Title: The InterVitaminK Trial - Effects of Vitamin K Supplementation on Cardiovascular, Metabolic, and Bone Health

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Analysis plan for the InterVitaminK trial

A randomized, double-blinded, placebo-controlled trial of the effects of vitamin K supplementation on cardiovascular, metabolic, and bone health in the general population

1. General analysis principles

1.1 Participant population

All primary analyses will be conducted following an intention-to-treat approach. The intention-to-treat population is defined as the InterVitaminK participants, who were eligible for enrolment (according to the inclusion and exclusion criteria) at the time of enrolment, and who were randomised to either Vitamin K supplementation or placebo, independent of adherence to treatment.

Some sensitivity analyses will be conducted on the per-protocol population, this will be specified in the analysis plan section 2. The per-protocol population is defined as the InterVitaminK participants, who have adhered to the allocated treatment (defined as compliance equal to or above 80%) until the time of the assessed outcome or study end for outcomes assessed at end of study. Compliance will be assessed through 3-monthly telephone interviews (self-reported) and through counting of tablets at yearly follow-up visits.

1.2 Missing data

For outcomes with missing values, we will assess the pattern of missingness and impute accordingly. Missing values will be imputed based on the outcome variable (for other participants) and baseline characteristics using multiple imputation.

The imputed outcome will be used as the primary outcome, but both complete-case analyses and analyses with imputed values will be reported.

1.3 Multiple testing and outcome definition

We will use two-sided p-values of ≤ 0.05 to indicate statistical significance for the main outcome. All outcomes will be reported with 95% confidence intervals. Supportive outcomes are used as support for other outcomes (i.e., if analyses of the primary outcome indicate a beneficial effect of vitamin K supplementation, beneficial effects on outcomes supportive for the primary outcome will lend support to the conclusion that vitamin K supplementation may reduce cardiovascular disease). Supportive outcomes will not be used to make individual claims, and hence p-values will not be corrected for multiple testing. Outcomes supportive of the primary outcome are classified as secondary outcomes. Other secondary outcomes (section 2.3) are testing separate hypotheses, and thus, p-values are not corrected for multiple testing. Outcomes supportive of secondary outcomes are classified as exploratory outcomes.

2 Outcomes

2.1 Primary outcome

2.1.1 CAC score

Our primary outcome is change in total CAC score assessed by CT scans (using Agatson score units) at baseline and 3-year follow-up.

2.2 Secondary outcomes supportive of the primary outcome

2.2.1 Coronary plaque composition

Change in total coronary plaque composition (calcified, non-calcified subcomponents) assessed by contrast Cardiac CT scans (unit: cubic millimeters) at baseline and 3-year follow-up.

2.2.2 Arterial stiffness

Change in arterial stiffness assessed by carotid-femoral pulse wave velocity measurement (unit: meters/second) at baseline, 1-year follow-up, 2-year follow-up, and 3-year follow-up.

2.2.3 Blood pressure
Change in blood pressure (unit: millimeter of mercury) assessed by a digital blood pressure device at baseline, 1-year follow-up, 2-year follow-up, and 3-year follow-up. Both systolic and diastolic blood pressure will be evaluated.

2.2.4 Aortic valve calcifications
Change in aortic valve calcifications assessed by non-contrast Cardiac CT scans (unit: Agatston score) assessed at baseline and 3-year follow-up

2.3 Other secondary outcomes (with supportive outcomes)
2.3.1 Bone mineral density
Change in bone mineral density assessed by quantitative CT scan of the columna thoracalis (unit: milligrams/cubic centimeter) at baseline and 3-year follow-up.

2.3.1.1 Biomarkers of bone resorption (supportive)
Change in bone metabolism reflected by biomarkers of bone resorption including C-terminal telopeptide of type I collagen (CTX) (unit: picograms/milliliter) assessed at baseline, 1-year follow-up, 2-year follow-up, and 3-year follow-up.

