SUPPLEMENTAL MATERIAL

DATA S1.

SUPPLEMENTAL METHODS

Cardiovascular phenotyping

All study-related actions including medical and technical measurements were performed according to standard operating procedures (SOP) by specifically trained and certified medical technical assistants. Digital Imaging data were recorded on a server with an integrated multi-modality image management system (Xcelera, Royal Philips Electronics, Amsterdam, The Netherlands).

Intima media thickness (IMT) was assessed with an iE33 ultrasound system (Philips Medical Systems, Best, The Netherlands) using a computerized edge detection system (Qlab software, Royal Philips, The Netherlands) with triggering according to the Q wave in electrocardiography. IMT was measured at both common carotid arteries (CCAs). Mean IMT was recorded 1 cm proximal to the carotid bulb over a length of 1 cm at the far wall and only in vessel segments without plaques.

Vascular function by ultrasound (flow-mediated dilation; FMD) was measured according to a standard protocol: after a 5-minute supra-systolic upper arm occlusion, diameter measurements of the brachial artery were performed on two-dimensional high-resolution ultrasound images recorded on a Philips HD11XE CV ultrasound machine (Philips, Best, Netherlands) using a linear array broadband probe, L12–5 (38 mm). Participants were in resting conditions of at least 5 minutes before the measurements. Diameters were measured offline using the commercially available Brachial Analyzer software package, version 5.0 (Medical Imaging Applications LLC, Iowa City, US). The means of three measurements at baseline and at 60 seconds after cuff release were taken for analysis.

Reflection index reflecting vascular tone of arteries and stiffness index reflecting artery stiffness were measured by PulseTrace 2000 device (Cardinal Health/Micro Medical Limited, Rochester, United Kingdom). The digital volume pulse was obtained by averaging the transmission of infrared light of ten pulse waves through the pulp of the right ring finger.

Peripheral Arterial Tonometry (PAT) was recorded by the Endo-PAT2000 fingertip device (Itamar Medical, Caesarea, Israel). Baseline pulse amplitude was measured electronically in both index fingers, with the left index finger serving as a control. The PAT-ratio was automatically calculated using a computerized algorithm.

All subjects underwent multimodal echocardiography with an iE33 echocardiography system with an S5–1 sector array transducer (Royal Philips Electronics, Amsterdam, The Netherlands). Cardiac structure was assessed by two-dimensional guided M-mode measurements of the parasternal long axis view of the left ventricle (LV). Left ventricular

mass and relative wall thickness (RWT) were calculated from LV diastolic internal dimension (LVDD), intraventricular septum diameter (IVSD) and LV posterior wall thickness (LVPWD). Cardiac function was assessed by biplane LV ejection fraction (LVEF in %) according to the modified Simpson method in 4- and 2-chamber views. The E/E' ratio as surrogate for diastolic function was calculated by dividing the early filling velocity of transmitral Doppler (E) by the early relaxation velocity on tissue Doppler (E').

Definitions of Cardiovascular Risk Factors and Comorbidities

Arterial hypertension was stated for participants, who at least hold one of the following conditions: a) Intake of hypertensive drugs b) mean systolic blood pressure ≥ 140 mm Hg c) mean diastolic blood pressure ≥ 90 mm Hg or d) definite diagnosis of hypertension by physician. Antidiabetic drug treatment, a fasting blood glucose level ≥ 126 mg/dl after overnight fasting of at least 8 hours, a blood glucose level of ≥ 200 mg/dl after a fasting period of at least 8 hours at the baseline examination or a physician diagnosis of diabetes lead to the diagnosis of diabetes. Dyslipidemia was defined on intake of lipid lowering drugs, a LDL / HDL-ratio of ≥ 3.5 or a definite diagnosis of dyslipidemia by a physician. Smoking was dichotomized into smokers (occasional smokers and smokers) and non-smokers (never smokers and ex-smokers). Anthropometric measurements were taken with calibrated digital scales (Seca 862, Seca, Hamburg, Germany), a measuring stick (Seca 220, Seca, Hamburg, Germany) and a non-stretching waist measuring tape. Waist circumference was measured midway between the lower rib margin and the superior anterior iliac spine. Body height and weight were measured without shoes in underwear and the body mass index (BMI) was calculated as weight in kilograms divided by the scare of height in meters. Obesity was determined as BMI ≥ 30 kg/ m² according to WHO criteria. A positive family history of myocardial infarction or stroke was defined as at least one myocardial infarction or stroke in a female first-degree relative before 65 years of age and in a male first-degree relative before 60 years of age.

