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Relationships between Lipophilic Micronutrient Biomarkers and Serum Persistent Organic Pollutant Concentrations in NHANES 2003 – 2004

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Relationships between Lipophilic Micronutrient Biomarkers and Serum Persistent Organic Pollutant Concentrations in NHANES 2003 – 2004

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Abstract

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By Kaitlin R. Taibl

Background: Dietary antioxidants pose the potential to mitigate and remediate persistent organic pollutant (POP) bioaccumulation. However, lipophilic micronutrients share many biochemical properties and physiological targets with POPs, which may promote sequestration. Since the relationships between lipophilic micronutrients and POPs may be healthy or unhealthy, a systematic examination is needed.
Objective: To determine if biomarkers for vitamins A, D, and E and carotenoids are associated with common circulating POPs by sex, age, and body mass index (BMI) in a nationally representative sample of the United States population.
Methods: We used data collected from 2,766 participants ≥ 12 years old in the 2003 – 2004 National Health and Nutrition Examination Survey (NHANES). Multivariate linear regressions stratified by sex estimated the relationships of twenty-two micronutrient biomarker levels against serum concentrations of eight polyhalogenated POPs, including 2,2',4,4'-tetrabromodiphenyl ether (PBDE-47), perfluorooctanoic acid (PFOA), and polychlorinated biphenyls (PCBs). All analytes were natural log-transformed, and potential effect measure modification by age and BMI was assessed. The Benjamini-Hochberg procedure for false discovery rate was used to address testing multiple comparisons.
Results: In this cross-sectional analysis, β-carotene was directly related to PCB-153 in normal weight (NW) males yet the relationship weakened with aging (both $q<0.10$). α-Tocopherol was inversely related to PBDE-47 among NW males (β=-1.8, p=0.003, q=0.07) and directly related to PCB-153 among females with obesity, the latter relationship attenuated by age (β=-0.01, p=0.003, q=0.07). Lycopene was directly related to PFOA among females with obesity (β=0.4, p=0.01) and inversely related to PCB-153 among NW males (β=-0.4, p=0.02), the latter relationship strengthened with aging (β=0.01, p=0.001) (all $q<0.10$).
Conclusion: Lipophilic micronutrient levels were differentially associated with POP concentrations according to sex, age, and obesity status in humans. Future studies should explore if associations are linked to sex-specific health outcomes, particularly among younger people with greater adiposity who may have limited antioxidant capacity to combat environmental pollutants.

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Table of Contents

1. Introduction

Environmental toxicants that are fat-soluble and have homeostasis-disrupting properties are collectively known as persistent organic pollutants (POPs) (Mullerova and Kopecky 2007). The pervasive tendency of POPs to bioaccumulate in the environment is compounded by their interaction with endogenous lipids and lipid-rich tissues. Adipose tissue will typically sequester POPs if they are not already bound to cholesterol, triglycerides, or lipoproteins in the circulatory system (La Merrill et al. 2013; Spratlen et al. 2020). Accordingly, these toxicants have been linked to prevalent metabolic diseases as well as disturbances to the reproductive, immune, and nervous systems (Cano-Sancho et al. 2017; Dusanov et al. 2018; Heindel et al. 2017; Iszatt et al. 2016; Klocke et al. 2020; Lee et al. 2014; Robledo et al. 2015; Wang et al. 2019). The health effects which arise from POP exposures differ according to sex, age, and fat mass and distribution (Arrebola et al. 2013; Salihovic et al. 2012; Valvi et al. 2020; Wahlang 2018; Warner et al. 2018; Zong et al. 2015). Diet is another determinant, since the most common POP exposure pathway is the consumption of meat, dairy, and fish products (Arrebola et al. 2018; Ax et al. 2015; Chiu et al. 2004; Christensen et al. 2017; Duarte-Davidson and Jones 1994; Fisher 1999). Since diet is a modifiable risk factor, public health scientists have increasingly become interested in identifying nutritional strategies to prevent and treat POP bioaccumulation, including antioxidant vitamin supplementation (Guo et al. 2016; Hennig et al. 2007).

In animals, especially apex predators with substantial fat mass, POP concentrations vary according to vitamin A, D, and E levels and vice versa (Elabbas et al. 2014; Molde et al. 2013; Murvoll et al. 2007; Pedro et al. 2019; Reiner et al. 2016;

Rogstad et al. 2017; Routti et al. 2005). Vitamins A, D, and E and carotenoids are fat-soluble and vital to optimal health including vision, immunity, fecundity, musculoskeletal strength, metabolism, and cognition (Bates 1995; Bouillon 2018; Miyazawa et al. 2019; Xavier and Perez-Galvez 2016). These lipophilic micronutrients are also regulatory signalers for adipose tissue where POPs elicit metabolic, hormonal, and developmental health effects (Landrier et al. 2012). A considerable number of observational studies in humans have demonstrated a relationship between a greater body burden of persistent environmental pollutants with fat-soluble vitamin deficiencies (Morales et al. 2013; Zile 1992). However, it remains unclear if POP concentrations are impacted by dietary lipophilic micronutrients, which equip the body with handling prooxidants.

To date, few investigations have considered the effect of micronutrient levels on POPs, including their role in sequestration or detoxication. Evidence from the limited available literature on whether nutrients weaken or strengthen POP concentrations are also conflicting (Hennig et al. 2005; Jin et al. 2014; Moses et al. 2009). Vitamins A, D, and E and carotenoids may be involved in the absorption, distribution, metabolism, or excretion of POPs, such as neutralizing reactive oxygen species (ROS) (Hoffman and Hennig 2017). Thus, they may serve to attenuate POP bioaccumulation via antioxidative properties. Alternatively, select biomarkers for vitamins A, D, and E and carotenoids, such as β-carotene, may potentiate POP bioaccumulation through mutual lipophilicity or via synergistic toxicokinetic mechanisms, particularly those involving paracrine and autocrine signaling and nuclear hormone receptors (Hennig et al. 2012). Hence, rigorous and robust research is needed to determine if the relationships between lipophilic micronutrients and POPs are beneficial or detrimental to human health.

