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Assessment of the effect of esterified propoxylated glycerol (EPG) on the status of fat-soluble vitamins and select water-soluble nutrients following dietary administration to humans for 8 weeks

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ABSTRACT

This double-blind, randomized, controlled study assessed the effect of esterified propoxylated glycerol (EPG) on fat-soluble vitamins and select nutrients in human subjects. For 8 weeks, 139 healthy volunteers consumed a core diet providing adequate caloric and nutrient intakes. The diet included items (spread, muffins, cookies, and biscuits) providing EPG (10, 25, and 40 g/day) *vs.* margarine alone (control). EPG did not significantly affect circulating retinol, α-tocopherol, or 25-OH D_2, but circulating β-carotene and phylloquinone were lower in the EPG groups, and PIVKA-II levels were higher; 25-OH D_3 increased but to a lesser extent than the control. The effect might be related to EPG acting as a lipid "sink" during gastrointestinal transit. No effects were seen in secondary endpoint measures (physical exam, clinical pathology, serum folate, RBC folate, vitamin B_{12}, zinc, iron, calcium, phosphorus, osteocalcin, RBP, intact PTH, PT, PTT, cholesterol, HDL-C, LDL-C, triglycerides). Gastrointestinal adverse events (gas with discharge; diarrhea; oily spotting; oily evacuation; oily stool; liquid stool; soft stool) were reported more frequently by subjects receiving 25 or 40 g/day of EPG. In general, the incidence and duration of these symptoms correlated directly with EPG dietary concentration. The results suggest 10 g/day of EPG was reasonably well tolerated.

1. Introduction

Esterified propoxylated glycerols (EPGs) represent a family of fat- and oil-like substances, resembling triglycerides in structure and appearance, but modified to prevent or limit their digestion when consumed in food. They consist of multiple propylene glycol units inserted between the glycerol and fatty acid moieties of fats and oils. Their poor absorption results in a low- to no-calorie profile when substituted for fat in the diet.

The present study evaluated the possible effects of dietary EPG on the circulating levels of fat-soluble vitamins and select nutrients, along with its tolerability, when administered to healthy volunteers for 8 weeks.

2. Materials and methods

This study was sponsored by ARCO Chemical Company, Newton Square, Pennsylvania, and conducted at Chicago Center for Clinical Research, Chicago, Illinois, between January and March of 1997. The study was conducted under the principles of the World Medical Assembly Declaration of Helsinki and its amendments. The study protocol, amendments, and written informed consent were reviewed and approved by the Schulman Associates Institutional Review Board, Inc. (Cincinnati, OH), in conformance with 21 CFR 50 and 21 CFR 56. A signed informed consent statement was obtained from each subject at the first visit prior to any study procedures.

2.1. Selection of study subjects

Generally healthy male and female volunteers age 18–50 years within −10%/+30% of the ideal body weight for height, based on the Metropolitan Life Insurance Company tables, 1983 without significant abdominal disorders.

Abbreviations: EPG, esterified propoxylated glycerol; 25-OH D_2, vitamin D, ergocalciferol; 25-OH D_3, vitamin D, cholecalciferol; PIVKA II, proteins induced in vitamin K absence; RBC, red blood cell; RBP, retinol-binding protein; PTH, parathyroid hormone; PT, prothrombin time; PTT, partial thromboplastin time; HDL-C, high-density lipoproteins; LDL-C, low-density lipoproteins.

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2.2. Study design

The study, which lasted 8 weeks, was conducted using a double-blind, randomized and controlled design with four parallel groups. To maintain balance among the treatment groups, the groups were stratified by age (18–29 and 30+), sex, and estimated caloric need (1800–2400 and 2600–3000). At least 139 healthy adult male and female eligible subjects were enrolled and were randomized to one of the following treatment groups: control (ordinary triglycerides from margarine); 10 g/day EPG (EPG 10); 25 g/day EPG (EPG 25); or 40 g/day EPG (EPG 40).

For the evaluation of the response to treatment, the following outcome variables were measured at baseline and regular intervals:

- Primary Endpoints
  Serum β-carotene, retinol (vitamin A), α-tocopherol (vitamin E), 25-OH D_2 (vitamin D, ergocalciferol), 25-OH D_3 (vitamin D, cholecalciferol), phylloquinone (vitamin K_1), and PIVKA-II (proteins induced in vitamin K absence).
- Secondary Endpoints
  Changes from baseline in serum folate, RBC (red blood cell) folate, vitamin B_{12}, zinc, iron, calcium, phosphorus, osteocalcin, RBP (retinol-binding protein), intact PTH (parathyroid hormone), PT (prothrombin time), PTT (partial thromboplastin time), cholesterol, HDL-C (high-density lipoproteins), LDL-C (low-density lipoproteins), and triglycerides.
  Changes from baseline in urine concentrations of zinc, sodium, potassium, creatinine, calcium, and phosphorus.
  Secondary endpoints also included tolerability and nutrient intake [% calories from fat (saturated, monounsaturated, polyunsaturated), carbohydrate, and protein; α-tocopherol; calcium; cholesterol; dietary fiber; energy; folacin; insoluble and water-soluble dietary fiber; iron; phosphorus; potassium; total protein; selenium; total carbohydrate; total fat (saturated, monounsaturated, polyunsaturated); vitamin A; vitamin B_{12}; vitamin D; and zinc].
  Tolerability was assessed by the incidence of 14 specific gastrointestinal adverse events: passing gas; gas with discharge; abdominal bloat/cramp; heartburn; diarrhea; constipation; urgency of bowel movement; fecal incontinence; oily spotting; oily evacuation; oily stool; liquid stool; soft stool; and hard stool.

2.3. Test material

The version of EPG tested in this study was EPG-05 HR/ST 45:55 [lot numbers 850308 and 900445]. EPG was incorporated into spreads, muffins, cookies and biscuits, prepared and coded so that neither the subject nor the staff member administering the food was aware of its composition.

2.4. Treatment

From Days 1 through 56, study subjects received study meals that incorporated two biscuits and two pats of spread (margarine or EPG) at breakfast; one muffin, one cookie, and one pat of spread at lunch; and one biscuit, two cookies, and one pat of spread at dinner. These items were intended to provide a total of 0 (control), 10, 25, or 40 g of EPG daily, divided approximately equally among the study meals (breakfast, lunch, and dinner on weekdays; brunch and dinner on weekends). In an effort to maintain a stable body weight for each subject, supplemental snacks were also provided, as needed, as a source of additional calories. Drinks such as diet/low-sodium soft drinks, regular, sparkling, and flavored water were provided for consumption off-site; water was consumed *ad libitum*.

A "core diet" was developed to achieve target intakes of 80–120% of the RDA for vitamins A, D, and K, β-carotene, folate, zinc, iron, and calcium, and at the same time meet the different total energy needs of the individual male and female subjects. The core diet, which included the EPG food vehicles, provided 2200 kcal of energy per day.

Because the core diet may not have satisfied the energy needs of all subjects in the study, 6 variations of the "core diet" were used to adjust energy intake by ±200 kcal of energy per day. Each of the 6 variations of the "core diet" included the food vehicles. Subjects with lower energy needs and micronutrient recommended dietary allowances (RDA) received smaller portions of the core diet that provided 1800–2000 kcal of energy per day. Subjects with higher energy needs and micronutrient RDAs were given larger portions of the core diet that provided 2400–3000 kcal of energy per day. Using this approach, the intakes of nutrients of interest were controlled, while providing essentially *ad libitum* intake of energy.

In addition, subjects were weighed on a weekly basis to check the need for adjustment of their dietary intake. Subjects were fed to maintain enrollment weight (±5%) throughout the study. Subjects were also asked to report feelings of hunger or excessive fullness during the study, and calories were adjusted based on this subjective evaluation.

The (7-day rotating) menu provided approximately 35–40% of calories from fat, 15% from protein, and 50% from carbohydrate. The diets were formulated to ensure that the total digestible fat content was comparable across the 4 study groups. The 35–40% fat level was the lowest practically achievable because of the need to supplement the EPG-containing diets with 10, 25, or 40 g/day of regular triglyceride fats (*e.g.*, margarine) to match the fat content of the control diet. In other words, the triglycerides replaced by EPG in muffins, biscuits, cookies, and margarine were supplemented by adding margarine in other parts of the diet. For example, compared to the control group, the 10 g/day EPG study group consumed 10 g/day less of ordinary triglyceride fat because it had been replaced by the EPG in the select dietary food vehicles; therefore, an additional 10 g/day of triglycerides was added elsewhere in the daily menu to keep the triglyceride levels equivalent to the control group. The diets for the 25 and 40 g/day EPG study groups were adjusted in the same manner.

The menu targeted between 80 and 120% of the RDA for vitamins A, D, and K, β-carotene, folate, zinc, and calcium. The levels of vitamin B_{12}, vitamin E, and phosphorus were consistent across study groups. A vitamin D_2 supplement (ergocalciferol, 400 IU) was provided to each subject every morning.

In order to remain eligible, subjects were expected to consume a minimum of 90% of the scheduled study meals. No more than six consecutive study meals could have been missed at any given time during the study without approval from the study director. Subjects were not allowed to miss dinner on the day immediately prior to blood/urine sampling.

Alcohol intake was discouraged and was documented by a dietitian during daily interviews, along with consumption of any other non-study food or drink. The portion size and nutrient value of any such food or drink was assessed by the dietitian using the Minnesota Nutrition Data System, version 2.9.

2.5. Measurement schedule

All subjects began the study on the same calendar day, except for 31 subjects that began within 2 days thereafter. For the purposes of limiting endogenous synthesis of vitamin D_3, exposure of subjects to sunlight was limited by conducting the study in Chicago during the mid-late winter months.

2.6. Laboratory analyses

All blood and urine analyses were performed by Quest Diagnostics (1355 Mittel Boulevard Wood Dale, IL), except for: vitamin D analyses, which were performed by Dr. Bruce W. Hollis, Medical University of South Carolina; and vitamin K analyses, which were performed by Dr. John W. Suttie, University of Wisconsin.

