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Serum β-carotene concentrations are associated with self-reported fatty acid intake in United States adults from the National Health and Examination Surveys

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Abstract

Bioavailability of dietary β-carotene (BC) is dependent on dose, quantity, dispersion, and presence of fat in the diet. Fats are comprised of a variety of fatty acids, which may impact the bioavailability of carotenoids. However, there is a gap in research on whether specific fatty acid classes affect serum BC concentrations in population samples. The primary objective of this study was to assess the association between reported fat and fatty acid intake and serum BC concentrations utilizing data from the National Health and Nutrition Examination Surveys (NHANES) 2003–2006. Data from 3278 NHANES participants 20–85 years old were analyzed to estimate the relationships between serum BC concentrations and reported saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acid intakes. Multiple linear regression estimated ln(serum BC) based on reported fatty acid intakes adjusted for age, sex, race/ethnicity, and reported dietary BC intakes. Mean and standard error (SE) for serum BC concentrations were 14.31 ± 0.05 μg/dl. Means and SE for total fat, SFA, MUFA, and PUFA were 85.7 ± 1.3, 26.9 ± 0.4, 31.1 ± 0.5, and 17.8 ± 0.4 g, respectively. There was a significant trend for association between serum BC and reported total fat intakes ($r = -0.002$, $p < 0.0001$), but the association was not strong. Multiple linear regression showed positive associations between serum BC concentrations and higher reported dietary PUFA consumption. PUFA alpha-linolenic acid intakes are positively associated with serum BC concentrations, while MUFA palmitoleic acid and SFA stearic acid were inversely associated with serum BC. The inverse association between MUFA and SFA suggests there may be multiple post-digestion factors affecting serum carotenoid concentrations.

KEYWORDS
dietary fat, fatty acids, human nutrition, MUFA, PUFA

INTRODUCTION

The dietary carotenoid, β-carotene (BC), is a fat-soluble antioxidant found in fruits and vegetables. The 2020–2025 Dietary Guidelines for Americans recommend most adults consume 2 cups of fruits and 2.5 cups of

ABBREVIATIONS: aLNA, alpha-linolenic acid; AMDR, Acceptable Macronutrient Distribution Range; BC, β-carotene; DRI, dietary reference intake; FA, fatty acid; HS, high school; LA, linoleic acid; ln, natural log; MEC, mobile examination center; MUFA, monounsaturated fatty acids; NH, non-Hispanic; NHANES, National Health and Nutrition Examination Survey; PUFA, polyunsaturated fatty acids; SE, standard error; SFA, saturated fatty acids; US, United States; WWEIA, What We Eat in America.

vegetables daily if daily caloric intake is approximately 2000 calories, as research has tied consumption to a reduced risk for many chronic diseases (U.S. Department of Agriculture and U.S. Department of Health and Human Services, 2020). Serum BC concentrations are a useful marker for predicting fruit and vegetable intake (Souverein et al., 2015). Dietary fats are comprised of a variety of fatty acids, which may impact the bioavailability of carotenoids (Failla et al., 2014). Further understanding of the relationship between serum BC and dietary fat intakes regarding bioavailability in the body is vital to chronic disease prevention on a population level.

The bioavailability of carotenoids is complex and dependent on dose (Evans et al., 2013; Novotny et al., 2010; Tang et al., 2003), quantity and dispersion throughout the day (Goltz et al., 2013), and presence of fat in the diet (Goltz et al., 2012, 2013; Granado-Lorencio et al., 2007; Mashurabad et al., 2017; van Vliet et al., 1995). Brown et al. reported that carotenoid absorption was highest when consumed with fat, with a 40-fold increase in post-prandial BC when consuming a salad with 28 g of fat versus 0 g (Brown et al., 2004). Additionally, the intestinal absorption of carotenoids varies by the chemical structure of the carotenoid (Courraud et al., 2013), release of the carotenoid from the food matrix (Fleshman et al., 2012) and intestinal cleavage of BC to retinol (Fleshman et al., 2012; Goltz et al., 2013). BC still bound to its food matrix and not solubilized in a micelle limits absorption, ultimately affecting circulating concentrations of BC (Tyssandier et al., 2003). Research conducted by Failla et al. using Caco-2 cells to assess bioaccessibility of BC found that dietary oils promote partitioning of total BC in simulated digestion, showing significant differences between fatty acid types (Failla et al., 2014).

