Novel effects of diets enriched with corn oil or with an olive oil/sunflower oil mixture on vitamin K metabolism and vitamin K-dependent proteins in young men

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Abstract **Little is known of how the fat components of diets influence the absorption and metabolism of vitamin K and the possible consequences to the synthesis of vitamin K-dependent (VKD) proteins in different target organs. We have evaluated the effects of two diets on circulating phylloquinone (K_1) and triacylglycerols (TAG). One diet was enriched with corn oil (CO) (also rich in γ-tocopherol) and the other with an olive/sunflower (O/SO) mixture (rich in α-tocopherol). Effects on γ-carboxylation were assessed from coagulation assays and sensitive assays for undercarboxylated prothrombin (ucFII) and osteocalcin (ucOC). Total plasma matrix Gla-protein (MGP) was also measured. After an initial adjustment diet, 26 healthy young men were fed, in a crossover design, the O/SO or CO diet for 2 weeks. Mean intakes of K_1 during consumption of adjustment, O/SO, and CO diets were 225 μg/day, 291 μg/day, and 291 μg/day, respectively. Mean fasting levels of TAG and K_1 were both significantly reduced by the CO diet, but not by the O/SO diet. Neither diet reduced FII activity but ucFII became detectable in nine subjects, eight of whom showed this abnormality with both diets. The CO diet induced a rise in ucOC ($P < 0.05$), which was negatively correlated to ucFII ($r = -0.71$, $P < 0.03$). The CO but not O/SO diet induced a decrease of total circulating MGP. We conclude that both oils, notably CO, affected vitamin K absorption and/or metabolism which may increase the requirements for γ-carboxylation. The mechanism is unclear but may result from interactions of vitamin K with PUFA and/or other lipid components such as vitamin E.**—Schurgers, L. J., M. J. Shearer, B. A. M. Soute, I. Elmadfa, J. Harvey, K-H. Wagner, R. Tomasch, and C. Vermeer. **Novel effects of diets enriched with corn oil or with an olive oil/sunflower oil mixture on vitamin K metabolism and vitamin K-dependent proteins in young men.** ***J. Lipid Res.*** **2002.** 43: **878–884.**

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The nutritional need for vitamin K is most commonly associated with the four vitamin K-dependent (VKD) coagulation factors (FII, FVII, FIX, and FX) that are all synthesized in the liver (1). Other VKD proteins are synthesized in extrahepatic tissues. They include the bone protein osteocalcin (OC), and matrix Gla-protein (MGP), which has mRNA that is expressed by various tissues and cell types. There is good evidence that OC plays a regulatory role in bone turnover (2) and that MGP is essential to prevent calcification of arteries (3). All these proteins require vitamin K for a post-translational modification in which selective glutamate residues are transformed to γ-carboxyglutamate (Gla) residues. The necessity of Gla residues for functional activity is known for the VKD coagulation proteins (1, 4) and is probable for others. When the supply of vitamin K is insufficient or when there is a metabolic blockade (e.g., by oral anticoagulants), undercarboxylated species of Gla-proteins are released into the circulation and provide a measure of the vitamin K status at their site of synthesis (5).

The major dietary and circulating form of vitamin K is phylloquinone (vitamin K_1) (1). After intestinal absorption, phylloquinone, like other fat-soluble vitamins, is carried via the chylomicron pathway. However, a major difference from other fat-soluble vitamins is that triacylglycerol-rich (TAG-rich) lipoproteins continue to be the major carriers of phylloquinone in both the postprandial (6) and fasting (7) states. This association with TAG-rich lipoproteins is

Abbreviations: AU/l, arbitrary units per liter; CO, corn oil; K1, phylloquinone; MGP, matrix Gla-protein; O/SO, olive/sunflower oil; PT, prothrombin times; TAG, triacylglycerols; ucOC, undercarboxylated osteocalcin; ucFII, undercarboxylated prothrombin; VKD, vitamin K-dependent.

