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ORIGINAL ARTICLE

Steady-state vitamin K_2 (menaquinone-7) plasma concentrations after intake of dairy products and soft gel capsules

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BACKGROUND: In a previous human intervention study, we observed an improved vitamin K status after 8 weeks of intake of a yogurt that was fortified with vitamin K_2 (as menaquinone-7, MK-7) and enriched with vitamins C and D_3, magnesium and polyunsaturated fatty acids. It was hypothesized that the added nutrients contributed to this improvement. Here we report on a study in which we compared the fasting plasma concentrations of MK-7 from (a) yogurt enriched with MK-7, vitamins D_3 and C, magnesium, n-3 poly unsaturated fatty acids (n-3 PUFA) and fish oil (yogurt Kplus), (b) yogurt fortified with MK-7 only (yogurt K) and (c) soft gel capsules containing only MK-7.

SUBJECTS/METHODS: For 42 days, healthy men and postmenopausal women between 45 and 65 years of age daily consumed either yogurt K, yogurt Kplus or capsules. Circulating MK-7, 25-hydroxy vitamin D (25(OH)D) and markers for vitamin K status (uncarboxylated osteocalcin (ucOC) and desphospho-uncarboxylated matrix Gla-protein (dp-ucMGP)) were assessed. Plasma MK-7 was also measured during the washout period of 2 weeks. MK-7 and dp-ucMGP were measured in citrated plasma, and 25(OH)D_3 and ucOC were measured in the serum.

RESULTS: The increase in plasma MK-7 with the yogurt Kplus product was more pronounced than the increase in MK-7 with the capsules. Circulating dp-ucMGP and ucOC were significantly lowered after consumption of the yogurt products and the MK-7 capsules, reflecting vitamin K status improvement. No significant differences in fasting plasma concentrations of various biomarkers between the yogurts were found.

CONCLUSIONS: Dairy matrix and nutrient composition may affect MK-7 delivery and improvement of vitamin K status. Yogurt fortified with MK-7 is a suitable matrix to improve the nutritional status of the fat-soluble vitamins.

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INTRODUCTION

Dietary forms of vitamin K are phylloquinone (vitamin K_1) and the group of menaquinones (menaquinone-*n* (MK-*n*), vitamin K_2). Food sources of phylloquinone are green vegetables and several plant oils,[1–3] whereas menaquinones are primarily found in meat and egg yolk (short-chain MK-4) and in fermented foods such as cheese and curd (MK-7 through MK-10, also referred to as the long-chain menaquinones).[3] Recently, it has been shown that individuals who regularly consume dairy foods, particularly low-fat products, are less likely to develop hypertension and arterial stiffness than those with lower intakes.[4–6] The underlying mechanism is thought to be based on the vitamin K-dependent matrix Gla-protein (MGP), which is regarded as the most important local inhibitor of vascular calcification.[7–9] To acquire its calcification-inhibitory activity, MGP must be activated in a vitamin K-dependent post-translational carboxylation reaction. Therefore, vitamin K is thought to have an active role in preventing vascular calcification. Vascular vitamin K insufficiency results in increased plasma concentrations of inactive MGP, that is, desphospho-uncarboxylated MGP (dp-ucMGP), which has been recognized as a marker for vascular vitamin K status.[10–15]

Potentially, a high cheese consumption might increase vitamin K_2 serum concentrations, as cheese is a good source of vitamin K_2 (long-chain menaquinones); a drawback, however, is that most cheeses are rich in animal (saturated) fat. The pivotal role of vitamin K in vascular health has been confirmed in several population-based studies, showing an inverse association between dietary vitamin K_2 intake and the risk of cardiovascular disease.[16,17] Remarkably, this effect was most prominent for the long-chain menaquinones, whereas vitamin K_1 intake was not associated with cardiovascular disease. Therefore, attention for long-chain menaquinones is increasing worldwide, and MK-7 is marketed (mostly as a food supplement) for promoting cardiovascular and bone health.