We sought to investigate whether lipophilic micronutrient biomarker levels are associated with concentrations of common circulating POPs, and if sex, age, and/or body mass index (BMI) modify any of the relationships. Our hypothesis was that vitamins A, D, and E and carotenoids are inversely associated with brominated flame retardants, dioxins, polychlorinated biphenyls (PCBs), organochlorine pesticides, and per- and poly-fluoroalkyl substances (PFAS) among normal weight individuals yet positively associated with legacy POPs among people with obesity. Further, we hypothesized these relationships would differ in males versus females and younger versus older individuals based on sexually distinct health effects elicited by POPs and windows of susceptibility, respectively.

2. Methods

2.1 Study design and population

The Centers for Disease Control and Prevention (CDC) conducts the National Health and Nutrition Examination Survey (NHANES) on a representative sample of non-institutionalized Americans. A multi-stage probability sampling protocol is used to identify eligible individuals for the cross-sectional survey which entails nutritional and health assessments administered through household interviews and medical examinations in the Mobile Examination Center (MEC). This study used de-identified data made publicly available through the CDC NHANES database for the 2003 – 2004 cycle. For this reason, it was not considered human subjects research and exempt from Institutional Review Board (IRB) approval according to 45 CFR 46.101 Subpart A b(4).

2.2 Micronutrient biomarker measurements

In our analyses, we included three vitamin A biomarkers (retinol, retinyl palmitate, retinyl stearate), one vitamin D biomarker [25-hydroxyvitamin D (25(OH)D)], three vitamin E biomarkers (α-tocopherol, δ-tocopherol, γ-tocopherol), and fifteen carotenoid biomarkers (lutein, zeaxanthin, cis-lutein/zeaxanthin, combined lutein/zeaxanthin, α-cryptoxanthin, β-cryptoxanthin, trans-lycopene, cis-lycopene, total lycopene, α-carotene, trans-β-carotene, cis-β-carotene, total β-carotene, phytoene, phytofluene). The serum concentrations of vitamins A and E and carotenoids were measured using high-performance liquid chromatography and reported in μg/dL units (CDC 2007a). Absorbance maxima for each biomarker was taken into consideration to achieve high sensitivity and specificity (CDC 2007a). Total serum 25(OH)D was measured with the Diasorin assay and reported in nmol/L units (CDC 2007b).

2.3 Pollutant measurements

A total of eight POPs were selected for our analyses from the following NHANES laboratory panels: brominated flame retardants [2,2',4,4'-tetrabromodiphenyl ether (PBDE-47)], dioxin and coplanar polychlorinated biphenyls [PCB-74, PCB-105, 2,3,7,8-tetrachlorodienzo-p-dioxin (TCDD)], non-dioxin-like polychlorinated biphenyls (PCB-153), organochlorine pesticides [p,p'-dichlorodiphenyldichloroethylene (DDE)], and per- and poly-fluoroalkyl substances [perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS)]. All analyte concentrations were measured in serum samples by the Division of Environmental Health Laboratory Sciences, National Center for Environmental Health, CDC (Atlanta, Georgia). TCDD (fg/g), PBDE-47 (pg/g), DDE

(ng/g), and the PCBs (ng/g) were measured with high-resolution gas chromatography/isotope-dilution high-resolution mass spectrometry (HRGC/ID-HRMS) (Patterson et al. 1991; Sjodin et al. 2004). PFOA and PFOS (ng/mL) were measured by solid phase extraction-high performance liquid chromatography-turboionspray ionization-tandem mass spectrometry (SPE-HPLC-TCI-MS/MS) (Kuklenyik et al. 2004).

2.4 Covariates

Previous research was referenced to identify variables with potential confounding effects on the relationship between lipophilic micronutrients and circulating POPs. Also, we decided *a priori* to explore potential effect measure modification (EMM) for sex, age, and BMI since the exposure-outcome relationship may be different based on these characteristics (Heindel et al. 2017). An interview-based questionnaire captured information on each participant's age (years), sex (male or female), race and ethnicity (non-Hispanic white, non-Hispanic Black, Other Hispanic, Mexican American, Other race and ethnicity), and family poverty-income ratio (PIR). We categorized PIR into a six-level ordinal variable to best capture household economic differences.

Overall adiposity was assessed using BMI, calculated as weight (kilograms) divided by height (meters^2). Since growth depends on pubertal development in children and adolescents, we calculated BMI-for-age percentiles by sex using the 2000 CDC growth charts (CDC 2017). Participants aged 12 – 19 years old were considered underweight (<5th percentile), normal weight (5th – <85th percentile), overweight (85th – <95th percentile), or having obesity (≥ 95th percentile) accordingly. Adults aged 20 years old and older were classified as follows: underweight (<18.5 kg/m^2), normal weight (18.5

– <25.0 kg/m^2), overweight (25.0 – <30.0 kg/m^2), or having obesity (≥ 30.0 kg/m^2). Also, instead of modeling the lipid-adjusted POP concentrations provided by NHANES, we calculated total serum lipids (mg/dL) based on total cholesterol and triglyceride laboratory results using Bernert's equation for greater precision (Bernert et al. 2007). Serum cotinine, a well-established biomarker for tobacco use, was used to classify people who currently smoke tobacco (≥10 ng/mL) from people who do not (<10 ng/mL) (Crinnion 2010). Lastly, seasonal effects on vitamin D status (expected higher 25(OH)D levels in spring and summer months) were approximated by the date of MEC examination, which is dichotomized into two six-month periods, November 1st, 2003 – April 30th, 2004 or May 1st – October 31st, 2004 (Norman 2008).

2.5 Statistical analyses

Of the total 10,122 participants in the 2003 – 2004 cycle, one-third of those aged 12 years and older were randomly selected for three subsamples used for the environmental pollutant assays as part of the NHANES protocol. We excluded participants with missing covariates (N=4,066), missing micronutrient levels (N=29), and missing POP concentrations (N=3,132). Also, we excluded participants with an underweight BMI given the small sample size (N=129). The remaining 2,766 participants, of which the subsample totals varied, were stratified by sex before proceeding with the analyses.

To assess linearity and normality, we examined the distribution of each analysis variable along with the bivariate relationship among covariates, exposures, and outcomes using t-tests for categorical variables and Pearson correlation coefficients for continuous

variables. The POP concentrations were natural log-transformed to adjust for their right-skewed distribution and micronutrient levels were natural log-transformed for ease of interpretation. All analytes remained on a continuous scale for the analyses.

