ORIGINAL RESEARCH

Intake of Vitamin K Antagonists and Worsening of Cardiac and Vascular Disease: Results From the Population-Based Gutenberg Health Study

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***Background*—**Preclinical data have indicated a link between use of vitamin K antagonists (VKA) and detrimental effects on vascular structure and function. The objective of the present study was to determine the relationship between VKA intake and different phenotypes of subclinical cardiovascular disease in the population.

***Methods and Results*—**Clinical and laboratory data, as well as medical–technical examinations were assessed from 15 010 individuals aged 35 to 74 years during a highly standardized 5-hour visit at the study center of the population-based Gutenberg Health Study. In total, the study sample comprised 287 VKA users and 14 564 VKA nonusers. Multivariable analysis revealed an independent association between VKA intake and stiffness index (β=+2.54 m/s; [0.41/4.66]; P=0.019), ankle-brachial index (β=−0.03; [−0.04/−0.01]; P<0.0001), intima-media thickness (β=+0.03 mm [0.01/0.05]; P=0.0098), left ventricular ejection fraction (β=−4.02% [−4.70/−3.33]; P<0.0001), E/E′ (β=+0.04 [0.01/0.08]; P=0.014) left ventricular mass (β=+5.34 g/m$^{2.7}$ [4.26/6.44]; P<0.0001), and humoral markers of cardiac function and inflammation (midregional pro-atrial natriuretic peptide: β=+0.58 pmol/L [0.50/0.65]; P<0.0001; midregional pro-adrenomedullin: β=+0.18 nmol/L [0.14/0.22]; P<0.0001; N-terminal pro B-type natriuretic peptide: β=+1.90 pg/mL [1.63/2.17]; P<0.0001; fibrinogen: β=+143 mg/dL [132/153]; P<0.0001; C-reactive protein: β=+0.31 mg/L [0.20/0.43]; P<0.0001). Sensitivity analysis in the subsample of participants with atrial fibrillation stratified by intake of VKA demonstrated consistent and robust results. Genetic variants in *CYP2C9*, *CYP4F2*, and *VKORC1* were modulating effects of VKA on subclinical markers of cardiovascular disease.

***Conclusions*—**These data demonstrate negative effects of VKA on vascular and cardiac phenotypes of subclinical cardiovascular disease, indicating a possible influence on long-term disease development. These findings may be clinically relevant for the provision of individually tailored antithrombotic therapy. **(*J Am Heart Assoc*. 2018;7:e008650. DOI: 10.1161/JAHA.118.008650.)**

Key Words: cardiovascular disease • oral anticoagulation • pharmacogenomic variants • vitamin K antagonists

Vitamin K antagonists (VKA) are recommended to patients with an indication for oral anticoagulation therapy to prevent thromboembolic complications.[1] Recent data imply that the intake of VKA involves effects beyond the well-known inhibition of the vitamin K–dependent coagulation factors (F) II, VII, IX, and X.[2] Experimental data suggest that VKA may

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Accompanying Data S1 and Tables S1 through S8 are available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.118.008650

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Clinical Perspective

What Is New?

- Detrimental effects of vitamin K antagonists on vascular and cardiac phenotypes of subclinical cardiovascular disease were observed in a large population-based cohort.
- The analysis demonstrated a relationship of vitamin K antagonists treatment with increased arterial stiffness, higher left ventricular mass, and decreased cardiac systolic function independent of the concomitant clinical profile.

What Are the Clinical Implications?

- Noncanonical effects of vitamin K antagonists merit critical consideration against the background of frequently coprevalent atherosclerosis and cardiovascular disease and may be relevant for the long-term management of patients with oral anticoagulation.
- Evaluating the findings in contrast to the treatment with direct-acting oral anticoagulants will be crucial to develop clinical implications for an individualized anticoagulation therapy.

decrease the activity of the vitamin K–dependent proteins matrix Gla protein (MGP) and growth arrest–specific gene 6 (Gas-6) by inhibiting the γ-carboxylation process.[3] Unlike coagulation factors, which are synthesized and carboxylated within the liver, MGP and Gas-6 are carboxylated within the vasculature.[4] Increased levels of undercarboxylated MGP have been associated with vascular calcification.[5] The deficiency of active MGP and Gas-6 provokes cell death, decreased contractility of vascular smooth muscle cells, and accelerated vascular calcification.[4,6] Studies have also demonstrated that VKA therapy is associated with vascular calcification.[7,8] Given the important role of vascular calcification in the pathophysiology of vascular stiffness and the correlation of calcification with increased serum levels of inflammatory markers, hypertension, and incident cardiovascular disease (CVD), VKA may exert clinically relevant noncoagulant effects.[9]

Since alternatives to VKA therapy are available for most indications, noncoagulant effects of VKAs on the development and progression of atherosclerosis and CVD may have clinically relevant implications.

Against this background, the present study investigated the interrelation between the intake of VKA and the progress and development of CVD in the setting of a large population-based cohort study.

Methods

The analysis presents clinical data of a large-scale population-based cohort with ongoing follow-up examinations. This project constitutes a major scientific effort with high methodological standards and detailed guidelines for analysis and publication to ensure scientific analyses on highest level. Therefore, data are not made available for the scientific community outside the established and controlled workflows and algorithms. To meet the general idea of verification and reproducibility of scientific findings, we offer access to data at the local database in accordance with the ethics vote upon request at any time. The GHS (Gutenberg Health Study) steering committee, which comprises a member of each involved department and the head of the GHS, convenes once a month. The steering committee decides on internal and external access of researchers and use of the data and biomaterials based on a research proposal to be supplied by the researcher. Interested researchers make their requests to the head of the GHS (Philipp S. Wild; philipp.wild@unimedizin-mainz.de).

Study Sample

We investigated data of 15 010 individuals (age range 35–74 years) enrolled in the GHS, a population-representative, prospective, observational, single-center cohort study in the Rhine-Main region in Midwestern Germany. Participants were enrolled between April 2007 and April 2012 and underwent a detailed 5-hour medical–technical examination in the study center. The sample was drawn randomly from the local registration offices with equal strata for sex, residence (urban and rural), and age decades. Details of the study design have been published elsewhere.[10] The study complies with the principles outlined in the Declaration of Helsinki. The study protocol, study documents, and sampling design were approved by the Ethics Committee of the State Chamber of Physicians of Rhineland-Palatinate, Germany (reference number 837.020.07 (5555)) and by local institutional review boards. All study participants provided written informed consent.

