The Journal of Nutrition
Nutrition and Disease

Concentrations of Circulating Phylloquinone, but Not Cerebral Menaquinone-4, Are Positively Correlated with a Wide Range of Cognitive Measures: Exploratory Findings in Centenarians

Jirayu Tanprasertsuk,[1,2] Guylaine Ferland,[3] Mary Ann Johnson,[4] Leonard W Poon,[5] Tammy M Scott,[2] Aron K Barbey,[6] Kathryn Barger,[1] Xiang-Dong Wang,[1] and Elizabeth J Johnson[1]

[1]Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA, USA; [2]Gerald J and Dorothy R Friedman School of Nutrition Science and Policy, Tufts University, Boston, MA, USA; [3]Department of Nutrition, University of Montreal, Montreal, Canada; [4]Department of Nutrition and Health Sciences, University of Nebraska Lincoln, Lincoln, NE, USA; [5]Institute of Gerontology, University of Georgia, Athens, GA, USA; and [6]Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana–Champaign, Urbana, IL, USA

ABSTRACT

Background: Vitamin K (VK) exists in the form of phylloquinone (PK) and menaquinones (MKs). Roles of VK on cognitive health in the elderly are emerging, but there is limited evidence on VK uptake and metabolism in human brain.

Objectives: The primary objective of this study was to characterize VK distribution in brains of an elderly population with varied cognitive function. In addition, associations among circulating (a biomarker of VK intake) and cerebral VK concentrations and cognition were investigated.

Methods: Serum or plasma ($n = 27$) and brain samples from the frontal cortex (FC; $n = 46$) and the temporal cortex (TC; $n = 33$) were acquired from 48 decedents (aged 98–107 y; 25 demented and 23 nondemented) enrolled in the Georgia Centenarian Study. Both circulating and brain VK concentrations were measured using HPLC with fluorescence detection. Cognitive assessment was performed within 1 y prior to mortality. Partial correlations between serum/plasma or cerebral VK concentrations and cognitive function were performed, adjusting for covariates and separating by dementia and antithrombotic use.

Results: MK-4 was the predominant vitamer in both FC (mean ± SD = 4.92 ± 2.31 pmol/g, ≥89.15% ± 5.09% of total VK) and TC (4.60 ± 2.11 pmol/g, ≥89.71% ± 4.43% of total VK) regardless of cognitive status. Antithrombotic users had 34.0% and 53.9% lower MK-4 concentrations in FC ($P < 0.05$) and TC ($P < 0.001$), respectively. Circulating PK was not correlated with cerebral MK-4 or total VK concentrations. Circulating PK concentrations were significantly associated with a wide range of cognitive measures in nondemented centenarians ($P < 0.05$). In contrast, cerebral MK-4 concentrations were not associated with cognitive performance, either before or after exclusion of antithrombotic users.

Conclusions: Circulating VK concentrations are not related to cerebral MK-4 concentrations in centenarians. Cerebral MK-4 concentrations are tightly regulated over a range of VK intakes and cognitive function. Circulating PK may reflect intake of VK-rich foods containing other dietary components beneficial to cognitive health. Further investigation of VK uptake and metabolism in the brain is warranted. *J Nutr* 2019;00:1–9.

Keywords: vitamin K, phylloquinone, menaquinone, cognition, older adults

Introduction

Vitamin K (VK) is an essential fat-soluble vitamin that exists in 2 forms: phylloquinone (PK) and menaquinones (MKs). PK accounts for 90% of dietary VK in the American diet, in which green leafy vegetables and vegetable oils are significant sources (1–3). MKs are composed of molecules with varying length of unsaturated side chains (MK-*n*, where *n* is the number of 5-carbon units), from MK-4 to MK-13 (4). A varied amount of MK-4 is found in meats, organ meats, eggs, and dairy products, whereas MK-7 to MK-10 are found in fermented foods and are also synthesized by gut microbiota (1, 2). MK-4 is also the primary form of VK in certain extrahepatic tissues of humans, including the brain (5). It has been demonstrated that tissue MK-4 is primarily derived from dietary PK with an endogenous conversion from PK to MK-4, independently of gut microbiota

Manuscript received May 5, 2019. Initial review completed May 16, 2019. Revision accepted July 25, 2019.
First published online 0, 2019; doi: https://doi.org/10.1093/jn/nxz200.

(3, 6–9). The intestine is also capable of cleaving dietary PK to menadione and releases it into the circulation (7, 9–11). It is still unclear which metabolite(s) of circulating VK the brain acquires and uses as a substrate for MK-4 production.

VK's classical function is acting as a coenzyme in the activation of coagulant factors (12). Anticoagulants, such as warfarin, inhibit the recycling of VK back from VK epoxide to its active reduced form and thus inhibit VK's activity on the coagulation process. However, VK has recently been proposed to play an important role in cognitive health in the aging population (13). VK-dependent proteins (VKDPs) have been characterized in brain tissues and proposed to mediate the effect of VK on cognitive functioning (14). Higher VK intake has been reported to be associated with better cognitive performance or lower risk of Alzheimer's disease in at least 4 different cohorts of older adults (15–18). Another study demonstrated that cognitively intact older adults with higher plasma PK concentrations performed better on cognitive assessments related to consolidation processes of the memory trace (19).

Despite consistent relation between VK status and cognitive health across animal and human studies, very little is known regarding VK uptake and metabolism in human brain. Only 1 study reported MK-4 being the most predominant form of VK among 3 middle-aged individuals, accounting for 86% of the total VK content (5). The primary objective of this study was to characterize VK forms and their distribution in the frontal cortex (FC) and the temporal cortex (TC) in a cohort of older adults whose cognitive status ranged from intact cognition to severe dementia. Additional analyses were performed to further characterize the relation among circulating and brain VK concentrations and also cognitive performance, with a goal of better understanding VK's distribution, metabolism, and its role in cognitive health among the aging population. To our knowledge, this is the first report on the assessment of VK status in human brain tissues in a population with varied cognitive status, as well as the relation between brain VK concentrations and premortem cognitive function in humans.

