Dietary phyloquinone intake and risk of type 2 diabetes in elderly subjects at high risk of cardiovascular disease

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ABSTRACT

Background: Limited evidence from human and animal studies has suggested that vitamin K has a potentially beneficial role in glucose metabolism and insulin resistance.

Objective: We analyzed the cross-sectional and longitudinal associations between dietary phyloquinone intake and type 2 diabetes in elderly subjects at high cardiovascular risk.

Design: Cross-sectional associations were tested in 1925 men and women in the Prevention with the Mediterranean Diet trial. A longitudinal analysis was conducted on 1069 individuals free of diabetes at baseline (median follow-up: 5.5 y). Biochemical and anthropometric variables were obtained yearly. Dietary intake was collected during each annual visit by using a food-frequency questionnaire, and phyloquinone intake was estimated by using the USDA database. The occurrence of type 2 diabetes during follow-up was assessed by using American Diabetes Association criteria.

Results: Dietary phyloquinone intake at baseline was significantly lower in subjects who developed type 2 diabetes during the study. After adjustment for potential confounders, risk of incident diabetes was 17% lower for each additional intake of 100 μg phyloquinone/d. Moreover, subjects who increased their dietary intake of vitamin K during the follow-up had a 51% reduced risk of incident diabetes compared with subjects who decreased or did not change the amount of phyloquinone intake.

Conclusion: We conclude that dietary phyloquinone intake is associated with reduced risk of type 2 diabetes. This trial was registered at http://www.controlled-trials.com as ISRCTN35739639. Am J Clin Nutr doi: 10.3945/ajcn.111.033498.

INTRODUCTION

Vitamin K is a fat-soluble vitamin that occurs in the following 2 biologically active forms: vitamin K₁ or phylloquinone and vitamin K₂ or menaquinone. Phyloquinone is predominantly in leafy green vegetables and certain vegetable oils, whereas menaquinone is in animal products such as poultry, meat, egg yolk, and cheese (1–3).

Interest was first shown in vitamin K largely because of its role in blood coagulation. However, recent evidence suggested that the physiologic role of vitamin K goes beyond that of coagulation, and it may have an effect on inflammatory modulation, bone metabolism, and the prevention of osteoporotic fractures. Moreover, limited evidence from human and animal studies suggested that vitamin K has a potentially beneficial role in glucose metabolism and insulin resistance. Rats fed a low phyloquinone-containing diet had a delayed insulin response to glucose infusion and decreased plasma glucose (4). Similar findings were observed in a short-term study conducted in young men (5). High phyloquinone intake has been cross-sectionally associated with improved glycemic control and insulin sensitivity in the Framingham Offspring Cohort (6). However, long-term interventional studies have reported contradictory results. Thus, whereas 12 mo of phyloquinone administration was not associated with changes in insulin secretion in 21 postmenopausal women (7), a 36-mo randomized controlled trial showed an improvement in insulin sensitivity after phyloquinone intake in men but not in women (8).

To our knowledge, only one epidemiologic study has prospectively evaluated whether dietary vitamin K intake is associated with risk of type 2 diabetes after 10.3 y of follow-up. A nonsignificant relation between baseline phyloquinone intake and a reduced risk of type 2 diabetes was demonstrated, whereas menaquinone intake was significantly associated with decreased risk of type 2 diabetes.

Because there is limited evidence on the association between dietary phyloquinone intake and risk of type 2 diabetes, we investigated the cross-sectional and longitudinal associations between dietary phyloquinone intake and type 2 diabetes in a cohort of individuals at high risk of cardiovascular disease.

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SUBJECTS AND METHODS

Study design and subjects

The present cross-sectional and longitudinal analyses have been conducted in a total of 1925 subjects within the framework of the Prevention with the Mediterranean Diet (PREDIMED) trial (http://www.controlled-trials.com; ISRCTN35739639) trial being carried out in Spain at the Reus-Tarragona and Navarra centers. PREDIMED is a multicenter, randomized, parallel-group clinical trial that aims to assess the effect of the Mediterranean diet on the primary prevention of cardiovascular disease (http://www.predimed.es). The design of the PREDIMED trial has been described elsewhere (9). The trial is currently taking place with 7447 participants with high risk of coronary artery disease (CAD) assigned to the following 3 intervention groups: a traditional Mediterranean diet with virgin olive oil group, a traditional Mediterranean Diet with mixed nuts group, and a control group in which a low-fat diet was recommended according to American Heart Association guidelines. Candidates were community-dwelling men (55–80 y of age) and women (60–80 y of age) without cardiovascular disease, but they met at least one of the 2 following criteria: type 2 diabetes or ≥3 CHD risk factors (smoking, hypertension, dyslipidemia, obesity, or a family history of CHD). Exclusion criteria were a history of cardiovascular disease, severe chronic illness, drug or alcohol addiction, difficulties with or low predicted likelihood of following the Prochaska and DiClemente stages of change in behavior, a history of a food allergy or intolerance to olive oil or nuts, and any condition that might have impaired participation in the study. The institutional review boards of recruitment centers approved the study protocol, and participants signed an informed consent form.