Besides food supplements, fortified foods are a good alternative to increase the nutritional intake of MK-7 as demonstrated in a human intervention trial using MK-7-fortified yogurt.[18] This fortified yogurt has been developed to the specific nutritional needs of elderly and is enriched with MK-7 and n-3 polyunsaturated fatty acids (n-3 PUFA) from fish oil to support vascular health. High intakes of fish oil, which is one of the richest sources of n-3 PUFA, were shown to be associated with a lower risk of cardiovascular disease.[19–21] Magnesium (Mg), vitamin D_3 and the anti-oxidant vitamin C were added to support general health. Already after short-term treatment of 8 weeks, a significant improvement of the vitamin K status (as concluded from the decrease in dp-ucMGP and uncarboxylated osteocalcin (ucOC)) was seen with this yogurt.[18] Addition of the PUFAs might stimulate the uptake and delivery of the fat-soluble vitamins K and D, and, as vitamin D_3 can increase transcription of the vitamin K-dependent MGP and OC,[22,23] its addition might increase the efficacy of MK-7. Mg is required for the binding of vitamin D to its transport protein and for the conversion

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of vitamin D into its active, hormonal form 1,25(OH)2D,[24,25] which increases both calcium absorption and bone strength.[26] We therefore hypothesized that the matrix and/or the added nutrients of the dairy product significantly contributed to optimal absorption and efficacy of MK-7. To test this hypothesis, we undertook a new study in which we investigated and compared the uptake and efficacy of MK-7 from this fortified yogurt that had also been enriched with vitamins C and D_3, Mg and n-3 PUFA (as detailed in the Materials and methods section), with that of a standard MK-7-fortified yogurt and an MK-7-containing food supplement. This should allow for isolation of the effects of MK-7 from those of the addition of the other vitamins, Mg and n-3 PUFA. The comparison of the yogurts with soft gel capsules should allow for assessment of the contribution of matrix composition. We hypothesized that the fasting plasma concentrations of MK-7 after ingesting the fully enriched yogurt would be higher compared with after taking the yogurt containing only MK-7 or after taking supplements.

MATERIALS AND METHODS

Study products

The MK-7-containing soft gel capsules were commercially available (Puritan's Pride, Oakdale, NY, USA). On the basis of high-performance liquid chromatography analysis, the capsules contained 58.3 ± 1.1 μg MK-7; other ingredients were sunflower seed oil, gelatin, vegetable glycerin, natural caramel color and titanium dioxide color. The MK-7 content of both yogurts was 34 μg per carton, but taking into account that 10% of the content was not consumed (see below), the actual MK-7 intake was 30.6 μg per serving (2 servings per day). Both in the capsules and the yogurts MK-7 was MenaQ7 from NattoPharma (Høvik, Norway). The yogurt products were provided by FrieslandCampina (Wageningen, The Netherlands). The dairy products were delivered in 250 -ml-coded cartons and were delivered as complete ready-to-use end products. The yogurts were manufactured according to standard procedures. Per 100 ml, the yogurts contained 3 g of protein, 1.5 g of fat and 10 g of carbohydrates. Fully enriched yogurt also contained per 100 ml the following: n-3 PUFA (40 mg), Mg (56 mg), calcium (108 mg), vitamin C (12 mg) and vitamin D_3 (0.75 μg). The source of n-3 PUFA was Omega-360 Pure 22-3, which is a pure, natural, minimally processed, non-encapsulated marine oil rich in long-chain n-3 PUFA as eicosapentaenoic acid (10% w/w) and docosahexaenoic acid (12% w/w) from Denomega (Sarpsborg, Norway). The active biological compound MK-7 (MenaQ7) was from NattoPharma (Høvik, Norway). To verify the stability of MK-7 within the dairy products, samples of the study products were analyzed at the start and end of the intervention period. The MK-7 content of the fortified yogurts and capsules was found to be stable during the entire study.

