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The effect of six-month oral vitamin K supplementation on calcification propensity time in individuals with type 2 diabetes mellitus: A *post hoc* analysis of a randomized, double-blind, placebo-controlled trial

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ABSTRACT

Background and aims: Experimental studies suggested that vitamin K supplementation may retard arterial calcification. Recently, serum calcification propensity time (T_{50}) has been suggested as a functional biomarker for arterial wall calcification propensity. In this *post-hoc* analysis of a clinical trial, we evaluated the effect of six-month oral vitamin K supplementation on T_{50} and assessed the correlation between T_{50} and imaging arterial calcification parameters in people with type 2 diabetes (T2DM).

Methods: This double-blind, randomized, placebo-controlled trial included 68 participants (age = 69 ± 8 years, 76% male) with T2DM. Participants were assigned to menaquinone-7 (360 μg/day; n = 35) or placebo (n = 33). T_{50} was measured via nephelometry in serum collected at baseline, three and six months. Arterial calcification was measured at baseline and six months via ^{18}F–Na PET-CT and conventional CT using Target-to-Background ratio (TBR) and Agatston score. Longitudinal analysis of covariance adjusted for baseline T_{50} was used to study the treatment effect. Spearman's correlation was used to assess the correlation between T_{50} and imaging calcification parameters.

Results: Median baseline T_{50} was similar in the vitamin K (350 [321–394] minutes) and placebo groups (363 [320–398]). There was no significant difference in T_{50} between treatment arms over time (ß = 1.00, 95%C.I. = 0.94–1.07, $p = 0.982$). The correlation coefficient of T_{50} with TBR and Agatston score at baseline were −0.185 ($p = 0.156$) and −0.121 ($p = 0.358$), respectively.

Conclusions: No effect of vitamin K supplementation on T_{50} was observed in T2DM. Moreover, T_{50} did not correlate with TBR and Agatston score. Further research on vitamin K in arterial calcification and on the validity of T_{50} as arterial calcification marker is warranted.

1. Introduction

Cardiovascular disease (CVD) is the main cause of mortality in people with type 2 diabetes mellitus (T2DM) [1]. Moreover, arterial calcification is associated with a three to four-fold increased CVD risk and is highly prevalent in people with T2DM [1,2]. As such, interventional studies should focus on interfering with the arterial calcification process, which may ultimately reduce CVD risk.

The process of arterial calcification is complex and involves an interplay between stimulators and inhibitors [3]. Matrix Gla protein (MGP) is the most potent inhibitor of arterial calcification in active form [4]. Vitamin K is a cofactor in the carboxylation of inactive MGP to its active form. Observational and experimental studies have shown that high vitamin K intake is associated with reduced arterial calcification

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[5], and CVD risk [6–9]. As such, it is hypothesized that vitamin K may delay the progression of arterial calcification through conversion of inactive MGP to active MGP.

Various trials have been conducted to evaluate the effect of vitamin K on arterial calcification [5,10–18]. However, results are inconclusive. Most of these trials measured arterial calcification either via ^{18}F–Na photon emission computed tomography (PET-CT) [10,11,17] or via conventional computed tomography (CT) [5,12,14–18]. Disadvantages of these imaging modalities are the high costs and the radiation dose [19]. These disadvantages limit the applicability of calcification quantification, especially by PET-CT, in routine clinical care and in large epidemiological studies [20]. Therefore, there is need for a low-cost, radiation-free and sensitive marker of arterial calcification.

Recently, serum calcification propensity time (T_{50}) has been suggested as a novel marker of arterial calcification propensity. This parameter is measured in serum using nephelometry and represents the formation rate of calciprotein particles (CPPs) responsible for ectopic calcification, including arterial wall calcification [21]. This assay is relatively cheap and radiation-free, making it more suitable for routine clinical care and large epidemiological studies than advanced imaging techniques [20]. However, there is lack of validity of T_{50} as marker of arterial calcification and subsequent CVD risk. Recent evidence shows that a longer T_{50} is associated with reduced CVD risk [22]. Two studies investigated the association between T_{50} and CT-derived parameters of arterial calcification. A recent study observed that T_{50} was correlated with calcification mass score ($r = -0.557$, $p < 0.05$) and Agatston score ($r = -0.534$, $p < 0.05$) of the abdominal aorta of patients with primary aldosteronism [23]. A similar finding was observed in another study in which every standard deviation shorter T_{50} was significantly associated with a 21% higher coronary Agatston score among people with chronic kidney disease [24]. However, the association between T_{50} and PET-CT-derived parameters of arterial calcification has not been studied before.

Over the last years, more attention has been devoted to the role of vitamin K in arterial calcification, as previous studies have shown that vitamin K is a promising, low-cost and safe treatment option for improving cardiovascular health [10,18,25]. Thus far, only one short-term study evaluated the effect of oral vitamin K supplementation on T_{50} in vitamin K-deficient kidney transplant recipients [26]. No significant effect of vitamin K on T_{50} was observed.

The aim of this *post-hoc* analysis was to evaluate the effect of six-month oral vitamin K supplementation on T_{50} in people with T2DM. Moreover, we investigated whether T_{50} correlated with imaging calcification parameters measured via ^{18}F–Na PET-CT and conventional CT. These aims are secondary analyses of a randomized, double-blind and placebo-controlled trial of which the results have been previously published [17]. Results of this research may shed light upon the modifiability of T_{50} by vitamin K in T2DM, and on the validity of T_{50} as marker of arterial calcification.

2. Patients and methods

2.1. Study design and population

This study is a *post-hoc* analysis of a double-blind, randomized, placebo-controlled trial conducted at the University Medical Center Utrecht (UMCU). The design has been described elsewhere [17]. Participants were diagnosed with T2DM and were recruited through the pre-existing Diabetes Pearl String Initiative cohort [27], the Julius Center database of subjects who showed interest in participation in clinical trials, and the outpatient clinics of the UMCU and Diakonessenhuis Utrecht. Individuals were eligible for participation when they were older than 40 years at recruitment, had pre-existing CVD and had an estimated glomerular filtration rate (eGFR) above 30 mL/min/1.73 m^2. Individuals were excluded from participation in the parent trial when they used vitamin K antagonists, multivitamins containing vitamin K, showed unwillingness to stop vitamin K supplementation before randomisation or had known coagulation problems. Randomisation was stratified by sex and was performed in a 1:1 ratio by the UMCU data management department. The randomisation key was safeguarded by the data management and the pharmacy departments. The clinical trial lasted six months with measurements performed at baseline, at three months and at six months. All participants provided written informed consent before participation. This trial was reviewed and approved by the institutional board of the UMC Utrecht and registered in clinical trial registries (NCT02839044/NTR5287).

