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Race-ethnicity is a strong correlate of circulating fat-soluble nutrient concentrations in a representative sample of the US population^{1,2,3}

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Abstract

Sociodemographic and lifestyle factors exert important influences on nutritional status; however, information on their association with biomarkers of fat-soluble nutrients is limited, particularly in a representative sample of adults. Serum or plasma concentrations of vitamin A (VIA), vitamin E (VIE), carotenes (CAR), xanthophylls (XAN), 25-hydroxyvitamin D (25OHD), saturated- (SFA), monounsaturated- (MUFA), polyunsaturated- (PUFA) and total fatty acids (tFA) were measured in adults (20 y) during all or part of NHANES 2003–2006. Simple and multiple linear regression were used to assess 5 sociodemographic variables (age, sex, race-ethnicity, education, income) and 5 lifestyle behaviors (smoking, alcohol consumption, BMI, physical activity, supplement use) and their relation to biomarker concentrations. Adjustment for total serum cholesterol and lipid-altering drug use was added to the full regression model. Adjustment for latitude and season was added to the full model for 25OHD.

Based on simple linear regression, race-ethnicity, BMI and supplement use were significantly related to all fat-soluble biomarkers. Sociodemographic variables as a group explained 5–17% of biomarker variability, whereas together, sociodemographic and lifestyle variables explained 22–23% (25OHD, VIE, XAN), 17% (VIA), 15% (MUFA), 10–11% (SFA, CAR, tFA) and 6% (PUFA). Although lipid adjustment explained additional variability for all biomarkers except 25OHD, it appeared to be largely independent of sociodemographic and lifestyle variables. After adjusting for sociodemographic, lifestyle and lipid-related variables, major differences in biomarkers were associated with race-ethnicity (from –44% to 57%); smoking (up to –25%); supplement use (up to 21%); and BMI (up to –15%). Latitude and season attenuated some race-ethnic differences. Of the sociodemographic and lifestyle variables examined, with or without lipid-adjustment, most fat-soluble nutrient biomarkers were significantly associated with race-ethnicity.

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³Supplemental Tables 1–5 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at <http://jn.nutrition.org>

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INTRODUCTION

Vitamins A, D and E, and the fruit and vegetable pigments, carotenes and xanthophylls, are well-known examples of fat-soluble micronutrients. Lipophilic macronutrients such as fatty acids are generally abundant as components of simple and complex lipids. Whether available in micro- or macro-amounts, these compounds play an essential or a beneficial role in human health. Fat-soluble nutrients may act as cofactors in enzymatic processes; or antioxidants in the body's intra- and extra-cellular fluid compartments; or components of membranes adding structure, antioxidant protection or bioactive signaling capability; or they may act like hormones via nuclear receptors turning genes on and off; or as substrates for production of hormone-like substances such as prostaglandins; or they may merely provide fuel for metabolic processes. For the essential fat-soluble nutrients, deficiency states have been defined based on clinical signs and symptoms and biomarker concentrations.

Nutritional biomarkers are commonly employed as objective indicators of nutritional status. Blood and urine are collected in the US National Health and Nutrition Examination Surveys (NHANES)⁴ to measure health and nutritional biomarkers; at the same time, information on sociodemographic and lifestyle parameters is provided by participants. Although dietary intake and supplements are the primary determinants for most biomarker concentrations, non-dietary factors may also show strong associations. From NHANES, we know that age, sex, race-ethnicity, lipids and alcohol consumption are associated with serum retinol (VIA) (1), as are education and Poverty Income Ratio (PIR) (2). Serum *alpha*-tocopherol (VIE) concentrations are associated with age, sex, race-ethnicity (3,4) and education (4). Carotene (CAR) concentrations in adults depend on age (5), sex (5,6), race-ethnicity (6–8), income (5,7), education (7), lipids (5) and cotinine (5,6). Xanthophylls (XAN) vary with race-ethnicity (7). Serum concentrations of 25-hydroxyvitamin D (25OHD) are associated with age (9–11), sex (9,11,12), race-ethnicity (7,9,11), education (7,12), BMI (9,11), income (11), supplement use (11) and season-latitude (9–11). Fatty acid (FA) concentrations are associated with age, sex and race-ethnicity (13). For some biomarkers, such as VIE, CAR and XAN, knowledge gaps exist in understanding their correlates and determinants (14).

In the present study, our interest was to expand upon the descriptive data from NHANES 2003–2006 that were recently published in the *Second National Report on Biochemical Indicators of Diet and Nutrition in the US Population* (13), which were stratified by age, sex and race-ethnicity. We were aware that some significant differences noted in this report may have been due to unmeasured association with other variables. By applying a systematic regression approach to assess the relative importance of common sociodemographic and lifestyle variables across the fat-soluble nutrient class of biomarkers, we wished to investigate similarities and differences within this class using the same comparative variables. From our review of the literature, this has not been done before. Other papers in this journal supplement are applying a similar approach to other classes of nutritional biomarkers. Our overarching goal for this series of papers is to provide researchers with a foundation of knowledge about these variables with which to develop more predictive models. Monitoring the nutritional status of the US population to inform public health policy is one of the key goals of NHANES and understanding the impact of sociodemographic,

behavioral and lifestyle factors on nutritional biomarker data is essential to interpret risk factors and trends.

SUBJECTS AND METHODS

Analytic sample

NHANES 2003–2006 was a complex, multistage, area probability sample representative of the US non-institutionalized civilian population during this period of time (15). All respondents gave their informed consent, and the NHANES protocol was reviewed and approved by the National Center for Health Statistics (NCHS) Research Ethics Review Board. Data collection consisted of: 1) a screening visit, during which sample persons were identified; 2) an interview during which a wide battery of health related questions were asked; and 3) an examination consisting of direct standardized physical examinations, including body measurements and blood and urine collection, carried out in a mobile examination center (MEC). Serum or EDTA-plasma specimens were prepared at the MEC, stored frozen at -20°C for several days to weeks, then shipped to CDC in Atlanta where they were stored at -70°C until tested. For fatty acids, in-house stability studies assured minimal instability under short-term (<3 mo) storage at -20°C and longer-term (up to 3 y) storage at -70°C ; fat-soluble micronutrients showed no instability at these times and temperatures (unpublished data). Depending on the analyte, between approximately 1,500 and 9,000 adults, age 20 y, from NHANES 2003–2006 had concentrations of fat-soluble nutrients measured (see Supplemental Table 1). Because the number of fat-soluble nutritional biomarkers measured in NHANES and available for this analysis is large, selected fat-soluble biomarkers were summed to create composite variables representing CAR, XAN, saturated- (SFA), monounsaturated- (MUFA), polyunsaturated- (PUFA), and total fatty acids (tFA). Composite variables were calculated only for those persons who had non-missing values across all corresponding biomarkers. All MEC-examined participants, 20 y, with at least 1 of the 9 fat-soluble biomarkers available were eligible for inclusion in the study.

