BRIEF COMMUNICATION:
RESEARCH REPORT

CURRENT DEVELOPMENTS IN NUTRITION

Vitamins and Minerals

Associations between Circulating Lipids and Fat-Soluble Vitamins and Carotenoids in Healthy Overweight and Obese Men

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ABSTRACT

Inconsistent associations between lipids and circulating markers of fat-soluble vitamin and carotenoid status have been reported. The aim of this hypothesis-generating study was to examine the contribution of the LC-MS-based lipidome, characterized by lipid class, carbon count, and the number of unsaturated bonds, to the interindividual variability in circulating concentrations of retinol, carotenoids, 25-hydroxyvitamin D_3, α-tocopherol, γ-tocopherol, and phylloquinone in 35 overweight and obese, but healthy men. A sparse partial least-squares method was used to accomplish this aim. Highly abundant phospholipids and triglycerides (TGs) contributed to the interindividual variability in phylloquinone, α-tocopherol, and γ-tocopherol. Interindividual variability in lycopene concentrations was driven by concentrations of low-abundant TG. 25-Hydroxyvitamin D_3, retinol, and the other carotenoids were not influenced by lipids. Except for lycopene, evaluation of lipids beyond class does not appear to further explain the interindividual variability in circulating concentrations of fat-soluble vitamins and carotenoids. *Curr Dev Nutr* 2020;4:nzaa089.

Keywords: lipids, fat-soluble vitamins, carotenoids, phylloquinone, tocopherol, retinol, 25-hydroxyvitamin D_3, lipidomics, micronutrients, liquid chromatography-mass spectrometry

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Abbreviations used: ChE, cholesterol ester; DG, diglyceride; FFA, free fatty acid; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; sPLS, sparse partial least-squares; SPM, sphingomyelin; TG, triglyceride.

Introduction

Validated biomarkers of micronutrient status are important tools in observational studies linking micronutrient exposure to disease risk (1). In the case of hydrophobic fat-soluble vitamins and carotenoids, the associations between various lipid classes and the interindividual variability of these circulating biomarkers are not consistent (2–6).

In the fasted state, retinol and 25-hydroxyvitamin D_3 circulate on specific binding proteins, but vitamin E and vitamin K forms and carotenoids are transported with lipids on lipoproteins (2, 7–10). Blood lipid profiles are typically described by triglycerides (TGs), total cholesterol, LDL cholesterol, and HDL cholesterol. However, thousands of distinct lipid molecular species have been identified in the circulation (11). Whereas TGs, free cholesterol, and cholesterol esters (ChEs) are the most abundant constituents of lipoproteins, glycerophospholipids, diglycerides (DGs), and free fatty acids (FFAs) constitute other important lipid classes (11, 12). Furthermore, esterified fatty acids can differ in carbon-chain length and the number of unsaturated bonds (11). With technological advances in characterization of the lipidome, there is an opportunity to broaden the study of lipids and their influence on these micronutrient biomarkers.

Following work by van den Broek et al. (13), in which micronutrients were correlated with molecules measured across multiple metabolomic and proteomic platforms, the objective of this hypothesis-generating study was to examine the contribution of the lipidome, characterized by lipid class, carbon-chain length, and number of unsaturated bonds, to the interindividual variability in circulating fat-soluble vitamins and carotenoids.

Methods

Study design and participants

This was a secondary analysis of data from the placebo arm of a randomized, double-blind, placebo-controlled, 5-wk intervention trial (NCT00655798) conducted from December 2006 to June 2007, to investigate the anti-inflammatory effects of nutritional interventions in 36 overweight and obese men with low-grade inflammation (14). The men (mean ± SD age: 47 ± 10 y) were otherwise healthy, with BMI 25.6–34.7 kg/m^2. Exclusion criteria included high fasting total cholesterol; acute inflammation (C-reactive protein >10 mg/L); a chronic disease related to inflammation (e.g., arthritis or inflammatory bowel disease); use of anticoagulant, antiplatelet, or anti-inflammatory medication; smoking; unexplained weight loss; alcohol intake >28 units/wk; following a weight-reduction diet; or use of dietary supplements. Placebo capsules contained 365 mg microcrystalline cellulose (Microz Food Supplements) or 1360 mg soy lecithin (Solgar Vitamin and Herb). The study protocol was approved by the independent Medical Ethics Committee (METOPP) (Tilburg, Netherlands).