Data Assessment

For the current analysis, information was obtained during the baseline visit at the study center. All study participants underwent comprehensive cardiovascular phenotyping at the study platform (see Data S1 for a detailed description of definitions for traditional cardiovascular risk factors and comorbidities used in the present analysis). Current medication use including medication on demand was recorded digitally by scanning the drug identification bar code from drug packages or alternatively established on the basis of self-reported information from participants (eg prescription plan). History of drug intake and the type of prescription (self-medication versus prescription by a physician) were recorded for the medication. Central pharmaceutical numbers were translated into the Anatomical Therapeutic Chemical code of the current

pharmaceutical index. Individuals with regular or current VKA use (Anatomical Therapeutic Chemical Code: B01AA) were defined as users and nonusers as the reference group.

Laboratory Analyses

Routine laboratory parameters (ie blood glucose, creatinine, lipids, fibrinogen, and blood count) were measured using standardized methods from fresh venous blood samples in all 15 010 study participants at enrollment in the central laboratory of the University Medical Center. For biobanking, samples were aliquoted and stored at −80°C immediately after blood draw. Specific biomarkers were analyzed in the subsample of the first 5000 participants: midregional pro-atrial natriuretic peptide (BRAHMS AG), midregional pro-adrenomedullin (BRAHMS AG), N-terminal pro B-type natriuretic peptide (Roche Diagnostics), high-sensitivity (hs) D-dimer (Instrumentation Laboratory), thrombomodulin (Sekisui Diagnostics), high-sensitivity C-reactive protein (hs-CRP) (Abbott), IL-18 (MBL), IL-1 receptor antagonist (R&D Systems), and myeloperoxidase (Prognostix) levels were measured using commercially available assays according to the manufacturer's recommendation. FII, FVII, FVIII, FIX, FX, FXI, tissue factor, and von Willebrand factor measurements were performed on a Siemens BCS-XP device. Collection, processing, handling, and storage of blood specimens were performed according to specific standard operating procedures.

Single Nucleotide Polymorphisms for VKA Metabolism

Single selected single nucleotide polymorphisms (SNPs) in the presently available genetic variant data set were used for this analysis. Detailed description of SNP selection is given in the Supplemental Material. Genetic information was available from 4175 of the first 5000 subjects enrolled. Genome-wide genotyping was performed using Affymetrix Genome-Wide Human SNP array 6.0 (Affymetrix, Santa Clara, CA), which assays 925.939 SNPs. SNPs contributing to the dose variability of vitamin K antagonists were selected from the genome-wide association studies catalogue (https://www.ebi.ac.uk/gwas/) maintained by the National Human Genome Research Institute using the following search terms: "warfarin," "phenprocoumon," "anticoagulants," and "vitamin K antagonist".

Data Management and Statistical Analysis

A central data management unit was in charge of quality control including the performance of plausibility tests and review for completeness by predefined algorithms. Descriptive statistics were generated for all variables. For comparisons of binary and continuous variables, prevalence ratios and relative differences were calculated, respectively. In linear regression models, surrogate parameters of clinical and subclinical CVD were related to VKA treatment. Skewness was evaluated by density plot and log-transformed where appropriate. Covariates were selected on the basis of known cardiovascular risk factors and significant findings from the univariate analysis. Linear regression models were used to screen for interaction by including the interaction terms age×VKA and sex (women)×VKA in the model. Since antihypertensive drugs and statins are known to influence cardiovascular function and structure, multivariable linear models were adjusted accordingly. To evaluate a potential time-dependent effect of VKA use on surrogate markers of CVD, VKA treatment was stratified according to treatment length with a cutoff point of 3 years. To investigate a homogeneous subsample of VKA users, a subgroup analysis was conducted in the individuals diagnosed with atrial fibrillation and a CHA_2DS_2-VASc score of ≥1 (as such an indication for treatment with oral anticoagulation). Inverse probability of treatment weighting using the propensity score was performed in individuals with diagnosed atrial fibrillation/venous thromboembolism only under consideration of the cardiovascular profile (ie presence of traditional cardiovascular risk factors and history of cardiovascular diseases). Multivariable regression models with surrogate markers of CVD as dependent variables were conducted to investigate their relationship with SNPs encoding genes involved in the metabolism of VKA (as independent variable). Because of the explorative character of the analysis, a significance threshold was not defined for *P* values. *P* values were interpreted as continuous measure of statistical evidence. Statistical data analyses were conducted using the software program R, version 3.3.1 (http://www.r-project.org).

Results

Comparisons of Clinical and Biochemical Characteristics According to VKA Intake

Of 15 010 study participants, 287 (1.9%) received VKA (282 phenprocoumon and 5 warfarin) at the time of examination. A total of 159 subjects were excluded from the analysis (156 individuals with missing information on medication intake and 3 individuals receiving novel, direct-acting anticoagulants), resulting in a sample size of 14 851 individuals for the present analysis. Table 1 displays cardiovascular risk factors and comorbidities according to VKA use. Subjects with current VKA use were about 12 years older and more likely to be male. As expected, VKA users had a higher cardiovascular burden. The strongest inequalities in prevalence were seen for the entities with indication for treatment with oral anticoagulation, atrial fibrillation, and pulmonary embolism. The

