THE ASSOCIATION BETWEEN REPORTED DIETARY β-CAROTENE INTAKES, SERUM β-CAROTENE, ANTHROPOMETRIC FACTORS, AND DIETARY FAT IN UNITED STATES ADULTS

A DISSERTATION

SUBMITTED TO THE FACULTY OF THE

UNIVERSITY OF MINNESOTA

BY

AMBRIA CRUSAN

IN PARTIAL FULLFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

SUSAN RAATZ, PhD, MPH, RD, Advisor

MARLA REICKS, PhD, RD, Advisor

RYAN DEMMER, PhD, MPH

DANIEL GALLAHER, PhD, Chair

SEPTEMBER 2020

ACKNOWLEDGEMENTS

I have a lengthy list of individuals and institutions to thank. First, I would like to thank my advisor, Dr. Susan Raatz, for taking me on as her student and challenging me in many ways. I quickly grew to appreciate her editorial skills and straightforwardness, and truly appreciate the role model she is as a scientist in the field of nutrition and all of the support she has provided. Dr. Marla Reicks has been extremely supportive, provided NHANES expertise, and has provided many resources for me along the way. I would not be where I am today without her thoughtful analysis and support in my path. To my committee members collectively, I appreciate the time, willingness, and suggestions towards my research project and your formative feedback, which has truly made me a better scientist. I have endless gratitude for Dr. Ryan Demmer, who has graciously spent hours grueling over SAS as I navigated the foreign waters of this statistical beast. Your mentoring in the NHANES and epidemiology piece of this project was truly unparalleled. I thank Dr. Dan Gallaher for being the carotenoid expert and the depth of knowledge he has shared in this content area. Additionally, I would like to thank Dr. David Jacobs Jr. who supported my statistics journey and jump-started this entire project, for his attentiveness and willingness to help me learn. I am grateful for all the investments each of you have made in my growth as a scientist.

Next, to my support system outside of academia. Words cannot express how grateful I am for Mike and Thira, who have been my primary support as I spent time focused on school and endured the journey to learn and grow. In addition, a thank you to all of my family

and friends who have encouraged my passion and guided me to this point; without all of you I wouldn't have made it where I am today. My last words of gratitude go out to all of the institutions that have made this possible. Starting with University of Minnesota, Duluth, thank you for funding my coursework and supporting me though my teaching experience. I thank each of my colleagues at UMD for convincing me this was a good idea and reminding me to keep moving forward. To the University of Minnesota Food Science and Nutrition Department for being flexible with a student who also had a full-time job and for funding travel opportunities to present my work. Lastly, thank you to my current institution, St. Catherine University, which has given me the opportunity to further my education while working and has funded opportunities to present this work. My colleagues at St. Kate's, especially Holly and Megan, have challenged me, reviewed my work, and been some of my biggest cheerleaders. Thank you. Without this financial and emotional support, none of this would have been possible.

ABSTRACT

Obesity prevalence continues to increase in the United States (US), therefore, understanding preventative measures is increasingly important for population health. The US has one the world's highest rates of overweight and obesity with at least 70% of adults categorized as overweight or obese, with an increasing prevalence of morbid obesity. Obesity is of concern because increasing rates are positively correlated with multiple comorbidities. The 2015-2020 Dietary Guidelines for Americans recommend adults consume approximately 2 cups of fruits and 2.5 cups of vegetables daily, as research has tied consumption to a reduced risk for many chronic diseases. Presumably, these recommendations are made because low serum carotenoid status, a marker of fruit and vegetable intake, is associated with increased cardiometabolic disease risk. Investigating the associations between the carotenoid β-carotene (BC) and obesity are pivotal in understanding obesity as a diet-related condition. This work is the first to assess multiple factors that might influence the association between dietary BC and serum BC concentrations utilizing population-based data collected in the US. Secondary data analysis of the 2003-2006 NHANES dataset, which utilized cross-sectional survey methods to obtain a unique collection of nationally-representative, health- and nutrition-related data on non-institutionalized civilians in the US, was conducted. Weighted variables were created in SAS statistical software to accommodate the complex survey design. There is a normal distribution across sex, ethnicity, age, and body mass index (BMI), however, we natural log transformed serum BC concentrations, reported dietary BC, high-sensitivity C-reactive protein

(hsCRP), and reported dietary lycopene due to skewing. Pearson correlation and partial correlation coefficients were used to assess variable correlation. Multivariable linear regression estimated relationships between serum BC concentrations and inflammation, reported dietary BC intake, BMI, total reported dietary fat intake, and total reported dietary fatty acid (FA) intakes. Notable associations were present between serum BC concentrations and BMI, hsCRP, reported dietary BC intakes, android body fat percentage, gynoid body fat percentage, saturated FAs, monounsaturated FAs, and polyunsaturated FAs. The findings of this project suggest a protective effect of increased serum BC concentrations against low-grade, systemic inflammation often associated with adipose tissue dysfunction present in obese individuals. The association present between serum BC and anthropometric factors related to higher adiposity, suggests individuals with an increased BMI and/or body fat percentage may have a greater risk of lower serum BC concentrations despite dietary BC intake. Additionally, dietary FA, polyunsaturated FA alpha-linolenic acid, is associated with increased 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.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AC = α-carotene

AF% = android body fat percentage

ALA = alpha-linolenic acid

AMDR = Acceptable Macronutrient Distribution Range

APP = acute phase protein

APR = acute phase response

BC = β-carotene

BCMO1 = 15'15-monoxygenase type 1

BF% = body fat percentage

BMI = body mass index

CMD = cardiometabolic diseases

CRP = C-reactive protein

DRI = Dietary Reference Intake

DXA = dual-energy X-ray absorptiometry

FA = fatty acids

FFQ = food frequency questionnaires

GDP = gross domestic product

GF% = gynoid body fat percentage

GLUT-4 = glucose-regulated glucose transporter 4

HDL = high-density lipoprotein

HEI = Healthy Eating Index

HS = high school

hsCRP = high-sensitivity C-reactive protein

HTN = hypertension

IL-1 = interleukin- 1β

IL-6 = interleukin- 6

LA = linoleic acid

ln = natural log

LPL = lipoprotein lipase

LYC = lycopene

MEC = mobile examination center

MetS = metabolic syndrome

MUFA = monounsaturated fatty acids

NH = non-Hispanic

NHANES = National Health and Nutrition Examination Survey

PUFA = polyunsaturated fatty acids

ROS = reactive oxygen species

SE = standard error

SES = socioeconomic status

SFA = saturated fatty acids

T2DM = type 2 diabetes mellitus

TG = triglycerides

TNF-α = tumor necrosis factor α

US = United States

WAT = white adipose tissue

WC = waist circumference

WHO = World Health Organization

WWEIA = What We Eat in America

INTRODUCTION

The prevalence of obesity in the United States is increasing steadily, therefore, understanding preventative measures is increasingly important for population health. Obesity is of concern because increasing rates are positively correlated with multiple comorbidities. The 2015-2020 Dietary Guidelines for Americans recommend adults consume approximately 2 cups of fruits and 2.5 cups of vegetables daily, as research has tied consumption to a reduced risk for many chronic diseases. Serum carotenoids are used as a marker of fruit and vegetable consumption. Low serum carotenoid status is associated with increased cardiometabolic disease risk. Investigating the associations between the carotenoid β-carotene (BC) and obesity are pivotal in understanding obesity as a diet-related condition.