Methods

Study population and sample collection

Blood and matched brain samples from FCs and TCs were collected from 48 centenarians (aged ≥98 y) who were enrolled in phase III of the Georgia Centenarian Study (GCS) (20, 21). Phase III was a population-based study conducted in 44 counties in northern Georgia from 2001 to 2009. Its long-term goal was to identify and elucidate the roles of biological, psychological, and social factors that are pertinent to survival and functioning in older adults. Recruitment and sample collection procedures for the GCS have previously been described in detail (21). After the enrollment, blood was collected in the participants' residences by a skilled phlebotomist every 6 mo. Participants were not required to be fasted at the time of blood draw in an effort to lessen the burden of sample collection. Serum or plasma samples for VK analysis ($n = 27$) were derived from blood samples from the time point closest to a participant's death. As previously described, 211 participants were approached for the opportunity to participate in the brain donation, and 66 participants agreed to donate brain tissues upon death (**Supplemental Figure 1**) (22). Samples from FC ($n = 46$) and TC ($n = 33$) available for VK analysis were collected during autopsy (**Supplemental Figure 2**). Among 33 subjects who provided samples from both FC and TC, 21 subjects also provided matched serum or plasma. All biological samples were stored at −80°C until the analysis. Samples were coded to maintain confidentiality, and there was no access to subject identification. The study was approved by the Institutional Review Board on Human Subjects from the University of Georgia and by Tufts University/Tufts Medical Center for the use of de-identified samples and data.

VK analyses

PK and MK-4 in brain.

Brain PK and MK-4 were quantified by reverse-phase HPLC as previously described (23). Briefly, 60–100 mg tissue samples were pulverized in anhydrous Na_2SO_4 and extracted with acetone containing an internal standard [2-methyl-3-(3,7,11,15,19-pentamethyl-2-eicosenyl)-1,4 naphthalenedione] (GL Synthesis). Dried extracts were then re-extracted with a mixture of hexane and water before being further purified by solid-phase extraction on silica gel columns (ThermoFisher Scientific). Quantitative analysis was performed using a C-18 reverse-phase column and fluorescence detection. The calibration standard consisted of a mixture of PK, MK-4, and 2-methyl-3-(3,7,11,15,19-pentamethyl-2-eicosenyl)-1,4-naphthalenedione at 2 ng in 50 μL. Limit of detection (LOD) for both vitamers was 0.03 pmol per injection. The percentage recovery for the samples was calculated from the internal standard and found to be >75%. The intra-assay CVs for PK and MK-4 were 5.1% and 4.6%, respectively.

Serum/plasma PK.

Circulating PK was quantified using an HPLC procedure that involves similar steps as those described for brain tissues (24, 25). Assessments were conducted on 150–250 μL of serum/plasma, and limit of detection of the assay was 0.02 nmol/L. Percentage recovery calculated from the internal standard was ~80%, and intra-assay CV was 7.6%.

Assessment of cognition, depression, and activities of daily living

After enrollment, a battery of cognitive tests was administered by geriatric psychologists at baseline and every 6 mo until mortality (21). All cognitive tests administered were described previously (26). Cognitive tests included the Global Deterioration Scale (GDS); Mini-Mental State Examination (MMSE); Severe Impairment Battery (SIB); Fuld Object Memory Evaluation (FOME); Controlled Oral Word Association Test (COWAT); Wechsler Adult Intelligence Scale–Third Edition (WAIS-III) Similarities; Behavioral Dyscontrol Scale (BDS); and the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) battery, which included Verbal Fluency (VF), Boston Naming Test (BNT), Constructional Praxis (CP), and Word List Memory Test (WLMT). The Geriatric Depression Scale–Short Form (GDSSF) was used as a measure of depression, and the Direct Assessment of Function Status (DAFS) was used as a measure of activities of daily living. Assessment from the time point closest to death (within 1 y prior to death for all subjects) was used for the analysis. GDS was used as an assessment of the presence of dementia (27). Subjects who scored 1–3 on the GDS were clinically free of dementia: GDS 1 represented intact cognition, GDS 2 represented age-associated memory impairment, and

Vitamin K analysis was funded by Abbott Nutrition.
Author disclosures: JT, GF, MAJ, LWP, TMS, AKB, KB, X-DW, and EJJ, no conflicts of interest.
Supplemental Figures 1–4 and Supplemental Tables 1 and 2 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn.
Address correspondence to EJJ (e-mail: Elizabeth.Johnson@tufts.edu)
Abbreviations used: BDS, Behavioral Dyscontrol Scale; BNT, Boston Naming Test; CERAD, Consortium to Establish a Registry for Alzheimer's Disease; COWAT, Controlled Oral Word Association Test; CP, Constructional Praxis; DAFS, Direct Assessment of Functional Status; FC, frontal cortex; FDR, false discovery rate; FOME, Fuld Object Memory Evaluation; GCS, Georgia Centenarian Study; GDS, Global Deterioration Scale; GDSSF, Geriatric Depression Scale–Short Form; LOD, limit of detection; MK, menaquinone; MMSE, Mini-Mental State Examination; PK, phylloquinone; SIB, Severe Impairment Battery; TC, temporal cortex; VF, Verbal Fluency; VK, vitamin K; VKDP, vitamin K-dependent protein; WAIS, Wechsler Adult Intelligence Scale; WLMT, Word List Memory Test.

GDS 3 represented mild cognitive impairment. Scores of 4–7 on the GDS represented increasing severity of dementia. Cognitive test scores except GDSSF and the CERAD battery were available for all subjects ($n = 41$ for GDSSF, $n = 36$ for VF, $n = 33$ for BNT, $n = 32$ for CP, and $n = 27$ for WLMT).

Assessment of cerebral atrophy

Assessment of cerebral atrophy was performed at the University of Kentucky Alzheimer's Disease Center as previously described (28). Cerebral atrophy is a measure of neuronal loss, ranked as absent, mild, moderate, or severe.

Demographics and health history

Variables included age at death, sex, race, living condition, BMI (in kg/m^2), education, history of smoking and alcohol use, dietary supplement use, number and type of medications, the presence of hypertension and diabetes at death, total serum cholesterol, and *ApoE* genotype.

Statistical methods

Data are presented as means ± SDs or frequency (%). Differences between VK concentrations in the FC and TC were evaluated using paired Student's t test. Evaluation of the association between serum/plasma PK or cerebral MK-4 concentrations with covariates was performed using Wilcoxon's rank sum test, Kruskal–Wallis test, or Spearman's correlation. Pearson's correlation was performed to assess the relation between MK-4 concentrations in 2 brain regions. Spearman's correlation analysis was performed to assess the relation between serum/plasma and brain VK concentrations. Additional adjustment for covariates (sex, race, GDS, BMI, hypertension, diabetes, and time since last meal) was also performed. Spearman's partial correlation analysis was also performed to assess the relation between serum/plasma PK concentrations and cognitive measures, as well as between FC MK-4 concentrations and cognitive measures with an adjustment for covariates (sex, BMI, education, time since last meal, and cerebral atrophy). Statistical significance level was set at $\alpha = 0.05$. Additional adjustment of significance level was performed for 13 comparisons with different cognitive tests using Benjamini and Hochberg's (29) false discovery rate (FDR). FDR-adjusted P values (q values) are also reported. FDR was set at 5%, which allows 5% of significant findings to be false positives. Subgroup analysis in nondemented (GDS 1–3, $n = 23$) and demented subjects (GDS 4–7, $n = 25$) was performed to investigate any impact of nutrition on cognition before or during earlier stages of dementia (30). Because antithrombotics interfere with VK metabolism, subgroup analysis was also carried out among those who did not use antithrombotics. All statistical computations were performed using R version 3.3.3 software.

