Dietary phylloquinone and menaquinones intake and risk of type 2 diabetes

Joline WJ Beulens PhD1,2, Daphne L van der A PhD3, Diederick E. Grobbee MD 1, Ivonne Sluijs MSc1, Annemieke MW Spijkerman PhD2, Yvonne T van der Schouw PhD1

1 Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, the Netherlands
2 Center for Prevention and Health Services Research, National Institute for Public Health and the Environment, Bilthoven, the Netherlands
3 Center for Nutrition and Health, National Institute for Public Health and the Environment, Bilthoven, the Netherlands

Corresponding author/request for reprints:
Joline Beulens
E-mail: J.Beulens@umcutrecht.nl

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Objectives: To investigate whether dietary phylloquinone and menaquinones intake are related to risk of type 2 diabetes.

Research Design & Methods: We used data of a prospective cohort study in 38,094 Dutch men and women, aged 20 to 70 years. Dietary phylloquinone and menaquinones intake was assessed using a validated food frequency questionnaire. Diabetes cases were mainly ascertained via self report and verified against medical records.

Results: During 10.3 years follow-up, 918 incident diabetes cases were documented. In a multivariate model adjusting for diabetes risk factors and dietary factors, phylloquinone intake tended to be associated (p=0.08) with a reduced risk of type 2 diabetes with a hazard ratio of 0.81 (95%-CI: 0.66-0.99) for the highest versus the lowest quartile. For menaquinones intake, a linear, inverse association (p=0.038) with risk of type 2 diabetes was observed with a hazard ratio of 0.93 (0.87-1.00) for each 10 µg increment in the multivariate model.

Conclusion: This study shows that both phylloquinone and menaquinones intake may be associated with a reduced risk of type 2 diabetes.
Vitamin K is a fat-soluble vitamin occurring in two biologically active forms; vitamin K1 (phylloquinone) and vitamin K2 (menaquinones). Phylloquinone, the most common form, is present in green, leafy vegetables and certain vegetable oils(1), while menaquinones occur in animal products like meat, eggs, and cheese(2). However, strong regional differences in amount and forms of menaquinones intake exist(2). Vitamin K functions as a cofactor in the $\gamma$-carboxylation of certain glutamic acid (Gla) residues of vitamin K-dependent proteins for their activation(3). Vitamin K was mainly known as a co-factor to carboxylate clotting factors like prothrombin(3). More recently, it became apparent that vitamin K also carboxylates other proteins like osteocalcin, a regulator of bone mineral maturation(3).

A recent study showed that osteocalcin concentrations may also affect insulin sensitivity and type 2 diabetes by regulating the expression of insulin genes and $\beta$-cell proliferation markers(4). In mice, osteocalcin was shown to increase insulin secretion and insulin sensitivity and decrease severity of type 2 diabetes(4). This animal study suggested a specific role for the uncarboxylated form of osteocalcin(4), contradicting a role for vitamin K. Subsequent human studies, however, observed relations between high total or carboxylated osteocalcin and improved insulin sensitivity(5,6). These latter studies suggest that vitamin K could reduce insulin resistance and risk of type 2 diabetes by carboxylating osteocalcin.

To date, no studies have investigated the relation between vitamin K intake and risk of type 2 diabetes and only few explored relations with insulin sensitivity. In rats, vitamin K deficiency delayed the insulin response and decreased plasma glucose(7). Similar results have been shown in small-scale human studies among young men with low risk of diabetes(8). Recently, two larger studies investigated the relation between dietary phylloquinone and insulin sensitivity. An observational study showed that a high phylloquinone intake was associated with improved insulin sensitivity and glycemic control(9). A randomized controlled trial showed improved insulin sensitivity after supplementing phylloquinone among men(10). Whether dietary phylloquinone or menaquinones intake are associated with a reduced risk of type 2 diabetes is unknown. Therefore, we investigated whether dietary phylloquinone and menaquinones intake are inversely associated with type 2 diabetes in a prospective cohort of Dutch men and women. Because previous studies suggested relations between vitamin K and inflammatory factors or blood lipid profile(11-14), we explored relations between phylloquinone and menaquinones intake and high-sensitive C-reactive protein, blood lipid profile and HbA1c as a marker of diabetes risk.

