Lifelong Low-Phylloquinone Intake Is Associated with Cognitive Impairments in Old Rats1–3

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Abstract

In a previous report, we showed vitamin K to preferentially accumulate in brain regions rich in white matter and to positively correlate with certain sphingolipids. In rodents, pharmacological vitamin K deficiency has resulted in behavioral perturbations. To gain insight on the role of vitamin K status on brain function, we investigated learning abilities (Morris water maze), motor activity (open field), and anxiety (elevated plus maze) in distinct groups of 6-, 12-, and 20-mo-old female Sprague-Dawley rats that had been fed diets containing low (L; ∼80 μg/kg diet), adequate (A; ∼500 μg/kg diet), or high (H; ∼2000 μg/kg diet) levels of phylloquinone (μg/kg diet; n = 9–12/diet) since weaning. In 20-mo-old rats, sphingolipids (cerebroside, sulfatide, sphingomyelin, ceramide, and gangliosides), phylloquinone, and menaquinone-4 were also assessed in cerebellum, midbrain, pons medulla, striatum, and hippocampus. Lifetime consumption of a low-vitamin K diet resulted in cognitive deficits in the 20-mo-old rats, with those in the L group having longer latencies than those in the H group (P < 0.05); this was associated with higher concentrations of ceramides in the hippocampus (P < 0.05) and lower gangliosides in the pons medulla and midbrain (P < 0.05). The low-vitamin K diet did not affect cognition at 6 and 12 mo of age, nor did it affect motor activity or anxiety at any age. Although much remains to be elucidated about the mechanism of action of vitamin K in cognition, this report points to vitamin K as an important nutritional factor contributing to cognitive health during aging. J. Nutr. 141: 1495–1501, 2011.

Introduction

Research conducted in the past decades has clearly established a role for nutrition in cognitive health and pathology during aging (1,2). Historically discovered for its role in blood coagulation, there is now convincing evidence that vitamin K has important actions in the nervous system (3,4). As a unique cofactor to the γ-glutamyl carboxylase enzyme, vitamin K contributes to the biological activation of the protein Gas6, a ligand for the receptor tyrosine kinases of the Tyro3, Axl, and MerTK family. In brain, Gas6 has been involved in a wide range of cellular processes that include cell growth, survival, and apoptosis (5).

The first indication that vitamin K was involved in the nervous system came from reports that administration of the vitamin K antagonist warfarin to growing rats results in reductions of brain sphingolipids and perturbation of their key synthetic enzymes (6–8). Sphingolipids are a group of complex lipids present in high concentrations in brain cell membranes, the principal sphingolipids being ceramide, sphingomyelin, ganglioside, cerebroside, and sulfatide (9–11) (Supplemental Fig. 1). In addition to their important structural functions, sphingolipids are currently considered key bioactive molecules that modulate processes such as proliferation, differentiation, senescence, cell-cell interaction, and transformation (10,12).

Recently, the role of vitamin K in sphingolipid metabolism has been further investigated. Unlike most other tissues where vitamin K is present as phylloquinone and menaquinone-4 (MK-4), vitamin K in brain is predominantly in the form of MK-4 (13). Our team reported that in 6-mo-old female Sprague-Dawley rats, MK-4 accounts for >98% of total cerebral vitamin K and is present in higher concentrations in myelinated (pons medulla and midbrain) than in nonmyelinated regions. Furthermore, we observed brain tissue MK-4 to be strongly correlated to the sphingolipids, sulfatides, sphingomyelin, and gangliosides (14). Positive correlations between MK-4 and sulfatide concent-
trations in the hippocampus and cortex of Fisher 344 male rats were recently reported (15).