2.7. Statistical analyses

2.7.1. Determination of sample size

Sample size and power calculations were based on the number of subjects required to detect clinically important differences in serum or plasma concentrations of β-carotene, retinol, α-tocopherol (absolute and cholesterol-adjusted), and 25-OH vitamins D_2 and D_3. Based on the results of a prior fat substitute feeding study conducted at the test facility, the standard deviations for changes in these indicators of vitamin status were each expected to be within the range of 12–20%. With at least 25 evaluable subjects per group, the power (1-β) to detect a difference between groups of one standard deviation (20% or less) was expected to be at least 85% at the 0.05 level of statistical significance. Assuming an attrition rate of less than 15%, a total sample size of 120 subjects (30 per treatment group) should have been sufficient.

2.7.2. Criteria for data exclusion

Data from all subjects that entered the treatment portion of the study were included in the analysis. Data from subjects that dropped out before the treatment portion, *i.e.*, before Day 1, were excluded from analysis.

2.7.3. Demographic and baseline medical characteristics

Demographic variables were summarized and tested for treatment group differences. Sex, race, and estimated energy needs were tabulated by number (*N*) and percent, and tested using Pearson's Chi-Square test. Continuous variables (age, height, weight, body mass, and eating attitude score) were summarized by number, mean, standard deviation, minimum value, median value, and maximum value, and tested using an analysis of variance (ANOVA). Treatment was the only term in the model.

Baseline laboratory assessments for the primary outcome variables, as well as baseline vital sign (blood pressure, heart rate, respiration rate, and temperature) measurements were summarized by number, mean, standard deviation, minimum value, median value, and maximum value; and tested using an analysis of variance procedure. Treatment was the only term in the model.

Baseline physical and perianal examination measurements, and baseline medical history were tabulated by treatment group.

2.7.4. Data sets analyzed

Two data sets were analyzed. The *primary outcome* analysis (*i.e.*, target vitamin and nutrient status) included only the outcome evaluable population, *i.e.*, those study subjects that: (1) received the study product at least once; (2) consumed a minimum of 90% of the scheduled study meals; and (3) completed the Day 56 assessment. The *intent-to-treat* group consisted of all subjects that gave consent to participate in the study, were randomized to a treatment group, and consumed any of the study product; these subjects were included in the analysis of changes from baseline for β-carotene, retinol, α-tocopherol, 25-OH D_2, 25-OH D_3, phylloquinone (K_1), and PIVKA II.

The analysis based on the outcome evaluable population was treated as the primary analysis; statistical significance for this analysis was declared at $p \leqslant 0.05$. The safety analysis was conducted on the intent-to-treat population only.

For each of the outcome parameters, the number of subjects, mean, median, standard deviation, and minimum and maximum values are presented by treatment group and visit. The differences between Day 56 and baseline values were calculated for each subject individually and reported using descriptive statistics: Number of differences, mean difference, median difference, and standard deviation for each of the four treatment groups. Ninety-five percent confidence intervals were also constructed for these differences. The ANOVA output was documented. The group-wise concentration time profiles are depicted graphically.

Between Days 42 and 56 of the study, there was a change in the standard used in the assay for blood vitamin levels, which required application of a correction factor of (÷1.404) to the results obtained during that period.

2.7.5. Primary endpoint analysis

The primary outcome endpoint was the comparison among the four treatment groups with respect to change from baseline to end of feeding period for serum β-carotene, retinol, α-tocopherol, 25-OH D_2, 25-OH D_3, phylloquinone, and PIVKA-II. Baseline values were defined as the average of the non-missing pretreatment values obtained on Days −7 and −1. However, in the statistical analyses presented on tables and listings, baseline is referred to as Day −1. End of feeding period values were defined as the Day 56 measurement.

Dunnett's test for multiple comparisons was used to compare the mean change from baseline to Day 56 for each of the three active treatment groups (EPG 10, EPG 25, EPG 40) *vs.* control. Statistical significance was declared at the 0.05 level.

The primary outcome variables were summarized by age, body mass, sex and race using descriptive statistics.

2.7.6. Secondary endpoint analysis

With the exception of serum concentrations of iron, calcium, and phosphorus, all laboratory parameters were measured at Days −7, −1, 14, 28, 42, and 56. Serum concentrations of iron, calcium, and phosphorus were measured at Days −42 to −15, −1, 14, 28, 42, and 56. The results are presented using 95% confidence intervals for change from baseline to Days 14, 28, and 42, as well as for the comparison to control at each time point.

2.7.7. Nutrient intake

Nutrient intakes were determined from food consumption data using the Minnesota Nutrition Data System, version 2.9. The data were analyzed using daily averages over each of the four 2-week periods, and over the entire 8-week study. The 95% confidence intervals for the mean differences between each of the three EPG groups (10, 25, and 40 g/day) minus control were computed.

2.7.8. Safety and tolerability

Adverse events were summarized by body system. Further summarization was performed for severity and relation to treatment. The number and percent of subjects with at least one of the 14 gastrointestinal adverse events (GI AEs) evaluated was tabulated. The incidence of adverse events for each of the three EPG treatment groups was compared to placebo by computing the difference in 95% confidence intervals (CIs). Fisher's exact test was used to assess overall treatment effect for each of the individual 14 GI AEs. Tolerability data were also tabulated by age group (18–29 and 30+), sex, race (white and non-white), body mass index (low and high), and estimated caloric need (1800–2400 and 2600–3000).

Other data (clinical laboratory test results, vital signs, physical exam results, *etc.*) were classified as normal or abnormal, and presented using descriptive statistics.

3. Results

3.1. Disposition and evaluability of subjects

The overall disposition of subjects is summarized in Table 1. A total of 139 subjects were enrolled (consumed study product); 107 (77%) completed the study. The percentage of subjects that consumed study product and that completed the study was comparable among groups. Across groups, the number of subjects that withdrew from the study ranged from 6 (17.6%) subjects in the EPG 10 group to 9 subjects in each the EPG 25 (25.7%) and control (25.0%) groups. The two primary reasons for premature termination from the study were non-compliance and adverse event occurrence. Overall, 28 (20.1%) subjects were terminated for noncompliance. Four (2.9%) subjects were terminated due to adverse events; one subject each from the control and EPG 10 groups, and two from the EPG 25 group. No statistically significant differences were detected across groups in the number of subjects that complied with the 90% consumption of study meals requirement.

Two subjects from the placebo group and one subject from the EPG 25 became pregnant during the study; these subjects were not using oral contraceptives. All three of the subjects were terminated from the study, having received the last study meal between study Days 15 and 30.

3.2. Demographics

Demographic characteristics of the subjects evaluable for the outcome analysis are provided in Table 2. No significant differences in demographic and baseline characteristics were noted; these parameters were similar to the all-subject population.

3.3. Primary outcome

Tables 3–9 summarize the changes in mean circulating β-carotene, retinol, α-tocopherol, 25-OH D_2, 25-OH D_3, phylloquinone, and PIVKA-II from baseline to the end of the study, respectively (subjects evaluable for outcome analysis); Figs. 1–7 show the means over time (intent-to-treat population).

3.3.1. β-Carotene

Mean circulating β-carotene levels declined over time in all subjects, including the control group (Fig. 1). As Table 3 illustrates, based on the calculated 95% confidence intervals (CIs), the within-group changes from baseline were statistically significant in all groups and all time points, with two exceptions: the increases from baseline by 3.5 and 0.1 μg/dL in the control group at Days 14 and 42. In addition, compared to the changes seen in the control group, the declines from baseline levels among subjects receiving EPG were significantly greater (p value of less than the adjusted $0.05 \div 3 = 0.017$) at all time points. However, there was no apparent relationship to EPG concentration, since the declines were consistently greater in the groups receiving 10 g/day and 40 g/day than in the 25 g/day group.

3.3.2. Retinol (vitamin A)

Despite declining from baseline to Day 14 across all groups, mean retinol levels increased steadily from Day 14 to Day 56 to levels approaching or surpassing baseline values (Fig. 2). Within groups, the changes from baseline were statistically significant only for EPG 10 at Days 42 and 56, and at endpoint (increases of 5.2, 6.7, and 6.7 μg/dL, respectively), and for EPG 25 at Days 14 and 28 (decreases of 8.4 and 6.4 μg/dL, respectively). There was no apparent relationship to EPG concentration. Overall, the changes from baseline retinol levels were not significantly different between the control and EPG groups, except for EPG 25 which exhibited a decline from baseline to Day 14 that was significantly ($p = 0.0141$) greater than the baseline to Day 14 change in the control (Table 4).

3.3.3. α-Tocopherol (vitamin E)

All groups had an initial decrease from baseline in mean circulating α-tocopherol levels (Fig. 3), followed by a gradual increase,

Table 1
Overall subject disposition.

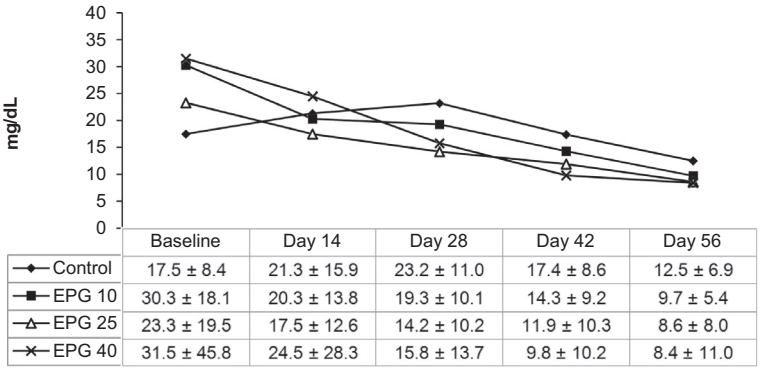
	Control	EPG (g/day)			Total
		10	25	40	
Consumed study product	36 (100%)	34 (100%)	35 (100%)	34 (100%)	139 (100%)
Prematurely terminated	9 (25%)	6 (17.6%)	9 (25.7%)	8 (23.5%)	32 (23%)
Primary reason					
Adverse event[a]	1 (2.8%)	1 (2.9%)	2 (5.7%)	0	4 (2.9%)
Non-compliance	8 (22.2%)	5 (14.7%)	7 (20%)	8 (23.5%)	28 (20.1%)
Missed study meals	3 (8.3%)	2 (5.9%)	2 (5.7%)	4 (11.8%)	11 (7.9%)
Withdrew consent	2 (5.6%)	2 (5.9%)	1 (2.9%)	2 (5.9%)	7 (5%)
Out of town	1 (2.8%)	0	2 (5.7%)	1 (2.9%)	4 (2.9%)
Scheduling conflict	0	1 (2.9%)	1 (2.9%)	1 (2.9%)	3 (2.2%)
Pregnancy	2 (5.6%)	0	1 (2.9%)	0	3 (2.2%)
Completed study	27 (75%)	28 (82.4%)	26 (74.3%)	26 (76.5%)	107 (77%)
Evaluable for outcome analysis?					
Yes	27 (75%)	27 (79.4%)	24 (68.6%)	25 (73.5%)	103 (74.1%)
No	9 (25%)	7 (20.6%)	11 (31.4%)	9 (26.5%)	36 (25.9%)

