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Fat Soluble Vitamins in Cystic Fibrosis and Pancreatic Insufficiency: Efficacy of a Nutrition Intervention

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Abstract

Objectives—To assess the impact of LYM-X-SORB™ (LXS), an organized lipid matrix that has previously been shown to be absorbable without pancreatic enzyme therapy on fat soluble vitamin status in children with CF and PI.

Methods—Children with CF and PI were randomized to daily LXS or an iso-caloric placebo comparison supplement for 12 months. Serum vitamins A (retinol), D (25-hydroxyvitamin D[25D]), E (α-tocopherol, α-tocopherol:cholesterol ratio) and K (%undercarboxylated osteocalcin [%ucOC] and plasma proteins induced by vitamin K absence factor II [PIVKA II]) were assessed at baseline and 12 months. Dietary intake was determined using 3-day weighed food records, and supplemental vitamin intake by a comprehensive questionnaire.

Results—58 subjects (32 males, age 10.3±2.9 yrs [mean±SD]) with complete serum vitamin, dietary and supplemental vitamin data were analyzed. After adjusting for dietary and supplemental vitamin intake, serum retinol increased 3.0±1.4 μg/dL (coefficient±SE) (Adj R^2=0.02, p=0.03) and vitamin K status improved as demonstrated by a decreased %ucOC of −6.0±1.6% by 12 months (Adj R^2=0.15, p<0.001). These changes occurred in both the LXS and placebo comparison groups. No changes in serum 25D or α-tocopherol were detected. Both nutrition interventions increased caloric intake a mean of 83±666 kcal/d by 12 months.

Conclusions—Vitamin A and K status improved, while vitamin D and E status was unchanged over 12 month of LXS and iso-caloric placebo comparison supplement in children with CF and PI.

Keywords

Cystic fibrosis; children; vitamin A; vitamin D; vitamin E; vitamin K

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Clinical Trial Registration: Study of LYM-X-SORB™ to Improve Fatty Acid and Choline Status in Children with Cystic Fibrosis and Pancreatic Insufficiency, NCT00406536

Introduction

About 90% of patients with cystic fibrosis (CF) in the US have pancreatic insufficiency (PI) and are at risk for fat malabsorption and fat soluble vitamin deficiency (1). Each fat soluble vitamin has multiple and essential metabolic functions for human health. Vitamin A is essential for normal vision, epithelial cell integrity, epithelial proliferation and immunity (2). Vitamin D is required for bone health and has a role in immune function and incidence of cancer, type 1 diabetes, autoimmune disease and heart disease (3–6). Vitamin E prevents cell membrane oxidation, maintains neurological functions, and studies reported a role in cognitive function in infants with CF (7, 8). Vitamin K is essential for bone calcification, coagulation, energy metabolism and modulation of inflammation (2, 9). Even with effective pancreatic enzyme medications and CF specific vitamin and mineral supplements, a portion of patients with CF and PI have suboptimal fat soluble vitamin status (10–14). LYM-X-SORB™ (LXS, Avanti Polar Lipids, Alabaster, AL) is a choline rich structured lipid matrix that was shown to be absorbed without pancreatic enzymes in subjects with CF and PI and improve growth and vitamin A status using a first generation LXS formulation (15).

The primary aim of this report was to assess the effect of second generation LXS on fat soluble vitamin status. These data were collected as a part of the randomized, placebo controlled trial to evaluate choline status in children with CF and PI with LXS supplementation compared to an iso-caloric placebo comparison supplement. The secondary aim of this report was to compare fat soluble vitamin status in this current sample to that of participants from a series of CF nutrition studies over the past decade.

Materials and Methods

Subjects with CF, PI and mild to moderate lung disease aged 5.0 to 17.9 yrs were recruited from ten CF Centers beginning March 2007 (last study visit May 2011) to participate in the CF Avanti Study, a placebo controlled double blind study evaluating the impact of second generation LXS on choline status. Exclusion criteria included FEV_1 <40% predicted, residual pancreatic lipase activity (fecal elastase >15ug/g stool), liver disease (serum GGT >3x reference range) or other chronic health conditions that may affect nutrient absorption or growth. The fecal elastase inclusion criteria (<15 ng/g) was selected for this study to increase the likelihood of participants with more complete PI. The GGT exclusion value was selected as a study specific screening criteria and does not reflect that used in clinical care to diagnose liver disease. Subjects were randomized to 12 months of LXS or placebo comparison supplementation; 2 packets/day (64 g powder) for ages 5.0 to 11.9 yrs, and 3 packets/day (96 g powder) for ages 12.0 to 17.9 yrs. This second generation LXS had improved palatability and solubility characteristics, and was designed to be mixed with a variety of foods and beverages. LXS is composed of lysophosphatidylcholine, triglycerides and fatty acids which form an organized choline rich lipid matrix complexed to wheat flour and sugar. The comparison intervention (placebo) had similar calories, total fat and macronutrient distribution (protein 6%, carbohydrate 58%, lipid 34% of kcal), and only about 10% as much choline. LXS and the placebo comparison intervention had the same calories, 157 kcal per packet and contained no fat soluble vitamins except for vitamin E as α-tocopherol (4.8 and 1.6 mg per packet in LXS and placebo comparison intervention

