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Vitamin K1 intake is associated with lower risk for all-cause and cardiovascular disease mortality in community-dwelling older Australian women

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Abstract *Background and aims:* Assessing the relationship between vitamin K1 intakes, using region-specific food databases, with both all-cause, and cardiovascular disease (CVD) mortality warrants further investigation to inform future preventative strategies. Consequently, we examined the aforementioned associations in the Perth Longitudinal Study of Ageing Women (PLSAW).
Methods and results: 1436 community-dwelling older Australian women (mean ± SD age 75.2 ± 2.7 years) completed a validated food frequency questionnaire at baseline (1998). Vitamin K1 intake was calculated based on an Australian vitamin K food database, supplemented with published data. All-cause and CVD mortality data was obtained from linked health records. Associations were examined using restricted cubic splines within Cox-proportional hazard models, adjusted for a range of cardiovascular and lifestyle related risk factors. Over 15 years of follow-up, 601 (41.9%) women died, with 236 deaths (16.4%) due to CVD. Compared to women with the lowest vitamin K1 intakes (Quartile 1, median 49.1 μg/day), those with the highest intakes (Quartile 4, median 119.3 μg/day) had lower relative hazards for all-cause mortality (HR 0.66 95%CI 0.51–0.86) and CVD mortality (HR 0.61 95%CI 0.41–0.92). A plateau in the inverse association was observed from vitamin K1 intakes of approximately ≥80 μg/day.
Conclusion: Higher vitamin K1 intakes were associated with lower risk for both all-cause and CVD mortality in community-dwelling older women, independent of CVD related risk factors. A higher intake of vitamin K1 rich foods, such as leafy green vegetables, may support cardiovascular health.

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1. Introduction

Cardiovascular disease (CVD) remains the leading global cause of mortality, accounting for 32% of deaths in 2019 [1]. Adding to this burden, CVD risk is known to increase with age [2], with poorer prognosis and higher rates of CVD-related mortalities reported in older women [3]. Previous studies have observed inverse associations between cruciferous (e.g. broccoli and cauliflower) and leafy green (e.g. spinach and kale) vegetable intake with risk of subclinical atherosclerosis [4], CVD-related [5–8], and all-cause mortalities [5]. These findings may be partially explained by nutrients within these vegetable types, such as vitamin K1 [9].

Vitamin K refers to a group of fat-soluble vitamins that are best known for their role in blood coagulation and haemostasis [10]. Vitamin K exists in two main forms: phylloquinone (PK, vitamin K1) and menaquinone (MK, vitamin K2). Vitamin K1 is the primary dietary source of vitamin K, and is found abundantly in leafy green and cruciferous vegetables and various vegetable oils [11]. Vitamin K2 refers to a group of isoprenologs, ranging from MK4 through MK13, found in animal meats and products, such as eggs, cheeses, yogurts and butter [11–13].

Higher vitamin K1 intakes have been reported to be associated with a lower risk of various clinical and subclinical atherosclerotic cardiovascular diseases (ASCVDs) [14,15]. Additionally, higher vitamin K1 intakes, but not vitamin K2 intakes, have been associated with a lower risk of both all-cause and CVD mortality in a large Danish cohort [16]. The cardioprotective role of vitamin K may be explained by the carboxylation of vitamin K-dependent proteins (VKDPs), which is associated with reduced inflammation, vascular calcification processes and glycaemic control [17–19]. Despite the hypothesised cardioprotective role of vitamin K, there is ambiguity surrounding the optimal intake amount and/or isoform of vitamin K (K1 or K2) for a range of health outcomes, including CVD [12].

Dietary vitamin K intake recommendations differ internationally [20–22]. For example, the adequate intake (AI) recommendations in Australia (60–70 μg/day) and the United States (90–120 μg/day) include total vitamin K [20,21], while the recently revised Nordic nutrition recommendations are specified for vitamin K1, as expressed by a person's body mass (1.0 μg/kg/day) [22]. These recommendations are typically set based on the role of vitamin K in relation to coagulation [20,21]. Despite this, the more recent Nordic guidelines have acknowledged that the amount of vitamin K required for optimal functioning of extrahepatic VKDPs, associated with bone and vascular health, is unknown [22]. Setting AI recommendations is further complicated by the lack of data available on vitamin K food content, and the regional variation of vitamin K in foods of up to 40% [11,12]. Despite this, current Australian vitamin K AI recommendations are still derived from American food databases [12]. To overcome this, region-specific vitamin K food databases that evaluate vitamin K content in commonly consumed foods in Australia is required to allow the evaluation of vitamin K intakes from food records. This data has recently become available in Australia in 2021 for vitamin K1 [11], with detailed data on vitamin K2 (e.g. menaquinones 4–13) still lacking.