We modeled the average change in natural log-transformed POP concentrations as a function of natural log-transformed micronutrient biomarker levels using crude and multivariate linear regressions. In total, 176 separate models were constructed to examine the association for every combination of micronutrients and POPs stratified by sex. The multivariate models included race and ethnicity, PIR, and serum cotinine as potential confounders. Total serum lipids were excluded when modeling adjusted PFOA and PFOS concentrations since these pollutants preferentially bind to albumin over other endogenous lipids and are not believed to affect both the exposure and outcome (Butenhoff et al. 2012). Similarly, the six-month examination period was only included in the multivariate models with 25(OH)D as the main predictor to account for seasonal differences in biosynthesis (Norman 2008). We also included two-way multiplicative interaction terms in our models to examine if any of the associations between micronutrients and POPs were modified by age and BMI. First-order terms for age and BMI remained in the models as well.

All descriptive and linear regression analyses accounted for the complex survey design by using the appropriate weight, cluster, and stratum for each subsample per CDC analytic recommendations (CDC 2013). Study population characteristics are reported as the sample-weighted mean ($\bar{x}$) $\pm$ standard error (SE) or count (n) and percentage (%). The analytes are reported as the sample-weighted geometric mean (GM) and corresponding (SE). To address a potentially inflated type I error rate with testing

multiple comparisons, q-values were obtained by adjusting p-values using the Benjamini-Hochberg false discovery rate (FDR) procedure (Benjamini and Hochberg 1995). The statistical significance level for p-values was set to 0.05 and for q-values to 0.10. All two-sided tests were performed in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1 Study population demographics and characteristics

A total of 1,395 males and 1,371 females were included in our analyses (**Table 1**). The sample-weighted study population was predominantly comprised of middle-aged adults ($\bar{x}$=41.3 – 43.5 years) who self-reported their race and ethnicity as non-Hispanic white (67.8 – 76.0%) and were examined between May 1st and October 31st, 2004 (57.0 – 66.5%). The majority also had an overweight or obese BMI (57.2 – 72.6%), a serum cotinine level less than 10 ng/mL (60.4 – 80.7%), and PIR ≥ 5 (22.5 – 34.0%). For all POP subsamples, the average amount of total serum lipids was slightly elevated for males and within normal limits for females based on reference ranges in NHANES 1999 – 2002 [6 – 19 years old: triglyceride (79 – 84 mg/dL) and total cholesterol (163 – 166 mg/dL); ≥ 20 years old: triglyceride (119 – 127 mg/dL) and total cholesterol (201 – 205 mg/dL)] (Carroll et al. 2012; Kit et al. 2012). Finally, males and females were approximately evenly distributed across the POP subsamples with TCDD having the fewest participants and PFOA and PFOS having the most. For the sample-weighted micronutrient and POP GM concentrations within each sex stratum see **Table S1**.

3.2 Micronutrients and POPs

The relationships between lipophilic micronutrient and POP blood levels stratified by sex and significantly modified by age and BMI are presented in **Table 2** and **Table 3** (see **Table S2** and **Table S3** for all regression estimate coefficients).

3.2.1 Associations specific to males

The significant inverse association between α-tocopherol levels and concentrations of the brominated flame retardant PBDE-47 in normal weight males was the strongest observed throughout the analyses (β=-1.81, p=0.003, q=0.07). Similarly, total lycopene was significantly inversely related to non-dioxin-like PCB-153 in normal weight males (β=-0.35, p=0.02, q=0.07). This relationship was significantly positively modified by age, which indicates the modeled effect of total lycopene levels on PCB-153 concentrations strengthens with every year older (β=0.01, p=0.001, q=0.01). In comparison, total β-carotene was significantly positively related to PCB-153 in normal weight males (β=0.45, p=0.04, q=0.09) yet weakened with aging (β=-0.01, p=0.04, q=0.09). Lastly, 25(OH)D was positively related to PCB-153 in overweight males, which was significantly attenuated by older age (β=-0.02, p=0.03, q=0.08).

3.2.2 Associations specific to females

Females with obesity were found to have a positive association between α-tocopherol levels and PCB-153 concentrations, which was significantly negatively modified by age (β=-0.01, p=0.003, q=0.07). α-Carotene and total β-carotene were also directly related to PBDE-47 among females with obesity (both q=0.13). In regard to the fluorinated pollutants, a significant positive association between total lycopene and

PFOA was observed for females with obesity (β=0.40, p=0.01, q=0.08), and PFOS displayed a similar positive association (p<0.05).

3.2.3 Associations present in both sexes

The direct relationship between lutein and PCB-153 was similar in males and females with a normal weight BMI (males β=0.63, p=0.01, q=0.07; females β=0.57, p=0.045, q=0.25). Neither association was significantly modified by age. Also, TCDD concentrations were lower on average in relation to vitamin A biomarker levels in normal weight participants. Among males, retinyl palmitate and retinyl stearate were significantly inversely associated with TCDD (both q<0.10). Among females, only retinyl stearate was inversely associated with TCDD (β=-0.17, p=0.02, q=0.14)

4. Discussion

Based on the limited available literature, we hypothesized vitamins A, D, and E and carotenoids may amplify or abate serum POP concentrations through shared lipid-rich tissue targets or antioxidative properties, respectively, depending on one's sex, age, and overall adiposity. Our results suggest a combination of these phenomena are plausible for flame retardants, surfactants, manufacturing byproducts, industrial oils, and pesticides. Of the total twenty-two lipophilic micronutrient biomarkers examined in this study, we found thirteen among males and two among females were significantly associated common circulating POP concentrations according to age and BMI.