Results

Subject characteristics

Subject characteristics are shown in Table 1. Briefly, subject age ranged from 98 to 107 y, with an average age of 102.2 y at death. Most were women, Caucasian, and had normal BMI (18–25 kg/m^2). Approximately half of the cohort did not finish high school, and 52% were diagnosed with dementia (GDS 4–7) at the time point closest to death. On average, each subject was on 7.8 medications. Antithrombotics and antibiotics were used among 23% and 15% of the cohort, respectively. Seventy-three percent took at least 1 form of dietary supplement. Among subjects whose data were available, most never smoked or consumed alcohol and had an average total serum cholesterol concentration of 167 mg/dL. Only 4 subjects (8%) had diabetes, whereas 25 subjects (52%) had hypertension at death. The *ApoE* ε2 allele was present in 8 subjects (17%), and the ε4

TABLE 1 Characteristics of subjects from the Georgia Centenarian Study[1]

Characteristics	Values
Age, y	102.2 ± 2.5
Sex	
Male	5 (10)
Female	43 (90)
Race	
Caucasian	43 (90)
African American	5 (10)
Living	
Community dwelling	15 (31)
Institutionalized	33 (69)
BMI,[2] kg/m^2	22.0 ± 3.9
Education	
<high school	23 (50)
High school	12 (26)
>high school	11 (24)
No data	2
GDS	
1 (no cognitive decline)	5 (10)
2 (age-associated memory impairment)	7 (15)
3 (mild cognitive impairment)	11 (23)
4 (mild dementia)	6 (13)
5 (moderate dementia)	9 (19)
6 (moderately severe dementia)	5 (10)
7 (severe dementia)	5 (10)
Smoking	
Never	31 (86)
Past	4 (11)
Present	1 (3)
No data	12
Alcohol use	
Never	22 (61)
Past	6 (17)
Present	8 (22)
No data	12
Dietary supplement use	
Yes	35 (73)
No	13 (27)
No. of medications	7.8 ± 3.8
Antithrombotic use	11 (23)
Antibiotic use	7 (15)
Hypertension	
Yes	25 (52)
No	23 (48)
Diabetes	
Yes	4 (8)
No	44 (92)
Serum/plasma total cholesterol,[3] mg/dL	167 ± 31
ApoE genotype	
ε3/ε3	32 (68)
ε2/ε3	7 (15)
ε3/ε4	7 (15)
ε2/ε4	1 (2)
No data	1
Cerebral atrophy[4]	
Absent	15 (33)
Mild	25 (56)
Moderate	3 (7)
Severe	2 (4)

(*Continued*)

TABLE 1 (*Continued*)

Characteristics	Values
No data	3
Time interval between meal and blood draw,[5] h	2.52 ± 0.64

[1]Values are means ± SDs for continuous variables or frequency (%) for categorical variables; $n = 48$. GDS, Global Deterioration Scale.
[2]Data on BMI excluded 1 subject who was a double amputee.
[3]Data on serum/plasma total cholesterol concentration were available for 34 subjects.
[4]Assessment of cerebral atrophy has been previously described by Neltner et al. (28).
[5]Applicable to those providing serum/plasma samples ($n = 27$).

allele was in 8 subjects (17%). No subjects were homozygous carriers of $\varepsilon 2$ or $\varepsilon 4$ alleles. Subject characteristics for those with serum/plasma VK data ($n = 27$) were not significantly different from those without data ($n = 21$), although all 5 male decedents provided serum/plasma samples (**Supplemental Table 1**).

VK distribution in FC and TC

MK-4 was the most predominant vitamer in all subjects in both FC and TC. Only MK-4 and PK were detected and quantified with our analysis. Whereas MK-4 was detected in all samples, PK was not detected in 39% of FC samples and 55% of TC samples. The VK extraction recovery rate was >70% for all except 1 TC sample (59%). One individual also had a very high MK-4 concentration in both FC (127.66 pmol/g) and TC (139.05 pmol/g). Therefore, these 3 samples from 2 individuals were excluded from subsequent analyses.

MK-4 concentrations were 4.92 ± 2.31 pmol/g in FC and 4.60 ± 2.11 pmol/g in TC (**Figure 1**A). MK-4 concentrations from matched FC and TC samples do not statistically differ. There was a strong correlation between FC and TC MK-4 concentrations (**Supplemental Figure 3**; $r = 0.89$, $P < 0.001$). Among samples in which PK was greater than the LOD, PK concentrations were 0.64 ± 0.30 pmol/g in FC and 0.58 ± 0.35 pmol/g in TC, and they accounted for 10.85% ± 5.09% of VK vitamers in FC and 10.29% ± 4.43% in TC. Samples with PK concentrations less than the LOD also had significantly lower MK-4 concentrations in FC (3.85 ± 2.14 pmol/g compared with 5.64 ± 2.17 pmol/g, Student's t test, $P = 0.009$) and a trend in TC (3.98 ± 1.60 pmol/g compared with 5.46 ± 2.47 pmol/g, Student's t test, $P = 0.07$) than those with PK greater than the LOD. Among samples with PK concentration greater than the LOD, there was no significant correlation between PK and MK-4 concentrations in either FC ($n = 27$) or TC ($n = 13$).

FC and TC MK-4 concentrations did not statistically differ by sex, BMI, education, diabetes, hypertension, the presence of *ApoE* allele $\varepsilon 2$ or $\varepsilon 4$, or cerebral atrophy. MK-4 concentrations were lower among individuals using antithrombotic medications in both FC (5.32 ± 2.36 and 3.51 ± 1.49 pmol/g for nonusers compared with users, respectively; $P = 0.02$) and TC (5.14 ± 1.98 and 2.37 ± 0.61 pmol/g for nonusers compared with users, respectively; $P < 0.001$). African Americans had significantly lower MK-4 concentrations (2.27 ± 0.23 pmol/g) compared with Caucasians (4.85 ± 2.07 pmol/g) in TC (Wilcoxon's rank sum test, $P = 0.02$) but not in FC, although 2 out of 4 African-American subjects who provided TC samples also took antithrombotic medications. Both FC and TC MK-4 concentrations were not statistically different between antibiotic users and nonusers.