respectively). This protocol was approved by the Institutional Review Board at The Children's Hospital of Philadelphia (CHOP) and in each participating Center. Verbal assent was obtained from all subjects and written informed consent was obtained from their parents or guardians.

Dietary intake was assessed at baseline and 12 months with 3-day weighed food records and analyzed (Nutrition Data System for Research, National Coordinating Center, University of Minnesota, Minneapolis, MN) (16). Supplemental intake of vitamins and minerals including product, frequency and dose was assessed by a comprehensive questionnaire. The Dietary Reference Intake (DRI) was used to evaluate nutrient intakes based on Recommended Dietary Allowance (RDA) and Adequate Intake (AI) recommendations. The DRI are nutrient-based reference values used to assess diets of the general population (2,3,7). DRI intakes are designed to reduce the incidence of deficiency diseases and to help individuals optimize health and prevent disease. Specifically, the RDA is the evidence-based daily nutrient intake that is sufficient to meet requirements of nearly all (97.5%) healthy individuals by age and sex groups. Intakes of a few essential nutrients are evaluated by the AI value. The AI is an average daily intake based on less evidence and is an estimated nutrient intake assumed to be adequate. The %RDA for vitamins A (retinol activity equivalents [RAE]), D (calciferol) and E (α-tocopherol) and as %AI for vitamin K (phylloquinone) were calculated (2,3,7). Energy intake was also assessed and expressed as percent Estimated Energy Requirement (%EER) for active children, as previously determined for children with CF and PI (17, 18). Adherence to LXS or placebo comparison intervention intake was calculated as a percentage of packets consumed/total packets prescribed every 28 days over the 12 month study. The percentage adherence that was used to adjust calories and vitamin intake from LXS or placebo comparison intervention was based upon the 28 day period within which the diet record assessment occurred.

Fasting serum vitamin A (retinol), vitamin D (25-hydroxyvitamin [25D]), vitamin E (α-tocopherol, and α-tocopherol:cholesterol ratio), and vitamin K (percent of total osteocalcin as undercarboxylated osteocalcin [%ucOC]) were assessed at baseline and 12 months. For vitamin K status, plasma proteins induced by vitamin K absence factor II [PIVKA II] was also assessed in a subsample of participants. Retinol was analyzed by high performance liquid chromatography (Craft Technologies, Inc. Wilson, NC). Serum retinol was considered low based on NHANES 1999–2002 data for the 5^{th} percentile (< 30 ug/dL). 25D was determined using a radioimmunoassay with a radio-iodinated tracer (Hollis Laboratory, Medical University of South Carolina, Charleston, SC) (19) and by CHOP Clinical Laboratory using liquid chromatography-tandem mass spectrometry. Levels of 25D <30 ng/mL were considered low based on current literature related to non-bone health outcomes (20–25). α-Tocopherol was assessed by quantitative high performance liquid chromatography (ARUP Laboratories, Salt Lake City, UT). Total cholesterol was assessed by standard methods at CHOP Clinical Laboratory. Low levels of α-tocopherol were defined from clinical laboratory reference ranges as <3 mg/L for children ages 1 to 12 yrs and < 6 mg/L for 13 to 19 yrs (26–28). When using α-tocopherol:cholesterol ratio, the cut point of <5.4 mg/g was used to define low levels in children based on CF specific results (29). For vitamin K, serum concentrations of total osteocalcin and ucOC were determined using a hydroxyapatite-binding radioimmunoassay and expressed as the percentage not bound

(undercarboxylated osteocalcin [%ucOC]) and normalized to total osteocalcin (Gundberg Laboratory, Yale School of Medicine, New Haven, CT) (30). PIVKA II was determined using an enzyme-linked immunoassay (Booth Laboratory, Tufts University, Boston, MA) (31). For vitamin K status using %ucOC, >50% was defined as low and for PIVKA II, >2.0 ng/mL was defined as low (32–34).

