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Association of dietary vitamin K and risk of coronary heart disease in middle-age adults: the Hordaland Health Study Cohort

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ABSTRACT

Objective The role of vitamin K in the regulation of vascular calcification is established. However, the association of dietary vitamins K1 and K2 with risk of coronary heart disease (CHD) is inconclusive.

Design Prospective cohort study.

Setting We followed participants in the community-based Hordaland Health Study from 1997 - 1999 through 2009 to evaluate associations between intake of vitamin K and incident (new onset) CHD. Baseline diet was assessed by a past-year food frequency questionnaire. Energy-adjusted residuals of vitamin K1 and vitamin K2 intakes were categorised into quartiles.

Participants 2987 Norwegian men and women, age 46–49 years.

Methods Information on incident CHD events was obtained from the nationwide Cardiovascular Disease in Norway (CVDNOR) Project. Multivariable Cox regression estimated HRs and 95% CIs with test for linear trends across quartiles. Analyses were adjusted for age, sex, total energy intake, physical activity, smoking and education. A third model further adjusted K1 intake for energy-adjusted fibre and folate, while K2 intake was adjusted for energy-adjusted saturated fatty acids and calcium.

Results During a median follow-up time of 11 years, we documented 112 incident CHD cases. In the adjusted analyses, there was no association between intake of vitamin K1 and CHD (HR_{Q4vsQ1} = 0.92 (95% CI 0.54 to 1.57), p for trend 0.64), while there was a lower risk of CHD associated with higher intake of energy-adjusted vitamin K2 (HR_{Q4vsQ1} = 0.52 (0.29 to 0.94), p for trend 0.03). Further adjustment for potential dietary confounders did not materially change the association for K1, while the association for K2 was slightly attenuated (HR_{Q4vsQ1} = 0.58 (0.28 to 1.19)).

Conclusions A higher intake of vitamin K2 was associated with lower risk of CHD, while there was no association between intake of vitamin K1 and CHD.

Trial registration number NCT03013725

Strengths and limitations of this study

- The study had a long follow-up time with minimal competing risk from other causes of death.
- Linkage to a nationwide database assured complete cohort follow-up.
- We had information on history of coronary heart disease (CHD) at baseline which enabled us to evaluate incident (new onset) CHD.
- The Food Frequency Questionnaire was not validated for intake of vitamin K.
- It was not possible to differentiate the various subtypes of vitamin K2.

INTRODUCTION

Vitamin K is a fat-soluble vitamin including vitamin K1 (K1; phylloquinone) from green leafy vegetables and vegetable oils as the main dietary sources, and vitamin K2 (K2; menaquinones) from dairy products, meat and egg yolk as the main dietary sources in Europe.[1–3] K2 has a longer half-life in the circulation than K1.[4] Both are absorbed from the small ileum and jejunum. K1 and K2 are incorporated into chylomicrons and delivered to the liver. K2 is also transported via low-density lipoprotein and high-density lipoprotein (HDL) particles to extrahepatic tissue.[4 5]

Vitamin K functions as a cofactor for the enzyme gamma-glutamyl carboxylase which converts protein-bound glutamate residues into gammacarboxyglutamate (Gla).[6 7] Gla-containing proteins are involved in, for example, the coagulation of blood,[8] inhibition of arterial calcification (Matrix Gla Protein) and vascular smooth muscle cell apoptosis and movement that is considered protective against vascular injury (Gas-6).[9] Matrix Gla Protein is involved in both medial and intimal calcification, and low vitamin K status has been associated with both types of calcification.[10–14] In addition, a study that examined the effect of warfarin (a vitamin K antagonist) on medial and intimal plaque

calcification in apoE$^{-/-}$ mice concluded that warfarin accelerates both medial and intimal calcification of atherosclerotic plaque.[15] Patients with both medial and intimal calcification have a higher cardiovascular risk when compared with similar patients without calcification.[16 17] Therefore, an inverse association between vitamin K intake and coronary heart disease (CHD) could be expected. Results from observational studies on the association between intake of vitamin K and CHD are inconsistent.[18–24] Among the identified studies, three found reduced risk of CHD in multivariable adjusted analyses at higher dietary K2[19 20] or K1.[24]

Nordic Nutrition Recommendations include a provisional recommended intake of vitamin K of 1 µg/kg body weight per day,[3] while adequate intake is 90 µg/day for women and 120 µg/day for men.[25] However, these recommendations may not be sufficient to attain complete carboxylation of extrahepatic vitamin K-dependent proteins.[26 27]

Given the limited number of epidemiological studies,[18–24] and the fact that dietary vitamin K sources and content differ between countries,[28–31] further research is warranted. The purpose of the current study was to evaluate the association between intake of both K1 and K2 and subsequent CHD events among community-living middle-age adults in Norway.

SUBJECTS AND METHODS

Study population

The current study is a prospective, community-based cohort study of participants living in Hordaland County, Norway (known as The Hordaland Health Study (HUSK); https://husk.w.uib.no/). The recruitment was based on a cohort from 1992 - 1993 (The Hordaland Homocysteine Study), where eligible subjects (Hordaland County residents born 1950–1951) were identified from the National Population Register on 31 December 1992.[32 33] In 1997–1999, all living Homocysteine Study cohort members born 1950–1951 and residing in the city of Bergen or the neighbouring suburban municipalities were invited to participate in HUSK. The baseline examinations were conducted during 1997–1999 as a collaboration between the National Health Screening Service (now The Norwegian Institute of Public Health), The University of Bergen and local health services. Participation rate was 77%. Participants underwent a brief health examination and provided a non-fasting blood sample. Information on lifestyle was collected via self-administered questionnaires. A semiquantitative Food Frequency Questionnaire (FFQ) was completed by 87% of the participants yielding 3107 men and women age 47–49 years eligible for the current study.