Correlations between circulating and cerebral VK concentrations

Compared with MK-4 being the primary form of VK in the brain, PK was the only vitamer detected in the circulation with an average of 1.35 ± 0.82 nmol/L, and it ranged from 0.21 to 3.00 nmol/L among 23 subjects whose circulating PK concentration was greater than the LOD (85% of 27 samples). Subjects whose circulating PK concentration was less than the LOD were imputed with 0 for subsequent analyses, and its distribution is shown in Figure 1B. Serum/plasma PK concentrations were not associated with sex, race, BMI, diabetes, hypertension, *ApoE* genotype, and antithrombotic and

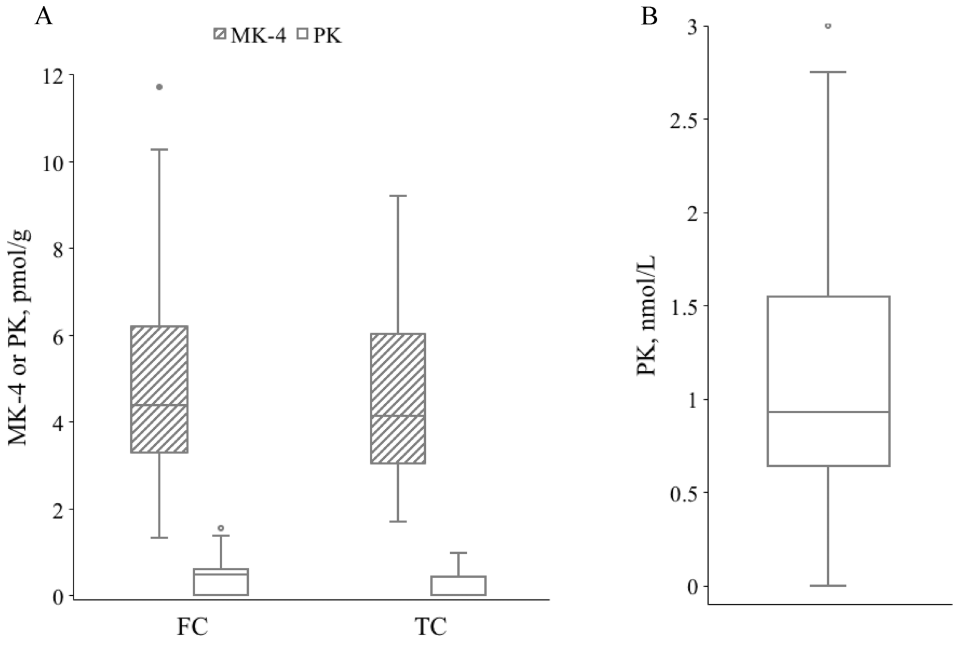

FIGURE 1 PK and MK-4 distribution in (A) FC ($n = 45$) and TC ($n = 31$) and (B) serum/plasma ($n = 27$). Brain and serum samples with PK concentrations below the limit of detection (0.30 pmol/g for brain PK and MK-4 and 0.02 nmol/L for serum PK) were imputed with 0. FC, frontal cortex; MK-4, menaquinone-4; PK, phylloquinone; TC, temporal cortex.

antibiotic use. Subjects who did not finish high school had significantly lower serum/plasma PK concentrations compared with those of subjects who had more than a high school education (0.74 ± 0.60 nmol/L compared with 1.84 ± 0.96 nmol/L, respectively; Wilcoxon's rank sum test, $P = 0.02$), and the difference remained significant after adjustment for pairwise comparisons at 5% FDR.

A correlation between serum/plasma PK and FC MK-4 or total VK concentrations was not observed ($n = 24$; **Supplemental Figure 4**), nor between serum/plasma PK and TC MK-4 or total VK concentrations ($n = 19$), either before or after adjusting for covariates (sex, race, GDS, BMI, hypertension, diabetes, time since last meal). A subgroup analysis by antithrombotic use or dementia status did not significantly change any unobserved associations.

Circulating PK and cerebral MK-4 and PK concentrations and premortem cognitive measures

Concentrations of serum/plasma PK from 27 subjects who provided samples were significantly associated with better performance on MMSE, SIB, COWAT, DAFS, and VF while controlling for sex, BMI, education, and time since last meal ($P < 0.05$ for all; **Supplemental Table 2**). After adjustment for multiple comparisons (FDR adjustment), there was a trend for DAFS ($q = 0.073$) at 5% FDR. Serum/plasma PK concentrations were not significantly different between demented and nondemented subjects. A subgroup analysis among nondemented subjects (GDS 1–3) demonstrated that those with GDS 1 had significantly higher serum/plasma PK concentrations (2.13 ± 0.83 nmol/L) compared with those with GDS 3 (0.80 ± 0.51 nmol/L) (Wilcoxon's rank sum test, $P = 0.048$). However, the difference became nonsignificant after FDR adjustment. Among 14 nondemented subjects (GDS 1–3) whose serum or plasma samples were available, concentrations of serum/plasma PK were significantly associated with better performance on MMSE, FOME Recall, COWAT, DAFS, VF, and CP and lower levels of depression on the GDSSF ($P < 0.05$ for all; **Table 2**). After FDR adjustment, COWAT, DAFS, CP, and GDSSF remained statistically significant ($q < 0.05$), whereas a trend was observed for MMSE ($q = 0.062$), FOME Recall ($q = 0.065$), and VF ($q = 0.062$). Among nondemented centenarians, serum/plasma PK concentrations were not correlated with SIB, FOME Retention, WAIS-III Similarities, BDS, BNT, and WLMT. Among demented centenarians (GDS 4–7), serum/plasma PK concentrations were positively associated with MMSE and SIB, but none remained statistically significant after FDR adjustment (data not shown). Therefore, observed associations between serum PK concentrations and cognitive performance in all subjects were primarily driven by nondemented subjects.

No significant difference was observed for FC and TC MK-4 concentrations between subjects with and without dementia or among different GDS scores. FC MK-4 concentrations were not correlated with any test scores in all subjects combined with an adjustment for sex, BMI, and education (Supplemental Table 2). After the exclusion of antithrombotic users, associations between FC MK-4 and VF and CP were observed but not after FDR adjustment. Additional adjustment for cerebral atrophy also did not change correlations either before or after the exclusion of antithrombotic users (data not shown). Among nondemented centenarians (GDS 1–3, $n = 23$), FC MK-4 concentrations were also not related to GDS or any other tests except GDSSF (Table 2), which became nonsignificant after FDR adjustment. A subgroup analysis among nonusers of antithrombotic medications and/or additional adjustment for cerebral atrophy did not change these results. In demented subjects (GDS 4–7), FC MK-4 concentrations were positively associated with MMSE, COWAT, BDS, and DAFS, but not after FDR adjustment (data not shown).