Weight was measured to the nearest 0.1 kg using an electronic scale (Scaletronix, White Plains, NY) and height to the nearest 0.1 cm using a stadiometer (Holtain, Crymych, UK). Age- and sex-specific standard deviation scores (Z-scores) for weight (WAZ), height (HAZ) and body mass index (BMIZ) were calculated (35).

Descriptive statistics were calculated for the study sample using means, standard deviations, medians, and ranges (as appropriate) for continuous variables, and frequency distributions for categorical variables. Vitamin intake and serum levels were analyzed for each group (LXS and placebo comparison group) separately and then for the total sample. Differences in serum vitamin levels between LXS and placebo comparison groups at baseline and at 12 months were assessed with unpaired t-tests or Mann Whitney tests, as appropriate for skewness. For longitudinal changes in growth status from baseline to 12 months, paired t-tests were used. Significant changes from baseline in serum vitamin status was explored for each vitamin outcome variable separately using longitudinal mixed effects (LME) models adjusting for both dietary and supplemental vitamin intake as % RDA or % AI (depending upon the vitamin) with time as the coefficient for the 12-month change. LME models were run separately by group (LXS and placebo comparison group) and then for the total sample. An interaction term for group x time was also explored to determine if time trends in vitamin status differed by supplementation group. All statistical analyses were performed with STATA 12.0 (STATA Corp., College Station, TX) and significance was defined as p value < 0.05. Data are presented as mean ± SD, unless otherwise indicated.

Results

A total of 58 subjects (32 males, age 10.3 ± 2.9 yrs, range 5.1to17.8) had baseline and 12 month serum vitamin, diet and supplemental vitamin intake results. Dietary and supplemental vitamin intakes at baseline and 12 months are presented in Table 1. for the total sample and by group as % RDA and % AI. For the total sample, caloric intake increased from a mean of 2569 ± 752 kcal/d at baseline to a mean of 2653 ± 660 kcal/d at 12 months resulting in a net increase of 83 ± 666 kcal/d. Baseline mean % EER was 126% ± 33 and was similar for each group. Growth status improved significantly over the 12 months: HAZ from −0.49 ± 0.93 to −0.42 ± 0.92 (p=0.019) and WAZ from −0.38 ± 0.70 to −0.29 ± 0.83 (p=0.043). Median supplemental intake vitamin A was high, approximately five times the RDA. Dietary intake of vitamin D was <50% RDA, and supplemental vitamin D was approximately two times the RDA. Total vitamin E was primarily from supplemental intake and this was 13 to 16 times the RDA. Dietary intake of vitamin K was similar to the AI, while supplemental intake was five times the AI at baseline and 13 times the AI at 12 months. The placebo comparison group had a higher supplemental vitamin K intake at 12 months.

Serum vitamin levels at baseline and 12 months are presented for the total sample and separately by supplementation group in Table 2. LME models show that vitamin A status improved significantly from baseline to 12 months by 3.0 ± 1.4 μg/dL (Adjusted R^2=0.02, p=0.03), as did serum vitamin K status, as indicated by a decline in %ucOC of −6.0 ± 1.6% by 12 months (Adjusted R^2=0.15, p<0.001). Serum vitamin D and E status did not change over 12 months, and vitamin K indicated by PIVKA II (subsample n=42) also did not change. The improvements in vitamin A (retinol) and K (%ucOC) status over 12 months were evident in both supplementation groups, although serum retinol was lower at both time points in the comparison group. The α-tocopherol:cholesterol ratio was lower in the comparison group at 12 months. Supplemental intake of vitamins D, E and K, but not A, significantly predicted serum vitamin status, while dietary vitamin intake was not predictive of serum status for any vitamin.

Table 3. provides a comparison of serum vitamin status for the present CF Avanti study sample with previous samples of children and young adults with CF and PI who participated in two CHOP nutrition studies over 12 years, the CF Nutrition (1998–2000) (11,12) and CF Bones (2000–2002) studies (10,13,14). There is a greater proportion of subjects with low serum retinol status in the present study (20%) than in the past studies (4% and 0%). In contrast, vitamin K status improved with only 5% in the present study having low vitamin K status based upon %ucOC compared to 19% in the past, and 19% in present study based upon PIVKA II compared to 50% in the past. Vitamin D status was low in 50% subjects in the present study, an improvement from 90% in the CF Bones study. For vitamin E status, 13% were low with little change from the past.