Assessing the relationship between vitamin K1 intakes and both all-cause, and CVD mortality in multiple populations, using appropriate food databases, is required to inform the development of novel preventative strategies to reduce the burden of CVD. Consequently, this study examined the associations between dietary vitamin K1, using a recently published Australian vitamin K1 database, with all-cause and CVD mortality in older Australian women.

2. Methods

2.1. Study population

Participants from the Perth Longitudinal Study of Ageing Women (PLSAW), which included 1500 community-dwelling older women from Western Australia (aged ≥70 years), were considered. Women were recruited in 1998 (baseline) for The Calcium Intake Fracture Outcome Study (CAIFOS), which was a five-year, double-blind, randomised controlled trial (RCT) that assessed daily calcium supplementation for fracture prevention [23]. A subset of women (n = 40) were randomised into a third trial arm of both vitamin D and calcium supplementation [23]. Exclusion criteria included serious medical conditions that made it unlikely participants would survive the 5-year trial. Out of the 1222 women excluded at initial screening due to CAIFOS eligibility criteria [23], 199 (16.3%) were due to the prevalence of serious medical conditions. The inclusive nature of the CAIFOS trial was designed to ensure results would be generalisable to older Australian women [23]. After the completion of CAIFOS, women were subsequently enrolled into two successive, five-year observational studies (2003–2013). The entire study over 15 years is known as the PLSAW, which was approved by the University of Western Australia's Human Research Ethics Committee (PLSAW trial registration number #ACTRN12617000640303) and complied with the Declaration of Helsinki [7,24]. Written, informed consent was obtained from each participant. Human ethics approval for use of data linkage was provided by the Western Australian Department of Health Human Research Ethics Committee (project #2009/24) [7].

2.2. Participants

At baseline, 15 women did not complete the food frequency questionnaire (FFQ). An additional 17 women with implausible total energy intake (<2100 kJ or >14,700 kJ per day) [4] were also excluded. As warfarin is a vitamin K antagonist [25], women taking warfarin were also excluded (n = 8). A further 21 women with missing covariate data, including smoking status (n = 8), physical

activity ($n = 2$) and residential postcode ($n = 11$) were excluded from the analysis. As three deaths were under coronial inquiry at the time of data extraction, no primary cause of death was able to be ascertained for these cases, thus, these participants were excluded from the analysis ($n = 3$). After exclusions, 1436 women were included in the current study (Supplementary Fig. 1).

2.3. Demographic and clinical assessment

Participant demographics and clinical assessments were assessed at baseline. Body weight was measured to the nearest 0.1 kg using digital scales and height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. Body mass index (BMI, kg/m^2) was then calculated. Physical activity levels were obtained at baseline using a questionnaire that assessed participation in sport, recreation and/or regular physical activity in the three months prior. Physical activity was quantified in kJ/day by accounting for primary activity undertaken, as described previously [26]. Smoking status was obtained from a questionnaire and was coded as non-smoker or former/current smoker (defined as smoking >1 cigarette per day for greater than 3 months over the lifespan). Alcohol intakes were calculated using the NUTTAB 95 food composition database [27] and categorised, by Australian standard drink serves [28], into one of three groups; no alcohol consumed, less than one standard drink per day (<10 g/day), and one or more standard drinks per day (≥10 g/day). A list of prescription medications was obtained from participants and was coded using the International Classification of Primary Care (ICPC) Plus method to assess diabetes prevalence (ICPC-2 Plus medication codes T89001–T90009) [29]. Medical histories and medication use were verified by the participants general practitioner, where possible. Prevalent CVD and cancer were obtained from the principal hospital discharge diagnoses from the Western Australian Data Linkage System, and the Western Australian Hospital Morbidity Data Collection. Diagnosis codes were recorded for study participants over the 18-year period prior to baseline (1980–1998). Disease coding was based on the International Statistical Classification of Diseases (ICD) codes; ICD-9-CM 390–459 for prevalent CVD, and 140–239, excluding 210–229, for prevalent cancer [30]. The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine-derived equation was used to estimate glomerular filtration rate (eGFR) in 1297 women with available data [31]. The socioeconomic indexes for areas, developed by the Australian Bureau of Statistics was used to calculate socioeconomic status (SES) [32]. Participants residential postcodes were ranked by socioeconomic advantage and disadvantage into six groups, ranking from top 10% most highly-disadvantaged, to top 10% least-disadvantaged [32].