Study design

Healthy men and postmenopausal women between 45 and 65 years of age were recruited from the southern region of Limburg, the Netherlands, through advertisements in local newspapers. Exclusion criteria were < 2 years postmenopausal, body mass index (BMI) between 20 and 30 kg/m^2, hypertension, hypercholesterolemia, metabolic or gastrointestinal diseases, chronic degenerative or inflammatory diseases, diabetes mellitus, coagulation disorders, cow's milk allergy and/or lactose intolerance, abuse of drugs and/or alcohol, use of corticosteroids, oral anticoagulants, blood pressure-lowering medication, cholesterol-lowering medication, use of vitamin K supplements and high dietary intake of vitamin K. After a final health check (interviews and questionnaires), 107 participants (43 men and 64 women) were included and randomly assigned to daily receive either two MK-7-fortified yogurts enriched with vitamin D_3, vitamin C, Mg and n-3 PUFA from fish oil (fully enriched yogurt Kplus), two standard MK-7-fortified yogurts (yogurt K) or one MK-7-containing food supplement (MK-7 soft gel capsules) for 42 days. The study had a partly single-blind and partly open-label design, that is, participants receiving the yogurts did not know whether they received yogurt K or Kplus. Measurements of the samples and statistical analyses were performed by investigators who were blinded with regard to the treatment group that the samples were from. Participants were not allowed to consume the study products together with milk or yogurt. In the period from 2 weeks before the start of the study until the final blood sampling, participants were instructed to restrict their intake of vitamin K-rich foods: no curd products, a maximum of one slice of cheese (25 g) per day and a maximum of 200 g of green vegetables. Adherence to this regimen was checked by interviews on all blood sampling days. Participants visited the research site at baseline and after 14, 28 and 42 days for measurements of body weight and height, and blood sampling. During their visits at the research site, study products were handed out to the participants for a period of 2 weeks. Remaining study products (capsules or yogurt containers) had to be returned to assess compliance. Measurements showed that on average ~10% of the yogurt was left in the containers after consumption. After the intervention period of 42 days, a washout period of 2 weeks took place in which blood was sampled at days 45, 49 and 56 (days 3, 7 and 14 of the washout period) to study potential differences in postintervention effects (return-to-baseline patterns). Participants were instructed to report any signs of illness, medication used and any deviations from the study protocol. In addition, subjects were urged not to change their level of physical exercise or alcohol consumption during the study.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Medical Ethics Committee of the Maastricht University Medical Center (Maastricht, The Netherlands). Written informed consent was obtained from all subjects before entering the study. The trial registration code on clinicaltrials.gov is NCT01873274.

Blood sampling

Venous blood was collected after an overnight fasting period of at least 12 h by venipuncture for the preparation of serum and plasma (Vacutainer, Greiner Bio-One BV, Alphen a/d Rijn, The Netherlands). All blood samples were drawn between 0800 and 1000 hours by experienced research nurses. For plasma preparation, blood (10 ml) was collected in citrate tubes, centrifuged for 15 min at 3000 *g*, subsampled and stored at − 80 °C until analysis. For serum preparation, blood (10 ml) was allowed to clot for 30 min at room temperature and centrifuged and stored as described above.

Circulating markers

Serum ucOC concentrations were determined with a commercial dual-antibody ELISA test (Takara Shuzo Co. Ltd, Otsu, Shiga, Japan). Inter- and intra-assay variations for this test were 8.3% and 5.2%, respectively. An in-house control serum pool was run on all ELISA plates. Plasma dp-ucMGP concentrations were measured by an in-house dual-antibody ELISA test with inter- and intra-assay variations of 10% and 6%, respectively.[7] A control plasma pool was run on all ELISA plates. To minimize interassay variation, different time-point samples of each subject were analyzed on the same ELISA plates. Plasma MK-7 concentrations were measured using a standard high-performance liquid chromatography technique;[3] R&D Group VitaK participates in the KEQAS quality scheme for vitamin K analysis to minimize analytical bias. Serum 25-hydroxy vitamin D (25(OH)D) was quantified using the iSYS immunoassay system based on the chemoluminescence technology (IDS, Boldon, UK). The inter- and intra-assay variations for this test were 8.3% and 12.1%, respectively. Vitamin C (ascorbic acid) levels in serum were measured with a commercial kit (Abcam, Cambridge, UK); inter- and intra-assay variations were 11% and 5%, respectively. Serum Mg concentrations were assessed routinely on a COBAS 8000 (Roche Diagnostics, Indianapolis, IN, USA) based on a colorimetric endpoint method.