Dietary assessment

Dietary intake was repeatedly measured at each annual visit by using a 137-item food-frequency questionnaire (FFQ) (10). Detailed information about the development of the FFQ and reproducibility and validity of the questionnaire in the PREDIMED cohort has been previously reported (11). Spanish food-composition tables were used to derive nutrient compositions (12), whereas phylloquinone intake was obtained by analyzing the FFQ with the database of the USDA (http://www.nal.usda.gov/fnic/foodcomp/search/). A 14-point food-item questionnaire, which was an extension of a previously validated questionnaire, albeit not for phylloquinone, was used to assess adherence to the traditional Mediterranean diet in a Mediterranean diet score.

Other measurements

All measurements were taken at baseline and again every year by using the same procedures. Information was collected on subject medical history, use of medication, sociodemographic variables, lifestyle, health conditions, and medical diagnoses. Physical activity was evaluated by using the validated Spanish version of the Minnesota Leisure Time Physical Activity Questionnaire. Weight, height, and waist circumference were measured by using standardized procedures. Blood pressure was measured by using a validated semiautomatic oscillometer (Omron HEM-705CP; Omron Healthcare) in duplicate. Centralized laboratory biochemical analyses were performed on blood samples obtained in fasting conditions. Plasma glucose, serum cholesterol, HDL-cholesterol and triglyceride concentrations were measured by using standard enzymatic automated methods. In patients with triglyceride concentrations <400 mg/dL, LDL-cholesterol concentrations were estimated by using Friedewald’s formula. Laboratory technicians were blinded to the intervention group. The occurrence of type 2 diabetes during follow-up was assessed according to the following American Diabetes Association criteria (13): fasting plasma glucose concentration ≥7.0 mmol/L or 2-h plasma glucose concentration ≥11.1 mmol/L after a 75-g oral glucose load measured during each annual visit. A second test that used the same criteria was required for confirmation. Cases of new-onset of diabetes were ascertained by the PREDIMED Clinical Event Committee, whose members were blinded to the intervention group.

Statistical analyses

To analyze the baseline characteristics of the population as a function of whether diabetes was present or absent or whether the subjects developed incident diabetes or not, we used a t test for continuous variables and a chi-square test for categorical variables. To assess the association of baseline phylloquinone intake with fasting plasma glucose concentrations at the end of the follow-up, we applied a linear regression model adjusted for potential confounders (sex, age, BMI, fasting glucose concentrations at baseline, total energy intake, intervention group, and smoking). We fitted Cox regression models to estimate HRs for type 2 diabetes for each additional 100-µg increment in dietary phylloquinone intake. Cox regression models were also fitted to estimate HRs for incident diabetes for individuals who increased their dietary phylloquinone intake during the follow-up (compared with their respective counterparts). Both Cox regressions models were adjusted for several potential confounding factors by using 2 different models to allow for different levels of adjustment. The first model was adjusted for sex, age, and BMI, and the second model was also adjusted for total energy intake, fasting glucose concentration at baseline, intervention group, and smoking. We additionally adjusted Cox regression models by adherence to the Mediterranean diet score to minimize the potential effect of a healthy diet. The time variable was the interval between random assignment and the date of the last follow-up, death, or diabetes diagnosis, whichever occurred first. Participants who were free of diabetes or were lost during follow-up were censored at the date of the last visit. We checked for a statistical interaction between dietary phylloquinone intake and confounding variables by including the interaction terms in the models. Because no significant interactions were shown, interaction terms were removed, and models were checked again. All statistical tests were 2-tailed, and the significance level was set at \( P \leq 0.05 \). Analyses were performed with SPSS software (version 17.0; SPSS Inc).

RESULTS

Of 2912 eligible candidates from the Tarragona-Reus and Navarra centers, 1925 subjects fulfilled the PREDIMED inclusion...
The energy-adjusted intake of protein, fat, and fiber was significantly lower in type 2 diabetes subjects, including intake of green leafy vegetables, which are the primary dietary source of phylloquinone. In a regression analysis, intakes of lettuce, escarole, endive chard, and spinach contributed to the 96% of the total dietary vitamin K. Thus, the dietary phylloquinone intake was also higher in type 2 diabetes subjects than in nondiabetic subjects (mean ± SD: 327 ± 141 and 307 ± 141 μg/d, respectively; P = 0.003). See “Supplemental data” in the online issue for a presentation of baseline characteristics of participants according to the development of type 2 diabetes or not.