2.4. Dietary intake assessment

Dietary intake was assessed at baseline (1998) via a 74-question, self-administered, semiquantitative FFQ that was developed and validated by the Cancer Council of Victoria [27,33]. The FFQ has been designed to assess habitual nutrient intake over the prior 12-month period. Calculations of energy (kJ/day) and nutrient intakes (g/day) were performed using the NUTTAB 95 food composition database and were supplemented by other data where necessary [27]. Participants were supervised by a research assistant while completing the FFQ, with food charts, models, measuring cups and spoons provided to participants to aid accuracy of reported consumption [27].

2.5. Vitamin K1 assessment

Dietary vitamin K1 intake was calculated from all the listed food items in the FFQ ($n = 101$), by multiplying the food item consumed (quantified as g/day) by its mean vitamin K1 value (μg/g). Vitamin K1 values for food items were quantified using two databases. The primary database was an Australian vitamin K database that assessed vitamin K1 content in various food products ($n = 55$), obtained from Australian supermarkets [11]. The main food groups included in this database were vegetables ($n = 19$), fruit ($n = 3$), animal products ($n = 16$), dairy products ($n = 14$) and fermented foods ($n = 3$) [11]. Where vitamin K1 data was not available for a specific food product in the Australian database, the United States Department of Agriculture (USDA) Food and Nutrient Database for Dietary Studies (2017–18) was adopted ($n = 46$) [34].

2.6. Mortality

Mortality records were obtained from Western Australia Data Linkage System (Department of Health, Western Australia, East Perth, Australia) for each study participant, from the date of their baseline clinical visit, across the 15-year study duration (1998–2013). Primary cause of death data was used for CVD mortality (ICD-9-CM codes 390–459 and ICD-10-AM codes I00–I99), cancer mortality (ICD-9-CM codes 140–239, excluding 210–229, and ICD-10-AM codes C00-D48 excluding D10-D36) and other mortalities (all other mortality codes not associated with CVD or cancer). Causes of death were obtained from the coded death certificate, using information in parts 1 and 2 of the death certificate, or all diagnosis text fields from the death certificate when coded deaths were not yet available.

2.7. Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics, version 29.0 (IBM Corporation, Armonk, New York) and R software, version 3.4.2 (R Foundation for Statistical Computing, Vienna, Austria). To explore potential non-linear associations, restricted cubic splines were adopted using the 'rms' R package [35]. The associations were presented graphically using the 'effects' R package [36]. Hazard ratio (HR) estimates are relative to the reference value, set as the median vitamin K1 intake of participants with the lowest intake (Quartile 1 [Q1]) and were

plotted against each outcome with 95% confidence bands provided. Wald tests were used to obtain p-values and the x-axis was truncated at 3 SD above the mean, for visual simplicity only. Schoenfeld residuals indicated that the assumptions of proportional hazards were not violated. Three models of adjustment were adopted: Model 1: Adjusted for age, treatment group (calcium vs. placebo vs. calcium + vitamin D) and BMI; Model 2: Model 1 plus physical activity, energy intake, alcohol intake, smoking history, and SES, and; Model 3: Model 2 plus prevalent diabetes, CVD, and cancer. Potential confounding factors were selected on a priori basis as potential risk factors for CVD and all-cause mortality.

2.8. Additional analyses

We also investigated the association between vitamin K1 with both cancer and other causes of mortality. As vegetables are a major source of vitamin K1, higher vitamin K1 intakes may represent a healthy diet. As such, an adherence measure of diet to the 2013 Australian Dietary Guidelines [37], the Dietary Guideline Index (DGI) [38], was adopted to assess diet quality [37,38], and was included as an additional covariate to all models of adjustment, when examining the associations between vitamin K1 intakes and mortality outcomes.