Biomarker laboratory methods

Plasma concentrations of FA were measured in fasted (8 h) adults using surplus EDTA plasma from NHANES 2003–2004 several years after collection. Individual FA were measured and classified as saturated (SFA: myristic [14:0], palmitic [16:0], stearic [18:0], arachidic [20:0], docosanoic [22:0] and lignoceric [24:0]), monounsaturated (MUFA: myristoleic [14:1n5], palmitoleic [16:1n7], *cis*-vaccenic [18:1n7], oleic [18:1n9], eicosenoic [20:1n9], docosenoic [22:1n9] and nervonic [24:1n9]), or polyunsaturated (PUFA: linoleic [18:2n6], *alpha*-linolenic [18:3n3], *gamma*-linolenic [18:3n6], eicosadienoic [20:2n6], *homo-gamma*-linolenic [20:3n6], arachidonic [20:4n6], eicosapentaenoic [20:5n3], docosatetraenoic [22:4n6], docosapentaenoic-3 [22:5n3], docosapentaenoic-6 [22:5n6] and docosahexaenoic [22:6n3]). MUFA and PUFA were the sums of each respective class of fatty acids. Total fatty acids (tFA) was defined as the sum of 24 individual FA. As part of NHANES 2005–2006, concentrations of VIA, VIE, CAR (sum of *alpha*- and *beta*-carotene and *cis*- and *trans*-lycopene), and XAN (sum of lutein, zeaxanthin and *beta*-cryptoxanthin) were measured in serum from fasted and non-fasted persons. Similarly, 25OHD was

measured in serum from fasted and non-fasted persons for NHANES 2003–2006. The analytical methods used were as follows: HPLC separation with UV-visible light detection for VIA, VIE, CAR and XAN (16); DiaSorin (Stillwater, MN) radioimmunoassay for 25OHD (17); and GC separation with mass spectrometry detection for individual FA, including free FA and those hydrolyzed from more complex lipids (18). Westgard-type QC multi-rules were used to judge assay performance (19).

Sociodemographic, lifestyle and lipid-related variables

A review of the literature, focusing on NHANES, suggested several variables that would likely explain non-diet-related sources of variability for nutritional biomarkers. All sociodemographic variables, alcohol intake, leisure activity, medication and supplement use data were self-reported. Age (20–39 y, 40–59 y, 60 y), race-ethnicity (Mexican American, non-Hispanic black, non-Hispanic white), and educational attainment (<high school, high school, >high school) groupings were used. PIR was calculated by dividing total family income by the poverty threshold index adjusted for family size at year of interview (20) and categorized as low (0–1.85), medium (>1.85–3.5), or high (>3.5). Alcohol intake was categorized based on the average daily number of drinks: non-drinker, <1 (not 0), 1–<2, or 2. Adiposity was assessed using BMI (kg/m²) (21). Classification as a smoker was based on serum cotinine >10 µg/L (22). Physical activity was calculated as total metabolic equivalent task (MET) in MET-min/wk from self-reported leisure time physical activities and categorized as: none reported, 0–<500, 500–<1000, or 1000 MET-min/wk (23). Dietary supplement users reported taking any dietary supplement within the 30 d preceding the household interview. As the literature suggested that adjustment for lipids is beneficial to interpret VIE, CAR and XAN (24,25), although patterns of sociodemographic variation for VIE vs. VIE adjusted for cholesterol were similar in an earlier analysis (3), we provided an additional model for the fat-soluble nutrients that adjusted for total serum cholesterol, which was measured using a cholesterol oxidase method (26,27), and for the participant's use of lipid-altering drugs. Lipid-altering drug user was defined as having taken at least one prescribed lipid-altering drug during the 30 d preceding the interview. Drugs included cholesterol synthesis inhibitors (statins), fibric acid derivatives, bile acid resins, cholesterol absorption inhibitors, miscellaneous anti-hyperlipidemic drugs (niacin or probucol), prescription fish oil, or combination-type drugs such as a statin and niacin.

Statistical methods

As we used the same statistical methods for the series of papers presented in this supplement, the reader is referred to Sternberg *et al.* (28) for a detailed description of the methods and for a discussion of compromises taken in developing the multiple regression model due to the limited degrees of freedom, such as the number of covariates considered, the chosen form of continuous covariates, and the consideration of interactions between covariates. In short, we explored bivariate associations between each biomarker and selected study variables by calculating Spearman correlations (for continuous variables) and by presenting the geometric means (arithmetic mean for 25OHD as its distribution was reasonably symmetric) and 95% CI across the variable categories. We used multiple linear regression to assess the impact of confounding and determine whether statistical significance persists after adjusting for differences in key variables. We arranged the independent

variables into 2 sets or “chunks”: 1) sociodemographic variables (age, sex, race-ethnicity, education level, and PIR) and 2) lifestyle variables (dietary supplement use, smoking, alcohol consumption, BMI, and physical activity level). We tested each chunk simultaneously to determine whether the independent variables (as a group) were related to the dependent variable; followed by a test for each individual variable while controlling for the other variables. We present the results of 3 regression models for each biomarker: simple linear regression (model 1), multiple linear regression model with the sociodemographic chunk (model 2), and multiple linear regression model with both the sociodemographic and lifestyle chunk (model 3). For the fat-soluble nutrient analysis, we created an additional model by adding lipid-altering drug use and total serum cholesterol to model 3 to assess the effects of lipid-related factors (model 4). To assess the effect of latitude and season specifically on 25OHD, we created a model in which latitude and season at the time of the participant’s MEC examination were added to model 3 (model 5 for 25OHD only). For each model we present the estimated percent change (absolute unit change for 25OHD) in biomarker concentrations with change in each covariate holding all other remaining covariates constant. Two-sided *P*-values were flagged as statistically significant if <0.05 .

RESULTS

Population characteristics

Characteristics of the study population with weighted percentages of adults in each category of each variable examined in the study are shown in Supplemental Table 2. Proportions of respondents in each category were similar in each survey period.

Correlations

The majority of the continuous sociodemographic, lifestyle or lipid-related variables (PIR, smoking, alcohol, BMI, physical activity) were not strongly significantly correlated ($|r|<0.3$) with concentrations of fat-soluble biomarkers. Significant correlations were strongest between total cholesterol and any FA class ($|r|=0.53-0.75$), total cholesterol and VIE ($|r|=0.57$), and age and VIE ($|r|=0.41$) (Table 1).

Simple linear regression

With few exceptions, based on simple linear regression (model 1), we found significant associations between biomarker concentrations and age (except for 25OHD), sex (except for SFA, MUFA and tFA), race-ethnicity, education (except for PUFA and tFA) and PIR (except for SFA, MUFA, PUFA and tFA) (Table 2). Among individual sociodemographic variables, age explained variability in biomarkers considerably for VIE (12%) and modestly for VIA and MUFA (5–6%). Sex accounted for almost 4% of the variability of VIA. Race-ethnicity explained 16% of variation in 25OHD and 5–6% variation in XAN and VIA. Other sociodemographic variables, such as PIR and education accounted for 3–4% of the variability in CAR concentrations.

Based on simple linear regression (Table 3), we found significant associations between biomarker concentrations and smoking (except for VIA, SFA, MUFA and tFA), alcohol consumption (except for VIE and PUFA), BMI, physical activity (except for VIE, PUFA

and tFA), and supplement use. Among individual lifestyle variables, smoking explained a considerable amount of variability for XAN (8%) while alcohol intake explained at most 3% for VIA. BMI accounted for 4–5% of the variability of XAN and 25OHD. Physical activity accounted for 4% of the variability of CAR and 25OHD, and supplement use accounted for 14% of the variability of VIE and 4% of 25OHD.