[a] Adverse events leading to discontinuation were as follows:Subject # 508, a 37-year-old female from the control group (normal triglycerides) experienced recurring moderate to severe constipation from Days 6 to 27, along with mild to moderate passing gas (Days 11 and 20), and moderate diarrhea (Day 11). All adverse events were judged by the investigator to be possibly related to the study meals. The subject's constipation was treated with herbal and vegetable laxatives (Metamucil and Colace). The last study meal was on Day 30.
Subject # 142, a 22-year-old female receiving EPG 10 g/day, experienced moderate and mild nausea on Days 2 and 4, respectively. The investigator judged both episodes to be possibly related to the test substance. The nausea was treated with antacids (Tums, 2 tablets per day). The last study meal was on Day 6.
Subject # 106, a 22-year-old female, receiving EPG 25 g/day, experienced severe abdominal bloating and cramping, diarrhea, and fatigue on Day 7; the subject had reported a history of constipation over about 5 years. The investigator judged the abdominal bloating, cramping, and diarrhea as possibly related to the test substance. The fatigue was judged to be unrelated. No therapy was required. The last study meal was on Day 6.
Subject # 148, a 21-year-old female receiving EPG 25 g/day, experienced moderate oily urine and stools, and mild headache on Day 5. The oily urine and stools were judged by the investigator to be possibly related to the test substance. The headache was judged to be unrelated. No therapy was required. The last study meal was on Day 6.

Table 2
Demographics of subjects evaluable for outcome analysis.

	Control	EPG(g/day)			Total	*p*-Value
		10	25	40		
Sex						
Male	12 (44.4%)	9 (33.3%)	9 (37.5%)	8 (32%)	38 (36.9%)	0.7845
Female	15 (55.6%)	18 (66.7%)	15 (62.5%)	17 (68%)	65 (63.1%)	
Race						
Asian	2 (7.4%)	5 (18.5%)	6 (25%)	5 (20%)	18 (17.5%)	0.5769
Caucasian	6 (22.2%)	7 (25.9%)	7 (29.2%)	3 (12%)	23 (22.3%)	
Black	16 (59.3%)	10 (37%)	10 (41.7%)	14 (56%)	50 (48.5%)	
Hispanic	3 (11.1%)	4 (14.8%)	1 (4.2%)	3 (12%)	11 (10.7%)	
Other	0	1 (3.7%)	0	0	1 (1%)	
Age (years)						
N	27	27	24	25	103	0.3761
Mean (SD)	30.1 (8.4)	30.3 (9.1)	30.7 (8.8)	27 (7.4)	29.5 (8.5)	
Median	29	28	28	24	28	
Min–max	18–47	19–48	20–48	18–42	18–48	
Weight (lbs)						
N	25	27	24	24	100	0.6216
Mean (SD)	153.6 (22.6)	159 (28.6)	157.7 (33.4)	148.3 (38.1)	154.8 (30.9)	
Median	155.5	156	155	138	153.5	
Min–max	105–210	109–213	110–245	100–234	100–245	
Height (in)						
N	26	27	24	24	101	0.9220
Mean (SD)	65.7 (3.5)	65.6 (2.5)	66 (3.6)	65.3 (3.3)	65.7 (3.2)	
Median	66	66	66	65.3	66	
Min–max	59.8–72.5	59.3–70.3	58.5–73	58.8–73	58.5–73	
Body mass index						
N	25	27	24	24	100	0.5200
Mean (SD)	25.1 (3.5)	25.9 (4)	25.4 (4.6)	24.2 (4.6)	25.2 (4.2)	
Median	25.2	25.3	25.5	23.4	25	
Min–max	19.7–32.6	19–32.7	18.4–32.7	17.7–34.1	17.7–34.1	
Eating attitude survey[a] score						
N	27	27	24	25	103	0.7741
Mean (SD)	9 (8.2)	8.3 (4.6)	8.2 (4.4)	7.4 (4.7)	8.2 (5.7)	
Median	7	7	8	6	7	
Min–max	2–41	1–20	2–22	1–22	1–41	
Estimated energy needs						
1800–2400	23 (85.2%)	23 (85.2%)	21 (87.5%)	21 (84%)	88 (85.4%)	0.9885
2600–3000	4 (14.8%)	4 (14.8%)	3 (12.5%)	4 (16%)	15 (14.6%)	

[a] Subjects were required to have a score of less than 30 points in the eating attitude survey to qualify for participation in the study.



	Baseline	Day 14	Day 28	Day 42	Day 56
Control	17.5 ± 8.4	21.3 ± 15.9	23.2 ± 11.0	17.4 ± 8.6	12.5 ± 6.9
EPG 10	30.3 ± 18.1	20.3 ± 13.8	19.3 ± 10.1	14.3 ± 9.2	9.7 ± 5.4
EPG 25	23.3 ± 19.5	17.5 ± 12.6	14.2 ± 10.2	11.9 ± 10.3	8.6 ± 8.0
EPG 40	31.5 ± 45.8	24.5 ± 28.3	15.8 ± 13.7	9.8 ± 10.2	8.4 ± 11.0

Fig. 1. Circulating β-carotene levels over time. Values represent mean ± standard deviation (SD).

such that at the end of the study, the levels were past baseline levels. However, the decline was greater and more persistent for the EPG 25 and EPG 40 groups. Based on 95% CIs (Table 5), EPG 25 and EPG 40 showed significantly greater declines at Day 14 (−1.9 and −1.7 mg/L, respectively), Day 28 (−1.4 and −0.9 mg/L), and Day 42 (−1 and −0.3 mg/L). There were no statistically significant declines in α-tocopherol at Day 56 for any of the EPG groups.

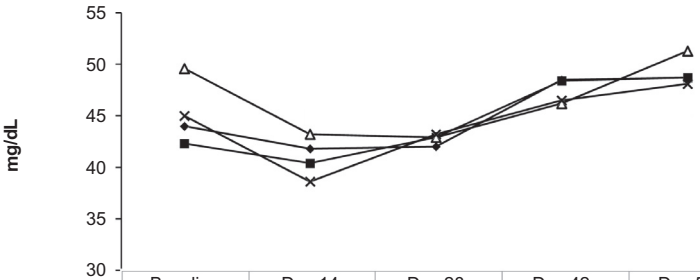
3.3.4. 25-OH D_2 (vitamin D, ergocalciferol)

As Fig. 4 shows, all study groups had a gradual increase in mean circulating 25-OH D_2 levels from baseline until Day 42, with a slight attenuation at Day 56. The increases were statistically significant from Day 28 onward in all groups, except for the EPG 25 group, which had an increase that was only slightly greater at Day 28 than it was at Day 14 (1.4 *vs.* 1.2 ng/mL). The Wilcoxon Rank Sum Test (Table 6) revealed no significant differences at the

Table 3
Summary statistics for changes from baseline in blood β-carotene (μ/dL).

	N	Change	SD	95% CI*	Comparison to control		
					Difference	95% CI	*p*-Value**
Day 14							
Control	22	3.5	12.1	[−1.6, 8.5]			
EPG 10	22	−10.3	8.1	[−13.6, −6.9]	−13.7	[−19.8, −7.6]	0.0001
EPG 25	19	−7.7	11.4	[−12.8, −2.5]	−11.1	[−18.4, −3.9]	0.0002
EPG 40	16	−17.6	27.3	[−30.9, −4.2]	−21.0	[−33.8, −8.2]	0.0001
Day 28							
Control	26	5.9	8.1	[2.8, 9.0]			
EPG 10	26	−10.4	11.6	[−14.9, −6.0]	−16.3	[−21.8, −10.9]	0.0001
EPG 25	24	−9.0	12.9	[−14.2, −3.9]	−14.9	[−20.8, −9.0]	0.0001
EPG 40	24	−16.7	37.6	[−31.8, −1.7]	−22.7	[−37.5, −7.8]	0.0001
Day 42							
Control	25	0.1	6.3	[−2.4, 2.6]			
EPG 10	26	−16.6	14.3	[−22.1, −11.1]	−16.7	[−22.8, −10.6]	0.0001
EPG 25	24	−11.4	13.0	[−16.6, −6.2]	−11.5	[−17.1, −5.8]	0.0001
EPG 40	24	−14.0	17.0	[−20.8, −7.2]	−14.1	[−21.2, −7.0]	0.0001
Day 56							
Control	25	−4.6	5.0	[−6.6, −2.7]			
EPG 10	27	−20.6	14.2	[−26.0, −15.3]	−16.0	[−21.9, −10.1]	0.0001
EPG 25	24	−14.7	13.4	[−20.0, −9.3]	−10.1	[−15.7, −4.4]	0.0001
EPG 40	23	−24.1	37.0	[−39.2, −9.0]	−19.5	[−34.1, −4.9]	0.0001
Endpoint***							
Control	26	−4.4	5.1	[−6.4, −2.5]			
EPG 10	27	−20.6	14.2	[−26.0, −15.3]	−16.2	[−22.0, −10.4]	0.0001
EPG 25	24	−14.7	13.4	[−20.0, −9.3]	−10.3	[−15.8, −4.8]	0.0001
EPG 40	25	−23.1	35.6	[−37.1, −9.2]	−18.7	[−32.5, −4.9]	0.0001

* 95% confidence intervals were used to compare the change from baseline to Days 14, 28, 42, and 56, and endpoint.
** Two-sided *t*-tests were used to compare the three EPG groups *vs.* control at Day 56. Bonferroni correction was used to adjust for multiple comparisons; statistical significance was declared at 0.017 (*i.e.*, 0.05 ÷ 3) level.
*** Subjects with data on or after Day 14 were included in the endpoint analysis, using the last observation carried forward (LOCF).