Sample size

The primary outcome measure was circulating MK-7 levels in the plasma. On the basis of preliminary estimates of standard deviation in circulating MK-7 in healthy subjects of 25%, we determined that 38 participants were required in each group to have a statistical power of 80% to detect a 20% increase in the peak-absorption value of circulating MK-7 in the yogurt Kplus group, while allowing for a withdrawal of 10%.

Statistics

Normal distribution of the data was checked by normal probability and residual plots and by histograms. At baseline, one-way analysis of variance was used to analyze the differences between the treatment groups in the total group and in men and women separately, as well as the differences between men and women. For the categorical variables sex and smoking, the Pearson's χ^2 test was performed.

Multivariate linear regression was used to determine the contribution of sex (m/f), BMI, age, habitual dairy intake (cheese and curd), YSM (women only) and serum 25(OH)D concentrations on the dependent variables (circulating MK-7, ucOC, dp-ucMGP, 25(OH)D, vitamin C and Mg).

The linear mixed model for repeated measurements was used to analyze the dose–response curve for circulating MK-7 concentrations during the intervention and washout periods. Treatment regimen and time were included as fixed effects and subject was included as random effect. Bonferroni *post hoc* correction was used to adjust for multiple comparisons.

This model was also used to assess the within- and between-subjects effect after 42 days of treatment on ucOC, dp-ucMGP, 25(OH)D, vitamin C and Mg. To determine the difference between men and women, sex was also included as fixed-effect besides regimen and time.

Mean values are given ± s.d. or stated otherwise. A $P < 0.05$ (two-sided significance level) was considered to be statistically significant. SPSS 19.0 was used to perform statistical analyses (SPSS Inc., Chicago, IL, USA).

RESULTS

Baseline characteristics

Baseline characteristics of the total group of men and women separately and for each study arm are shown in Table 1. No between-group differences of the study arms were found at baseline, except for BMI in the total group ($P = 0.04$) and dp-ucMGP in the group of women ($P = 0.04$). These significances were because of the borderline significance between the capsules and yogurt K groups for BMI ($P = 0.07$) and between the yogurt Kplus and capsules groups for dp-ucMGP ($P = 0.06$).

At baseline, weight and height of the women were significantly lower compared with that in men ($P < 0.0001$), resulting in a significant lower BMI for women ($P = 0.043$). Serum ucOC concentrations ($P < 0.0001$) as well as vitamin C concentrations ($P < 0.0001$) were significantly higher in women compared with that in men.

Contributing covariates were age for dp-ucMGP (β-coefficient ± s.e.: 11.3 ± 3.5 pmol/l, $P = 0.002$) and sex for ucOC (1.3 ± 0.3 ng/ml, $P < 0.0001$) and for vitamin C (12 ± 2.4 nmol/ml, $P < 0.0001$). Serum 25(OH)D did not contribute to the outcomes of the dependent variables and was not included as covariate in the analyses. The contribution of habitual dairy (cheese and curd) intake and years since menopause were not significantly contributing covariates (data not shown).

There were no dropouts during the study, all 107 included participants completed the study. However, two participants started with interfering medication during the trial and were therefore excluded from further analyses. A few complaints ($n = 7$) arose during the study after consuming yogurt K or Kplus, namely complaints of satiated feeling, heartburn, stomach ache, abdominal cramps, diarrhea and nausea. The complaints were equally divided over both yogurt groups: four in yogurt K and three in yogurt Kplus. On the basis of counts of the remaining study products, 96 ± 3%, 97 ± 2% and 96 ± 3% compliance was reached in the soft gel capsules, yogurt K and yogurt Kplus groups, respectively.

Differences in plasma vitamin K levels after taking dairy products and capsules

In 105 participants, increased intake of MK-7 was accomplished through consumption of the study products. The consumption of the yogurt Kplus resulted in higher plasma MK-7 levels during the 42 days of intervention compared with that in the yogurt K and capsules groups (Figure 1). Mean plasma MK-7 levels during the intervention period were statistically significant between the three treatment groups: (mean estimate ± s.e.) 2.29 ± 0.08 ng/ml (yogurt Kplus), 2.17 ± 0.09 ng/ml (yogurt K) and 2.00 ± 0.09 ng/ml (capsules); $P = 0.047$. This was due to the difference between the yogurt Kplus and the capsules group ($P = 0.042$). The differences in MK-7 levels between both yogurt groups and between the yogurt K and capsules groups were not significant ($P = 0.95$ and $P = 0.48$, respectively).