We investigated the association of 25OHD with where and when participants were examined using latitude and time of year as proxies for sun exposure. As noted previously (10), the NHANES MECs spend the winter months in the warmer lower latitudes and the summer months in the cooler higher latitudes, which has an impact on assessment of vitamin D status. Without stratifying for race-ethnicity, a bivariate analysis showed that mean 25OHD was different in different latitudes or in different months (Supplemental Table 4) by as much as about 15–25%.

Multiple linear regression

Multiple linear regression demonstrated that sociodemographic variables as a group (model 2) explained 17% (VIE, 25OHD), 13% (VIA), 10% (MUFA, XAN), 6% (CAR, SFA, tFA) and 5% (PUFA) of the biomarker variability (Supplemental Table 3). In model 3, the chunks of sociodemographic and lifestyle variables explained 23% (VIE, 25OHD), 22% (XAN), 17% (VIA), 15% (MUFA), 11% (SFA), 10% (CAR, tFA) and 6% (PUFA) of the variability. In some cases, adjusting for lifestyle variables diminished the impact of sociodemographic variables as described by the *beta* coefficients, suggesting that lifestyle factors captured some unmeasured confounding associated with sociodemographic variables. For example, the association of a high school education on CAR concentration was attenuated when other sociodemographic variables were adjusted (model 2 vs. model 1) and was further attenuated by adjusting for lifestyle variables (model 3 vs. model 1).

It has been suggested that interpretation of some fat-soluble nutrient concentrations is not appropriate in the absence of lipid adjustment (25), thus we developed a model 4, in which we adjusted for the use of lipid-altering drugs and total serum cholesterol after adjustment for sociodemographic and lifestyle variables. While the addition of a lipid-related factors chunk to a model with both sociodemographic and lifestyle variables was statistically significant for all of the fat soluble outcomes, the R^2 did not substantially change for 25OHD. On the other hand, the R^2 increased from model 3 to model 4 for VIE (from 23% to 55%), XAN (from 22% to 32%), CAR (from 10% to 26%), VIA (from 17% to 24%), SFA (from 11% to 47%), MUFA (from 15% to 40%), PUFA (from 6% to 55%) and tFA (from 10% to 53%).

Because log transformation may obscure the interpretation of the *beta* coefficients, we estimated the percent change in biomarker concentrations (except change in nmol/L for 25OHD) associated with each covariable (Table 4). As can be seen with the *beta* coefficients, the estimated association of most of these variables changed between models 1 and 4, suggesting that at least some of the association measured in the unadjusted model may be a result of confounding. Even after adjusting for sociodemographic, lifestyle and lipid-related factors, race-ethnicity remained significantly correlated with biomarkers of fat-soluble nutrient status. Specifically, of 9 fat-soluble biomarkers, 4 (25OHD, XAN, MUFA,

VIA) were most strongly associated with race-ethnicity. All other things being equal, NHB had lower estimated concentrations of 25OHD, MUFA, VIA compared to NHW (9% to ~44% lower); in contrast, XAN was estimated to be 33% higher in NHB. Comparing MA with NHW, XAN was 57% higher while the concentration of 25OHD was ~24% lower. All FA classes were 8–10% higher in MA compared with NHW.

With the exception of CAR and 25OHD, age was significantly associated with higher fat-soluble micro-nutrient biomarker concentrations (Table 4). On the other hand, the association of age with SFA, PUFA and tFA lost significance after lipid adjustment. Sex differences, when significant, were associated with lower biomarker concentrations (VIA and XAN) in women. Higher SFA and PUFA in women lost significance with lipid adjustment. In general, with the exception of the higher CAR in NHB than NHW, race-ethnic differences were not much different with or without lipid adjustment. Largest effects of PIR in model 4 were on XAN and CAR which were 3–4% lower with every 2 unit decrease in PIR. In the full regression model, the strongest association of education was with CAR and XAN which were estimated to be 8–9% lower in those without, compared to those with some higher education. The negative association of smoking with several biomarkers (VIE, CAR and XAN) was substantially attenuated for VIE but only slightly attenuated for CAR and XAN after adjustment; smoking and MUFA were positively associated. VIA, SFA and MUFA were about 5–6% higher in drinkers versus non-drinkers. Increasing BMI was negatively associated with CAR, XAN and 25OHD after adjustment for sociodemographic, lifestyle and lipid-related factors; SFA, MUFA and tFA maintained a positive association with BMI after adjustment. Supplement use was significantly associated with all fat-soluble micro-nutrients in the full regression model.

Considering that for most persons the primary source of vitamin D comes from the action of sunlight on skin, we added season and latitude to assess their independent association with 25OHD after controlling for sociodemographic and lifestyle. We found that latitude and time of year continued to be significantly associated with concentration of 25OHD and had limited impact on *beta* coefficients of the other variables. The only *beta* coefficient that changed by more than 20% was that associated with the comparison between MA and NHW (Supplemental Table 5). The R^2 after adjusting for latitude and season (model 5) was 26.2%.

DISCUSSION

After adjusting for lipid-related factors, 4 variables (race-ethnicity, smoking, BMI and supplements) had strong associations with multiple fat-soluble nutritional biomarkers. Race-ethnicity was a major correlate of fat-soluble nutrient concentrations showing large differences for most biomarkers, even after adjustments were made for income, education, adiposity, and other variables that have been suggested to contribute to race-ethnic differences. Smoking and high BMI were frequently associated with lower fat-soluble micro-nutrient concentrations. As might be expected, supplement use was consistently associated with higher fat-soluble micro-nutrient biomarker concentrations. Our main aim was to expand on the *Second National Report on Biochemical Indicators of Diet and Nutrition in the US Population* (13), which provided nutritional biomarkers data tables stratified by age, sex and race-ethnicity but did not adjust for other sociodemographic or any

lifestyle variables. It is noteworthy that after controlling for other sociodemographic and lifestyle variables, significant race-ethnic differences (8/9) and age differences (6/9) persisted for most fat-soluble biomarkers; however, the picture for sex differences was mixed with only 2/5 differences being retained.

Race-ethnicity

As shown in other studies (9,29), race-ethnicity was strongly associated with 25OHD. In the present analysis, even after adjustments for other sociodemographic, lifestyle and lipid-related factors, MA and NHB had 25OHD concentrations that were 12 and 24 nmol/L lower, respectively, than NHW. Sun exposure is undoubtedly an unmeasured factor that is responsible for a substantial fraction of vitamin D biomarker variability. The ability of melanin to interfere with cutaneous synthesis of vitamin D₃ (30) and the much lower intake of vitamin D (50% less from diet and supplements) by NHB vs. NHW adults in the general population (31) most likely underlie the black-white difference in 25OHD. Although season and latitude continued to be important for 25OHD even after controlling for demographic and lifestyle factors, these variables had a limited effect on the *beta* coefficients of the other variables in the model. Overall, model 3 accounted for 23% of the variability in 25OHD, which is close to 24% of the variability shown in a US community-based sample using a model that included intake (29) and higher than 11% of the variability modeled in a representative sample of German adults that also included intake (32). Adding latitude and season provided only a small improvement (26%).