In humans, studies on the impact of vitamin K deficiency in brain function are limited. Fetal exposure to warfarin derivatives during the first trimester of pregnancy has long been known to result in a wide range of physical anomalies collectively known as warfarin embryopathy. This syndrome includes optic atrophy, dilatation of the cerebral ventricles, blindness, microencephaly, and mental retardation (16,17). More recently, our group published a report in which community-dwelling patients in the early stage of Alzheimer’s disease were found to have significantly lower vitamin K intakes than age- and gender-matched healthy participants (18). Regarding the role of vitamin K in cognition, data are even more limited with only 1 rat study currently available. In this study, it was reported that vitamin K deficiency induced by administration of a vitamin K-deficient diet or warfarin treatment was associated with hypoactivity and a lack of exploratory behavior in rats (19).

To gain additional insight on the role of vitamin K status on cognition and other behavioral components, female Sprague-Dawley rats that had been fed diets containing either low (L), adequate (A), or high (H) levels of phylloquinone since weaning were investigated at different life stages.

**Materials and Methods**

**Rats and diets.** This study was approved by the Animal Care Committee of the University of Montreal in compliance with the guidelines of the Canadian Council on Animal Care. Four-week-old female Sprague-Dawley rats were obtained from Charles Rivers Canada and housed 3 cage in suspended stainless steel wire-bottom cages (to prevent coprophagy) in a room kept at 22°C with a 12-h-light/dark cycle. Rats were kept in the same housing conditions and rat facility throughout the experimental period. Rats had free access to water and food. After acclimatization with standard rodent nonpurified diet for 1 wk, rats were randomly assigned to an AIN 76-based diet (20) containing L (~80 µg/kg diet), A (~500 µg/kg diet), or H (~2000 µg/kg diet) amounts of phylloquinone (Sigma Chemicals) (n = 9–12/diet) until the time of testing. The powder diets were prepared in our laboratory and phylloquinone concentrations evaluated by HPLC analysis were: (mean ± SEM, n = 18) 83.7 ± 4.3 µg/kg diet (L), 550 ± 21.7 µg/kg diet (A), and 1944 ± 32.0 µg/kg diet (H). Food intake and body weights were recorded every 2 wk and the health of the rats was regularly monitored by a veterinarian throughout the experimental period.

**Behavioral analysis.** Distinct 6-, 12-, and 20-mo-old rats from the 3 dietary groups (n = 9–12/diet group) were subjected to the Morris water maze [cognitive abilities (21)], the open field [motor activity (22)], and the elevated plus-maze [anxiety (23)]. Rats from the different age groups underwent testing only once, that is, at the designated age of testing. Because female rats were used in the present experiment and to account for potential influence of the estrous cycle on behavior, a male rat was housed in the same housing room as the females 1 wk prior to the start and throughout the experimental period to promote cyclicity (24). Estrous synchronicity was then monitored through daily vaginal smears and inspection of cell types under a light microscope. Testing was conducted when females were in comparable stages of estrous cycles.

**Morris water maze.** The maze consisted of a large circular pool (1.6-m diameter, 45-cm depth) filled with 22°C water placed in a room with external cues (posters, light, experimenter) visible to the swimming rats. The platform (10 cm) was submerged 2 cm below the water surface and was placed in the center of 1 of the 4 quadrants of the pool. Testing was conducted daily over 5 consecutive days, each rat being given 3 trials/d. Once the rat located the platform, it was allowed to remain on it for ~30 s. If a rat did not find the platform after 120 s of swimming, it was gently put on it by the experimenter. Learning performance was based on the mean of 3 daily trials. Latencies to find the platform, swim speed, and time in each quadrant were recorded using a HVS 2020 tracker and were later analyzed using the Water 2020 software (HVS Image).

**Probe trial.** On d 6, the spatial accuracy of the rats was further assessed by removing the platform from the pool and allowing the rats to swim freely during 30 s. The pool was divided into 4 equal quadrants and the percent time spent in each quadrant was computed. Rats were allowed 2 trials.

**Cue test.** A cue test was conducted to ensure that poor performances were not due to visual deficits. In this test, rats had to find the platform that had been rendered visible by lowering the pool water level (2 cm below the top of the platform). Rats were allowed 2 trials.