Discussion

The aim of this study was to assess the impact on fat soluble vitamin absorption of LXS and a placebo comparison group with similar calorie and fat composition in children with CF and PI. Serum vitamin A and K status, adjusted for dietary and supplemental intakes improved overall, while vitamin D and E were unchanged. The Lepage et al. (15) randomized placebo controlled trial with first generation LXS in children ages 6 to 17 yrs, reported an improvement in serum vitamin E in the LXS group only and no changes in serum vitamin A, D and K (PIVKA II). In the present study the improvements in vitamin A and K status occurred in both LXS and placebo comparison groups. These improvements may be related to the sustained increase in caloric intake, or better adherence to CF care while enrolled in this research study or other unidentified factors. Vitamin A status improvement is not explained by dietary or supplemental intake. Vitamin K status improvement may be related to the greater use of higher dose supplemental vitamin K with increasing participant age (two vitamins per day in older patients) and with increased vitamin K content of commercially available CF-specific vitamin products (i.e. SourceCF®, AquADEKS®).

Changes over recent years in vitamin status in CF can be considered by comparing the present study to two older studies conducted with similar populations and methods as summarized in Table 3. Serum vitamin status and both dietary and supplemental vitamin intakes were assessed using similar methods. Intake from supplements was consistently

higher than dietary intake in each vitamin evaluated. In this current CF Avanti study, dietary intake of vitamins were similar to those found in the CF Nutrition and CF Bones studies, with the exception of dietary vitamin D intake which was lower in the present study than CF Bones (median 264 vs. 329 IU/d) (14). Mean supplemental intake of vitamins A and E were similar to those found in previous studies, while intakes of D and K were somewhat higher in the present study compared to the CF Bones study (13, 14), likely due to increased D and K dose per pill in the CF specific vitamin products.

Mean baseline serum retinol (Table 3) was lower in the present study than in the CF Nutrition (11) and CF Bones studies (10). In the present sample, 20% had low serum retinol levels compared to 4% and 0% in the CF Nutrition and CF Bones studies. The decrease in serum retinol in the present study compared to previous studies was likely due to vitamin A changes in CF-specific vitamin formulation over the last 10 years. Preformed vitamin A content has decreased and carotenoids content has increased. Carotenoids are inefficiently converted to serum retinol (2). Mean baseline serum retinol in the current study was similar to retinol levels reported by Brei et al. in a 2011 observational study in Germany in subjects ages 4 to 27 yrs (39 μg/dL) (36) and higher than those reported in 2007 by Hakim et al. in Israel in subjects ages 1.5 to 27 yrs (24 μg/dL) (37).

Serum vitamin D status is typically low in children and adults with CF and PI. The median baseline serum 25D (31 ng/ml) in this study was comparable to levels observed in children ages 1 to 17 yrs in the UK in 2010 (median 29 ng/mL), and modestly improved compared to older reports from the US and UK from similar populations (21 to 26 ng/ml) (14, 38–41). In the present sample, 50% had low (<30 ng/mL) 25D, which was an improvement from the 90% of children and young adults found insufficient in CF Bones Study. It was similar to the 46 to 56% insufficiency found in more recent studies in the US, UK and Australia (14, 38, 41–43). The serum 25D improvement over time was likely due to enhanced clinical monitoring and increased vitamin D supplementation in patients with CF and PI.

There has been little change in vitamin E status over this time interval. The median serum α-tocopherol:cholesterol ratio was 7.7 mg/g in the present study, comparable to that found in our previous CF Nutrition study (median: 8.2 mg/g) (12) and somewhat improved compared to James et al. (median: 5.7mg/g) conducted in the UK in a similar population two decades ago (29). Using the cutoff value of 5.4 mg/g for vitamin E deficiency, 13% of both our present sample and the previous CF Nutrition sample (12) were deficient.