Chronic kidney disease (CKD) is associated with impaired vitamin K metabolism and increased mortality [39–41]. As such, we examined if the relationships between vitamin K1 intake with all-cause and CVD mortality were influenced by including eGFR to the primary analysis, as well as by undertaking interaction testing to determine if the presence of CKD (eGFR <60 mL/min [n = 408] or an eGFR ≥60 mL/min [n = 889]) influenced these relationships.

In light of the recently revised Nordic vitamin K1 recommendations being expressed by body mass (1 μg/kg/day) [22], we examined the associations between vitamin K1 intakes, quantified per kilogram of body mass, with both all-cause, and CVD mortality.

3. Results

Baseline characteristics for the 1436 women by vitamin K1 intake quartiles are presented in Table 1. Mean ± SD age was 75.2 ± 2.7 years and BMI was 27.2 ± 4.7 kg/m^2, the mean ± SD intake for vitamin K1 was 82.3 ± 30.7 μg/day. Women with the highest vitamin K1 intake (Q4) appeared to have higher energy intakes, physical activity levels and DGI score compared to those in Q1 (Table 1). They were also less likely to have a history of smoking.

3.1. Vitamin K, all-cause and CVD mortality

Over 15 years (mean ± SD; 12.8 ± 3.5 years) of follow-up (18,385 person-years), 41.9% (n = 601/1436) of women died from any cause, and in 16.4% (n = 236/1436) of women, the primary cause of death was attributed to CVD. The multivariable adjusted (Model 2), non-linear relationship between vitamin K1 intakes with both all-cause, and CVD mortality showed lower relative hazards, compared to Q1, as intakes increased, with a plateau in benefit appearing at approximately 80 μg/day (Fig. 1). Compared to Q1, women in Q2, Q3 and Q4 had lower relative hazards for all-cause (26%, 32% and 34%) and CVD (30%, 39% and 39%) mortality, respectively (*Model 2,* Table 2). Similar associations were observed across all models of adjustment.

3.2. Additional analyses

Over 15 years, 10.7% (154/1436) and 14.7% (211/1436) of women died due to cancer or any other cause, respectively. A diagrammatic representation of the relationships between vitamin K1, cancer and other mortalities are presented in Supplementary Fig. 2. For other mortalities, only women in Q2 and Q3 of vitamin K1 intake, recorded statistically significantly lower relative hazards compared to Q1 (25% and 28%, respectively) (Model 2, Supplementary Table 1, and Supplementary Fig. 2). No such relationships were observed for cancer mortality over 15 years (Model 2, Supplementary Table 1, and Supplementary Fig. 2).

The addition of the DGI to Model 2 did not change the relationship between vitamin K1 intakes with both all-cause and CVD mortality. Specifically, compared to women in Q1, those in Q2, Q3 and Q4 had lower hazards for all-cause (25%,30 % and 31%, respectively) and CVD mortality (29%, 38% and 36%, respectively) (Supplementary Table 2). When considering other mortality, only women in Q2, but not Q3 and Q4, recorded 24 % lower hazards compared to Q1 (Supplementary Table 2).

The addition of eGFR to Model 2 did not alter the relationships between vitamin K1, all-cause and CVD mortality. Compared to women in the lowest quartile (Q1), women with higher vitamin K1 intakes (those in Q2, Q3 and Q4) had lower hazards for all-cause (25%, 30% and 33%, respectively) and CVD mortality (29%, 39% and 38%, respectively) (Supplementary Table 3). No interaction was observed between the presence of CKD and vitamin K1 when considering all-cause ($p_{interaction}$ = 0.393) and CVD mortality ($p_{interaction}$ = 0.693).

When vitamin K1 intake was expressed in μg/kg/day, there was a non-linear inverse association between vitamin K1 with both all-cause and CVD mortality (Supplementary Table 4, and Supplementary Fig. 3). Specifically, compared to Q1, women with higher vitamin K1 intakes (those in Q2, Q3 and Q4) had a lower hazard for all-cause (27%, 36% and 38%, respectively) and CVD mortality (30%, 40% and 40%, respectively) (Model 2, Supplementary Table 4). For the aforementioned analysis, the benefits appeared to plateau once intakes of approximately 1.3 μg/kg/day were achieved (Supplementary Fig. 3).

Table 1 Baseline characteristics of all participants, and by quartiles of vitamin K1 intake.