The aim of this project was to a) describe the relationship between carotenoids and adipose tissue development; b) examine the associations between adiposity, inflammatory markers, dietary carotenoid intakes, serum carotenoid concentrations, and other factors in the diet influencing serum carotenoid concentrations. Background information regarding obesity, carotenoids, and inflammation is discussed in the review of literature (Chapter 1). Chapters 2 to 4 present the objectives and research conducted in this project.

CHAPTER 1

Literature Review

I. Introduction to Obesity and Its Implications

a. Increased prevalence of overweight and obesity

The prevalence of overweight adults worldwide in 2016 was 39% and obesity was 13%, tripling since 1975[1]. Moreover, the American Medical Association officially recognized obesity as a disease for improved health interventions and insurance coverage. This was done in efforts to reduce the adverse medical effects of obesity[2]. Currently, there are 25 countries throughout the world with greater than two-thirds of their population considered overweight or obese[3,4]. The United States (US) has one the world's highest rates of overweight and obesity with at least 70% of adults categorized as overweight or obese in 2018. More specifically, 27.6% of adults have a body mass index (BMI) between 25 kg/m^2 and 30 kg/m^2 and 42.4% of adults have a BMI over 30 kg/m^2, with an increasing prevalence of morbid obesity[4–6]. Understanding the health consequences related to obesity becomes more important as this worldwide epidemic increased mortality rates and chronic disease prevalence[7–10].

Obesity does not target or solely present itself in a specific population; however, there are differences in prevalence based on age, sex, race/ethnicity, education level

and socioeconomic status (SES). In the US, the prevalence of obesity was highest among middle-aged adults (40-59 years) at 44.8%, followed by adults over 60 years of age at 42.8%, and adults 20-39 at 40.0%[5,6]. Globally, the proportion of overweight by sex has risen equally since 1980, with an 8.1% increase in rates for men and 8.2% for women. Different patterns of overweight and obesity were found in different countries, with developing countries displaying higher rates of overweight and obesity in women, and developed countries with higher rates in men. However, the rates of overweight and obesity increased more rapidly for women in developed countries[3]. The prevalence of a woman being obese in the US, a developed country, is 4% greater than that of men[5], and women also have a significantly higher prevalence of morbid obesity than men[6].

Obesity was found to be most prevalent in non-Hispanic black adults (49.6%), followed by Hispanic (44.8%), non-Hispanic White (42.2%), and lowest among non-Hispanic Asian adults (17.4%) in the US[5]. Independent of race, individuals that have obtained a college degree had lower prevalence of obesity those who had less education[5]. However, when assessing SES, there was a difference in obesity prevalence by race. Hispanic and non-Hispanic white men and women in the high-income group had lower rates of obesity compared to those in the middle-income group. Black men in the high-income group also had lower rates of obesity compared to the middle- and low-income groups. Black women, however, showed no significant difference in obesity prevalence by income group[5]. When

implementing dietary recommendations for different populations, communities, families, or individuals, there is growing importance in understanding the patterns present in overweight and obesity trends.

Within family units, dual-burden paradoxes exist where obesity is present in at least one family member, but not others, which creates a challenge to understand how to feed a family to support healthy body weight. Doak *et al* assessed dual burden households in seven countries of differing socioeconomic status, finding that countries with a median gross domestic product (GDP) had the highest prevalence of dual burden households (e.g. Krgyzstan- 15.5%). The lowest prevalence of dual burden households was present in the lowest GDP country assessed, Vietnam (3.7%), and was the highest in the US (5.4%) for the high GDP countries assessed[11]. Socioeconomic status not only affected the food choices individuals made but was also linked to higher prevalence of disease. When using the National Health Interview Survey to assess sociodemographic and behavioral determinants of obesity and BMI (n=23,434), Shaikh *et al* found that an income of >500% of the poverty threshold had a significantly lower prevalence of obesity than those at all other income levels ($p<0.001$). Those with the highest prevalence of obesity were between 100-199% of the poverty threshold[12].

To aid in understanding nutrient needs in overweight and obesity, the World Health Organization (WHO) and the Food and Agriculture Organization of the United

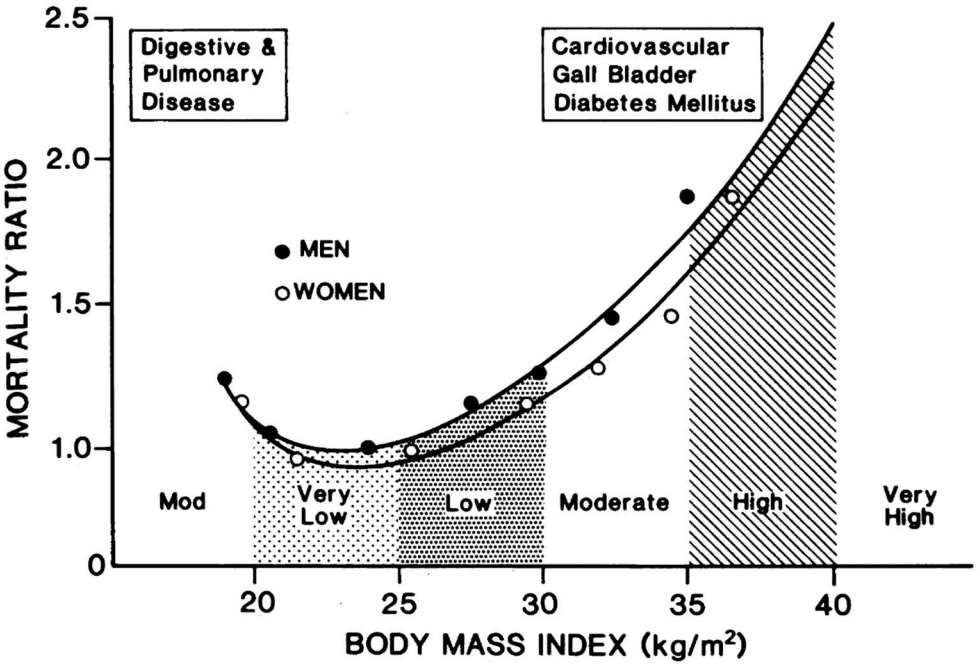
Nations developed guidelines for human nutrient requirements for countries to adopt and build their own nutritional guidelines[13]. These guidelines were set to prevent obesity, but do not address some of the individual complications that may be present regarding nutrition for those who were already overweight or obese or have comorbid health conditions.