We excluded from the analyses 27 men and 35 women who reported extreme energy intakes (below the first percentile: <1125 kcal for men and <705 kcal for women; or above the 99th percentile: >4519 kcal for men and >3571 kcal for women). Further, we excluded 27 participants (22 men and 5 women) who had prior CHD based on self-reported information and/or prior CHD hospitalisations during 1994–1999. Additionally, those with missing information on self-reported myocardial infarction from the Homocysteine Study (1992–1993; 4 men and 19 women) were excluded. Further, we excluded two participants (one man and one woman) who reported use of warfarin and six participants (two men and four women) with missing measurement on dietary vitamin K intake. The final study population thus included 1279 men and 1708 women.

Patient and public involvement

Participants were not involved in designing the research question, conducting the study, or in the interpretation, or writing of the results. There are no plans to involve participants or relevant patient communities in dissemination of results. Results are disseminated to study participants via website (https://husk.w.uib.no).

Dietary assessment

Information on food intake was obtained at baseline (1997–1999) using a slightly modified version of a previously described[34] past-year 169-item semiquantitative FFQ. The FFQ was handed out on the health examination day, filled out at home and returned by mail to the HUSK project centre. The questionnaire included frequency alternatives (from once a month to several times per day), the number of units consumed and portion sizes (eg, slices, glasses, spoons) to capture the habitual diet during the past year. The dietary information presented includes individual food or beverage items, food groups and nutrient intakes. Daily nutrient intakes were computed from a database and software system developed at the Department of Nutrition, University of Oslo (KBS, V.3.2). The nutrient database is primarily based on the official Norwegian food composition table[35] and available literature.[28] Data for K1 are mostly developed by public authorities in Finland,[36] Sweden[37] and USA.[38] For some Norwegian food products, analyses were performed using high performance liquid chromatography of fermented foods.[39] K2 was evaluated as one entity with no distinction between the different menaquinones. At time of study, the most commonly used dietary supplements in Norway did not contain vitamin K. Thus, vitamin K intake reflects only dietary sources. Measurements used as independent variables in this study are the total dietary amount of K1 and K2, expressed as energy-adjusted residuals.[40]

Health examination and health habits

Baseline examinations included measurements of height, weight, waist circumference, resting blood pressure (Dinamap 845 XT equipment (Criticon)) and non-fasting venous blood samples for evaluation of serum lipids and glucose. Serum samples of total cholesterol, HDL cholesterol, triglycerides and glucose were analysed within 7 days at the department of Clinical Chemistry, Ullevål University Hospital, Oslo, using enzymatic methods with

reagents from Boehringer Mannheim (Roche, Basel, Switzerland).

Information on educational level and medication use was collected through self-administered questionnaires.

Hypertension was considered present if the mean of at least two consecutive measurements of systolic blood pressure was ≥140 mm Hg or of diastolic blood pressure ≥90 mm Hg or if use of medication for hypertension was reported.

Diabetes was diagnosed according to diagnostic criteria at the time of the screening/survey. Participants taking diabetic medications or who reported a diagnosis of diabetes were defined as having diabetes mellitus. Also, participants with a serum glucose level >7 mmol/L who had not eaten a meal during the last 8 hours, or with glucose level >11.1 mmol/L and less than 8 hours since their last meal, were defined as having diabetes. Prediabetes was defined as having glucose levels between 5.6 and 7 mmol/L at least 8 hours after their last meal or between 7.8 and 11 mmol/L less than 8 hours after their last meal.

Participants answered one categorical question on past-year vigorous physical activity resulting in sweating or breathlessness (none, <1 h/week, 1–2 h/week, or ≥3 h/week). This variable was treated as a categorical variable with none as the reference.

Participants were classified as non-smokers, former smokers or current smokers and this variable was treated as a categorical variable with non-smokers as reference.

Outcome

The study endpoints were incident (first time) hospitalisation with CHD (International classification of diseases (ICD)9 codes 410–414, ICD10 codes I20–I25) as primary or secondary diagnosis or death with CHD as the underlying cause of death. Participants were followed from baseline through 31 December 2009 for CHD events through the Cardiovascular Disease in Norway project database (CVDNOR, www.cvdnor.no)[41 42] and The Cause of Death Registry. Follow-up time represented time from baseline (1997–1999) until CHD, death from other causes, emigration or 31 December 2009, whichever came first. During follow-up, there were 107 non-fatal and 5 fatal events of interest while 60 participants died due to other causes and were censored at date of death.

Statistical analyses

Energy-adjusted residuals were obtained from linear regression models with total energy intake as independent variable and K1 or K2 as dependent variables. The residuals measure the difference between actual intake and expected intake predicted by total energy intake[40] and thereby provides an assessment of K1 and K2 intake relative to energy consumed. Residuals were then categorised into sex-specific quartiles.

Descriptive characteristics included counts with percents and medians (interquartile range) for categorical and continuous variables, respectively. Trends in dichotomous, categorical and continuous baseline characteristics across energy-adjusted quartiles of K1 and K2 were evaluated using logistic, ordinal logistic and linear regression analyses, respectively. The median residuals for each quartile group was specified as a continuous independent variable in the regression models.

Cox proportional hazards models were used to calculate adjusted HR and 95% CIs for CHD associated with sex-specific energy-adjusted quartiles and per 10 µg increments of K1 or K2 intake. The covariates included were either those associated with intake of K1 or K2 and with CHD, or those that modified the association of either K1 or K2 with CHD when included in the multivariable model. Analyses included adjustments for sex, age (years) and total energy intake (kcal/day) (model 1), with additional adjustment for categories of vigorous physical activity (none vs <1 hour/week, 1–2 hours/week and ≥3 hours/week), smoking habits (previous smokers and current smokers, respectively vs non-smokers) and education (high school or vocational school, and any college or university, respectively vs primary school(≤10 years)) (model 2). In a third model, K1 was adjusted additionally for energy-adjusted intake of fibre (g/day) and folate (mg/day); K2 was additionally adjusted for energy-adjusted intake of saturated fatty acids (SFA) (g/day) and calcium (mg/day) (model 3). The following additional potential confounders were also evaluated but not included in the tables as they did not noticeably alter the vitamin K1 or K2 coefficients for CHD: family history of myocardial infarction and energy-adjusted alcohol intake (g/day). Further, adjusting for the following intermediate factors: body mass index (BMI, kg/m^2), diabetes mellitus (pre-diabetes and diabetes, respectively, vs no diabetes), hypertension, serum total cholesterol (mmol/L) and statin use, only attenuated the association to a small degree.