**Open field.** The open field consisted of a black wooden arena measuring 60 × 50 × 50 cm with the floor divided in 25 × 10 cm squares. After 30 min of adaptation to the room, rats were placed individually into the open field and allowed to explore it freely for 5 min. Motor activity was determined based on the total path traveled and the duration of time spent in the inner squares (i.e., those at least 1 square removed from the walls). This procedure was followed once per day for 3 consecutive days. Motor activity components were recorded using a HVS 2020 tracker and were analyzed by the Field 2020's software (HVS Image).

**Elevated plus maze.** Anxiety was measured using the elevated plus maze, a test that builds on the natural aversion of rodents for open spaces and heights (23). The elevated plus maze was constructed of wood, painted gray, consisted of 4 arms (90 × 8 cm) shaped in the form of a cross, and was elevated 70 cm from the floor. Two opposite arms were enclosed by side end walls (10 cm high) and the other 2 arms were open. The connecting area measured 10 × 10 cm. Following transport to the test room and a 30-min acclimatization period, each rat was placed into the center area of the plus maze facing a closed arm. The rat was then permitted to explore the maze freely for a 5-min period. During this period, the number of open arm entries, the total number of arm entries, and the total time spent in the open arms were measured. This procedure was followed for 1 d.

**Biochemical analyses**

The following biochemical analyses were conducted in 20-mo-old rats after completion of behavioral testing.

**Vitamin K analysis.** Rats were anesthetized with pentobarbital and bled from the abdominal aorta. Whole brains were quickly removed and dissected on ice into cerebellum, pons medulla, midbrain, hippocampus, and striatum. Following dissection, tissue samples were immediately frozen in liquid nitrogen and stored at −80°C until analysis.

Phylloquinone and MK-4 were quantified (n = 4–6/diet group) by HPLC as previously described (14). Briefly, tissue samples were pulverized in anhydrous Na2SO4 and extracted with acetone containing an internal standard [2-methyl-3-(3,7,11,15,19-pentamethyl-2-ecosynyl)-1,4-naphthalenedione] (1,4-Naphthalenedione) (GL-Synthesis). Dried extracts were then reextracted with a mixture of hexane and water before being further purified by solid phase extraction on silica gel columns (JT Baker). Quantitative analysis of K1 and MK-4 was performed by reverse-phase HPLC using a C-18 reverse phase column and fluorescence detection. The calibration standard consisted of a mixture of phylloquinone, MK-4, and 2-methyl-3-(3,7,11,15,19-pentamethyl-2-ecosynyl)-1,4-naphthalenedione at 2 ng in 50 µL. The percent recovery for the samples was calculated from the internal standard and found to be 85–90%.

**Sphingolipid analysis.** The sphingolipids sulfatides, cerebrosides, sphingomyelin, ceramides, and gangliosides were assessed (n = 4–6/diet group) in the different brain regions as described in Carrié et al. (14). Briefly, lipids were extracted from the brain regions using chloroform: methanol (2:1, v:v) and partitioned according to the method of Folch et al. (25). Gangliosides were eluted according to the method of Williams and McCluer (26) and were measured by quantification of free sialic acids according to Jourdain et al. (27). Ceramides, cerebrosides, sulfatides, and sphingomyelin were loaded onto LC-NH2 columns...
(Supelco) and eluted sequentially. The sulfatide fraction was further applied to a C-18 silica column. Each fraction was evaporated and suspended in chloroform:methanol (2:1, v:v). Ceramides, cerebrosides, and sphingomyelin were quantified by determination of sphingosine with fluorescamine according to the method of Naoi et al. (28) and sulfatides with azure A according to the method of Kean (29).