A relationship between obesity and risk for all-cause mortality has been well established (Figure 1.1)[7,10,14–17]. Additionally, overweight or obesity in any population is a concern due to the positive correlation between obesity and comorbidities such as insulin resistance, Type 2 diabetes mellitus (T2DM), dyslipidemia, hypertension (HTN), and visceral adiposity. When an individual displays three or more of these conditions, it is defined as metabolic syndrome (MetS), a disease that places one at higher risk for cardiometabolic diseases (CMD) and increased all-cause mortality[17]. MetS has also been associated with an increase in low-grade, systematic inflammation[10,18]. Researching associations between diet and obesity has been pivotal in understanding this diet-related condition.

Obesity is considered a complex health issue associated with increased mortality risk, poorer mental health, and decreased quality of life[19]. Not only does obesity place physical and emotional burden on individuals and families, but it has also generated related medical expenses, placing a financial burden on the family and economy, both directly and indirectly. Using an instrumental variable approach,

Cawley *et al* found a $67.2 billion dollar underestimation in the nation's medical expenses from obesity-related complications in 2008[20], greater than the estimated $147 billion dollars spent[19]. Interestingly, they also found a similar pattern between obesity and mortality risk, with the most significant medical expenses associated with Class II and III obesity. For individuals with a BMI >31 kg/m^2, significant reductions in medical spending related to obesity were found with as little as a 5% reduction in BMI[21].

Figure 1.1. All-cause mortality ratios for men and women with varying BMI. (Figure from Gray, D. (1989). Diagnosis and Prevalence of Obesity. *Obesity*, 73(1), 1-13[17])



b. Defining overweight and obesity

A measure often used to categorize overweight and obesity based on tissue mass is the BMI or Quetelet index. BMI has served as a crude index allowing a quick assessment of mortality risk and is determined by the equation: $\frac{weight\ (kg)}{heigh\ \ (m)^2}$, which factors in height (m) and weight (kg) of an individual over 18 years of age, and not other markers of health such as body fat percentage, physical activity level, or lean body mass[22]. Based on the interpretation of BMI, categorization as underweight, normal, overweight, or a level of obesity occurs (Table 1.1)[23]. However, some issues have been presented with using BMI for the health assessment of an individual's weight. For example, Deurenberg *et al* found significant differences in predicted body fat percentage based on BMI versus measured body fat percentage via underwater weighing ($p<0.05$). However, the significant difference was <0.5%, which was nominal in regards to body fat percentage[22]. In contrast, research has shown that physical activity level and BMI together are better indicators of mortality risk than BMI alone[15].

Other markers of adiposity such as body fat percentage (BF%) and waist circumference (WC) are often considered helpful anthropometric assessments in addition to BMI. Obesity in terms of BF% has been defined as a BF% of greater than 25% for men and greater than 30% for women[17]. Assessments such as skinfold measurements, underwater weighing, bioelectric impedance, and dual-energy X-ray absorptiometry (DXA) scan, are used to estimate BF%, with the DXA scan considered the most accurate[24]. WC has been another measurement that is easy to

obtain by trained professionals and can depict an accurate representation of visceral fat accumulation. A WC of greater than 40" for men and 35" for women has been indicative of increased visceral fat accumulation, placing individuals at high risk for CMD[17,25].

Although obesity is considered a preventable disease, its etiology is multifactorial. Causes of obesity are commonly associated with individuals who have consumed a higher energy intake in comparison to their expenditure[26]. There has been a strong positive relationship between populations with higher supply of energy and rates of overweight and obesity[4]. However, at least 70% of obesity has been attributed to the expression of genes in the current environment, particularly appetite regulation via the hormones leptin and ghrelin[27,28], metabolic factors[29], and the effects of aging[28]. Through the emerging study of epigenetics, there has been growing evidence that environmental factors such as food or lifestyle exposures may also influence the genome[28]. Other environmental factors such as medications, sedentary lifestyles, access to food, socioeconomic status, and peer influence have contributed to obesity[7,30].

Alexander *et al* evaluated the effects of aging on BMI with the National Health and Nutrition Examination Survey (NHANES) data finding that for each component of MetS evaluated, there was a complex interaction between BMI and age. This interaction showed that there were components, such as markers of inflammation,

that were linearly associated with obesity, but not aging, whereas fasting blood glucose showed associations with both BMI and aging[31].

Due to the increased prevalence of obesity and its related comorbidities, it is crucial to understand the physiology of adipose tissue development and measures to ensure disease management[32].

Table 1.1. Classifications of BMI per the World Health Organization

BMI Range	***Classification***
< 18.5 kg/m^2	Underweight
18.5 -24.9 kg/m^2	Normal
25- 29.9 kg/m^2	Overweight
30 – 34.9 kg/m^2	Obesity Class I
35- 39.9 kg/m^2	Obesity Class II
> 40 kg/m^2	Obesity Class III

II. Associations between overweight/obesity and inflammation

a. White adipose tissue development

As the body's main long-term energy storage site, accumulation of fat mass as white adipose tissue (WAT) is the result of energy surplus[26]. Alternative to the WHO definition of obesity, obesity is defined as excess adiposity with body fat percentages of greater than 37% for women and 24% for men, overweight ranges from 32-36.9% in women and 21-23.9% in men[1,28]. However, adipose tissue has

important functions in the body besides energy storage of triglycerides (TG). Other major roles of WAT include: a) functioning as an endocrine organ by releasing cytokines that regulate inflammatory responses and energy balance, b) regulation of energy metabolism through release of fatty acids (FA) into circulation, and c) protecting major organs from damage by serving as cushion[26,32–37].

b. Storage of triglycerides

Excess stored TG in response to a fed state can contribute to two different forms of adipocyte growth: hypertrophy and hyperplasia. Hypertrophy, or enlargement by volume of existing adipocytes, differs from hyperplasia, which is an increase in number of adipocytes and is characteristic of growing children[17]. Both forms of growth, hyperplasia and hypertrophy, increase the total adipose tissue size, which increases the functional capacity of the adipose tissue[33]. Hyperplasia is often coined "metabolically healthy" expansion, as hyperplasia is associated with appropriate angiogenesis, vascular remodeling, and extracellular matrix remodeling[33]. Hypertrophy is favored when there is a dramatic surplus of energy in the body. In a fasting state, adipocytes will decrease in size to maintain energy homeostasis[32,34]. Interestingly, research shows that obese individuals were found to have an increased number of hypertrophic adipocytes, but weight loss did not decrease cell number, only cell size[38]. However, hypertrophy poses a higher health risk because it is related to systemic inflammation and insulin resistance compared to hyperplasia[26]. The increased inflammation present in hypertrophic adipocytes is

exacerbated by hypoxia resulting from poor access to vascularization and macrophage recruitment as a result of dysfunction from an increase in size of the adipocytes[26,33,34].