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reflected in a positive correlation of plasma phylloquinone and triacylglycerol concentrations (8–10). This suggests that dietary and other factors that influence the metabolism of TAG-rich lipoproteins may also affect the transport, tissue distribution, and metabolism of vitamin K, possibly affecting γ-carboxylation of the target Gla proteins. Indeed it was recently suggested that decreases in FII and FVII in rats produced by diets enriched with n-3 PUFA may have resulted from a lipid-lowering effect which affected the lipoprotein transport and delivery of vitamin K to the liver (11).

As far as we are aware there have been no human studies that have directly examined whether dietary manipulation of the fat components of diets can influence the intestinal absorption and/or metabolism of vitamin K and thereby the γ-carboxylation of VKD proteins. The present study was facilitated by our access to a dietary cross-over study in healthy young men that had initially been designed to compare the effects of γ-tocopherol-rich corn oil (CO) and α-tocopherol-rich olive/sunflower (O/SO) oil on DNA damage in healthy young men (12). This study was also of interest to us because high dietary intake of vitamin E has been associated with an inhibitory effect on vitamin K action (13). Apart from measurements of plasma phylloquinone, vitamin K status was assessed from functional assays of VKD coagulation proteins, sensitive assays for undercarboxylated prothrombin (14), and undercarboxylated osteocalcin (15), and a new assay for total MGP (16).

MATERIALS AND METHODS

Subjects and study design

Twenty-six healthy men (aged 19–31 years) were recruited from the population of Vienna. None were taking medications or vitamin supplements for at least 2 weeks before entering the study. During an randomly assigned initial 2-week period all subjects consumed the same adjustment diet followed by diets enriched with either an olive/sunflower oil mixture (O/SO diet) or corn oil (CO diet) in a cross-over design of 2 weeks per diet. Weighed dietary records were kept for each participant for the entire study period to calculate nutrient intakes. Venous blood samples were collected in trisodium citrate (after an overnight fast) at 2-weekly intervals coinciding with the end of each dietary regime. Plasma samples were stored in aliquots at −80°C until analysis. The study protocol was approved by the Medical Ethics Committee of the University of Vienna and all subjects gave their informed consent in writing.

Dietary oils and experimental diets

The sources of the oils used for the diets were: corn oil, CPC-Bestfoods (Heilbronn, Germany); olive oil, Linea Natura (Milan, Italy); sunflower oil, Nestle (Karlsruhe, Germany). Their SAFA-MUFA-PUFA compositions were: olive oil 14:77:9 (v/v/v), sunflower oil 12:24:64 (v/v/v), and corn oil 13:33:54 (v/v/v). Their vitamin E contents per 100 g oil were: olive oil 20 mg α-tocopherol and 1.7 mg γ-tocopherol, sunflower oil 85.3 mg α-tocopherol and 8.8 mg γ-tocopherol, and corn oil 24.6 mg α-tocopherol and 126.2 mg γ-tocopherol (12). The K_1 contents of olive, sunflower, and corn oil were 54.8, 5.7, and 2.7μg per 100 g, respectively.

All food was prepared at the Institute of Nutritional Sciences of the University of Vienna as three daily meals and offered to the subjects in the presence of one of the investigators. On weekdays all subjects ate their mid-day meal in the Institute. Breakfast and evening meals were prepacked and given to the subjects on a daily basis. Weekend meals were also prepared beforehand and given to the subjects on Fridays. The subjects reported no side effects while on these diets and all maintained their weight throughout the study. Also, there is strong experimental evidence of compliance from the measured changes in plasma concentrations of α-tocopherol and γ-tocopherol in the same study and published separately (12). Typical foodstuffs for breakfasts comprised rye and wheat bread, muesli, buttermilk, and occasionally yogurt and fruit. Lunches comprised soup, fish or meat with vegetables, and usually a fresh salad. Common food items for evening meals were bread, yogurt, fruit, and cheese.