**Statistical analysis.** All data were expressed as the mean ± SEM. Survival rates in the 3 diet groups were analyzed with Kaplan-Meier curves and compared by the logrank test. Body weight was assessed by repeated-measures ANOVA, with diet the main effect tested and month the repeated measure. Morris water maze and open field tests were analyzed, in each age group, by repeated-measures ANOVA, with diet the main effect tested and day the repeated measure. Cue and probe trials (Morris water maze) and elevated plus maze were analyzed, in each age group, by 1-way ANOVA. Phylloquinone, MK-4, and sphingolipids were assessed in 20-mo-old rats with respect to diet and regional distribution using 2-way ANOVA with diet as a between-subject factor and region as a repeated-measure factor. In all cases, the Tukey’s test was used in post hoc analyses. Differences were considered significant at \( P < 0.05 \). All analyses were performed with SPSS 16.0 for Windows.

**Results**

**Body weight and survival**

Body weights increased sharply between 1 and 3 mo of age and more gradually thereafter in the 3 diet groups. The diets did not affect body weight gain (Supplemental Fig. 2) or survival (Fig. 1).

**Behavioral assessment**

**Morris water maze.** The time to find the hidden platform decreased over the successive learning trials for all age groups \( (P < 0.0001) \) (Fig. 2). At 6 and 12 mo, there was no effect of diet or diet × days interaction, suggesting that at these ages, diet did not influence learning abilities. In contrast, at 20 mo of age, group L had longer latencies than both the A \( (P = 0.054) \) and the H \( (P < 0.05) \); post hoc test d 2 and 5, \( P < 0.05 \) groups. However, the probe trial and cue test results were not affected by diet (data not shown).

**Open field.** The motor activity assessed by the path traveled by the rats decreased during the 3 d for the 3 age groups \( (P < 0.01) \) but was not affected by diet \( (P = 0.21) \) (Fig. 3). Furthermore, the time spent in the center squares of the open field did not differ, suggesting comparable exploratory behavior among the 3 diet groups at a given age (data not shown).

**Elevated plus maze.** The time spent in the open arms and the percentage of open:total entries ratio, considered an index of lower anxiety, did not differ among the groups at any age \( (P = 0.43) \) (Supplemental Table 1).

**Vitamin K.** MK-4 represented the major form (~99%) of vitamin K in brain in all brain regions investigated (Table 1). Furthermore, tissue phylloquinone and MK-4 concentrations increased with vitamin K intake \( (P < 0.001) \). MK-4 concentrations in group H were 3- and 7-fold higher than those in groups A and L, respectively. MK-4 was unevenly distributed in the brain with the highest concentrations being observed in the midbrain and pons medulla \( (P < 0.05) \). In the cerebellum, hippocampus, and striatum, MK-4 concentrations were comparable. Of note, differences in regional MK-4 distribution were more pronounced in the L \( (P = 0.006) \) than in the A \( (P = 0.016) \) and H \( (P = 0.091) \) dietary groups.

**Sphingolipids.** Each class of sphingolipid concentrations varied according to brain regions \( (P < 0.001) \) (Table 2). Sulfatides, cerebrosides, and sphingomyelin were present in higher concentrations in the pons medulla and midbrain, whereas ceramides and gangliosides were higher in the striatum and hippocampus \( (P < 0.05 \) in all cases).
Discussion

This study has shown that lifetime low-vitamin K intakes leads to a deficit in spatial learning ability in old age, a finding associated with higher concentrations of ceramides in the hippocampus and lower levels of gangliosides in the pons medulla and midbrain. To our knowledge, this is the first study to report on the long-term impact of vitamin K on behavioral components.

In the present study, the impact of vitamin K on behavior was investigated with respect to spatial learning and memory, motor activity, and anxiety. Aged (20 mo) rats fed a diet low in phylloquinone since weaning acquired spatial learning more slowly than those fed diets containing adequate or high amounts. Learning impairments in old rats were not related to motor or visual deficits, because performance on the cue and probe trials was identical across groups. Motor activity and exploratory behavior were also assessed using the open field test and performance did not vary as a function of diet. Similarly, anxiety, as assessed by the elevated plus maze test, was not affected by diet. Thus, the impairment observed in the water maze is not likely attributable to differences in either motor ability or emotionality, but rather reflects a true cognitive deficit. Interestingly, a low-vitamin K diet had no significant impact on cognition in rats aged 6 and 12 mo, which suggests that vitamin K is particularly important to brain function in the more vulnerable aging state.