Adipose tissue expansion is also related to downstream effects of insulin. The release of insulin from the pancreas occurs in response to elevated glucose circulating in the blood. Additionally, insulin activates hepatic lipogenesis to reduce high concentrations of glucose present in the blood. This upregulates glycolytic and lipogenic gene expression in the short-term, increasing the production of FA. Moreover, insulin also promotes lipogenesis in adipose tissue, further promoting FA storage in adipose tissue[34]. These storage sites are fluid. For example, at rest, fat serves as the primary source of fuel, providing up to 65% of energy for the body, therefore, the homeostatic mechanisms and optimal functioning of adipose tissue is necessary[39].

The location of the WAT depots is an important factor in the promotion of inflammation. In humans, adipose can be found in two major depots, the subcutaneous and visceral adipose tissue, but it can also be stored intramuscularly[27,33]. Visceral adipose tissue is characterized by higher turnover rates than subcutaneous adipose tissue, but is a significant risk factor for comorbidities and complications of obesity when in excess[27]. The subcutaneous depot is associated with fat storage beneath the skin, whereas the visceral depot is

located around the organs and intra-abdominally. These depots differ in their adipogenic capability, secretory function, and inflammatory response[26]. Visceral adipose tissue is often related to a higher lipogenic activity, whereas subcutaneous depots have a lower lipid turnover[37].

Studies indicate that fat distribution can be controlled by multiple factors including sex, due to differences in hormone secretion and patterning[15,22,27,40]. Men tend to accumulate fat viscerally and women, subcutaneously[27]. Age and physical activity level are also linked to depot-specific adiposity, with increasing age and decreased activity levels contributing to increased visceral adiposity[39].

c. White adipose tissue and inflammation

The association between WAT development and systemic inflammation is crucial in understanding the relationship between obesity and the role of WAT as an endocrine organ. WAT is a plastic storage site for lipid droplets via hypertrophy of mature adipocytes or hyperplasia involving precursor cells present in the adipose tissue. Adipose tissue is formed though the activity of adipocytes that actively take up FA and glucose from the blood through the action of lipoprotein lipase (LPL) and insulin with FA and glucose subsequently stored as TG in the adipocyte. This mechanism of fat storage is mediated by glucose-regulated glucose transporter 4 (GLUT-4) located on the outer membrane of adipocytes[32,34,38].

Low-grade, systemic inflammation is related to increased adiposity which increases the risk for chronic diseases[8,14–17,41]. Excess adiposity is positively correlated with concentrations of circulating inflammatory markers such as the acute phase protein (APP), C-reactive protein (CRP)[42,43], or the proinflammatory cytokines, tumor necrosis factor α (TNF-α), interleukin- 1β (IL-1) and interleukin- 6 (IL-6)[37,41,43–45]. Increases in these proinflammatory cytokines exacerbates the production of reactive oxygen species (ROS), resulting in oxidative stress. Longitudinal studies analyzing the relationship between circulating inflammatory cytokines and progression of chronic disease show that cytokine concentrations are a predictive biomarker of CMD[41].

The WHO assessed the usefulness of CRP as a marker of inflammation versus marker of infection. Of importance, CRP levels are known to rise rapidly in response to infection, but concentrations decline quicker than other APPs[46]. Additionally, changes in CRP occur independent of nutrient status[42], allowing for differentiation between dietary effects and inflammation. A diet high in antioxidants, especially dietary carotenoids, is a modality recognized to reduce low-grade inflammation as a result of adipose tissue dysfunction[47,48].

Although titled “acute” phase response (APR), APP such as CRP are associated with both acute and chronic inflammatory conditions[49]. The APR is a complex series of reactions mediated by the liver involving a range of pathological responses

on both local and systemic levels[50]. Proinflammatory cytokines IL-6 and IL-1, which are present as a result of obesity, upregulate the APR, working as messengers between the body's site of damage and hepatocytes. One of the pathological responses to increased cytokine signaling by hepatocytes is an increase in production of APP[49–51]. Kramer *et al* found that transcription of CRP is induced by IL-6 in Hep3B-cells and IL-1 in primary human hepatocytes[51]. The major biological functions of CRP are to mediate complement activation and phagocytosis in the host defense from microbes and to induce the release of ROS and cytokines from macrophages as a response to inflammation[49]. This is an important reaction in the inflammatory response, however, when continued for long periods of time, such as in low-grade inflammation caused by obesity, the reaction can be detrimental.

Associations between BMI and CRP were examined in a number of studies, mostly reporting positive associations between BMI and CRP[45,52–54]. Visser *et al* found a positive correlation between BMI and CRP levels with obese men and women being 2.13 and 6.21 times, respectively, more likely to have elevated CRP compared to the normal-weight participants[52]. Similar results were ascertained in a study by Ishii *et al* assessing the relationship of sex, obesity, and CRP. CRP was more likely to be elevated in women than in men, and in individuals that were categorized as obese (BMI >30 kg/m^2)[55]. Moreover, a study assessing the correlation between CRP and morbid obesity found a positive linear relationship ($p<0.0001$)[53]. The evidence in support of a relationship between obesity and CRP

signify the importance of finding solutions to mitigate the effects of low-grade inflammation and oxidative stress as a result of obesity.

The relationship between oxidative stress caused by ROS and free radicals, adiposity, and inflammation forms a positive feedback cycle that constitutes further investigation. Research supports the hypothesis that individuals with higher adiposity have higher oxidative stress than their normal-weighted counterparts[56–59]. Once adipocytes have shown increased proliferation, differentiation, and growth, the cycle between inflammation and oxidative stress begins. Increased systemic ROS triggers the release of proinflammatory cytokines via increased transcription of nuclear factor-κB (NF-κB) and activator protein-1, which are reduction-oxidation sensitive[58]. These proinflammatory cytokines contribute to the chronic, low-grade inflammation often present in obese individuals.