The composition of the diets is shown in **Table 1**. The adjustment diet provided the daily intakes of 35 g olive oil, 4.4 g sunflower oil, and 13.8 g of butter. The O/SO and CO test diets were identical apart from their different fat contents. The O/SO diet provided the daily intakes of 68 g olive oil and 12 g sunflower oil (SAFA-MUFA-PUFA = 28:49:23, v/v/v) and the CO diet the equivalent intake of 80 g corn oil (SAFA-MUFA-PUFA = 29:33:38, v/v/v). The 80 g total daily intakes of dietary oils represented 73% of the total daily fat intake. Both O/SO and CO diets provided similar intakes of α-tocopherol but the CO diet provided a high intake of γ-tocopherol (100 mg/day) compared with the O/SO diet (2.4 mg/day) (Table 1). Dietary intakes of phylloquinone (K_1) were calculated from the weighed dietary records of the participants and the K_1 content of foods taken from newly available databases from The Netherlands and the UK (17–19). Based solely on the K_1 values for their component oils the O/SO and CO diets provided daily intakes of 15.4 μg and 2.8 μg K_1, respectively. However, this difference was negligible compared with the intakes of K_1 from other food components, especially green leafy vegetables. With the cross-over design the average daily intakes of vitamin K were closely similar for both groups, but there was considerable day to day variation (mean: 291 μg, SD ± 213), which was due to the variability of consumption of green vegetables. The average daily intakes of K_1 calculated for each phase of the study were 225 μg (adjustment phase), 238 μg (cross-over phase I), and 343 μg (cross-over phase II).

Various assays

All biochemical analyses were performed in duplicate, and mean values are given throughout this paper. Prothrombin times (PT) were determined by automated assay using Thromborel S (Behringwerke, Marburg, Germany) as thromboplastin reagent.

TABLE 1. Composition of the diets

Diet Content	Adjustment Diet	Olive/ Sunflower Oil Diet	Corn Oil Diet
Mean energy (MJ)	11.6	11.6	11.6
Carbohydrate (energy%)	50–55	50–55	50–55
Protein (energy%)	15	15	15
Fat (energy%)	30–35	30–35	30–35
SAFA (energy%)	11–13	8–10	9–10
MUFA (energy%)	13–15	15–17	10–12
PUFA (energy%)	6–7	7–8	11–13
α-Tocopherol (mg/day)	12	24	20
γ-Tocopherol (mg/day)	—	2.4	100
Phylloquinone (μg/day)	225	291	291

Table shows the range of daily consumption of macronutrients and the mean daily consumption of tocopherol and phylloquinone (vitamin K_1) micronutrients.

FII concentrations were assessed assay using Thromborel S and human clotting factor II-deficient plasma from Behringwerke. Species of undercarboxylated FII (ucFII) were measured using a conformation-specific monoclonal antibody in an ELISA-based assay (14). Results are expressed as arbitrary units per liter (AU/l) because in states of vitamin K deficiency circulating ucFII may comprise multiple forms of partially carboxylated FII and neither their relative abundance in plasma nor their relative affinity for the antibody is known. Using electrophoretic techniques 1 AU is equivalent to 1 μg of purified ucFII (14). The detection limit was 150 AU/l plasma.

Total immunoreactive OC was measured using the two-site N-mid hOsteocalcin ELISA (Osteometer A/S, Copenhagen, Denmark). ucOC was determined with the Glu-OC kit from Takara Shuzo (Tokyo, Japan). MGP was determined with an ELISA-based assay recently developed at the Maastricht Biochemistry Department (16). Serum MGP concentrations were calculated with the aid of a reference curve from pooled normal serum, and expressed as AU/l.

Plasma K_1 concentrations were measured by HPLC and fluorescence detection after on-line, post column electrochemical reduction of the effluent, which converted the quinone forms of vitamin K compounds to their fluorescent quinol forms (20). Plasma TAG concentrations were determined by an automated enzymatic procedure using commercial reagents (Boehringer Mannheim, Germany) and a Beckmann Synchron CX 7-2 autoanalyser (Fullerton, CA).