2.2. Intervention

Participants were randomly allocated to treatment arms using oral vitamin K supplementation or placebo. Daily vitamin K supplementation consisted of 360 μg menaquinone-7 (Nattopharma). Placebo (Legosan) was similar in taste and appearance. Participants were instructed to return leftover tablets during the study visit at three months and six months. Subsequently, compliance was calculated.

2.3. Serum calcification propensity time (T_{50})

The primary outcome was the difference in change of T_{50} from baseline to six months between vitamin K and placebo. T_{50} was measured at baseline, at three months and at six months. During these three study visits, non-fasting serum samples were collected via venepuncture. The serum samples were frozen and stored at −80 °C in the UMCU biobank. Four years after trial completion, the frozen serum samples were shipped to Switzerland for determination of T_{50}. This measurement was performed via nephelometry, as described by Pasch [21]. A shorter T_{50} reflects a higher formation rate of CPP, involved in ectopic calcification, including arterial wall calcification. Frozen samples are suitable for this assay and the coefficient of variation was 6% [21].

2.4. Imaging tests

Arterial calcification was measured using low-dose, full-body ^{18}F–Na PET-CT scans (Siemens Biograph 40 scanner; Siemens Healthcare, Erlangen, Germany) at baseline and at six months.

On CT, lower-extremity CT arterial calcification was quantified using a locally developed program (iX Viewer, Image Science Institute, Utrecht, the Netherlands). A calcific lesion was defined as a dense lesion in the arterial wall with Hounsfield Units (HU) > 130. Calcification from the bifurcation of the femoral arteries to the femur condyles was measured using the Agatston method [28]. The inter-rater reliability was 0.998 (95%C.I. = 0.995–0.999).

On PET, arterial calcification was quantified using the Target-to-Background ratio (TBR). The injected tracer (2.0 MBq/kg ^{18}F–Na, with a maximum dosage of 200 MBq) binds to hydroxyapatite within the femoral arterial wall. This target signal was detected by PET-CT scan and was measured as maximal SUV (SUV_{max}) of each slice of the left and right femoral artery. All the slices were averaged. As background, the mean blood pool activity was determined in the superior vena cava on three slices (SUV_{mean}). The inter-rater reliability was 0.98 (95%C.I. = 0.94–0.99).

2.5. Biochemical measurements

Non-fasting serum samples were drawn at each study visit and analysed for measurement of total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides (all mmol/L), HbA1c (mmol/mol) and creatinine levels (μmol/L) using standard laboratory techniques. The CKD-EPI formula was used to estimate the eGFR (mL/min/1.73 m^2) [29]. Levels of dp-ucMGP (pmol/L) were measured using the sandwich ELISA method via the IDS Automated Analyser MGP assay (Maastricht

University).

2.6. Covariates

Data on covariates were obtained at all three study visits. The paper by Zwakenberg et al. describes how information related to demography, anthropometry, lifestyle, CVD history and medication use was collected [17].

Vascular measurements were performed in duplicate using an automated oscillomat (Omron HEM-907) to measure systolic blood pressure (SBP; mmHg), diastolic blood pressure (DBP; mmHg) and Ankle-Brachial Index (ABI; unitless). ABI is a measure of vascular stiffness and was only measured at baseline. ABI was calculated by dividing the highest average arm SBP by the highest ankle SBP.

Finally, data on intakes of energy (kcal/day), vitamin K1, vitamin K2 and total vitamin K (all mg/day) were estimated using a three-day food diary. The diary included two weekdays and one weekend day, all non-consecutive, and requested data on breakfast, lunch, dinner and snacks. Data from the diary were analysed using Evry (Ensemble BV), the 2013 Dutch national food composition table [30], and the vitamin K food content database [31]. The energy-adjusted intakes of total vitamin K, vitamin K1 and vitamin K2 were calculated using the regression residual method.

2.7. Statistical analysis

Baseline characteristics were described for both intervention arms. Continuous variables were reported depending on their distribution, using mean ± standard deviation for normally distributed variables, or using median [interquartile range] for non-normally distributed variables. Normality of covariates was assessed using the Shapiro Wilks test. Categorical variables were presented as frequency (percentage). We did