The diagnosis of Atrial Fibrillation (AF) was based on the history of AF reported by the participant during the computer assisted interview and/or the evidence of AF on the resting electrocardiogram (ECG) and/or the documentation of AF on the echocardiogram performed during the study. At least two physicians with training and experience in ECG reading had to confirm the diagnosis. Cancer, chronic obstructive pulmonary disease, congestive heart failure, coronary artery disease, deep vein thrombosis, liver disease, myocardial infarction, peripheral arterial disease, pulmonary embolism, and stroke were assessed by self-report of the participants and were collected by computer-assisted personal interviews. Chronic kidney

disease was defined as an estimated glomerular filtration rate of less than 60 ml/min/1.73m² assess by a urine sample taken at the study center.

Genotyping and imputation of single nucleotide polymorphism

The search in the GWAS catalogue, accessed on 25th July 2015, resulted in four studies with 11 warfarin metabolism related SNPs. The genetic variants have been investigated in detail; inconsistent results were identified and not considered for further analysis. Two SNPs were present on the Affymetrix array used in this study. The other eight SNPs were tested with SNP Annotation and Proxy Search (http://www.broadinstitute.org/mpg/snap/ldsearch.php) for one or several proxies in linkage disequilibrium (LD) on the array. The following search options were used: r2 threshold = 0.9, distance limit = 500, population panel = CEU, SNP data set = 1000 Genomes Pilot 1. Overlapping SNPs were excluded from the analysis. For two GWAS SNPs no matching proxy could be found on the Affymetrix 6.0 Array, resulting in five warfarin maintenance dose related SNPs (**Table 7**).

Table S1. Use of medication according to VKA intake

	No VKA intake (N= 14,564)	Intake of VKA (N=287)	Prevalence ratio
Antidiabetics	6.0 (879)	15.3 (44)	2.6
Antiplatelet agents	10.3 (1499)	9.1 (26)	0.9
Beta-blockers	16.2 (2357)	61.7 (177)	3.8
Calcium channel blockers	7.0 (1024)	22.6 (65)	3.2
Diuretics	4.8 (705)	29.3 (84)	6.1
Lipid modifying drugs	12.8 (1862)	41.5 (119)	3.2
Statins	11.2 (1628)	39.7 (114)	3.3
Fibrates	0.7 (100)	2.1 (6)	3.0
Other lipid modifying agents	1.2 (168)	5.9 (17)	3.2
Low-molecular-weight heparins	0.1 (18)	1.7 (5)	17.0
Renin angiotensin aldosterone system inhibitors	23.1 (3358)	64.1 (184)	2.8

Data are expressed as median with 25th/75th percentile. The prevalence ratio was calculated as the ratio of the frequency for “Intake of VKA” to the one for “No VKA intake”.

Table S2. Vitamin K dependent proteins and laboratory tests according to VKA intake

	No VKA intake (N= 14,564)	Intake of VKA (N=287)	Difference
FII [%]	117.5 (105.5/130.1)	33.0 (27.9/39.9)	-72%
FVII [%]	111.8 (99.7/129.3)	33.9 (29.7/43.8)	-70%
FIX [%]	111.8 (101.5/122.4)	58.7 (49.8/70.6)	-47%
FX [%]	115.9 (103.6/130.1)	18.0 (13.6/22.8)	-84%
Protein C [%]	117.5 (105.0/131.0)	52.6 (47.2/61.5)	-55%
Protein S [%]	101.8 (90.2/114.2)	49.8 (42.2/56.4)	-51%
aPTT [s]	30.2 (28.4/32.3)	46.2 (40.6/50.5)	+53%
INR	1.00 (1.00/1.00)	2.50 (2.10/2.90)	+150%

Data are expressed as median with $25^{th}/75^{th}$ percentile. Significant difference between the groups ($P < 0.0001$) was detected for all outcomes. The percentage differences represent the change between “Intake of VKA” compared to “No VKA intake”.