METHODS

EPIC-NL consists of the two Dutch contributions to the EPIC study, the Prospect-EPIC and MORGEN-EPIC cohorts. These cohorts were set up simultaneously in 1993–1997 and merged into one Dutch EPIC cohort. The design and rationale of EPIC-NL are described elsewhere(15). The Prospect-EPIC study includes 17,357 women aged 49–70 years living in Utrecht and vicinity. The MORGEN-EPIC cohort consists of 22,654 adults aged 21–64 years selected from random samples of the Dutch population in three Dutch towns. All participants provided informed consent before study inclusion. The study complies with the Declaration of Helsinki and was approved by the institutional board of the University Medical Center Utrecht (Prospect) and the Medical...
Ethical Committee of TNO Nutrition and Food Research (MORGEN).

After exclusion of prevalent diabetes cases (n=615), individuals with abnormal energy intake (kcal < 600 or > 5000; n=108), missing nutritional data (n=213), and missing follow-up (n=981), 38,094 participants were left for analysis.

**Intake of phylloquinone, menaquinones and other nutrients.** Daily nutritional intake was obtained from a food frequency questionnaire (FFQ) containing questions on the usual frequency of consumption of 79 main food items during the year preceding enrolment. This questionnaire allows the estimation of the mean daily consumption of 178 foods. The FFQ has been validated against 12 24-hour dietary recalls(16). The 1996 Dutch food consumption table was used to calculate energy and nutrient intakes. This table does not contain information on vitamin K contents of foods. Therefore the concentrations of phylloquinone and menaquinones (menaquinone subtypes menaquinone-4 (MK4) through menaquinone-10(MK10)) in a series of Dutch foods were assessed at the Biochemistry Laboratory, Maastricht University(2). For some foods, published data by others were used to update the dietary database for vitamin K(2,17-19). In total, vitamin K contents of 260 foods were collected and tabulated to estimate phylloquinone and menaquinones intake. We used data from our validation study to estimate reliability of the FFQ to estimate vitamin K intake against 12 24-h recalls in 58 women and 63 men(16). We observed a low relative validity of phylloquinone and MK10 intake with correlations of the FFQ against 24-hour recalls of 0.24 and 0.23, respectively. Relative validity for intake of menaquinones and MK4 to MK9 was reasonable to good with correlation ranging from 0.51 for MK7 to 0.73 for MK5. Intakes of nutrients were adjusted for energy intake by the regression residual method.

**Diabetes.** Occurrence of diabetes during follow-up was self-reported in two follow-up questionnaires with 3- to 5-year intervals. Participants were asked whether diabetes was diagnosed, in what year, and by whom and what treatment was received. In the Prospect study, incident cases of diabetes were detected via a urinary glucose strip test, sent out with the first follow-up questionnaire, for detection of glucosuria. Diagnoses of diabetes were also obtained from the Dutch Centre for Health Care Information, which holds a standardized computerized register of hospital discharge diagnoses. In this register, admission files have been entered continuously from all general and university hospitals in the Netherlands from 1990 onward. All diagnoses were coded according to the ICD-9-CM. Follow-up was complete on 1 January 2006. Potential cases identified by any of these methods were verified against participants' general practitioner or pharmacist information through mailed questionnaires. Diabetes was defined as being present when either of these confirmed the diagnosis. For 89% of participants with potential diabetes, verification information was available, and 72% were verified as having type 2 diabetes and were used for the analysis.

**Other measurements.** At baseline, participants filled in a general questionnaire containing questions on demographics, presence of chronic diseases, and risk factors for chronic diseases. Smoking was categorized into current, past and never smoker and parental history of diabetes in none, one or two parents. Systolic and diastolic blood pressure measurements were performed twice in supine position on the right arm using a Boso Oscillomat (Bosch & Son, Jungingen, Germany) (Prospect) or on the left arm using a random zero Sphygmomano-meter (MORGEN) and the mean was taken. Hypertension was defined present based on diastolic blood pressure ≥ 90
mm Hg, systolic blood pressure $\geq 140$ mm Hg, self reported use of antihypertensive medication, or self-reported presence of hypertension. Physical activity was assessed using a questionnaire validated in an elderly population, and the Cambridge Physical Activity Score was calculated and used to categorize physical activity(20). Because we could not calculate a total physical activity score for 14% of all participants, we imputed missing scores by means of single linear regression modeling (SPSS MVA procedure). Waist circumference, height and weight were measured and BMI was calculated (kg/m$^2$). A 6.5% random sample (n=2604) of the baseline cohort was drawn for more detailed biochemical measurements. The baseline characteristics of this random sample were similar to the baseline characteristics of the entire cohort(15). Thus, the random sample is representative of the full baseline cohort and can therefore be used to explore relations between risk markers and other exposures. HbA1c was measured in erythrocytes using an immunoturbidimetric latex test, total cholesterol and triglycerides were measured using enzymatic methods, high-sensitive CRP was measured with a turbidimetric method and HDL-cholesterol and LDL-cholesterol were measured using homogeneous assay with enzymatic endpoint(15).