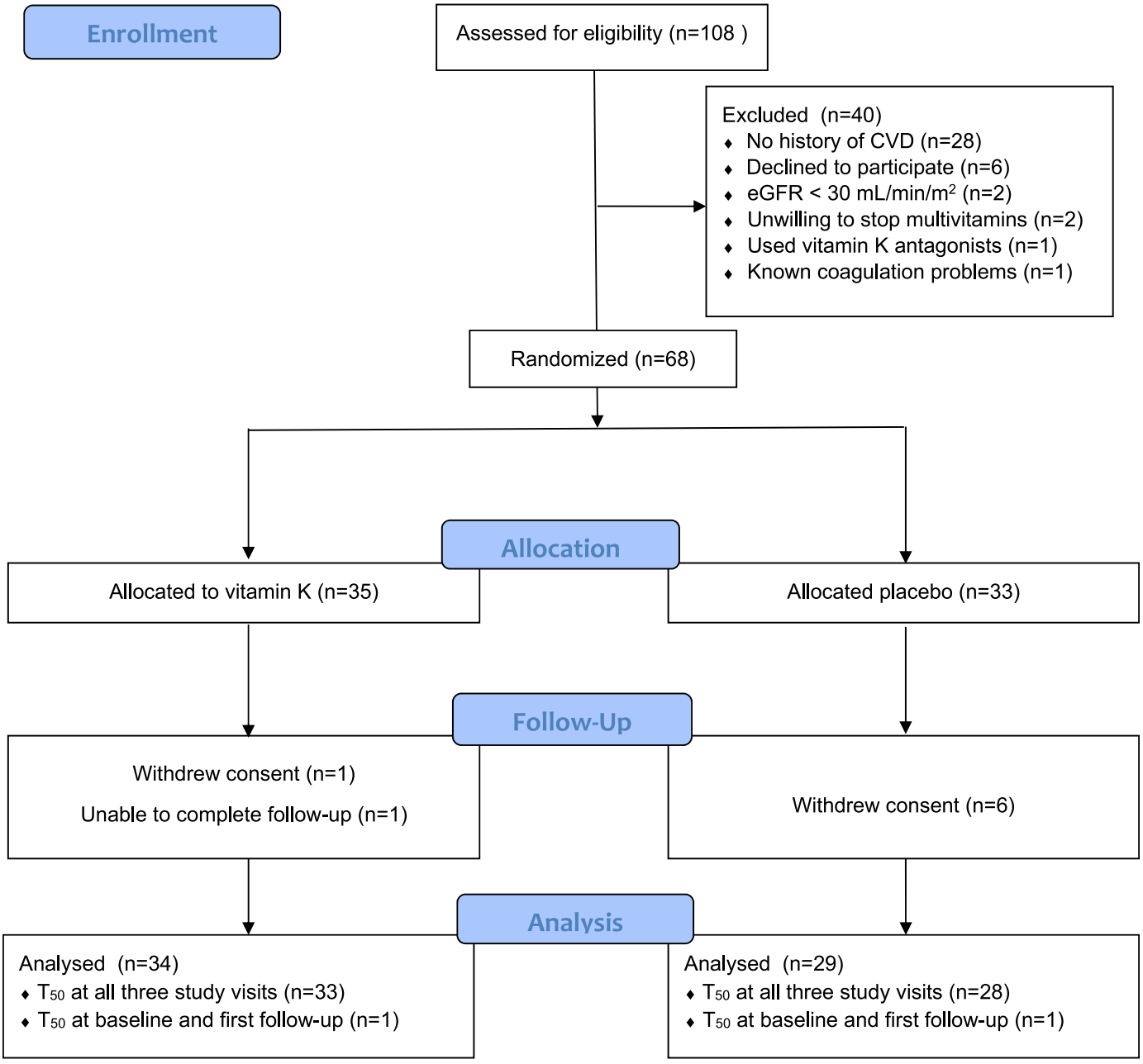

Fig. 1. CONSORT 2010 flow diagram of study participation.
CVD = cardiovascular disease, eGFR = estimated glomerular filtration rate, T_{50} = serum calcification propensity time.

not test for significant differences across intervention arms as suggested by the CONSORT 2010 statement [32].

In an intention-to-treat analysis, the effect of vitamin K *versus* placebo on T_{50} was evaluated using a longitudinal analysis of covariance with adjustment for baseline T_{50}. This regression-based method is capable of dealing with missing data. Moreover, three different effect estimates were determined: the overall intervention effect over time and the intervention effect at both follow-up measurements. Standard errors and *p*-values at first and second follow-up were obtained with different reference categories for time. T_{50} levels were log-transformed due their skewed distribution. Back transformation was performed by raising e to the power of the regression coefficient of the treatment variable. Normality of residuals was assessed by inspection of residual plots.

In addition, correlations between T_{50} with TBR and Agatston score were assessed using the Spearman's Rank Correlation Test as the normality assumption for Pearson's Correlation test was violated. These correlations were assessed at baseline, at six month follow-up and for the change between sixth months follow-up and baseline. Results were pooled for both intervention arms. Moreover, correlation was visually represented with scatterplots.

Finally, the main analysis focusing on treatment effect of vitamin K on T_{50} was repeated using a per-protocol approach. All individuals with a compliance lower than 80% were removed from the analysis.

No imputation of missing data was performed as longitudinal analysis of covariance can appropriately deal with missing data. All analyses were carried out using R software (Version 4.0., RStudio, Boston, Massachusetts, USA) and SPSS (Version 28., IBM SPSS Statistics, Chicago, IL, USA). A *p*-value <0.05 was regarded as statistically significant.

3. Results

3.1. Baseline characteristics

68 participants were eligible for participation in the study (Fig. 1). T_{50} data was available for 34/35 participants and 29/33 participants in the vitamin K and placebo group, respectively. In both groups, one individual had missing T_{50} data at last follow-up visit. Minimal pill compliance was 74% and 89% in the vitamin K group and placebo group, respectively. Only one participant in the trial was considered non-compliant, as compliance was below 80%. No serious adverse events were reported during the trial.

The study included 26 men (74% in vitamin K; 79% in placebo) with a mean age of 69.1 ± 8.4 years in both arms (Table 1). No differences were observed in baseline inactive MGP (dp-ucMGP) levels between vitamin K (613 [513–684] pmol/L) and placebo (615 [489–743] pmol/L). However, the vitamin K group tended to comprise more current (17% *vs* 12%) and former smokers (69% *vs* 55%) and consumed more vitamin K1 (124 [100–225] *vs* 94 [73–149] milligrams per day). Moreover, the prevalence of ABI ≤0.9 was higher in the vitamin K group (46%) compared to the placebo group (27%). Finally, the vitamin K group showed higher Agatston score (752 [106–1520] *vs* 169 [37–1365] Agatston units). Finally, T_{50} (350 [321–394] *vs* 363 [320–398] minutes) and TBR (2.1 [1.7–2.8] *vs* 2.1 [1.7–2.4] were similar in both groups.

3.2. Inactive MGP status

Circulating inactive MGP status declined in the vitamin K group and remained stable in the placebo group. This underlines the good compliance and biological effectiveness of vitamin K on inactive MGP. Detailed information on this marker is described elsewhere [17].

3.3. The effect of vitamin K on T_{50} (intention-to-treat)

In the vitamin K group, T_{50} changed from baseline (350 [321–394] minutes) to first follow-up (336 [314–391]) and was (345 [288–382]) at second follow-up (Fig. 2). Similarly, T_{50} in the placebo group changed from baseline (363 [320–398]) to first follow-up (337 [320–385]) and was (363 [320–378]) at second follow-up. There was no significant difference in T_{50} between the vitamin K and placebo groups over the whole trial period (ß = 1.00, 95%C·I. = 0.94–1.07, $p = 0.982$; after back-transformation), at first follow-up (ß = 1.00, 95%C·I. = 0.93–1.07, $p = 0.899$) and second follow-up (ß = 1.00, 95%C·I. = 0.93–1.08, $p = 0.923$).