The highest increase in plasma MK-7 was seen during the first 14 days of treatment (2.19 ± 0.11 ng/ml, $P < 0.0001$). In the next 28 days, the increase in MK-7 was less pronounced: between 14 and 28 days 0.18 ± 0.15 ng/ml ($P = 1.0$) and between 28 and 42 days 0.18 ± 0.17 ng/ml ($P = 0.18$), reaching a level of 2.92 ± 0.13 ng/ml. After 14 days of postintervention (day 56), plasma MK-7 had

Table 1. Baseline characteristics of the total group and the study groups

	Total group	*Capsules*	*Yogurt Kplus*	*Yogurt K*
Sex (M/F)				
Total	107	37	36	34
M	43	15	15	13
F	64	22	21	21
Age (years)				
Total	57 ± 6	57 ± 6	57 ± 6	57 ± 6
M	57 ± 7	56 ± 8	56 ± 7	58 ± 6
F	57 ± 5	57 ± 6	57 ± 5	58 ± 5
Weight (kg)				
Total	74 ± 12	71 ± 11	77 ± 13	75 ± 13
M	83 ± 12	78 ± 7	86 ± 12	86 ± 15
F	68 ± 9***	65 ± 8***	70 ± 9***	70 ± 9**
Height (m)				
Total	1.71 ± 0.09	1.69 ± 0.08	1.72 ± 0.09	1.70 ± 0.09
M	1.78 ± 0.06	1.76 ± 0.04	1.80 ± 0.07	1.79 ± 0.06
F	1.66 ± 0.06***	1.65 ± 0.06***	1.66 ± 0.01***	1.65 ± 0.07***
BMI (kg/m^2)				
Total	25.4 ± 2.8	24.4 ± 2.4	25.8 ± 2.5	26.0 ± 3.2
M	26.1 ± 2.6	25.2 ± 2.0	26.4 ± 2.8	26.6 ± 2.9
F	24.9 ± 2.8*	24.0 ± 2.6	25.3 ± 2.2	25.6 ± 3.3
Smoking (n)				
Total	12	6	3	3
M	5	2	1	2
F	7	4	2	1
Circulating markers				
ucOC (ng/ml)				
Total	4.1 ± 1.5	4.2 ± 1.6	4.0 ± 1.7	4.1 ± 1.3
M	3.3 ± 1.5	3.7 ± 1.5	2.8 ± 1.5	3.7 ± 1.4
F	4.6 ± 1.3***	4.6 ± 1.5	4.8 ± 1.2***	4.4 ± 1.2
dp-ucMGP (pmol/l)				
Total	523 ± 207	489 ± 181	582 ± 225	490 ± 202
M	528 ± 203	529 ± 228	538 ± 155	513 ± 245
F	519 ± 211	464 ± 144	613 ± 263	477 ± 179
MK-7 (ng/ml)				
Total	0.40 ± 0.23	0.46 ± 0.23	0.38 ± 0.19	0.35 ± 0.26
M	0.37 ± 0.21	0.43 ± 0.19	0.35 ± 0.23	0.32 ± 0.19
F	0.41 ± 0.24	0.46 ± 0.25	0.41 ± 0.17	0.37 ± 0.30
25(OH)D (ng/ml)				
Total	32 ± 9	34 ± 10	30 ± 7	32 ± 9
M	33 ± 10	34 ± 12	31 ± 6	31 ± 10
F	32 ± 8	33 ± 8	30 ± 8	33 ± 9
Vitamin C (nmol/ml)				
Total	43 ± 13	41 ± 13	45 ± 12	44 ± 12
M	36 ± 11	35 ± 12	38 ± 11	36 ± 10
F	48 ± 11***	45 ± 13*	50 ± 10**	49 ± 11**
Mg (mmol/l)				
Total	0.87 ± 0.05	0.87 ± 0.05	0.86 ± 0.05	0.88 ± 0.06
M	0.88 ± 0.05	0.87 ± 0.05	0.88 ± 0.05	0.88 ± 0.05
F	0.87 ± 0.06	0.87 ± 0.06	0.87 ± 0.07	0.86 ± 0.05

Abbreviations: BMI, body mass index; dp-ucMGP, desphospho-uncarboxylated matrix Gla-protein; Mg, magnesium; MK, menaquinone; 25(OH)D, 25-hydroxy vitamin D; ucOC, uncarboxylated osteocalcin. All values are means ± s.d. Differences between men and women: *$P < 0.05$, **$P < 0.005$ and ***$P < 0.0001$.