Table 1. Cardiovascular Risk Profile of the Study Sample According to VKA Intake

	No Intake of VKA (N=14 564)	Intake of VKA (N=287)
Age, y	55.0 (46.0/64.0)	67.0 (61.0/71.0)
Sex (female), % (n)	50.0 (7282)	30.3 (87)
Traditional cardiovascular risk factors, % (n)		
Diabetes mellitus	7.4 (1069)	18.2 (52)
Dyslipidemia	29.3 (4257)	42.7 (122)
Family history of myocardial infarction and/or stroke	22.1 (3221)	24.0 (69)
Hypertension	49.5 (7208)	72.5 (208)
Obesity	25.0 (3638)	41.6 (119)
Smoking	19.5 (2834)	14.8 (42)
Comorbidities, % (n)		
Atrial fibrillation	2.2 (315)	58.1 (161)
Cancer	9.0 (1305)	16.8 (48)
Chronic kidney disease	3.1 (450)	14.7 (42)
Chronic obstructive pulmonary disease	5.0 (721)	8.7 (25)
Congestive heart failure	1.1 (158)	14.0 (40)
Coronary artery disease	4.1 (582)	21.3 (58)
Deep vein thrombosis	3.4 (498)	28.6 (80)
Liver disease	0.7 (107)	1.0 (3)
Myocardial infarction	2.7 (394)	15.7 (44)
Peripheral artery disease	3.1 (448)	18.4 (52)
Peripheral vascular bypass surgery	0.2 (31)	6.3 (18)
Pulmonary embolism	0.1 (13)	4.5 (13)
Stroke	1.6 (238)	14.4 (40)

Data are expressed as the relative and absolute frequencies for binary variables, for continuous variables as median with 25th/75th percentiles. Information on medication-based Anatomical Therapeutic Chemical code was available for 14 851 individuals. A total of 3 individuals received direct-acting anticoagulants and were therefore excluded from the analysis. VKA indicates vitamin K antagonists.

smallest differences were observed for current smoking and family history of myocardial infarction or stroke. With regard to indication for oral anticoagulation therapy, the majority of study participants (58.1%) had atrial fibrillation, followed by venous thromboembolism (33.1%). Subjects with oral anticoagulation were more likely to be using antidiabetic medications, antihypertensive drugs, diuretics, and lipid-modifying drugs. The intake of antiplatelet drugs did not differ between both groups (9.1% and 10.3% for VKA users and nonusers, respectively; Table S1).

Surrogate markers of clinical and subclinical CVD according to VKA intake are summarized in Table 2. As expected from the clinical characteristics, subjects with VKA treatment had a higher augmentation index, stiffness index, baseline brachial artery diameter, intima-media thickness, E/E′-ratio, left ventricular (LV) mass/height$^{2.7}$ and relative wall thickness as well as lower flow-mediated dilatation, reactive hyperemia index, and LV ejection fraction compared with individuals not taking VKA.

Accordingly, the concentrations of biomarkers related to cardiac function were higher in anticoagulated compared with nonanticoagulated subjects and there was greater inflammatory activity, as strongly reflected by the elevated concentration of hs-CRP. As proof for the VKA drug effect, activity of vitamin K-dependent coagulation factors was reduced in VKA users and concentrations of hs-D-dimer were ≈50% lower than in VKA-naïve participants (Tables S2 and S3). Concentrations of fibrinogen, however, were increased by 56%.

VKA Intake and Cardiovascular Status in Multivariable Regression Models

In a fully adjusted regression model, controlled for age, sex, and cardiovascular risk factors, the strongest independent associations with VKA use were observed for stiffness index (β=2.54 m/s [0.41; 4.66], *P*=0.019), ankle–brachial index (β=−0.03 [−0.04; −0.01], *P*<0.0001), mean intima-media thickness of the carotid artery (β=0.03 mm [0.01; 0.05], *P*=0.0098), LV ejection fraction of the heart (β=−4.02% [−4.70; −3.33], *P*<0.0001), E/E′ (β=0.04 [0.01; 0.08], *P*=0.014), and LV mass/height$^{2.7}$ (β=5.34 g/m^2 [4.26; 6.44], *P*<0.0001). Vascular function measured by flow-mediated dilatation, reactive hyperemia index, or reflection index was not associated with anticoagulation use (Table 3).

VKA Intake and Humoral Biomarkers in Multivariable Regression Models

A significant positive association with anticoagulation therapy was revealed for midregional pro-adrenomedullin, midregional pro-atrial natriuretic peptide, and N-terminal pro B-type natriuretic peptide as surrogates for the presence of heart failure. hs-CRP and fibrinogen concentrations were positively linked with anticoagulation use (β=0.31 mg/L [0.20; 0.43], *P*<0.0001 and β=143 mg/dL [132; 153], *P*<0.0001, respectively). Again, as proof of the VKA effect, activity levels of FVIII, von Willebrand factor were positively related with VKA use, whereas it was inversely related with FXI activity and hs-D-dimer concentration (Table 4).

Relationship Between Genetic Variants of VKA Metabolism and Surrogate Markers of CVD

Table 5 and Table S4 display the relationship between systematically selected SNPs associated with warfarin

Table 2. Surrogate Parameters of Clinical and Subclinical Cardiovascular Disease

		No Intake of VKA (N=14 564)	Intake of VKA (N=287)
Vasculature	Arterial stiffness		
	Augmentation index, %*	14.43 (3.12/29.03)	16.73 (6.74/31.48)
	Stiffness index, m/s	7.29 (5.78/9.13)	7.72 (6.36/9.26)
	Endothelial function		
	Flow-mediated dilation, %	7.4 (4.6/10.9)	6.0 (3.6/8.4)
	log (reactive hyperemia index)†	0.67 (0.33/0.94)	0.41 (0.13/0.78)
	Reflection index	68 (55/77)	69 (57/78)
	Endothelial structure		
	Baseline BA diameter, mm	4.32 (3.68/4.94)	4.81 (4.19/5.33)
	Intima-media thickness, mm‡	0.63 (0.56/0.73)	0.72 (0.66/0.85)
	Peripheral arterial disease		
	Ankle-brachial index	0.99 (0.93/1.04)	0.97 (0.88/1.06)
Heart	Cardiac function		
	Diastolic function—E/E′ ratio	7.18 (5.90/8.94)	8.33 (6.57/11.31)
	Systolic function—LV ejection fraction, %	63.5 (60.0/67.1)	60.8 (55.0/65.4)
	Cardiac structure		
	LV mass/height$^{2.7}$, g/m$^{2.7}$	36.5 (30.7/43.5)	45.9 (38.6/55.6)
	Relative wall thickness	0.395 (0.345/0.455)	0.424 (0.366/0.490)

