol may have been mediated by PPARα. Due to inhibition of inflammatory response by repressing nuclear factor kappa B and interleukin-1 as well as reduction of vascular cell adhesions molecule-1, PPARα maintain normal endothelial function [25]. Therefore, the activation of PPARα due to retinoid-activated RXR receptor can result in cardio-protective effects.

The results of the present study furthermore showed that lower RBP4 plasma concentrations were linked to a higher mortality and a higher risk for CVE and SCD. Kalousova *et al.* [16] demonstrated a positive effect of high RBP4 concentrations on overall and cardiovascular mortality in haemodialysis patients. Furthermore, inverse correlations of RBP4 with carotid artery echogenicity were observed in the elderly supposing an involvement of RBP4 in the development of atherosclerosis [26]. In contrast, high rather than low RBP4 levels were associated with co-morbidities including arteriosclerosis in patients with type 2 diabetes [11]. Therefore, the role of RBP4 is not clear in diabetic patients on haemodialysis. Our study provides new information, identifying low RBP4 as an important risk factor for adverse outcomes including combined CVE, mortality and specifically SCD in this patient population.

In conclusion, this investigation shows, for the first time, significant associations between low retinol plasma concentrations and a higher risks for all-cause mortality, SCD and infectious deaths in a large well-characterized cohort of haemodialysis patients with T2DM. Furthermore, similar associations were seen for low RBP4, being a strong risk factor for death, particularly SCD in these patients. Further investigations are needed to explore the underlying pathways in detail.

Acknowledgements. We thank all patients, investigators and study nurses who participated in the 4D Study. We are furthermore grateful to the Else-Kröner-Fresenius foundation for the support with a research grant. We also thank K. Blouin, L. Haeussler and A. Hurtienne for their excellent technical support.

Conflict of interest statement. None declared.

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Received for publication: 2.12.10; Accepted in revised form: 7.3.11