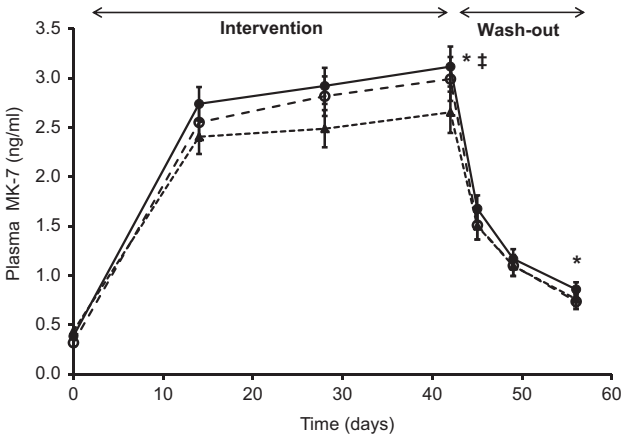

Figure 1. The effect on plasma MK-7 levels during and after consumption of MK-7-enriched dairy products and MK-7 capsules. Plasma MK-7 levels were assessed during the intervention period of 42 days and the washout period of 14 days in the yogurt Kplus group (closed circles), yogurt K group (open circles) and in the capsules group (triangles). *Significant increase between baseline and 42 days of intervention ($P < 0.0001$) and between baseline and after 14 days washout ($P < 0.0001$). ‡Significant effect between treatment regimens ($P < 0.05$). Error bars represent s.e.

decreased to 0.79 ± 0.05 ng/ml, still being significantly higher compared with that at the start ($P < 0.0001$).

Differences in vitamin K status after taking dairy products and capsules

After 42 days of treatment, overall plasma dp-ucMGP had decreased to 445 ± 18 pmol/l ($P = 0.005$): in the capsules group to 434 ± 31 pmol/l, in the yogurt Kplus group to 485 ± 30 pmol/l and in the yogurt K group to 417 ± 33 pmol/l. Between-group comparisons showed an overall significant effect of the treatment regimens on plasma dp-ucMGP ($P = 0.019$). The mean estimates were 535 ± 22 pmol/l (yogurt Kplus), 450 ± 24 pmol/l (yogurt K) and 464 ± 23 pmol/l (capsules). The mean change of plasma dp-ucMGP in the yogurt Kplus and the yogurt K group was statistically significant ($P = 0.030$) and that between the yogurt Kplus and the capsules group was borderline significant ($P = 0.078$). No effect was seen between capsules and yogurt K treatment ($P = 1.0$).

The overall change in circulating ucOC after 42 days of treatment was −0.48 ± 0.19 to 3.6 ± 0.1 ng/ml ($P = 0.012$). For ucOC, no significant differences were found between the estimated group means: 3.8 ± 0.2 ng/ml (yogurt Kplus), 3.9 ± 0.2 ng/ml (yogurt K) and 4.0 ± 0.2 ng/ml ($P = 1.0$).

Effects of yogurt Kplus on circulating 25(OH)D, vitamin C and Mg concentrations

At baseline, the mean serum 25(OH)D concentrations were >30 ng/ml (75 nmol/l) in the three groups (Table 1), indicative of vitamin D sufficiency. After 42 days of treatment, the overall 25 (OH)D concentration had increased by 5.3 ± 1.2 to 37.4 ± 0.8 ng/ml ($P < 0.0001$). The mean estimates were 34.5 ± 1.0 ng/ml (yogurt Kplus), 34.0 ± 1.1 ng/ml (yogurt K) and 35.6 ± 1.0 ng/ml (capsules). No effect was found between the treatment groups ($P = 0.52$).