For continuous variables, data are expressed as median with 25th/75th percentile. Data were available in >85% of participants, unless otherwise indicated. BA indicates brachial artery; LV, left ventricular; VKA, vitamin K antagonists.
*Measured in a sample of 11 250 participants.
†Measured in a sample of 10 512 participants.
‡Measured in a sample of the first 5000 participants.

maintenance dose and surrogate markers of CVD. *CYP2C9* polymorphisms mainly affected cardiac structure and function among VKA users. Carriers of the minor SNP allele had increased thrombomodulin levels and lower LV mass/height$^{2.7}$ in VKA users, but not in VKA nonusers. Rs2108622 was significantly correlated with an elevated concentration of fibrinogen in VKA users but not in VKA nonusers. The mutant allele of *CYP4F2* rs2108622 was linked to elevated F-XI concentrations and increased LV mass/height,$^{2.7}$ both in VKA users and nonusers, with higher estimates in VKA users.

Subgroup Analysis

In order to evaluate the results in a more homogeneous subgroup, linear regression models controlled for age, sex, and traditional cardiovascular risk factors were performed in individuals with atrial fibrillation and a CHA_2DS_2-VASc score of ≥1 only (see Table S5 for clinical characteristics). The results in this subgroup confirmed the earlier findings (Table S6): VKA therapy was independently related to LV ejection fraction (β=−2.12% [−3.85; −0.38], *P*=0.017), LV mass/height$^{2.7}$ (β=3.61 g/m^2 [0.90; 6.31], *P*=0.0089), midregional pro-atrial natriuretic peptide (β=0.42 pmol/L [0.25; 0.60], *P*<0.0001), N-terminal pro B-type natriuretic peptide (β=1.19 pg/mL [0.73; 1.65], *P*<0.0001), fibrinogen (β=135 mg/dL [112; 157], *P*<0.0001), F-XI (β=−10.8% [−17.5; −4.14], *P*=0.0015), hs-D-dimer (β=−1.07 µg/L [−1.31; −0.84], *P*<0.0001), and hs-CRP (β=0.20 mg/L [0.01; 0.39], *P*=0.035). With regard to arterial stiffness, VKA intake showed a similar effect on stiffness index in this sensitivity analysis as was demonstrated in the unrestricted analysis (β=3.45 m/s [−1.60; 8.50], *P*=0.18). A further propensity score analysis weighted for traditional cardiovascular risk factors and history of cardiovascular diseases in individuals with atrial fibrillation/venous thromboembolism confirmed the robustness of the observations made in the regression analysis (Table S7). To assess possible effects of the duration of VKA treatment on the interrelation between VKA intake and subclinical CVD phenotypes, data were analyzed stratified by the history of drug intake. In brief, hs-CRP concentrations were elevated by 11% when comparing patients with VKA treatment >3 years as opposed to those with shorter treatment. In line with the previous results, arterial stiffness, measured by augmentation index and stiffness index, was higher in individuals with long-term

Table 3. Multivariable Linear Regression Models on the Relationship Between Surrogate Parameters of Clinical and Subclinical Cardiovascular Disease and Therapy With VKA

		β-Estimates for VKA Therapy			
		Adjusted for Age and Sex		Additionally Adjusted for Cardiovascular Risk Factors*	
		β [95% CI]	*P* Value	β [95% CI]	*P* Value
Vasculature	Arterial stiffness				
	Augmentation index, %	−2.12 [−4.61; 0.37]	0.095	−1.23 [−3.66; 1.20]	0.32
	Stiffness index, m/s†	3.39 [1.24; 5.54]	0.0020	2.54 [0.41; 4.66]	0.019
	Endothelial function				
	Flow-mediated dilation, %	−0.11 [−0.75; 0.53]	0.73	0.09 [−0.55; 0.73]	0.79
	log (reactive hyperemia index)	−0.07 [−0.13; −0.01]	0.014	−0.05 [−0.11; 0.01]	0.078
	Reflection index	−0.87 [−2.74; 1.00]	0.36	−0.96 [−2.84; 0.91]	0.31
	Endothelial structure				
	Baseline BA diameter, mm	0.04 [−0.03; 0.11]	0.27	0.005 [−0.07; 0.08]	0.90
	Intima-media thickness, mm	0.03 [0.01; 0.05]	0.0048	0.03 [0.01; 0.05]	0.0098
	Peripheral arterial disease				
	Ankle–brachial index	−0.03 [−0.04; −0.01]	0.00012	−0.03 [−0.04; −0.01]	<0.0001
Heart	Cardiac function				
	Diastolic function—log (E/E′-ratio)	0.06 [0.02; 0.09]	0.0012	0.04 [0.01; 0.08]	0.014
	Systolic function—LV ejection fraction, %	−4.11 [−4.79; −3.43]	<0.0001	−4.02 [−4.70; −3.33]	<0.0001
	Cardiac structure				
	LV mass/height$^{2.7}$, g/m$^{2.7}$	6.27 [5.08; 7.45]	<0.0001	5.34 [4.26; 6.44]	<0.0001
	Relative wall thickness	3.6×10^{-3} [-6.2×10^{-3}; 13.4×10^{-3}]	0.47	-2.7×10^{-3} [-12.3×10^{-3}; 6.9×10^{-3}]	0.58

Effect estimates presented are β-values for VKA use (yes/no) derived from general linear models for each outcome. BA indicates brachial artery; CI, confidence interval; LV, left ventricular; VKA, vitamin K antagonists.

*Cardiovascular risk factors are diabetes mellitus, dyslipidemia, hypertension, obesity, smoking, family history of stroke/myocardial infarction, and estimated glomerular filtration rate.

†Displayed estimates are given for mean age of 55 years; model was additionally adjusted for age×VKA interaction.

exposure to VKA compared with those with short-term exposure, supporting a dose–response relationship (Table 6). For further evaluation of a time-dependent, cumulative effect of VKA intake on subclinical phenotypes of CVD, treatment duration was stratified in <1, 1 to 3, and >3 years as illustrated in Table S8. In brief, the analysis confirmed a dose-dependent interrelation between intake of VKA and specific biomarkers identified in the prior analysis (eg stiffness index, E/E′ ratio, and LV mass).