**Data analysis.** Baseline characteristics by quartiles of energy-adjusted dietary phylloquinone and menaquinones were inspected using analysis of variance for continuous variables and a chi-square test for categorical variables. We calculated person-years of follow-up for each participant from the date of return of the questionnaire to the date of type 2 diabetes, the date of death, or January 1 2006. We used Cox regression to estimate hazard ratios (HRs) for type 2 diabetes for quartiles of either energy-adjusted phylloquinone or menaquinones intake and for each 50 $\mu$g increment of energy-adjusted phylloquinone intake and for each 10 $\mu$g increment of energy-adjusted menaquinones intake. These increments were based on an approximately half standard deviation for phylloquinone (SD: 98) and menaquinones (SD: 17). We adjusted for type 2 diabetes risk factors and dietary factors using three models. The first model was adjusted for age, gender and waist circumference. In a second model, we included smoking status (non/current/former), physical activity (4 categories), education (3 categories), hypertension, alcohol consumption and total energy intake (both continuous). In the final multivariate model, we also adjusted for diet by including energy-adjusted intake of saturated, poly- and monounsaturated fat, protein, fibre, calcium, vitamin C and vitamin E in the model (all continuous). We checked whether interaction of gender, waist and BMI with phylloquinone and menaquinones intake was present by including the interaction terms in the model. Presence of a non-linear association of phylloquinone or menaquinones intake was explored by including the quadratic term of phylloquinone and menaquinones in the model with the linear term. The possibility of a non-linear relation was further examined non-parametrically with restricted cubic splines(21). Tests for non-linearity used the likelihood-ratio test, comparing the model with only the linear term to the model with the linear and cubic spline terms. Associations between risk markers and phylloquinone and menaquinones intake (both modelled continuously per 50 and 10 $\mu$g) were assessed using linear regression using the third multivariate model in the baseline random sample. In these analyses, we excluded those with CRP $>10$ mg/L (indicating active infection; n=72) and HbA1c $>6.5\%$ (indicating presence of diabetes; n=60). Data analysis was performed using SPSS version 15.0 for Windows and SAS 9.1 for Windows.

**RESULTS**
Intake of phylloquinone was 200 ±98 µg/day and of menaquinones 31 ± 7 µg/day in our study population. Vegetables contributed 78% of phylloquinone intake, while cheese contributed 53%, milk products 19% and meat 17% of menaquinones intake. Age and energy-adjusted intake of protein were higher with higher phylloquinone and menaquinones intake, while percentage males and prevalence of physical inactivity were lower with higher intake of phylloquinone and menaquinones (table 1). With higher phylloquinone intake, we observed higher intakes of energy-adjusted fibre and vitamin C, while alcohol consumption was lower. Alcohol consumption and energy-adjusted intake of saturated fat and calcium were higher with higher menaquinones intake and prevalence of smoking was lower with higher menaquinones intake (table 1).

During a median follow-up of 10.3 years, we documented 918 verified cases of type 2 diabetes. In an age, sex and waist-adjusted model, phylloquinone intake was not associated with risk of type 2 diabetes with a hazard ratio of 1.00 (95%-CI: 0.97-1.03) for each 50 µg increment (table 2). However, adjusting for diabetes risk factors and dietary factors, quartiles of phylloquinone intake tended to be associated (p=0.08) with a reduced risk of type 2 diabetes with a hazard ratio of 0.81 (0.66-0.99) for the highest versus the lowest quartile. This association, however, remained non-significant when modeled linearly per 50 µg. This was due to presence of a non-linear relation as indicated by a significant quadratic term (p=0.016). Spline regression indeed showed evidence of a non-linear relation (p=0.053) between phylloquinone intake and type 2 diabetes with a linear risk reduction at lower levels, reaching a plateau at higher levels of intake with a hazard ratio of approximately 0.65 (figure 1).