However, there is a gap in research on whether total fat intakes and specific fatty acid (FA) classes affect serum BC concentrations for optimal absorption in population samples. Determining the relationship between serum carotenoid concentrations, reported dietary intake of carotenoids, and both reported fat quantity and type of FA will provide a better understand of the bioavailability of carotenoids in foods. The primary objective of this study was to assess the association between serum BC concentrations and reported intake of total fat and specific FA classes in United States (US) adults, utilizing the National Health and Nutrition Examination Surveys (NHANES) data.

MATERIALS AND METHODS

Design overview

Cross-sectional evaluation of data from the demographic, anthropometric, laboratory, dietary, and questionnaire components of NHANES were analyzed to determine associations between serum BC, total reported fat intakes and reported intake of specific fatty acid classes. Multivariable linear regression was used to examine how reported fat intakes would affect serum BC concentrations among US adults.

Participants and dataset

The cross-sectional data collected by NHANES is publicly available to allow for research on the health and nutritional status of the non-institutionalized US population. Approximately 5000 people per year were selected from 15 locations across the US (Ahluwalia et al., 2016) with data released on a 2-year cycle, with the most current serum carotenoid collection done in 2003–2004 and 2005–2006. The data collection process conducted by NHANES is well documented in literature (Sondik et al., 2012). NHANES used trained personnel to conduct dietary interviews using the What We Eat in America (WWEIA) survey in partnership with the U.S. Department of Agriculture and the U.S. Department of Health and Human Services (Sondik et al., 2012), perform clinical examinations, and obtain laboratory measurements in Mobile Examination Centers (MEC) to compile the data collected (Beydoun et al., 2011). The reported dietary intakes for BC, total fat, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) were acquired from two 24-h dietary recalls administered by a trained interviewer (Center for Disease Control, 2015).

There were 20,470 individuals enrolled in the NHANES survey between 2003 and 2006. This analysis includes males and non-pregnant females aged 20–85 years who (a) had recorded demographic data on race/ethnicity, sex, age, smoking status, (b) had reliable day 1 dietary recalls for reported dietary, and (c) participated at the MEC to obtain a blood draw for laboratory analysis of serum BC concentrations. Exclusions were made for individuals who were <20 years of age ($n = 10{,}450$) and missing data for serum BC ($n = 5567$), reported dietary intake of total fat ($n = 1052$), SFA ($n = 1052$), MUFA ($n = 1052$), PUFA ($n = 1052$), dietary BC ($n = 1052$), and current smoking status ($n = 2956$). Due to the effects of smoking on serum carotenoid concentrations (Andersen et al., 2006), participants were also excluded if they indicated they were current every day smokers ($n = 1844$) or smoked some days ($n = 376$). There were 3278 participants included in the current analysis after all exclusions. The University of Minnesota Institutional Review Board determined the secondary analysis of this de-identified dataset to be exempt (study #6976).