Table 1
Baseline characteristics of the trial population.

Baseline characteristics [a]	Vitamin K (n = 35)	Placebo (n = 33)
Demographic variables		
Male sex (yes)	26 (74.3)	26 (78.8)
Age (years)	69.1 ± 8.4	69.1 ± 8.4
Duration of diabetes (years)	13.0 (6.5–17.0)	16.0 (10.0–21.0)
Educational level	15 (42.9)	13 (39.4)
University	5 (14.3)	7 (21.2)
Vocational school	10 (28.6)	6 (18.2)
High school	18 (51.4)	18 (54.5)
Lower school	2 (5.7)	2 (6.1)
Lifestyle variables		
BMI (kg/m^2)	31.2 ± 5.6	31.1 ± 5.0
Alcohol intake (glasses/week)		
>21	1 (2.9)	1 (3.0)
15-21	3 (8.6)	3 (9.1)
7-14	5 (14.3)	5 (15.2)
1-6	5 (14.3)	7 (21.2)
<1	8 (22.9)	5 (15.2)
0	13 (37.1)	12 (36.4)
Smoking		
Current (yes)	6 (17.1)	4 (12.1)
Former (yes)	24 (68.6)	18 (54.5)
Never (yes)	5 (14.3)	11 (33.3)
Physical activity (hours/week)		
>8	11 (31.4)	10 (30.3)
5-8	6 (17.1)	5 (15.2)
3-4	5 (14.3)	12 (36.4)
1-2	10 (28.6)	2 (6.1)
<1	3 (8.6)	4 (12.1)
Medication use		
Antihypertensive medication (yes)	30 (85.7)	30 (90.9)
Lipid-lowering medication (yes)	16 (45.7)	15 (45.5)
Glucose-lowering medication (yes)	30 (85.7)	30 (90.9)
Manifest cardiovascular disease		
Cerebrovascular disease (yes)	12 (34.3)	11 (33.3)
Coronary artery disease (yes)	23 (65.7)	18 (54.5)
Abdominal aortic aneurysm (yes)	2 (5.7)	4 (12.1)
Peripheral artery disease (yes)	6 (17.1)	10 (30.3)
Diet		
Daily caloric intake (kcal)	2082 (1774–2402)	2199 (1972–2580)
Total vitamin K (mg)	167 (138–288)	141 (117–193)
Vitamin K1 (mg)	124 (100–225)	94 (73–149)
Vitamin K2 (mg)	49 (35–57)	48 (33–52)
Laboratory measurements		
HbA1c (mmol/mol)	55.0 (46.5–63.0)	56.0 (49.0–67.0)
eGFR (ml/min/1.73 m^2)	84.2 ± 25.1	81.2 ± 18.0
Total cholesterol (mmol/L)	4.1 (3.7–5.1)	4.0 (3.3–4.9)
HDL-cholesterol (mmol/L)	1.0 (0.9–1.2)	1.1 (0.9–1.3)
LDL-cholesterol (mmol/L)	2.0 (1.6–2.5)	1.8 (1.4–2.4)
Triglycerides (mmol/L)	2.8 (1.8–3.4)	1.9 (1.5–2.7)
dp-ucMGP (pmol/L)	613 (513–684)	615 (489–743)
Vascular measurements		
Systolic blood pressure (mmHg)	136 ± 21	138 ± 14
Diastolic blood pressure (mmHg)	70 ± 11	74 ± 10
Low ABI (≤0.9) (yes)	16 (45.7)	9 (27.3)
Calcification parameters		
T50 (minutes)	350 (321–394)	363 (320–398)
TBR (unitless)	2.1 (1.7–2.8)	2.1 (1.7–2.4)
Calcification mass score (unitless)	196 (33–424)	45 (10–410)
Agatston score (Agatston units)	752 (106–1520)	169 (37–1365)

[a] Values are mean ± SD, median (IQR) or n (%). BMI = body mass index, HbA1c = glycated haemoglobin, eGFR = estimated glomerular filtration rate, HDL = high density lipoprotein, LDL = low density lipoprotein, dp-ucMGP = dephosphorylated uncarboxylated matrix G1a protein, ABI = ankle-brachial index, T50 = serum calcification propensity time, TBR = target-to-background ratio.

3.4. Correlation between T_{50} and imaging markers of arterial calcification

There was no correlation between T_{50} and TBR at baseline ($\rho = -0.185$, $p = 0.156$), at six months ($\rho = -0.191$, $p = 0.147$) and for the changes between six months and baseline ($\rho = -0.033$, $p = 0.805$) (Fig. 3). Similarly, there was no correlation between T_{50} and Agatston score at baseline ($\rho = -0.121$, $p = 0.358$), at six months ($\rho = -0.206$, $p = 0.117$) and for the changes between six months and baseline ($\rho = -0.076$, $p = 0.566$).

3.5. The effect of vitamin K on T_{50} (per-protocol)

In the vitamin K group, one participant was considered incompliant and was excluded from the per-protocol analysis. Results of the per-protocol analysis did not yield large differences with the results of the intention-to-treat analysis (data not shown).

4. Discussion

This study showed that six months oral vitamin K supplementation had no effect on T_{50} levels in people with T2DM. Moreover, T_{50} levels did not correlate with imaging arterial calcification parameters TBR and Agatston score, as measured with ^{18}F–Na PET-CT and conventional CT.