Discussion

The present study investigated, for the first time, the link between the use of VKA and a comprehensive set of clinical and subclinical measures of CVD in a large population-based sample. The analysis demonstrated a relationship of VKA treatment with increased arterial stiffness, higher LV mass, and decreased cardiac systolic function independent of the clinical profile. Correspondingly, anticoagulation use was also linked with increased concentrations of humoral biomarkers of cardiac function and inflammation. Subgroup analysis confirmed these data: a homogeneous subsample of subjects with atrial fibrillation with indication for oral anticoagulation based on the CHA_2DS_2-VASc score showed consistent results for the comparison of VKA users to anticoagulation-naïve individuals. As an indicator for a dose–response effect, levels of arterial stiffness and hs-CRP were higher in long-term VKA users compared with individuals with shorter intake.

The results from this large population sample are supported by data from the literature: an observational study reported that warfarin administration is associated with a rapid progression of aortic stiffness in patients undergoing hemodialysis.[11] Arterial stiffness is influenced by the calcification of the elastic components of the artery wall, leading to hypertension. In humans, studies of limited sample size have reported an association of VKA treatment with calcification of the coronary arteries.[8,12,13] Also, carotid intima-media

Table 4. Multivariable Linear Regression Models on the Relationship Between Humoral Biomarkers and Therapy With VKA

	β-Estimates for VKA Therapy			
	Adjusted for Age and Sex		Additionally Adjusted for Cardiovascular Risk Factors*	
	β [95% CI]	*P* Value	β [95% CI]	*P* Value
Biomarkers of cardiac function				
log (MR-proANP), pmol/L	0.59 [0.51; 0.67]	<0.0001	0.58 [0.50; 0.65]	<0.0001
log (MR-proADM), nmol/L	0.22 [0.18; 0.27]	<0.0001	0.18 [0.14; 0.22]	<0.0001
log (Nt-proBNP), pg/mL†	1.93 [1.66; 2.19]	<0.0001	1.90 [1.63; 2.17]	<0.0001
Biomarkers of coagulation				
Fibrinogen, mg/dL†	147 [136; 158]	<0.0001	143 [132; 153]	<0.0001
F-VIII, %	15.8 [8.99; 22.7]	<0.0001	13.4 [6.7; 20.2]	<0.0001
F-XI, %	−9.01 [−12.7; −5.30]	<0.0001	−9.63 [−13.31; −5.95]	<0.0001
log (hs-D-dimer), μg/L	−0.89 [−1.02; −0.76]	<0.0001	−0.92 [−1.05; −0.79]	<0.0001
log (thrombomodulin), %	0.04 [−0.04; 0.12]	0.31	0.01 [−0.07; 0.09]	0.76
Tissue factor, %	16.9 [−3.89; 37.7]	0.11	14.7 [−6.0; 35.4]	0.16
vWF, %	13.4 [5.9; 20.9]	0.00047	11.2 [3.8; 18.7]	0.0032
Biomarkers of inflammation				
log (hs-CRP), mg/L	0.42 [0.30; 0.55]	<0.0001	0.31 [0.20; 0.43]	<0.0001
IL-18, pg/mL	23.0 [−0.91; 46.9]	0.059	17.9 [−5.7; 41.5]	0.14
IL-1RA, pg/mL†	36.0 [−10.1; 82.2]	0.13	17.2 [−26.0; 60.3]	0.43
Leukocyte count, /nL	0.03 [−0.001; 0.06]	0.058	0.01 [−0.02; 0.04]	0.39
MPO, pmol/L	33.9 [−1.58; 69.4]	0.061	30.6 [−5.0; 66.2]	0.092

Effect estimates presented are β-values for VKA use (yes/no) derived from general linear models for each outcome. All biomarkers were measured in 5000 participants, except CRP and leukocyte count (available for 15 010 participants). CI indicates confidence interval; hs-CRP, high sensitivity C-reactive protein; MPO, myeloperoxidase; Nt-proBNP, N-terminal pro B-type natriuretic peptide; MR-proADM, midregional pro-adrenomedullin; MR-proANP, midregional pro-atrial natriuretic peptide; VKA, vitamin K antagonists; vWF, von Willebrand factor.
*Cardiovascular risk factors are diabetes mellitus, dyslipidemia, hypertension, obesity, smoking, family history of stroke/myocardial infarction, and estimated glomerular filtration rate.
†Displayed estimates are given for men; model was additionally adjusted for sex (women)×VKA interaction; the estimates for women have to be corrected by adding the following values: Nt-proBNP, −0.95; fibrinogen, +39.0; IL-1RA, +111.

thickness is known to be correlated with atherosclerotic calcification.[14] Interestingly, in the present analysis anticoagulation therapy remained independently related to higher intima-media thickness after adjustment for the clinical profile.

Experimental data indicated that VKA may lead to calcification via inhibition of MGP, a vitamin K-dependent protein produced by vascular smooth muscle cells, which is considered to be a strong inhibitor of vascular calcification.[15] MGP-knockout mice developed soft-tissue calcification resulting in vascular stiffening and died of vascular rupture 8 weeks after birth.[16] Furthermore, both valvular and arterial calcification have been reported in animals on warfarin treatment.[17] By contrast, limited data from experimental animal studies have indicated a potentially beneficial effect of novel, direct-acting anticoagulants on the development and progression of atherosclerosis.[18–20]

Aortic and cardiac valve calcification as well as the abnormal pressure caused by calcification increase cardiac afterload and therefore may promote the development of systolic and diastolic cardiac dysfunction, LV hypertrophy, aortic stenosis, and subsequently congestive heart failure.[21] In the present study, these findings were substantiated by demonstrating an aggravation of cardiac dysfunction in VKA patients compared with the reference group without VKA. Linear regression analyses suggest a link between anticoagulation therapy and an increased LV mass/height$^{2.7}$ ratio, potentially caused by arterial hypertension because of higher stiffness or valve resistance.[22]