The addition of vitamin C and Mg to the yogurt Kplus did not result in significant differences after 42 days of treatment ($P = 0.46$ and $P = 0.69$ respectively). However, a statistical difference was found for circulating vitamin C in the various treatment groups ($P = 0.042$), due to the (borderline) significance of the changes in circulating vitamin C of the yogurt Kplus group and the capsules group (5 ± 2 nmol/ml, $P = 0.057$). No effect on circulating Mg was seen between the treatment regimens ($P = 0.49$).

Differences between men and women

The overall mean plasma MK-7 concentration in the group of women during the 42 days of treatment was 2.3 ± 0.06 ng/ml and in men 1.9 ± 0.08 ng/ml ($P < 0.0001$). In the group of women, the change in plasma MK-7 between the yogurt Kplus and the capsules was more pronounced (0.41 ± 0.15 ng/ml, $P = 0.025$) compared with that between both yogurt regimens (0.33 ± 0.15 ng/ml, $P = 0.11$) and between yogurt K and capsules (0.08 ± 0.16 ng/ml, $P = 1.0$). In the group of men, the changes in plasma MK-7 were not statistically significant between the treatment groups ($P = 0.26$).

In women, overall dp-ucMGP decreased by 78 ± 35 pmol/l ($P = 0.026$), whereas in men the decrease was 71 ± 46 pmol/l ($P = 0.13$) after 42 days. The differences in plasma dp-ucMGP between the three treatment regimens were statistically significant in women ($P = 0.020$) but not in men ($P = 0.83$). The significant effect among women was because of the difference in dp-ucMGP between the yogurt Kplus group and the capsules group (105 ± 42 pmol/l, $P = 0.039$) and between both dairy products (102 ± 43 pmol/l, $P = 0.057$).

Overall serum ucOC after treatment was higher in women compared with that in men (4.4 ± 0.1 and 3.1 ± 0.2 ng/ml, respectively, $P < 0.0001$). Within the group of women, ucOC decreased significantly by 0.5 ± 0.2 ng/ml ($P = 0.040$), whereas in men the decrease was not significant (0.5 ± 0.3 ng/ml, $P = 0.15$). Between treatment effects were not significant for women ($P = 0.67$) nor for men ($P = 0.16$).

DISCUSSION

In this paper, we compare the fasting plasma concentrations of MK-7 after oral intake using three delivery systems: an MK-7-fortified yogurt also enriched with Mg, vitamins D_3 and C and n-3 PUFA (yogurt Kplus), a standard MK-7-fortified yogurt (yogurt K) and an MK-7-containing supplement (soft gel capsules). Uptake of MK-7 both from capsules and from fortified yogurts resulted in an increase in plasma MK-7. Highest plasma MK-7 concentrations were found in the Kplus group; consistently, the improvement of MGP carboxylation was largest in this group, but it should be noticed that the effect was mainly because of the response in women. The decline of postintervention concentrations during the washout period showed a biphasic decline of MK-7 with a plasma half-life time of about 3 days during the first phase and over 8 days during the second phase. Two weeks after intervention, plasma MK-7 concentrations were still significantly higher compared with the corresponding baseline concentrations. In general, the yogurts were well tolerated, only seven participants had minor complaints, which might have resulted from increased sensitivity of the intestinal system by the relatively large volume of yogurt consumption (~500 ml per day) during a relatively long study period.

A recently published intervention study with MK-7-containing supplements showed similar circulating concentrations as reported here, but higher daily doses (90 and 180 μg) of MK-7 were required to reach these concentrations.[27] It should be noted that the participants in that study were young adults instead of older adults as in the study presented in this paper. It cannot be excluded that the older age of our population positively affected absorption of MK-7, as was shown before for phylloquinone.[28] Next to higher phylloquinone plasma and intake levels in elderly, Booth *et al.*[29] showed a higher response to phylloquinone-rich oil in older (60–80 years) as compared with younger (20–40 years) adults. In another study by Booth *et al.*,[29] this age effect of higher plasma phylloquinone area under the curve values in older adults disappeared when plasma phylloquinone concentrations were adjusted for the higher TAG concentrations, suggesting that the higher fasting plasma concentrations observed in elderly reflect the increase in TAG concentrations with age. Increasing age with