Statistical analysis

Blood samples taken at the end of the adjustment phase, cross-over phase I, and cross-over phase II, and were analyzed as repeated measurements in the cross-over design to determine whether there were any significant trends. The analysis was used to examine statistical differences between both the oil-regimens and the adjustment phase or between both oil-regimens themselves. The statistical significance of differences between the oil-regimens and the adjustment phase were examined using the SPSS 9.0 Student's paired *t*-tests (two-tailed). Differences between the regimens in either cross-over phase I or cross-over phase II were examined with the Wilcoxon matched pairs test. Differences were considered to be statistically significant at $P < 0.05$.

RESULTS

The results of coagulation assays together with plasma concentrations of TAG, K_1, and VKD proteins at the end of each dietary period are shown in **Table 2**. Compared with the adjustment phase diet, plasma levels of both TAG and K_1 were significantly lowered by the CO diet ($P < 0.01$ for TAG, $P < 0.05$ for K_1) but not by the O/SO diet. The effect of both diets on hepatic synthesis of VKD procoagulants was assessed by three different assays with varying sensitivities: the PT (an overall coagulation assay that is insensitive to large decreases in the Gla-content of VKD coagulation factors) (11), the FII assay (specific for functional Gla forms of prothombin), and an in-house ELISA to detect low concentrations of ucFII. No diet-induced change in hepatic VKD coagulation proteins was detected by either the PT or FII assay. After the adjustment diet, ucFII was below the limit of detection in all subjects but elevated levels were found in eight subjects (also defined as ucFII positive) after the O/SO diet, and in nine subjects after the CO diet (**Fig. 1**). Eight of these had an elevated ucFII after both diets that first presented at the end of the first dietary cross-over phase (phase I) with the O/SO diet and which continued to remain elevated during phase II while taking the CO diet (subject group B in Table 2). One subject showed an elevated ucFII at the end of cross-over phase I while taking the CO diet, which then dropped below the detectable limit after phase II with the O/SO diet. In the remaining 17 subjects ucFII remained undetectable throughout the study.

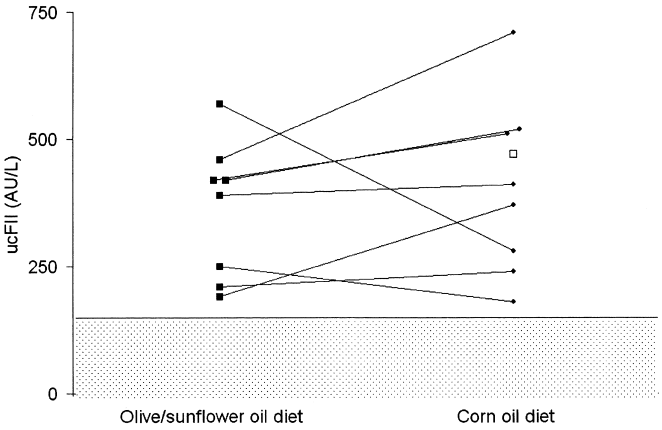

Fig. 1. Diet induced increase of undercarboxylated factor II (ucFII). Closed square, positive responders after the olive/sunflower (O/SO) diet; closed circle, positive responders (paired samples) after the corn oil (CO) diet (connecting lines indicate same subjects); open circle, subject only positive in the CO diet. The shaded area shows the lower detection limit (150 arbitrary units per liter).

In the ucFII positive subjects, there was no significant difference in the magnitude of ucFII after O/SO or CO diets (Table 2), although in six of eight subjects who had an elevated ucFII after phase I (O/SO diet), the levels had increased slightly by the end of phase II (CO diet) (Fig. 1).

There was no correlation between ucFII and plasma K_1 concentrations: for the O/SO diet the mean K_1 concentrations in subjects with undetectable or detectable ucFII were 0.53 ± 0.16 nmol/l (n = 18) and 0.63 ± 0.47 nmol/l (n = 8), respectively ($P = 0.57$). Similarly, for the CO diet, the mean plasma K_1 concentrations were 0.39 ± 0.18 nmol/l in ucFII negative subjects (n = 17) and 0.48 ± 0.31 nmol/l in ucFII positive subjects (n = 9) ($P = 0.47$).