The proportion of children with low vitamin K status in the present study was only about one quarter that found in CF Bones (5% vs. 19%). Recent studies of subjects ages 8 to 25 years in the US and Canada have found better vitamin K status (35 to 47% ucOC) (13, 44, 45) than earlier studies of children ages 5 to18 years in Greece and the UK (59 to 71%) (46, 47). Despite this improvement in vitamin K status children in the present study remain suboptimal (74% with >20% ucOC), which was comparable to the 64 to 100% found in other more recent samples of children with CF (13, 44, 45). Using PIVKA II, the median was 1.2 ng/mL in the present study. These PIVKA II levels were lower than the 2 to 5 ng/mL found in more recent studies of children with CF (13, 39), and much lower than the 11 to 23 ng/mL found in earlier studies of children and adults in Canada and the US (45, 48–

50). Others have observed higher rates of low vitamin K status than in the present sample based upon PIVKA II, from 42 to 80% (13, 47, 49, 50). The improvement in vitamin K status was likely the result of an increased use of CF specific products and a higher vitamin K dose in the products. From this review of fat soluble vitamin status in patients with CF and PI, there is evidence that the situation has changed over 12 years. Improvement in both vitamin D and K status are demonstrated, with a possible decline in vitamin A status and little change in vitamin E status.

In summary the nutritional intervention described in this study provided similar additional calories to both treatment groups and was associated with improvement in growth status. Vitamin A and K status improved over 12 months while vitamin D and E remained unchanged in both the LXS and placebo comparison supplement groups. The current vitamin products and age adjusted dosing approach have not resulted in optimal fat soluble vitamin status in CF and PI.

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Abbreviations

25D	Serum 25 Hydroxyvitamin D
AI	Adequate Intake
CF	Cystic Fibrosis
CHOP	The Children's Hospital of Philadelphia
DRI	Dietary Reference Intake
FEV_1	Forced Expiratory Volume
GGT	Serum Gamma Glutamyltransferase
LXS	LYM-X-SORB™
NHANES	National Health and Nutrition Examination Survey
PI	Pancreatic Insufficiency

PIVKA II	Serum Proteins induced by vitamin K absence factor II
RAE	Retinol Activity Equivalent
RDA	Recommended Dietary Allowance
ucOC	Serum Undercarboxylated Osteocalcin

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Table 1

Dietary and supplemental vitamin intake as median [range] by Dietary Reference Intake criteria

	Baseline			12 Months		
	All n=58	**LYX n=23**	**Placebo Comparison Group n=35**	**All**	**LYX**	**Placebo Comparison Group**
Vitamin A, % RDA						
Diet	165 [46 – 500]	163 [70 – 296]	176 [46 – 500]	128 [44 – 438] *	126 [61 – 337]	131 [44 – 438] *
Supplement	555 [0 – 1284]	548 [0 – 1284]	616 [130 – 1232]	456 [0 – 1235]	492 [130 – 840]	420 [0 – 1235]
Total	723 [105 – 1722]	718 [105 – 1442]	765 [315 – 1722]	673 [120 – 1462] *	676 [211 – 1168]	654 [120 – 1462] *
Vitamin D, % RDA						
Diet	44 [12 – 171]	46 [22 – 171]	41 [12 – 111]	37 [9 – 136]	38 [9 – 112]	36 [11 – 136]
Supplement	133 [44 – 1500]	133 [67 – 1433]	133 [44 – 1500]	167 [0 – 1500]	167 [67 – 333]	167 [0 – 1500]
Total	195 [71 – 1611]	194 [92 – 1477]	196 [71 – 1611]	207 [30 – 1624]	207 [87 – 409]	207 [30 – 1624]
Vitamin E, % RDA						
Diet	88 [27 – 893]	86 [50 – 247]	89 [27 – 893]	71 [31 – 574]	71 [48 – 255]	71 [31 – 574]
Supplement	1619 [0 – 3810]	1273 [0 – 3810]	1818 [182 – 3810]	1273 [0 – 3111]	1212 [182 – 2424]	1905 [0 – 3111]
LXS/Placebo	0	0	0	72 [3 – 160]	100 [3 – 160] §	56 [12 – 89] §
Total	1851 [62 – 4703]	1345 [62 – 3910]	1896 [350 – 4703]	1494 [71 – 3178]	1302 [331 – 2660]	2025 [71 – 3178]
Vitamin K, % AI						
Diet	95 [27 – 397]	95 [55 – 252]	87 [27 – 397]	96 [30 – 1233]	117 [47 – 1233]	85 [30 – 285]
Supplement	500 [0 – 9404]	500 [0 – 3000]	667 [0 – 9404]	1333 [0 – 9455]	1067 [0 – 2667] §	2333 [0 – 9454] §
Total	689 [63 – 9535]	578 [63 – 3094]	1064 [85 – 9535]	1430 [43 – 9593]	1292 [125 – 2797] §	2395 [43 – 9593] §