Due to the complex relationship between oxidative stress and obesity, Dandona *et al* investigated the effects of dietary restriction and weight loss on the generation of ROS in nine obese individuals. Four weeks of a 1,000-calorie diet regimen resulted in an average weight loss of 4.5 ± 2.8 kg and significant reduction in generated ROS ($p<0.001$) compared to controls. Three months after the study with no energy restrictions, participants' ROS returned to concentrations higher than baseline. Unfortunately, weights were not obtained at the 3-month follow-up[59]. However,

their results suggest a positive association between obesity and ROS generation and the importance of weight loss in decreasing ROS concentrations.

d. Energy exchange of adipose tissue

When in excess, stored TG contribute to adipocyte hypertrophy, and are effectively mobilized in situations of energy deficit, such as fasting[34]. The uptake of fatty acids into adipose tissue is regulated by the action of LPL and facilitated into cells by an insulin-regulated GLUT-4 in a fed state. The liver is also responsible for the production of very-low density lipoproteins, which serve to distribute dietary fatty acids throughout the body. Because the liver is primarily responsible for the distribution of fats, any disruptions to normal function of the liver can alter the fat metabolism, favoring fat deposition and contributing to obesity. This is highly associated with both insulin resistance and metabolic disease. Most stored TG contribute to hypertrophy, which can cause low-grade inflammation without proper vascularization in the adipocyte.

III. Defining vitamin A and carotenoids

a. Defining carotenoids

In humans, all vitamin A must be obtained from the diet from two primary sources: 1) preformed vitamin A sourced from animal products as retinol or retinyl esters or 2) carotenoids from plant products, or provitamin A compounds, that can be enzymatically converted to vitamin A via dioxygenase[60]. When carotenoids are

cleaved to vitamin A in the body, they form one of three chemical formulations of vitamin A[61]. Retinal can be oxidized to retinol and retinoic acid (Figure 1.3). Retinol can be esterified and stored as retinyl esters in the hepatocytes or adipose tissue. Retinol is reversibly oxidized to retinal by aldehyde dehydrogenases and microsomal short-chain dehydrogenases, then irreversibly oxidized to retinoic acid by retinaldehyde dehydrogenases[32].

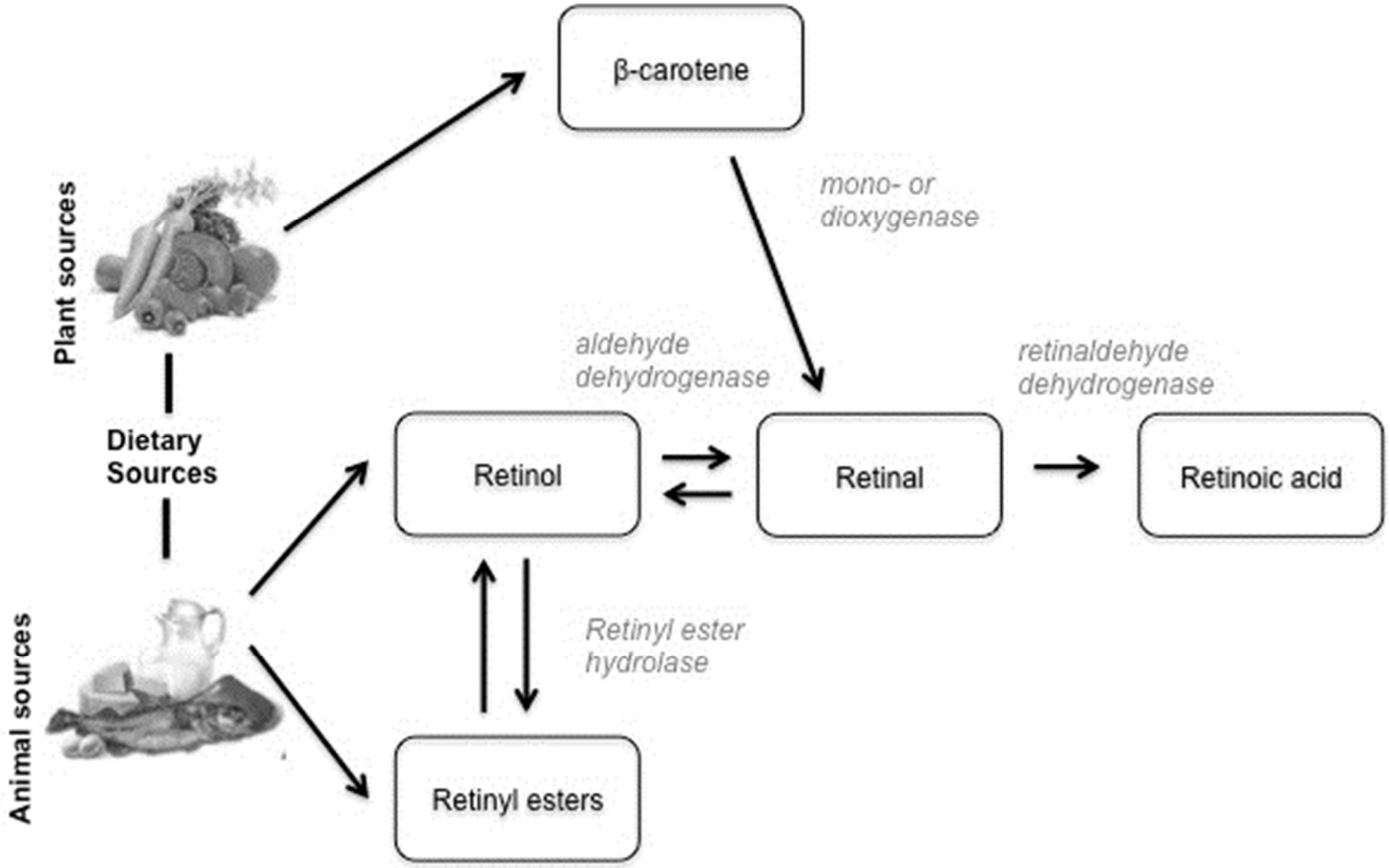
Synthesis and degradation of retinoic acid by the enzyme CYP26 largely regulates homeostasis of retinoic acid[62]. Retinoic acid, which is a conversion product of dietary carotenoids, has a large effect on gene expression by binding and activating retinoic acid receptors and retinoid X receptors[63]. The main functions of vitamin A in the body are in facilitating normal vision, supporting placental development and maintenance, regulation of cell differentiation, maintenance of skin and epithelial cells, serving in the maintenance and regulation of immunity, and as an antioxidant[32,34,38,62].