Study variables

Sociodemographic factors assessed included sex, race/ethnicity (self-identified as non-Hispanic [NH]

Whites, NH Blacks, Mexican Americans, other Hispanic, and other ethnicities), age, and education level (less than high school diploma, high school diploma or equivalent, and any post-secondary education). Trained individuals used protocols developed by NHANES to obtain anthropometric, dietary, and laboratory data at the MEC (Ahluwalia et al., 2016; Ajani et al., 2004; Beydoun et al., 2011). The serum samples from non-fasted participants were collected using standard phlebotomy procedures to determine serum BC concentrations. Serum samples of 0.3–1.0 ml were stored in properly sealed vials and frozen at −70°C until analysis (Laboratory Procedure Manual: Fat Souble Micronutrients, 2008). Serum *trans*-BC and *cis*-BC concentrations were determined via high performance liquid chromatography with multiwavelength photodiode-array absorbance detection (Laboratory Procedure Manual: Fat Souble Micronutrients, 2008). Serum BC concentrations were evaluated as the sum of *cis*- and *trans*-BC in this assessment (LBXBCC in μg/dl). The day 1 dietary data for BC, fat, and fatty acids was recorded via 24-h recall collected in the MEC (Center for Disease Control, 2007). Day 2 dietary recalls were collected via telephone in the post-MEC interview (Ahluwalia et al., 2016). BC was the carotenoid of focus as it has both highest quantities in serum (Prince & Frisoli, 1993), with normal serum BC concentrations ranging from 2.2 to 122.7 mg/dl (Institute of Medicine, 2000), and has the highest consumption in the diet in comparison to other carotenoids (Tourniaire et al., 2009). There is no identified dietary reference intake (DRI) for BC, therefore, reported dietary BC intake was noted on a continuous scale (Institute of Medicine, 2000). Reported dietary fat, SFA, MUFA, and PUFA was assessed on a continuous scale.

Statistical methods

SURVEY procedures in SAS statistical software (version 9.4, Cary, NC, USA) were used for all analyses to account for the complex stratified, clustered design. Survey weights were created according to the guidelines for analysis published by the Center for Disease Control (Ahluwalia et al., 2016; Rothwell et al., 2013) to account for oversampling, survey non-response, and post-stratification adjustment to match total population counts from the Census Bureau.

Reported fat intakes (total, SFA, MUFA, PUFA) were normally distributed. The data were not normally distributed for serum BC and reported dietary BC, therefore were natural log (ln) transformed. Using the SURVEYMEANS procedure, the mean and standard errors (SEs) were used for continuous variables and percentages for categorical variables. PROC RANK was used to establish quartiles of serum BC concentrations and reported dietary BC; variables were compared across quartiles using ANOVA and Rao-Scott χ^2 analysis. Multivariable linear regression was used via SURVEYREG to estimate adjusted mean ln(serum BC) concentrations according to total fat or fatty acid intakes adjusted for age, sex, and race/ethnicity. Outcomes including variables with potential to confound such as reported intakes of other carotenoids, reported intakes of other fat-soluble vitamins, reported total caloric intake, and reported alcohol consumption were reviewed. Pearson correlations were used to estimate the association between serum BC and specific fatty acids, using partial correlations to adjust for age, sex, and race/ethnicity. Statistically significant results were reported as $p < 0.05$. The University of Minnesota Institutional Review Board determined the secondary analysis of this de-identified dataset to be exempt.

RESULTS

Of the 3278 participants in this analysis, there were 1493 men (45.55%) and 1785 women (54.55%) with a mean age of 48.2 ± 0.5 years. Other participant demographics are shown in Table 1. Mean and SE was

TABLE 1 Weighted demographic characteristics for the 3278 US adults 20+ from NHANES 2003–2006

Variable	Number of participants	Percent sample
Sex		
Men	1493	45.6
Women	1785	54.4
Ethnicity		
Mexican American	684	20.9
Other Hispanic	101	3.1
Non-Hispanic White	1768	53.9
Non-Hispanic Black	595	18.1
Other-multiracial	130	4.0
Age in years		
20–30	539	16.44
31–50	960	29.29
51–70	930	28.37
70+	849	25.90
Education		
Less than HS diploma	905	27.61
HS diploma or equivalent	759	23.15
More than HS	1608	49.05
Unknown/refused	6	0.19
Income to poverty ratio		
<1	490	15.79
1–5	2035	65.56
>5	579	18.65

14.31 ± 0.05 μg/dl (range: 0.4–422.6 μg/dl) for serum BC (Table 2) and reported dietary BC intake was 827.0 ± 1.1 μg. There was an 8-fold difference between the lowest quartile of serum BC concentrations and the highest quartile, with a significant difference in proportions across the quartiles for sex and age ($p < 0.0001$). For example, women have almost double the number of participants in the highest quartile of intakes compared to men. Additionally, the 70+ year age group had 3.6-times the participants in the highest quartile compared to the 20–30 year age group. Other demographic characteristics by quartiles of serum BC concentrations are also shown in Table 2.