To test for linear trends across energy-adjusted quartiles of K1 and K2 intakes, the median value of the residuals within each quartile group was entered as a continuous independent variable. Supplementary analyses re-evaluated K1 and K2 intake as sex-specific quartiles of absolute intake rather than energy-adjusted residuals.

Missing data on physical activity (3.8%), education (0.8%) and smoking habits (2.1%) were handled with listwise deletion in all analyses included in the main manuscript. In supplementary analyses, missing values for physical activity, smoking and education were imputed using ordinal logistic regression as the imputation model in MICE (multiple imputation using chained equation) with 20 imputations. All variables in the Cox regression models were included as imputation variables together with total cholesterol, HDL cholesterol, triglycerides and BMI as auxiliary variables due to their correlation with physical activity, smoking and education.

The proportional hazards assumption was evaluated using Schoenfeld's test and log–log test.

In Cox regression with penalised splines, the functional form of the association between absolute K2 intake (not

residuals) and risk of CHD was estimated by smoothing splines, in which the estimated smooth functions were used to plot the relative hazards of CHD.[43] Intakes above the 95th percentile and below the 5th percentile are excluded in the figure.

To test for sex interactions between K1 and K2, we compared models with and without an interaction term using likelihood-ratio test.

Statistical analyses were performed using Stata V.15 (Stata Corp LP) and R V.3.4.0 (https://www.r-project.org/, The R Foundation for Statistical Computing, Vienna, Austria). P<0.05 were considered statistically significant.

Consent to participate

All subjects gave their written consent to participate in the study.

RESULTS

Reported intake of energy-adjusted K1 ranged from 8 to 1063 µg/day/1000 kcal (median 48 µg/day/1000 kcal) and were higher for women compared with men. Intake of energy-adjusted K2 ranged from 1 to 31 µg/day/1000 kcal (median 7 µg/day/1000 kcal) and were slightly higher for women compared with men.

The major dietary sources of K1 were vegetables (64%), fruits and berries (6%) and milk and milk products (6%), while sources of K2 were cheese (40%), other dairy products (14%), meat (24%) and eggs (13%).

In the evaluation of baseline characteristics associated with K1 intake, the concentration of HDL cholesterol and the proportion of participants who were highly educated and reported at least 1 hour of vigorous physical activity per week were higher with higher quartiles of K1 intake (table 1). Further, intakes of energy-adjusted total vitamin K, folate and fibre were higher with higher K1 intake quartiles. In contrast, a lower proportion with a family history of CHD and lower energy-adjusted K2, SFA and carbohydrate intakes were noted with higher K1 intake. In addition, intake of fruit and berries and vegetables were higher with higher K1 intake quartiles, while intake of cheese, milk and milk products and soft drinks with sugar were lower with higher intake quartiles of energy-adjusted K1 intake.

Evaluation of baseline characteristics by quartiles of energy-adjusted K2 intake identified that the proportion of participants highly educated, and the concentration of HDL cholesterol were higher with higher quartiles of K2 intake, while the concentration of triglycerides was lower (table 2). Further, intake of energy-adjusted total fat, SFA and calcium was higher with higher K2 intake quartiles. In contrast, lower intake of energy-adjusted K1 and carbohydrates was noted with higher K2 intake. In addition, intake of butter, eggs, cheese, meat and minced meat were higher with higher quartiles, while intake of soft drinks with sugar and fruit and berries were lower with higher quartiles of energy-adjusted K2 intake.

Association between dietary vitamin K1 and CHD

During a mean 10.8 (SD 1.3) years follow-up, representing 32 362 person years among 2987 participants, we documented 112 incident CHD events. Due to listwise deletion of missing values (2.1% for smoking habits, 0.8% for education and 3.8% for physical activity), multivariable-adjusted analyses included 6.5% fewer participants compared with model 1 analyses (ie, 2792 (1213 men and 1579 women) participants and 100 CHD events).

When adjusting for age, sex and total energy intake, there was no association between intake of energy-adjusted K1 and CHD comparing the fourth to the first quartile and there was no trend (table 3, model 1). The results were similar when further adjusting for physical activity, smoking habits and education ($HR_{Q4vs.Q1}$=0.92 (0.54 to 1.57), p for trend 0.64; table 3, model 2). In analyses of energy-adjusted K1 intake as a continuous variable (per 10 µg increase), there was no association between K1 and CHD in the adjusted analysis (table 3, model 2). Additional adjustments for energy-adjusted fibre and folate did not materially change the results (table 3, model 3). In supplementary analyses, where missing data were handled with multiple imputation, results were similar to those presented in table 3 (online supplementary table 1, models 2 and 3).

Results were consistent with the above analyses in the supplemental analyses evaluating sex-specific quartiles of absolute K1 intake rather than energy-adjusted residuals (online supplementary table 2).

Association between dietary vitamin K2 and CHD

When adjusting for age, sex and total energy intake, there was a lower risk of CHD with energy-adjusted K2 in the fourth compared with the first quartile ($HR_{Q4vs.Q1}$=0.50 (0.28 to 0.88), p for trend 0.02; table 3, model 1). Results were consistent when further adjusting for physical activity, smoking habits and education ($HR_{Q4vs.Q1}$=0.52 (0.29 to 0.94), p for trend 0.03; table 3, model 2). Consistency in results was observed in analyses of K2 intake as a continuous variable (per 10 µg increase; HR=0.74 (0.52 to 1.05), p=0.09). Additional adjustments for energy-adjusted SFA and calcium slightly attenuated the risk estimates for the association between K2 intake and CHD ($HR_{Q4vs.Q1}$=0.58 (0.28 to 1.19), p for trend 0.16; table 3, model 3). Similar results were found in supplementary analyses where missing data were handled with multiple imputation (model 2: $HR_{per\ 10\mu g\ increase}$=0.70 (0.50 to 0.98), p=0.04) (online supplementary table 1, models 2 and 3).