Carotenoids are a large family of fat soluble, plant-derived pigments that have the ability to function *in-vivo* as a precursor for vitamin A[64,65]. There are over 700 identified species of carotenoids and those with provitamin A activity in the body are β-carotene (BC), α-carotene (AC), and β-cryptoxanthin[66]. The xanthophyllic carotenoids lutein, lycopene, and zeaxanthin are also utilized in organs such as the eyes, but are not cleaved in the intestine to be converted to vitamin A[67].

b. Dietary sources of carotenoids

Carotenoids are known markers of fruit and vegetables in the diet. Many studies have been conducted to assess carotenoids as biomarkers of fruit and vegetable intakes, but often times are conducted on self-reported dietary recall. Couillard *et al* utilized a fully controlled dietary study over 4-5 weeks in 264 male and female participants age 42.6±14 years to validate serum carotenoid concentrations as markers for fruit and vegetable intakes. Interestingly, when consuming the provided the 12±6 servings of fruits and vegetables daily, they found negative associations between fruit and vegetable intakes and serum AC and lycopene ($p<0.001$), and no associations to serum BC concentrations. Their research did support β-cryptoxanthin, zeaxanthin, and lutein as biomarkers of mixed fruit and vegetable intakes, due to the significant positive association with fruit and vegetable intakes ($p<0.005$)[68]. Their results concurred with other research using food frequency questionnaires (FFQ) that support β-cryptoxanthin and lutein as biomarkers of fruit and vegetable intakes[69].

Figure 1.2. Overview of vitamin A and carotenoid conversion. Preformed vitamin A as retinol and retinyl esters are sourced from animal products in the diet. Carotenoids, such as β-carotene, are obtained via plant sources and converted to retinal via mono- or dioxygenases. Retinal is reversibly oxidized to retinol or irreversibly oxidized to retinoic acid, dependent on the body's needs.



A meta-analysis aiming to determine biomarkers of interventions for adequate consumption of fruits and vegetables found that carotenoids AC, BC, β-cryptoxanthin, and lutein were increased following fruit and vegetable consumption, meeting the WHO guidelines of 400 grams of fruits and vegetables daily. Lycopene did not display similar results, as plasma concentrations showed no significant difference before and after interventions[70]. Souverein *et al* made similar conclusions in a meta-analysis assessing serum carotenoid concentrations as a marker of fruit and vegetable intake, finding all individual carotenoids have a positive correlation to reported fruit and vegetable intakes[71]. These results may be attributed to AC and BC being the two most abundant and bioavailable carotenoids in the diet. Research also suggests the sum of carotenoids may be the best predictor of fruit and vegetable intake[71] as it is most representative of diet in its entirety.

A study conducted by Jansen *et al* assessed plasma carotenoid concentrations in 591 Dutch men and women ages 20-59 years relative to fruit and vegetable consumption. The study utilized national FFQ data to obtain information on reported fruit and vegetable intakes and reported fruit and vegetable juice consumption followed by a physical examination as part of the nation-wide survey. Serum carotenoid concentrations were assessed finding higher carotenoid concentrations in women compared to men. Lycopene contributed most to total serum carotenoid concentrations in men, and AC and β-cryptoxanthin in women. Additionally, all carotenoids, with the exception of lycopene were positively

correlated with serum concentrations. The strongest correlation between fruit and vegetable intakes and serum carotenoid concentrations in both men and women was seen for β-cryptoxanthin (r=0.35 and r=0.41, respectively)[69].

An indicator of carotenoids in food is a red, yellow-orange, or green color; however, carotenoids are widespread in other sources. Foods rich in carotenoids BC and AC are: carrots (13,485μg/100g) and sweet potatoes (9,488μg/100g), whereas leafy green vegetables such as spinach (17,535 μg/100g) are rich in lutein and zeaxanthin (Table 1.2)[72]. Lycopene is highly associated with cooked tomato products, as the best sources of lycopene in the diet are ketchup (17,008 μg/100g), marinara sauce (15,990 μg/100g), and other canned tomato products such as paste (29,330 μg/100g), soup (10, 920 μg/100g), and puree (16,670 μg/100g)[72].

When evaluating dietary intakes of carotenoids, it is important to understand the bioavailability of the carotenoids as it is dependent on dose[67,73,74], quantity and dispersion throughout the day[65], stability of the compound when cooked versus raw[66], and presence of other foods consumed in the diet, specifically fat[65,75–77]. Carotenoids are fat soluble, therefore, dietary fat is important regarding bioavailability; fat aids in the release of BC from the food matrix and incorporation into the mixed micelle[78]. Additionally, the intestinal absorption of carotenoids varies by the chemical structure of the carotenoid[66], release of the carotenoid from the food matrix[79] and intestinal cleavage of BC to retinol[65,79]. If carotenoids are still

bound to their food matrix and not solubilized in a micelle, carotenoid absorption is limited, ultimately affecting circulating concentrations of carotenoids[80].

In the Western diet, it is proposed that 25-50% of vitamin A is sourced by provitamin A carotenoids[81], making their consumption an important part of meeting recommended dietary allowances of vitamin A. BC is the primary carotenoid in plant sources, in the diet, and has the highest quantities in serum[81–83]. However, most foods containing BC also contain other carotenoids[72]. Positive relationships between co-consumption of BC and other carotenoids have been noted, however, other studies have found that the carotenoid lycopene can reduce the absorption of BC[81].

Table 1.2. Carotenoid content in raw foods in μg/100 g edible portion[72].

	α-carotene	*β-carotene*	*β-cryptoxanthin*	*Lutein + zeaxanthin*	*Lycopene*
Fruits					
Apricots	0	2554	0	0	5
Cherries	242	1048	27	105	N/A
Grapefruit	5	603	12	13	1462
Mangos	17	445	11	0	N/A
Raspberries	12	8	0	0	N/A
Strawberries	5	9488	0	0	N/A
Tomatoes	112	393	0	130	3025
Watermelon	0	295	103	17	4868
Vegetables					
Green beans	68	377	0	640	0
Broccoli	1	779	0	2445	0
Carrots	4649	8836	0	0	0
Swiss chard	49	3954	0	0	0
Red peppers	59	2379	2205	0	N/A
Pumpkin	4795	6940	0	0	0
Spinach	0	5597	0	11938	0
Butternut squash	834	4226	0	0	N/A

c. Consumption of carotenoids with dietary fat

The relationship between BC and the presence of dietary fat is important for understanding carotenoid bioavailability. Multiple factors have an influence on 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[84]. Research indicates that the addition of fat to a carotenoid-containing meal improves intestinal absorption of BC[65,75–78,85]. 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 28g of fat versus 0g[78].

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 (150g) versus 1.5 eggs (75g) significantly increased BC absorption 10 hours post consumption ($p<0.001$)[86]. Another study assessing the effectiveness of avocado or avocado oil reported significant differences in areas under the curve for BC in the plasma triacylglycerol-rich lipoprotein fraction 9.5 hours after consumption of 300g salsa with 150g avocado ($p<0.003$) and 200g salad with 75g avocado ($p<0.01$), 150g avocado ($p<0.01$), or 24g avocado oil ($p<0.01$)[87]. White *et al* found similar results when adding 0, 2, 4, 8, 16 and 32g of soybean oil to a salad containing 11.54±0.5mg BC. There was a positive linear relationship between BC and total grams of soybean oil between 0-8g, with highest BC absorption with 32g of oil[88].

