Supplementary Material

Vitamin K1 and Progression of
Cardiovascular Calcifications in Hemodialysis Patients:
The VitaVasK Randomized Controlled Trial

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Table S1: VitaVasK Trial Synopsis

Protocol Title	Vitamin K1 to slow vascular calcification in hemodialysis patients (VitaVasK)
Study Code	VitaVasK
EudraCT No.	2010-021264-14
Study No.	10-003
Principal Investigators	• Prof. Dr. Jürgen Floege, RWTH Aachen University Hospital, Germany • Dr. Christoph Kopp, University of Erlangen, Germany • Prof. Dr. Michel Jadoul, University of Brussels, Belgium • PD Dr. Orfeas Liangos, Clinical Center of Coburg, Germany • Prof. Dr. Peter Stenvinkel, Karolinska University Hospital, Stockholm, Sweden • PD Dr. Ralf Westenfeld, University of Düsseldorf, Germany • Prof. Dr. Pieter Evenepoel, UZ Leuven, Belgium
Study Period	Recruitment Period: 6 years Intervention Period: 18 months
Phase	III
Objectives	The aim of the study is to show that a vitamin K1-based therapy attenuates the progression of thoracic aortic and coronary artery calcification compared to standard treatment
Study Design	Prospective, randomized, multicenter, multinational, controlled clinical trial using a two-arm parallel group design and 18-month treatment phase
Inclusion Criteria	• Male or female ≥ 18 years of age • Not less than 6 months on hemodialysis • Cardiovascular calcification present (coronary artery volume score > 100) • Written consent to take part in the study • Life expectancy not less than 18 months
Exclusion Criteria	• Known hypersensitivity against vitamin K1 • Intake of Vitamin K • History of thrombosis (except shunt occlusion) • Intake of vitamin K antagonists at baseline or in the 3 months prior to baseline • Inflammatory bowel disease • Short-bowel syndrome • Significant liver dysfunction • Any condition likely to impair vitamin K absorption (i.e. chronic pancreatitis) • Malignancy other than non-melanoma skin tumors • More than one stent in one coronary artery plus one or more stents in an additional artery

	- Hemoglobin < 70 g/L - Pulse > 100/min (resting heart rate) - Women who are pregnant or breastfeeding - Women without sufficient contraception - Alcohol or drug abuse - Mental condition rendering the subject unable to understand the nature, scope and possible consequences of the study - Subject unlikely to comply with protocol, e.g. uncooperative attitude, inability to return for follow-up-visits and unlikelihood of completing the study - Participation in a parallel clinical trial or participation in another clinical trial within the previous 3 months - Subjects who are in any state of dependency to the sponsor or the investigators - Employees of the sponsor or the investigators - Subjects who have been committed to an institution by legal or regulatory order
Treatment	Arm 1: Standard treatment (usual care) Arm 2: Vitamin K1 (phylloquinone), thrice weekly p.o. (5 mg)
Efficacy	Primary Endpoints: 1. Progression of thoracic aortic calcification (absolute change in the volume score at the 18-month MSCT versus the baseline MSCT) 2. Progression of coronary artery calcification (absolute change in the volume score at the 18-month MSCT versus the baseline MSCT) Secondary Endpoints: 1. Progression of thoracic aortic calcification (absolute change in the Agatston score at the 18-month MSCT versus the baseline MSCT) 2. Progression of coronary artery calcification (absolute change in the Agatston score at the 18-month MSCT versus the baseline MSCT) 3. Progression of aortic valve calcification (absolute change in the Agatston and volume scores at the 18-month MSCT versus the baseline MSCT) 4. Progression of mitral valve calcification (absolute change in the Agatston and volume scores at the 18-month MSCT versus the baseline MSCT) 5. Mortality from any cause within 18 months after start of treatment 6. Major adverse cardiovascular events: myocardial infarction, stroke, acute coronary syndrome, embolism, symptom-driven revascularization, death from cardiovascular cause within 18 months after start of treatment
Safety	Clinical and laboratory safety variables include:

	- History and physical examination - Frequency, type, severity and duration of adverse events - Assessment of laboratory parameters (sodium, potassium, calcium, phosphate, glucose, pH, triglycerides, AST, ALT, iPTH, 25-OH vitamin D_3)

Table S2. Calcification mass (in mg) at baseline of all participants included in the analysis of the VitaVask study.