Mean and SE for reported total fat intake was 85.7 ± 1.3 g. The first and second quartiles of serum BC concentrations showed significantly higher mean reported total fat consumption (91.2 ± 1.7 and 87.6 ± 1.5 g, respectively) in comparison to individuals in the highest quartile of serum BC concentrations 77.0 ± 1.5 g ($p < 0.05$). Quartile 3 had a mean intake of 80.0 ± 1.5 g, which did not show a significant difference in mean intakes from individuals in quartile 4. A multivariable linear regression model assessed the relationship of serum BC and total fat adjusted for age, sex, race/ethnicity, and reported dietary BC was significant (as shown Table 3). For each 10 g increase in reported total fat intake, serum BC concentrations decreased by 0.02 μg/ml ($p < 0.0001$), which is a nominal decrease for the increase in reported grams of fat consumed. Moreover, the relationship between serum BC concentrations and total fat remained significant with all adjustments made to the model, even considering reported dietary BC, an indicator of dose of BC.

For reported SFA intake, the mean and SE was 26.9 ± 0.4 g. Additionally, intakes for mean SFA were significantly higher in quartile 1 (30.8 ± 0.6 g) and 2 (28.1 ± 0.5 g) versus quartile 4 (24.4 ± 0.5 g) of serum BC concentrations ($p < 0.0001$). The relationship between serum BC concentrations and reported SFA intake was assessed using a multivariable linear regression model, adjusting for age, sex, race/ethnicity, and reported dietary BC. Significant negative associations were found between the aforementioned variables (as shown in Table 3). For each 1 g increase in reported SFA intake, which is 3.72% of the mean intake of SFA, serum BC concentrations decreased by 0.006 μg/ml ($p < 0.0001$). Additionally, the associations between serum BC concentrations and specific fatty

TABLE 2 Weighted demographic characteristics by BC concentration in quartiles from NHANES 2003–2006 ($n = 3278$)

Variables	All ($n = 3278$)	Q1 ($n = 819$)/ (% quartile)	Q2 ($n = 820$)/ (% quartile)	Q3 ($n = 820$)/ (% quartile)	Q4 ($n = 819$)/ (% quartile)	p-value[b]
Serum BC (μg/dl)[a]	14.31 ± 0.05	5.26 ± 0.01	11.03 ± 0.01	19.01 ± 0.01	42.10 ± 0.02	<0.0001
Sex						<0.0001
Men	1493	453 (14.4)	414 (13.6)	347 (9.9)	279 (8.2)	
Women	1785	366 (11.2)	406 (13.0)	473 (14.0)	540 (15.8)	
Race/ethnicity						0.44
Mexican American	684	186 (2.3)	186 (2.0)	185 (2.1)	127 (1.4)	
Other Hispanic	101	28 (1.1)	19 (0.7)	26 (0.9)	28 (1.0)	
Non-Hispanic White	1768	394 (17.6)	456 (20.3)	435 (17.3)	483 (17.9)	
Non-Hispanic Black	595	182 (3.3)	134 (2.5)	140 (2.4)	139 (2.3)	
Other-multiracial	130	29 (1.3)	25 (1.1)	34 (1.2)	42 (1.4)	
Age in years						<0.0001
20–30	539	207 (6.2)	129 (3.9)	120 (3.7)	83 (2.3)	
31–50	960	257 (10.6)	273 (11.7)	237 (8.8)	193 (8.1)	
51–70	930	237 (7.1)	229 (7.7)	220 (7.1)	244 (7.8)	
70+	849	118 (1.7)	189 (3.3)	243 (4.3)	299 (5.8)	
Education						0.01
Less than HS diploma	905	242 (5.2)	233 (4.8)	241 (4.6)	189 (3.6)	
HS diploma or equivalent	759	217 (8.3)	196 (8.1)	187 (6.2)	159 (5.3)	
More than HS	1608	213 (12.1)	253 (14.1)	256 (13.1)	313 (14.5)	
Income to poverty ratio						<0.0001
<1	490	163 (3.5)	134 (2.6)	120 (2.2)	73 (1.6)	
1–5	2035	513 (17.8)	521 (17.9)	500 (14.9)	501 (14.3)	
>5	579	91 (4.1)	129 (6.2)	152 (6.4)	207 (8.4)	