The relation between brain PK concentrations and cognitive status was also explored. Subjects were divided into 2 groups based on their PK concentrations in FC and TC: 1 group with PK detected in both FC and TC ($n = 17$) and the other group with PK <LOD in either FC or TC ($n = 28$). Cognitive performance between the 2 groups was not significantly different (Wilcoxon's rank sum test, $P > 0.05$ for all cognitive tests). A subgroup analysis in nondemented older adults found that those with PK <LOD in FC or TC ($n = 15$) had significantly lower scores on MMSE ($P = 0.046$), SIB ($P = 0.046$), COWAT ($P = 0.034$), WAIS-III Similarities ($P = 0.017$), and DAFS ($P = 0.032$) compared with those with PK detected in both FC and TC ($n = 6$) (Wilcoxon's rank sum test). However, all associations became nonsignificant after adjustment for multiple comparisons ($q > 0.10$ for all tests).

Discussion

This is the first report on VK distribution in brain tissues from FC and TC from human subjects with a wide range of cognitive status—from intact cognition to severe dementia. MK-4 was the most predominant vitamer in all brain tissues analyzed in both demented and nondemented subjects. Importantly, cerebral MK-4 concentrations were significantly lower among antithrombotic users. Circulating PK concentrations were not associated with cerebral MK-4 or total VK concentrations, regardless of antithrombotic use or dementia status. We also demonstrated that circulating PK concentrations were positively related to a wide range of cognitive tests among nondemented older adults. On the other hand, cerebral MK-4 concentrations were not related to any tests in nondemented subjects except GDSSF, an assessment of depression, which became nonsignificant after an adjustment for multiple comparisons.

Previously, VK distribution had been described in rodent brain (31–33). It was observed that MK-4 represented ~98% of total cerebral VK in adult rats, and the concentrations varied by age and sex (34, 35). Similarly, MK-4 accounted for 86% of total VK in brain tissues acquired from 3 middle-age individuals (aged 30, 50, and 54 y) (5). In the current study, we also observed that MK-4 is the most predominant form of VK, accounting for ≥89.2% and ≥89.7% of total VK vitamers in FC and TC, respectively. Except for PK and MK-4, other forms of VK were not detected in any brain tissues in both human studies (5).

Serum/plasma PK concentrations were not associated with cerebral MK-4 or total VK concentrations among antithrombotic users and nonusers and also among subjects with and without dementia. To date, the molecular mechanism of VK transport across the blood–brain barrier has not been demonstrated, but its presence in the brain suggests that PK can reach this organ. Our findings suggest a tight regulation of MK-4 concentrations in the central nervous system. It has been proposed that human brain obtains PK or menadione from the circulation and converts them to MK-4 by the enzyme UBIAD1 (6–8, 10). Serum PK concentrations have been widely used as a biomarker of VK status, and its concentrations in this population were comparable to those in other populations,

TABLE 2 Spearman's partial correlation, *P* value, and false discovery rate between serum/plasma PK concentrations and cognitive measures and between FC MK-4 concentrations and cognitive measures among nondemented centenarians (Global Deterioration Scale 1–3) adjusted for covariates[1]

	Serum/plasma PK				FC MK-4				FC MK-4 antithrombotic nonusers			
Cognitive test	ρ	*P*	*q*	*n*	ρ	*P*	*q*	*n*	ρ	*P*	*q*	*n*
MMSE	0.60	0.025	0.062	14	0.07	0.767	0.967	21	0.16	0.534	0.806	17
SIB	0.42	0.141	0.178	14	−0.01	0.967	0.967	21	0.05	0.854	0.925	17
FOME Recall	0.57	0.035	0.065	14	0.16	0.495	0.967	21	0.32	0.212	0.689	17
FOME Retention	0.41	0.151	0.178	14	0.11	0.625	0.967	21	0.23	0.383	0.806	17
COWAT	0.74	0.004	0.047	14	0.28	0.217	0.967	21	0.46	0.068	0.442	17
WAIS-III Similarities	0.39	0.170	0.184	14	−0.20	0.389	0.967	21	−0.06	0.824	0.925	17
BDS	0.47	0.094	0.152	14	0.08	0.737	0.967	21	0.10	0.701	0.911	17
GDSSF	−0.65	0.015	0.047	14	−0.48	0.028	0.364	21	−0.59	0.014	0.182	17
DAFS	0.68	0.009	0.047	14	0.05	0.824	0.967	21	0.16	0.547	0.806	17
CERAD												
VF	0.62	0.029	0.062	13	0.02	0.945	0.967	19	0.25	0.375	0.806	15
BNT	0.47	0.128	0.178	12	−0.19	0.467	0.967	17	−0.02	0.964	0.964	13
CP	0.68	0.014	0.047	13	0.11	0.674	0.967	18	0.38	0.186	0.689	14
WLMT	−0.44	0.204	0.204	10	−0.08	0.803	0.967	15	0.20	0.558	0.806	11

[1]Covariates for serum/plasma PK include sex, BMI, education, and time since last meal. Covariates for FC MK-4 include sex, BMI, and education. BDS, Behavioral Dyscontrol Scale; BNT, Boston Naming Test; CERAD, Consortium to Establish a Registry for Alzheimer's Disease; COWAT, Controlled Oral Word Association Test; CP, constructional praxis; DAFS, Direct Assessment of Functional Status; FC, frontal cortex; FOME, Fuld Object Memory Evaluation; GDSSF, Geriatric Depression Scale–Short Form; MK-4, menaquinone-4; MMSE, Mini-Mental State Examination; PK, phylloquinone; *q*, false discovery rate; SIB, Severe Impairment Battery; VF, Verbal Fluency; WAIS-III, Wechsler Adult Intelligence Scale–Third Edition; WLMT, Word List Memory Test; ρ, Spearman's partial correlation.

as previously reported (2, 36). Similar to γ-tocopherol (a form of vitamin E), the major portion (>50%) of PK is transported on triglyceride-rich lipoprotein particles, with each of the LDL and HDL fractions carrying ∼15% of PK in the circulation (37, 38). The scavenger receptor class B type I on the blood–brain barrier facilitates the uptake of HDL-associated vitamin E in porcine brain capillary endothelial cells, but whether the VK uptake mechanism is the same for vitamin E remains to be investigated (39). Moreover, postprandial triglyceride concentrations are influenced by meal composition and may confound an association between circulating and brain VK concentrations (36). A major limitation in our study was an absence of triglyceride measure with the use of nonfasting serum/plasma samples. However, we have previously shown that serum levels of γ-tocopherol were reflective of its brain concentrations in this population (40). Therefore, an absence of the association between circulating and cerebral VK concentrations was unlikely to be confounded by triglyceride concentrations.