Calcification mass (mg)	Control (N = 23)	Vitamin K1 (N = 17)
Thoracic aorta calcification mass (mg)	1444.5 ± 1419.7	1218.8 ± 3091.8
Coronary artery calcification mass (mg)	334.1 ± 255.7	317.8 ± 266.7
Aortic valve calcification mass (mg)	31.1 ± 53.2	25 ± 32.2
Mitral valve calcification mass (mg)	317 ± 722.7	347.4 ± 684.4

Values are expressed as mean values ± standard deviation.

Table S3: Primary study endpoints: P-values yielded by the sensitivity analyses.

Progression of thoracic aortic calcification volume score			
Parameter:	**Model 1**	**Model 2**	**Model 3**
Time	<.0001	<.0001	<.0001
Group	0.1228	0.2296	0.0664
Time & group	0.0850	0.0903	0.0966
Center	0.5678	0.0689	0.5806
Gender	.	0.3601	0.3746
Age	.	0.0186	0.0123
Smoker	.	.	0.3045
Diabetes mellitus	.	.	0.1335
Progression of coronary artery calcification volume score			
Parameter:	**Model 1**	**Model 2**	**Model 3**
Time	0.0025	0.0034	0.0054
Group	0.1457	0.3183	0.3952
Time & group	0.1913	0.2067	0.2564
Center	0.5279	0.5784	0.7678
Gender	.	0.5202	0.8483
Age	.	0.8196	0.6795
Smoker	.	.	0.0365
Diabetes mellitus	.	.	0.0241

Model 1: adjusted for center. Model 2: adjusted for center, gender and age. Model 3: adjusted for center, gender, age, smoking status and diabetes mellitus.

Table S4. Secondary endpoint thoracic aortic calcification Agatston score: changes in Agatston scores between baseline, 12 and 18 months in participants of the VitaVask study

		Model 1	Model 2	Model 3
Changes in Agatston score versus baseline within groups				
Vitamin K	**Baseline vs. 12 months**	601.0 (334.3), 0.0774	578.6 (343.6), 0.0976	578.3 (344.3), 0.0987
	Baseline vs. 18 months	885.5 (420.5), 0.0396	853.7 (438.7), 0.0566	859.9 (439.5), 0.0555
Control	**Baseline vs. 12 months**	1102.7 (279.3), 0.0002	1101.9 (281.3), 0.0002	1082.7 (295.8), 0.0006
	Baseline vs. 18 months	2051.6 (356.7), <.0001	2058.5 (358.9), <.0001	2035.8 (368.9), <.0001
Changes in Agatston score versus baseline between groups				
Vitamin K vs. control	**Baseline vs. 12 months**	501.7 (435.6), 0.2542	523.3 (444.0), 0.2435	504.4 (453.9), 0.2713
	Baseline vs. 18 months	1166.1 (551.4), 0.0388	1204.7 (566.8), 0.0379	1175.9 (573.8), 0.0452

Data are linear mixed model estimates (standard error, SE) and p-value.
Model 1: adjusted for center. Model 2: adjusted for center, gender and age. Model 3: adjusted for center, gender, age, smoking status and diabetes mellitus.

Progression of thoracic aortic calcification Agatston score. P-values yielded by the sensitivity analyses.

Parameter:	Model 1	Model 2	Model 3
Time	<.0001	<.0001	<.0001
Group	0.1122	0.2095	0.0566
Time & group	0.0993	0.0975	0.1107
Center	0.5751	0.0676	0.5701
Gender	.	0.3355	0.3496
Age	.	0.0196	0.0124
Smoker	.	.	0.2846
Diabetes mellitus	.	.	0.1219

Model 1: adjusted for center. Model 2: adjusted for center, gender and age. Model 3: adjusted for center, gender, age, smoking status and diabetes mellitus.