[a]Mean and SE.
[b]Variables were compared across quartiles of serum BC using ANOVA and Rao-Scott χ^2 analysis.

TABLE 3 Linear regression modeling for the association between ln(serum BC concentrations) and reported total fat, and saturated, monounsaturated, and polyunsaturated fatty acids; regression coefficients and SE for ln-transformed serum BC from NHANES 2003–2006 ($n = 3278$)

	Total fat		SFA		MUFA		PUFA	
Models[a]	β	95% CI	β	95% CI	β	95% CI	β	95% CI
1	-0.002 ± 0.0003	$(-0.003; -0.002)$[b]	-0.008 ± 0.001	$(-0.01; -0.006)$[b]	-0.006 ± 0.001	$(-0.008; -0.004)$[b]	-0.001 ± 0.002	(2.57; 2.80)
2	-0.001 ± 0.0003	$(-0.002; -0.001)$[c]	-0.006 ± 0.001	$(-0.008; -0.003)$[b]	-0.004 ± 0.001	$(-0.006; -0.002)$[b]	0.001 ± 0.001	$(-0.002; 0.004)$
3	-0.001 ± 0.0003	$(-0.001; -0.0001)$[d]	-0.004 ± 0.001	$(-0.006; -0.002)$[c]	-0.002 ± 0.001	$(-0.004; -0.001)$[e]	0.004 ± 0.001	$(0.0003; 0.007)$[d]
4	-0.001 ± 0.0003	$(-0.001; -0.0001)$[d]	-0.004 ± 0.001	$(-0.006; -0.002)$[c]	-0.002 ± 0.001	$(-0.004; -0.001)$[e]	0.004 ± 0.001	$(0.0003; 0.007)$[d]
5	-0.002 ± 0.0002	$(-0.002; -0.001)$[b]	-0.006 ± 0.001	$(-0.008; -0.004)$[b]	-0.005 ± 0.001	$(-0.006; -0.003)$[b]	-0.002 ± 0.002	$(0.006; -0.002)$

[a]1: crude; 2: age adjusted; 3: age and sex adjusted; 4: age, sex, and race/ethnicity adjusted; 5: age, sex, race/ethnicity, and reported dietary BC intake adjusted.
[b]p for trend is <0.0001.
[c]p for trend is <0.001.
[d]p for trend is ≤0.05.
[e]p for trend is ≤0.01.

TABLE 4 Pearson partial correlations between serum BC concentrations and reported intakes of individual saturated, monounsaturated, and polyunsaturated fatty acids adjusted for age, sex, and race/ethnicity from NHANES 2003–2006 ($n = 3278$)