Our results concerning the treatment effect of vitamin K are in agreement with a recently conducted trial in vitamin K-deficient kidney transplant recipients [26]. Vitamin K-deficiency was defined as plasma dp-ucMGP levels >500 pmol/L (according to this criterion, 78% of our study population is vitamin K-deficient). These patients were subjected to twelve weeks of oral menaquinone-7 supplementation (also 360 μg/day) and T_{50} was measured at baseline and after twelve weeks. The treatment effect was not statistically significant (vitamin K: +2.3 ± 27.4 *vs* placebo: +0.8 ± 34.4 min; $p = 0.88$). There are different possible explanations for the observed null-effect within both trials. First, T_{50} was used as outcome and it reflects the formation time of CPPs in serum. However, the carboxylation of inactive to active MGP occurs in tissue. It is unlikely that activated MGP plays a prominent role in retarding the formation of CPP in serum. This process is one of the many facets in the complex arterial calcification pathway, and our intervention likely affects a different path from the one that is reflected by T_{50}. Second, the vitamin K group in our study showed higher arterial calcification and contained more smokers at baseline compared to the placebo group. It is possible that differences in these factors (like smoking) and unmeasured factors of relevance in the formation of vascular calcification nullified the effects of T_{50}. Also, it is possible that the vitamin K group had a higher intrinsic tendency to calcify and the effect of vitamin K on T_{50} could therefore be obscured. An observational study indeed showed that T_{50} has a normal distribution in the general population suggesting that some individuals have a high tendency to calcify in multiple vascular beds beyond atherosclerosis burden [22]. Finally, no significant effect of vitamin K on TBR and Agatston score was observed in the primary analysis of this trial, which would also explain why no significant treatment effect was observed within this analysis [16,17].

Since T_{50} is proposed as a novel marker of arterial calcification, various cross-sectional studies have examined whether T_{50} is associated with established CT-derived parameters of arterial calcification. A recent study assessed the association between T_{50} with calcification mass score and Agatston score within the abdominal aorta of patients with primary aldosteronism [23]. This research group observed that a higher T_{50} was statistically significantly associated with a lower calcification mass score ($r = -0.557$, $p < 0.05$) and Agatston score ($r = -0.534$, $p < 0.05$). A similar finding was observed in another study in which every standard deviation lower T_{50} was significantly associated with a 21% higher coronary Agatston score among people with chronic kidney disease [24]. Although this study's findings were not statistically significant, and the strength of the associations was much weaker compared to previous studies, the associations were in the same direction, i.e. a negative correlation between T_{50} and the imaging parameters of arterial calcification. The statistical insignificance may be attributed to the low sample size for this analysis or the homogeneous distribution of T_{50} within the study population as the population was subjected to several inclusion criteria for the trial. Another explanation is that we studied the association between T_{50} and calcification cross-sectionally. It is possible that in the period antedating vascular imaging, differences in T_{50} may have existed, and changed over time. To the best of our knowledge, no studies investigated the correlation and/or association between T_{50} and TBR.

Our study has several limitations. First, the placebo group showed a relatively high number of drop-outs, which may have affected the statistical power in an already fairly small study. However, baseline characteristics of participants with complete follow-up were comparable to

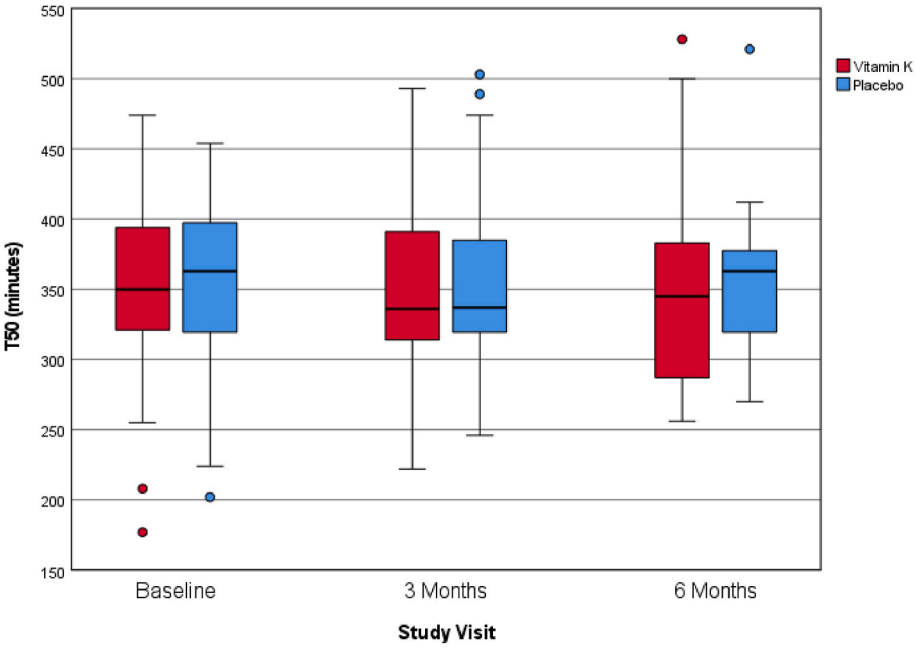

Fig. 2. T_{50} levels per intervention arm at baseline, at first follow-up after three months and at second follow-up after six months.