4.1 Relationships between vitamin A and POPs

Vitamin A biomarkers were associated with decreased TCDD concentrations in both normal weight and overweight males. Retinyl palmitate and retinyl stearate are primarily acquired by consuming animal proteins and products, then are distributed to distant organs, adipose tissue, and the liver, where they are stored as retinol (Bates 1995). Once present in its active form, retinoic acid possesses potent ROS quenching capabilities. Accordingly, vitamin A supplementation mitigates TCDD-mediated oxidative stress in experimental laboratory studies (Alsharif and Hassoun 2004; Hilscherova et al. 2003; Yang et al. 2005). An appreciable amount of research has been performed on the perturbation of vitamin A homeostasis due to dioxins, too. Crosstalk between retinoid receptor and aryl hydrocarbon receptor (AhR) pathways is believed to be the link underlying vitamin A's relationship with TCDD and their subsequent health cascades (Novak et al. 2008). Retinoid species may effectively initiate phase I, II, and III metabolism to detoxify TCDD in non-obese individuals (Shmarakov 2015). The abundance of retinoid receptors and functions in adipose tissue compounded by TCDD's paradoxical dose-response, with low doses considered obesogenic and high doses resulting in wasting syndrome, support BMI as a modifier of this relationship (Brulport et al. 2017; Frey and Vogel 2011; Grun and Blumberg 2006). As such, focused epidemiological investigations are needed to clarify how vitamin A and TCDD are associated in humans while accounting for sex and sex-related variables such as adiposity.

4.2 Relationships between vitamin D and POPs

Unexpectedly, 25(OH)D, the serum biomarker for vitamin D status, was associated with higher concentrations of PCB-105, PCB-153, and PFAS, with older age attenuating the 25(OH)D-PCB relationships in normal weight males and the 25(OH)D-PFOS relationship in overweight females. In contrast, previous studies have demonstrated a relationship between vitamin D deficiency and elevated POP concentrations (Etzel et al. 2019; Morales et al. 2013), while other studies with younger cohorts have found inconclusive results (Di Nisio et al. 2020) or null associations between PFOA and PFOS with bone health outcomes (Khalil et al. 2018). As sun exposure is a major contributor to circulating 25(OH)D levels and sunscreens are known to contain POPs (Fujii et al. 2013), it is possible that our findings reflect confounding from personal care product practices and dermal absorption of POPs. In light of the conflicting results, more investigations are warranted to determine the true nature of the relationship between total serum 25(OH)D and circulating POPs.

4.3 Relationships between vitamin E and POPs

We identified differential relationships between vitamin E levels and POP concentrations by BMI, age, and in part, isomeric configuration, but not sex. Humans mostly consume γ-tocopherol, though the body prefers α-tocopherol since it readily conjugates with peroxyl free radicals (Mustacich et al. 2007). In our analysis, α-tocopherol was uniformly associated with lower POPs in normal weight participants and higher POPs in those with an overweight or obese BMI, except for PFOA. Age modified the positive relationship between α-tocopherol and PCB-153 in females with obesity, suggesting a stronger relationship in younger individuals. *In vitro* work in human

trophoblasts supports the strong, inverse association between α-tocopherol and PBDE-47 (Park and Loch-Caruso 2015). Likewise, α-tocopherol has been shown to protect against TCDD or reverse its health effects in experimental animal studies and human cell cultures (Hirai et al. 2002; Rosinczuk and Calkosinski 2015). In contrast, α-tocopherol concentrations were positively associated with PCBs in an Inuit population as well as in Greenland sharks and Baltic seals, all of which had a greater average fat mass than our sample (Belanger et al. 2008; Molde et al. 2013; Nyman et al. 2003). It is possible that, in the setting of excess adiposity, the redox capacity of α-tocopherol is insufficient to combat the additive ROS generated by lipophilic pollutants, particularly in children and adolescents who may not consume enough antioxidants. Additional experimental and prospective studies are required to better elucidate α-tocopherol's potential to remediate common POPs across ranging amounts of adiposity and age groups.

4.4 Relationships between carotenoids and POPs

The carotene biomarker lycopene was associated with lower PCB-153 concentrations in older males with a normal weight or obese BMI. Lycopene is the primary carotenoid in adults and distributed throughout the body, with the highest levels present in the adrenal glands followed by the male sex organs (Rao et al. 2006). The micronutrient treats PCB-induced infertility (Krishnamoorthy et al. 2013) and epididymis toxicity (Raj et al. 2014) in male rats**.** Lycopene has also been shown to have an ameliorative effect on prostate cancer risk, prognosis, and progression in cell-, animal-, and population-based studies (Rao et al. 2006). Although the exact etiology of prostate cancer remains unknown and is likely multi-factorial, aggressive forms of prostate cancer

have been associated with PCB-153 exposures (Ali et al. 2016). Our results support the idea of lycopene protecting against POPs in the context of aging male health.

In our study, lycopene was not universally predictive of reduced serum POPs. Females with obesity were found to have positive relationships among all three lycopene biomarkers (trans-, cis-, total) and serum PFOA and PFOS. Adipose tissue has intrinsic roles in metabolic capacity and the endocrine system, all of which differ between sexes (Le Magueresse-Battistoni 2020), and may help to explain the sexually dimorphic findings presented. For example, lycopene is considered a dietary hormone since it is believed to modulate steroid receptor activity, respond to insulin-like growth factor I signals, as well as possess anti-estrogenic properties (Wang 2012). However, people with obesity are more likely to have lower carotenoid levels compared to their non-diseased counterparts (Garcia et al. 2009; Harari et al. 2020). The altered setpoint may allow a window of opportunity such that, when present together in females with unhealthy adiposity, lycopene is no longer robust to xenoestrogens and metabolism disrupting chemicals (MDCs) like PFAS (White et al. 2011).This area of research is ongoing and future prospective studies should investigate the molecular dynamics of lycopene with POPs in females by body composition phenotype.

Finally, all three β-carotene biomarkers (trans-, cis-, total) were positively associated with PCB-153 concentrations in normal weight males and PBDE-47 concentrations in females with obesity. Although this lipophilic micronutrient has known ROS scavenging functions in the human body, assigned supplementation of high dose β-carotene has been implicated in cancer cases among male tobacco smokers and male asbestos workers (Alpha-Tocopherol Beta Carotene Cancer Prevention Study Group

1994; Milani et al. 2017; Omenn et al. 1996). In line with this prior research, the results presented here indicate β-carotene has a positive relationship with environmental pollutants in the body, suggestive of promoting their concentrations in both sexes. Additionally, the xanthophyll biomarker lutein produced distinct relationships for DDE and PCB-153 versus TCDD and PCB-105, though only normal weight participants experienced FDR-significant positive associations with PCB-153 in the analyses. To the best of our knowledge, this is the first report of such relationships in the epidemiological literature, as lutein and its isomer zeaxanthin are understudied in this space of research. Among neonates and seniors, the micronutrient is known to have critical roles in cognitive development and maintenance, respectively (Stringham et al. 2019). Forthcoming studies may consider focusing on lutein's interactions with lipophilic pesticides and industrial oils in relation to neurological health outcomes.