Table S5: Secondary endpoint coronary artery calcification Agatston score: changes in Agatston scores between baseline, 12 and 18 months in participants of the VitaVask study.

		Model 1	Model 2	Model 3
Changes in Agatston score versus baseline within groups				
Vitamin K	**Baseline vs. 12 months**	210.1 (154.2), 0.1783	209.9 (158.3), 0.1899	209.5 (159.3), 0.1938
	Baseline vs. 18 months	224.9 (189.5), 0.2398	225.2 (197.7), 0.2592	228.6 (198.5), 0.2543
Control	**Baseline vs. 12 months**	502.6 (129.9), 0.0003	502.6 (130.9), 0.0003	473.0 (138.1), 0.0011
	Baseline vs. 18 months	790.8 (166.1), <.0001	791.3 (167.5), <.0001	764.6 (172.6), <.0001
Changes in Agatston score versus baseline between groups				
Vitamin K vs control	**Baseline vs. 12 months**	292.5 (201.6), 0.1521	292.6 (205.4), 0.1596	263.5 (210.8), 0.2165
	Baseline vs. 18 months	565.8 (252.0), 0.0284	566.1 (259.1), 0.0329	536.0 (263.0), 0.0462

Data are linear mixed model estimates (standard error, SE) and p-value.
Model 1: adjusted for center. Model 2: adjusted for center, gender and age. Model 3: adjusted for center, gender, age, smoking status and diabetes mellitus.

Progression of coronary artery calcification Agatston score. P-values yielded by the sensitivity analyses.

Parameter:	Model 1	Model 2	Model 3
Time	0.0006	0.0008	0.0014
Group	0.1554	0.3292	0.3688
Time & group	0.0863	0.0978	0.1266
Center	0.5898	0.6469	0.8037
Gender	.	0.4858	0.8424
Age	.	0.7619	0.7482
Smoker	.	.	0.0321
Diabetes mellitus	.	.	0.0291

Model 1: adjusted for center. Model 2: adjusted for center, gender and age. Model 3: adjusted for center, gender, age, smoking status and diabetes mellitus.

Table S6: Secondary endpoint aortic valve volume score: changes in volume scores (mm^3) between baseline, 12 and 18 months in participants of the VitaVask study.

		Model 1	Model 2	Model 3
Changes in volume score versus baseline within groups				
Vitamin K	**Baseline vs. 12 months**	5.2 (15.2), 0.7325	4.4 (15.6), 0.7785	4.4 (14.7), 0.7630
	Baseline vs. 18 months	19.0 (18.5), 0.3107	17.7 (19.3), 0.3648	17.3 (18.1), 0.3431
Control	**Baseline vs. 12 months**	19.5 (12.4), 0.1208	19.5 (12.5), 0.1236	28.9 (12.3), 0.0224
	Baseline vs. 18 months	55.1 (15.9), 0.0010	55.0 (16.1), 0.0011	64.5 (15.5), 0.0001
Changes in volume score versus baseline between groups				
Vitamin K vs control	**Baseline vs. 12 months**	14.3 (19.6), 0.4690	15.1 (20.0), 0.4531	24.4 (19.1), 0.2070
	Baseline vs. 18 months	36.1 (24.4), 0.1446	37.4 (25.1), 0.1424	47.2 (23.8), 0.0527

Data are linear mixed model estimates (standard error, SE) and p-value.
Model 1: adjusted for center. Model 2: adjusted for center, gender and age. Model 3: adjusted for center, gender, age, smoking status and diabetes mellitus.

Progression of aortic valve volume score. P-values yielded by the sensitivity analyses.

Parameter:	Model 1*	Model 2 **	Model 3 ***
Time	0.0059	0.0084	0.0025
Group	0.4696	0.3172	0.8730
Time & group	0.2982	0.2960	0.1439
Center	0.5443	0.4226	0.0958
Gender	.	0.3948	0.6272
Age	.	0.3009	0.1137
Smoker	.	.	0.2237
Diabetes mellitus	.	.	0.3243

Model 1: adjusted for center. Model 2: adjusted for center, gender and age. Model 3: adjusted for center, gender, age, smoking status and diabetes mellitus.