In the sole publication that has examined the relationship between vitamin K and behavior, feeding rats a vitamin K-deficient diet for 11 wk had no impact on short-term memory as tested using the radial-arm maze but was associated with a significant reduction in locomotor activity. In an independent experiment, short-term treatment of rats with the vitamin K antagonist warfarin resulted in reduced exploratory behavior. However, because no information was provided regarding the vitamin K content of the deficient diet and/or vitamin K status of the rats, it is difficult to firmly establish that the reported behavior perturbations were a consequence of a low-vitamin K status (19).

As observed in younger rats (14), vitamin K in brain of aged rats was largely in the form of MK-4, with this K vitamer accounting for ~99% of total vitamin K. Furthermore, brain vitamin K content increased as a function of intake, with rats having consumed the H diet presenting significantly higher MK-4 concentrations than those of the L and A groups. The finding that MK-4 is the principal K vitamer in brains of aged rats confirms similar observations by other groups (13, 15, 30).

An important finding of the present study concerns the fact that lifetime consumption of a low-vitamin K diet is associated

![FIGURE 3](image)

**FIGURE 3**Motor activity in the open field of 6- (A), 12- (B), and 20-mo-old rats fed a L, A, or H vitamin K diet since weaning. Values are means ± SEM, n = 9–12. Motor activity decreased over time in all age groups, P < 0.01, but was not affected by diet.

In 20-mo-old rats, lifetime consumption of the H diet resulted in lower concentrations of ceramides in the hippocampus compared with the A and L groups (P < 0.05). At this age, cerebrosides, sulfatides, and gangliosides were also modulated by diet in the pons medulla and midbrain. Specifically, consumption of the H diet was associated with lower cerebroside and sulfatide concentrations in the pons medulla compared with the A and L groups (P < 0.05) and higher ganglioside concentrations in the pons medulla and midbrain compared with the L group (P < 0.05).

### TABLE 1 Phylloquinone and MK-4 concentrations in brain regions of 20-mo-old rats fed L, A, or H vitamin K diets since weaning

<table>
<thead>
<tr>
<th>Regions</th>
<th>L (pmol/g)</th>
<th>A (pmol/g)</th>
<th>H (pmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phylloquinone</td>
<td>MK-4</td>
<td>Phylloquinone</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.5 ± 0.1b,x</td>
<td>121 ± 159b,x</td>
<td>3.7 ± 0.4y</td>
</tr>
<tr>
<td>Pons medulla</td>
<td>Tracea,x</td>
<td>162 ± 173b,x</td>
<td>6.8 ± 0.9b,y</td>
</tr>
<tr>
<td>Midbrain</td>
<td>Tracea,x</td>
<td>175 ± 187c,x</td>
<td>9.1 ± 1.0b,y</td>
</tr>
<tr>
<td>Striatum</td>
<td>1.3 ± 0.5b,c,x</td>
<td>101 ± 167b,x</td>
<td>8.8 ± 0.7b,y</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>2.2 ± 0.3a,x</td>
<td>102 ± 125a,x</td>
<td>8.4 ± 0.7b,y</td>
</tr>
</tbody>
</table>

Values are means ± SEM, n = 4–6. Means in a column without a common letter (a, b, c) differ, P < 0.05; means in a row for each vitamer without a common letter (x, y, z) differ, P < 0.05.
with higher levels of ceramides in the hippocampus, a key brain region in spatial memory and navigation (31). Ceramides, which can be generated from de novo synthesis or from sphingomyelin through the action of sphingomyelinase (Smases), have been shown to mediate processes such as differentiation, growth arrest, apoptosis, and senescence (12). Specifically, when present in high concentrations, ceramides have been involved in inflammatory processes (production of cytokines IL-2 and IL-6 (32)) and in the generation of reactive oxygen species from mitochondria (33). Ceramides have also been shown to inhibit the neuronal survival pathway regulated by phosphatidil-inositol-3-kinase/Akt (34) and activate the caspase-3/caspase-3 pathway (35,36). Finally, numerous studies have reported elevated levels of ceramides in neurodegenerative diseases such as Alzheimer’s disease (37). In light of the actions of ceramides, i.e. inflammation, generation of reactive oxygen species, etc., all of which have been linked to neuronal vulnerability during aging (38), their presence in lower concentrations in the hippocampus of rats having consumed the high-vitamin K diet could have contributed to their better spatial memory performances.