Table S3. Profile of humoral biomarkers by VKA use

	No VKA intake (N= 14,564)	Intake of VKA (N=287)	Difference
Biomarkers of cardiac function			
MR-proANP [pmol/L]	65.3 (48.7/88.8)	145.6 (98.9/229.7)	+123%
MR-proADM [nmol/L]	0.46 (0.39/0.54)	0.64 (0.55/0.78)	+39%
Nt-proBNP [pg/mL]	60.4 (27.8/119.6)	514.4 (141.9/1256.2)	+752%
Biomarkers of coagulation			
Fibrinogen [mg/dl] ‡	320 (277/372)	500 (428/586)	+56%
F-VIII [%]	120.60 (100.30/141.28)	139.25 (120.84/161.06)	+15%
F-XI [%]	109.0 (97.5/122.5)	101.5 (89.2/112.6)	-7%
hs-D-dimer [µg/L]	229 (149/352)	122 (66/210)	-47%
Thrombomodulin [%]	1.99 (1.62/2.45)	2.03 (1.73/2.65)	+2%
Tissue factor [%]	200 (160 /250)	237 (192/280)	+18%
vWF [%]	106.8 (81.5/137.2)	138.6 (106.1/159.0)	+30%
Biomarkers of inflammation			
hs-CRP [mg/l] ‡	1.50 (0.54/3.20)	2.70 (1.30/5.40)	+80%
IL-18 [pg/ml]	217 (168/283)	243 (193 /337)	+12%
IL-1RA [pg/ml]	319 (239/425)	367 (285/487)	+15%
Leukocyte count [10^9/L] *	6.90 (5.82/8.26)	7.20 (6.07/8.33)	+4%
MPO [pmol/L]	296 (234/371)	345 (265/428)	+17%

MR-proANP, Mid-regional Pro-Atrial Natriuretic Peptide; MR-proADM, Midregional Pro-Adrenomedullin; Nt-proBNP, N-terminal pro-brain natriuretic peptide; vWF, von Willebrand factor; hs-CRP, high sensitivity C-reactive protein; IL-18, interleukin-18; IL-1RA, interleukin-1 receptor antagonist; MPO, myeloperoxidase;

Data are expressed as median with 25^{th}/75^{th} percentile. The percentage differences represent the change between “Intake of VKA” compared to “No VKA intake”.

All biomarkers were measured in a sample set of the first 5,000 participants, unless otherwise indicated. * measured in a sample set of 15,010 participants

Table S4. SNPs identified in GWAS catalogue known to influence warfarin dose requirements

Selected SNPs form GWAS catalogue	Chr	Position (Mb) ‡	Gene	Tag SNP on Affymetrix 6.0 with $r^2 > 0.9$	r^2 between lead SNP and tag SNP	Distance between lead SNP and tag SNP (KB)	Minor allele	MAF †	Effect of minor allele
rs10509680	10	96734339	*CYP2C9*	rs9332245	1.00	14842	A	0.058	Lower dose requirement
rs12777823	10	96405502	*CYP2C9*	n.a	n.a	n.a	A	0.169	Lower dose requirement
rs4086116	10	96707202	*CYP2C9*	n.a	n.a	n.a	T	0.205	Lower dose requirement
rs10871454	16	31048079	*VKORC1*	rs11150604	1,00	11059	T	0.398	Lower dose requirement
rs2108622	19	15990431	*CYP4F2*	n.a	n.a	n.a	T	0.232	Higher dose requirement

SNP, single nucleotide polymorphism; GWAS, genome-wide association studies; MAF, Minor allele frequency