When evaluating sex-specific absolute K2 intake rather than energy-adjusted residuals, HRs were similar to those observed in the primary analyses ($HR_{Q4vs.Q1}$=0.72 (0.36 to 1.45), p for trend 0.25; online supplementary table 2, model 2). Similarly, the penalised spline figure for absolute K2 intake and its association with CHD adjusting for model 2 covariates showed a tendency towards lower risk of CHD with higher K2 intake (figure 1).

Table 1 Baseline characteristics by sex-specific quartiles of energy-adjusted residuals of vitamin K1 intake: the Hordaland Health Study

	Total	Q1	Q2	Q3	Q4	P trend*
Subjects, n	2987	746	747	748	746	
Age, years	48 (47 to 48)	48 (47 to 48)	48 (47 to 48)	48 (48 to 49)	48 (47 to 49)	0.135
Men	1279 (42.8)	319 (42.8)	320 (42.8)	321 (42.9)	319 (42.8)	0.993
Any college and/or university education	1136 (38.3)	265 (36.0)	288 (38.9)	289 (38.8)	294 (39.6)	0.018
Family history of CHD	1183 (40.9)	314 (43.3)	309 (42.6)	286 (39.3)	274 (38.4)	0.034
Smoking habits						0.104
Previous smokers	914 (31.3)	214 (29.4)	227 (30.8)	237 (32.4)	236 (32.5)	
Current smokers	978 (33.5)	239 (32.8)	231 (31.4)	261 (35.7)	247 (34.0)	
Physical activity						<0.001
None	741 (25.8)	198 (27.8)	201 (28.0)	188 (26.0)	154 (21.4)	
<1 hour/week	810 (28.2)	226 (31.7)	192 (26.7)	201 (27.8)	191 (26.5)	
1–2 hours/week	907 (31.6)	207 (29.0)	239 (33.3)	226 (31.3)	235 (32.6)	
≥3 hours/week	415 (14.4)	82 (11.5)	86 (12.0)	107 (14.8)	140 (19.4)	
Hypertension	707 (23.7)	172 (23.1)	176 (23.6)	185 (24.7)	174 (23.3)	0.945
Glucose intolerance						0.875
Pre-diabetes	66 (2.2)	24 (3.2)	12 (1.6)	14 (1.9)	16 (2.2)	
Diabetes	27 (0.9)	8 (1.1)	4 (0.5)	5 (0.7)	10 (1.4)	
Body mass index, kg/m^2	24.9 (22.8 to 27.4)	25.0 (22.8 to 27.4)	25.0 (22.8 to 27.6)	24.9 (22.9 to 27.5)	24.7 (22.6 to 27.2)	0.148
Waist circumference, cm	85.0 (77.0 to 94.0)	85.0 (77.0 to 94.0)	85.0 (77.0 to 94.0)	85.0 (77.0 to 93.0)	85.0 (76.0 to 93.0)	0.266
Serum cholesterol, mmol/L	5.65 (5.06 to 6.30)	5.58 (5.05 to 6.25)	5.70 (5.11 to 6.44)	5.66 (5.05 to 6.27)	5.65 (5.03 to 6.25)	0.331
Serum LDL-C, mmol/L	3.56 (3.01 to 4.17)	3.53 (2.98 to 4.14)	3.65 (3.06 to 4.29)	3.56 (3.02 to 4.11)	3.54 (2.94 to 4.10)	0.072
Serum HDL-C, mmol/L	1.28 (1.06 to 1.53)	1.27 (1.04 to 1.52)	1.28 (1.07 to 1.51)	1.27 (1.05 to 1.53)	1.31 (1.07 to 1.58)	0.004
Serum triglycerides, mmol/L	1.40 (1.01 to 2.03)	1.43 (1.01 to 2.02)	1.39 (1.00 to 1.99)	1.39 (1.04 to 2.11)	1.38 (0.98 to 2.05)	0.462
Energy intake, kcal/day	2057 (1690 to 2550)	2152 (1712 to 2671)	1944 (1608 to 2353)	2032 (1627 to 2485)	2171 (1775 to 2687)	<0.001
Dietary intake						
Total vitamin K, µg/day	120 (85 to 175)	78 (58 to 99)	95 (76 to 118)	137 (111 to 161)	234 (189 to 301)	<0.001
Total vitamin K, µg/day/1000 kcal	56 (43 to 79)	36 (31 to 42)	49 (44 to 55)	65 (57 to 76)	105 (84 to 144)	<0.001
Vitamin K2, µg/day	15 (11 to 21)	16 (12 to 22)	14 (11 to 20)	15 (11 to 20)	15 (12 to 20)	0.336
Vitamin K2, µg/day/1000 kcal	7 (6 to 9)	8 (6 to 9)	7 (6 to 9)	7 (6 to 9)	7 (6 to 9)	<0.001
Vitamin K1, µg/day	103 (69 to 157)	61 (44 to 77)	81 (63 to 101)	121 (99 to 143)	218 (172 to 282)	<0.001
Vitamin K1 µg/day/1000 kcal	48 (35 to 71)	29 (23 to 33)	41 (37 to 47)	58 (50 to 67)	98 (77 to 136)	<0.001
Total fat, E%	32 (29 to 36)	32 (28 to 35)	33 (29 to 36)	33 (30 to 37)	33 (29 to 36)	<0.001
SFA, E%	13 (11 to 14)	13 (11 to 14)	13 (11 to 14)	13 (11 to 14)	12 (11 to 14)	0.004
PUFA, E%	7 (6 to 8)	6 (5 to 7)	7 (6 to 8)	7 (6 to 9)	7 (6 to 9)	<0.001
MUFA, E%	10 (9 to 12)	10 (9 to 11)	10 (9 to 12)	11 (9 to 12)	10 (9 to 12)	0.357
Protein, E%	16 (14 to 17)	16 (14 to 17)	16 (14 to 17)	16 (14 to 17)	16 (15 to 18)	<0.001
Carbohydrates, E%	49 (46 to 53)	50 (47 to 54)	50 (46 to 53)	49 (45 to 52)	48 (45 to 52)	<0.001
Alcohol, E%	1 (0 to 3)	1 (0 to 3)	1 (0 to 3)	2 (0 to 3)	2 (1 to 3)	0.001
Folate, µg/day/1000 kcal	110 (95 to 133)	99 (88 to 113)	105 (93 to 121)	112 (98 to 131)	135 (113 to 164)	<0.001
Fibre, g/day/1000 kcal	11 (10 to 13)	10 (9 to 12)	11 (10 to 13)	11 (10 to 13)	13 (11 to 16)	<0.001
Intake of food items, g/day/1000 kcal						
Butter†	0 (1.2)	0 (1.3)	0 (1.1)	0 (1.1)	0 (1.2)	0.749
Margarine	3 (2 to 8)	2 (1 to 3)	3 (2 to 8)	3 (2 to 11)	3 (2 to 9)	<0.001
Cheese	13 (7 to 21)	15 (8 to 24)	13 (7 to 22)	12 (7 to 19)	12 (6 to 20)	<0.001
Yoghurt	5 (0 to 18)	6 (0 to 21)	5 (0 to 16)	6 (0 to 18)	5 (0 to 17)	0.740
Milk and milk products	129 (65 to 205)	157 (86 to 230)	134 (71 to 220)	127 (57 to 193)	105 (51 to 174)	<0.001
Sausages	20 (2 to 49)	17 (1 to 47)	18 (1 to 47)	21 (4 to 53)	21 (5 to 53)	0.067
Meat	55 (41 to 70)	54 (39 to 68)	55 (42 to 73)	56 (43 to 70)	53 (40 to 67)	0.651