4.5 Strengths, limitations, and future directions

To the best of our knowledge, this is the first study to systematically examine a panel of lipophilic micronutrient biomarkers in blood and their relationships with common circulating POPs in humans. As such, a novel aspect of this analysis is the modeling strategy to determine if vitamin A, D, and E and carotenoid serum levels are associated with POP serum concentrations in a nationally representative sample of children, adolescents, adults, and seniors. The carefully selected covariates for each model, FDR correction, and assessment of EMM by age and BMI are other notable strengths of this analysis.

Our cross-sectional study is subject to several limitations. None of the analytes were measured longitudinally, so the temporality assumption for causation cannot be satisfied. While reverse causality may provide alternative explanations to the findings presented, the research question necessitated this exposure-outcome relationship in the analysis. Another limitation is only being able to use the 2003 – 2004 NHANES cycle because the examined POP concentrations are pooled for all of the following years, except for PFAS. Regional differences in pollutant exposures are also not readily available in the CDC NHANES database. By adjusting for six-month examination period this bias may have been partially addressed in the vitamin D models since NHANES conducts MEC examinations in the summer/fall months for northern states and in the winter/spring months for southern states. However, residual confounding is still a concern for vitamin D and unmeasured confounding for the other micronutrients. In addition, we did not account for organ pathologies, including liver diseases and diabetes, or form of micronutrient intake (e.g. supplement versus foodstuffs) due to significant missingness for the self-reported responses, which were only available for adults and seniors in our study population. BMI was also used as a proxy for overall adiposity. One methodological improvement would be to use precise measurements of adiposity, such as isolating fat depots with dual-energy X-ray absorptiometry (DEXA) scans, to elucidate how sex-specific adipose tissue distribution contributes to the identified relationships. Finally, the analytes were measured in blood and not adipose tissue, which may inaccurately represent their systemic concentrations even after adjusting for lipids in the specified models.

Collectively, these findings are important for generating hypotheses about the endogenous interactions between vitamins A, D, and E and carotenoids with POPs in the general population, which can be tested in subsequent studies. Repeated studies are also needed to validate our findings plus hone the associations present in underweight individuals and whether vitamin K, another lipophilic micronutrient, yields similar results.

5. Conclusion

In conclusion, we found evidence that levels of vitamins A, D, and E and the carotenoid lycopene are significantly inversely related to TCDD, PCB-153, and PBDE-47 in males. Alternatively, the carotenoids β-carotene and lutein were significantly positively related to PCB-153 in males. Among females, we identified significant positive relationships between the carotenoid lycopene and PFOA. Overall, normal weight and older males were observed to have the greatest negative change in POP concentrations whereas younger females with obesity tended to experience higher average POP concentrations. Forthcoming research should prospectively examine these pollutant profiles in relation to sex-specific health outcomes linked to lipophilic micronutrient status. Clinical trials designed to investigate POP bioremediation with dietary antioxidant supplementation should also consider the differential effects according to sex, age, and obesity status.

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Table 1. Sample-weighted demographics and characteristics of study population. National Health and Nutrition Examination Survey, 2003-2004, aged 12 years and older.

Characteristic	TCDD	PCB-74	PCB-105	PCB-153	PBDE-47	PFOA	PFOS	DDE
				Males				
Participants (n)	381	396	393	396	434	468	468	420
Age (y)	41.5 ± 1.1	41.4 ± 1.0	41.3 ± 1.0	41.4 ± 1.0	41.3 ± 0.9	41.5 ± 1.1	41.5 ± 1.1	41.3 ± 0.9
Total serum lipids	658.1 ± 12.5	657.1 ± 11.8	657.4 ± 12.0	657.1 ± 11.8	656.5 ± 14.8	643.0 ± 9.7	643.0 ± 9.7	660.7 ± 14.6
Body mass index (BMI)								
Normal weight	151 (30.4)	158 (29.8)	157 (30.1)	158 (29.8)	173 (31.6)	172 (27.4)	172 (27.4)	167 (31.4)
Overweight	116 (33.8)	120 (34.2)	120 (34.6)	120 (34.2)	134 (32.2)	169 (42.3)	169 (42.3)	129 (32.3)
Obese	114 (35.8)	118 (35.9)	116 (35.3)	118 (35.9)	127 (36.2)	127 (30.3)	127 (30.3)	124 (36.3)
Race and ethnicity								
Mexican American	84 (8.2)	85 (7.8)	85 (7.9)	85 (7.8)	105 (8.5)	124 (10.9)	124 (10.9)	103 (8.5)
Other Hispanic	14 (5.4)	14 (5.3)	14 (5.4)	14 (5.3)	13 (2.7)	12 (3.4)	12 (3.4)	12 (2.6)
Non-Hispanic White	172 (70.8)	176 (70.2)	174 (69.9)	176 (70.2)	196 (73.4)	195 (71.2)	195 (71.2)	188 (73.4)
Non-Hispanic Black	96 (9.9)	104 (10.5)	103 (10.6)	104 (10.5)	107 (10.4)	123 (10.8)	123 (10.8)	105 (10.7)
Other race and ethnicity	15 (5.6)	17 (6.2)	17 (6.2)	17 (6.2)	13 (4.9)	14 (3.6)	14 (3.6)	12 (4.8)
Poverty-income ratio (PIR)								
0 – < 1	83 (12.9)	89 (12.9)	89 (13.1)	89 (12.9)	90 (12.6)	94 (9.8)	94 (9.8)	85 (12.5)
1 – 1.9	93 (19.3)	94 (19.4)	92 (19.3)	94 (19.4)	104 (18.3)	118 (18.8)	118 (18.8)	103 (18.8)
2 – 2.9	60 (14.7)	63 (14.9)	63 (15.1)	63 (14.9)	65 (15.1)	90 (19.9)	90 (19.9)	62 (14.8)
3 – 3.9	34 (10.7)	36 (10.7)	36 (10.8)	36 (10.7)	38 (9.7)	49 (13.2)	49 (13.2)	37 (9.9)
4 – 4.9	27 (8.3)	30 (8.9)	30 (9.0)	30 (8.9)	35 (10.5)	30 (10.3)	30 (10.3)	35 (11.0)
≥ 5	84 (34.0)	84 (33.2)	83 (32.7)	84 (33.2)	102 (33.8)	87 (28.0)	87 (28.0)	98 (33.1)
Examination period								
November 1st – April 30th	166 (34.2)	170 (34.2)	167 (33.5)	170 (34.2)	209 (42.9)	227 (41.5)	227 (41.5)	205 (43.0)
May 1st – October 31st	215 (65.8)	226 (65.8)	226 (66.5)	226 (65.8)	225 (57.1)	241 (58.5)	241 (58.5)	215 (57.0)
Serum cotinine								
< 10 ng/mL	288 (73.4)	297 (72.2)	295 (72.1)	297 (72.2)	304 (61.1)	330 (64.4)	330 (64.4)	293 (60.4)
≥ 10 ng/mL	93 (26.6)	99 (27.8)	98 (27.9)	99 (27.8)	130 (38.9)	138 (35.6)	138 (35.6)	127 (39.6)
				Females				
Participants (n)	398	401	399	401	422	442	442	409
Age (y)	42.9 ± 1.1	42.7 ± 1.0	42.7 ± 1.0	42.7 ± 1.0	43.5 ± 0.9	42.1 ± 1.0	42.1 ± 1.0	43.2 ± 0.9
Total serum lipids	644.8 ± 13.1	640.5 ± 12.4	639.5 ± 12.4	640.5 ± 12.4	646.5 ± 8.8	649.6 ± 25.8	649.6 ± 25.8	645.4 ± 8.8
Body mass index (BMI)								
Normal weight	149 (38.9)	150 (38.7)	149 (38.9)	150 (38.7)	157 (34.8)	178 (42.8)	178 (42.8)	155 (35.6)
Overweight	114 (27.9)	118 (29.2)	117 (28.9)	118 (29.2)	112 (29.2)	125 (30.4)	125 (30.4)	110 (30.5)