2.3.1.2 Biomarkers of bone formation (supportive)
Change in bone metabolism reflected by biomarkers of bone formation including osteocalcin (with different phosphorylation and carboxylation forms) (unit: nanograms/milliliter), Fibroblast growth factor 23 (FGF23) (unit: nanograms/milliliter), osteoprotegerin (unit: Picomoles/liter), and Procollagen 1 Intact N-Terminal Propeptide (P1NP) (unit: milligram/liter) assessed at baseline, 1-year follow-up, 2-year follow-up, and 3-year follow-up.

2.3.2 Pulmonary function
Change in pulmonary function reflected by forced expiratory volume in one second (FEV1) (unit: Volume in Liter)

2.3.2.1 Pulmonary function (supportive)
Change in pulmonary function reflected by expiratory forced vital capacity (FVC) (unit: Volume in Liter)

2.3.2.2 Pulmonary function (supportive)
Change in pulmonary function reflected by the FEV1/FVC ratio

2.3.2.3 Lung tissue density (supportive)
Change in lung tissue density as a measure of lung fibrosis assessed by CT-scan

2.3.2.4 Respiratory infections (supportive)
Annual number of respiratory infectious disease episodes, both upper and lower respiratory infections including COVID-19 (registered through telephone interviews and yearly follow-up visits).

2.3.3 Insulin resistance

Change in insulin resistance assessed by Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) (unit: millimoles/liter * picomoles/liter) assessed at baseline, 1-year follow-up, 2-year follow-up, and 3-year follow-up.

2.3.3.1 Glucose control (supportive)

Change in glucose regulation reflected by Glycated hemoglobin A1c (HbA1c) (unit: millimole/mol) assessed at baseline, 1-year follow-up, 2-year follow-up, and 3-year follow-up.

2.4 Other explorative pre-specified endpoints

2.4.1 Biomarkers of lipid metabolism

Change in biomarkers of lipid metabolism (including total cholesterol, triglycerides, low-density lipoprotein, high-density lipoprotein) (unit: milligrams/deciliter) assessed at baseline, 1-year follow-up, 2-year follow-up, and 3-year follow-up.

2.4.2 Inflammation

Change in inflammatory biomarkers including interleukin-6 (IL-6) (unit: picograms/milliliter) and Tumor necrosis factor α (TNF-α) (unit: picograms/milliliter) assessed at baseline, 1-year follow-up, 2-year follow-up, and 3-year follow-up.

2.4.3 Body composition

Change in anthropometry reflected by body fat percentage (%) assessed by bioimpedance (unit is estimated per cent fat mass) assessed at baseline, 1-year follow-up, 2-year follow-up, and 3-year follow-up.

3 Planned analyses

3.1 Baseline comparison

Descriptive statistics:

We will describe participant flow by group allocation in a flowchart. For participants included in the main analysis, we will describe background characteristics. Distribution of background characteristics will be presented by group allocation. Background characteristics will be summarised by counts (percentages), means (standard deviation) or medians (interquartile range) as appropriate. Information on the number and proportion with missing information will be specified in Table 1.

Table 1: Summary of background characteristics by Vitamin K supplementation and placebo group will be presented in the main publication

- Sex
- Age
- BMI
- Comorbidities
- Smoking
- Biomarkers (Vitamin K status, lipid profile, glucose metabolism, vitamin D)
- Medicine (Statins, phosphate binding agents, metformin, insulin, sglt-2 inhibitors, anti-osteoporotic medicine)
- Estimated vitamin K intake
- Baseline CAC score and other CT-scan measures

- Baseline pulse wave velocity
- Baseline blood pressure

3.2 Analysis of primary outcome

The primary outcome will be analysed using mixed effects linear regression. The mixed effects linear regression will include a fixed effect for group allocation (intervention/control), a fixed effect for time point (baseline and 3-year follow-up), fixed effect for baseline CAC score and an interaction between group allocation and time point. Treatment at baseline will be modelled as a separate common treatment category constraining baseline measurements to no systematic treatment effect between the two arms, as suggested in the literature [1]. The mixed effects model will include a random intercept for each enrolled participant and a first order autoregressive correlation structure allowing correlation of measurements for the same participant with higher correlation for measurements closer in time.
The primary outcome will be reported as change between baseline and 3-year follow-up.
The primary analysis of the primary outcome is described in more detail in Table 2.