During a median follow-up of 5.5 y, we documented 131 verified cases of incident type 2 diabetes. At baseline, no significant differences in the energy-adjusted intake of macronutrients were observed between nonincident and incident type 2 diabetes subjects (data not shown), whereas the lower energy-adjusted intake of phylloquinone at baseline was shown in incident type 2 diabetes subjects (mean ± SD: 312 ± 132 mg/d in nonincident subjects and 285 ± 131 mg/d in incident type 2 diabetes subjects; P = 0.027). After regression analysis, in
a model adjusted for sex, age, BMI, fasting glucose concentrations at baseline, total energy intake, intervention group, and smoking. The baseline dietary phylloquinone intake was not associated with fasting plasma glucose concentrations at the end of follow-up ($B = -0.651$; 95% CI: $-1.531$, 0.230). However, the baseline phylloquinone intake was associated with a $17\%$ reduced risk of incident type 2 diabetes with an HR of 0.83 (95% CI: 0.712, 0.967; $P = 0.017$) for each additional intake of 100 µg phylloquinone/d (Table 2). Furthermore, a $51\%$ lower risk of incident diabetes was observed in subjects who increased their dietary intake of phylloquinone during the follow-up compared with subjects who decreased or did not change their intake (Table 3). These results were not modified after adjustment for adherence to a Mediterranean diet (data not shown). In addition, we analyzed the effect of phylloquinone intake on incident type 2 diabetes according to the intervention group. We observed a significant protective role of phylloquinone on the development of type 2 diabetes in both Mediterranean diet groups [HR (95% CI): 0.693 (0.504, 0.952) and 0.564 (0.392, 0.812) in the diet plus virgin olive oil group and the diet plus mixed nuts group, respectively]; however, this effect was not observed in the control group [HR (95% CI): 1.039 (0.839, 1.285)]. Whether or not this result was due to a lower bioavailability of vitamin K in the low-fat diet group could not be established in our study.

**DISCUSSION**

In this longitudinal study, we showed, for the first time to our knowledge, that a higher intake of phylloquinone was associated with a reduced risk of new-onset type 2 diabetes. Moreover, an increase in the amount of phylloquinone intake during the follow-up was associated with a $51\%$ lower risk of diabetes in elderly subjects at high cardiovascular risk after a median follow-up of 5.5 y.

Studies have suggested that vitamin K may improve insulin sensitivity and glycemic status. Men with lower phylloquinone intakes showed lower insulin and higher glucose concentrations after oral glucose loading than did men with higher phylloquinone intakes (5). However, in the same study, no associations were observed between phylloquinone intake and fasting glucose or insulin concentrations (5). Similar results have been observed in a cross-sectional analysis of 2719 men and women in the Framingham cohort (6). Once again, a positive association was shown between a higher phylloquinone intake and insulin sensitivity or a better glycemic status measured 2-h after an oral-glucose-tolerance test. No significant association was shown in the fasting state.

The results of clinical studies designed to evaluate the effect of phylloquinone intake on glucose and insulin metabolism are contradictory. In a small study conducted in 12 young men, a short-term (1-wk) menaquinone-4 supplementation improved the insulin response after an oral glucose loading (5). In a 36-mo controlled trial designed to assess the impact of daily phylloquinone supplementation on bone loss, the HOMA-IR significantly decreased during the intervention in men in the supplemented group, although no effect was shown in women (8). Similarly, women who received 1 mg phylloquinone/d for 12 mo did not show any significant improvement in either glucose concentrations or HOMA-IR (7). Unfortunately, circulating insulin concentrations were not measured in our study, and thus, we could not evaluate the effect of phylloquinone on insulin resistance.