Other variables have been reported to explain the variation in brain and circulating VK. Although only 10% of this cohort were men, brain MK-4 concentrations did not differ from those of women, as previously observed in rats (33, 35). Similarly, carriers of different *ApoE* alleles did not have statistically different cerebral or circulating VK concentrations as previously reported (41), although no subject was a homogyzous carrier of *ApoE* ε2 or ε4 in our study. We also observed that cerebral MK-4 concentrations were significantly lower among antithrombotic users. Anticoagulants are a subgroup of antithrombotics that inhibit VK's activity (42). When rats were administered warfarin, a VK antagonist and an anticoagulant, cerebral MK-4 concentrations decreased (31). Warfarin lowers brain MK-4 by inhibiting the recycling of MK-4 back from MK-4 epoxide, but it remains unclear whether it also inhibits the MK-4 biosynthetic activity of UBIAD1 (9, 43). Warfarin administration also increases microbleeds and the risk of intracerebral hemorrhage by 2- to 5-fold (44, 45). Altogether, these findings suggested that antithrombotics, specifically anticoagulants, interfere with VK metabolism in the brain to reduce MK-4 concentrations. However, types and doses of antithrombotics were not recorded in the GCS, thus limiting the interpretation of these results. In addition to antithrombotics, statins (a class of cholesterol-lowering medications) have been reported to affect VK metabolism through inhibition of UBIAD1's activity (11). However, none of 48 subjects in this cohort used statins.

The presence of VKDPs in the brain lends support to the relation between VK status and cognitive heath (13, 14, 46). In addition, VK has been implicated in sphingolipid metabolism and neuroprotection (34, 43, 46), and a lower concentration of brain MK-4 was associated with worse cognitive functioning in old rats fed a low VK diet (23). In our study, however, there was no consistent relation between cerebral MK-4 concentrations and performance on cognitive tests in subjects with or without dementia, even after controlling for sex, BMI, education, and cerebral atrophy. Findings from our study do not challenge VK's proposed roles in brain function. It may be a matter that once needs are met, more is not necessarily better. As previously reported, the majority of the GCS centenarians consumed green leafy vegetables ≥1 time per day (47), which likely exceeds the current recommendation of VK intake of 120 and 90 μg/d in both men and women ≥70 y (48).

This begs the question: Why were circulating VK concentrations consistently related to better cognitive performance while brain MK-4 was not? Consistent with our findings, higher plasma PK concentrations were related to a better performance on cognitive assessments related to consolidation processes of the memory trace in nondemented older adults from the NuAge study (19). Circulating PK concentrations were comparable in these studies (1.35 ± 0.82 nmol/L in GCS and 1.45 ± 1.80 nmol/L in NuAge). Dietary VK concentrations were also reported to be related to cognitive performance

among different older adult populations (15–18). This is the first study, however, that has evaluated the relation between brain concentrations of MK-4 and premortem cognitive function in humans. Apart from its proposed functions in the brain, VK is also essential for overall vascular health (49, 50). Because vascular diseases are major risk factors of cognitive decline (51), this may explain the observed relation between VK status (as measured by serum/plasma) and cognitive performance but not brain VK concentrations. Moreover, it is possible that circulating VK was a biomarker of habitual intake of vegetable oils and green leafy vegetables. Compared to animal fats, vegetable oils are relatively low in saturated fats, and their intake has been associated with higher risk of dementia (52, 53). Green leafy vegetables are significant sources of both PK and lutein, a carotenoid that is selectively taken up into neural tissue and associated with cognitive performance across the life span (54). In this group of centenarians, circulating PK and lutein concentrations were highly correlated ($r = 0.54$, $P = 0.004$), and serum lutein reflected brain lutein concentrations (40), which were also related to better cognitive performance (26). Hence, it is possible that the relation observed between serum/plasma PK concentrations and cognition is explained by the roles of other dietary components in VK-rich foods on cognitive function.

Despite strengths in the availability of matched brain and serum or plasma samples, the limited number of subjects and relatively liberal FDR adjustment require additional supportive evidence to confirm study findings. Homogeneity of subjects (advanced age, female, Caucasian, and low prevalence of diabetes) and longevity-associated characteristics (i.e., survivorship bias) also rendered the difficulty of extrapolating our findings to other populations (55). However, centenarians are the fastest-growing demographic group in many nations, and findings from this age group will become more relevant to the general population (56). Only nonfasting blood samples were available for VK analysis, and recent intakes might influence circulating PK concentrations from nonfasting samples, but they were likely to reflect habitual VK intake among very old populations as previously discussed (40). Cross-sectional study also did not imply causality and temporality. There was a possibility of reverse causation in which cognitive impairment could affect vitamin K uptake and metabolism. Plasma PK concentrations may simply be a biomarker of overall healthy lifestyle, such as an adherence to healthy dietary pattern and a regular physical activity (57, 58), both of which are strongly related to better cognitive performance and lower risk of dementia (59, 60). However, a causal relation between VK insufficiency and cognitive impairment demonstrated in animal studies supports a need for VK adequacy for cognitive health (23, 43).

In summary, MK-4 was the most predominant form of VK in human brain regardless of cognitive status. Serum/plasma PK concentrations did not reflect brain MK-4 or total VK concentrations, and brain MK-4 concentrations were not associated with premortem cognitive performance. Consistent with previous findings in other populations, we have characterized the cross-sectional relation between higher circulating PK concentrations and better cognitive performance among nondemented older adults but not among those with dementia. Circulating PK concentrations were likely to reflect habitual consumption of VK-rich foods that also contain other dietary components beneficial for cognitive health. Given its emerging roles in cognitive health, our findings warrant the urgency to investigate the uptake and metabolism of VK in the human brain, as well as the consideration of a dietary requirement and biomarkers of VK status that reflect the amount optimized for maintaining cognitive health during advanced aging.

Acknowledgments

The authors' responsibilities were as follows—JT, GF, MAJ, LWP, and EJJ: designed the study; JT, GF, MAJ, LWP, TMS, and EJJ: interpreted the data; MAJ and LWP: collected data and biological samples from the GCS; GF: analyzed VK concentrations in all samples; JT: performed statistical analysis, wrote the paper, and had primary responsibility for final content; and all authors: read and approved the final manuscript.