Lifetime consumption of a low-vitamin K diet was also associated with modulation of sphingolipids in pons medulla and midbrain, 2 structures that relay information between the forebrain and spinal cord (31). Specifically, levels of gangliosides decreased in both regions in rats from the L group, whereas sulfatides and their precursors, cerebrosides, increased in pons medulla. Gangliosides and sulfatides are also important for the nervous system. Gangliosides are abundant on neuronal cell surfaces and are actively involved in the maintenance and repair of nervous tissues (39). Sulfatides and cerebrosides have functional roles in the regulation of oligodendrocyte differentiation and myelination (33) and are essential for the maintenance of myelin and axon structures of the central nervous system (40). In addition to their structural roles, gangliosides and sulfatides are important modulators of membrane receptors and downstream signaling (41). For instance, both the ganglioside subtype GM1 and sulfatides have been implicated in the modulation of uptake of the neuronal mediator dopamine by striatal neurons (42).

As illustrated in Supplemental Figure 1, sulfatides and gangliosides are synthesized from ceramides through 2 distinct pathways. Gangliosides, which make up a family of compounds, are synthesized from ceramide through the actions of the enzymes glucosylceramide synthase, galactosyltransferase, and lactosylceramide synthase, whereas sulfatides are produced through the actions of galactosylceramide synthase and galactocerebroside sulfotransferase. Our finding of increased ganglioside and decreased sulfatide concentrations in pons medulla and midbrain of rats that have consumed a high-vitamin K diet tends to suggest that in these 2 brain regions, a high-vitamin K diet leads to a prioritizing of the ganglioside pathway over that of sulfatides. It could be that a higher ganglioside concentration in the pons helps maintain the integrity of the signal relays that this structure sustains with the cerebellum, a brain structure involved in motor and spatial learning processes (43,44). Such a hypothesis needs to be verified, but in the present study, higher gangliosides in pons medulla and midbrain were associated with improved cognition.

In addition to its modulatory effect on sphingolipid metabolism, the beneficial impact of a high-vitamin K diet on cognition could have been through other mechanisms, notably some specific actions of vitamin K in inflammation and oxidative stress. In vitro, MK-4 has been shown to limit the production of IL-6 in cultured human fibroblasts (45) and of prostaglandins (46). In animal studies, MK-4 has been observed to limit inflammation in models of encephalomyelitis (47), whereas phylloquinone has been reported to suppress LPS-induced inflammation in the rat (48). In a recent study, the antiinflammatory activity of vitamin K, notably MK-4, was shown to be mediated via the inhibition of the NF-kB signaling pathway (49). Furthermore, recent epidemiological cohort studies have reported that a high-vitamin K nutritional status is associated with lower levels of the proinflammatory markers IL-6, intracellular adhesion molecule-1, TNF receptor 2, and C-reactive protein (30,51). Finally, Li

Table 2: Sphingolipid concentrations in brain regions of 20-mo-old rats fed a L, A, or H vitamin K diet since weaning