SFA	r	MUFA	r	PUFA	r
Butyric acid (4:0)	−0.01	Palmitoleic acid (16:1)	−0.09[a]	Linoleic acid (18:2)	0.04[b]
Caproic acid (6:0)	−0.01	Oleic acid (18:1)	−0.04[b]	Alpha-linolenic acid (18:3)	0.09[a]
Caprylic acid (8:0)	−0.02	11-Eicosenoic acid (20:1)	−0.03	Stearidonic acid (18:4)	0.02
Capric acid (10:0)	−0.01	Erucic acid (22:1)	−0.02	Arachidonic acid (20:4)	−0.02
Lauric acid (12:0)	−0.03			Eicosapentaenoic acid (20:5)	0.05[c]
Myristic acid (14:0)	−0.05[c]			Docosapentaenoic acid (22:5)	0.05[c]
Palmitic acid (16:0)	−0.07[a]			Docosahexaenoic acid (22:6)	0.05[c]
Stearic acid (18:0)	−0.09[a]				

Note: Unmarked values are not significant.
[a]p for trend is <0.0001.
[b]p for trend is ≤0.05.
[c]p for trend is ≤0.01.

acids within the fatty acid classes were assessed using Pearson correlation. The partial correlation, when adjusted for age, sex, and race/ethnicity, between serum BC concentrations and reported intakes of specific SFA is reported in Table 4. Mean reported dietary intake was highest for long chain SFA, palmitic acid (14.62 ± 0.2 g), followed by long chain SFA, stearic acid (6.96 ± 0.1 g), which were reflective of typical intakes (Iggman & Risérus, 2011; Raatz et al., 2017).

Mean and SE for reported MUFA intake was 31.1 ± 0.5 g, showing similar trends to SFA, as mean MUFA intakes were significantly higher in quartile 1 (34.8 ± 0.7 g) and 2 (33.4 ± 0.6 g) versus quartile 4 (28.6 ± 0.6 g) of serum BC concentrations($p < 0.001$). In modeling the multivariable linear regression between serum BC concentrations and reported MUFA intakes, similar results to reported total fat and SFA intakes were obtained. For each 1 g increase in reported MUFA intake, serum BC concentrations decreased by 0.005 μg/ml ($p < 0.0001$). A 1 g increase in MUFA is 3.21% of the mean MUFA intake. Moreover, Table 4 shows the partial correlations between specific MUFA and serum BC concentrations adjusted for age, sex, and race/ethnicity. The non-significant associations between serum BC concentrations and very long-chain MUFA 11-eicosenoic acid (20:1) and erucic acid (22:1) were likely due to minimal reported mean quantities of these fatty acids. The mean reported dietary intake for oleic acid was 129 times higher than 11-eicosenoic acid and 740 times higher than erucic acid (29.02 ± 0.5 g versus 0.24 ± 0.01 and 0.04 ± 0.002 g). Mean reported intakes of oleic acid were also over 22 times higher than palmitoleic acid (1.31 ± 0.03 g), however, palmitoleic acid showed a stronger association to serum BC concentrations.

For reported PUFA intakes the mean and SE were 17.8 ± 0.4 g, with no significant differences of PUFA intakes between the quartiles of serum BC concentrations. Results were inconsistent, although PUFA was the only fatty acid class to show positive associations with serum BC concentrations after adjusting for demographic factors (Table 3). However, the multivariable linear regression model assessing the relationship of serum BC concentrations and reported PUFA intakes adjusted for age, sex, race/ethnicity, and reported dietary BC intake was not significant. The association between specific PUFA and serum BC concentrations determined by Pearson partial correlation are presented in Table 4. Means and SE for omega-3 fatty acid alpha-linolenic acid (aLNA) (18:3) and Omega-6 fatty acid linoleic acid (LA) (18:2) were 1.55 ± 0.04 and 15.58 ± 0.40 g, respectively.

DISCUSSION

Multiple factors influence the bioavailability of BC including efficient transfer from food to mixed micelles, incorporation to chylomicrons for transport to the lymph and serum, and distribution to tissues (Failla et al., 2014). The findings of this study indicate that there are significant associations between serum BC concentrations and reported dietary fat intakes. However, this study suggests that the reported quantity of total fat consumed is not a factor dictating serum BC concentrations in population samples, as a significant, inverse relationship is present when adjusted for participant demographics and is unaffected by confounding factors. This trend is likely due to adequate absorption even at lowest levels of reported fat consumption (Mashurabad et al., 2017). Current research supports the relationship between consuming BC containing foods with a fat; however, there is not research showing the effects of total fat reported on a usual basis on serum BC concentrations or BC bioavailability when BC and the source of fat may not be consumed at the same time.