Race-ethnicity was also an important correlate of FA concentrations. Little is known about non-dietary sources of variation in plasma FA concentrations; this study provides the first nationally representative data on this subject. Of interest, these data show that after adjustment for sociodemographic, lifestyle and lipid-related factors, NHB had lower plasma concentrations of SFA (−6%), MUFA (−15%) and tFA (−5%) than NHW, whereas MA had higher plasma concentrations of all FA classes (8–10%) compared with NHW. Race-ethnic differences for total triglycerides are consistent with the race-ethnic pattern seen in SFA and MUFA with lower concentrations in NHB and higher concentrations in MA (33). The large difference in MUFA between NHB and NHW suggests that important variables that may closely associate with race-ethnicity have been omitted from the full regression model, e.g., genetic differences in fatty acid metabolism. It is interesting to note that expression of stearoyl CoA desaturase-1 (SCD-1), the rate-limiting enzyme catalyzing the conversion of SFA to MUFA, is 54%–72% lower in adipose tissue and muscle of black compared with white women (34).

Race-ethnic differences were apparent in XAN and CAR, which are currently the best biomarkers for consumption of fruits and vegetables (14). In an earlier NHANES, non-whites generally had higher lipid-adjusted xanthophyll and carotene (except lycopene) concentrations than other race-ethnic groups (6); similarly, in the present study, MA and NHB had much higher XAN concentrations but only NHB had higher CAR concentrations than NHW.

VIA concentrations were shown to be higher in NHW than in NHB and MA in an earlier NHANES survey (1). In analyses of several NHANES data sets, VIE was shown to be

higher in whites than in African Americans, but the difference between MA and whites was of variable significance (3,4). Kant *et al.* (7) used sex-specific models on NHANES data and showed that race-ethnic differences in VIE were limited to men and that NHB men had lower concentrations than NHW or MA men. In comparison, our analysis of the most recent NHANES data set using the full regression model showed that NHW had higher VIE concentrations than either NHB or MA.

Smoking

Among lifestyle variables, smoking was significantly associated with (20–29% reduction) CAR and XAN which showed little attenuation after adjustment. Smoking had previously been shown to be negatively associated with serum concentrations of *alpha*- and *beta*-carotene (6), lutein/zeaxanthin (6,35) and *beta*-cryptoxanthin (6) in NHANES III, and with low serum carotene and xanthophyll concentrations in the UK National Diet and Nutrition Survey (NDNS) (36) suggesting either the intake of these anti-oxidants by smokers is lower than by nonsmokers, and/or they are oxidized by free radicals in cigarette smoke. Many studies have found lower CAR and XAN in smokers after adjusting for dietary intake (14) suggesting smoke-related oxidation of CAR and XAN occurs. In an analysis of a community-based sample of adults in the US, race-ethnicity, education and smoking had the strongest associations with serum lutein concentrations (37). Similarly, we found race-ethnicity, smoking and BMI followed by education to have the significant associations with XAN in this representative sample of US adults. In the cross-sectional MRFIT study, higher plasma concentrations of two major MUFA, namely, palmitoleic (16:1n7) and oleic (18:1n9), were reported in smokers after multivariate adjustment (38); in agreement, we found higher total MUFA in smokers, with or without lipid adjustment. In the *Dietary Reference Intakes* (14), the Institute of Medicine recommended thorough investigation of the determinants of VIE, including the relationship between oxidative stress and vitamin E status. Smoking and physical activity, two forms of oxidative stress, were examined in the present study. Physical activity, which is expected to produce free radicals through increased metabolic activity and thereby consume VIE, had little association with VIE. In contrast, the association of smoking with VIE was substantially attenuated from 13% to 5% lower in smokers in the full regression model and remained statistically significant. An analysis of NHANES 1988–1994 suggested that VIE was not different in smokers and nonsmokers after adjusting for multiple covariates including other nutritional biomarkers (4). Similarly, in the NDNS (36), smoking was not related to VIE or lipid-adjusted VIE in older adults. Thus, the relationship between smoking and VIE remains to be clarified in future studies.

Body Mass Index

BMI, a measure of adiposity, was negatively associated with XAN (15% decrease for a 25% increase in BMI) in the full regression model, as were CAR and 25OHD. In our analysis, a 25% increase in BMI was associated with about 4 nmol/L decrease in 25OHD which is consistent with 6 nmol/L decrease in 25OHD in obese versus normal weight women reported in a New Zealand national survey (39). Conversely, VIE, SFA, MUFA and tFA were positively associated with increasing BMI. BMI has been shown to be associated with fasting serum triglycerides (40) which are made-up primarily of SFA and MUFA, so this

positive relationship with BMI is not unexpected. A complex association of BMI with fat-soluble nutrient concentrations has been shown previously (5,29,41).

Supplement use

As anticipated, supplement use was positively associated with most biomarkers, in particular VIE and CAR, even after full regression adjustment. Interview data from NHANES indicate that approximately 20–40% of adults used supplements containing vitamins A (or pro-vitamin A carotenoids) and E during 2003–2006 (42).

Other variables

Aside from the main variables race-ethnicity, smoking, BMI and supplement use, we also found some smaller but significant associations with other variables (Table 4). Age remained a significant correlate for most biomarkers after adjusting for sociodemographic, lifestyle and lipid-related factors. Several US surveys indicate that intake of fruits and vegetables increases with age (43,44) which could help to explain some biomarker increases. However, to study the relationship between fruit and vegetable intake and age, specific carotenes and xanthophylls would need to be investigated as the composite variables CAR and XAN were differentially affected by age. Lipids (e.g., cholesterol, triglycerides) increase with age in young or middle-aged adults (33) which could explain some attenuation of age with the lipid adjustment. VIA (1) and VIE (3,4) were shown in earlier NHANES to be positively correlated with age and lipids.

It is interesting to note that physical activity was positively associated with CAR, XAN and 25OHD. Exercise has previously been shown to be positively associated with lutein and zeaxanthin intake (29). It seems plausible that leisure-time physical activity is positively associated with fruit and vegetable intake and outdoor activities, and that the significant association with CAR, XAN and 25OHD captures this shared association.

No significant association of sex was apparent in the full regression model except for VIA and XAN, which were 10% and 3% lower in women, respectively. Among the remaining lifestyle variables, alcohol consumption was moderately positively associated with VIA, 25OHD, SFA, MUFA and tFA. The ability of alcohol to acutely increase serum VIA has been known for decades (45) and a positive association between VIA and alcohol intake was also demonstrated in an earlier NHANES survey (1). Alcohol-related increases in FA may be related to increases in triglycerides which have been shown to occur even with moderate alcohol intake (46). Alcohol has been reported to be inversely associated with most carotenes and xanthophylls (14) but its association was not significant in the current analysis. In general, PIR and education were relatively weakly associated with fat-soluble biomarkers and, in agreement with a previous analysis (7), did not appear to account for race-ethnic differences.

Lipid adjustment

In this analysis, we adjusted for cholesterol because others have shown that a large proportion of the variability for some nutritional biomarkers, particularly VIE, can be explained when serum lipid concentrations are included in the model (4,29). Several

approaches have been suggested (25) but adjustment using total serum cholesterol was shown to be nearly equivalent to more complex adjustment (3,24). Adjusting for lipids as we did provided insight into where the lipid adjustment has impact. We found that aside from the lack of any effect of lipid adjustment on 25OHD, there was general attenuation of the effect of age and sex by lipid adjustment and, with few exceptions involving CAR and XAN and either age, sex or race-ethnicity, lipid adjustment did not mediate the effects other variables on fat-soluble nutritional biomarkers, i.e., adjusting for lipids showed relatively little impact on the remaining sociodemographic and lifestyle variables. However, the R^2 for the full regression model accounted for more of the variability of the biomarkers when lipid adjustment was added. This suggests that lipids are independent predictors of most fat-soluble biomarkers, including VIA, which is transported to the liver as retinyl esters by lipids after absorption but thereafter is transported in the plasma by a specific binding protein not associated with lipids.