‡ based on genome built 105

† based on HapMapCEU data (http://www.ncbi.nlm.nih.gov.snp)

Table S5. Baseline characteristic in the subgroup of participants with atrial fibrillation and indication for oral anticoagulation stratified for VKA use (diagnosis of atrial fibrillation and CHA2DS2-VASc Score of ≥ 1)

	No VKA intake (N= 263)	Intake of VKA (N=158)
Age [years]	68.0 (63.0/71.0)	68.0 (64.0/72.0)
Sex (Female)	33.8 (89)	28.5 (45)
Traditional cardiovascular risk factors		
Diabetes mellitus	18.6 (49)	19.0 (30)
Dyslipidemia	43.7 (114)	44.9 (71)
Family history of myocardial infarction /stroke	27.8 (73)	22.2 (35)
Hypertension	81.4 (214)	80.4 (127)
Obesity	37.6 (99)	39.9 (63)
Smoking	12.2 (32)	12.8 (20)
Comorbidities		
Cancer	16.8 (44)	17.7 (28)
Chronic kidney disease	9.9 (26)	17.7 (28)
Chronic obstructive pulmonary disease	11.4 (30)	11.4 (18)
Congestive heart failure	9.9 (26)	20.3 (32)
Coronary artery disease	23.3 (58)	27.3 (41)
Deep vein thrombosis	8.1 (21)	12.8 (20)
Liver disease	1.1 (3)	1.3 (2)
Myocardial infarction	15.7 (41)	19.0 (29)
Peripheral artery disease	8.6 (22)	10.8 (17)
Pulmonary embolism	0 (0)	0.6 (1)
Stroke	5.7 (15)	16.3 (25)

Data are expressed as the relative and absolute frequencies for binary variables, for continuous variables as median with $25^{th}/75^{th}$ percentiles. The prevalence ratio was calculated as the ratio of the frequency for “Intake of VKA” to the one for “No VKA intake”.

Table S6. Multivariable linear regression models on the relationship between surrogate parameters of subclinical cardiovascular disease and humoral biomarkers in the subgroup of participants with atrial fibrillation and a CHA_2DS_2-VASc Score of ≥ 1

		β - estimates with corresponding 95% CI for VKA use		
		Adjusted for age, sex and cardiovascular risk factors *		
		β	95% CI	*P*-Value
Vascular structure and function	*Arterial stiffness*			
	Augmentation index [%]	-0.81	-4.93; 3.32	0.70
	Stiffness index [m/s] †	3.45	-1.61; 8.51	0.18
	Endothelial function			
	Flow- mediated dilation [%]	-0.76	-1.73; 0.21	0.12
	log (Reactive hyperemia index)	**-0.11**	**-0.21**; **-0.01**	**0.025**
	Reflection-Index	-0.03	-3.77; 3.71	0.99
	Endothelial structure			
	Baseline brachial artery diameter [mm]	-0.02	-0.15; 0.12	0.80
	Intima-media thickness [mm]	0.01	-0.03; 0.05	0.65
	Peripheral arterial disease			
	Ankle brachial-index	-0.01	-0.04; 0.02	0.58
Cardiac structure and function	*Cardiac function*			
	log (E/E´-ratio)	0.03	-0.04; 0.11	0.39
	LV ejection fraction [%]	**-2.12**	**-3.85**; **-0.38**	**0.017**
	Cardiac structure			
	LV mass/ height$^{2.7}$ [g/m$^{2.7}$]	**3.61**	**0.90**; **6.31**	**0.0089**
	Relative wall thickness	0.01	-0.01; 0.03	0.45
Humoral biomarkers	*Biomarkers of cardiac function*			
	log (MR-proANP) [pmol/L]	**0.42**	**0.25**; **0.60**	**< 0.0001**
	log (MR-proADM) [nmol/L]	0.06	-0.01; 0.14	0.085
	log (Nt-proBNP) [pg/mL] ‡	**1.19**	**0.73**; **1.65**	**< 0.0001**
	Biomarkers of coagulation			
	Fibrinogen [mg/dl] ‡	**135**	**112**; **157**	**< 0.0001**
	FVIII [%]	3.18	-9.75; 16.1	0.63
	FXI [%]	**-10.8**	**-17.5**; **-4.14**	**0.0015**
	log (hs-d-Dimer) [µg/L]	**-1.07**	**-1.31**; **-0.84**	**< 0.0001**
	log (Thrombomodulin) [%]	0.10	-0.06; 0.26	0.22
	Tissue factor [%]	2.4	-32.1; 36.9	0.89
	vWF [%]	5.3	-9.1; 19.8	0.47