References

1. Booth SL. Vitamin K: food composition and dietary intakes. Food Nutr Res 2012;56(1):5505.
2. Fusaro M, Gallieni M, Rizzo MA, Stucchi A, Delanaye P, Cavalier E, Moysés RMA, Jorgetti V, Iervasi G, Giannini S, et al. Vitamin K plasma levels determination in human health. Clin Chem Lab Med 2017;55(6):789–99.
3. Booth SL, Suttie JW. Dietary intake and adequacy of vitamin K. J Nutr 1998;128(5):785–8.
4. Sakano T, Nagaoka T, Morimoto A, Hirauchi K. Measurement of K vitamins in human and animal feces by high-performance liquid chromatography with fluorometric detection. Chem Pharm Bull (Tokyo) 1986;34(10):4322–6.
5. Thijssen HHW, Drittij-Reijnders MJ. Vitamin K status in human tissues: tissue-specific accumulation of phylloquinone and menaquinone-4. Br J Nutr 1996;75(1):121–7.
6. Davidson RT, Foley AL, Engelke JA, Suttie JW. Conversion of dietary phylloquinone to tissue menaquinone-4 in rats is not dependent on gut bacteria. J Nutr 1998;128(2):220–3.
7. Thijssen HHW, Vervoort LMT, Schurgers LJ, Shearer MJ. Menadione is a metabolite of oral vitamin K. Br J Nutr 2006;95(2):260–6.
8. Hirota Y, Tsugawa N, Nakagawa K, Suhara Y, Tanaka K, Uchino Y, Takeuchi A, Sawada N, Kamao M, Wada A, et al. Menadione (vitamin K3) is a catabolic product of oral phylloquinone (vitamin K1) in the intestine and a circulating precursor of tissue menaquinone-4 (vitamin K2) in rats. J Biol Chem 2013;288(46):33071–80.
9. Nakagawa K, Hirota Y, Sawada N, Yuge N, Watanabe M, Uchino Y, Okuda N, Shimomura Y, Suhara Y, Okano T. Identification of UBIAD1 as a novel human menaquinone-4 biosynthetic enzyme. Nature 2010;468(7320):117–21.
10. Okano T, Shimomura Y, Yamane M, Suhara Y, Kamao M, Sugiura M, Nakagawa K. Conversion of phylloquinone (vitamin K1) into menaquinone-4 (vitamin K2) in mice: two possible routes for menaquinone-4 accumulation in cerebra of mice. J Biol Chem 2008;283(17):11270–9.
11. Hirota Y, Nakagawa K, Sawada N, Okuda N, Suhara Y, Uchino Y, Kimoto T, Funahashi N, Kamao M, Tsugawa N, et al. Functional characterization of the vitamin K2 biosynthetic enzyme UBIAD1. PLoS One 2015;10(4):e0125737.
12. Danziger J. Vitamin K-dependent proteins, warfarin, and vascular calcification. Clin J Am Soc Nephrol 2008;3(5):1504–10.
13. Alisi L, Cao R, De Angelis C, Cafolla A, Caramia F, Cartocci G, Librando A, Fiorelli M. The relationships between vitamin K and cognition: a review of current evidence. Front Neurol 2019;10:239.
14. Ferland G. Vitamin K and the nervous system: an overview of its actions. Adv Nutr Int Rev J 2012;3(2):204–12.
15. Chouet J, Ferland G, Féart C, Rolland Y, Presse N, Boucher K, Barberger-Gateau P, Beauchet O, Annweiler C. Dietary vitamin K intake is associated with cognition and behaviour among geriatric patients: the CLIP study. Nutrients 2015;7(8):6739–50.
16. Soutif-Veillon A, Ferland G, Rolland Y, Presse N, Boucher K, Féart C, Annweiler C. Increased dietary vitamin K intake is associated with less severe subjective memory complaint among older adults. Maturitas 2016;93:131–6.