Vitamin K1					
Participant demographics	All participants n = 1436	Quartile 1 (<61.1 μg/d), *n = 359*	Quartile 2 (61.1 to <78.7 μg/d), *n = 359*	Quartile 3 (78.7 to <99.1 μg/d), *n = 359*	Quartile 4 (≥99.1 μg/d), *n = 359*
Age, y	75.2 ± 2.7	75.2 ± 2.8	74.9 ± 2.7	75.2 ± 2.7	75.3 ± 2.7
BMI, kg/m^2	27.2 ± 4.7	27.2 ± 4.8	26.9 ± 4.5	27.4 ± 4.9	27.2 ± 4.7
Treatment, n (%)					
Calcium, n (%)	697 (48.5)	163 (45.4)	169 (47.1)	182 (50.7)	183 (51.0)
Calcium & vitamin D, n (%)	39 (2.7)	11 (3.1)	8 (2.2)	12 (3.3)	8 (2.2)
Physical activity, kcal/d	111.2 (23.1–203.6)	93.2 (0.0–200.8)	112.7 (43.3–199.6)	102.6 (38.3–206.2)	118.1 (50.9–207.1)
Vitamin K1, μg/d	82.3 ± 30.7	47.3 ± 10.0	70.1 ± 5.2	87.6 ± 5.6	124.1 ± 21.3
Alcohol intake, g/d	1.8 (0.3–9.8)	1.6 (0.2–9.9)	2.1 (0.3–10.2)	1.7 (0.3–9.1)	1.6 (0.3–9.4)
Energy intake, kJ/d	7102 ± 2083	5614 ± 1351	6706 ± 1607	7363 ± 1804	8725 ± 2156
Smoking history, n (%)	535 (37.3)	148 (41.2)	136 (37.8)	129 (35.9)	122 (33.9)
Prevalent cancer, n (%)	148 (10.3)	24 (6.7)	43 (11.9)	39 (10.9)	42 (11.7)
Prevalent CVD, n (%)	330 (22.9)	79 (22.0)	82 (22.8)	88 (24.5)	81 (22.5)
Prevalent diabetes, n (%)	87 (6.1)	20 (5.6)	24 (6.7)	21 (5.8)	22 (6.1)
eGFR, mL/min/1.73m^2 [a]	66.8 ± 13.2	66.3 ± 13.7	67.0 ± 13.1	67.3 ± 13.1	66.5 ± 13.1
DGI score	34.9 ± 8.8	32.7 ± 8.5	33.5 ± 9.1	35.4 ± 8.2	37.8 ± 8.4
Socioeconomic status, n (%)					
Top 10 % most highly disadvantaged	62 (4.3)	18 (5.0)	12 (3.3)	17 (4.7)	15 (4.2)
Highly disadvantaged	172 (11.9)	41 (11.4)	44 (12.3)	40 (11.1)	47 (13.1)
Moderate - highly disadvantaged	234 (16.3)	51 (14.2)	60 (16.7)	51 (14.2)	72 (20.1)
Low - moderately disadvantaged	219 (15.3)	62 (17.3)	41 (11.4)	61 (17.0)	55 (15.3)
Low disadvantaged	304 (21.2)	75 (20.9)	77 (21.4)	78 (21.7)	74 (20.6)
Top 10 % least disadvantaged	445 (30.9)	112 (31.2)	125 (34.8)	112 (31.2)	96 (26.7)

Data is expressed as mean ± SD, median (IQR), or n (%).
Abbreviations: body mass index (BMI), cardiovascular disease (CVD), estimated glomerular filtration rate (eGFR), dietary guideline index (DGI).
[a] eGFR data was available for n = 1297 women.