Biochemical analyses

At the end of the 5-wk placebo period, blood samples were collected 4 h after a light standard breakfast (1597 kJ, % energy: 8.6 protein, 17.0 fat, 72.4 carbohydrates) preceded by an overnight fast (14). Samples for plasma lipids and micronutrient analysis were collected in lithium-heparinized tubes and tubes containing tri-potassium salts of EDTA (K3-EDTA), respectively. Serum and plasma were centrifuged for 15 min at 2000 × *g* at 4°C and separated within 30 min of collection and stored at −80°C in the dark until analysis.

Fat-soluble vitamins and carotenoids.

Retinol, α-tocopherol, γ-tocopherol, 25-hydroxyvitamin D_3, α-carotene, β-carotene, β-cryptoxanthin, and lycopene concentrations were measured in 2013, as previously described (13, 15, 16), in a laboratory that adheres to international bioanalytical guidelines for daily and long-term performance and participates in ring-tests. Serum phylloquinone was measured in 2019 from archived serum samples stored for ≤12 y at −80°C using reversed-phase HPLC with fluorometric detection in the Vitamin K Laboratory at the Human Nutrition Research Center on Aging at Tufts University, which participates in the vitamin K external quality assurance scheme (17, 18).

Plasma lipids.

Plasma lipids were analyzed directly after study conclusion (2006/2007) with electrospray LC-MS using a Thermo Linear Trap Quadrupole equipped with a Thermo Surveyor HPLC pump, described previously (19). As described, the LC-MS platform performance was assessed by quality control sample (pooled plasma from all participants) analysis, placed after every 10 samples, and method performance was monitored using 5 internal standards and duplicate sample analysis (20). Concentrations of 131 lipids including 6 lysophosphatidylcholines (LPCs), 19 phosphatidylcholines (PCs), 11 sphingomyelins (SPMs), 14 ChEs, 55 TGs, 4 DGs, and 22 FFAs were quantified. Baseline TGs, total cholesterol, and HDL cholesterol were measured with enzymatic techniques. LDL cholesterol was determined by the Friedewald calculation (14, 21).

Statistical analyses

Placebo-phase micronutrient and lipid data were not available for 1 participant so 35 of the 36 participants were included in this analysis. Circulating micronutrient and plasma lipid concentrations were described by means ± SDs. Given the small sample size and large number of highly correlated lipid exposures, we modeled the variability in circulating concentrations of each micronutrient using sparse partial least-squares (sPLS) regression. This method avoids overfitting when there may be few underlying or latent factors (components) that account for the variability in exposures and response variables (22). Using this technique, components are constructed to account for as much variability in the exposures as possible while maximizing the explained variability of the outcome (micronutrient). Loadings reflect the contribution of exposures (lipids) to the component (22).

The *spls* function from the mixOmics R package (23) in *regression mode* was used to decompose information from the 131 lipids into fewer components. The number of components for each model was determined by maximizing the predictability of the model with the fewest number of components that explained variability in both the predictor and response variables. A component was selected if its Q^2 value, a parameter used to assess the goodness of prediction, was ≥0.0975, a cutoff proposed by Tenenhaus (24). R^2 is regarded as a measure of explained variability and Q^2 is equivalent to R^2 when the training set model is applied to a test set. A Q^2 that approximates R^2 is a qualitative indicator that the sPLS model performance was not unique to the training data set.