Research supports the addition of fat to a carotenoid-containing meal improves intestinal absorption of BC (Brown et al., 2004; Goltz et al., 2012, 2013; Granado-Lorencio et al., 2007; Mashurabad et al., 2017; van Vliet et al., 1995). However, BC micellarization is enhanced if as little as 1%–2.5% dietary fat is present, though micellarization was found to be dose dependent (Mashurabad et al., 2017). Goltz et al. determined that adding 20 g of lipids to a meal containing BC significantly affected the absorption rates of BC, independent of the type of lipid consumed ($p < 0.01$) (Goltz et al., 2012). White et al. found similar results when adding 0, 2, 4, 8, 16 and 32 g of soybean oil to a salad containing 11.54 ± 0.5 mg BC. There was a positive linear relationship between BC and total grams of soybean oil between 0 and 8 g, with highest BC absorption with 32 g of oil (White et al., 2017). This indicates that, on its own, BC has poor bioavailability and the presence of fat is necessary for absorption.

Other studies assessing co-consumption of fat-containing foods and BC showed significant increases in BC absorption. A study by Kim et al. assessed co-consumption of eggs and carotenoids within a meal and found that a meal of three eggs (150 g) versus 1.5 eggs (75 g) significantly increased BC absorption 10 h post consumption ($p < 0.001$) (Kim et al., 2015). Another study assessed the effectiveness of avocado or avocado oil and reported significant differences in areas under the curve for BC in the plasma triacylglycerol-rich lipoprotein fraction 9.5 h after consumption of 300 g salsa with 150 g avocado ($p < 0.003$) and 200 g salad with 75 g avocado ($p < 0.01$), 150 g avocado ($p < 0.01$), or 24 g avocado oil ($p < 0.01$) (Unlu et al., 2005).

Our data indicates that the SFAs with the highest mean concentrations in the diet, including stearic acid and palmitic acid, show the strongest negative correlations with serum BC concentrations. However, a long-chain MUFA, palmitoleic acid, showed higher reported mean intake compared to oleic acid, but oleic acid has a stronger negative association to serum BC concentrations. Similar patterns were found with PUFA, LA, which had reported dietary intakes 10 times higher than those of aLNA, but aLNA had a stronger positive association to BC concentrations (Mashurabad et al., 2017). This suggests that even though aLNA is consumed in small quantities, it may have stronger biologic effects with regards to BC absorption. However, we do not know if this is an effect of micellarization prior to intestinal absorption, which can be affected by the food matrix and/or physiochemical aspects of BC or is a result of intake of foods that contain both aLNA and BC, such as leafy greens (Mashurabad et al., 2017; Unlu et al., 2005).

The relationship between serum BC concentrations and specific fatty acids has been assessed in other studies. Mashurabad et al. studied the effects of different types of dietary oils on BC uptake in Caco-2 intestinal cells, using the aqueous micellar fraction obtained after digestion of fruits and vegetables. When comparing olive oil (highest proportion of MUFA oleic acid), soybean oil (highest proportion of PUFA LA + aLNA), sunflower oil (highest proportion of PUFA LA), peanut oil (highest proportion of MUFA oleic acid + SFA palmitic acid), and coconut oil (highest proportion of SFA lauric acid), BC micellarization was significantly higher in the MUFA and PUFA rich oils than the SFA rich oils ($p < 0.05$) (Mashurabad et al., 2017). BC uptake was dependent on the type of fat, suggesting the food matrix, BC polarity, and type of dietary fat determine BC bioavailability (Mashurabad et al., 2017). Similar results were obtained by Failla et al. finding BC micellarization and cellular uptake was significantly

different between fatty acid types (soybean oil > olive > canola > butter) ($p < 0.05$) (Failla et al., 2014).