Additionally, the intestinal absorption of carotenoids varies by the chemical structure of the carotenoid[66], release of the carotenoid from the food matrix[79] and intestinal cleavage of BC to retinol[65,79]. If BC is still bound to its food matrix and not solubilized in a micelle, absorption is limited, ultimately affecting circulating concentrations of BC[80]. 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[84]. Goltz *et al* determined that adding 20g of lipids to a meal containing BC significantly affected the absorption rates of BC, independent of the type of lipid consumed ($p<0.01$)[10]. This indicates that, on its own, BC has poor bioavailability and the presence of fat is necessary for absorption.

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, (which contains a high proportion of monounsaturated fatty acids (MUFA)), soybean oil (high proportion of polyunsaturated fatty acids (PUFA)), sunflower oil (high proportion of PUFA), peanut oil (high proportion of MUFA + saturated fatty acids (SFA)), and coconut oil (highest proportion of SFA), BC micellarization was significantly higher in the MUFA and PUFA rich oils than the SFA rich oils ($p<0.05$)[85]. BC uptake was dependent on the type of fat, suggesting the food matrix, BC polarity, and type of dietary fat determine BC bioavailability[85]. Similar results were obtained

by Failla *et al* finding BC micellarization and cellular uptake were significantly different between fatty acid types (soybean oil > olive > canola > butter) ($p<0.05$)[84]. This suggests that unsaturated fatty acids MUFA and PUFA are better promoters of BC micellarization and cellular uptake than SFA.

d. Carotenoid digestion and absorption

It is often assumed that there are minor between-person differences in digestion and absorption, however, biologically, there are significant differences both within-person and between persons in how people digest, absorb, and metabolize nutrients. Research supports serum carotenoid concentrations as short-term markers of fruit and vegetable consumption, however, tissue carotenoid concentrations, especially adipose tissue, are more stable markers of long-term carotenoid intakes[89]. Johnson and Russel assessed serum carotenoid concentrations for 10 days after a 120 mg dose of BC and found that mean serum carotenoid concentrations significantly increased after six and 12 hours, however, serum concentrations peaked between 16 and 36 hours[90]. Interestingly, major differences were seen between participant peak response, suggesting serum status must be maintained over time with long-term dietary intake[90]. Mathews-Roth researched the effects of long-term consumption of 180 mg of BC per day for 11 weeks on serum BC concentrations. Mean serum carotenoid concentrations reached a plateau between 1.5 and 4 weeks, however, between-person plateau times varied[91].

The relationship between dietary intakes of carotenoids and serum carotenoid concentrations is complex, especially taking into account the bioavailability and bioaccessibility of carotenoids[92]. Some research hypothesizes that food matrices and intestinal saturation reduce carotenoids absorption[93]. Kopec *et al* hypothesized that low bioavailability and bioaccessibility of carotenoids, compared to other dietary antioxidants, is attributed to oxidation occurring during the digestive process. Carotenoids are temperature and pH sensitive. During digestion, carotenoids would be exposed to both increased temperatures and low pH.

Additionally, co-consumption of oxidizing agents, such as iron, can influence the pro-oxidative state of carotenoids, oxidizing them before they have reached the intestine for absorption[92]. Therefore, the researchers studied initial, post-gastric, post-duodenal and post-jejunal carotenoid (BC, lutein and lycopene) stability to better understand how digestion affects postprandial carotenoid concentrations. Their research indicated that the percent carotenoid transferred from lipid droplets to the micelle during digestion is proportional to the degree of hydrophobia of the carotenoid. This was evident in the results as lycopene is the least hydrophobic between lycopene, BC, and lutein and had the least loss (20%) throughout digestion compared to BC and lutein, which saw a 40% loss. Interestingly, lutein and lycopene loss was most significant following the jejunal phase ($p<0.05$), whereas BC loss was most significant following the gastric phase[92].

The intestine is the main site of carotenoid cleavage to retinal, however, the liver, adipose tissue, lungs and kidneys are also capable of converting BC to retinal[73] . There is a low rate of activity in the intestinal cells for carotene oxygenases, the enzymes responsible for the cleavage of carotenoids, therefore much of the carotenoids consumed in the diet are absorbed and remain intact in circulation[94]. Cooperstone *et al* assessed within person variability of carotenoid cleavage and absorption when assessing the contributions of BC and AC to postprandial concentrations of vitamin A in twelve healthy subjects. Their findings show that the range of cleavage for both BC (9-68%) and AC (6-63%) was highly variable, and there was no correlation between preference in cleavage and preference in absorption within subject. However, there was a strong, positive relationship between BC conversion and AC conversion (R=0.82), suggesting that individuals who convert more BC also convert more AC[95]. Ultimately, there are multiple factors that contribute to serum carotenoid concentrations, with a wide variability within-person and between-persons. This makes it difficult to make firm conclusions on the relationship between dietary carotenoid intakes and serum carotenoid concentrations.

Once absorbed into the intestinal cell, carotenoids can be 1) converted to retinol, esterified, and packaged to chylomicrons or 2) secreted directly into chylomicrons[81]. For delivery to the body tissues, chylomicrons are dependent on saturation of the absorptive intestinal cells and concentrations of BC, as a concentration gradient must be present for passive diffusion to occur[60,74,94,96].

O'Neill *et al* explored the TG-rich fraction of plasma to assess conversion to retinol and esterification rates post-consumption of a standard meal and supplementation of 40 mg BC, 31.2 mg lutein, or 38 mg lycopene in twelve healthy subjects ages 20-25. They obtained interesting results as the plasma concentrations of BC increased in all subjects, however, plasma concentrations of BC also showed large between-subject variability with a 2-fold variation. Lycopene and lutein showed similar patterns, with a more significant variation (2-3 fold difference) between subjects[81].

Other research assessed the relationship between postprandial chylomicron carotenoid response and age. Their participants fell into one of two groups: 20-35 years (younger) or 60-75 years (older), and all participants were non-obese, non-smoking individuals. In the younger participants, chylomicron TG concentration of BC, lycopene, and lutein maximized 2 hours after a meal, whereas in the older group TG concentration maximized 2-3 hours after a meal, indicating more variability between persons. The only significant difference in chylomicron carotenoid response between the younger and older group was observed in lycopene concentrations (41.2±17.9 versus 24.9±8.0, $p=0.04$), however, the older group had a 27% higher chylomicron TG response. They proposed that these effects are seen as age affects gastrointestinal function, bioavailability of micronutrients, efficiency of carotenoid absorption, provitamin A conversion to vitamin A, and modifies chylomicron metabolism and transport of carotenoids[97].