Table S7: Secondary endpoint mitral valve volume score: changes in volume scores (mm^3) between baseline, 12 and 18 months in participants of the VitaVask study.

		Model 1	Model 2	Model 3
Changes in volume score versus baseline within groups				
Vitamin K	**Baseline vs. 12 months**	80.9 (71.6), 0.2630	87.4 (73.4), 0.2387	87.5 (73.5), 0.2389
	Baseline vs. 18 months	159.1 (88.8), 0.0784	170.6 (92.4), 0.0700	170.0 (92.5), 0.0714
Control	**Baseline vs. 12 months**	160.5 (63.2), 0.0138	160.4 (63.6), 0.0145	146.7 (65.3), 0.0287
	Baseline vs. 18 months	258.4 (79.9), 0.0020	259.6 (80.4), 0.0021	247.0 (81.6), 0.0037
Changes in volume score versus baseline between groups				
Vitamin K vs control	**Baseline vs. 12 months**	79.6 (95.5), 0.4079	73.1 (97.1), 0.4551	59.3 (98.3), 0.5490
	Baseline vs. 18 months	99.3 (119.4), 0.4089	89.0 (122.5), 0.4704	77.1 (123.4), 0.5347

Data are linear mixed model estimates (standard error, SE) and p-value.
Model 1: adjusted for center. Model 2: adjusted for center, gender and age. Model 3: adjusted for center, gender, age, smoking status and diabetes mellitus.

Progression of mitral valve volume score. P-values yielded by the sensitivity analyses.

Parameter:	Model 1*	Model 2 **	Model 3 ***
Time	0.0039	0.0037	0.0054
Group	0.7810	0.4245	0.3456
Time & group	0.6731	0.7318	0.8065
Center	0.6488	0.0652	0.7818
Gender	.	0.5533	0.7484
Age	.	0.3665	0.2293
Smoker	.	.	0.1697
Diabetes mellitus	.	.	0.3883

Model 1: adjusted for center. Model 2: adjusted for center, gender and age. Model 3: adjusted for center, gender, age, smoking status and diabetes mellitus.

Table S8. Changes in Vitamin K and dp-ucMGP levels between baseline, 4 weeks, 12 and 18 months in participants of the VitaVask study.

| Groups	Time points	Vitamin K level	Dp-ucMGP level
		Parameter estimates (standard error, SE)	
Vitamin K	**Baseline**	0.8 (0.4)	1574.8 (112.7)
	4 weeks	3.9 (0.4)	574.3 (114.9)
	12 months	3.2 (0.5)	534.2 (152.4)
	18 months	5.3 (0.6)	487.4 (185.1)
Control	**Baseline**	0.8 (0.3)	1448.0 (115.6)
	4 weeks	0.6 (0.3)	1579.9 (109.2)
	12 months	0.5 (0.3)	1673.4 (110.1)
	18 months	0.7 (0.4)	1472.3 (139.8)
Groups	**Time points**	**Vitamin K level**	**Dp-ucMGP level**
		Changes in levels versus baseline between groups	
Vitamin K vs control	**Baseline to 4 weeks**	-3.2 (0.5), <.0001	1132.4 (173.9), <.0001
	Baseline to 12 months	-2.6 (0.8), 0.0006	1265.9 (233.6), <.0001
	Baseline to 18 months	-4.6 (0.9), <.0001	1111.7 (265.6), <.0001

Data are linear mixed model estimates (standard error, SE) and p-values.
The model was adjusted for center.