Obese	135 (33.1)	133 (32.1)	133 (32.3)	133 (32.1)	153 (36.0)	139 (26.8)	139 (26.8)	144 (33.9)
Race and ethnicity								
Mexican American	90 (6.7)	91 (7.0)	91 (7.0)	91 (7.0)	97 (7.7)	97 (6.7)	97 (6.7)	92 (7.7)
Other Hispanic	11 (1.3)	11 (1.3)	11 (1.3)	11 (1.3)	17 (4.8)	7 (2.1)	7 (2.1)	17 (4.9)
Non-Hispanic White	189 (75.2)	193 (76.0)	192 (76.0)	193 (76.0)	179 (68.0)	193 (70.7)	193 (70.7)	173 (67.8)
Non-Hispanic Black	92 (11.3)	91 (10.8)	90 (10.9)	91 (10.8)	107 (13.6)	120 (13.3)	120 (13.3)	106 (13.7)
Other race and ethnicity	16 (5.5)	15 (4.8)	15 (4.8)	15 (4.8)	22 (5.9)	25 (7.2)	25 (7.2)	21 (5.9)
Poverty-income ratio (PIR)								
0 – < 1	84 (11.4)	87 (11.8)	86 (11.8)	87 (11.8)	117 (16.6)	109 (15.9)	109 (15.9)	110 (15.2)
1 – 1.9	108 (21.7)	105 (21.0)	105 (21.1)	105 (21.0)	100 (21.7)	122 (21.3)	122 (21.3)	98 (21.9)
2 – 2.9	48 (12.4)	52 (13.4)	52 (13.5)	52 (13.4)	48 (13.1)	58 (13.7)	58 (13.7)	48 (13.5)
3 – 3.9	42 (12.7)	42 (12.6)	41 (12.2)	42 (12.6)	41 (10.2)	42 (14.5)	42 (14.5)	42 (10.7)
4 – 4.9	34 (13.4)	33 (13.2)	33 (13.2)	33 (13.2)	27 (9.4)	37 (12.1)	37 (12.1)	25 (8.8)
≥ 5	82 (28.3)	82 (28.1)	82 (28.3)	82 (28.1)	89 (29.0)	74 (22.5)	74 (22.5)	86 (29.8)
Examination period								
November 1st – April 30th	187 (38.3)	189 (38.2)	188 (38.0)	189 (38.2)	205 (43.0)	192 (35.4)	192 (35.4)	197 (42.1)
May 1st – October 31st	211 (61.7)	212 (61.8)	211 (62.0)	212 (61.8)	217 (57.0)	250 (64.6)	250 (64.6)	212 (57.9)
Serum cotinine								
< 10 ng/mL	340 (80.7)	338 (79.4)	336 (79.3)	338 (79.4)	348 (75.6)	368 (80.1)	368 (80.1)	336 (75.2)
≥ 10 ng/mL	58 (19.3)	63 (20.6)	63 (20.7)	63 (20.6)	74 (24.4)	74 (19.9)	74 (19.9)	73 (24.8)

Table 1. Categorical variables presented as count and percentage [n (%)] and continuous variables as mean and standard error ($\bar{x} \pm$ SE). Values may not sum to 100% due to rounding. Abbreviations for persistent organic pollutants (POPs) are as follows: 2,3,7,8-tetrachlorodienzo-p-dioxin (TCDD), polychlorinated biphenyl 74 (PCB-74), polychlorinated biphenyl 105 (PCB-105), polychlorinated biphenyl 153 (PCB-153), polybrominated diphenyl ether 47 (PBDE-47), perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS), p,p'-dichlorodiphenyldichloroethylene (DDE). Body mass index (BMI) was categorized for participants aged 12 – 19 years old with BMI-for-age percentiles by sex using the 2000 Centers for Disease Control and Prevention (CDC) growth charts [underweight (<5th percentile), normal or healthy weight (5th – <85th percentile), overweight (85th – <95th percentile), or having obesity (≥ 95th percentile)]. BMI was categorized for participants 20 years old and older by weight in kilograms divided by weight (kilograms) divided by height (meters^2) [underweight (<18.5 kg/m^2), normal or healthy weight (18.5 – <25.0 kg/m^2), overweight (25.0 – <30.0 kg/m^2), or having obesity (≥ 30.0 kg/m^2)]. Participants considered underweight were excluded due to small sample size.