Strengths and weaknesses

The present study has as its primary strength that a unified approach was used to assess the impact of sociodemographic and lifestyle variables on fat-soluble nutrient biomarker concentrations using a representative sample of the US population. As the models were built based on current research data and with the NHANES focus, they should be generalizable to the US population. This same approach was applied to other types of nutritional indicators (see other papers in this issue) such that comparisons can be made among biomarkers using equivalent modeling strategies. As noted by others (7), relatively little is known about the components of variance for most nutritional biomarkers, particularly relative differences among biomarkers, thus, this analysis fills-in gaps in knowledge.

There are several limitations to the analytical approach used in this series of papers. First, observational studies such as NHANES can be used to find associations, but they cannot be used to demonstrate causality. We did not aim to develop a predictive model but rather to summarize general patterns with respect to a selected set of sociodemographic and lifestyle variables to determine how these covariates, jointly, are related to fat-soluble nutrient concentrations. Dietary intake data were not included in this analysis and neither was information on the intake of *specific* dietary supplements although these are likely to be important variables. For this series of papers, we did not include intake as a predictor or confounder because we could not devise a consistent set of variables across all the classes of biomarkers and because *df* were limited for some biomarkers. Analysis of nutrient intakes, which is problematic for the fat-soluble nutrient group for various reasons including poor correlation of dietary intake with biomarker concentration, homeostatic control of nutrient availability, endogenous synthesis and inter-conversion of nutrients, will require more in-depth analysis than could be accommodated in an already large inquiry. Another limitation of this analysis is that interactions could not be examined. The size of the multiple regression model was limited by the available *df*, as many of the variables were categorical. This limited the opportunity to fit higher order interactions and include more variables in the full model. Fasting status was not controlled except for FA, which were measured only if participants were fasted for at least 8 h. However, in a separate analysis of fasting as a preanalytical variable (47), we found no effect of fasting on 25OHD, VIA and XAN, and

only a modest effect of fasting on VIE and CAR (4–5% higher if <3 h). Lastly, in this analysis, composite variables (CAR, XAN, SFA, MUFA, PUFA, tFA) were used which provided useful summary information; however, individual nutrients within a composite may not be represented by these findings.

In conclusion, after controlling for 10 sociodemographic and lifestyle variables, race-ethnicity continued to be strongly associated with most fat-soluble nutrient biomarker concentrations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

25OHD	25-hydroxyvitamin D
CAR	carotenes
FA	fatty acid
MA	Mexican American
MET	metabolic equivalent task
MUFA	monounsaturated fatty acids
NCHS	National Center for Health Statistics
NDNS	UK National Diet and Nutrition Survey
NHANES	US National Health and Nutrition Survey
NHB	non-Hispanic black
NHW	non-Hispanic white
PIR	Poverty Income Ratio
PUFA	polyunsaturated fatty acids
SFA	saturated fatty acids
tFA	total fatty acids (sum of SFA, MUFA, PUFA)
VIA	vitamin A (retinol)
VIE	vitamin E (<i>alpha</i> -tocopherol)

XAN xanthophylls

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Spearman correlation coefficients between concentrations of fat-soluble nutritional biomarkers and selected continuous sociodemographic, lifestyle or lipid-related variables for adults 20y, NHANES 2003–2006^{1,2}

Table 1

Continuous variable	VIA	VIE	CAR	XAN	25OHD	SFA	MUFA	PUFA	tFA
Age	0.25*	0.41*	-0.08*	0.09*	-0.03	0.21*	0.27*	0.17*	0.22*
PIR ³	0.13*	0.15*	0.18*	0.09*	0.16*	0.01	-0.03	0.04	0.02
Cotinine ⁴	-0.06	-0.20*	-0.16*	-0.29*	-0.07*	-0.01	0.01	-0.10*	-0.04
Alcohol consumption ⁵	0.22*	0	0.07*	0.03	0.13*	0.10	0.05	-0.01	0.06
BMI	-0.01	0.14*	-0.16*	-0.22*	-0.23*	0.18*	0.15*	0.08	0.14*
Physical activity ⁶	0.07*	0.02	0.12*	0.10*	0.12*	-0.06	-0.09	-0.02	-0.06
Total cholesterol	0.22*	0.57*	0.35*	0.29*	0.06*	0.62*	0.53*	0.75*	0.68*

¹ 25OHD, 25-hydroxyvitamin D; CAR, carotenes; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; tFA, total fatty acids (sum of SFA, MUFA, PUFA); VIA, vitamin A (retinol); VIE, vitamin E (*alpha*-tocopherol); XAN, xanthophylls

² Biomarker data available for analysis: plasma concentrations of SFA, MUFA, PUFA and tFA (NHANES 2003–2004); serum concentrations of 25OHD (NHANES 2003–2006); and serum concentrations of VIA, VIE, CAR and XAN (NHANES 2005–2006)

³ Family Poverty Income Ratio (PIR)

⁴ Serum biomarker of cigarette smoke exposure

⁵ Alcohol consumption: calculated as average daily number of “standard” drinks [(quantity×frequency) / 365.25]; 1 drink ≈ 15 g ethanol

⁶ Physical activity: calculated as total metabolic equivalent task (MET) in MET-min/wk from self-reported leisure-time physical activities

* $P < 0.05$

Unadjusted fat-soluble biomarker concentrations stratified by sociodemographic variable categories for adults 20 y, NHANES 2003–2006^{1,2,3,4}