The effect of both diets on the synthesis of extrahepatic VKD proteins was assessed for OC and MGP. Neither diet affected circulating concentrations of total immunoreactive OC. However, differences were seen with a monoclonal-based assay specific for ucOC. When absolute concentrations of ucOC were compared there were no significant differences between the O/SO and CO diets, but the mean plasma ucOC concentrations after the CO diet were significantly higher compared with the adjustment phase. When ucOC concentrations were expressed as a percentage of total OC, the increases were magnified and, compared with the adjustment diet, the %ucOC was significantly different for both CO and O/SO diets (Table

TABLE 2. Effects of O/SO and CO tests diets on vitamin K-dependent proteins, vitamin K, and triglycerides

	Adjustment Diet			O/SO Test Diet			CO Test Diet		
Measurement	All Subjects	Group A	Group B	All Subjects	Group A	Group B	All subjects	Group A	Group B
Prothrombin time (s)	14.4 ± 0.8	14.3 ± 1.0	14.4 ± 0.7	14.2 ± 0.9	14.0 ± 1.0	14.5 ± 0.9	14.5 ± 0.8	14.8 ± 0.9	14.1 ± 0.4
Factor II (% normal)	88.7 ± 7.5	88.7 ± 7.6	88.8 ± 7.7	92.1 ± 9.7	89.6 ± 10.0	94.6 ± 9.0^{b}	90.9 ± 11.8	89.2 ± 13.5	92.7 ± 10.1^{a}
ucFII (AU/l)	<150	<150	<150	220 ±120^{b}	<150	280 ±150^{b}	240 ±160^{b}	180 ± 90	310 ± 180^{b}
ucFII (prevalence)	0/26	0/13	0/13	8/26	0/13	8/13	9/26	1/13	8/13
Total osteocalcin (μg/l)	22.3 ± 12.9	23.5 ± 13.7	21.2 ± 12.5	19.1 ± 9.1	18.6 ± 8.7^{a}	19.5 ± 9.9	18.6 ± 10.5^{a}	16.4 ± 8.4^{b,c}	20.8 ± 12.3
ucOC (μg/l)	5.9 ± 2.4	6.0 ± 2.4	5.8 ± 2.5	6.3 ± 2.4	6.8 ± 2.8	5.9 ± 1.9	6.3 ± 2.3^{a}	6.3 ± 2.3	6.3 ± 2.3
ucOC (%)	30.2 ± 11.6	28.3 ± 8.9	32.0 ± 14.2	35.9 ± 12.2^{b}	38.7 ± 14.4^{b}	33.0 ± 9.4	38.5 ± 13.9^{b}	42.9 ± 16.3^{b}	34.0 ± 9.6
MGP (U/l)	93 ± 8	92 ± 9	94 ± 9	98 ± 10	94 ± 11	101 ± 9	86 ± 11^{b,d}	88 ± 12	83 ± 10^{a,d}
Vitamin K_1 (nmol/l)	0.59 ± 0.37	0.64 ± 0.35	0.54 ± 0.40	0.56 ± 0.29	51 ± 0.18	0.61 ± 0.37	0.42 ± 0.23^{a,c}	0.43 ± 0.19	0.41 ± 0.27^{c}
Triglycerides (mmol/l)	0.85 ± 0.46	0.91 ± 0.44	0.79 ± 0.49	0.79 ± 0.44	0.72 ± 0.33	0.86 ± 0.53	0.64 ± 0.34^{b,c}	0.57 ± 0.30^{b,c}	0.71 ± 0.38

The results (mean ± SD) are shown for measurements made at the end of each 2-week dietary phase after the subjects had received the adjustment, the olive/sunflower oil (O/SO), and corn oil (CO) diets, respectively. Values for each diet are shown for all subjects after completion of the cross-over study and under groups A and B according to the sequence in which the two randomized groups of 13 subjects received the O/SO and CO test diets after the adjustment diet. Subjects in group A received the CO diet in phase I and the O/SO diet in phase II while subjects in group B received the O/SO diet in phase I and the CO diet in phase II of the cross-over design. Significant differences between adjustment diet and either O/SO or CO diets are denoted by $^{a}P < 0.05$, and $^{b}P < 0.01$. Significant differences between O/SO and CO diets are denoted by $^{c}P < 0.05$, and $^{d}P < 0.01$.