The current study demonstrated the presence of elevated concentrations of inflammatory biomarkers (ie hs-CRP, fibrinogen) in individuals on VKA treatment. Previous reports on the inflammation profile of anticoagulated subjects are rare and rather inconsistent. Studies have demonstrated anti-inflammatory effects at low-dose warfarin concentrations in animals[23] and showed little or no effect at the concentration that is used in the clinical setting to reduce hypercoagulability.[24] These studies, however, are prone to

Table 5. SNPs Identified in GWAS Catalogue Known to Influence Warfarin Dose Requirements and Their Relationship to Surrogate Parameters of Atherosclerosis

Selected SNPs From GWAS Catalogue	Chr	Position (Mb)*	Gene	Tag SNP on Affymetrix 6.0 With r^2>0.9	Effect of Minor Allele	Effect Under VKA Use	β Estimate for VKA User†	Effect Under No VKA Use	β Estimate for VKA Nonuser†
rs10509680	10	96734339	*CYP2C9*	rs9332245	Lower	Baseline BA diameter ↑	0.153	Baseline BA diameter ↓	−0.057
					dose	E/E′ ↓	−0.230	E/E′ →	No effect
					requirement				
rs12777823	10	96405502	*CYP2C9*	n.a.	Lower	Fibrinogen ↑	8.7	Fibrinogen ↑	5.6
					dose	Flow-mediated dilation ↓	−0.44	Flow-mediated dilation ↓	−0.46
					requirement	Relative wall thickness ↑	0.05	Relative wall thickness →	No effect
						Ejection fraction ↑	5.54	Ejection fraction →	No effect
						IL-18 ↑	100.8	IL-18 →	No effect
rs4086116	10	96707202	*CYP2C9*	n.a.	Lower	Baseline BA diameter ↓	−0.144	Baseline BA diameter ↓	−0.034
					dose	MR-proADM ↓	−0.057	MR-proADM ↑	0.013
					requirement	IL-18 ↓	−16.6	IL-18 ↑	8.4
						Ejection fraction ↓	−4.27	Ejection fraction →	No effect
						LV mass/height$^{2.7}$ ↑	4.59	LV mass/height$^{2.7}$ →	No effect
rs10871454	16	31048079	*VKORC1*	rs11150604	Lower	Thrombomodulin ↑	0.199	Thrombomodulin ↓	−0.021
					dose	LV mass/height$^{2.7}$ ↓	−2.42	LV mass/height$^{2.7}$ →	No effect
					requirement				
rs2108622	19	15990431	*CYP4F2*	n.a.	Higher	LV mass/height$^{2.7}$ ↑	1.68	LV mass/height$^{2.7}$ ↑	0.48
					dose	F-XI ↑	5.5	F-XI ↑	1.0
					requirement	Fibrinogen ↑	30.47	Fibrinogen →	No effect

BA indicates brachial artery; Chr, chromosome; GWAS, genome-wide association studies; IL-18, interleukin 18; LV, left ventricular; MR-proADM, midregional pro-adrenomedullin; n.a., not available; SNP, single nucleotide polymorphism.
*Based on genome built 105.
†Estimated change per allele.

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Table 6. Surrogate Parameters of Clinical and Subclinical Cardiovascular Disease and Humoral Biomarkers According to History of VKA Treatment Length

		3 Y or Less (N=130)	More Than 3 Y (N=130)	Difference
Vascular structure and function	Arterial stiffness			
	Augmentation index, %	14.6 (6.2/29.2)	19.7 (7.6/33.2)	+35%
	Stiffness index, m/s	7.56 (6.12/8.92)	7.89 (6.41/9.58)	+4%
	Endothelial function			
	Flow-mediated dilation, %	6.21 (3.61/8.99)	5.90 (3.63/8.12)	−5%
	Log (reactive hyperemia index)	0.43 (0.10/0.88)	0.34 (0.15/0.74)	−21%
	Reflection-index	66.5 (54.0/77.0)	72.0 (60.3/80.0)	+8%
	Endothelial structure			
	Baseline BA diameter, mm	4.73 (4.08/5.33)	4.95 (4.25/5.33)	+5%
	Intima-media thickness, mm	0.72 (0.63/0.84)	0.73 (0.68/0.85)	+2%
	Peripheral arterial disease			
	Ankle–brachial index	0.97 (0.89/1.04)	0.97 (0.88/1.07)	0%
Cardiac structure and function	Cardiac function			
	E/E′-ratio	8.02 (6.32/10.03)	8.73 (6.80/12.38)	+9%
	Ejection fraction, %	61.1 (55.4/66.4)	60.0 (53.7/65.1)	−2%
	Cardiac structure			
	LV mass/height$^{2.7}$, g/m$^{2.7}$	45.6 (37.2/54.5)	47.6 (38.9/56.9)	+4%
	Relative wall thickness	0.429 (0.371/0.489)	0.417 (0.365/0.507)	−3%
Humoral biomarker	Biomarker of coagulation			
	Fibrinogen, mg/dL	506 (438/584)	500 (428/588)	−1%
	Biomarker of inflammation			
	hs-CRP, mg/L	2.65 (1.20/5.50)	2.95 (1.30/5.42)	+11%
	Leukocytes, /nL	7.08 (5.93/8.20)	7.30 (6.19/8.50)	+3%

For continuous variables, data are expressed as median with 25th/75th percentile. The percentage differences represent an increase or decrease going from "VKA use of 3 y or less" to "VKA use of more than 3 y." BA indicates brachial artery; hsCRP, high-sensitivity C-reactive protein; LV, left ventricular; VKA, vitamin K antagonists.

