VITAMIN K STATUS IS NOT ASSOCIATED WITH COGNITIVE DECLINE IN MIDDLE AGED ADULTS

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Abstract: Objectives: The aim of this study was to examine the association between dephospho-uncarboxylated matrix Gla protein (dp-ucMGP), an indicator of vitamin K status, and cognitive decline, and the modifying role of 25(OH)D. Design: Longitudinal study with six years follow-up. Setting: Community based. Participants: 599 participants of the Longitudinal Aging Study Amsterdam (aged 55-65 years). Measurements: Information processing speed and a composite Z-score by combining three domains of cognition reflecting general cognitive functioning. Results: Generalized estimating equations (GEE) showed no significant associations between dp-ucMGP and decline in general cognitive functioning. Vitamin D modified the association between dp-ucMGP and speed of information processing (p<0.05). In the group with a 25(OH)D concentration > 50 nmol/l, the highest tertile of dp-ucMGP (>406 pmol/l), which corresponds to lower vitamin K levels, was associated with 1.5 higher score on information processing speed (p=0.023) as compared to the lowest tertile of dp-ucMGP. Conclusion: In contrast to our hypothesis, a suboptimal vitamin K was not associated with cognitive decline in middle-aged adults.

Key words: Cognitive decline, speed of information processing, vitamin K status, desphospho-uncarboxylated matrix Gla-protein.

Introduction

Cognitive functioning declines during life, however, the rate of cognitive decline differs considerably among persons. Previous studies have focused on modifiable factors that influence the rate of decline, such as nutrition, yet little is known about a possible effect of suboptimal vitamin K levels on cognitive decline. A cross-sectional study showed an association between higher phylloquinone concentration and better verbal episodic memory performances in healthy older adults aged 70-85 (1). Circulating phylloquinone concentrations reflect recent intakes, since the plasma half-lifetime of phylloquinone is 1-2 hours (2). This is not the case when measuring vitamin K-dependent uncarboxylated Gla-proteins, which reflect vitamin K status at the tissue level (3). Since the tissue half-life of vitamin K is much longer than in blood, uncarboxylated Gla-proteins reflect better long-term dietary habits and actual vitamin K insufficiency. Increased levels of desphospho-uncarboxylated matrix Gla protein (dp-ucMGP), one of the vitamin K-dependent uncarboxylated Gla-proteins, reflecting a low vitamin K status, were shown to be associated with coronary artery calcification and cardiovascular disease in relatively healthy adults (4, 5). As cardiovascular disease are also associated with cognitive decline (6) dp-ucMGP might also be associated to cognitive decline.

When studying the association between vitamin K and cognitive decline it is important to take vitamin D status in account for vitamin D, also a fat-soluble vitamin, increases MGP transcription in vascular smooth muscle cells of the blood vessel wall (7). Furthermore, low levels of vitamin D were associated with reduced cognitive functioning (8, 9).

Vitamin K occurs in two natural forms in the diet: menaquinones (MK-n; vitamin K2) which occurs in meat and eggs as MK-4, and in fermented foods, like cheese and curds as long-chain menaquinones (MK-7, MK-8, MK-9) (10, 11). Phylloquinone (vitamin K1) is mainly found in leafy green vegetables and in some plant oils (12). Therefore, vitamin K insufficiency is highly treatable by promoting adequate food intake or by medication, which may enable prevention of cognitive decline very easily. Hence it is important to study whether there is an association between Dp-ucMGP and cognitive decline. We examined this association and a possible modifying effect of 25(OH)D in middle aged adults in a longitudinal design. We hypothesized that a suboptimal vascular vitamin K status, as determined by circulating dp-ucMGP is associated with cognitive decline over a period of 6 years.

Subjects and methods

Data for this study were collected within the framework of the Longitudinal Aging Study Amsterdam (LASA) (13). In 2002/2003, a cohort of 1002 men and women, aged between 55 and 65 years, were enrolled for the baseline examination; 995 (99.3%) completed the main interview. Of these the following participants were excluded: no or insufficient blood
sampling (n=248), no established vitamin K status (n=52), and vitamin K antagonist use (ATC-code B01AA; n=20), and missing values on important covariates (n=48). Of these 627 respondents, 596 (95.1%) and 542 (86.4%) participated in the 3- and 6-year follow-up interview, respectively. As we were interested in longitudinal association between vitamin K status and cognition, analyses were based on persons of whom data on cognition on at least two occasions was available (n=599). Compared to the study sample, the persons who were excluded or lost to follow up had poorer cognitive functioning, were more often current smokers, had more cardiovascular diseases and diabetes, and had lower dp-ucMGP levels at baseline (all \(p\)'s <0.05).