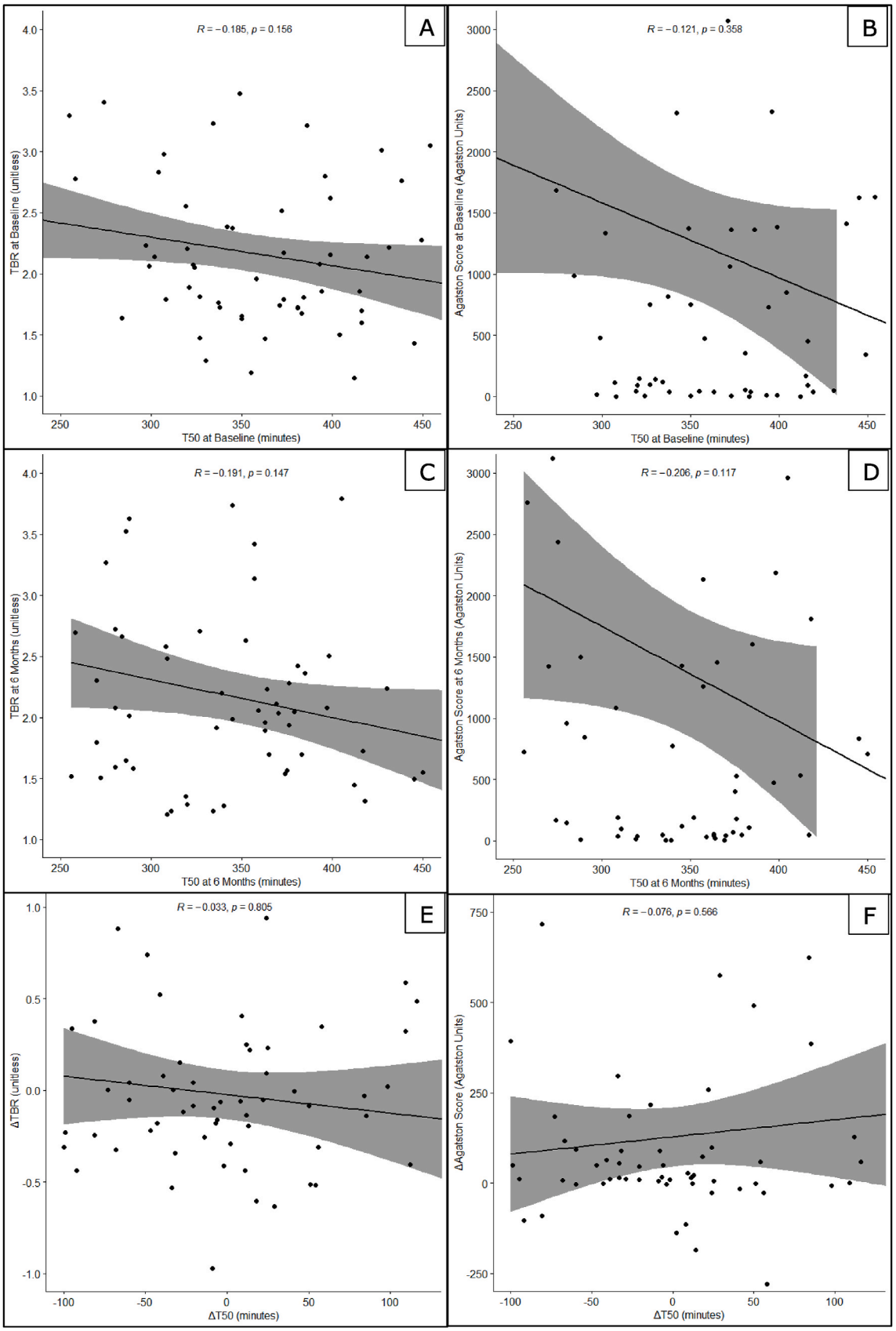

Fig. 3. Two-way scatterplots of T_{50} with Target-to-Background Ratio (A, C and E) and Agatston score (B, D and F) at baseline (A and B), at six months (C and D) and the difference between six months and baseline (E and F).

all randomized participants. Therefore, the drop-outs may have reduced precision but did not introduce selection bias. Moreover, the numerical difference between vitamin K and placebo treatment in T_{50} was very small, making it unlikely that this null result is due to insufficient power. Second, arterial calcification, smoking status and low ABI prevalence at baseline were higher in the vitamin K group compared to the placebo group, which indicate that randomisation did not lead to completely balanced groups. Furthermore, this was a *post-hoc* analysis and therefore our study can be seen only as explorative, although it adds to the body of literature on vitamin K and T_{50}. Finally, although our measurement methods are used in the research setting, their clinical role in treatment stratification and monitoring is absent.

Besides the limitations, this trial also has several strengths. First, this trial was double-blind and placebo-controlled, which is the golden standard to evaluate a treatment effect. Second, the compliance was high, with only one participant considered incompliant. This high compliance is also reflected in the similarity of the results of the intention-to-treat and per-protocol analysis. Third, there was exhaustive and complete data on arterial calcification parameters and covariates. Finally, the coefficient of variation of the T_{50} measurement was low and the inter-rater reliability for the PET-CT-derived and CT-derived parameters of arterial calcification was high.

Future research should focus on improving active MGP status and T_{50} in parallel. However, since vitamin K does not seem to affect T_{50} at all, distinct interventions are needed for both targets. Moreover, more attention needs to be guided to the formation of new calcific lesions next to existent calcific lesions to understand the role of vitamin K on arterial calcification progression. Finally, more large-scale longitudinal studies are needed to assess the validity of T_{50} and its change over time as marker of arterial calcification.

In conclusion, six-month oral vitamin K supplementation did not improve T_{50} in people with T2DM. Moreover, T_{50} did not correlate with established parameters of arterial calcification measured with PET-CT and conventional CT. Hence, our data do not support the use of T50 as a proxy for arterial calcification. Further research on the role of vitamin K in arterial calcification in T2DM and on the validity of T_{50} as measure of arterial calcification is warranted.

Declaration of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

R. Meer: contributed to conception and design, Formal analysis, interpretation, drafted the manuscript and critically revised it. M.L. Romero Prats: contributed to conception and design, Formal analysis, interpretation, drafted the manuscript and critically revised it. M.G. Vervloet: contributed to conception and design, Funding acquisition, interpretation and critically revised the manuscript. In order to ensure integrity and accuracy, all authors gave final approval and agreed to be accountable for all aspects of this work. Y.T. van der Schouw: contributed to conception and design, Funding acquisition, interpretation and critically revised the manuscript. In order to ensure integrity and accuracy, all authors gave final approval and agreed to be accountable for all aspects of this work. P.A. de Jong: contributed to conception and design, Funding acquisition, interpretation and critically revised the manuscript. In order to ensure integrity and accuracy, all authors gave final approval and agreed to be accountable for all aspects of this work. J.W.J. Beulens: contributed to conception and design, Funding acquisition, interpretation and critically revised the manuscript. In order to ensure integrity and accuracy, all authors gave final approval and agreed to be accountable for all aspects of this work.

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References

[1] C.K. Kramer, B. Zinman, J.L. Gross, L.H. Canani, T.C. Rodrigues, M.J. Azevedo, R. Retnakaran, Coronary artery calcium score prediction of all cause mortality and cardiovascular events in people with type 2 diabetes: systematic review and meta-analysis, BMJ 346 (2013) f1654, https://doi.org/10.1136/bmj.f1654.