Table 2

Variable	VIA µg/dL	VIE mg/dL	CAR µg/dL	XAN µg/dL	25OHD nmol/L	SFA mmol/L	MUFA mmol/L	PUFA mmol/L	tFA mmol/L
Sociodemographic									
Age, y									
20–39	54.3 (53.3–55.3)	1.02 (1.00–1.04)	60.6 (58.7–62.7)	22.3 (21.3–23.3)	59.8 (57.5–62.1)	3.44 (3.35–3.52)	2.35 (2.28–2.42)	4.63 (4.54–4.72)	10.50 (10.3–10.7)
40–59	58.7 (57.7–59.7)	1.23 (1.19–1.26)	61.1 (57.9–64.4)	22.6 (21.2–24.0)	58.2 (56.0–60.3)	3.800 (3.67–3.94)	2.66 (2.56–2.76)	4.92 (4.80–5.04)	11.4 (11.1–11.7)
60	64.4 (62.8–66.1)	1.40 (1.36–1.44)	56.0 (52.7–59.5)	24.0 (22.8–25.2)	58.3 (56.7–60.0)	3.91 (3.80–4.02)	2.90 (2.81–3.00)	4.98 (4.85–5.12)	11.8 (11.5–12.1)
P-value ⁵	<0.0001	<0.0001	0.0419	0.0380	0.43	<0.0001	<0.0001	<0.0001	<0.0001
r ² , % ⁶	5	12	<1	<1	<1	4	6	2	4
Sex									
Men	61.6 (60.6–62.6)	1.15 (1.13–1.18)	57.2 (55.4–59.1)	22.2 (21.4–23.2)	58.9 (57.0–60.9)	3.64 (3.50–3.79)	2.61 (2.50–2.72)	4.73 (4.61–4.85)	11.1 (10.7–11.4)
Women	55.3 (54.0–56.6)	1.20 (1.17–1.24)	62.1 (60.1–64.1)	23.3 (22.3–24.3)	58.7 (56.8–60.7)	3.72 (3.61–3.82)	2.56 (2.47–2.65)	4.90 (4.80–5.01)	11.2 (10.9–11.5)
P-value	<0.0001	<0.0001	0.0001	0.0031	0.0021	0.37	0.51	0.0103	0.52
r ² , %	4	<1	<1	<1	0	<1	<1	1	<1
Race-ethnicity									
MA	51.7 (50.9–52.6)	1.12 (1.09–1.15)	55.1 (52.3–58.1)	30.9 (28.2–33.7)	49.4 (46.5–52.2)	3.89 (3.66–4.12)	2.78 (2.55–3.02)	5.05 (4.84–5.26)	11.9 (11.4–12.5)
NHB	51.6 (50.5–52.8)	1.00 (0.98–1.02)	57.8 (54.0–61.8)	24.5 (23.4–25.7)	37.6 (35.8–39.4)	3.38 (3.23–3.54)	2.19 (2.10–2.28)	4.66 (4.48–4.85)	10.3 (9.76–10.8)
NHW	60.7 (59.7–61.7)	1.22 (1.20–1.25)	60.2 (58.5–62.0)	21.3 (20.5–22.1)	64.1 (62.6–65.6)	3.70 (3.60–3.81)	2.61 (2.53–2.70)	4.81 (4.70–4.92)	11.2 (10.9–11.4)
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	<0.0001	0.0344	0.0004
r ² , %	6	3	<1	5	16	2	3	1	2
Education									
< High school	55.2 (53.4–57.0)	1.11 (1.08–1.14)	50.4 (48.5–52.4)	23.1 (21.3–25.0)	52.9 (50.0–55.7)	3.75 (3.64–3.87)	2.69 (2.56–2.83)	4.90 (4.81–4.99)	11.2 (10.9–11.6)
High school	58.8 (57.3–60.2)	1.16 (1.11–1.21)	54.6 (52.0–57.4)	20.1 (19.0–21.1)	58.9 (57.1–60.8)	3.79 (3.59–4.00)	2.66 (2.50–2.83)	4.83 (4.68–4.98)	11.3 (10.8–11.8)

Variable	VIA μg/dL	VIE mg/dL	CAR μg/dL	XAN μg/dL	25OHD nmol/L	SFA mmol/L	MUFA mmol/L	PUFA mmol/L	tFA mmol/L
> High school	59.0 (57.7–60.3)	1.21 (1.18–1.24)	65.4 (63.4–67.4)	24.0 (23.1–24.9)	60.7 (58.7–62.6)	3.61 (3.53–3.68)	2.52 (2.45–2.59)	4.79 (4.68–4.90)	11.0 (10.8–11.3)
<i>P</i> -value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0038	0.0166	0.16	0.31
<i>r</i> ² , %	1	1	4	2	2	1	1	<1	<1
PIR ⁷									
Low	55.5 (54.1–56.9)	1.10 (1.06–1.14)	53.5 (51.6–55.6)	21.9 (20.7–23.2)	53.9 (51.5–56.3)	3.71 (3.62–3.79)	2.66 (2.56–2.76)	4.79 (4.69–4.89)	11.2 (10.9–11.5)
Medium	58.5 (57.0–60.1)	1.16 (1.12–1.21)	57.6 (55.4–59.8)	22.0 (20.9–23.1)	58.7 (56.7–60.7)	3.69 (3.55–3.84)	2.57 (2.46–2.70)	4.80 (4.67–4.93)	11.0 (10.7–11.4)
High	59.9 (58.7–61.1)	1.25 (1.22–1.27)	65.8 (63.4–68.2)	23.8 (22.8–24.8)	62.3 (60.3–64.3)	3.68 (3.55–3.82)	2.57 (2.45–2.68)	4.88 (4.76–5.01)	11.3 (10.9–11.6)
<i>P</i> -value	<0.0001	<0.0001	<0.0001	0.0060	<0.0001	0.92	0.23	0.26	0.52
<i>r</i> ² , %	1	2	3	1	2	<1	<1	<1	<1

¹ Values are geometric means (95% CI) with the exception of 25OHD for which arithmetic means are displayed

² 25OHD, 25-hydroxyvitamin D; CAR, carotenes; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; tFA, total fatty acids (sum of SFA, MUFA, PUFA); VIA, vitamin A (retinol); VIE, vitamin E (*α*-tocopherol); XAN, xanthophylls. SI conversion factors are as follows: VIA, ×0.03491 (μmol/L); VIE, ×23.218 (μmol/L)

³ Biomarker data available for analysis: plasma concentrations of SFA, MUFA, PUFA and tFA (NHANES 2003–2004); serum concentrations of 25OHD (NHANES 2003–2006); and serum concentrations of VIA, VIE, CAR and XAN (NHANES 2005–2006)

⁴ Sample sizes for each biomarker stratified by variable can be found in Supplemental Table 1

⁵ *P*-value based on the Wald *F* test

⁶ *r*² based on model 1 (simple linear regression) using categories as shown

⁷ Family Poverty Income Ratio (PIR); categorized as low: 0–1.85; medium: >1.85–3.5; or high: >3.5

Unadjusted fat-soluble biomarker concentrations stratified by lifestyle variable categories for adults 20 y, NHANES 2003–2006^{1,2,3,4}