Table S6. (continued)

	β - estimates with corresponding 95% CI for VKA use		
	Adjusted for age, sex and cardiovascular risk factors *		
	β	95% CI	*P*-Value
Biomarkers of inflammation			
log (hs-CRP) [mg/l]	**0.20**	**0.01**; **0.39**	**0.035**
IL-18 [pg/ml]	29.6	-24.2; 83.3	0.28
IL-1RA [pg/ml] ‡	26.9	-77.1; 131	0.61
log (Leukocyte count) [10^9/L]	0.0008	-0.05; 0.05	0.97
MPO [pmol/L]	43.8	-27.0; 115	0.23

* Cardiovascular risk factors include diabetes mellitus, dyslipidemia, hypertension, obesity, smoking, family history of stroke/myocardial infarction, eGFR

† displayed estimates are given for mean age of 64.5 years; model was additionally adjusted for age*VKA interaction

‡ displayed estimates are given for men; model was additionally adjusted for sex(women)*VKA interaction; consequently the estimates for women have to be corrected by adding the following values: Nt-proBNP, +0.42; fibrinogen, +28.2; IL-1RA, +99.9. LV, left ventricular.

Table S7. Parameters of cardiovascular function and structure by VKA intake in propensity score weighted sample of individuals with atrial fibrillation or venous thrombosis

		No intake of VKA (N= 226)	**Intake of VKA (N=224)**
Vasculature	*Arterial stiffness*		
	Augmentation index [%]	18.91 (8.49/31.75)	14.73 (6.46/26.61)
	Stiffness index [m/s]	8.25 (6.55/9.96)	7.91 (6.50/9.40)
	Endothelial function		
	Flow- mediated dilation [%]	6.69 (4.10/8.97)	6.16 (3.90/8.59)
	log (Reactive hyperemia index)	0.47 (0.23/0.82)	0.41 (0.12/0.77)
	Reflection-Index	72.00 (57.54/79.00)	67.00 (55.92/78.00)
	Endothelial structure		
	Baseline brachial artery diameter [mm]	4.83 (4.30/5.32)	4.80 (4.16/5.33)
	Intima-media thickness [mm]	0.72 (0.65/0.83)	0.73 (0.66/0.85)
	Peripheral arterial disease		
	Ankle brachial-index	0.99 (0.91/1.05)	0.98 (0.89/1.06)
Heart	*Cardiac function*		
	log (E/E´-ratio)	8.37 (6.65/10.29)	8.26 (6.50/10.79)
	LV ejection fraction [%]	62.5 (58.7/66.7)	60.9 (55.2/65.4)
	Cardiac structure		
	LV mass/ height$^{2.7}$ [g/m$^{2.7}$]	43.0 (35.8/51.2)	45.7 (38.4/55.4)
	Relative wall thickness	0.42 (0.37/0.49)	0.43 (0.37/0.50)
Humoral biomarkers	*Biomarkers of cardiac function*		
	log (MR-proANP) [pmol/L]	95.7 (65.9/141.0)	151.0 (106.6/240.4)
	log (MR-proADM) [nmol/L]	0.61 (0.50/0.72)	0.63 (0.55/0.75)
	log (Nt-proBNP) [pg/mL]	132 (58/292)	554 (208/1270)
	Biomarkers of coagulation		
	Fibrinogen [mg/dl]	349 (295/411)	498 (431/591)
	FVIII [%]	133.5 (112.1/154.1)	138.4 (120.1/152.7)
	FXI [%]	108.5 (96.9/120.4)	101.0 (87.4/112.6)
	log (hs-d-Dimer) [µg/L]	374.8 (230.9/624.3)	120.0 (62.5/213.7)
	log (Thrombomodulin) [%]	2.09 (1.83/2.64)	2.04 (1.72/2.64)
	Tissue factor [%]	206.9 (167.5/259.1)	230.8 (195.5/276.2)
	vWF [%]	125.0 (95.1/153.4)	139.4 (106.1/159.9)
	Biomarker of inflammation		
	Hs-CRP [mg/l]	2.30 (1.20/4.30)	2.60 (1.24/5.50)
	IL-18 [pg/ml]	246.1 (185.3/311.8)	248.7 (199.5/338.6)
	IL-1RA [pg/ml]	350.0 (260.8/458.1)	366.2 (279.7/489.8)
	Leukocytes [/nl]	7.10 (6.13/8.47)	7.16 (6.08/8.32)
	MPO [pmol/L]	320.8 (244.8 /380.9)	333.7 (249.9/412.7)