Continued

Table 1 Continued

	Total	Q1	Q2	Q3	Q4	P trend*
Minced meat	24 (16 to 34)	25 (15 to 35)	25 (16 to 35)	25 (16 to 34)	22 (14 to 31)	<0.001
Soft drinks with sugar	25 (0 to 65)	31 (3 to 76)	28 (2 to 69)	24 (0 to 61)	18 (0 to 51)	<0.001
Fruit and berries	104 (65 to 154)	91 (56 to 132)	103 (67 to 150)	105 (68 to 167)	114 (71 to 164)	<0.001
Vegetables	85 (54 to 131)	49 (35 to 72)	74 (53 to 101)	98 (67 to 134)	154 (104 to 212)	<0.001

Values are presented as N (%) and median (interquartile range) for continuous and categorical data, respectively.
*Logistic regression for dichotomous categories, ordered logistic regression when more than two categories and linear regression for continuous variables where median residuals within each quartile group was used as the independent variable in the analyses.
†Mean (median) are reported due to a large proportion with zero intake.
CHD, coronary heart disease; E%, energy per cent; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; Q, quartile; SFA, saturated fatty acids.

DISCUSSION

Among community-dwelling middle-age adults in Western Norway, a higher energy-adjusted reported intake of K2 was associated with a lower risk of subsequent CHD events, whereas intake of K1 was not associated with incident CHD. Similar direction of associations was observed when further adjusting for potential dietary confounders.

Strengths and weaknesses

Strengths of our study include a long follow-up time with minimal competing risk from other causes of death in this relatively young study population. Linkage to the CVDNOR project database assured complete follow-up. In addition, we had information on several possible confounders including baseline health status, medication use, health habits and history of CHD which enabled us to evaluate incident CHD.

Weaknesses include self-reported information on dietary intake, health habits and medication use which may lead to misclassification in covariates used in the analyses. Furthermore, we lack information on changes in diet including intake of vitamin K, medications and risk factors over time. Inherent problems with FFQs are systematic under-reporting and over-reporting. However, the FFQ is well suited to rank individuals when adjusting for total energy intake.[44] This FFQ is not validated specifically for intake of vitamin K. Vitamin K content of foods differs according to production conditions, and the bioavailability is dependent on preparation, fat content of meals, the food matrix and subtypes of vitamin K. Therefore, although the rank ordering of participants may be valid, the absolute vitamin K intake based on FFQ is likely inaccurate.[5 29 30 45 46] In addition, K2 can be produced by intestinal gut microbiota, but little is known about its contribution to vitamin K status since the majority is located in bacterial membranes in the colon and is probably not available for absorption.[26]

Further, vitamin K2 intake may be underestimated in this study population since much of the information on vitamin K content of food used in this study comes from a Dutch study,[28] and recent research have shown that Norwegian cheeses are especially rich in vitamin K2.[29 47] In addition, we could not differentiate the subtypes of vitamin K2[19] in our study.

Although we performed multivariable analyses, residual confounding may still be present.