2). Suggestive for a differential carboxylation of FII in the liver and OC in bone was the observation that for both O/SO and CO diets, the mean ucOC concentrations in ucFII positive subjects were 20% lower than in ucFII negative subjects (ucOC 5.4 μg/l vs. 6.8 μg/l in positive and negative subjects respectively). This relatively small difference did not attain statistical significance, however. A test of the degree of correlation between ucOC and ucFII concentrations was only possible in about a third of the subjects with detectable ucFII, and as shown in **Fig. 2**, for the CO diet there was a negative correlation between ucOC and ucFII ($r = -0.71$, $P = 0.03$). In the O/SO group this correlation was also negative ($r = -0.35$) but not statistically significant. We also measured the circulating levels of MGP, which were significantly lower after the CO diet than after either the adjustment or O/SO diets (**Fig. 3**). These measurements represent total MGP (assays discriminating between MGP and ucMGP are presently not available).

Since previous studies have reported that circulating VKD coagulation factors may be positively related to plasma lipids, we examined the relationship between plasma TAG and the VKD proteins. FII was positively correlated with TAG during all diets (pooled data: $R = 0.385$; $P < 0.01$) and also separately within each dietary phase with the strongest correlation for the CO diet ($R = 0.536$; $P < 0.01$). The extrahepatic VKD proteins OC and MGP did not correlate with TAG.

DISCUSSION

The present study provides evidence that altering the lipid component of the diet has the potential to reduce plasma levels of K_1 and/or impair the γ-carboxylation of representative hepatic (FII) and extrahepatic (OC) Gla-proteins. It was noteworthy that the PUFA-rich CO diet induced a significant reduction in plasma K_1 and TAG compared with both adjustment and O/SO diets. This is consistent with the carriage of K_1 by TRL (6) and with the well-established ca-

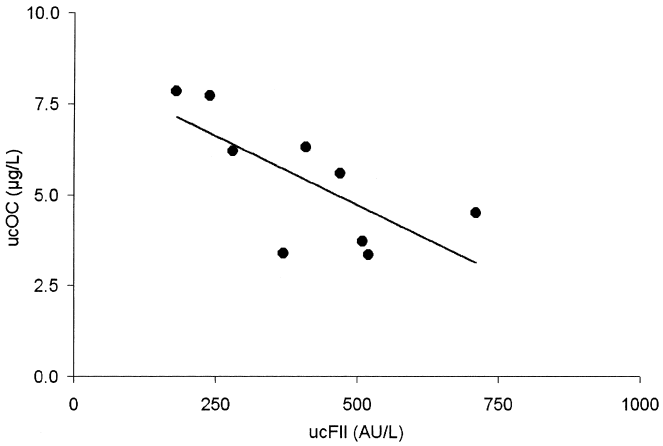

Fig. 2. Inverse correlation between ucFII and undercarboxylated osteocalcin during the CO diet. The Pearson correlation coefficient (r) was −0.71 and level of significance (P) was 0.03.