<table>
<thead>
<tr>
<th>Sphingolipid</th>
<th>Cerebellum</th>
<th>Pons medulla</th>
<th>Midbrain</th>
<th>Hippocampus</th>
<th>Striatum</th>
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<tbody>
<tr>
<td>Cerebrosides, μmol sphingosine/g</td>
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<tr>
<td>L</td>
<td>8.49 ± 1.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.1 ± 1.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.0 ± 1.97&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.34 ± 0.20&lt;sup&gt;d&lt;/sup&gt;</td>
<td>14.3 ± 1.53&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>A</td>
<td>7.50 ± 2.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.4 ± 1.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.5 ± 1.67&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.82 ± 0.23&lt;sup&gt;d&lt;/sup&gt;</td>
<td>12.6 ± 1.14&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>H</td>
<td>9.11 ± 0.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.7 ± 0.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.2 ± 1.47&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.93 ± 0.20&lt;sup&gt;d&lt;/sup&gt;</td>
<td>13.5 ± 3.24&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
<td>Sphingomyelin, μmol sphingosine/g</td>
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<td></td>
<td></td>
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<tr>
<td>L</td>
<td>5.46 ± 0.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.9 ± 1.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.64 ± 0.41&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.84 ± 0.10&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.00 ± 0.07&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>A</td>
<td>4.79 ± 0.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.5 ± 1.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.66 ± 0.85&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.92 ± 0.25&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.08 ± 0.05&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
<td>H</td>
<td>4.62 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.5 ± 0.56&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.95 ± 0.27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.08 ± 0.20&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.09 ± 0.16&lt;sup&gt;e&lt;/sup&gt;</td>
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<td>Sulfatides, μmol cerebroside sulfate/g</td>
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<td></td>
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<tr>
<td>L</td>
<td>2.26 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.09 ± 0.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.38 ± 0.66&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.63 ± 0.17&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>A</td>
<td>2.03 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.79 ± 0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.89 ± 0.39&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>3.42 ± 0.33&lt;sup&gt;e&lt;/sup&gt;</td>
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<td>H</td>
<td>2.01 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.62 ± 0.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.27 ± 0.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.93 ± 0.21&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.94 ± 1.15&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
<td>Ceramides, μmol sphingosine/g</td>
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<td>L</td>
<td>0.12 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.18 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.19 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.26 ± 0.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.50 ± 0.02&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>A</td>
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<td>0.17 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.18 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>0.47 ± 0.01&lt;sup&gt;e&lt;/sup&gt;</td>
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<td>H</td>
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<td>0.17 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.16 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.17 ± 0.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.50 ± 0.07&lt;sup&gt;e&lt;/sup&gt;</td>
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<td>Gangliosides, μmol sialic acid/g</td>
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<tr>
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<td>0.74 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.87 ± 0.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.38 ± 0.10&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>A</td>
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<td>0.89 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>1.84 ± 0.28&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.14 ± 0.49&lt;sup&gt;e&lt;/sup&gt;</td>
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<td>1.06 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.85 ± 0.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.96 ± 0.20&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.58 ± 0.99&lt;sup&gt;e&lt;/sup&gt;</td>
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Superscripts without a common letter differ, P < 0.05.

1 Values are mean ± SEM, n = 4–6. Means in a column (a, b, c) or row (x, y, z) with superscripts without a common letter differ, P < 0.05.
et al. (52) showed that both phylloquinone and MK-4 suppressed the cell death of oligodendrocytes depleted of glutathione. In a more recent report, this group provided additional evidence that the protective effect of vitamin K against oxidative cell death was by inhibiting the activation of enzyme 12-lipoxygenase (53).

In conclusion, we show for the first time to our knowledge that lifetime low-vitamin K intakes lead to cognitive deficits in old age, a finding associated with higher concentrations of ceramides in the hippocampus and lower levels of gangliosides in the pons medulla and midbrain. Although much remains to be elucidated about the mechanism of action of vitamin K in cognition, this report points to vitamin K as an important nutritional factor of cognitive health during aging.

Acknowledgments
G.F. designed the study; I.C. and E.B. conducted the research and performed statistical analysis; I.C., J.P. J.R., and G.F. interpreted the data; I.C. and G.F. wrote the paper; and G.F. had primary responsibility for final content. All authors read and approved the final manuscript.

Literature Cited