Inverse probability of treatment weighting using the propensity score was applied. The underlying propensity model included age, sex, diabetes mellitus, obesity, smoking, arterial hypertension, dyslipidemia, family history of stroke or myocardial infarction and history of cardiovascular diseases. All standardized differences for those variables between treatment groups after weighting were <0.1.

Table S8. Parameters of cardiovascular function and structure by VKA exposure time

		< 1 year (N=64)	**1-3 years (N=66)**	**> 3 years (N=130)**
Vasculature	*Arterial stiffness*			
	Augmentation index [%]	14.5 (6.3 /26.6)	16.7 (5.7 /31.1)	19.7 (7.6/33.2)
	Stiffness index [m/s]	7.47 (6.25/8.82)	7.65 (6.06/9.20)	7.89 (6.41/9.58)
	Endothelial function			
	Flow- mediated dilation [%]	5.54 (3.65/9.06)	7.04 (3.56/8.96)	5.90 (3.63/8.12)
	Log (Reactive hyperemia index)	0.63 (0.29/0.92)	0.26 (0.06/0.72)	0.34 (0.15/0.74)
	Reflection-Index	65.0 (54.0/75.0)	67.0 (55.2/77.0)	72.0 (60.3/80.0)
	Endothelial structure			
	Baseline BA diameter [mm]	4.68 (4.02/5.32)	4.75 (4.20/5.33)	4.95 (4.25/5.33)
	Intima-media thickness [mm]	0.78 (0.56/0.94)	0.71 (0.66/0.77)	0.73 (0.68/0.85)
	Peripheral arterial disease			
	Ankle brachial- index	0.98 (0.88/1.05)	0.97 (0.90/1.04)	0.97 (0.88/1.07)
Heart	*Cardiac function*			
	E/E´-ratio	7.90 (6.22/9.96)	8.25 (6.37/10.51)	8.73 (6.80/12.38)
	Ejection fraction [%]	59.2 (53.7/64.5)	62.3 (57.0/66.7)	60.0 (53.7/65.1)
	Cardiac structure			
	LV mass/ height$^{2.7}$ [g/m$^{2.7}$]	41.9 (36.2/52.2)	48.7 (42.5/59.2)	47.6 (38.9/56.9)
	Relative wall thickness	0.43 (0.35/0.50)	0.43 (0.38/0.48)	0.42 (0.37/0.51)
Humoral Biomarker	*Biomarker of coagulation*			
	Fibrinogen [mg/dl]	484 (429/570)	518 (456/593)	500 (428/588)
	Biomarker of inflammation			
	Hs-CRP [mg/l]	2.55 (1.14/4.80)	2.70 (1.59/6.43)	2.95 (1.30/5.42)
	Leukocytes [/nl]	7.28 (6.00/8.90)	6.78 (5.86/7.80)	7.30 (6.19/8.50)