Table 2: Primary analysis of primary outcome

Population	a) Intention-to-treat population
Observation period	From: enrolment To: 3-years follow-up examination
Outcome definition	Development in CAC score defined as CAC score at 3-years follow-up adjusted for CAC-score at enrolment (unit: Agatson score)
Statistical tool	Mixed effects linear regression
Random intercept	Individual
Fixed effects	Group allocation, time point, baseline CAC
Model check	Normality assumptions: Inspection of histograms and quantile-quantile plots
Stata code	# Change in outcome from baseline to end of follow-up mixed outcome i.treatment#i.time i.time || ivk:, ar(1) # Calculation of mean change from baseline at 36 months lincom 3.time (controls) lincom 1.treatment#3.time+3.time (intervention) # Calculation of treatment effect at 36 months lincom 1.treatment#3.time (treatment effect) *ivk: InterVitaminK study number

Suggested table output for all continuous outcomes

Examples of outcomes	Time period	Intervention	Control	Treatment effect (95% CI)	P-value
		Mean change from baseline[1] (95% CI)	Mean change from baseline[1] (95% CI)		
CAC score	0–36m				

Pulse wave velocity	0–36m				
	0–12m				
	12–24m				
	24–36m				

[1] Change from baseline will be calculated as the mean of the individual changes from baseline to follow-up (36 months for the primary outcome and 12, 24, and 36 months for outcomes with yearly assessments) CI: confidence interval.

3.2.1.1 Potential effect modifiers

We will assess whether the effect of vitamin K supplementation on the primary outcome is modified by the following potential effect modifiers including an interaction term in the primary model:

- sex
- baseline vitamin K status (stratifying on quartiles of dp-ucMGP)
- baseline CAC score (in four groups: [10–100[, [100–400[, [400–1000[, [1000–)

3.2.2 Sensitivity analyses

In sensitivity analyses, we will assess whether the conclusions are robust to

- limiting the population to the per-protocol population (defined in section 1.1)
- allowing systematic treatment effects at baseline (removing the separate common treatment category constraining baseline measurements to no systematic treatment effect between the two arms)

3.3 Analysis of numerical outcomes

Numerical secondary and/or supportive outcomes will be analysed using the same approach as for the primary analysis (using mixed effects linear regression). Some outcomes are measured at several time points (baseline, 1 year follow-up, 2-year follow-up, and 3-year follow-up). In all analyses, time point will be included for all measurements.

As for the primary outcome, the mixed effects linear regression will include a fixed effect for group allocation (intervention/control), a fixed effect for time point, a fixed effect for the baseline value of the outcome and an interaction between group allocation and time point. Treatment at baseline will be modelled as a separate common treatment category constraining baseline measurements to no systematic treatment effect between the two arms, as suggested in the literature [1]. All mixed effects models will include a random intercept for each enrolled participant and a first order autoregressive correlation structure allowing correlation of measurements for the same participant with higher correlation for measurements closer in time.

All outcomes will be reported as change between baseline and 3-year follow-up. In addition, yearly change will be reported for outcomes with yearly measurements.

3.3.1 Potential effect modifiers of secondary and/or supportive outcomes

For all outcomes, we will assess whether the effect of vitamin K supplementation on the outcome is modified by baseline vitamin K status (stratifying on quartiles of dp-ucMGP, as for the primary outcome) by including an interaction term in the model.

4 References

1 Twisk J, Bosman L, Hoekstra T, *et al.* Different ways to estimate treatment effects in randomised controlled trials. *Contemp Clin Trials Commun* 2018;**10**:80–5. doi:10.1016/j.conctc.2018.03.008