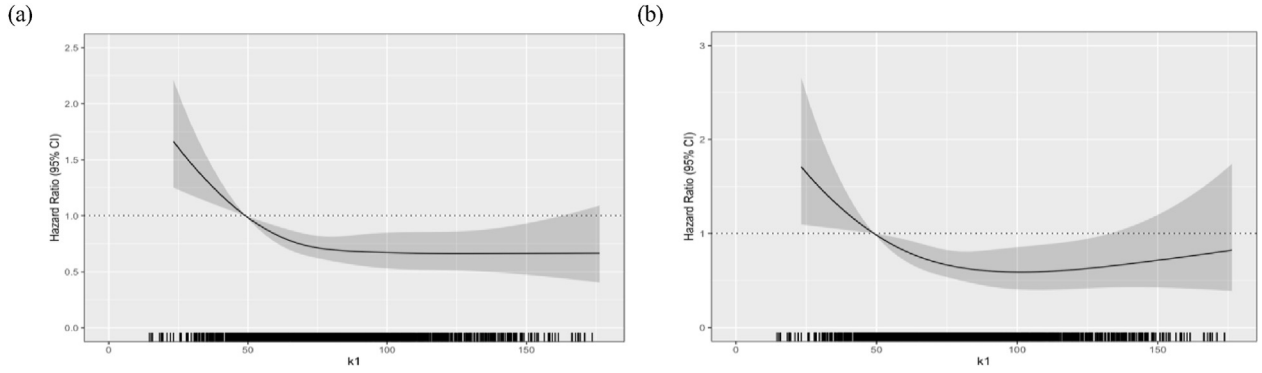

Figure 1 Multivariable-adjusted hazard ratios for the relationship between vitamin K1 and all-cause (a) and cardiovascular disease (b) mortality, over 15 years. Model adjusted for age, treatment, body mass index, smoking history, energy intake, alcohol intake, socioeconomic status, and physical activity (Model 2). Solid lines are the estimated hazard ratio and shaded areas represent the 95% confidence intervals. The rug-plot along the x-axis represents each individual.

4. Discussion

The results of this prospective cohort study in 1436 community-dwelling older women demonstrate that moderate to high intakes of vitamin K1 (approximately ≥80 μg/day) are associated with lower risk of all-cause (up to 34%) and CVD mortality (up to 39%), independent of a range of lifestyle and CVD risk factors. Notably, these associations were non-linear, with a plateau in the relative hazard at intake levels from approximately 80 μg/day.

To our knowledge, only few studies have assessed vitamin K1 intakes and all-cause mortality, with two studies reporting no associations [15,42]. The first reported no relationship between vitamin K1 intakes and both all-cause, and cause specific (CVD, cancer and other cause) mortality over 16.8 years (*n* = 33,289, mean age

Table 2 Hazard ratio (95% CI) for all-cause and cardiovascular disease mortality, over 15 years, by quartiles of vitamin K1 intake.

		Quartiles for vitamin K1 intake			
		Quartile1 < 61.1 μg/d	Quartile 2 61.1 to <78.7 μg/d	Quartile 3 78.7 to <99.1 μg/d	Quartile 4 ≥ 99.1 μg/d
All-cause mortality	Events, n (%)	184 (51.2)	137 (38.2)	146 (40.7)	134 (37.3)
	Model 1	Ref.	**0.73 (0.65–0.83)**	**0.69 (0.58–0.80)**	**0.69 (0.56–0.85)**
	Model 2	Ref.	**0.74 (0.65–0.84)**	**0.68 (0.57–0.82)**	**0.66 (0.51–0.86)**
	Model 3	Ref.	**0.74 (0.65–0.84)**	**0.67 (0.56–0.81)**	**0.65 (0.51–0.84)**
CVD mortality	Events, n (%)	75 (20.9)	60 (16.7)	50 (13.9)	51 (14.2)
	Model 1	Ref.	**0.68 (0.56–0.81)**	**0.58 (0.45–0.76)**	**0.58 (0.42–0.82)**
	Model 2	Ref.	**0.70 (0.58–0.86)**	**0.61 (0.45–0.82)**	**0.61 (0.41–0.92)**
	Model 3	Ref.	**0.69 (0.57–0.85)**	**0.60 (0.45–0.81)**	**0.60 (0.40–0.91)**

Estimated hazards ratio and 95% CI from Cox proportional hazards analysis, comparing the median vitamin K1 intake from each quartile (Q) compared to Q1. Median vitamin K1 intake for Q1, Q2, Q3 and Q4 was 49.1, 69.9, 87.5 and 119.3 μg/d, respectively. Bolded indicates $p < 0.05$ compared to Q1. Model 1: Adjusted for age, treatment, and body mass index. Model 2: Model 1 plus smoking history, energy intake, alcohol intake, physical activity, and socioeconomic status. Model 3: Model 2 plus prevalent cardiovascular disease, prevalent cancer, and prevalent diabetes Abbreviations: cardiovascular disease (CVD).