17. Presse N, Shatenstein B, Kergoat M-J, Ferland G. Low vitamin K intakes in community-dwelling elders at an early stage of Alzheimer's disease. J Am Diet Assoc 2008;108(12):2095–9.
18. Morris MC, Wang Y, Barnes LL, Bennett DA, Dawson-Hughes B, Booth SL. Nutrients and bioactives in green leafy vegetables and cognitive decline: prospective study. Neurology 2018;90(3): e214–22.
19. Presse N, Belleville S, Gaudreau P, Greenwood CE, Kergoat M-J, Morais JA, Payette H, Shatenstein B, Ferland G. Vitamin K status and cognitive function in healthy older adults. Neurobiol Aging 2013;34(12):2777–83.
20. Poon LW, Clayton G, Martin P, Johnson MA, Courtenay B, Sweaney A, Merriam SB, Pless BS, Thielman SB. The Georgia Centenarian Study. Int J Aging Hum Dev 1992;34(1):1–17.
21. Poon LW, Jazwinski M, Green RC, Woodard JL, Martin P, Rodgers WL, Johnson MA, Hausman D, Arnold J, Davey A, et al. Methodological considerations in studying centenarians: lessons learned from the Georgia Centenarian Studies. Annu Rev Gerontol Geriatr 2007;27(1):231.
22. Shaw K, Gearing M, Davey A, Burgess M, Poon LW, Martin P, Green RC. Successful recruitment of centenarians for post-mortem brain donation: results from the Georgia Centenarian Study. J Biosci Med 2012;2(1):124.
23. Carrie I, Belanger E, Portoukalian J, Rochford J, Ferland G. Lifelong low-phylloquinone intake is associated with cognitive impairments in old rats. J Nutr 2011;141(8):1495–501.
24. Davidson KW, Sadowski JA. Determination of vitamin K compounds in plasma or serum by high-performance liquid chromatography using postcolumn chemical reduction and fluorimetric detection. In: McCormick DB, Suttie JW, Wagner C , editors. Methods in enzymology. Cambridge (MA): Academic Press; 1997. p. 408–21. (Vitamins and coenzymes Part L, vol. 282).
25. Wang LY, Bates CJ, Yan L, Harrington DJ, Shearer MJ, Prentice A. Determination of phylloquinone (vitamin K1) in plasma and serum by HPLC with fluorescence detection. Clin Chim Acta 2004;347(1):199–207.
26. Johnson EJ, Vishwanathan R, Johnson MA, Hausman DB, Davey A, Scott TM, Green RC, Miller SL, Gearing M, Woodard J, et al. Relationship between serum and brain carotenoids, α-tocopherol, and retinol concentrations and cognitive performance in the oldest old from the Georgia Centenarian Study. J Aging Res 2013;2013: 1–13.
27. Reisberg B, Ferris S, de Leon M, Crook T. The Global Deterioration Scale for assessment of primary degenerative dementia. Am J Psychiatry 1982;139(9):1136–9.
28. Neltner JH, Abner EL, Jicha GA, Schmitt FA, Patel E, Poon LW, Marla G, Green RC, Davey A, Johnson MA, et al. Brain pathologies in extreme old age. Neurobiol Aging 2016;37:1–11.
29. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Statist Soc B 1995;57(1):289–300.
30. Vauzour D, Camprubi-Robles M, Miquel-Kergoat S, Andres-Lacueva C, Bánáti D, Barberger-Gateau P, Bowman GL, Caberlotto L, Clarke R, Hogervorst E, et al. Nutrition for the ageing brain: towards evidence for an optimal diet. Ageing Res Rev 2017;35:222–40.
31. Thijssen H, Drhtu-Reijnders M, Fischer M. Phylloquinone and menaquinone-4 distribution in rats: synthesis rather than uptake determines menaquinone-4 organ concentrations. J Nutr 1996;126(2):537–43.
32. Thijssen HH, Drittij-Reijnders MJ. Vitamin K distribution in rat tissues: dietary phylloquinone is a source of tissue menaquinone-4. Br J Nutr 1994;72(3):415–25.
33. Huber AM, Davidson KW, O'Brien-Morse ME, Sadowski JA. Tissue phylloquinone and menaquinones in rats are affected by age and gender. J Nutr 1999;129(5):1039–44.
34. Carrié I, Portoukalian J, Vicaretti R, Rochford J, Potvin S, Ferland G. Menaquinone-4 concentration is correlated with sphingolipid concentrations in rat brain. J Nutr 2004;134(1):167–72.
35. Ferland G, Doucet I, Mainville D, Ferland G, Doucet I, Mainville D. Phylloquinone and menaquinone-4 tissue distribution at different life stages in male and female Sprague–Dawley rats fed different VK levels since weaning or subjected to a 40% calorie restriction since adulthood. Nutrients 2016;8(3):141.
36. Shea M, Booth S. Concepts and controversies in evaluating vitamin K status in population-based studies. Nutrients 2016;8(1):8.
37. Lamon-Fava S, Sadowski JA, Davidson KW, O'Brien ME, McNamara JR, Schaefer EJ. Plasma lipoproteins as carriers of phylloquinone (vitamin K1) in humans. Am J Clin Nutr 1998;67(6): 1226–31.
38. Jiang Q, Christen S, Shigenaga MK, Ames BN. γ-Tocopherol, the major form of vitamin E in the US diet deserves more attention. Am J Clin Nutr 2001;74:714–22.
39. Goti D, Hrzenjak A, Levak-Frank S, Frank S, van der Westhuyzen DR, Malle E, Sattler W. Scavenger receptor class B, type I is expressed in porcine brain capillary endothelial cells and contributes to selective uptake of HDL-associated vitamin E. J Neurochem 2001;76(2):498–508.
40. Tanprasertsuk J, Mohn ES, Matthan NR, Lichtenstein AH, Barger K, Vishwanathan R, Johnson MA, Poon LW, Johnson EJ. Serum carotenoids, tocopherols, total n–3 polyunsaturated fatty acids and n–6/n–3 polyunsaturated fatty acid ratio reflect brain concentrations in a cohort of centenarians. J Gerontol Ser A 2019;74(3):306–14.
41. Yan L, Zhou B, Nigdikar S, Wang X, Bennett J, Prentice A. Effect of apolipoprotein E genotype on vitamin K status in healthy older adults from China and the UK. Br J Nutr 2005;94(6):956–61.
42. Ansell J, Hirsh J, Hylek E, Jacobson A, Crowther M, Palareti G. Pharmacology and management of the vitamin K antagonists: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th edition). Chest 2008;133(6 Suppl):160S–98S.
43. Tamadon-Nejad S, Ouliass B, Rochford J, Ferland G. Vitamin K deficiency induced by warfarin is associated with cognitive and behavioral perturbations, and alterations in brain sphingolipids in rats. Front Aging Neurosci 2018;10:213.
44. Lee G-H, Kwon SU, Kang D-W. Warfarin-induced intracerebral hemorrhage associated with microbleeds. J Clin Neurol Seoul Korea 2008;4(3):131–3.
45. Flaherty ML, Tao H, Haverbusch M, Sekar P, Kleindorfer D, Kissela B, Khatri P, Stettler B, Adeoye O, Moomaw CJ, et al. Warfarin use leads to larger intracerebral hematomas. Neurology 2008;71(14): 1084–9.
46. Ferland G. Vitamin K and brain function. Semin Thromb Hemost 2013;39(8):849–55.
47. Johnson MA, Davey A, Hausman DB, Park S, Poon LW. Dietary differences between centenarians residing in communities and in skilled nursing facilities: the Georgia Centenarian Study. Age 2006;28(4):333–41.
48. Institute of Medicine Panel on Micronutrients. Vitamin K. Washington (DC): National Academies Press; 2001.
49. Villa JKD, Diaz MAN, Pizziolo VR, Martino HSD. Effect of vitamin K in bone metabolism and vascular calcification: a review of mechanisms of action and evidences. Crit Rev Food Sci Nutr 2017;57(18): 3959–70.
50. van den Heuvel EG, van Schoor NM, Lips P, Magdeleyns EJ, Deeg DJ, Vermeer C, den Heijer M. Circulating uncarboxylated matrix Gla protein, a marker of vitamin K status, as a risk factor of cardiovascular disease. Maturitas 2014;77(2):137–41.
51. Reitz C, Luchsinger JA, Mayeux R. Vascular disease and cognitive impairment. Expert Rev Neurother 2008;8(8):1171–4.
52. Orsavova J, Misurcova L, Vavra Ambrozova J, Vicha R, Mlcek J. Fatty acids composition of vegetable oils and its contribution to dietary energy intake and dependence of cardiovascular mortality on dietary intake of fatty acids. Int J Mol Sci 2015;16(6):12871–90.
53. Barnard ND, Bunner AE, Agarwal U. Saturated and trans fats and dementia: a systematic review. Neurobiol Aging 2014;35:S65–73.
54. Mohn ES, Johnson EJ. Lutein and cognition across the lifespan. Nutr Today 2017;52(4):183–9.
55. Wilcox DC, Willcox BJ, Poon LW. Centenarian studies: important contributors to our understanding of the aging process and longevity. Curr Gerontol Geriatr Res 2010;2010:484529.
56. Robine JM, Cubaynes S. Worldwide demography of centenarians. Mech Ageing Dev 2017;165(Pt B):59–67.
57. Braam L, McKeown N, Jacques P, Lichtenstein A, Vermeer C, Wilson P, Booth S. Dietary phylloquinone intake as a potential marker for a heart-healthy dietary pattern in the Framingham Offspring cohort. J Am Diet Assoc 2004;104(9):1410–4.

58. Erkkilä AT, Booth SL, Hu FB, Jacques PF, Lichtenstein AH. Phylloquinone intake and risk of cardiovascular diseases in men. Nutr Metab Cardiovasc Dis 2007;17(1):58–62.
59. Solfrizzi V, Custodero C, Lozupone M, Imbimbo BP, Valiani V, Agosti P, Schilardi A, D'Introno A, La Montagna M, Calvani M, et al. Relationships of dietary patterns, foods, and micro- and macronutrients with Alzheimer's disease and late-life cognitive disorders: a systematic review. J Alzheimers Dis 2017;59(3):815–49.
60. Song D, Yu DSF, Li PWC, Lei Y. The effectiveness of physical exercise on cognitive and psychological outcomes in individuals with mild cognitive impairment: a systematic review and meta-analysis. Int J Nurs Stud 2018;79:155–64.