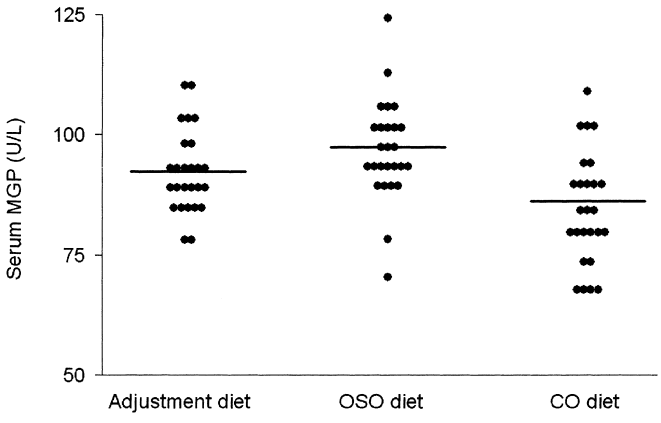

Fig. 3. Serum matrix Gla-protein concentrations after the three different dietary regimes. The mean value after the CO diet was statistically significantly lower than the values after the adjustment phase and the O/SO diet ($P < 0.001$).

pacity of diets rich in PUFA to reduce post-absorptive concentrations of plasma TAG (21), probably by reducing the post-prandial accumulation of TRL (22). The accompanying evidence of slight increases in undercarboxylation of FII and osteocalcin by the CO diet imply a lowered vitamin K status that may, however, be unrelated to the lowering of plasma TAG and K_1. Possible explanations include a reduced intestinal absorption of vitamin K, a reduced extracellular or intracellular delivery of vitamin K to target proteins, an increased turnover rate of the vitamin, or an inhibitory effect on VKD γ-carboxylation.

The possibility that the CO diet reduced the intestinal absorption of vitamin K is supported by in vivo studies in rats that showed that the PUFA linoleic acid (18:2) caused a marked decrease in the intestinal absorption rate of vitamin K_1 (23). This inhibition was not seen with the MUFA oleic acid (18:1). The same authors briefly commented that linoleic acid also inhibited the absorption of vitamin A. Other in vivo studies have shown that feeding diets with increasing linoleic acid contents to rats depressed the intestinal lymphatic absorption of vitamin E (24). Taken together, these studies in rats suggest a general inhibitory effect of PUFAs on the absorption of fat-soluble vitamins that share a similar pathway of absorption and lymphatic transport.

Previous studies in healthy adults have indicated that the levels of VKD FVII are more influenced by the total fat content of the diet rather than the SAFA-MUFA-PUFA composition (25). In our study we also found no effects of the O/SO or CO diets on the PT or FII concentrations, but we did find a significant correlation of FII levels with plasma TAG (O/SO diet $r = 0.453$; $P < 0.01$ and CO diet $r = 0.536$; $P < 0.01$). This is in agreement with previous studies showing associations of one or more VKD coagulation factors with TAG in patients with hyperlipidemia (26, 27) and in healthy young men (28). The reason for this association of VKD proteins with lipids is still unclear, but the recent finding of the in vivo binding of all VKD coagulation proteins to TRL (29) provides one explanation. Our data support the view that circulating levels of VKD coagulation proteins are linked to lipid metabolism in normal physiology (28) with the possibility that part of this interaction with lipids may be mediated via an effect on vitamin K metabolism (11).

To assess possible effects of the diets on the γ-carboxylation of FII, we used a sensitive immunoassay specific to ucFII. Hitherto, plasma ucFII concentrations in freeliving, healthy adults with this assay have always been below the detection limit. The same was true in the present study for all 26 subjects after the adjustment phase, but both test diets induced detectable ucFII. That this was a genuine effect of the diets is supported by the fact that the analyses of ucFII were blind and carried out in random order. It should be emphasized that the absolute increases in ucFII were relatively low and that the presence of such low concentrations (200–700 AU/l) did not affect the PT or FII assay. In patients on stable anticoagulant therapy ucFII values with this assay range from 6,900–99,500 AU/l (mean 40,000 AU/l) (M. J. Shearer, unpublished results). It seems unlikely, therefore, that this small increment in ucFII, and presumably also in ucFVII, ucIX, and ucX, would lead to a hypocoagulable state. On the other hand, evidence that minor reductions in VKD coagulation factors may result in a clinically significant decreased coagulability comes from studies of low dose warfarin regimes. Thus, daily doses of 1 mg warfarin reduced the incidence of deep vein thrombosis after surgery with no appreciable prolongation of the PT (30), and prevented thrombosis in central venous catheters with no changes in overall coagulation assays or decreases in specific VKD factors (31). This suggests that a hypocoagulable state can be induced by relatively minor perturbations of the carboxylation status of VKD coagulation proteins.