Results in relation to other studies

This study did not find an association between intake of K1 and CHD, in line with results from most previous studies.[19 20 22 23] However, Erkkilä *et al* concluded that high K1 intake may be a marker of low CHD risk, but that dietary patterns associated with K1 intakes, rather than intake of K1 itself might account for this association.[21] Similarly, Juanola-Falgarona *et al* studied the association between dietary K1 and K2 with mortality in a cohort with high cardiovascular disease (CVD) risk, and found that an increase in dietary intakes of both K1 and K2 were associated with a reduced risk of all-cause mortality, while only K1 was associated with a reduced risk of CVD mortality.[24] However, since participants came from a Mediterranean country in which the consumption of fruit, vegetables and vegetable oils was quite high, K1 could be regarded as a marker of adherence to a healthy diet.[24]

Regarding vitamin K2, Geleijnse *et al* found a 41% lower risk of CHD comparing the highest with the lowest tertile of K2 intake in the Rotterdam study,[20] similar to our results. Gast *et al* found a dose–response relationship with a 9% lower CHD risk with each 10 µg higher K2 intake, with the strongest association shown for long-chain K2.[19] Opposite from this study where the association attenuated when further adjusting for SFA and calcium, the association became stronger in both these studies in their multivariable models.[19 20] Adjusting for SFA and calcium, however, may be an overadjustment as dairy products rich in SFA and calcium are also major sources of K2.[24] Zwakenberg *et al* found that only intake of long-chain K2 (per10µg) was borderline significantly inversely associated with CHD mortality (p for trend 0.06).[23] This discrepancy compared with our study may be because lifestyle factors such as diet may have a larger impact on total CHD events (fatal and non-fatal) given the underlying mechanisms of reducing calcification. Treatment, however, is probably of larger importance for CHD mortality.[48]

In the western diet, cheese is the most important source of long-chain K2, and hard cheese is, in general, richer in K2 than soft cheese.[28 29] Fu *et al* showed that vitamin K concentrations in cheese ranged from 40 µg to 850 µg per

Table 2 Baseline characteristics by sex-specific quartiles of energy-adjusted residuals of vitamin K2 intake: the Hordaland Health Study

	Total	Q1	Q2	Q3	Q4	P trend*
Subjects, n	2987	746	747	747	747	
Age, years	48 (47 to 48)	48 (47 to 49)	48 (47 to 48)	48 (47 to 49)	48 (47 to 48)	0.600
Men	1279 (42.8)	319 (42.8)	320 (42.8)	320 (42.8)	320 (42.8)	0.977
Any college and/or university education	1136 (38.3)	261 (35.3)	272 (36.8)	284 (38.3)	319 (42.9)	0.001
Family history of CHD	1183 (40.9)	292 (40.7)	308 (42.1)	284 (39.3)	299 (41.6)	0.904
Smoking habits						0.267
Previous smokers	914 (31.3)	217 (29.7)	241 (33.1)	229 (31.2)	227 (31.1)	
Current smokers	978 (33.5)	239 (32.7)	244 (33.5)	240 (32.7)	255 (34.9)	
Physical activity						0.114
None	741 (25.8)	185 (25.8)	181 (25.5)	194 (26.9)	181 (25.0)	
<1 hour/week	810 (28.2)	189 (26.3)	179 (25.2)	224 (31.0)	218 (30.2)	
1–2 hours/week	907 (31.6)	214 (29.8)	244 (34.4)	224 (31.0)	225 (31.1)	
≥3 hours/week	415 (14.4)	130 (18.1)	106 (14.9)	80 (11.1)	99 (13.7)	
Hypertension	707 (23.7)	185 (24.8)	180 (24.1)	184 (24.6)	158 (21.2)	0.100
Glucose intolerance						0.543
Pre-diabetes	66 (2.2)	18 (2.4)	15 (2.0)	17 (2.3)	16 (2.2)	
Diabetes	27 (0.9)	6 (0.8)	7 (0.9)	2 (0.3)	12 (1.6)	
Body mass index, kg/m^2	24.9 (22.8 to 27.4)	24.8 (22.7 to 27.4)	25.1 (23.0 to 27.5)	25.0 (22.9 to 27.3)	24.8 (22.6 to 27.4)	0.830
Waist circumference, cm	85 (77 to 94)	84 (77 to 93)	85 (77 to 94)	85 (77 to 94)	85 (76 to 93)	0.724
Serum cholesterol, mmol/L	5.65 (5.06 to 6.30)	5.66 (5.02 to 6.30)	5.64 (5.07 to 6.36)	5.7 (5.1 to 6.32)	5.57 (5.05 to 6.20)	0.124
Serum LDL-C, mmol/L	3.56 (3.01 to 4.17)	3.57 (3.00 to 4.14)	3.58 (2.99 to 4.19)	3.60 (3.07 to 4.24)	3.52 (2.97 to 4.09)	0.227
Serum HDL-C, mmol/L	1.28 (1.06 to 1.53)	1.27 (1.03 to 1.52)	1.27 (1.07 to 1.54)	1.30 (1.06 to 1.55)	1.30 (1.06 to 1.54)	0.043
Serum triglycerides, mmol/L	1.40 (1.01 to 2.03)	1.44 (1.04 to 2.16)	1.40 (1.00 to 2.05)	1.39 (1.01 to 1.99)	1.36 (0.97 to 1.96)	0.022
Energy intake, kcal/day	2057 (1690 to 2550)	2098 (1682 to 2645)	1976 (1603 to 2398)	2014 (1653 to 2469)	2215 (1795 to 2637)	0.002
Dietary intake						
Total vitamin K, µg/day	120 (85 to 175)	119 (82 to 179)	116 (78 to 169)	114 (83 to 165)	133 (94 to 186)	0.728
Total vitamin K, µg/day/1000 kcal	56 (43 to 79)	56 (40 to 80)	55 (42 to 81)	55 (43 to 76)	59 (46 to 80)	0.011
Vitamin K2, µg/day	15 (11 to 21)	10 (8 to 13)	13 (11 to 16)	16 (14 to 19)	24 (21 to 29)	<0.001
Vitamin K2, µg/day/1000 kcal	7 (6 to 9)	5 (4 to 5)	7 (6 to 7)	8 (8 to 9)	11 (10 to 13)	<0.001
Vitamin K1, µg/day	103 (69 to 157)	109 (72 to 167)	101 (67 to 155)	97 (67 to 149)	104 (71 to 156)	0.329
Vitamin K1, µg/day/1000 kcal	48 (35 to 71)	51 (36 to 78)	49 (35 to 74)	46 (35 to 67)	47 (34 to 68)	0.038
Total fat, E%	32 (29 to 36)	30 (27 to 34)	31 (29 to 35)	33 (30 to 36)	35 (32 to 38)	<0.001
SFA, E%	13 (11 to 14)	11 (19 to 12)	12 (11 to 13)	13 (12 to 14)	14 (13 to 16)	<0.001
PUFA, E%	7 (6 to 8)	7 (6 to 9)	7 (6 to 8)	7 (6 to 8)	6 (6 to 8)	<0.001
MUFA, E%	10 (9 to 12)	10 (8 to 11)	10 (9 to 11)	11 (10 to 12)	11 (10 to 12)	<0.001
Protein, E%	16 (14 to 17)	15 (13 to 16)	16 (14 to 17)	16 (15 to 17)	16 (15 to 18)	<0.001
Carbohydrates, E%	49 (46 to 53)	53 (49 to 57)	50 (47 to 53)	48 (45 to 51)	46 (43 to 49)	<0.001
Alcohol, E%	1 (0 to 3)	1 (0 to 3)	1 (0 to 3)	1 (0 to 3)	1 (0 to 3)	0.387
Calcium, mg/day/1000 kcal	385 (313 to 468)	332 (264 to 410)	373 (302 to 448)	389 (329 to 464)	443 (374 to 538)	<0.001
Fibre, g/day/1000 kcal	11 (10 to 13)	12 (11 to 15)	12 (10 to 13)	11 (9 to 13)	11 (9 to 12)	<0.001
Intake of food items, g/day/1000 kcal						
Butter†	0 (1.2)	0 (0.5)	0 (1.0)	0 (1.3)	0 (1.9)	<0.001
Margarine	3 (2 to 8)	3 (2 to 10)	3 (2 to 7)	3 (2 to 8)	3 (2 to 5)	<0.001
Egg	8 (4 to 11)	5 (3 to 8)	8 (5 to 11)	8 (5 to 12)	8 (4 to 11)	<0.001
Cheese	13 (7 to 21)	7 (3 to 12)	10 (6 to 15)	14 (9 to 20)	25 (17 to 34)	<0.001
Yoghurt	5 (0 to 18)	4 (0 to 15)	5 (0 to 17)	6 (0 to 19)	5 (0 to 19)	0.456
Milk and milk products	129 (65 to 205)	127 (51 to 209)	144 (82 to 213)	135 (69 to 209)	112 (55 to 181)	<0.001