Table S9: Evolution of serum phosphate, intact parathyroid hormone and bone-specific alkaline phosphatase concentrations during the study period.
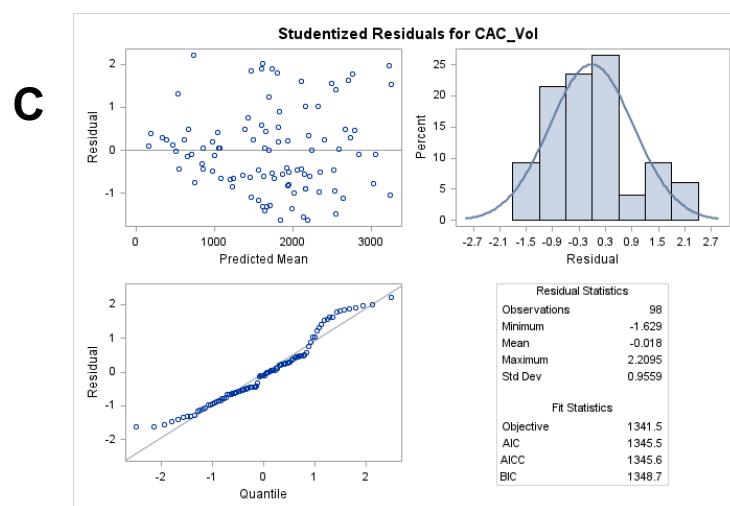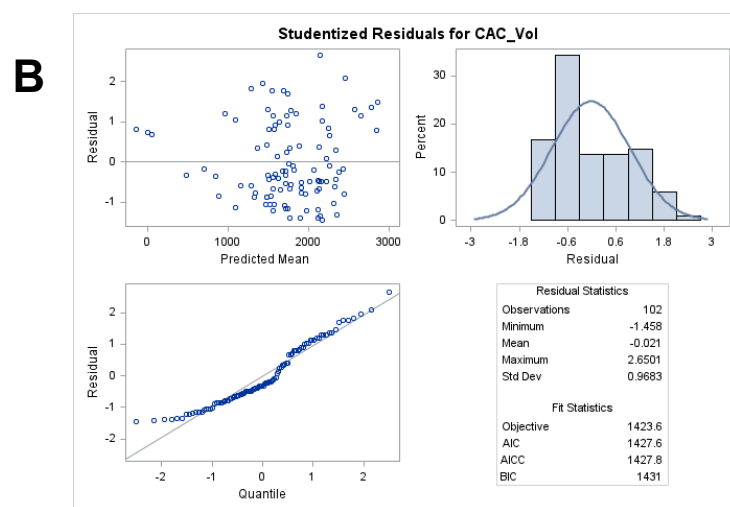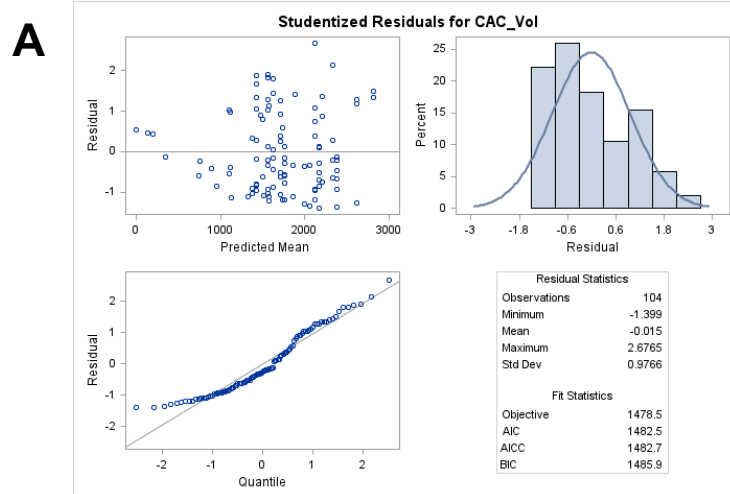
Parameter	Time Point	Control group	Vitamin K1 group
Serum phosphate, mmol/L	Baseline	1.8 ± 0.5	1.9 ± 1.2
	12 months	1.7 ± 0.6	1.6 ± 0.5
	18 months	1.6 ± 0.5	1.6 ± 0.4
Serum-intact parathyroid hormone, ng/L	Baseline	265 ± 307	536 ± 427
	12 months	246 ± 208	263 ± 314
	18 months	204 ± 141	520 ± 307
Serum bone-specific alkaline phosphatase, U/I	Baseline	27 ± 23	36 ± 33
	12 months	24 ± 16	35 ± 40
	18 months	20 ± 11	28 ± 29

Data are means ± SD.