Menaquinones intake tended to be inversely associated (p=0.060) with risk of type 2 diabetes with a hazard ratio of 0.95 (0.91-1.01) for each 10 µg increment in an age-, sex-, and waist-adjusted model. In the final multivariate model, an inverse association (p=0.038) was observed with a hazard ratio of 0.93 (0.87-1.00) for each 10 µg increment of menaquinones intake. These results were similar using quartiles of menaquinones intake. Spline regression showed a linear inverse association (p=0.035) between menaquinones intake and type 2 diabetes without evidence for a non-linear relation.

The interaction between gender and phylloquinone or menaquinones intake was not significant, nor was the interaction with waist circumference or BMI. If waist circumference was replaced with BMI in the model, similar results were observed for menaquinones (HR= 0.93; 0.86-0.99) and phylloquinone (HR= 0.98; 0.95-1.02). Adjustment for parental history of type 2 diabetes did not change the results for phylloquinone (HR= 0.99; 0.95-1.02) or menaquinones (HR= 0.94; 0.87-1.00) intake, nor did adjustment for vitamin D intake (menaquinones: HR=0.93; 0.87-1.00; phylloquinone: HR=0.98; 0.95-1.02).

In the baseline random sample (n=1604), used for hypothesis-generating analysis, HDL cholesterol tended to be higher with higher menaquinones intake (β=0.01 ±0.007; p=0.11). The HDL to total cholesterol ratio was higher with higher menaquinones intake (β=0.003 ±0.001; p=0.034). High-sensitive CRP was inversely associated with menaquinones intake (β=-0.10 ±0.04; p=0.016). None of the other risk markers (HbA1c, total, LDL-cholesterol, triglycerides) were associated with menaquinones intake. Phylloquinone intake was not associated with any of the risk markers (data not shown).

**DISCUSSION**

In this large cohort of 38,094 Dutch men and women, both dietary phylloquinone and
menaquinones intake were associated with a reduced risk of type 2 diabetes. This association was linear inverse for menaquinones, while a significant risk reduction for phylloquinone was particularly observed at higher intakes. High dietary menaquinones intake was associated with lower C-reactive protein concentrations and improved blood lipid profile. The strengths of this study include its prospective design, long follow-up, large sample size and verification of type 2 diabetes against medical records. However, certain limitations need to be addressed. The main limitation was the determination of dietary intake of phylloquinone and menaquinones intake using a FFQ. Although the FFQ was not validated for phylloquinone and menaquinones at that time, we estimated relative validity of phylloquinone and menaquinones using the data of the validation study. Relative validity of our FFQ for phylloquinone intake was low, but for menaquinones intake it was reasonable with correlation coefficients well in line with many other nutrients estimated using FFQ’s(16). In addition, FFQ’s are valid tools to rank participants according to nutritional intake, but are not designed to estimate absolute intakes. Therefore, we can only use our data to show the shape of associations, but exact amounts for a threshold cannot be determined.

Secondly, as in any observational study, our results could in part be influenced by differences in factors other than vitamin K intake. Phylloquinone and menaquinones intake are associated with different, almost opposite, lifestyle behaviours. Vegetable intake contributes mostly to phylloquinone intake and is associated with a healthy lifestyle. Residual confounding in this case could therefore lead to an inverse association between phylloquinone intake and type 2 diabetes. In contrast, consumption of meat, milk products and cheese contribute to menaquinones intake and are related to an unhealthier lifestyle and higher risk of type 2 diabetes. Residual confounding in this case would therefore attenuate our results for menaquinone intake. Although we simultaneously adjusted for several diabetes risk factors and these dietary factors in our analysis, residual confounding may be present.

Finally, relations between phylloquinone and menaquinones intake and risk markers were explored cross-sectionally in a baseline random sample. These results should therefore be regarded as hypothesis-generating to explain the relation with type 2 diabetes and not as a causal relation.
Phylloquinone intake from an FFQ has been previously reported (22).