48.6 ± 12.0 years) in the Dutch contribution to the European Prospective Investigation into Cancer and Nutrition cohort (EPIC-NL) [42]. Interestingly, the authors reported very high mean vitamin K1 intakes (87.7 ± 17.5 μg/day) in individuals within the lowest intake quartile. This is higher than the AI guidelines of 70 μg/day of total vitamin K in both Europe, and Australia [20,43,44], possibly explaining these null findings. The Rotterdam study also reported no association between vitamin K1 intakes and all-cause mortality (n = 4,807, aged >55 years) [15]. A high median intake of vitamin K1 in the lowest tertile (154.6 μg/day) was also reported, which was attributed to high vegetable consumption [15]. Conversely, a large Danish cohort study (n = 56,048, 52.4% female, aged 52–60 years) reported lower hazards of 23% for all-cause, and 28% for CVD mortality, between the lowest (median intake of 57 μg/day) and highest (median intake of 192 μg/day) vitamin K1 intake quintiles [16]. These results support the findings of the current study, where a 34% lower risk of all-cause mortality and 39% lower risk of CVD mortality were observed between the highest and lowest vitamin K1 intake quartiles. Comparable to our results, the authors reported that the gradient of this association plateaued when vitamin K1 intakes of approximately 100 μg/day were attained [16]. Regarding cancer mortality, this Danish study [16] identified a significant non-linear relationship between vitamin K1 intakes and cancer mortality, with up to 19% lower hazard when comparing the highest vs. lowest quintiles [16]. No such relationship was reported in the current study. Of importance, this relationship was only reported in only current/former smokers, which made up 64% (n = 35,871) of the cohort [16], compared to 37% in this current study. Consequently, further research is required before conclusions can be drawn regarding vitamin K1 intakes and cancer mortality.

Based on the aforementioned studies providing mixed results in relation to CVD mortality [15,16,42], it is evident that further investigation is warranted to address this ambiguity. Nevertheless, another study in the aforementioned Danish cohort reported lower risk of non-fatal CVD hospitalisations, with higher vitamin K1 intakes [14]. Specifically, a 21% lower risk of ASCVD hospitalisations, between the highest and lowest vitamin K1 quintiles was reported [14]. Collectively, these findings highlight the potential benefits of vitamin K1 for cardiovascular health, especially when dietary intake may be inadequate. Although VKDPs may have numerous cardioprotective roles (e.g. limiting inflammation), carboxylated matrix Gla protein may be crucial as it is reported to inhibit vascular calcification, a major risk factor for CVD [18]. Nevertheless, further research is required to understand how the carboxylation of matrix Gla protein by vitamin K may contribute to cardiovascular health. Noteworthy, the presence of CKD and vitamin K deficiency may result in exacerbation of abnormal mineralisation processes, including increased vascular calcification, leading to CVD mortality [15,39,40]. Despite this, no difference was observed in the relationships between vitamin K1 intakes, all-cause and CVD mortality with the addition of eGFR to the primary analysis. However, the benefit of vitamin K in patients with CKD was reported in the NHANES III study (n = 3401, ~66% female, mean age 61.9 years), in which total dietary vitamin K intakes (vitamins K1 and K2) above the locally recommended levels (90 μg/day for women and 120 μg/day for men) were associated with a 15% lower risk of all-cause mortality in individuals with CKD [45]. This is an area which requires further examination, especially since only 31% of women in our study presented with CKD. Interpretation and comparison of these results to the current study are also complicated by the examination of total vitamin K, which includes vitamin K2 that is estimated to provide ~10% of total dietary vitamin K in Western diets [12]. Of importance, there is currently limited international and Australian data available for the vitamin K2 content in foods (MK4 through MK13); hence, vitamin K2 was not considered in the present study.

Collectively, our results suggest that moderate to high vitamin K1 intakes may be linked to lower all-cause and CVD mortality risk. Current Australian AI guidelines recommend 60 and 70 μg/day of total vitamin K, for

women and men, respectively [20]. These are substantially lower (33–42%) than the AI of 90 and 120 μg/day promoted for adult women and men, respectively, in the United States [21]. In 2017, the ESFA Panel on Dietetic Products, Nutrition and Allergies stated that the currently available information on the absorption and function of MKs was insufficient to establish dietary recommendations, setting the AI for vitamin K1 only (1 μg/kg/day) [43]. Furthermore, the recently revised Nordic nutrition recommendations state that comprehensive assessment of vitamin K (especially K2) content in foods is required internationally. Consequently, an AI for vitamin K1 only was recommended, at 1 μg per kg of body weight, daily [22]. Albeit slightly lower, this aligns to the current study where vitamin K1 intakes from approximately 1.3 μg/kg/day appeared the most beneficial.