	Baseline	Day 14	Day 28	Day 42	Day 56
Control	44.0 ± 11.1	41.8 ± 9.8	42.0 ± 9.4	48.5 ± 14.2	48.7 ± 14.0
EPG 10	42.3 ± 10.6	40.4 ± 12.4	42.9 ± 8.6	48.4 ± 8.0	48.7 ± 11.2
EPG 25	49.6 ± 10.8	43.2 ± 10.3	42.9 ± 6.8	46.2 ± 7.5	51.3 ± 11.1
EPG 40	45.0 ± 12.5	38.6 ± 9.5	43.2 ± 8.4	46.5 ± 9.6	48.1 ± 10.7

Fig. 2. Circulating retinol (vitamin A) levels over time. Values represent mean ± standard deviation (SD).

primary endpoint (Day 56) in the mean change from baseline 25-OH D_2 levels in EPG *vs.* control subjects; similar results were obtained at Days 14, 28, and 42, and at the endpoint analysis.

3.3.5. 25-OH D_3 (vitamin D, cholecalciferol)

As Fig. 5 illustrates, in the control group, mean 25-OH D_3 levels rose over time to levels that far exceeded baseline levels, reaching a maximum on Day 42 and declining thereafter, but remaining higher than baseline. In general, circulating 25-OH D_3 levels also increased over time in the EPG groups, but to a lesser extent and at a slower pace. At the end of the study (Day 56), only EPG 25 and EPG 40 had 25-OH D_3 levels below baseline levels (−1.7 and −1.3 ng/mL, respectively, Table 7). The within-group differences from baseline were not statistically significant for any group, except for the control group (increases at all time points). Combined with only gradually rising levels in the EPG groups, this resulted in the differences from baseline in the EPG groups appearing to be mostly statistically significant declines (see Table 7, negative differences *vs.* control). In actuality, after the initial (Day 14) decline seen in all EPG groups, 25-OH D_3 levels were consistently higher than baseline in the EPG 10 group through the end of the study; levels were reduced only for the EPG 25 (after Day 42) and EPG 40 (Day 56 and endpoint) groups.

3.3.6. Phylloquinone (vitamin K_1)

Mean circulating phylloquinone levels were consistently higher among control subjects at all time points, including baseline (Fig. 6). With the exception of EPG 40, values declined initially among all groups from baseline to Day 14, increased until Day 42, and showed a slight attenuation by Day 56. In the control

Table 4
Summary statistics for changes from baseline in blood retinol (vitamin A) (μg/dL).

	N	Change	SD	95% CI*	Comparison to control		
					Difference	95% CI	p-Value**
Day 14							
Control	18	−1.5	5.2	[−3.9, 0.9]	0.59	[−5.1, 6.3]	0.5741
EPG 10	17	−0.9	11.1	[−6.2, 4.3]	−6.87	[−12.3, −1.5]	0.0141
EPG 25	14	−8.4	10.1	[−13.7, −3.1]	−5.78	[−12.6, 1.0]	0.2290
EPG 40	13	−7.3	13.4	[−14.6, 0]			
Day 28							
Control	23	−2.5	6.6	[−5.1, 0.2]			
EPG 10	23	1.0	7.8	[−2.2, 4.2]	3.46	[−0.7, 7.6]	0.1794
EPG 25	19	−6.4	10.4	[−11.1, −1.8]	−3.99	[−9.2, 1.2]	0.0827
EPG 40	22	−2.7	10.9	[−7.2, 1.9]	−0.23	[−5.5, 5.0]	0.3453
Day 42							
Control	22	2.6	8.3	[−0.8, 6.1]			
EPG 10	22	5.2	7.9	[1.9, 8.5]	2.57	[−2.2, 7.4]	0.4236
EPG 25	19	−2.2	9.6	[−6.6, 2.1]	−4.85	[−10.3, 0.6]	0.0648
EPG 40	22	0.9	12.8	[−4.5, 6.3]	−1.70	[−8.1, 4.7]	0.4808
Day 56							
Control	22	4.7	10.7	[0.2, 9.1]			
EPG 10	23	6.7	7.3	[3.7, 9.7]	2.04	[−3.3, 7.4]	0.3692
EPG 25	19	1.2	11.4	[−3.9, 6.4]	−3.42	[−10.2, 3.4]	0.5043
EPG 40	21	3.5	12.5	[−1.8, 8.9]	−1.14	[−8.1, 5.8]	0.7704
Endpoint***							
Control	23	4.1	10.9	[−0.4, 8.5]			
EPG 10	23	6.7	7.3	[3.7, 9.7]	2.63	[−2.7, 8.0]	0.2620
EPG 25	19	1.2	11.4	[−3.9, 6.4]	−2.83	[−9.6, 3.9]	0.6487
EPG 40	22	3.7	12.2	[−1.4, 8.8]	−0.34	[−7.1, 6.4]	0.9909

* 95% confidence intervals were used to compare the change from baseline to Days 14, 28, 42, and 56, and endpoint.
** Two-sided *t*-tests were used to compare the three EPG groups *vs.* control at Day 56. Bonferroni correction was used to adjust for multiple comparisons; statistical significance was declared at 0.017 (*i.e.*, 0.05 ÷ 3) level.
*** Subjects with data on or after Day 14 were included in the endpoint analysis, using the last observation carried forward (LOCF).

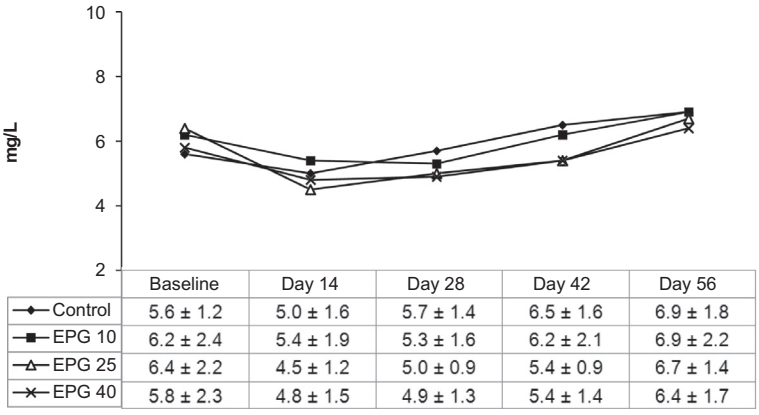


Fig. 3. Circulating α-tocopherol (vitamin E) levels over time. Values represent mean ± standard deviation (SD).

group, values were above baseline levels at all time points after Day 14, whereas the EPG groups had levels below baseline at all time points after Day 14. In the EPG 40 group, phylloquinone levels fluctuated between decreasing and increasing. Calculated 95% confidence intervals for within-group differences showed no trend in statistical significance. A comparison of the differences from baseline in EPG groups *vs.* control showed statistical significance (decline in phylloquinone levels) in nearly all EPG groups at nearly all time points. However, the declines did not exceed 0.1 ng/mL (Table 8).

3.3.7. PIVKA-II (proteins induced in vitamin K absence)

PIVKA-II was inadvertently dropped from the laboratory requisition form at various time points. Consequently, PIVKA-II values were available only for the baseline and Day 56 time points. Calculated 95% confidence intervals showed concentration-dependent increases from baseline PIVKA-II levels among EPG groups, reaching statistical significance only for the EPG 25 and EPG 40 groups (Table 9). Compared to control group, the changes from baseline in the EPG 25 and EPG 40 groups were also statistically significant ($p \leqslant 0.017$).

Subgroup analyses based on sex, race (black and non-black), age (18–29, and 30+), and body mass index (⩽25 and >25) revealed no notable differences for any of the primary outcome measures (retinol, α-tocopherol, 25-OH D_2, 25-OH D_3, phylloquinone, PIVKA-II), except β-carotene. EPG was associated with slightly greater decline in mean β-carotene levels among females (all dose levels) and among EPG 10 and EPG 40 subjects with a smaller body mass index (data not shown).

Table 5
Summary statistics for changes from baseline in blood α-tocopherol (vitamin E) (mg/L).

	N	Change	SD	95% CI*	Comparison to control		
					Difference	95% CI	p-Value**
Day 14							
Control	22	−0.5	1.2	[−1.0, 0]			
EPG 10	21	−1.0	1.9	[−1.8, −0.2]	−0.44	[−1.4, 0.5]	0.3430
EPG 25	19	−1.9	2.5	[−3.0, −0.7]	−1.33	[−2.5, −0.2]	0.0498
EPG 40	16	−1.7	2.0	[−2.6, −0.7]	−1.12	[−2.1, −0.1]	0.0166
Day 28							
Control	27	0.1	1.0	[−0.3, 0.4]			
EPG 10	26	−0.7	1.7	[−1.4, −0.1]	−0.79	[−1.5, 0]	0.0616
EPG 25	24	−1.4	2.0	[−2.2, −0.6]	−1.44	[−2.3, −0.6]	0.0014
EPG 40	25	−0.9	1.5	[−1.4, −0.3]	−0.90	[−1.6, −0.2]	0.0152
Day 42							
Control	26	0.8	1.1	[0.4, 1.3]			
EPG 10	26	−0.1	2.2	[−0.9, 0.8]	−0.92	[−1.9, 0]	0.1241
EPG 25	24	−1.0	1.8	[−1.7, 0.3]	−1.86	[−2.7, −1.0]	0.0001
EPG 40	25	−0.3	1.8	[−1.1, 0.4]	−1.19	[−2.0, −0.4]	0.0017
Day 56							
Control	26	1.3	1.1	[0.8, 1.7]			
EPG 10	27	0.8	1.8	[0.1, 1.4]	−0.48	[−1.3, 0.3]	0.4337
EPG 25	24	0.3	2.2	[−0.6, 1.2]	−0.96	[−1.9, 0]	0.2140
EPG 40	23	0.6	1.8	[−0.2, 1.3]	−0.68	[−1.5, 0.2]	0.0624
Endpoint***							
Control	27	1.2	1.1	[0.8, 1.6]			
EPG 10	27	0.8	1.8	[0.1, 1.4]	−0.45	[−1.2, 0.3]	0.4835
EPG 25	24	0.3	2.2	[−0.6, 1.2]	−0.93	[−1.9, 0]	0.2655
EPG 40	25	0.5	1.8	[−0.2, 1.2]	−0.71	[−1.5, 0.1]	0.0490

A correction factor of 1.404 was used for samples analyzed on Day 56.
* Assuming normal approximation.
** Two-sided *t*-tests were used to compare the three EPG groups *vs.* control at Day 56. Bonferroni correction was used to adjust for multiple comparisons; statistical significance was declared at 0.017 (*i.e.*, 0.05 ÷ 3) level.
*** Subjects with data on or after Day 14 were included in the endpoint analysis, using the last observation carried forward (LOCF).