**Vitamin K status**

Morning blood samples were collected in 2002/03 when subjects were in a non-fasting state and in a sitting position. Citrated plasma samples were stored at -80 °C until analyses. Circulating dp-ucMGP was measured as described by Cranenburg and colleagues (3).

**Cognitive functioning**

We included two cognitive measures in our analyses, information processing speed and general cognitive functioning. Information processing speed is hypothesized to be especially affected by vitamin K-induced changes in signaling events via its impact on brain sphingolipid metabolism within the brain (14). In addition, it has been shown to be associated with vitamin B12 and folate (15) and homocysteine (16) and therefore we expected this outcome to be the most sensitive. Information processing speed was measured by an adapted version of a letter substitution task, the Alphabet Coding Task-15 (17). General cognitive functioning was measured with a composite Z-score by combining three domains of cognition; information processing speed, episodic memory, and fluid intelligence. Episodic memory was measured with the abbreviated Auditory Verbal Learning Test (AVLT) (18), from which we obtained the total number of words the respondent could remember of a list of 15 words during the three trials (recall score) and the number of words that a person still remembered after 15 minutes (delayed recall score, range 0–15). As we included both recall score and delayed recall for general cognitive performance, these 2 parameters were given a coefficient of 0.5 in the formula. Fluid intelligence, defined as the ability to deal with new information, was assessed with the Raven’s Coloured Progressive Matrices (RCPM) (19).

**Covariates**

All analyses were adjusted for time and age. In addition, the following potential confounders were included in the analyses: sex, education (in years), hypertension, diabetes mellitus, cardiovascular disease, Body Mass Index (BMI), alcohol consumption, smoking, total cholesterol, and \(25(OH)D\). The time variable was defined as the number of follow-up years (i.e. 0, 3 and 6 years). Hypertension was defined by sitting blood pressure, >160/100 mm/Hg, use of antihypertensive medication or both. Diabetes mellitus and cardiovascular diseases were assessed by self-report, the use of disease related medicine or both. Lifestyle variables included smoking (never, former, current) and alcohol consumption (none, light, moderate, excessive). The ApoE phenotypes were determined by isoelectric focusing of delipidated serum samples, followed by immunoblotting (20). Total cholesterol was measured with a Hitachi 747 analyzer using enzymatic colorimetric assays (Roche diagnostics, Mannheim, Germany). Serum \(25(OH)D\) concentration was determined using a competitive binding protein assay (Diasorin, Stillwater, Minesota, USA).

**Statistical analyses**

Categorical data were expressed as number (percentage); continuous data were expressed as mean (SD) for normally distributed variables, or as median (interquartile range) for skewed variables.

Generalized estimating equations (GEE) with an exchangeable correlation structure were used (21) to analyze longitudinal associations between dp-ucMGP (tertiles and continuous variable), cognitive performance and rate of cognitive decline during 6 years of follow-up, and the possible modifying role of \(25(OH)D\). To be able to determine whether dp-ucMGP was associated with the rate of cognitive decline, the interaction with time was evaluated by adding the product term between dp-ucMGP and time to the models. Effect modification by \(25(OH)D\) was investigated by including product terms in the fully adjusted models. The first regression models were adjusted for time and age. The second models included all potential confounders.

All analyses were tested at the 0.05 level of significance, except for effect modification for which a level of significance of 0.10 was tolerated. Analyses were performed using the IBM SPSS 20 statistical package.

**Results**

**Baseline characteristics**

The baseline sample consisted of 599 older persons (274 men, 325 women) whose mean age was 59.9 (SD 3.0) year. The median (interquartile range) of plasma dp-ucMGP was 377.4 (234.0 – 460.0) pmol/L. The mean (SD) level of general cognitive functioning declined from 0.05 (0.71) at baseline to 0.01 (0.74) after 6 years (\(p=0.022\)). For information processing speed the levels declined from 29.61 (5.96) to 28.93 (6.40) during 6 years (\(p<0.001\)). Table 1 shows the baseline characteristics of the total study population stratified by tertiles of dp-ucMGP concentration.

Over the follow-up period of 6 years, no significant associations were found between dp-ucMGP (in tertiles) and general cognitive functioning or information processing speed (Table 2). The product term between dp-ucMGP and