Continued

Table 2 Continued

	Total	Q1	Q2	Q3	Q4	P trend*
Sausage	20 (2 to 49)	21 (2 to 53)	22 (3 to 53)	21 (3 to 50)	17 (2 to 42)	<0.001
Meat	55 (41 to 70)	47 (33 to 58)	56 (43 to 71)	61 (46 to 77)	58 (42 to 76)	<0.001
Minced meat	24 (16 to 34)	19 (12 to 28)	24 (16 to 33)	27 (18 to 37)	27 (17 to 36)	<0.001
Soft drinks with sugar	25 (0 to 65)	29 (1 to 72)	26 (0 to 66)	26 (2 to 64)	23 (0 to 54)	<0.001
Fruit and berries	104 (65 to 154)	116 (74 to 174)	105 (67 to 162)	100 (64 to 149)	93 (57 to 135)	<0.001
Vegetables	85 (54 to 131)	85 (50 to 133)	90 (58 to 139)	82 (55 to 125)	84 (53 to 122)	0.006

Values are presented as N(%) and median (interquartile range) for categorical and continuous variables, respectively.
*Logistic regression for dichotomous categories, ordered logistic regression when more than two categories and linear regression for continuous variables where median residuals within each quartile group was used as the independent variable in the analyses.
†Mean (median) are reported due to a large proportion with zero intake.
CHD, coronary heart disease; E%, energy per cent; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; Q, quartile; SFA, saturated fatty acids.

100 g, and that reduced-fat products contained 5%–22% of the vitamin K found in full-fat equivalents.[30] K2 in cheese originates from bacterial processes present at the start of the cheese-making process.[26] As different lactic acid bacteria are used in cheese making, a large variability in K2 content is found. Cheeses from Norway are among those with the highest long-chain K2 content.[29 47]

A meta-analysis including only two published studies of sufficient quality did not conclude that there is a lower risk of cardiovascular events with higher intake of K2.[49] However, a systematic review and meta-analysis found that supplementation with vitamin K (K1 and K2) significantly reduced vascular calcification, but not vascular stiffness, compared with controls.[50] However, Shea *et al* studied

Table 3 Associations between intake of energy-adjusted vitamin K1 and vitamin K2 and incident coronary heart disease (CHD)*

Exposure	Intake, µg/day mean (SD)	N	CHD, N(%)	Model 1 HR (95% CI)† n=2987	Model 2 HR (95% CI)‡ n=2792§	Model 3 HR (95% CI)¶ n=2792§
Vitamin K1						
		2987	112			
Q1	63 (25)	746	33 (4.4)	1 (ref)	1 (ref)	1 (ref)
Q2	83 (27)	747	18 (2.4)	0.47 (0.27 to 0.85)	0.50 (0.27 to 0.93)	0.48 (0.26 to 0.89)
Q3	122 (32)	748	31 (4.1)	0.84 (0.51 to 1.39)	0.89 (0.53 to 1.51)	0.83 (0.49 to 1.41)
Q4	269 (191)	746	30 (4.0)	0.91 (0.55 to 1.49)	0.92 (0.54 to 1.57)	0.69 (0.38 to 1.27)
P for trend**				0.64	0.64	0.59
Continuous, per 10 µg				1.00 (0.99 to 1.02), p=0.57	1.00 (0.99 to 1.02), p=0.62	0.99 (0.97 to 1.01), p=0.27
Vitamin K2						
		2987	112			
Q1	10 (4)	746	35 (4.7)	1 (ref)	1 (ref)	1 (ref)
Q2	13 (4)	747	30 (4.0)	0.79 (0.48 to 1.29)	0.79 (0.47 to 1.34)	0.83 (0.49 to 1.43)
Q3	17 (4)	747	29 (3.9)	0.77 (0.47 to 1.26)	0.77 (0.45 to 1.31)	0.84 (0.47 to 1.48)
Q4	26 (8)	747	18 (2.4)	0.50 (0.28 to 0.88)	0.52 (0.29 to 0.94)	0.58 (0.28 to 1.19)
P for trend**				0.02	0.03	0.16
Continuous, per 10 µg				0.71 (0.50 to 0.99), p=0.04	0.74 (0.52 to 1.05), p=0.09	0.82 (0.51 to 1.30), p=0.39