Table 2. False discovery rate corrected associations between lipophilic micronutrient levels and serum persistent organic pollutant concentrations in males, modified by body mass index and age. National Health and Nutrition Examination Survey, 2003-2004, aged 12 years and older.

Effect measure modifier	Lipophilic micronutrient biomarker	Persistent organic pollutant	β estimate	*p*-Value	*q*-Value
Normal weight	Retinyl palmitate	TCDD	-0.27	0.003	0.07*
	Retinyl stearate	TCDD	-0.21	0.01	0.07*
	α-Tocopherol	TCDD	-0.48	0.05	0.36
		PBDE-47	-1.81	0.003	0.07*
	δ-Tocopherol	PCB-74	-0.27	0.04	0.44
		PCB-153	-0.53	0.002	0.05*#
		PFOA	0.42	0.01	0.15
		PFOS	0.26	0.02	0.17
	γ-Tocopherol	PFOA	0.68	0.03	0.19
		PFOS	0.39	0.01	0.17
	trans-β-Carotene	PCB-153	0.45	0.04	0.09*#
	cis-β-Carotene	PCB-153	0.50	0.02	0.07*#
	total β-Carotene	PCB-153	0.45	0.04	0.09*#
	trans-Lycopene	PCB-153	-0.42	0.01	0.07*#
	cis-Lycopene	PFOA	0.41	0.03	0.19
	total Lycopene	PCB-153	-0.35	0.02	0.07*#
		PFOA	0.40	0.04	0.19
	Phytofluene	PCB-153	-0.21	0.03	0.08*#
	Lutein	PCB-153	0.63	0.01	0.07*
	cis-Lutein/Zeaxanthin	PCB-153	0.42	0.05	0.11
	Combined Lutein/zeaxanthin	PCB-153	0.72	0.01	0.07*
	α-Cryptoxanthin	PCB-74	0.19	0.01	0.29
Overweight	Retinol	TCDD	-0.66	0.01	0.14
	Retinyl stearate	DDE	-0.20	0.04	0.56
	25-Hydroxyvitamin D	PCB-105	0.74	0.02	0.43
		PCB-153	0.65	0.03	0.34#
	α-Tocopherol	TCDD	0.48	0.03	0.24
	δ-Tocopherol	PCB-74	-0.24	0.02	0.34
	γ-Tocopherol	TCDD	-0.35	0.004	0.08*
		PCB-74	-0.23	0.03	0.34
		PCB-153	-0.37	0.03	0.34#
	trans-Lycopene	PFOA	-0.24	0.048	0.23
	total Lycopene	PFOA	-0.23	0.047	0.23
	Zeaxanthin	PFOS	-0.33	0.03	0.28
	cis-Lutein/Zeaxanthin	PFOS	-0.31	0.03	0.28
	Combined Lutein/zeaxanthin	PFOS	-0.33	0.04	0.28
Obese	α-Tocopherol	TCDD	0.49	0.01	0.13

		PCB-74	0.54	0.01	0.14
		PCB-153	0.70	0.03	0.24
	γ-Tocopherol	TCDD	-0.36	0.01	0.13
	cis-Lycopene	PCB-74	-0.22	0.047	0.44
	total Lycopene	PCB-153	-0.38	0.04	0.24#
	Phytofluene	PCB-153	-0.32	0.01	0.14#
	Zeaxanthin	PBDE-47	-0.69	0.046	0.60
Age	25-Hydroxyvitamin D	PCB-105	-0.02	0.03	0.64
		PCB-153	-0.02	0.03	0.08*
		DDE	-0.02	0.04	0.88
	α-Tocopherol	PBDE-47	0.03	0.01	0.20
		PFOA	-0.01	0.03	0.23
	δ-Tocopherol	PCB-153	0.01	0.003	0.02*
	γ-Tocopherol	TCDD	0.01	0.01	0.13
		PCB-153	0.01	0.03	0.09*
	trans-β-Carotene	PCB-153	-0.01	0.04	0.09*
	cis-β-Carotene	PCB-153	-0.01	0.02	0.07*
	total β-Carotene	PCB-153	-0.01	0.04	0.09*
	trans-Lycopene	PCB-153	0.01	0.0004	0.01*
	cis-Lycopene	PCB-153	0.01	0.02	0.07*
	total Lycopene	PCB-153	0.01	0.001	0.01*
	Phytofluene	PCB-74	0.004	0.04	0.58
		PCB-153	0.01	0.002	0.01*
		PFOA	-0.005	0.04	0.23
	cis-Lutein/Zeaxanthin	PFOA	-0.01	0.04	0.23

Table 2. In 381 – 468 males, false discovery rate (FDR) corrected statistical significance values (regression estimate coefficient, p-value, q-value) using the Benjamini-Hochberg procedure presented by effect measure modifier and adjusted model. An asterisk (*) denotes value is statistically significant with p-values less than 0.05 and q-values less than 0.10. A hashtag (#) denotes the body mass index (BMI)-specific association is significantly modified by age. Abbreviations for persistent organic pollutants (POPs) are as follows: 2,3,7,8-tetrachlorodienzo-p-dioxin (TCDD), polychlorinated biphenyl 74 (PCB-74), polychlorinated biphenyl 105 (PCB-105), polychlorinated biphenyl 153 (PCB-153), polybrominated diphenyl ether 47 (PBDE-47), perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS), p,p'-dichlorodiphenyldichloroethylene (DDE). BMI was categorized for participants aged 12 – 19 years old with BMI-for-age percentiles by sex using the 2000 Centers for Disease Control and Prevention (CDC) growth charts and for participants 20 years old and older by weight (kilograms) divided by height (meters^2). All models adjusted for race and ethnicity, serum cotinine, and poverty-to-income ratio (PIR). PFOA and PFOS modeled without adjusting for total serum lipids. Only models with vitamin D biomarker as a predictor adjusted for 6-month exam period.