methodological limitations including small sample sizes. Importantly, it is well recognized that coagulation factors play a significant role in the process of inflammation and atherosclerosis.[25] The attenuation of protein C has been reported to reduce anti-apoptotic activity of the endothelial barrier and to promote local inflammation within the arterial wall,[25] which is accompanied, at least to a certain degree, by calcification.[26] In addition, infiltration of vascular tissue is characterized by increased oxidative stress and subsequently by endothelial dysfunction leading to increased vascular stiffness.[27] Coumarin derivatives including phenprocoumon have been shown to act as sepiapterin reductase inhibitors leading to intracellular tetrahydrobiopterin (BH_4) depletion.[28] Endothelial BH_4 depletion in turn may reduce vascular nitric oxide production or even cause endothelial nitric oxide synthase uncoupling associated with endothelial dysfunction and therefore higher vascular production of reactive oxygen species within the vascular wall.[29] Thus, the resulting reduction in vascular nitric oxide bioavailability may also contribute to increased vascular stiffness. VKA also inhibit the carboxylation of Gas-6, which protects vascular smooth muscle cells from calcification by inhibiting apoptosis.[30] This may have contributed to the enhanced inflammation observed in the present study. Therefore, one might speculate that VKA cause inflammation and apoptosis of vascular smooth muscle cells while simultaneously reducing endothelial nitric oxide production, which could potentially accelerate the process of vascular and cardiac damage. It merits consideration that the influence of VKA treatment may affect specific vascular beds differently (eg according to the content of VKA-dependent extracellular matrix protein Gla), which may also impact on the subsequent clinical outcome.[31] As an interconnecting link, the increased level of systemic inflammation upon VKA intake may serve as a potential explanation for VKA-induced propagation of (subclinical) atherosclerosis and cardiovascular disease.

Finally, the effect of genetic polymorphisms associated with VKA dosing surrogate markers for prevalent CVD was investigated. Genome-wide association studies have identified the association of CYP2C9, VKORC1, and CYP4F2 SNPs with stable VKA dosing.[32] Interestingly, comparable effects of gene variants on enzyme activity did not always match with homogeneous effects on cardiac structure and function, and inflammation. Among VKA users and VKA-naïve subjects, SNPs affecting the VKA dosing translated into different effects on subclinical and humoral markers of CVD. The influence of VKA metabolism indicates that VKA therapy has a link to CVD progression, rather than an underlying CVD. Although these analyses do not provide sufficient evidence to avoid the use of VKA in patients susceptible to deterioration of cardiac and vascular function, the potential implication for individualized antithrombotic therapy merits further investigation, especially in comparison to direct inhibition of FIIa and FXa, respectively.

Limitations

There are several limitations that need to be considered when interpreting the data of the present study. First, the cross-sectional design does not allow for making any inferences about cause and effect. Prospective data covering an adequate period of exposure to VKA is necessary to analyze the effects of VKA on atherosclerosis over time. Second, although a large panel of potential confounders was adjusted for in regression analysis, the possibility that unmeasured confounders might have contributed to the observed findings cannot be excluded. Third, information on serum concentrations of MGP and Gas-6 were not available for the present study, but rather likely biological sequelae were investigated. Fourth, individuals receiving novel, direct-acting anticoagulants for oral anticoagulation therapy were not available for comparison at a statistically adequate sample size in the study sample. Fifth, the limited sample size did not allow providing specific subgroup analysis for potentially vulnerable patient populations (eg patients with type 2 diabetes mellitus). Sixth, parts of the findings of the present study confirm prior investigations on the associations of VKA and subclinical markers of CVD. Finally, the results may not be extrapolated to populations of other ethnic backgrounds as allele frequencies of CYP2C9 and VKORC1 vary among ethnic groups.

Conclusions

In summary, the present investigation indicates an independent association between the use of VKA and surrogate parameters of arterial stiffness, vascular morphology, cardiac structure and function, and inflammation in the population. Given the high coprevalence of oral anticoagulation therapy with (sub)clinical atherosclerosis and the increasing need for antithrombotic agents in the future, these findings may have implications for individually tailored approaches for antithrombotic therapy.

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References

1. Guyatt GH, Akl EA, Crowther M, Gutterman DD, Schuunemann HJ. Executive summary: antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians evidence-based clinical practice guidelines. *Chest*. 2012;141:7s–47s.
2. Palaniswamy C, Sekhri A, Aronow WS, Kalra A, Peterson SJ. Association of warfarin use with valvular and vascular calcification: a review. *Clin Cardiol*. 2011;34:74–81.
3. Chatrou ML, Winckers K, Hackeng TM, Reutelingsperger CP, Schurgers LJ. Vascular calcification: the price to pay for anticoagulation therapy with vitamin K-antagonists. *Blood Rev*. 2012;26:155–166.
4. Danziger J. Vitamin K-dependent proteins, warfarin, and vascular calcification. *Clin J Am Soc Nephrol*. 2008;3:1504–1510.
5. Schurgers LJ, Teunissen KJ, Knapen MH, Kwaijtaal M, van Diest R, Appels A, Reutelingsperger CP, Cleutjens JP, Vermeer C. Novel conformation-specific antibodies against matrix gamma-carboxyglutamic acid (Gla) protein:

undercarboxylated matrix Gla protein as marker for vascular calcification. *Arterioscler Thromb Vasc Biol*. 2005;25:1629–1633.