[2] R.J. Rennenberg, A.G. Kessels, L.J. Schurgers, J.M. van Engelshoven, P.W. de Leeuw, A.A. Kroon, Vascular calcifications as a marker of increased cardiovascular risk: a meta-analysis, Vasc. Health Risk Manag. 5 (2009) 185–197, https://doi.org/10.2147/vhrm.s4822.

[3] D.S. Levy, R. Grewal, T.H. Le, Vitamin K deficiency: an emerging player in the pathogenesis of vascular calcification and an iatrogenic consequence of therapies in advanced renal disease, Am. J. Physiol. Ren. Physiol. 319 (2020) F618–F623, https://doi.org/10.1152/ajprenal.00278.2020.

[4] E.C. Cranenburg, L.J. Schurgers, H.H. Uiterwijk, J.W. Beulens, G.W. Dalmeijer, R. Westerhuis, E.J. Magdeleyns, M. Herfs, C. Vermeer, G.D. Laverman, Vitamin K intake and status are low in hemodialysis patients, Kidney Int. 82 (2012) 605–610, https://doi.org/10.1038/ki.2012.191.

[5] J.W. Beulens, M.L. Bots, F. Atsma, M.L. Bartelink, M. Prokop, J.M. Geleijnse, J. C. Witteman, D.E. Grobbee, Y.T. van der Schouw, High dietary menaquinone intake is associated with reduced coronary calcification, Atherosclerosis 203 (2009) 489–493, https://doi.org/10.1016/j.atherosclerosis.2008.07.010.

[6] J.W. Bellinge, F. Dalgaard, K. Murray, E. Connolly, L.C. Blekkenhorst, C. P. Bondonno, J.R. Lewis, M. Sim, K.D. Croft, G. Gislason, et al., Vitamin K intake and atherosclerotic cardiovascular disease in the Danish diet cancer and health study, J. Am. Heart Assoc. 10 (2021), e020551, https://doi.org/10.1161/JAHA.120.020551.

[7] C.L. Cheung, S. Sahni, B.M. Cheung, C.W. Sing, I.C. Wong, Vitamin K intake and mortality in people with chronic kidney disease from NHANES III, Clin. Nutr. 34 (2015) 235–240, https://doi.org/10.1016/j.clnu.2014.03.011.

[8] J.M. Geleijnse, C. Vermeer, D.E. Grobbee, L.J. Schurgers, M.H. Knapen, I.M. van der Meer, A. Hofman, J.C. Witteman, Dietary intake of menaquinone is associated with a reduced risk of coronary heart disease: the Rotterdam Study, J. Nutr. 134 (2004) 3100–3105, https://doi.org/10.1093/jn/134.11.3100.

[9] I.J. Riphagen, C.A. Keyzer, N.E.A. Drummen, M.H. de Borst, J.W.J. Beulens, R. T. Gansevoort, J.M. Geleijnse, F.A.J. Muskiet, G. Navis, S.T. Visser, et al., Prevalence and effects of functional vitamin K insufficiency: the PREVEND study, Nutrients 9 (2017), https://doi.org/10.3390/nu9121334.

[10] J.W. Bellinge, R.J. Francis, S.C. Lee, N.P. Bondonno, M. Sim, J.R. Lewis, G.F. Watts, C.J. Schultz, The effect of vitamin K1 on arterial calcification activity in subjects with diabetes mellitus: a post hoc analysis of a double-blind, randomized, placebo-controlled trial, Am. J. Clin. Nutr. 115 (2022) 45–52, https://doi.org/10.1093/ajcn/nqab306.

[11] J.W. Bellinge, R.J. Francis, S.C. Lee, A. Vickery, W. Macdonald, S.K. Gan, G. T. Chew, M. Phillips, J.R. Lewis, G.F. Watts, et al., The effect of vitamin-K(1) and colchicine on vascular calcification activity in subjects with diabetes mellitus (ViKCoVaC): a double-blind 2x2 factorial randomized controlled trial, J. Nucl. Cardiol. 29 (2022) 1855–1866, https://doi.org/10.1007/s12350-021-02589-8.

[12] A.C.P. Diederichsen, J.S. Lindholt, S. Moller, K.A. Ovrehus, S. Auscher, J. Lambrechtsen, S.E. Hosbond, D.H. Alan, G. Urbonaviciene, S.W. Becker, et al., Vitamin K2 and D in patients with aortic valve calcification: a randomized double-blinded clinical trial, Circulation 145 (2022) 1387–1397, https://doi.org/10.1161/CIRCULATIONAHA.121.057008.

[13] R. El Borolossy, M.S. El-Farsy, The impact of vitamin K2 and native vitamin D supplementation on vascular calcification in pediatric patients on regular hemodialysis. A randomized controlled trial, Eur. J. Clin. Nutr. 76 (2022) 848–854, https://doi.org/10.1038/s41430-021-01050-w.

[14] J.S. Lees, A.J. Rankin, K.A. Gillis, L.Y. Zhu, K. Mangion, E. Rutherford, G.H. Roditi, M.D. Witham, D. Chantler, M. Panarelli, et al., The ViKTORIES trial: a randomized, double-blind, placebo-controlled trial of vitamin K supplementation to improve vascular health in kidney transplant recipients, Am. J. Transplant. 21 (2021) 3356–3368, https://doi.org/10.1111/ajt.16566.