Table 3

Variable	VIA µg/dL	VIE mg/dL	CAR µg/dL	XAN µg/dL	25OHD nmol/L	SFA mmol/L	MUFA mmol/L	PUFA mmol/L	tFA mmol/L
Lifestyle									
Smoker ⁵									
No	58.2 (57.1–59.4)	1.23 (1.19–1.26)	63.5 (62.0–64.9)	25.0 (24.2–25.9)	59.1 (57.2–61.0)	3.66 (3.56–3.76)	2.54 (2.47–2.62)	4.87 (4.78–4.96)	11.1 (10.9–11.3)
Yes	58.3 (56.6–60.0)	1.07 (1.04–1.10)	51.1 (49.1–53.1)	17.9 (17.1–18.7)	58.2 (56.1–60.4)	3.74 (3.61–3.88)	2.69 (2.55–2.83)	4.70 (4.58–4.82)	11.2 (10.8–11.5)
P-value ⁶	0.94	<0.0001	<0.0001	<0.0001	0.0333	0.19	0.07	0.0013	0.74
r ² , % ⁷	0	3	3	8	<1	<1	<1	1	<1
Alcohol consumption ⁸									
None	55.7 (54.5–56.8)	1.20 (1.15–1.25)	55.7 (53.5–58.1)	23.0 (21.9–24.2)	55.5 (52.8–58.1)	3.68 (3.61–3.75)	2.59 (2.51–2.68)	4.83 (4.72–4.93)	11.2 (10.8–11.5)
<1 (not 0)	58.0 (56.9–59.1)	1.18 (1.15–1.21)	62.3 (60.2–64.4)	22.9 (21.8–24.1)	60.0 (58.2–61.9)	3.64 (3.50–3.79)	2.53 (2.42–2.64)	4.81 (4.68–4.94)	11.0 (10.7–11.4)
1–<2	65.6 (63.3–68.0)	1.19 (1.13–1.26)	63.3 (59.2–67.7)	23.6 (21.4–26.0)	63.1 (60.2–66.0)	3.73 (3.48–4.00)	2.61 (2.38–2.87)	4.82 (4.64–5.01)	11.4 (10.7–12.1)
2+	64.4 (62.4–66.3)	1.15 (1.08–1.22)	56.2 (52.1–60.5)	19.4 (17.8–21.1)	62.1 (59.0–65.2)	4.12 (3.78–4.49)	2.96 (2.70–3.26)	4.79 (4.48–5.13)	11.90 (11.20–12.80)
P-value	<0.0001	0.42	<0.0001	<0.0001	<0.0001	0.0285	0.0061	0.98	0.0375
r ² , %	3	<1	1	1	1	1	1	<1	1
BMI ⁹									
Underweight	50.2 (46.1–54.6)	0.99 (0.88–1.11)	60.6 (50.4–72.9)	25.1 (19.8–31.8)	59.3 (53.8–64.7)	NR	NR	NR	NR
Normal weight	57.6 (56.2–59.0)	1.13 (1.10–1.17)	65.8 (62.6–69.2)	25.2 (23.8–26.7)	64.1 (62.3–66.0)	3.43 (3.29–3.58)	2.39 (2.27–2.52)	4.71 (4.58–4.86)	10.6 (10.1–11.0)
Overweight	60.9 (59.8–62.0)	1.24 (1.20–1.28)	62.3 (59.8–64.9)	24.1 (23.2–25.1)	60.9 (58.7–63.0)	3.78 (3.68–3.88)	2.68 (2.59–2.77)	4.88 (4.78–4.98)	11.5 (11.2–11.8)
Obese	56.9 (55.6–58.3)	1.18 (1.15–1.21)	52.8 (51.3–54.3)	19.7 (18.8–20.5)	52.1 (50.0–54.3)	3.86 (3.69–4.04)	2.70 (2.55–2.85)	4.88 (4.70–5.07)	11.4 (10.9–12.0)
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0001	0.0023	0.0240	0.0004
r ² , %	2	2	3	4	5	3	3	1	3
Physical activity ¹⁰									

Variable	VIA µg/dL	VIE ng/dL	CAR µg/dL	XAN µg/dL	25OHD nmol/L	SFA mmol/L	MUFA mmol/L	PUFA mmol/L	tFA mmol/L
None reported	57.1 (55.7–58.5)	1.16 (1.12–1.20)	51.2 (49.3–53.2)	20.7 (19.5–21.8)	52.9 (51.1–54.8)	3.81 (3.69–3.93)	2.70 (2.62–2.79)	4.85 (4.71–5.00)	11.3 (11.0–11.6)
0–<500	57.2 (55.7–58.7)	1.18 (1.13–1.23)	59.9 (58.0–61.8)	22.2 (21.0–23.5)	58.6 (56.8–60.5)	3.67 (3.54–3.82)	2.61 (2.44–2.80)	4.82 (4.71–4.93)	11.2 (10.8–11.6)
500–<1000	58.7 (57.1–60.4)	1.20 (1.13–1.26)	61.0 (57.2–65.0)	23.3 (21.2–25.6)	61.5 (59.3–63.7)	3.66 (3.51–3.82)	2.54 (2.39–2.70)	4.86 (4.70–5.02)	11.2 (10.7–11.6)
1000+	60.1 (58.6–61.5)	1.20 (1.16–1.24)	68.7 (65.6–72.0)	25.3 (24.2–26.5)	64.7 (62.3–67.0)	3.56 (3.41–3.70)	2.44 (2.34–2.56)	4.77 (4.65–4.90)	10.9 (10.5–11.3)
P-value	<0.0001	0.42	<0.0001	<0.0001	<0.0001	0.0003	0.0057	0.69	0.29
r ² , %	1	<1	4	2	4	1	1	<1	<1
Supplement user ^{1/1}									
No	55.4 (54.4–56.5)	1.33 (1.30–1.37)	64.7 (62.7–66.8)	21.4 (20.4–22.4)	54.1 (51.8–56.4)	3.56 (3.42–3.71)	2.50 (2.39–2.61)	4.70 (4.57–4.84)	10.8 (10.4–11.2)
Yes	60.8 (59.5–62.0)	1.02 (1.00–1.04)	54.3 (52.8–55.8)	24.0 (23.1–25.0)	62.7 (61.1–64.4)	3.78 (3.68–3.88)	2.66 (2.59–2.72)	4.91 (4.79–5.05)	11.4 (11.2–11.7)
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0083	0.0106	0.0231	0.0179
r ² , %	2	14	3	1	4	1	1	1	1

^{1/1} Values are geometric means (95% CI) with the exception of 25OHD for which arithmetic means are displayed

² 25OHD; 25-hydroxyvitamin D; CAR, carotenes; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; tFA, total fatty acids (sum of SFA, MUFA, PUFA); VIA, vitamin A (retinol); VIE, vitamin E (*alpha*-tocopherol); XAN, xanthophylls. SI conversion factors are as follows: VIA, ×0.03491 (µmol/L); VIE, ×23.218 (µmol/L)

³ Biomarker data available for analysis: plasma concentrations of SFA, MUFA, PUFA and tFA (NHANES 2003–2004); serum concentrations of 25OHD (NHANES 2003–2006); and serum concentrations of VIA, VIE, CAR and XAN (NHANES 2005–2006)

⁴ Sample sizes for each biomarker stratified by variable can be found in Supplemental Table 1

⁵ “Smoker” defined by serum cotinine concentration >10 µg/L

⁶ P-value based on the Wald F test

⁷ r² based on model 1 (simple linear regression) using categories as shown

⁸ Alcohol consumption: calculated as average daily number of “standard” drinks [(quantity×frequency)/365.25]; 1 drink ~15 g ethanol

⁹ BMI (kg/m²): categorized as underweight (<18.5), normal weight (18.5–<25), overweight (25–<30), or obese (≥30)

¹⁰ Physical activity: categorized based on metabolic equivalent task (MET) in MET-min/wk from leisure-time physical activity

¹¹ “Supplement user” defined as one who took any dietary supplement during the 30 d preceding the household interview

NR Not reported; estimate suppressed because of inadequate cell size ($n < 42$)

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Estimated changes in biomarkers after adjusting for sociodemographic, lifestyle and lipid-related variables through chunk-wise modeling using data for adults 20 y, NHANES 2003–2006^{1,2,3,4,5}