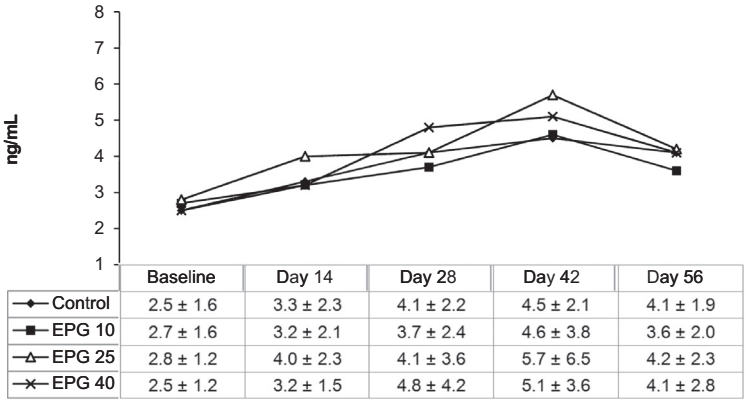


Fig. 4. Circulating 25-OH D_2 (ergocalciferol) levels over time. Values represent mean ± standard deviation (SD).

3.4. Secondary outcome

As Tables 10–12 show, there were no statistically significant differences (EPG *vs.* control) at any time point in the mean change from baseline in serum folate, vitamin B_{12}, zinc, iron, calcium, phosphorus, intact PTH, PT, PTT, RBP, osteocalcin, undercarboxylated osteocalcin, cholesterol, and triglycerides. The more limited (Day 28) data on RBC folate, HDL-C, and LDL-C showed statistically significant changes (declines) only for LDL-C in the EPG 25 and EPG 40 groups.

3.4.1. Overall safety and tolerability

Mean body weight values remained constant throughout the study; the majority of subjects having less than a 5% change from baseline weight (data not shown).

Some deviations from normal values were observed in mean hematology, serum chemistry, and urinalysis parameters during the study (data not shown). However, none of the deviations was considered clinically significant, having already been present at baseline and/or not resulting in any adverse effect. The results of 24-h urine sample analysis for zinc, sodium, potassium, calcium, creatinine, and phosphorus showed no evidence of any treatment-related effects (data not shown).

Physical examination at the end of the study, including vital signs, review of general body systems, and inspection of the perianal area, revealed no clinically-significant changes. The majority of subjects did not have any significant changes in health or medications during the study period.

As previously noted, three subjects were prematurely terminated from the study due to pregnancy. No complications with

Table 6
Summary statistics for changes from baseline in blood 25-OH D_2 (ergocalciferol) (ng/mL).

	N	Change	SD	95% CI*	Comparison to control		
					Difference	95% CI	p-Value**
Day 14							
Control	27	0.8	2.1	[0, 1.6]			
EPG 10	27	0.6	2.0	[−0.2, 1.3]	−0.26	[−1.4, 0.8]	0.8220
EPG 25	23	1.2	2.3	[0.3, 2.2]	0.41	[−0.8, 1.6]	0.5080
EPG 40	25	0.7	1.7	[0, 1.3]	−0.16	[−1.2, 0.9]	0.8980
Day 28							
Control	27	1.7	2.0	[0.9, 2.4]			
EPG 10	27	1.0	2.1	[0.2, 1.8]	−0.72	[−1.8, 0.4]	0.2795
EPG 25	23	1.4	3.5	[0, 2.8]	−0.28	[−1.8, 1.3]	0.3501
EPG 40	25	2.3	4.2	[0.6, 3.9]	0.62	[−1.2, 2.4]	0.5335
Day 42							
Control	25	2.0	2.3	[1.1, 3.0]			
EPG 10	27	1.9	3.3	[0.7, 3.2]	−0.11	[−1.7, 1.4]	0.5274
EPG 25	24	2.9	6.4	[0.4, 5.5]	0.88	[−1.8, 3.5]	0.9601
EPG 40	25	2.6	3.6	[1.2, 4.0]	0.56	[−1.1, 2.2]	0.7342
Day 56							
Control	27	1.7	1.9	[0.9, 2.4]			
EPG 10	27	0.9	2.2	[0.1, 1.7]	−0.79	[−1.9, 0.3]	0.2004
EPG 25	23	1.5	2.0	[0.7,2.3]	−0.17	[−1.3, 0.9]	0.5206
EPG 40	24	1.6	2.9	[0.4, 2.8]	−0.07	[−1.4, 1.3]	0.3956
Endpoint***							
Control	27	1.7	1.9	[0.9, 2.4]			
EPG 10	27	0.9	2.2	[0.1, 1.7]	−0.79	[−1.9, 0.3]	0.2004
EPG 25	24	2.8	6.4	[0.2, 5.3]	1.09	[−1.5, 3.6]	0.7128
EPG 40	25	1.5	2.9	[0.4, 2.7]	−0.15	[−1.5, 1.2]	0.3181

* Assuming normal approximation.
** Wilcoxon Rank Sum Test was used to compare the three EPG treatment groups *vs.* placebo.
*** Subjects with data on or after Day 14 were included in the endpoint analysis, using the last observation carried forward (LOCF).

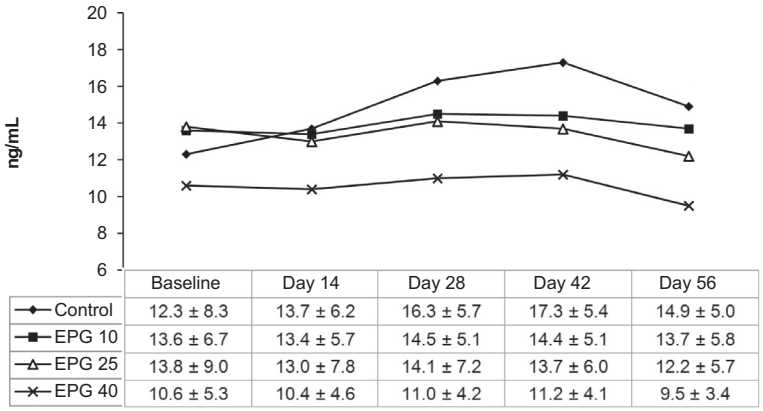


Fig. 5. Circulating 25-OH D_3 (cholecalciferol) levels over time. Values represent mean ± standard deviation (SD).

any of the pregnancies were reported to the study facility; however, further attempts to follow-up with the subjects were unsuccessful.

No serious adverse events were reported during this study, and only 4 (2.9%) subjects were terminated for adverse events (see Table 1). All adverse events reported by subjects in this study are summarized in Table 13. In general, with the exception of gastrointestinal tract symptoms, adverse events were not considered related to EPG.

Seven of the 14 pre-defined Gl AEs were reported with significantly ($p \leqslant 0.05$) greater frequency by subjects receiving 25 or 40 g/day of EPG: gas with discharge; diarrhea; oily spotting; oily evacuation; oily stool; liquid stool; and soft stool. In general, the frequency of GI AEs appeared to be directly related to EPG dietary concentration. However, with the exception of oily stool, there were no statistically significant differences between the EPG 10 group and the control group.

Post-hoc analyses revealed an overall increase in the mean percent days with any GI AE (days with any GI AE/days on trial × 100) with increasing EPG concentration (data not shown). The mean percent of days with any GI AE was 5.6 days in the control group, compared with 11, 12.7, and 15.9 in the EPG 10, EPG 25, and EPG 40 groups, respectively.

Post-hoc analysis for the number of subjects experiencing moderate to severe GI AEs showed statistical significance in the EPG *vs.* control groups for: gas with discharge ($p = 0.0043$); oily spotting ($p = 0.0072$); oily stool ($p = 0.0012$); and soft stool ($p = 0.0002$).

The most commonly reported GI AE, lasting for at least two successive days, was passing gas, with 3 (11.1%), 4 (14.8%), 4 (16.7%), and 5 (20%) subjects in each the control, EPG 10, EPG 25, and EPG 40 groups, respectively. For the control group, the number of subjects reporting GI AEs lasting at least two successive days was less than 5%, except for abdominal bloating/cramping, which was

Table 7
Summary statistics for changes from baseline in blood 25-OH D_3 (cholecalciferol) (ng/mL).

	N	Change	SD	95% CI*	Comparison to control		
					Difference	95% CI	*p*-Value**
Day 14							
Control	27	1.4	3.2	[0.2, 2.6]			
EPG 10	27	−0.2	3.7	[−1.6, 1.2]	−1.55	[−3.4, 0.3]	0.0582
EPG 25	23	−0.9	2.3	[−1.9, 0]	−2.29	[−3.9, −0.7]	0.0040
EPG 40	25	−0.2	1.8	[−0.9, 0.5]	−1.59	[−3.0, −0.2]	0.0243
Day 28							
Control	27	3.9	3.5	[2.6, 5.2]			
EPG 10	27	0.9	2.9	[−0.2, 2.0]	−2.98	[−4.7, −1.3]	0.0013
EPG 25	23	0.2	3.2	[−1.2, 1.5]	−3.75	[−5.6, −1.9]	0.0004
EPG 40	25	0.4	2.5	[−0.5, 1.4]	−3.48	[−5.1, −1.8]	0.0001
Day 42							
Control	25	4.4	5.1	[2.4, 6.4]			
EPG 10	27	0.8	3.3	[−0.4, 2.1]	−3.61	[−5.9, −1.3]	0.0033
EPG 25	24	−0.1	3.9	[−1.6, 1.5]	−4.48	[−7.0, −1.9]	0.0008
EPG 40	25	0.6	2.4	[−0.3, 1.6]	−3.80	[−6.0, −1.6]	0.0012
Day 56							
Control	27	2.6	5.4	[0.5, 4.6]			
EPG 10	27	0.1	3.9	[−1.3, 1.6]	−2.44	[−5.0, 0.1]	0.0127
EPG 25	23	−1.7	5.0	[−3.7, 0.4]	−4.25	[−7.2, −1.3]	0.0020
EPG 40	24	−1.3	4.0	[−2.9, 0.3]	−3.83	[−6.5, −1.2]	0.0017
Endpoint***							
Control	27	2.6	5.4	[0.5, 4.6]			
EPG 10	27	0.1	3.9	[−1.3, 1.6]	−2.44	[−5.0, 0.1]	0.0127
EPG 25	24	−1.6	4.9	[−3.5, 0.4]	−4.14	[−7.0, −1.3]	0.0019
EPG 40	25	−1.1	4.0	[−2.7, 0.5]	−3.66	[−6.3, −1.0]	0.0020

* 95% confidence intervals were used to compare the change from baseline to Days 14, 28, 42, and 56, and endpoint.
** Two-sided *t*-tests were used to compare the three EPG groups *vs.* control at Day 56. Bonferroni correction was used to adjust for multiple comparisons; statistical significance was declared at 0.017 (*i.e.*, 0.05 ÷ 3) level.
*** Subjects with data on or after Day 14 were included in the endpoint analysis, using the last observation carried forward (LOCF).