[15] T. Oikonomaki, M. Papasotiriou, T. Ntrinias, C. Kalogeropoulou, P. Zabakis, D. Kalavrizioti, I. Papadakis, D.S. Goumenos, E. Papachristou, The effect of vitamin K2 supplementation on vascular calcification in haemodialysis patients: a 1-year

follow-up randomized trial, Int. Urol. Nephrol. 51 (2019) 2037–2044, https://doi.org/10.1007/s11255-019-02275-2.
[16] J.W. Bartstra, F. Draaisma, S.R. Zwakenberg, N. Lessmann, J.M. Wolterink, Y. T. van der Schouw, P.A. de Jong, J.W.J. Beulens, Six months vitamin K treatment does not affect systemic arterial calcification or bone mineral density in diabetes mellitus 2, Eur. J. Nutr. 60 (2021) 1691–1699, https://doi.org/10.1007/s00394-020-02412-z.
[17] S.R. Zwakenberg, P.A. de Jong, J.W. Bartstra, R. van Asperen, J. Westerink, H. de Valk, R. Slart, G. Luurtsema, J.M. Wolterink, G.J. de Borst, et al., The effect of menaquinone-7 supplementation on vascular calcification in patients with diabetes: a randomized, double-blind, placebo-controlled trial, Am. J. Clin. Nutr. 110 (2019) 883–890, https://doi.org/10.1093/ajcn/nqz147.
[18] M.K. Shea, C.J. O'Donnell, U. Hoffmann, G.E. Dallal, B. Dawson-Hughes, J. M. Ordovas, P.A. Price, M.K. Williamson, S.L. Booth, Vitamin K supplementation and progression of coronary artery calcium in older men and women, Am. J. Clin. Nutr. 89 (2009) 1799–1807, https://doi.org/10.3945/ajcn.2008.27338.
[19] E.R. Smith, T.D. Hewitson, S.G. Holt, Diagnostic tests for vascular calcification, Adv. Chron. Kidney Dis. 26 (2019) 445–463, https://doi.org/10.1053/j.ackd.2019.07.001.
[20] M. Pluquet, S. Kamel, G. Choukroun, S. Liabeuf, S.M. Laville, Serum calcification propensity represents a good biomarker of vascular calcification: a systematic review, Toxins 14 (2022), https://doi.org/10.3390/toxins14090637.
[21] A. Pasch, S. Farese, S. Graber, J. Wald, W. Richtering, J. Floege, W. Jahnen-Dechent, Nanoparticle-based test measures overall propensity for calcification in serum, J. Am. Soc. Nephrol. 23 (2012) 1744–1752, https://doi.org/10.1681/ASN.2012030240.
[22] C. Eelderink, C.A. Te Velde-Keyzer, A.S. Frenay, E.A. Vermeulen, M. Bachtler, P. Aghagolzadeh, P.R. van Dijk, R.T. Gansevoort, M.G. Vervloet, J.L. Hillebrands, et al., Serum calcification propensity and the risk of cardiovascular and all-cause mortality in the general population: the PREVEND study, Arterioscler. Thromb. Vasc. Biol. 40 (2020) 1942–1951, https://doi.org/10.1161/ATVBAHA.120.314187.
[23] M. Kantauskaite, K. Bolten, M. Boschheidgen, C. Schmidt, T. Kolb, K.U. Eckardt, A. Pasch, L. Schimmoller, L.C. Rump, J. Voelkl, et al., Serum calcification propensity and calcification of the abdominal aorta in patients with primary aldosteronism, Front Cardiovasc Med 9 (2022), 771096, https://doi.org/10.3389/fcvm.2022.771096.
[24] J.D. Bundy, X. Cai, J.J. Scialla, M.A. Dobre, J. Chen, C.Y. Hsu, M.B. Leonard, A. S. Go, P.S. Rao, J.P. Lash, et al., Serum calcification propensity and coronary artery calcification among patients with CKD: the CRIC (chronic renal insufficiency cohort) study, Am. J. Kidney Dis. 73 (2019) 806–814, https://doi.org/10.1053/j.ajkd.2019.01.024.
[25] T. Li, Y. Wang, W.P. Tu, Vitamin K supplementation and vascular calcification: a systematic review and meta-analysis of randomized controlled trials, Front. Nutr. 10 (2023), 1115069, https://doi.org/10.3389/fnut.2023.1115069.
[26] C. Eelderink, D. Kremer, I.J. Riphagen, T.J. Knobbe, L.J. Schurgers, A. Pasch, D. J. Mulder, E. Corpeleijn, G. Navis, S.J.L. Bakker, et al., Effect of vitamin K supplementation on serum calcification propensity and arterial stiffness in vitamin K-deficient kidney transplant recipients: a double-blind, randomized, placebo-controlled clinical trial, Am. J. Transplant. (2023), https://doi.org/10.1016/j.ajt.2022.12.015.
[27] E. van't Riet, M.T. Schram, E.J. Abbink, W.M. Admiraal, M.W. Dijk-Schaap, F. Holleman, G. Nijpels, B. Ozcan, H. Pijl, N.C. Schaper, et al., The diabetes Pearl: diabetes biobanking in The Netherlands, BMC Publ. Health 12 (2012) 949, https://doi.org/10.1186/1471-2458-12-949.
[28] A.S. Agatston, W.R. Janowitz, F.J. Hildner, N.R. Zusmer, M. Viamonte Jr., R. Detrano, Quantification of coronary artery calcium using ultrafast computed tomography, J. Am. Coll. Cardiol. 15 (1990) 827–832, https://doi.org/10.1016/0735-1097(90)90282-t.
[29] A.S. Levey, L.A. Stevens, C.H. Schmid, Y.L. Zhang, A.F. Castro 3rd, H.I. Feldman, J. W. Kusek, P. Eggers, F. Van Lente, T. Greene, et al., A new equation to estimate glomerular filtration rate, Ann. Intern. Med. 150 (2009) 604–612, https://doi.org/10.7326/0003-4819-150-9-200905050-00006.
[30] Dutch national Institute for public health and the environment (RIVM) SN. Nederlands voedingsstoffen bestand (NEVO). https://www.rivm.nl/nederlands-voedingsstoffenbestand/nevo-online. 2013.
[31] S.R. Zwakenberg, A.I.P. Engelen, G.W. Dalmeijer, S.L. Booth, C. Vermeer, J. Drijvers, M.C. Ocke, E.J.M. Feskens, Y.T. van der Schouw, J.W.J. Beulens, Reproducibility and relative validity of a food frequency questionnaire to estimate intake of dietary phylloquinone and menaquinones, Eur. J. Clin. Nutr. 71 (2017) 1423–1428, https://doi.org/10.1038/ejcn.2017.121.
[32] M.R. de Boer, W.E. Waterlander, L.D. Kuijper, I.H. Steenhuis, J.W. Twisk, Testing for baseline differences in randomized controlled trials: an unhealthy research behavior that is hard to eradicate, Int. J. Behav. Nutr. Phys. Activ. 12 (2015) 4, https://doi.org/10.1186/s12966-015-0162-z.