Table 3. False discovery rate corrected associations between lipophilic micronutrient levels and serum persistent organic pollutant concentrations in males, modified by body mass index and age. National Health and Nutrition Examination Survey, 2003-2004, aged 12 years and older.

Effect measure modifier	Lipophilic micronutrient biomarker	Persistent organic pollutant	β estimate	*p*-Value	*q*-Value
Normal weight	Retinol	PFOA	0.69	0.03	0.32
		PFOS	0.74	0.049	0.15
	Retinyl stearate	TCDD	-0.17	0.02	0.14
	25-Hydroxyvitamin D	PCB-105	0.50	0.02	0.22
		PFOA	0.52	0.01	0.32
		PFOS	0.40	0.01	0.11
	α-Tocopherol	TCDD	-0.74	0.01	0.13
		PCB-105	-0.65	0.04	0.28
	α-Carotene	PFOS	-0.17	0.02	0.11
	trans-β-Carotene	PFOS	-0.20	0.02	0.11
	cis-β-Carotene	PFOS	-0.19	0.04	0.14
	total β-Carotene	PFOS	-0.20	0.02	0.11
	trans-Lycopene	PCB-74	-0.50	0.03	0.31
	cis-Lycopene	PCB-74	-0.54	0.04	0.31
	total Lycopene	PCB-74	-0.54	0.03	0.31
	Lutein	TCDD	-0.36	0.03	0.14
		PCB-153	0.57	0.045	0.25
	Zeaxanthin	TCDD	-0.34	0.049	0.21
	cis-Lutein/Zeaxanthin	PCB-153	0.53	0.03	0.25
		PBDE-47	0.86	0.01	0.16
		PFOS	-0.37	0.02	0.11
	Combined Lutein/zeaxanthin	TCDD	-0.43	0.01	0.14
		PCB-153	0.70	0.03	0.25
		PBDE-47	0.86	0.045	0.30
	α-Cryptoxanthin	PCB-105	-0.37	0.01	0.21
Overweight	Retinyl palmitate	PCB-153	0.24	0.04	0.43
	25-Hydroxyvitamin D	DDE	-0.49	0.04	0.34
	α-Tocopherol	PCB-74	0.32	0.03	0.58
	γ-Tocopherol	PBDE-47	-0.69	0.01	0.20
	α-Carotene	PFOS	0.19	0.02	0.39
	Lutein	PCB-105	-0.39	0.02	0.25
		DDE	0.60	0.01	0.18
	Zeaxanthin	PCB-105	-0.39	0.04	0.27
	Combined Lutein/zeaxanthin	PCB-105	-0.43	0.02	0.25
Obese	Retinyl palmitate	PCB-74	0.24	0.04	0.52
		PCB-153	0.32	0.02	0.24
		PBDE-47	0.38	0.01	0.13

	Retinyl stearate	PCB-153	0.31	0.04	0.27
		PFOA	-0.19	0.046	0.20
	α-Tocopherol	PCB-153	0.59	0.02	0.24
		PFOA	-0.37	0.02	0.14
	γ-Tocopherol	PBDE-47	-0.80	0.02	0.13
	α-Carotene	PBDE-47	0.35	0.04	0.13
	trans-β-Carotene	PBDE-47	0.55	0.03	0.13
	cis-β-Carotene	TCDD	0.27	0.01	0.19
		PBDE-47	0.56	0.02	0.13
	total β-Carotene	PBDE-47	0.55	0.03	0.13
	trans-Lycopene	PFOA	0.43	0.002	0.05*
		PFOS	0.26	0.04	0.19
	cis-Lycopene	PFOA	0.34	0.04	0.20
		PFOS	0.25	0.045	0.19
	total Lycopene	PFOA	0.40	0.01	0.08*
		PFOS	0.26	0.04	0.19
	Phytoene	PFOS	0.22	0.02	0.19
	cis-Lutein/Zeaxanthin	PFOS	0.31	0.049	0.19
Age	Retinol	TCDD	0.01	0.03	0.23
		PFOS	-0.02	0.05	0.51
	25-Hydroxyvitamin D	PFOS	-0.01	0.03	0.51
	α-Tocopherol	TCDD	0.01	0.01	0.14
		PCB-153	-0.01	0.003	0.07*
		DDE	-0.02	0.01	0.12
	γ-Tocopherol	DDE	0.01	<.0001	0.002*
	trans-Lycopene	PCB-74	0.01	0.03	0.52
		PCB-153	0.01	0.02	0.24
	total Lycopene	PCB-74	0.01	0.047	0.52
	Phytoene	PCB-105	0.01	0.02	0.33
	Zeaxanthin	TCDD	0.01	0.03	0.23
		PCB-105	0.01	0.04	0.33
	cis-Lutein/Zeaxanthin	PBDE-47	-0.02	0.01	0.14
	Combined Lutein/zeaxanthin	PBDE-47	-0.02	0.02	0.27

Table 3. In 399 – 442 females, false discovery rate (FDR) corrected statistical significance values (regression estimate coefficient, p-value, q-value) using the Benjamini-Hochberg procedure presented by effect measure modifier and adjusted model. An asterisk (*) denotes value is statistically significant with p-values less than 0.05 and q-values less than 0.10. A hashtag ($^{\#}$) denotes the body mass index (BMI)-specific association is significantly modified by age. Abbreviations for persistent organic pollutants (POPs) are as follows: 2,3,7,8-tetrachlorodienzo-p-dioxin (TCDD), polychlorinated biphenyl 74 (PCB-74), polychlorinated biphenyl 105 (PCB-105), polychlorinated biphenyl 153 (PCB-153), polybrominated diphenyl ether 47 (PBDE-47), perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS), p,p'-dichlorodiphenyldichloroethylene (DDE). BMI was categorized for participants aged 12 – 19 years old with BMI-for-age percentiles by sex using the 2000 Centers for Disease Control and Prevention (CDC) growth charts and for participants 20 years old and older by weight (kilograms) divided by

height (meters^2). All models adjusted for race and ethnicity, serum cotinine, and poverty-to-income ratio (PIR). PFOA and PFOS modeled without adjusting for total serum lipids. Only models with vitamin D biomarker as a predictor adjusted for 6-month exam period.