6. Koos R, Krueger T, Westenfeld R, Kuhl HP, Brandenburg V, Mahnken AH, Stanzel S, Vermeer C, Cranenburg EC, Floege J, Kelm M, Schurgers LJ. Relation of circulating matrix Gla-protein and anticoagulation status in patients with aortic valve calcification. *Thromb Haemost*. 2009;101:706–713.
7. Han KH, O'Neill WC. Increased peripheral arterial calcification in patients receiving warfarin. *J Am Heart Assoc*. 2016;5:e002665. DOI: 10.1161/JAHA.115.002665.
8. Rennenberg RJ, van Varik BJ, Schurgers LJ, Hamulyak K, Ten Cate H, Leiner T, Vermeer C, de Leeuw PW, Kroon AA. Chronic coumarin treatment is associated with increased extracoronary arterial calcification in humans. *Blood*. 2010;115:5121–5123.
9. Schurgers LJ, Aebert H, Vermeer C, Bultmann B, Janzen J. Oral anticoagulant treatment: friend or foe in cardiovascular disease? *Blood*. 2004;104:3231–3232.
10. Wild PS, Zeller T, Beutel M, Blettner M, Dugi KA, Lackner KJ, Pfeiffer N, Munzel T, Blankenberg S. [The Gutenberg health study]. *Bundesgesundheitsblatt, Gesundheitsforschung, Gesundheitsschutz*. 2012;55:824–829.
11. Mac-Way F, Poulin A, Utescu MS, De Serres SA, Marquis K, Douville P, Desmeules S, Lariviere R, Lebel M, Agharazii M. The impact of warfarin on the rate of progression of aortic stiffness in hemodialysis patients: a longitudinal study. *Nephrol Dial Transplant*. 2014;29:2113–2120.
12. Weijs B, Blaauw Y, Rennenberg RJ, Schurgers LJ, Timmermans CC, Pison L, Nieuwlaat R, Hofstra L, Kroon AA, Wildberger J, Crijns HJ. Patients using vitamin K antagonists show increased levels of coronary calcification: an observational study in low-risk atrial fibrillation patients. *Eur Heart J*. 2011;32:2555–2562.
13. Tantisattamo E, Han KH, O'Neill WC. Increased vascular calcification in patients receiving warfarin. *Arterioscler Thromb Vasc Biol*. 2015;35:237–242.
14. Allison MA, Tiefenbrun J, Langer RD, Wright CM. Atherosclerotic calcification and intimal medial thickness of the carotid arteries. *Int J Cardiol*. 2005;103:98–104.
15. Ueland T, Gullestad L, Dahl CP, Aukrust P, Aakhus S, Solberg OG, Vermeer C, Schurgers LJ. Undercarboxylated matrix Gla protein is associated with indices of heart failure and mortality in symptomatic aortic stenosis. *J Intern Med*. 2010;268:483–492.
16. Luo G, Ducy P, McKee MD, Pinero GJ, Loyer E, Behringer RR, Karsenty G. Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein. *Nature*. 1997;386:78–81.
17. Price PA, Faus SA, Williamson MK. Warfarin causes rapid calcification of the elastic lamellae in rat arteries and heart valves. *Arterioscler Thromb Vasc Biol*. 1998;18:1400–1407.
18. Hara T, Fukuda D, Tanaka K, Higashikuni Y, Hirata Y, Nishimoto S, Yagi S, Yamada H, Soeki T, Wakatsuki T, Shimabukuro M, Sata M. Rivaroxaban, a novel oral anticoagulant, attenuates atherosclerotic plaque progression and destabilization in ApoE-deficient mice. *Atherosclerosis*. 2015;242:639–646.
19. Kadoglou NP, Moustardas P, Katsimpoulas M, Kapelouzou A, Kostomitsopoulos N, Schafer K, Kostakis A, Liapis CD. The beneficial effects of a direct thrombin inhibitor, dabigatran etexilate, on the development and stability of atherosclerotic lesions in apolipoprotein E-deficient mice: dabigatran etexilate and atherosclerosis. *Cardiovasc Drugs Ther*. 2012;26:367–374.
20. Schurgers LJ, Spronk HM. Differential cellular effects of old and new oral anticoagulants: consequences to the genesis and progression of atherosclerosis. *Thromb Haemost*. 2014;112:909–917.
21. Demer LL, Tintut Y. Vascular calcification: pathobiology of a multifaceted disease. *Circulation*. 2008;117:2938–2948.
22. Ventura JE, Tavella N, Romero C, Petraglia A, Baez A, Munoz L. Aortic valve calcification is an independent factor of left ventricular hypertrophy in patients on maintenance haemodialysis. *Nephrol Dial Transplant*. 2002;17:1795–1801.
23. Kater AP, Peppelenbosch MP, Brandjes DP, Lumbantobing M. Dichotomal effect of the coumadin derivative warfarin on inflammatory signal transduction. *Clin Diagn Lab Immunol*. 2002;9:1396–1397.
24. Maclean PS, Tait RC, Rumley A, McMahon AD, Lowe GD. Anticoagulation with warfarin downregulates inflammation. *J Thromb Haemost*. 2003;1:1838–1839.
25. Borissoff JI, Spronk HM, ten Cate H. The hemostatic system as a modulator of atherosclerosis. *N Engl J Med*. 2011;364:1746–1760.
26. New SE, Aikawa E. Molecular imaging insights into early inflammatory stages of arterial and aortic valve calcification. *Circ Res*. 2011;108:1381–1391.
27. Wenzel P, Knorr M, Kossmann S, Stratmann J, Hausding M, Schuhmacher S, Karbach SH, Schwenk M, Yogev N, Schulz E, Oelze M, Grabbe S, Jonuleit H, Becker C, Daiber A, Waisman A, Munzel T. Lysozyme M-positive monocytes mediate angiotensin II-induced arterial hypertension and vascular dysfunction. *Circulation*. 2011;124:1370–1381.
28. Schoedon G, Blau N, Schneemann M, Flury G, Schaffner A. Nitric oxide production depends on preceding tetrahydrobiopterin synthesis by endothelial cells: selective suppression of induced nitric oxide production by sepiapterin reductase inhibitors. *Biochem Biophys Res Commun*. 1994;199:504–510.
29. Forstermann U, Munzel T. Endothelial nitric oxide synthase in vascular disease: from marvel to menace. *Circulation*. 2006;113:1708–1714.
30. Clarke MC, Talib S, Figg NL, Bennett MR. Vascular smooth muscle cell apoptosis induces interleukin-1-directed inflammation: effects of hyperlipidemia-mediated inhibition of phagocytosis. *Circ Res*. 2010;106:363–372.
31. Allison MA, Hsi S, Wassel CL, Morgan C, Ix JH, Wright CM, Criqui MH. Calcified atherosclerosis in different vascular beds and the risk of mortality. *Arterioscler Thromb Vasc Biol*. 2012;32:140–146.
32. Cooper GM, Johnson JA, Langaee TY, Feng H, Stanaway IB, Schwarz UI, Ritchie MD, Stein CM, Roden DM, Smith JD, Veenstra DL, Rettie AE, Rieder MJ. A genome-wide scan for common genetic variants with a large influence on warfarin maintenance dose. *Blood*. 2008;112:1022–1027.

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)