It is important to highlight that dietary guidelines for vitamin K have traditionally been developed in relation to coagulation [20,21]. Here, we provide insight that higher intakes (>80 ug/day) of vitamin K1 may be required to support cardiovascular health in older women. This higher requirement may be attributed to the vitamin K requirements of extrahepatic VKDPs, linked with anti-calcification processes (e.g. matrix Gla protein), compared to that of hepatic VKDPs, involved in blood coagulation (e.g. coagulation factors II,VII, IX, and X) [17]. Collectively, these findings suggest that vitamin K1 intakes higher than current Australian AI guidelines (60 μg/day) may be more beneficial for cardiovascular health in older women.

Considering vegetables are a primary source of dietary vitamin K1 [11], promotion of a vegetable-rich diet, comprising leafy green and cruciferous varieties, would be a simple and effective strategy to increase vitamin K1 intake. Specifically, to maximise any benefits, vitamin K1 quantities coinciding with those in the upper intake quartiles (Q3 and Q4) of the present study may be attained by consuming 1–2 serves (75–150 g) of broccoli, spinach, cabbage, or lettuce per day [11]. This is an achievable target as part of the recommended 5–6 serves per day of vegetables [37]. As vitamin K1 bioavailability is known to be enhanced in the presence of oils [46], the use of oils rich in vitamin K1, such as extra virgin olive oil [11] during cooking may further enrich vitamin K1 content and bioavailability. It must be acknowledged that our study is not without limitations. Firstly, as the cohort studied is primarily older, healthy, Caucasian women, the generalisability of these findings may be limited to this population. Further, due to the observational nature of this work, causality cannot be established. It must be also acknowledged that vitamin K1 intakes were calculated from self-reported FFQ's that can be impacted by recall and reporting bias. However, women were supported by a research assistant and provided with food charts and measuring cups to minimise error when completing the FFQ. Furthermore, we did not have measures of gastrointestinal diseases that may have affected nutrient absorption. However, this limitation would likely have led to a type II error (false negative) rather than a type I error (false positive). To minimise residual confounding, a large range of other factors were considered (e.g. prevalent disease, smoking status, alcohol intake and physical activity levels). Moreover, as vegetables are a major source for vitamin K1, higher vitamin K1 intakes may be indicative of a healthier dietary pattern. Nevertheless, to account for this we included DGI, a measure of diet quality, in our analysis, with our results remaining unchanged. Despite these limitations, our study has several strengths. This includes a long duration of follow up (15 years) in a population-based cohort, representative of community-dwelling older women, who often present with higher CVD burden compared to their male counterparts [2,47,48]. We also used linked health records, enabling the ascertainment of prevalent disease status, which were included as part of multivariable-adjusted analysis (Model 3). Finally, we adopted an Australia-specific vitamin K1 food composition database. Given regional differences in consumption and/or composition of food between countries, assessing vitamin K1 intakes using region specific databases enables greater precision [11].

5. Conclusion

The present study demonstrates non-linear, inverse relationships between vitamin K1 intakes with both all-cause and CVD mortality, in a cohort of community-dwelling older women. These benefits tended to plateau once intakes of approximately 80 μg/day were attained. As leafy green and cruciferous vegetables are a primary source of vitamin K1, promoting 1–2 servings of these vegetables, as part of the currently recommended 5–6 serves per day of total vegetables may provide adequate amounts of vitamin K1. Future research should seek to investigate the relationships between vitamin K1 intakes and non-fatal CVD events, including potential mechanisms, to further inform public health messaging.

Author contributions

MD, MS, JRL, JMH, SRB designed the research. RLP, JRL, conducted the research. MD, MS, JRL analysed the data. MS, MD, JRL, LZ, SRB drafted the paper. MD and MS has primary responsibility for the final content. All authors read and approved the final manuscript.

Disclaimers

All authors declare no conflicts of interest.

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Declaration of competing interest

The authors have nothing to disclose.

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Appendix A. Supplementary data

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