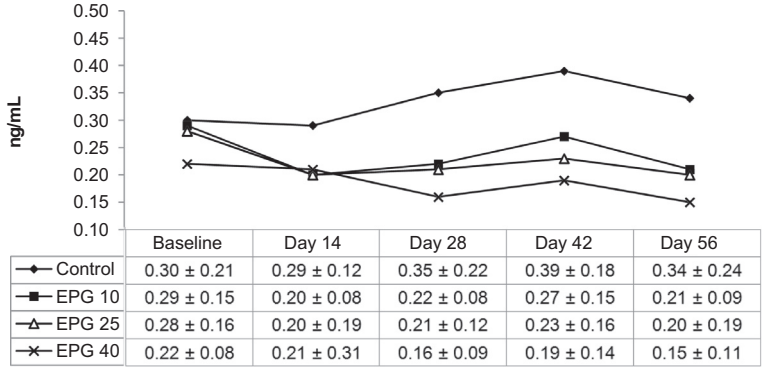


Fig. 6. Circulating phylloquinone (vitamin K_1) levels over time. Values represent mean ± standard deviation (SD).

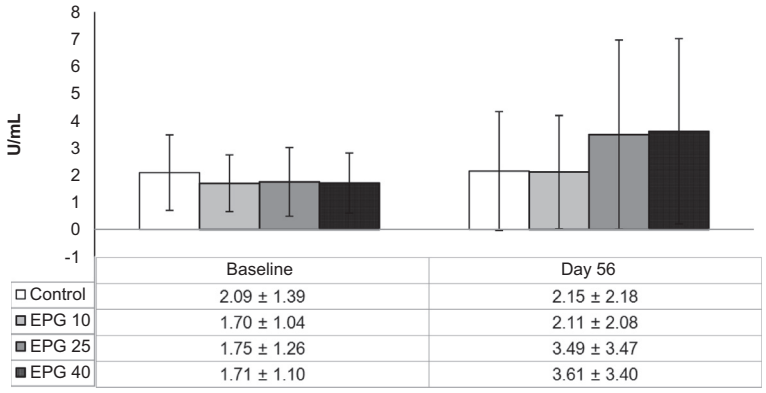


Fig. 7. Circulating PIVKA-II (proteins induced in vitamin K absence) levels at baseline and end of study. Values represent mean ± standard deviation (SD).

Table 8
Summary statistics for changes from baseline in blood phylloquinone (vitamin K_1) (ng/mL).

	N	Change	SD	95% CI*	Comparison to control		
					Difference	95% CI	*p*-Value**
Day 14							
Control	24	0	0.2	[−0.1, 0.1]			
EPG 10	25	−0.1	0.2	[−0.2, 0]	−0.09	[−0.2, 0]	0.0054
EPG 25	21	−0.1	0.2	[−0.2, 0]	−0.08	[−0.2, 0]	0.0098
EPG 40	21	0	0.3	[−0.2, 0.1]	−0.01	[−0.2, 0.1]	0.0140
Day 28							
Control	24	0.1	0.2	[0, 0.2]			
EPG 10	24	−0.1	0.1	[−0.1, 0]	−0.12	[−0.2, 0]	0.0024
EPG 25	20	−0.1	0.2	[−0.2, 0]	−0.15	[−0.3, 0]	0.0014
EPG 40	19	−0.1	0.1	[−0.1, −0.1]	−0.17	[−0.3, −0.1]	0.0001
Day 42							
Control	20	0.1	0.2	[0.1, 0.2]			
EPG 10	25	0	0.2	[−0.1, 0.1]	−0.16	[−0.3, −0.1]	0.0009
EPG 25	21	−0.1	0.2	[−0.2, 0]	−0.22	[−0.3, −0.1]	0.0001
EPG 40	18	−0.1	0.1	[−0.1, 0]	−0.22	[−0.3, −0.1]	0.0001
Day 56							
Control	23	0	0.2	[−0.1, 0.1]			
EPG 10	26	−0.1	0.2	[−0.1, 0]	−0.11	[−0.2, 0]	0.0242
EPG 25	22	−0.1	0.2	[−0.2, 0]	−0.12	[−0.3, 0]	0.0087
EPG 40	22	−0.1	0.1	[−0.1, −0.1]	−0.13	[−0.2, 0]	0.0027
Endpoint***							
Control	25	0	0.3	[−0.1, 0.1]			
EPG 10	27	−0.1	0.2	[−0.1, 0]	−0.12	[−0.2, 0]	0.0200
EPG 25	23	−0.1	0.2	[−0.2, 0]	−0.12	[−0.3, 0]	0.0093
EPG 40	22	−0.1	0.1	[−0.1, −0.1]	−0.14	[−0.2, 0]	0.0029

* 95% confidence intervals were used to compare the change from baseline to Days 14, 28, 42, and 56, and endpoint.
** Two-sided *t*-tests were used to compare the three EPG groups *vs.* control at Day 56. Bonferroni correction was used to adjust for multiple comparisons; statistical significance was declared at 0.017 (*i.e.*, 0.05 ÷ 3) level.
*** Subjects with data on or after Day 14 were included in the endpoint analysis, using the last observation carried forward (LOCF).

Table 9
Summary statistics for changes from baseline in blood PIVKA-II (proteins induced in vitamin K absence) (U/mL).

	N	Change	SD	95% CI*	Comparison to control		
					Difference	95% CI	*p*-Value**
Day 56							
Control	24	0.1	2.4	[−0.9, 1.0]			
EPG 10	25	0.4	1.7	[−0.2, 1.1]	0.36	[−0.8, 1.5]	0.0702
EPG 25	22	1.7	3.3	[0.3, 3.1]	1.68	[0, 3.3]	0.0032
EPG 40	19	1.9	3.6	[0.3, 3.5]	1.85	[0, 3.7]	0.0049

* 95% confidence intervals were used to compare the change from baseline to Day 56 and endpoint.
** Two-sided *t*-tests were used to compare the three EPG groups *vs.* control at Day 56. Bonferroni correction was used to adjust for multiple comparisons; statistical significance was declared at 0.017 (*i.e.*, 0.05 ÷ 3) level.

reported by 2 (7.4%) subjects. In the EPG 10 group, GI AEs lasting at least two successive days were reported by less than 10% of subjects, except for abdominal bloating/cramping in 4 (14.8%) subjects; constipation in 4 (14.8%) subjects; and urgency of bowel movement in 3 (11.1%) subjects. In the EPG 25 group, 3 (12.5%) subjects reported each of the following events: gas with discharge, oily evacuation, oily stool, and soft stool for two or more successive days. All other GI-related events in this group were reported by less than 10% of subjects. GI AEs lasting more than two successive days were reported by less than 10% of subjects from the EPG 40 group, except for abdominal bloating/cramping in 4 (16%) subjects, urgency of bowel movement in 3 (12%) subjects, and soft stool in 3 (12%) subjects.

Subgroup analyses based on sex, race (black and non-black), age (18–29, and 30+), and body mass index (⩽25 and >25) revealed that females consistently reported more GI AEs than males. No other statistically significant differences were noted among the subgroups.

3.4.2. Nutrient Intake

Nutrient intakes were determined from food consumption data after the study was completed, over 17 years ago. There are, however, some apparent inconsistencies in the nutrient intake results that cannot be resolved, because detailed food consumption records are no longer available. Specifically, the mean daily intakes of selenium and vitamin D appear on record to have been significantly greater (based on 95% CIs) among subjects receiving EPG (*vs.* control) at all time points, in a concentration-dependent manner. For vitamin D, intakes were approximately 1.1, 1.4, and 1.6 times as high as the control in each the EPG 10, EPG 25, and EPG 40 groups, respectively; for selenium intakes were approximately 1.1, 1.3, and 1.4 times greater. The source of the additional amounts of these nutrients in the EPG groups is uncertain, although it might have been due to human error when entering the data into the nutrient database.

Each of the six variations of the diet administered to subjects in this study included the food vehicles (spread, muffins, cookies, bis-

Table 10
Mean change from baseline to the end of the study in blood folate, vitamin B_{12}, zinc, iron, calcium, and phosphorus.