Each model was cross-validated using the *tune.spls* function with the *M-fold* method, which resamples the data into *n* (35 in this analysis) groups, replicated 100 times. With 1 independent outcome per model, we investigated the number of lipids (25, 50, 75, 100, 125, or 131) to retain for ≤10 components. Components were added to the model if there was a gain in performance based on a 1-sided *t* test. All variables were centered and scaled. Phylloquinone, α-tocopherol, γ-tocopherol, lycopene, α-carotene, and β-cryptoxanthin were skewed so these data were ln-transformed.

Results

Table 1 shows descriptive data for micronutrients and plasma lipids. ChEs, TGs, and PCs were the most abundant lipid classes. SPMs, FFAs, and DGs were present in very low quantities. ChEs (18:1) were ∼4 times more abundant than any other lipid. TGs with 50, 52, and 54 carbons were also highly abundant. These TGs contained 1–3, 1–4, and 3–5 unsaturated bonds, respectively. The top PCs had 34 and 36 carbons.

Table 1 shows the number of components, exposures, R^2, and Q^2 for each sPLS model. **Figure 1** and **Supplemental Tables 1–4** show the component 1 loadings for phylloquinone, α-tocopherol, γ-tocopherol, and lycopene, the micronutrients for which a robust model was established. Highly abundant LPCs (16:0) and PCs (34 or 36 carbons, 1–3 unsaturated bonds) explained the most variability in circulating phylloquinone. After these, several low-abundant ChEs and high-abundant TGs (50:2, 50:3, 52:1, 52:2) also contributed. The variability in γ-tocopherol was primarily explained by high-abundant TGs (52:4, 54:3–6, and 56:2–5) in addition to PCs (34:0) and few DGs. The

TABLE 1 Descriptive data for the fat-soluble vitamins, carotenoids, and total lipids with sparse partial least-squares model parameters for the micronutrients

			Explained variability, R^2			
	Mean ± SD	Components, *n*	Exposures, *n*	Vitamin or carotenoid	Lipids	Predictability, Q^2
Phylloquinone,[1] nM	1.7 ± 1.7	1	131	1.00	0.33	0.33
Retinol, μM	1.97 ± 0.31	1	131	1.00	0.34	0.03
α-Tocopherol,[1] μM	29.0 ± 6.2	1	75	1.00	0.30	0.27
γ-Tocopherol,[1] μM	1.74 ± 0.68	2	50, 131	1.00, 0.60	0.24, 0.21	0.20, 0.18
25-OH-vitamin D_3, nM	63.2 ± 32.8	9	50, 25, 131, 131, 131, 131, 100, 25, 50	1.00, 0.54, 0.45, 0.33, 0.26, 0.16, 0.11, 0.6, 0.5	0.10, 0.13, 0.10, 0.25, 0.11, 0.7, 0.3, 0.4, 0.2	0.17, −0.28, −0.38, −0.15, 0.26, 0.10, 0.21, −0.20, −0.34
Lycopene,[1] μM	0.62 ± 0.30	1	25	1.00	0.26	0.20
α-Carotene,[1] μM	0.06 ± 0.05	1	50	1.00	0.19	−0.17
β-Carotene, μM	0.40 ± 0.17	1	100	1.00	0.22	0.03
β-Cryptoxanthin,[1] μM	0.22 ± 0.20	1	25	1.00	0.25	−0.15
Total cholesterol,[2] mg/dL	231 ± 37					
LDL cholesterol,[2] mg/dL	152 ± 31					
HDL cholesterol,[2] mg/dL	47 ± 9					
Total triglycerides,[2] mg/dL	147 ± 72					

[1] Skewed distribution.
[2] Plasma lipids were measured at the baseline study visit.