Table 4

Variable	VIA	VIE	CAR	XAN	25OHD	SFA	MUFA	PUFA	tFA
Age: every 10 year increase									
Model 1	4.1*	8.3*	-1.2*	2.5*	-0.3	3.3*	5.4*	2.0*	3.0*
Model 2	3.7*	8.5*	-0.8*	4.6*	-1.1*	3.2*	5.3*	2.0*	3.0*
Model 3	4.0*	7.5*	-0.7*	4.6*	-0.7*	3.0*	5.5*	1.8*	3.0*
Model 4	2.1*	5.0*	-2.8*	2.5*	-0.8*	0.4	2.4*	-0.1	0.7
Sex: women vs men									
Model 1	-10.2*	4.4*	8.4*	4.6*	-0.2	2.0	-1.7	3.7*	1.4
Model 2	-10.3*	3.8*	8.8*	3.8*	0.4	1.8	-2.5	4.0*	1.0
Model 3	-9.0*	2.1	6.9*	0.3	0.3	4.2*	0.6	3.9*	2.3
Model 4	-9.6*	-1.2	2.7	-3.0*	0.1	2.0	-1.2	1.8	0.6
Race-ethnicity: NHB vs NHW ⁶									
Model 1	-14.9*	-18.1*	-4.0	15.2*	-26.5*	-8.7*	-16.2*	-3.0	-8.1*
Model 2	-12.4*	-12.5*	0.9	23.8*	-26.1*	-7.7*	-15.9*	-1.5	-7.0*
Model 3	-10.8*	-10.1*	5.1	28.7*	-23.8*	-8.5*	-16.7*	-1.4	-7.2*
Model 4	-9.4*	-6.6*	9.1*	33.1*	-23.6*	-6.3*	-15.0*	0.4	-5.3*
Race-ethnicity: MA vs NHW ⁶									
Model 1	-14.7*	-8.4*	-8.5*	44.9*	-14.8*	5.0	6.3	5.0*	6.6*
Model 2	-10.0*	5.0*	2.8	67.8*	-13.9*	7.4*	8.0*	8.3*	9.4*
Model 3	-8.7*	6.4*	0.2	58.9*	-11.9*	7.9*	10.5*	8.6*	11.0*
Model 4	-8.7*	5.2*	-1.4	57.2*	-12.0*	8.4*	10.0*	8.9*	10.1*
PIR: every 2 unit decrease ⁷									
Model 1	-4.4*	-7.7*	-11.4*	-5.9*	-4.5*	-0.0	2.0*	-1.4	-0.5
Model 2	-1.9*	-5.8*	-8.7*	-7.7*	-1.8*	-0.3	3.1*	-2.1*	-0.3

Variable	VIA	VIE	CAR	XAN	25OHD	SFA	MUFA	PUFA	tFA
Model 3	-1.1	-3.7*	-5.2*	-4.7*	-0.6	1.1	3.9*	-1.2	0.5
Model 4	-0.7	-2.5*	-3.7*	-3.4*	-0.6	0.4	3.7*	-1.6*	0.4
Education: HS vs >HS ⁸									
Model 1	-3.0*	-6.1*	-19.2*	-11.3*	-4.2*	4.7*	6.2*	1.4	1.9
Model 2	-1.8	-5.4*	-14.8*	-14.3*	-0.6	3.0	2.3	1.2	0.2
Model 3	-1.2	-2.8*	-9.4*	-8.0*	1.6*	1.3	0.3	1.3	-0.6
Model 4	-1.2	-2.8*	-9.3*	-7.9*	1.6*	2.2	0.9	1.7	0.6
Smoker: yes vs no ⁹									
Model 1	0.1	-12.9*	-19.6*	-28.6*	-0.9	2.3	5.6	-3.5*	0.4
Model 3	-0.1	-4.0*	-16.3*	-24.0*	-1.5	2.5	6.3*	-1.8	1.7
Model 4	-0.3	-4.8*	-17.3*	-24.8*	-1.5	1.5	5.4*	-2.4*	1.2
Alcohol: 1 vs 0 drinks/d ¹⁰									
Model 1	8.6*	-0.5	0.8	-4.1	3.5*	4.9*	5.2*	-0.1	3.1*
Model 3	6.8*	1.8	2.6	0.5	1.8*	7.9*	7.5*	1.9	5.1*
Model 4	6.1*	-0.4	0.1	-1.6	1.6*	4.8*	5.5*	-0.6	3.1*
BMI: 25% increase ¹¹									
Model 1	-0.6	1.0	-8.9*	-11.8*	-5.4*	5.0*	5.1*	1.6	3.2*
Model 3	-0.2	1.3*	-8.2*	-13.0*	-4.2*	6.2*	6.2*	1.5	3.8*
Model 4	-1.4*	-0.9*	-10.3*	-14.8*	-4.3*	4.4*	4.2*	0.3	2.0*
Physical activity: 750 vs 150 MET- min/wk ¹²									
Model 1	0.9*	0.6	5.6*	3.8*	2.3*	-1.4*	-2.0*	-0.3	-0.6
Model 3	0.7*	0.5	2.8*	2.5*	1.5*	-0.5	-0.5	0.2	0.2
Model 4	0.6*	0.5	2.8*	2.5*	1.5*	-0.3	-0.5	0.3	0.2
Supplement user: yes vs no ¹³									
Model 1	9.6*	30.7*	19.2*	12.4*	8.6*	6.3*	6.1*	4.5*	5.8*
Model 3	5.4*	21.1*	11.8*	6.1*	5.2*	5.3*	4.3	3.0	3.9
Model 4	5.4*	20.9*	11.7*	6.1*	5.2*	3.5*	2.0	1.3	2.2

- ¹ Changes represent percent change (%) in geometric mean for all biomarkers except for 25OHD where change in arithmetic mean represents concentration units (nmol/L)
- ² 25OHD, 25-hydroxyvitamin D; CAR, carotenes; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; tFA, total fatty acids (sum of SFA, MUFA, PUFA); VIA, vitamin A (retinol); VIE, vitamin E (*alpha*-tocopherol); XAN, xanthophylls
- ³ Model 1, simple linear regression; model 2, multiple linear regression adjusting for socio-demographic variables; model 3, multiple linear regression adjusting for socio-demographic and lifestyle variables; model 4, multiple linear regression adjusting for sociodemographic, lifestyle and lipid-related variables (total cholesterol and use of lipid-altering drugs); change in covariable was carried out while holding any other variables in the model constant
- ⁴ Biomarker data available for analysis: plasma concentrations of SFA, MUFA, PUFA and tFA (NHANES 2003–2004); serum concentrations of 25OHD (NHANES 2003–2006); and serum concentrations of VIA, VIE, CAR and XAN (NHANES 2005–2006)
- ⁵ Sample sizes for each biomarker by variable can be found in Supplemental Table 1 (model 1) and Supplemental Table 3 (models 2–4)
- ⁶ Non-Hispanic black (NHB); non-Hispanic white (NHW); Mexican American (MA)
- ⁷ Family Poverty Income Ratio (PIR); categorized as low: 0–1.85; medium: >1.85–3.5; or high: >3.5
- ⁸ High school (HS)
- ⁹ “Smoker” defined by serum cotinine concentration > 10 µg/L
- ¹⁰ Alcohol consumption: calculated as average daily number of “standard” drinks [(quantity×frequency)/365.25]; 1 drink ~15 g ethanol
- ¹¹ A 25% decrease in BMI (kg/m²) is comparable to a change from normal weight to overweight
- ¹² Physical activity: categorized based on metabolic equivalent task (MET) in MET-min/wk from leisure-time physical activity
- ¹³ “Supplement user” defined as one who took any dietary supplement during the 30 d preceding the household interview

* Change is significantly different from 0; $P < 0.05$