Metabolic alterations in dialysis patients

Drechsler, C.

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.

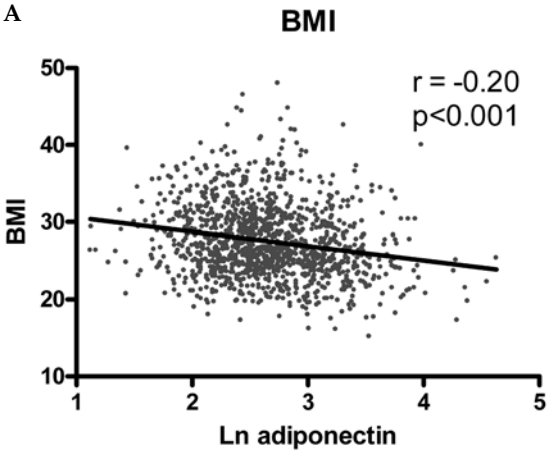
Subsequently, multivariate analyses were done with stepwise inclusion of confounders. Adjustments for age, gender, atorvastatin treatment, coronary heart disease, congestive heart failure, duration of type 2 diabetes mellitus, smoking status and blood pressure did not meaningfully change the association 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).

A



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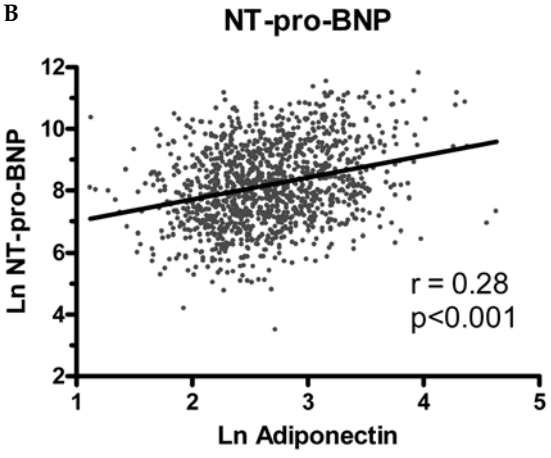


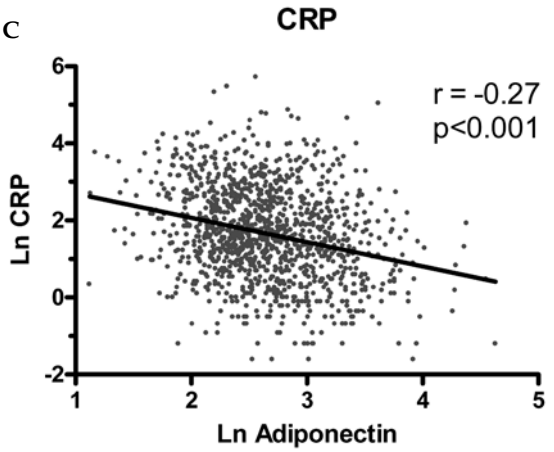


Figure 1a-c: Correlations of adiponectin (log transformed) with 1a) Body mass index, 1b) NT-pro-BNP (log transformed), 1c) C-reactive protein (log transformed)

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)

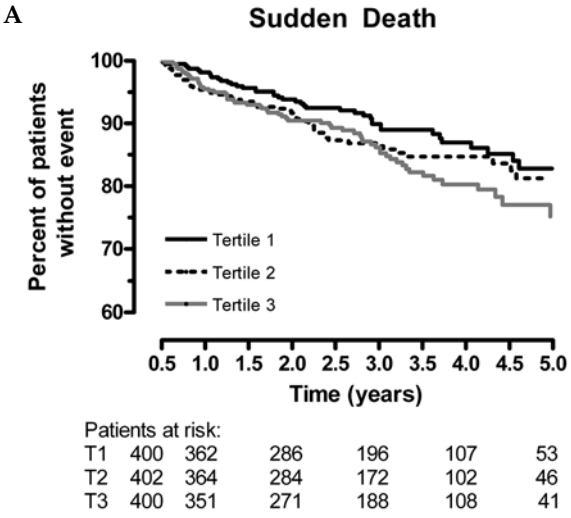
† Analyses were adjusted for age, gender, atorvastatin treatment, coronary heart disease, congestive heart failure, duration of type 2 diabetes mellitus, smoking status, systolic blood pressure, body mass index, N-terminal-pro-B-type natriuretic peptide‡

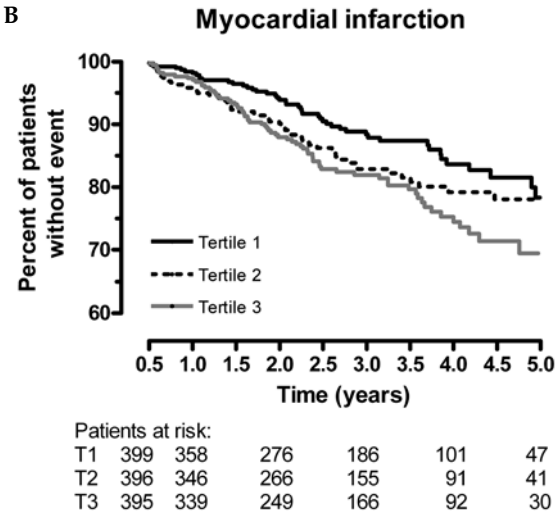
Change from baseline adiponectin and outcome

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.





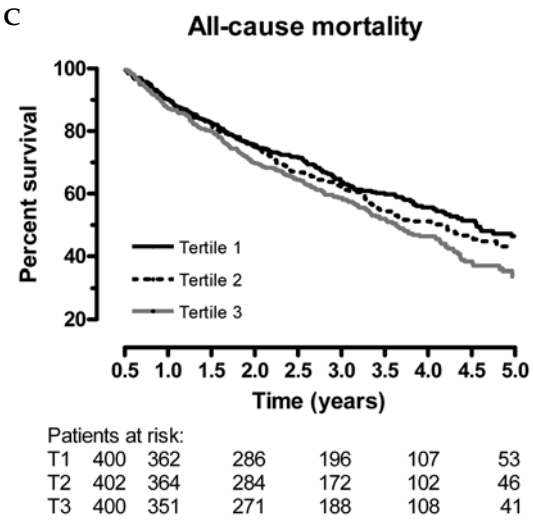


Figure 2a-c: Kaplan-Meier curves for the time to a) sudden death, b) myocardial infarction, and c) all-cause mortality by tertiles of the change in adiponectin from baseline. Tertile 1: patients with decreasing adiponectin greater than or equal to 12.8% (n=400), Tertile 2: patients with stable adiponectin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