RDA: Recommended Daily Allowance; AI: Adequate Intake

* $p<0.05$, the difference between baseline and 12 months within each group (all, LXS, or placebo);

§ $p<0.05$ for the difference between LXS and comparison group at 12 month

Note: LXS and comparison intakes are adjusted for adherence

Table 2

Serum fat soluble vitamin status as mean ± SD in all children and by group

	n	Baseline	12 months
Vitamin A, μg/dL (retinol)			
All	54	38.0 ± 12.0	40.8 ± 11.1 *
LXS	21	43.1 ± 16.4	46.4 ± 12.3
Placebo Comparison group	33	34.9 ± 6.6 §	37.1 ± 8.6 §
Vitamin D, ng/mL (25OHD)			
All	54	32.0 ± 8.2	30.3 ± 10.7
LXS	20	32.2 ± 7.6	28.6 ± 9.1
Placebo Comparison group	34	31.9 ± 8.7	31.4 ± 12.4
Vitamin E, mg/L (α-Tocopherol)			
All	55	10.9 ± 3.5	10.4 ± 3.9
LXS	21	11.6 ± 4.1	11.4 ± 4.1
Placebo Comparison group	34	10.4 ± 3.0	9.7 ± 3.6
αTocopherol:Cholesterol mg/g			
All	56	8.2 ± 2.2	7.6 ± 2.3
LXS	22	8.9 ± 2.6	8.4 ± 2.5
Placebo Comparison group	34	7.8 ± 1.8	7.1 ± 2.1 §
Vitamin K			
%ucOC			
All	54	28.7 ± 11.5	21.9 ± 13.2 **
LXS	21	29.9 ± 11.7	21.6 ± 13.2
Placebo Comparison group	33	27.9 ± 11.5	22.2 ± 13.3
PIVKA II ng/mL			
All	42	1.3 ± 0.7	1.7 ± 2.5
LXS	15	1.4 ± 0.6	1.4 ± 0.7
Placebo Comparison group	27	1.3 ± 0.8	1.8 ± 3.1

* $p \leq 0.05$;

** $p \leq 0.001$ the difference between 12 months and baseline within each group (all, LXS + comparison group) after adjustment for diet and supplement intake as %RDA or %AI;

§ $p < 0.05$ for the difference between LXS and comparison groups within each time point

Table 3

Fat soluble vitamin status as mean ± SD, or median [range] from previous and current studies in children with CF and PI

	CF Nutrition[a]	CF Bones[b]	CF Avanti
Study Years	1998–2000	2000–2002	2007–2010
CF Centers, n	13	3	9
Sample size, n[c]	78	101	58
Age, yrs	7.3 ± 0.9	14.8 ± 4.2	10.3 ± 2.9
Range	6.0 – 10.0	8.0 – 25.0	5.0 – 17.9
Vitamin A, ug/mL			
Mean	52.0 ± 13.0	82.0 ± 29.0	38.0 ± 12.0
Median	–	80 [33.0 – 208.0]	38.0 [12.9 – 89.6]
Low status (<30 ug/dL), %	4	0	20
Vitamin D, ng/mL			
Mean	42.6 ± 12.2[d]	20.7 ± 6.5	32.0 ± 8.2
Median	40.0 [18.9 – 79.2][d]	20.3 [4.5 – 34.9][d]	31.2 [9.3 – 77.0]
Low status (<30ng/mL), %	12	90	50
Vitamin E, αTocopherol:Cholesterol ratio			
Mean	8.8 ± 3.4[d]	11.4 ± 5.5[d]	8.2 ± 2.2
Median	8.2 [2.9 – 20.2]	10.7 [2.9 – 27.3][d]	7.7 [2.4 ± 15.5]
Low status (<5.4 mg/g), %	13	13	13
Vitamin K, % ucOC			
Mean	–	33.7 ± 17.9[d]	28.7 ± 11.5
Median	–	34.6 [3.1 – 76.1]	25.3 [3.8 – 99.3]
Low status (>50%), %		19	5
PIVKA II, ng/mL			
Mean	–	4.6 ± 6.5[d]	1.3 ± 0.7
Median	–	2.1 [0 – 41.6]	1.2 [0.3 – 16.7]
Low status (>2.0 ng/mL), %		50	19

[a] References #11, #12;

[b] References #10, #13, #14;

[c] sample size for each vitamin may be smaller based on number of completed assays;

[d] unpublished data