The potential mechanisms that underlie the role of vitamin K on glucose metabolism and insulin resistance remain to be elucidated. First, in vitro studies have shown an inhibitory role of phylloquinone on inflammatory cytokines related to insulin metabolism (14). Second, vitamin K acts as a cofactor in the γ-carboxylation of osteocalcin, which is a bone-derived protein that is traditionally involved in the mineralization of bone and calcium homeostasis and was recently related to insulin metabolism. Animal and in vitro studies suggested that only the uncarboxylated form of osteocalcin, not the carboxylated one, improved glucose tolerance and insulin sensitivity (15, 16), which suggested a deleterious effect of vitamin K on glucose metabolism. However, human studies have reported that both carboxylated osteocalcin and uncarboxylated osteocalcin exert a positive role in glucose metabolism and improved insulin sensitivity (17, 18). In this regard, we have recently shown a positive association between changes in serum osteocalcin or uncarboxylated osteocalcin concentrations and improvements in glucose metabolism, insulin resistance, and β cell dysfunction in the frame of the PREDIMED study (19), which supported the potential role of dietary vitamin K in type 2 diabetes prevention. The beneficial role of vitamin K in type 2 diabetes prevention observed in the current study could be apparently contradictory with the association between uncarboxylated osteocalcin and improvements of glucose metabolism previously described in

**TABLE 2**

Baseline phylloquinone intake and risk of incident type 2 diabetes during the follow-up ($n = 1065$)\(^1\)

<table>
<thead>
<tr>
<th>HR (95% CI) per intake of 100 µg phylloquinone/d</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude model</td>
<td>0.830 (0.712, 0.967)</td>
</tr>
<tr>
<td>Model 1</td>
<td>0.844 (0.725, 0.982)</td>
</tr>
<tr>
<td>Model 2</td>
<td>0.838 (0.713, 0.985)</td>
</tr>
</tbody>
</table>

\(^1\)Multivariable Cox regression was used for analyses. Model 1 was adjusted for sex, age, and BMI (in kg/m\(^2\)), and model 2 was adjusted for sex, age, BMI (in kg/m\(^2\)), fasting glucose concentrations at baseline, total energy intake (kcal/d), intervention group, and smoking.
our group. An explanation for this apparent contradiction relative to our previous results might be that total osteocalcin is highly correlated with uncarboxylated osteocalcin, and, therefore, the uncarboxylated osteocalcin tracks the total osteocalcin or vice versa (20).

In the cross-sectional analysis of the current study, the energy-adjusted vitamin K intake was paradoxically significantly higher in type 2 diabetic subjects; however, the longitudinal results suggest a protective effect of dietary vitamin K on incident diabetes. These apparent discrepancies could be attributed to a reverse causation bias. Thus, subjects already diagnosed with type 2 diabetes at the beginning of the study might have tended to eat healthier diets than did their nondiabetic counterparts. Indeed, subjects already diagnosed with type 2 diabetes at the beginning of the study reported higher intakes of other vitamins and fiber, such as leafy green vegetables, which are the main sources of vitamin K. Furthermore, according to longitudinal results, we observed that the baseline dietary intake of vitamin K was significantly lower in incident-diabetes subjects than in their counterparts, and there were no significant differences in the other dietary exposures evaluated.

Finally, our findings were consistent with, and a longitudinal extension of, those obtained in an earlier prospective cohort study conducted in Dutch subjects in which volunteers allocated to the upper quartile of vitamin K intake showed a nonsignificant 19% reduced risk of incident diabetes.

Our study had some limitations. First, the cohort studied was elderly and at high risk of cardiovascular disease, and thus, our findings cannot automatically be generalized to young and healthy individuals. Nevertheless, the beneficial effect of dietary phylloquinone intake on risk of diabetes may be similar in other populations. Second, we could not discount a slight overestimation of phylloquinone intake because of the use of the FFQ and USDA food-composition database. Moreover, phylloquinone could be converted into menaquinone-4 at an unknown rate, although the physiologic implications of this conversion are still unknown (21, 22). Third, because the subjects in our study generally consumed a Mediterranean-type diet meant that the effect of an increase in dietary phylloquinone intake on glucose metabolism and insulin sensitivity may have been masked, and in populations with lower consumptions of phylloquinone or poor nutrition, an increase would be much more beneficial. In contrast, our study had some strengths such as its longitudinal design that allowed for the suggestion of a cause-effect relationship between dietary phylloquinone and risk of developing type 2 diabetes and the repeated measurement of diet. Moreover, the diagnosis of diabetes was not self-reported as was the case in the previous study (23) and was verified by a second analytic test, which made more reliable the case ascertainment to identify the incidence of new cases of diabetes. However, some participants did not undergo an oral-glucose-tolerance test, and thus, diabetes could be diagnosed only by confirmation of a fasting blood sugar concentration ≥ 7.0 mmol/L using a second test, which might have falsely lowered overall incidence rates.

In conclusion, the results of this study show that dietary phylloquinone intake is associated with reduced risk of type 2 diabetes, which extends the potential roles of vitamin K in human health. Additional prospective studies and trials are needed to confirm these results.

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REFERENCES