	N	Change	SD	95% CI*	Comparison to control		
					Difference	95% Cl	*p*-Value**
Folate (μg/L)							
Control	25	2.2	3.5	[0.9, 3.6]			
EPG 10	22	1.8	4.3	[0, 3.6]	−0.44	[−2.7, 1.8]	0.9405
EPG 25	21	1.5	5.4	[−0.8, 3.8]	−0.75	[−3.3, 1.8]	0.6119
EPG 40	22	2.0	3.7	[0.5, 3.5]	−0.25	[−2.3, 1.8]	0.6088
RBC folate[a] (μg/dL)							
Control	25	41.1	59.5	[17.8, 64.5]			
EPG 10	27	42.6	46.0	[25.2, 59.9]	1.45	[−27.3, 30.2]	0.8260
EPG 25	24	23.3	64.0	[−2.3, 48.9]	−17.8	[−52.4, 16.7]	0.4237
EPG 40	24	39.8	43.9	[22.2, 57.3]	−1.39	[−30.8, 28.0]	0.8103
Vitamin B_{12} (pg/mL)							
Control	25	23.0	122	[−24.7, 70.6]			
EPG 10	22	−39.1	127	[−92.4, 14.1]	−62.1	[−133.0, 9.1]	0.2496
EPG 25	21	−28.7	75.1	[−60.8, 3.4]	−51.7	[−111.0, 8.1]	0.2048
EPG 40	22	−31.8	83.1	[−66.5, 3.0]	−54.7	[−115.0, 5.7]	0.2864
Zinc (μg/dL)							
Control	27	−12.3	16.4	[−18.5, −6.1]			
EPG 10	27	−13.2	12.2	[−17.8, −8.6]	−0.96	[−8.7, 6.7]	0.7162
EPG25	24	−9.5	13.0	[−14.7, −4.3]	2.82	[−5.4, 11.0]	0.5457
EPG40	24	−11.7	17.1	[−18.6, −4.9]	0.57	[−8.6, 9.8]	0.8134
Iron (μg/dL)							
Control	26	0.2	36.1	[−13.7, 14.1]			
EPG 10	27	−4.8	34.3	[−17.7, 8.1]	−4.99	[−24.0, 14.0]	0.7152
EPG 25	24	8.8	43.3	[−8.6, 26.1]	8.56	[−13.5, 30.6]	0.2479
EPG 40	24	1.6	37.3	[−13.3, 16.5]	1.41	[−19.0, 21.8]	0.9072
Calcium (mg/dL)							
Control	26	0.2	0.5	[0, 0.4]			
EPG 10	27	0	0.3	[−0.1, 0.1]	−0.15	[−0.4, 0.1]	0.3308
EPG25	24	0	0.5	[−0.2, 0.2]	−0.17	[−0.4, 0.1]	0.2800
EPG 40	24	0	0.4	[−0.2, 0.1]	−0.18	[−0.5, 0.1]	0.2385
Phosphorus (mg/dL)							
Control	26	0.1	0.4	[0, 0.3]			
EPG 10	27	0.2	0.3	[0.1, 0.3]	0.11	[−0.1, 0.3]	0.3396
EPG25	24	0.1	0.5	[−0.1, 0.3]	0.01	[−0.3, 0.3]	0.9302
EPG40	24	0.3	0.7	[0, 0.6]	0.16	[−0.2, 0.5]	0.2473

Data shown for subjects evaluable for outcome analysis (see Section 2).
[a] Data for RBC folate only available through Day 28.
* 95% confidence intervals were used to compare the change from baseline (Day-1) to Days 14, 28, 42, and 56, and endpoint.
** Two-sided *t*-tests were used to compare the three EPG groups *vs.* control at Day 56. Bonferroni correction was used to adjust for multiple comparisons; statistical significance was declared at 0.017 (*i.e.*, 0.05 ÷ 3) level.

cuits). However, because the target foods were not labeled as containing either EPG or margarine (*i.e.*, double-blind study design), the person(s) entering the data into the system would have needed additional information, such as a nutritional information label or a list of ingredients with amounts (flour, sugar, *etc.*) for each of the target foods; the former providing a more reliable measure of nutrient intake than the latter. If the EPG "spread" was erroneously classified as "margarine", for example, this might have resulted in miscalculated nutrient (*e.g.*, vitamin D) intakes.

Analysis of the existing nutrient intake data (95% CIs) otherwise showed no statistically significant differences between EPG groups and the control group for the average daily intake of calcium, cholesterol, dietary fiber, total energy, folacin, water-soluble and insoluble dietary fiber, iron, phosphorus, potassium, protein, total carbohydrate, and zinc.

In this study, subjects consuming EPG were provided with extra fat in the form of margarine to make up for the caloric deficit created by incorporating EPG into the study food products. However, the results of nutrient analysis suggest that subjects in the EPG 25 and EPG 40 groups were unable to consume all of the additional fat necessary to fully compensate for the EPG consumed. In addition, fat intake declined over time in the EPG 25 and EPG 40 groups, so that the discrepancy in daily fat consumption between these groups and the control (ordinary triglycerides) group was 1.5–2 times as large during the final 2 weeks of the study, compared with the first 2 weeks (data not shown).

4. Discussion

Overall, retinol (vitamin A), α-tocopherol, and 25-OH D_2 (vitamin D, ergocalciferol) were not significantly affected by EPG, although there were occasionally within-group differences from baseline, especially early in the study, that reached statistical significance. On the other hand, EPG intake was associated with declines in circulating β-carotene and phylloquinone (vitamin K_1), and an increase in PIVKA-II (proteins induced in vitamin K absence); 25-OH D_3 (vitamin D, cholecalciferol) increased slightly over time in the EPG groups.

Significant declines in β-carotene were seen at each time period, and the effect was more pronounced in the EPG groups. However, if the effect was indeed related to EPG intake, it is uncertain why there was no apparent relationship to EPG concentration (more severe at 10 g/day and 40 g/day than at 25 g/day).

The bioavailability of carotenoids such as β-carotene can vary considerably based on the food matrix, concurrent fat intake, and serum/tissue concentrations. A study that explored, among others, the fate of β-carotene from carrot purée when administered to

Table 11
Mean change from baseline to the end of the study in blood intact PTH, PT, PTT and RBP.

	N	Change	SD	95% CI*	Comparison to control		
					Difference	95% CI	*p*-Value**
Intact PTH							
Control	27	6.1	8.5	[3.0, 9.3]			
EPG 10	27	4.6	11.4	[0.3, 8.9]	−1.59	[−6.9, 3.8]	0.5858
EPG 25	23	9.0	11.2	[4.4, 13.6]	2.84	[−2.6, 8.3]	0.2548
EPG 40	24	9.1	12.3	[4.2, 14.0]	2.94	[−2.8, 8.7]	0.4733
PT (seconds)							
Control	27	−0.1	0.4	[−0.3, 0.1]			
EPG 10	27	0	0.3	[−0.1, 0.1]	0.11	[−0.1, 0.3]	0.5560
EPG 25	24	0.1	0.4	[0, 0.3]	0.22	[0, 0.4]	0.1538
EPG 40	24	0.1	0.3	[−0.1, 0.2]	0.18	[0, 0.4]	0.1330
PTT (seconds)							
Control	27	−1.7	1.7	[−2.4, −1.0]			
EPG 10	27	−1.3	2.1	[−2.1, −0.5]	0.42	[−0.6, 1.5]	0.3869
EPG 25	24	−1.0	1.6	[−1.7, −0.4]	0.67	[−0.2, 1.6]	0.2905
EPG 40	24	−1.3	1.7	[−2.0, −0.6]	0.42	[−0.5, 1.4]	0.9323
RBP (mg/dL)							
Control	24	0.1	0.6	[−0.1, 0.4]			
EPG 10	23	0.4	0.7	[0.2, 0.7]	0.31	[−0.1, 0.7]	0.1222
EPG 25	17	0.2	0.5	[−0.1, 0.4]	0.05	[−0.3, 0.4]	0.7007
EPG 40	22	0.5	1.0	[0.1, 0.9]	0.34	[−0.1, 0.8]	0.1689
Osteocalcin (ng/mL)							
Control	25	7.2	11.4	[2.7, 11.7]			
EPG 10	26	8.7	9.8	[4.9, 12.5]	1.48	[−4.3, 7.3]	0.6242
EPG 25	22	8.1	8.7	[4.4, 11.7]	0.86	[−5.0, 6.7]	0.8982
EPG 40	24	7.1	12.8	[2.0, 12.3]	−0.07	[−6.9, 6.7]	0.9045
Undercarboxylated osteocalcin (ng/mL)							
Control	25	0.8	1.8	[0.1, 1.5]			
EPG 10	26	1.3	1.8	[0.6, 2.0]	0.50	[−0.5, 1.5]	0.3708
EPG 25	22	1.6	1.9	[0.8, 2.4]	0.82	[−0.2, 1.9]	0.1029
EPG 40	24	1.1	2.1	[0.3, 2.0]	0.39	[−0.7, 1.5]	0.2891

Data shown for subjects evaluable for outcome analysis (see Section 2).
* 95% confidence intervals were used to compare the change from baseline (Day-1) to Days 14, 28, 42, and 56, and endpoint.
** Two-sided *t*-tests were used to compare the three EPG groups *vs.* control at Day 56. Bonferroni correction was used to adjust for multiple comparisons; statistical significance was declared at 0.017 (*i.e.*, 0.05 ÷ 3) level.

Table 12
Mean change from baseline to the end of the study in blood cholesterol, HDL-C, LDL-C, and triglycerides.

	N	Change	SD	95% CI*	Comparison to control		
					Difference	95% CI	*p*-Value**
Cholesterol (mg/dL)							
Control	26	4.9	16.3	[−1.4, 11.1]			
EPG 10	27	6.9	20.3	[−0.8, 14.5]	1.99	[−8.0, 11.9]	0.9291
EPG 25	24	−3.3	18.8	[−10.8, 4.2]	−8.18	[−17.9, 1.6]	0.1157
EPG 40	24	2.1	17.7	[−4.9, 9.2]	−2.74	[−12.1, 6.7]	0.4091
HDL-C[a] (mg/dL)							
Control	27	−3.2	5.9	[−5.4, −0.9]			
EPG 10	27	−4.9	6.4	[−7.3, −2.5]	−1.75	[−5.0, 1.5]	0.4211
EPG 25	24	−7.0	5.9	[−9.4, −4.7]	−3.90	[−7.1, −0.6]	0.0235
EPG 40	25	−4.4	5.0	[−6.4. −2.5]	−1.27	[−4.3, 1.7]	0.3181
LDL-C[a] (mg/dL)[a]							
Control	27	7.0	16.2	[0.9, 13.1]			
EPG 10	24	3.9	16.6	[−2.7, 10.5]	−3.14	[−12.2, 5.9]	0.6234
EPG 25	24	−7.6	12.3	[−12.5, −2.6]	−14.6	[−22.6, −6.6]	0.0001
EPG 40	25	−3.5	13.0	[−8.6, 1.6]	−10.6	[−18.6. −2.6]	0.0017
Triglycerides (mg/dL)							
Control	26	18.7	64.9	[−6.2, 43.7]			
EPG 10	27	−10.6	44.2	[−27.3, 6.0]	−29.4	[−59.2, 0.4]	0.1445
EPG 25	24	−0.3	28.7	[−11.8, 11.1]	−19.0	[−47.3, 9.2]	0.6136
EPG 40	24	1.7	26.5	[−8.9, 12.3]	−17.0	[−45.0, 10.9]	0.2032

Data shown for subjects evaluable for outcome analysis (see Section 2).
[a] Data for HDL-C and LDL-C only available through Day 28.
* 95% confidence intervals were used to compare the change from baseline (Day-1) to Days 14, 28, 42, and 56, and endpoint.
** Two-sided *t*-tests were used to compare the three EPG groups *vs.* control at Day 56. Bonferroni correction was used to adjust for multiple comparisons; statistical significance was declared at 0.017 (*i.e.*, 0.05 ÷ 3) level.