On the other hand, the differences might also be explained by differences in distribution and metabolism of both forms. Because phylloquinone is transported with the triacylglycerol-rich fraction and menaquinones both by triacylglycerol-rich lipoprotein and low-density lipoprotein, phylloquinone is more effectively cleared from the circulation by the liver for activation of clotting factors than menaquinones (23). An intervention study indeed suggested that MK7 more effectively carboxylated osteocalcin than phylloquinone (24). However, as both forms have the same function in carboxylation of proteins, both should have similar effects that could only differ in dosage. More research is, however, needed to assess if differences are indeed present between both form and at what dosage.

The underlying mechanism for the relation between vitamin K intake and type 2 diabetes is unknown, but several pathways may be involved. Firstly, vitamin K could influence insulin sensitivity and risk of type 2 diabetes by carboxylating osteocalcin. Osteocalcin was suggested to function as a hormone in energy metabolism, regulating insulin sensitivity through an effect on adiponectin (4). However, only the uncarboxylated form seems to function hormonally in energy metabolism (4), making it unlikely to explain effects through carboxylation by vitamin K.

Osteocalcin also functions as a regulator of bone mineral maturation by binding calcium (3). Since both calcium and vitamin D insufficiency are consistently associated with an increased risk of type 2 diabetes (25), vitamin K could reduce insulin resistance and risk of type 2 diabetes through effects on calcium metabolism. However, adjusting for calcium intake and vitamin D in our study did not affect the associations. Finally, preliminary studies have shown that vitamin K influences other diabetes risk factors. Both in vitro (13) and observational (14) studies showed that vitamin K intake may decrease inflammation, which could also improve insulin sensitivity. Previous studies showed that menaquinones supplementation improved blood lipid profile (12), while phylloquinone supplementation increased triglyceride and decreased HDL cholesterol concentrations after 6 weeks (11). These studies are confirmed by our exploratory results with risk markers and could explain the inverse relation between dietary menaquinones and type 2 diabetes.

In conclusion, the findings of this study show that both phylloquinone and menaquinones intake may be associated with a reduced risk of type 2 diabetes. For phylloquinone intake, these risk reductions occurred at higher levels of intake, while for menaquinones a linear inverse association was observed.

Author contributions: Study design: JWB, DEG, YTS; Data acquisition: JWB, DLA, IS, DEG, AMS, YTS; Data analysis: JWB; Interpretation of data: JWB, DLA, IS, DEG, AMS, YTS; Writing manuscript: JWB; Editing/reviewing manuscript: JWB, DLA, IS, DEG, AMS, YTS.

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Conflict of interest: None

Figure legends:
Figure 1: Association between phylloquinone intake and risk of type 2 diabetes modelled continuously using splines; hazard ratio (———) with grey 95% confidence limits.

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Table 1. Baseline characteristics according to quartiles of energy-adjusted phylloquinone and menaquinones intake of 38,094 Dutch adults.*