variability in α-tocopherol was also explained by TGs and select PCs and DGs, with an overlap in carbon count and the number of unsaturated bonds. The variability in circulating lycopene was explained by TGs with 40–52 carbons. All loadings were negative, indicating an inverse relation. The top contributors had 40–46 carbons and 0–2 unsaturated bonds. Q^2 was not comparable with R^2 for retinol, α-carotene, β-carotene, and β-cryptoxanthin so these models were not robust. For 25-hydroxyvitamin D_3, the model appeared overfitted, requiring 9 components, none of which sufficiently accounted for variability in the exposures and response variables with adequate performance. For example, component 1 maximized the variability in circulating 25-hydroxyvitamin D_3 with $Q^2 > 0.0975$, but only explained 10% of the variance in lipids. In contrast, component 4 explained more variance in lipids (25%), but limited variability in 25-hydroxyvitamin D_3 (33%) and had poor performance ($Q^2 = -0.15$).

Discussion

Using a sPLS technique, the relations between lipids (characterized by class, carbon-chain length, and the number of unsaturated bonds) and the interindividual variability in blood-based markers of fat-soluble vitamin and carotenoid status were assessed in healthy overweight and obese adult males. We found that lipids contributed to the variability in circulating phylloquinone, α-tocopherol, γ-tocopherol, and lycopene, but not 25-hydroxyvitamin D_3, retinol, α-carotene, β-carotene, or β-cryptoxanthin.

The largest interindividual variability in circulating concentrations was observed for phylloquinone. We observed that high relative abundance TGs contributed to the variability in serum phylloquinone, but the contribution of highly abundant PC and LPC was unexpectedly greater. Phylloquinone concentrates in the TG-rich lipoprotein fraction (10), but it is plausible phylloquinone and TGs share transport mechanisms without a causal relation between the molecules. That high-abundant lipids explained the most variability in circulating phylloquinone suggests that total PCs and TGs sufficiently account for the variability in circulating phylloquinone and differentiation by number of carbons and unsaturated bonds does not explain more. These data also suggest that variances in PC/LPC may be more indicative of circulating phylloquinone than variances in TGs. The association between circulating phylloquinone and PC/LPC is not well-studied. However, there is biological plausibility to our findings. PC is critical to VLDL synthesis and higher circulating phylloquinone has been associated with higher VLDL particle number, independently of TGs (25) (JM Kelly, unpublished results). In addition, based on the chemical structure of phylloquinone, we posit that phylloquinone resides at the surface of the lipoprotein with PC rather than in the core with TGs (26, 27). Further research is required to confirm this. Importantly, these findings should not be extrapolated to other forms of vitamin K because the transport of menaquinones in the body is not well understood.

Consistent with the report by van den Broek et al. (13), the interindividual variability in α-tocopherol concentrations was also sensitive to variances in highly abundant PC/LPC. We also observed contributions of TGs for both vitamin E vitamers. That vitamin K and vitamin E vitamers were similarly influenced by lipids is in line with evidence that suggests there is overlap in the metabolic pathways of these 2 fat-soluble vitamins (26, 28, 29).

In contrast to the observations for phylloquinone, α-tocopherol, and γ-tocopherol, lycopene was inversely related to variances in a few low-abundant TGs. That low-abundant TGs accounted for the most variability in lycopene suggests there is something unique about TGs with 40–46 carbons and few unsaturated bonds with respect to circulating lycopene. Interestingly, a robust sPLS model was established for lycopene, but not the other carotenoids. Variances in circulating retinol and 25-hydroxyvitamin D_3 were not explained by lipids. This was not