Table 13
Incidence of adverse events among all study subjects.[a]

Body system	Control ($N = 36$)	EPG 10 ($N = 34$)	EPG 25 ($N = 35$)	EPG 40 ($N = 34$)
Body as a whole	11 (30.6%)	9 (26.5%)	11 (31.4%)	10 (29.4%)
Central and peripheral nervous system disorders	19 (52.8%)	14 (41.2%)	16 (45.7%)	18 (52.9%)
Headache	19 (52.8%)	14 (41.2%)	16 (45.7%)	17 (50%)
Gastrointestinal (any)	22 (61.1%)	27 (79.4%)	26 (74.3%)	27 (79.4%)
Gastrointestinal (pre-defined)	21 (58.3%)	23 (67.6%)	24 (68.6%)	25 (73.5%)
Abdominal bloating/cramping	13 (36.1%)	15 (44.1%)	20 (57.1%)	17 (50%)
Constipation	8 (22.2%)	8 (23.5%)	8 (22.9%)	9 (26.5%)
Diarrhea	10 (27.8%)	10 (29.4%)	19* (54.3%)	21* (61.8%)
Fecal incontinence	4 (11.1%)	3 (8.8%)	6 (17.1%)	10 (29.4%)
Gas with discharge	4 (11.1%)	8 (23.5%)	11* (31.4%)	14* (41.2%)
Hard stool	5 (13.9%)	10 (29.4%)	4 (11.4%)	5 (14.7%)
Heartburn	5 (13.9%)	7 (20.6%)	4 (11.4%)	3 (8.8%)
Liquid stool	6 (16.7%)	8 (23.5%)	9 (25.7%)	18* (52.9%)
Oily evacuation	2 (5.6%)	6 (17.6%)	12* (34.3%)	14* (41.2%)
Oily spotting	3 (8.3%)	8 (23.5%)	14* (40%)	16* (47.1%)
Oily stool	1 (2.8%)	10* (29.4%)	16* (45.7%)	20* (58.8%)
Passing gas	15 (41.7%)	19 (55.9%)	19 (54.3%)	21 (61.8%)
Soft stool	6 (16.7%)	12 (35.3%)	15* (42.9%)	19* (55.9%)
Urgency of bowel movement	9 (25%)	11 (32.4%)	15 (42.9%)	19 (55.9%)
Gastrointestinal system disorders (other)	15 (41.7%)	14 (41.2%)	11 (31.4%)	14 (41.2%)
Abdominal pain	3 (8.3%)	2 (5.9%)	1 (2.9%)	6 (17.6%)
Dyspepsia	3 (8.3%)	2 (5.9%)	3 (8.6%)	0
Eructation	0	0	0	1 (2.9%)
Gastrointestinal disorder, nonspecific	0	1 (2.9%)	0	0
Gastroesophageal reflux	0	0	0	1 (2.9%)
Hemorrhoids	0	1 (2.9%)	0	0
Mouth dry	0	1 (2.9%)	0	0
Nausea	4 (11.1%)	5 (14.7%)	5 (14.3%)	3 (8.8%)
Tooth ache	0	2 (5.9%)	2 (5.7%)	1 (2.9%)
Tooth caries	0	0	1 (2.9%)	1 (2.9%)
Vomiting	7 (19.4%)	5 (14.7%)	3 (8.6%)	6 (17.6%)
Hearing and vestibular disorders	0	1 (2.9%)	0	1 (2.9%)
Metabolic and nutritional disorders	1 (2.8%)	0	1 (2.9%)	2 (5.9%)
Musculoskeletal system disorders	2 (5.6%)	1 (2.9%)	0	2 (5.9%)
Neoplasm	1 (2.8%)	0	0	0
Platelet, bleeding and clotting disorders	0	0	1 (2.9%)	0
Psychiatric disorders	1 (2.8%)	1 (2.9%)	0	3 (8.8%)
Reproductive disorders, female	6 (16.7%)	4 (11.8%)	5 (14.3%)	3 (8.8%)
Resistance mechanism disorders	3 (8.3%)	0	4 (11.4%)	1 (2.9%)
Respiratory system disorders	19 (52.8%)	20 (58.8%)	12 (34.3%)	14 (41.2%)
Rhinitis	14 (38.9%)	12 (35.3%)	9 (25.7%)	8 (23.5%)
Secondary terms	1 (2.8%)	0	1 (2.9%)	1 (2.9%)
Skin and appendages disorders	3 (8.3%)	2 (5.9%)	2 (5.7%)	5 (14.7%)
Urinary system disorders	2 (5.6%)	0	4 (11.4%)	2 (5.9%)
Vascular (extracardiac disorders)	0	1 (2.9%)	1 (2.9%)	0
Vision disorders	1 (2.8%)	0	0	0

[a] Includes 14 pre-defined gastrointestinal symptoms and others by body system and World Health Organization (WHO) terms. If a subject experienced more than one episode of an adverse event, he/she was counted only once for the total number of subjects experiencing that adverse event.
* Statistically significant difference *vs.* control ($p \leqslant 0.05$), Fisher's Exact Test with pairwise comparisons.

healthy human subjects, showed based on samples of blood, and stomach and duodenal contents, that the stomach initiates the transfer of carotenoids from the food matrix to the fat phase of a meal, but that the proportion of carotenoids recovered in the micellar phase of the duodenum is very low (<7%) (Tyssandier et al., 2003).

It is possible that the apparent effect of EPG on circulating β-carotene was related to the lower dietary fat intake among subjects receiving EPG. As previously mentioned, subjects might not have consumed all of the additional fat necessary to fully compensate for what EPG displaced in the diet. In this case, as a lipid-like material, EPG might have affected the absorption of these nutrients strictly through physicochemical processes, acting as a lipid "sink" during transit in the gastrointestinal tract. Substances known to reduce the bioavailability of carotenoids are lipid-lowering agents such as cholestyramine and probucol (Elinder et al., 1995), nonabsorbable fat substitutes such as sucrose polyester (olestra) (Peters et al., 1997; Schlagheck et al., 1997; Tulley et al., 2005; Neuhouser et al., 2006), plant sterol-enriched margarines (Gylling et al., 1999; Law, 2000; Hendriks et al., 2003), and dietary fiber supplementation (Rock and Swendseid, 1992). No dietary reference intakes (DRIs) *per se* have been proposed by the Institute of Medicine for carotenoids, although existing recommendations for increased consumption of carotenoid-rich fruits and vegetables are supported (IOM, 2000).

In experimental animal studies, EPG has been associated with declines in some fat-soluble vitamins, including vitamin D, but with no clinical signs of vitamin deficiency (unpublished data). In the present study, dietary EPG was not associated with any effects on circulating 25-OH-D_2 levels. At the end of the study, 25-OH-D_3 levels in the EPG 25 and EPG 40 groups were lower than baseline (also in EPG 25 at Day 42). However, it is difficult to determine whether EPG had any effect on circulating 25-OH-D_3 levels in this study for a number of reasons. First, the control group had an abnormal rise in circulating 25-OH-D_3 to levels that, at times, far exceeded baseline levels. This is an unexpected finding, since the study was conducted in the winter in Chicago, Illinois, USA, when endogenous 25-OH-D_3 synthesis would have been extremely low,

and the dietary intakes of 25-OH-D_3 by the control and EPG groups (from prepared meals consumed at the study facility) would have been comparable. As a result of this unusual occurrence, the control group showed a greater change (increase) from baseline than the EPG groups, which had only marginal increases/decreases from baseline. Secondly, the within-group differences from baseline were not statistically significant for any EPG group; in the EPG 10 group, 25-OH D_3 was consistently higher than baseline after Day 14. Therefore, any possible effect of EPG on circulating 25-OH-D_3 in this study was very small, evident only at 25 g/day and greater, and with no other clinical manifestation.

Whereas there was no change or a slight increase from baseline circulating phylloquinone levels in the control, EPG intake was associated with a slight decline across all groups. However, the declines did not exceed 0.1 ng/mL and were not statistically significant within any of the individual groups; statistical significance was observed only when the differences within each EPG group were compared to the differences (none or positive) in the control. In experimental animal studies, EPG has not been found to have any effect on vitamin K (unpublished data).

By the end of the study, the levels of proteins induced in vitamin K absence (PIVKA-II) had increased significantly in the EPG 25 and EPG 40 groups, compared to the control; in the EPG 10 group, the difference from baseline was comparable to the difference from baseline in the control. Combined with the phylloquinone results, these data suggest that EPG might have affected the synthesis of vitamin K-dependent clotting factors to some extent, but the changes were small, and there was no indication of any clinical manifestation. The changes in clotting parameters (PT and PTT) from baseline to the end of the study were comparable between the control and EPG groups.

With the exception of gastrointestinal discomfort, all adverse events reported by subjects in this study were considered unrelated to EPG. Seven of the 14 pre-defined gastrointestinal adverse events (gas with discharge; diarrhea; oily spotting; oily evacuation; oily stool; liquid stool; soft stool) were reported more frequently by subjects receiving 25 or 40 g/day of EPG, especially females. In general, the incidence and duration of these symptoms correlated with EPG dietary concentration and suggest that 10 g/day of EPG was reasonably well tolerated.

Conflict of interest statement

The authors are unaware of any conflicts of interest.

Funding sources statement

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