| Characteristics                          | Phylloquinone | Menaquinones |
|------------------------------------------|---------------|--------------|
|                                          | Q1 (low)      | Q2           | Q3           | Q4 (high)    | Q1 (low) | Q2 | Q3 | Q4 (high) |
| Participants (n)                         | 9523          | 9524         | 9524         | 9523         | 9486     | 9557 | 9557 | 9494      |
| Phylloquinone (µg)                       | 95.7 ± 24.6   | 155.8 ± 15.1 | 213.1 ± 19.1 | 332.9 ± 82.5 | 187.1 ± 97.3 | 200.6 ± 96.8 | 205.3 ± 99.5 | 204.5 ± 98.6 |
| Menaquinones (µg)                        | 29.4 ± 14.5   | 30.3 ± 13.6  | 30.9 ± 13.3  | 31.5 ± 13.5  | 15.4 ± 3.7  | 24.5 ± 2.2  | 33.0 ± 2.8  | 49.1 ± 10.8  |
| Male gender (n (%))                      | 3204 (33.6)   | 2738 (28.7)  | 2191 (23.0)  | 1607 (16.9)  | 3509 (37.0) | 2585 (27.0) | 2033 (21.3) | 1613 (17.0)  |
| Age (years)                              | 46.3 ± 12.4   | 48.2 ± 11.8  | 49.8 ± 11.6  | 52.2 ± 10.9  | 44.9 ± 12.9 | 49.0 ± 11.9 | 50.5 ± 11.2 | 52.0 ± 10.2  |
| BMI (kg/m²)                              | 84.7 ± 11.7   | 84.8 ± 11.4  | 85.0 ± 11.2  | 85.7 ± 11.3  | 25.3 ± 3.9  | 25.7 ± 4.0  | 25.8 ± 4.0  | 25.8 ± 4.1   |
| Waist circumference (cm)                 | 84.7 ± 11.7   | 84.8 ± 11.4  | 85.0 ± 11.2  | 85.7 ± 11.3  | 84.9 ± 11.7 | 85.4 ± 11.4 | 85.2 ± 11.2 | 84.8 ± 11.4  |
| Current smoker (n (%))                   | 3167 (33.3)   | 2860 (30.1)  | 2754 (29.0)  | 2849 (30.0)  | 3376 (35.7) | 2979 (31.3) | 2707 (28.4) | 2568 (27.1)  |
| Physically inactive† (n (%))             | 1079 (11.3)   | 808 (8.5)    | 785 (8.2)    | 829 (8.7)    | 1169 (12.3) | 871 (9.1)   | 750 (7.8)   | 711 (7.5)    |
| Higher education (n (%))                 | 2257 (23.7)   | 2030 (21.3)  | 1955 (20.5)  | 1588 (16.7)  | 1676 (17.7) | 1770 (18.5) | 2024 (21.2) | 2360 (24.9)  |
| Family history of diabetes (n (%))       | 1570 (16.5)   | 1674 (17.6)  | 1759 (18.5)  | 1860 (19.5)  | 1482 (15.6) | 1783 (18.7) | 1851 (19.4) | 1747 (18.4)  |
| Systolic BP (mmHg)                       | 124.3 ± 18.5  | 125.1 ± 18.1 | 126.7 ± 19.0 | 128.1 ± 19.5 | 123.6 ± 18.0 | 126.1 ± 18.6 | 127.0 ± 19.1 | 127.3 ± 19.3 |
| Diastolic BP (mmHg)                      | 77.4 ± 10.8   | 77.6 ± 10.4  | 77.9 ± 10.6  | 78.2 ± 10.7  | 77.1 ± 10.4 | 77.9 ± 10.7 | 78.0 ± 10.7 | 78.0 ± 10.7  |
| Hypertension (n (%))                     | 3225 (33.9)   | 3307 (34.7)  | 3605 (37.9)  | 3908 (41.0)  | 3096 (32.6) | 3592 (37.6) | 3664 (38.3) | 3693 (38.9)  |
| Hyperlipidemia (n (%))                   | 771 (8.1)     | 787 (8.3)    | 748 (7.9)    | 828 (8.7)    | 958 (10.1)  | 836 (8.7)   | 738 (7.7)   | 602 (6.3)    |
| Alcohol intake‡ (g/day)                  | 5.6 (16.7)    | 5.7 (15.3)   | 5.2 (14.3)   | 4.1 (13.7)   | 4.3 (15.7)  | 5.2 (15.4)  | 5.2 (14.1)  | 5.7 (14.9)   |
| **Diet**                                 | **Energy intake (kcal/day)** | 2060 ± 649  | 2103 ± 624  | 2071 ± 612  | 1984 ± 590  | 2087 ± 669  | 2064 ± 618  | 2043 ± 590  | 2024 ± 601  |
|                                          | **Saturated fat intake (g/day)** | 31.9 ± 5.9  | 32.5 ± 5.8  | 32.8 ± 5.7  | 33.1 ± 6.1  | 29.4 ± 5.4  | 31.7 ± 5.2  | 33.2 ± 5.1  | 35.9 ± 5.8  |
|                                          | **PUFA intake (g/day)**         | 14.5 ± 3.9  | 14.9 ± 3.7  | 15.1 ± 3.8  | 15.3 ± 4.1  | 15.5 ± 4.2  | 15.2 ± 3.8  | 14.9 ± 3.7  | 14.3 ± 3.7  |
|                                          | **MUFA intake (g/day)**         | 29.2 ± 5.2  | 29.5 ± 5.0  | 29.6 ± 5.1  | 29.5 ± 5.3  | 29.0 ± 5.6  | 29.4 ± 5.1  | 29.5 ± 4.9  | 30.0 ± 5.0  |
|                                          | **Protein intake (g/day)**      | 73.5 ± 11.3 | 75.0 ± 10.4 | 76.2 ± 10.5 | 78.2 ± 11.2 | 69.0 ± 10.3 | 74.1 ± 9.4  | 77.5 ± 9.5  | 82.3 ± 10.2 |
|                                          | **Fiber intake (g/day)**        | 21.1 ± 4.6  | 22.9 ± 4.4  | 23.9 ± 4.4  | 25.5 ± 4.7  | 23.0 ± 5.3  | 23.4 ± 4.8  | 23.6 ± 4.5  | 23.4 ± 4.6  |
|                                          | **Vitamin C intake (mg/day)**   | 94.2 ± 39.6 | 107.0 ± 42.9 | 113.6 ± 44.3 | 122.8 ± 49.2 | 101.5 ± 47.2 | 108.5 ± 44.3 | 113.0 ± 43.9 | 114.6 ± 44.8 |
|                                          | **Vitamin E intake (mg/day)**   | 11.5 ± 3.2  | 12.0 ± 3.1  | 12.4 ± 3.1  | 13.0 ± 3.4  | 12.6 ± 3.5  | 12.4 ± 3.3  | 12.2 ± 3.1  | 11.7 ± 3.0  |
|                                          | **Calcium intake (mg/day)**     | 1003 ± 370  | 1050 ± 347  | 1079 ± 343  | 1130 ± 349  | 804 ± 296  | 990 ± 285  | 1127 ± 283  | 1340 ± 320  |