Sex-specific quartiles with 1279 men and 1708 women. The Hordaland Health Study.
*HR are presented as Q2 versus Q1, Q3 versus Q1, Q4 versus Q1.
†Cox proportional hazards regression analysis adjusted for age, sex and total energy intake.
‡Adjusted in addition for physical activity, smoking habits and education.
§Analyses were based on a reduced number of participants (n=2792) and CHD events (n=100) due to listwise deletion when covariates were missing.
¶Vitamin K1 is adjusted in addition for energy-adjusted fibre and folate, while vitamin K2 is adjusted in addition for energy-adjusted calcium and saturated fatty acids.
**P trend, to test for linear trends across quartiles, we modelled the median intake of each quartile as a continuous variable.
N, number of participants; Q, quartile.

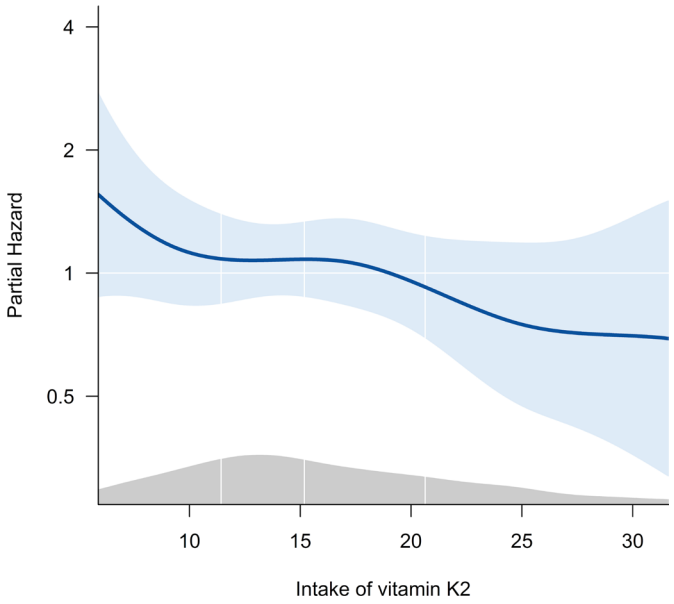

Figure 1 Cox proportional hazards regression with penalised splines, The Hordaland Health Study. Distribution of partial HR (solid line) with 95% CI (shadow) for coronary heart disease across the distribution of dietary vitamin K2 in µg per day (not energy-adjusted residuals). The model includes adjustment for age, sex, total energy intake, physical activity, smoking habits and education. Intakes above the 95th percentile and below the 5th percentile are excluded in the figure.

supplementation with K1 on coronary artery calcification progression in older men and women and found no difference between the control and treatment groups in the main analyses. Less progression of coronary artery calcification was found in participants who were ≥85% adherent to supplementation and in those with pre-existing coronary artery calcification.[51]

Potential mechanisms

The lower risk of CHD with a high intake of K2 may have different explanations. Jakobsen *et al* showed that intake of SFA seems to be preferable compared with intake of carbohydrates with high glycaemic index in order to reduce risk of myocardial infarction.[52] Further, intake of K2 correlates positively with intake of SFA, especially dairy sources as cheese, and negatively with intake of carbohydrates, especially sugar-rich sources as soft drinks with sugar and fruit and berries (both fresh and canned). Further, cheese has been associated with lower risk of CHD,[53] and the median intake of cheese more than triples between the first and fourth quartile of K2 intake. This may also partly explain why adjusting for SFA and calcium attenuated the association between intake of K2 and CHD (table 3, model 3).

The lower risk of CHD with a high intake of K2 may further be explained by carboxylation of vascular Matrix Gla Protein and consequently less arterial calcification.[54] Intimal calcification starts in the inner layer of large arteries, is associated with dyslipidaemia and may cause ischemia and arterial infarction, while calcification of the medial layer occurs even in small arteries and may lead to arterial stiffness, hypertension and left ventricular hypertrophy that further increases risk of CHD.[55]

The observed association for K2 only may be due to the fact that in addition to being cleared by the liver, it is also transported to extrahepatic tissues.[5] However, extrahepatic vitamin K-dependent proteins seem to be of lower priority compared with those in the liver.[26 27] Thus, one hypothesis is that intake of vitamin K has to be of a certain magnitude in order to have an effect on CHD. The different results on K1 and K2 may also be due to biological differences between K1 and K2 or to lower ability of the FFQ to estimate K1.[4 26 56] Due to different bioavailability, the contribution of K2 to vitamin K status is at least equal to that of K1 even though dietary K1 contributes to the majority of the total vitamin K intake.[26 46 57]

Alternatively, our findings may reflect that K2 may be a marker of another nutrient or food constituent that has heart-healthy properties.

Implications and future research

Our findings contribute to the sparse literature relating dietary vitamin K to future CHD risk. Current dietary guidelines are based on insufficient knowledge with regard to vitamin K metabolism and the different characteristics of K1 and K2. Therefore, our results indicate a need for more studies on the association between K2 and CHD. In addition, more knowledge about the absorption, transport and bioactivity of K2 is warranted.

Conclusion

In this Norwegian community-based study population, we observed that intake of K2 was associated with lower risk of CHD, while there was no association between intake of K1 and CHD. These results are considered generalisable to other middle-aged Western populations in which dairy products are the primary source of K2.

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