The strongest association between specific fatty acids and serum BC concentrations in this study was aLNA, showing a moderate, positive association. Interestingly, the strongest negative association was with long-chain SFA stearic acid and palmitic acid, which are most prevalent in a Westernized diet, high in red meat. These results are parallel with the recommendations to increase carotenoids and reduce SFA in the diet, especially stearic acid and palmitic acid for reduction of cardiometabolic diseases (Iggman & Risérus, 2011). Additionally, low serum BC status is associated with increased cardiometabolic disease risk (Beydoun et al., 2012; Liu et al., 2014). An inverse relationship was observed between serum BC and hypertension ($p < 0.01$) (Hozawa et al., 2009), dyslipidemia ($p < 0.029$) (Guerendiain et al., 2015), waist circumference ($p < 0.001$) and Metabolic Syndrome ($p < 0.001$) (Kabat et al., 2015). Moreover, research on mortality in US adults by Shardell et al. concluded that the mortality rate ratio for the lowest quartiles of carotenoid intakes was 1.83 times higher than individuals with the highest carotenoid intakes (Shardell et al., 2011).

A strength of this study was the use of a dataset that is representative of the US population, allowing the findings to be generalized to the US population. The NHANES dataset is large and organized by a stratified, multistage, probability sampling design to properly reflect the US demographics (Ahluwalia et al., 2016). In combining data from two 2-year cycles, statistically significant estimates were obtained for the subgroups of interest. Trained professionals collected the data, allowing use of objective data points, such as serum biomarkers and anthropometric data, versus using self-reported data.

However, a primary limitation of the study is that dietary intakes of foods containing fat and BC were self-reported through the 24-h recalls, which may increase both social desirability and recall biases in comparison to intakes being monitored or directly measured. Another limitation is that the current analysis was cross-sectional, limiting the temporality of the outcome. Moreover, several demographic and lifestyle factors were accounted for, however, the potential for effects of other confounding factors that may not have been captured is present. Last, oils and fats contain percentages of each type of fatty acid, with a higher proportion of one fatty acid over another. Therefore, it may be difficult to discern the effects of a specific fatty acid type on BC concentrations unless the fatty acid was isolated from a fat source. Future research to address some of these limitations and explore the relationship to diet quality would benefit the overall understanding of this relationship. For example, we would stratify the sample to better understand the relationship between serum BC and fatty acids for individuals with hypertension, dyslipidemia or metabolic syndrome compared to those without the aforementioned comorbidities. Additionally, we could adjust for a Healthy Eating Index score or physical activity to better understand the relationship to overall dietary and lifestyle habits.

This study is the first to explore the relationship between fat-soluble serum BC concentrations, a marker of fruit and vegetable intakes, and fatty acid intakes in a nationally-representative population sample. This study suggests that reported PUFA intake, specifically aLNA is associated with increased BC in circulation, whereas, reported SFA stearic acid and MUFA palmitoleic acid are associated with decreased BC in circulation. Moreover, the inverse association present between serum BC and other specific fatty acid classes suggests there may be multiple post-digestion factors affecting serum BC concentrations. Total fat intake was not strongly associated with serum BC concentrations likely due to adequate absorption even at lowest levels of reported fat consumption.

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ETHICS STATEMENT

The procedures involving human subjects were approved by the National Center for Health Statistics Institutional Review Board for the Ethics Review Board as NHANES adheres to guidelines set forth by the Declaration of Helsinki.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Ambria C. Crusan and Susan K. Raatz conceived and designed the study. Ambria C. Crusan carried out the research, analyzed the data, and wrote the first draft of the manuscript. Ambria C. Crusan, Susan K. Raatz, Marla Reicks, and Ryan T. Demmer contributed to and approved the final draft of the manuscript.

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