Abbreviations: PUFA: polyunsaturated fat; MUFA: monounsaturated fat; BMI: body mass index; CPAI: Cambridge physical activity index; BP: blood pressure.

* P-value for trend <0.001 except for waist circumference over menaquinones categories
† inactive according to CPAI
‡ median (interquartile range)
** all nutrients are energy-adjusted except energy intake.
Table 2: Energy-adjusted phylloquinone and menaquinones intake and risk of type 2 diabetes among 38,094 Dutch men and women.

|                        | Q1  | Q2  | Q3  | Q4  | P trend | per 50 µg     |
|------------------------|-----|-----|-----|-----|---------|---------------|
| Phylloquinone intake (µg/day) | 100.1 | 155.7 | 211.4 | 308.1 |         |               |
| Age-, sex-, waist-adjusted | 1.0  | 0.89 | 0.95 | 0.89 | 0.35    | 1.00 (0.97-1.03) |
| Multivariate adjusted*   | 1.0  | 0.89 | 0.94 | 0.88 | 0.26    | 0.99 (0.96-1.02) |
| Multivariate adjusted†   | 1.0  | 0.87 | 0.90 | 0.81 | 0.08    | 0.98 (0.95-1.02) |

|                        | Q1  | Q2  | Q3  | Q4  | P trend | per 10 µg    |
|------------------------|-----|-----|-----|-----|---------|--------------|
| Menaquinones intake (µg/day) | 16.0 | 24.5 | 32.9 | 46.1 |         |               |
| Age-, sex-, waist-adjusted | 1.0  | 1.03 | 0.95 | 0.86 | 0.07    | 0.95 (0.91-1.01) |
| Multivariate adjusted*   | 1.0  | 1.04 | 0.97 | 0.88 | 0.13    | 0.96 (0.91-1.02) |
| Multivariate adjusted†   | 1.0  | 0.99 | 0.89 | 0.80 | 0.04    | 0.93 (0.87-1.00) |

* Adjusted for age, sex, waist circumference, smoking status, physical activity, hypertension, education, alcohol consumption and total energy intake
† Adjusted for confounders in footnote 1 and energy-adjusted intake of saturated, poly- and monounsaturated fat, protein, fibre, calcium, vitamin C and vitamin E.
Figure 1: Association between phylloquinone intake and risk of type 2 diabetes modelled continuously using splines.

Adjusted for Sex age recr WalsL_1 CPAimp Smokestatus and other variables