Normal values for serum phosphate range from 0.81 to 1.45 mmol/L, for serum-intact parathyroid hormone range from 15 to 65 ng/L, and for serum bone-specific alkaline phosphatase range from 15 to 42 U/I.
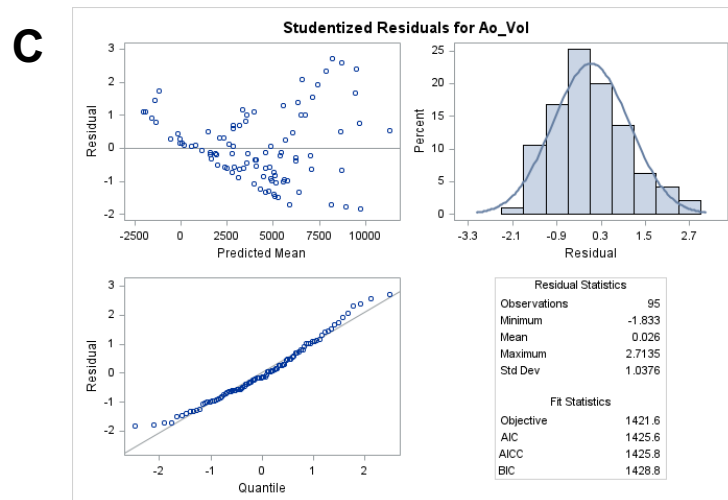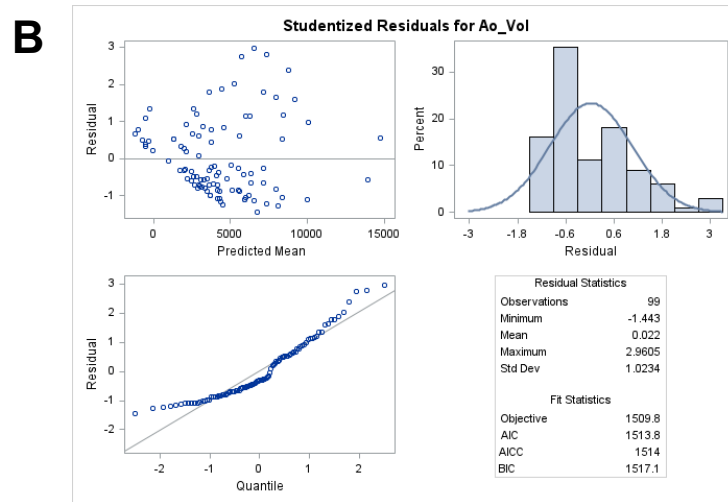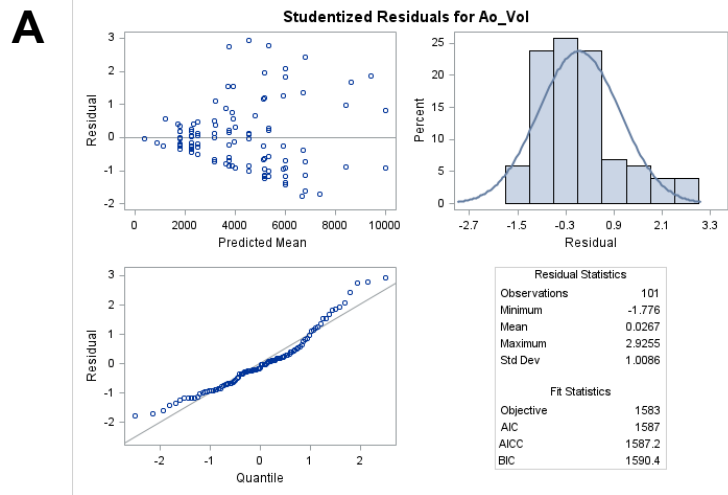
Supplemental Figure S1: Residual plots for the outcome parameter thoracic aortic calcification. A: adjusted for center. B: adjusted for center, gender and age. C: adjusted for center, gender, age, smoking status and diabetes mellitus.

Supplemental Figure S2: Residual plots for the outcome parameter coronary artery calcification. A: adjusted for center. B: adjusted for center, gender and age. C: adjusted for center, gender, age, smoking status and diabetes mellitus.