concomitant higher TAG concentrations seems to be an important factor influencing response to vitamin K intake. A recent study showed MK-7 concentrations of ~8 ng/ml in young adults after a 7-day administration of capsules consisting of 60 μg MK-7.[30] Such high concentrations of serum vitamin K_2 after nutritional doses have not been shown before, although direct comparison with our results is difficult because results on separate MK forms are lacking. Another study in adults using comparable nutritional doses of 45 and 90 μg MK-7 with a fortified olive oil showed MK-7 concentrations of 1.28 and 2.47 ng/ml, respectively, which is more in line with our results.[31]

Significant differences in fasting MK-7 plasma concentrations were found after 14, 28 and 42 days between the yogurts and the soft gel capsules in the complete study population. This is the first study demonstrating higher MK-7 steady-state concentrations after intake of a fortified food than a standard food supplement in a direct comparison. Both dairy products showed higher plasma concentrations and postintervention profiles of MK-7 in comparison with soft gel capsules, whereas the fully fortified yogurt performed slightly better than the MK-7-fortified yogurt. In terms of functionality, all test products significantly decreased plasma dp-ucMGP and serum ucOC, reflecting vascular and bone vitamin K status improvement, respectively. The fully enriched test product was significantly more effective in lowering dp-ucMGP compared with MK-7 capsules. No significant differences in change of dp-ucMGP and ucOC between yogurt K and capsules, nor between yogurt K and Kplus, were observed. In this study, women were better responders to MK-7 supplementation than men as shown by significantly larger increases in plasma MK-7 concentrations during intervention. Earlier studies showed no significant differences in vitamin K bioavailability between men and women.[29,32]

Vitamin D has important roles in calcium homeostasis and skeletal health. Its serum concentrations are strongly affected by nutritional vitamin D intake.[33,34] Vitamin D_3 was well absorbed from yogurt Kplus, as concluded from the significantly increased serum 25(OH)D concentrations. Also, in both other groups, there was a minor increase of serum vitamin D (possibly due to seasonal effects), but this increase was substantially lower than in the vitamin D-treated group (8 ng/ml vs 3 ng/ml).

We had hypothesized that addition of the PUFAs to yogurt Kplus might facilitate the uptake and delivery of the fat-soluble vitamins K and D, whereas Mg is known to be required for the conversion of vitamin D to its active form, $1,25(OH)_2D_3$. Although these additions did not result in significantly higher plasma concentrations of MK-7, the change in plasma dp-ucMGP, reflecting vascular vitamin K status, was more pronounced with the fully enriched yogurt Kplus and both the yogurt K group (significant) and the capsules group (borderline significant), whereas the change in dp-ucMGP in the yogurt K and the capsules group was comparable. This indicates that the additions in the fully enriched yogurt either improve the efficacy of MK-7 supplementation or have an independent additive effect.

Our study has some limitations. First, no data were available on the background diet of the participants. We collected information on their intake of vitamin K-containing food products but not on their general dietary habits. Intake of vitamin K-rich foods was restricted from 2 weeks before the intervention and during the intervention period to limit interference of background dietary vitamin K. Furthermore, our study population was limited to men and postmenopausal women between 45 and 65 years, and it is not certain that our findings can be extrapolated to other age groups. Although women were found to be better responders than men, it should be noted that the study was not powered to study differences between men and women. Finally, we have not measured TAGs for three reasons: (1) postprandial uptake of TAGs in the circulation peaks at 4 h after food intake, whereas we collected blood after an overnight fast and no substantial differences could be expected; (2) those who did not consume yogurt (capsules group) have replaced the daily 500 ml of yogurt by different foods, which also may have contained fats; (3) in two comparable studies that were completed last year, we did not find a measurable effect of 500 ml of yogurt on the blood lipid profile.[18]

In conclusion, dairy matrix and nutrient composition may affect MK-7 delivery and improvement of vitamin K status. Yogurt fortified with MK-7 is a suitable matrix to improve the nutritional status of this fat-soluble vitamin.

CONFLICT OF INTEREST

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