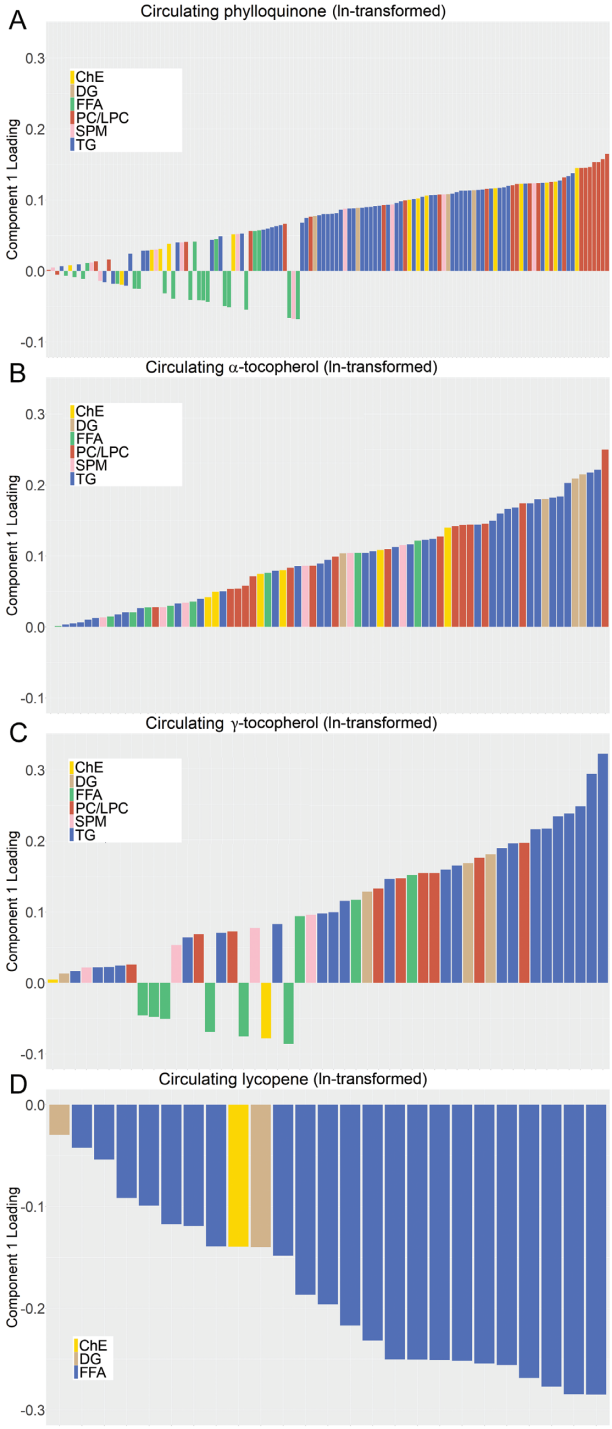

FIGURE 1 Plasma lipid loading plots of component 1 from cross-sectional sparse partial least-squares regression models for circulating concentrations of (A) phylloquinone (131 lipids), (B) α-tocopherol (75 lipids), (C) γ-tocopherol (50 lipids), and (D) lycopene (25 lipids). ChE, cholesterol ester; DG, diglyceride; FFA, free fatty acid; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; SPM, sphingomyelin; TG, triglyceride.

unexpected given that both fat-soluble vitamins are transported on specific binding proteins (7, 8).

Total lipids and the relative abundance of lipids according to the number of carbons and unsaturated bonds for these participants are consistent with previous reports (11) and micronutrient concentrations were within expected ranges for this population (13, 30). However, there are several limitations of this study. First, this was a cross-sectional analysis so it cannot be determined whether lipids influence micronutrients or vice versa. Second, the cohort was a small sample of healthy overweight and obese males and there was no control group of normal-weight males, which limits generalizability. Finally, for this pilot study, the cross-validation procedure was conducted in subsets of the same cohort rather than in separate training and validation data sets, so replication in larger and more diverse cohorts is needed.

This was a study to investigate the associations of lipids, categorized by lipid class, the number of carbons, and the number of unsaturated bonds, with circulating concentrations of fat-soluble vitamins and carotenoids. Except for lycopene, refinement of the lipidome beyond class did not further characterize the interindividual variability in these micronutrients. However, based on these analyses, future investigations of the role of PC/LPC in vitamin E and K metabolism are merited.

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