In contrast to the hepatic Gla-proteins, there is evidence that the bone protein OC is not fully carboxylated and is readily responsive to changes in dietary intakes of vitamin K (32). Compared with the adjustment diet, ucOC was significantly reduced only by the CO diet, but with the concomitant fall in total OC this effect was significant for both diets when ucOC was expressed as a percent of total OC. Total circulating OC is also a marker of osteoblastic activity, reflecting the rate of bone formation, but as this reduction of total OC was unexpected, further studies would be needed to address such a relationship. Unlike FII, there was no correlation of either OC or ucOC with TAG levels and there are no reports in the literature indicating such a relationship.

A direct inhibition of the VKD γ-glutamyl carboxylase or other enzymes of the vitamin K-epoxide cycle by non-TAG components also cannot be excluded. Compared with the adjustment diet, both the O/SO and CO diets provided about double the intake of α-tocopherol while the CO diet provided a high intake of γ-tocopherol. The high γ-tocopherol content of corn oil has already been forwarded as an explanation of the reduced frequency of sister chromatid exchange seen in the same subjects after the CO diet compared with the O/SO diet (12). Corn oil is also a rich source of ubiquinone-9. Vitamin E and ubiquinone compounds are structurally related to vitamin K, and have been reported to inhibit VKD carboxylase in vitro (33, 34).

The literature contains several reports that vitamin E compounds may interfere with vitamin K action in animals or humans but the mechanism remains unclear (35–39). An early study showed that the daily oral administration of large (100 mg) doses of α-tocopherolquinone to pregnant mice caused bleeding in the reproductive system that was reversible with vitamin K [Wooley et al., 1945, (36)] and a similar vitamin K-reversible coagulopathy was observed in chicks with large doses of vitamin E (38). Helson et al. (39) showed in patients with neuroblastoma that large doses of vitamin E caused a bleeding diathesis and that this was a systemic effect on vitamin K metabolism rather than an effect on absorption. More recently, Alexander and Suttie (40) have provided further evidence for cellular and whole animal interactions of vitamin E with vitamin K. First, cultured H-35 cells showed a significant decrease in FII production in the presence of both

α-tocopherol and α-tocopherolquinone. Secondly, rats fed a vitamin K deficient diet and increasing amounts of α-tocopherol in combination with a constant amount of phylloquinone showed a dose-dependent reduction in plasma FII and concomitant reductions in both plasma and liver phylloquinone. The effects on vitamin K and VKD proteins observed in our study were minor in comparison, but they raise the possibility of an interaction with the increased dietary intakes of vitamin E.

The strong inverse relationship between ucFII and ucOC seen in those subjects who had detectable ucFII after the CO diet is suggestive of some inter-subject variability in the relative bioavailability of vitamin K between the liver and bone. This could be mediated by difference(s) in lipoprotein metabolism that causes a differential organ/tissue uptake of TRL. One candidate is apolipoprotein E, which has common variants known to influence post-prandial TG metabolism and to mediate the uptake of TRL remnant particles (41). From this and other studies Kohlmeier et al. (7) generated the hypothesis that the liver and bone are competing organs for vitamin K, a hypothesis that may explain the inverse correlation of ucFII and ucOC.

Measurements of total plasma MGP with a recently available assay showed that the CO diet but not the O/SO diet caused a decrease in circulating MGP compared with the adjustment diet. This fall in MGP after the CO diet mirrored that in total OC, but the significance of this finding is unclear since little is yet known of the determinants of plasma MGP. There is strong interest in this VKD protein because recent studies show that vascular MGP synthesis is upregulated at sites of atherosclerotic calcification in the vessel wall (42) and that circulating MGP is increased during some stages of atherosclerosis (16).

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