IV. Associations between obesity and serum carotenoid concentrations

a. Carotenoids in the metabolism of adipocytes

More recently, the effects of provitamin A carotenoids and vitamin A in adipose tissue biology have been explored[32]. Carotenoids have been well studied for a proposed role in adiposity because WAT is the main storage site for carotenoids[16,34]. After retinol is taken up by adipose cells, it can be stored or converted to retinaldeyhde and retinoic acid. These metabolites have an anti-adipogenic effect on fat metabolism and adipocyte differentiation[60]. Some research supports the idea that uncleaved BC can also have an effect on the function of adipocytes. Using 3T3-L1 cells from mouse models, Kawada *et al* tested the effects of multiple vitamins on adipocyte differentiation. They found that retinoic acid, retinal, and retinyl palmitate not only inhibited adipocyte differentiation, but concentrations ranging from 2-50 μM BC also significantly reduced TG percentage in the adipocytes ($p<0.05$)[98]. Kameji *et al* investigated the effects of BC on 3T3-L1 cell models for four days during differentiation and found that cells cultured with 20 μM concentrations of BC had significantly higher expression of genes related to FA metabolism, insulin sensitivity, and anti-inflammatory proteins than those cultured without BC ($p<0.05$)[99].

Further research discovered that carotenoids, especially BC, regulators of adipocyte development, modulating adipocyte differentiation, lipogenesis, and lipolysis[35,100,101]. Research on the effects of BC supplementation of 1,500 IU in vivo on mice showed a 28% decrease in body fat, down regulation of gene expression in WAT, and reduction in adipocyte size ($p<0.05$)[100]. Moreover, Mercader *et al*

discovered a dose-dependent decrease in body weight ($p<0.001$) and WAT size ($p<0.005$) independent of energy intake in mice[35]. These rodent studies provide evidence in support of BC playing a role in adipocyte differentiation, lipid metabolism, and gene modulation of adipocytes.

Interestingly, Harari *et al* were able to detect the same carotenoids in the serum as in adipose tissue, but in differing proportions. BC was 40% of the proportion of carotenoids present in the serum, but 26% in adipose tissue, whereas AC was 11% in serum and 14% in adipose tissue. Lutein and lycopene also had a higher presence in serum than in adipose tissue[83]. Moreover, Chung *et al* analyzed carotenoid concentrations in biopsied human adipose tissue from the abdominal area, lateral buttock, and inner thigh. They found that mean concentrations of BC, AC, lutein + zeaxanthin, lycopene, and β-cryptoxanthin were highest in the abdomen ($p<0.05$), intermediate in the buttock, and lowest in the thigh[102]. Additionally, many carotenoid concentrations, including BC in the abdomen and buttock, lycopene in the thigh, and cryptoxanthin in the abdomen were positively correlated with age ($p<0.05$). A significant relationship with age may be present due to the association with higher carotenoid intakes with age[102].

b. Carotenoids and BMI

Lower correlations between dietary intakes and serum concentrations indicate that there are potential factors dictating serum carotenoid concentrations as they forego intestinal cleavage and absorption. One theorized factor is that carotenoids are stored and utilized in adipose tissue, as BMI was found to be associated with efficiency of conversion of carotenoids to retinal in women ($r=0.71$, $p<0.01$). The process in which carotenoids, especially BC, are stored in the body may explain this relationship between adiposity and carotenoid concentrations. The enzyme β-carotene 15'15-monoxygenase type 1 (BCMO1) is present in adipose tissue, promoting conversion of BC to retinaldehyde on site; some BC is also left intact and is stored in the adipose tissue. Previous studies concluded that carotenoids/retinoic acid promotes weight loss, glucose tolerance, and body fat loss in mice, independent of diet[101,103,104].

When comparing serum carotenoid concentrations with dietary intake of carotenoids in women ages 50-79, Kabat *et al* found significant associations between dietary intake and serum concentrations of BC ($r=0.19$, $p<0.001$), AC ($r=0.28$, $p<0.001$), lycopene ($r=0.20$, $p<0.001$), and β-cryptoxanthin ($r=0.32$, $p<0.001$)[16]. Vioque *et al* obtained similar results when evaluating the association between dietary intake and serum concentrations of carotenoids in 545 older men and women with findings reported by BMI category, to further understand the relationship between BMI and carotenoid digestion and absorption. Serum BC

concentrations were positively associated with dietary intakes of BC for normal ($r=0.35$, $p<0.05$), overweight ($r=0.15$, $p<0.05$), and obese ($r=0.20$, $p<0.01$) individuals. Similar results were obtained for lycopene and β-cryptoxanthin. However, AC and lutein + zeaxanthin did not show significant associations in obese individuals, but did in normal and overweight participants[105].

Burrows *et al* conducted similar research reporting on servings of fruits and vegetables consumed daily and its effect on BMI. They also assessed the relationship between BMI and serum carotenoid concentrations. Their findings show that there were no significant differences in the quantity of fruits and vegetables consumed between participants with a normal versus overweight BMI ($p<0.2$), however, participants that were classified as overweight had significantly lower concentrations of serum BC ($p=0.01$), AC ($p=0.05$), and lutein + zeaxanthin ($p=0.05$) compared to their counterparts with a normal BMI[106].

Recent research suggests that BMI and serum BC concentrations are inversely related. Östh *et al* found that those with higher adiposity also have a significantly lower level of BC stored in their adipose tissue compared to lean counterparts ($p<0.02$)[107]. Bovier *et al* assessed the relationship between lutein and zeaxanthin status, measured via macular pigment optical density, and body fat in 100 healthy subjects. Their findings indicated that there is a significant relationship between macular lutein and zeaxanthin status and body fat percentage in both men and

women ($r=-0.32$, $p<0.01$)[108]. Additionally, Harari *et al*. performed a comprehensive analysis of serum and adipose tissue BC in 80 non-diabetic, Caucasian subjects with BMI ranging between 30.1-48.5 kg/m^2. Serum BC concentrations correlated inversely with weight, BMI, WC, total BF%, body fat-free mass, and central body fat. Interestingly, adipose BC concentrations did not significantly correlate with the abdominal subcutaneous or visceral fat depots, whereas AC, lutein, and lycopene did ($p<0.05$). All serum carotenoids were significantly lower in individuals with a BMI >30 kg/m^2 than those with a normal or overweight BMI[83].

Most studies have examined the relationship between BMI and/or BF%, however, Wang *et al* assessed the associations between serum carotenoid concentrations and body fat distribution in 4,048 Chinese adults aged 40-75 years. There were significant, inverse associations between total serum carotenoid concentrations and all measured markers of adiposity: BMI, WC, waist-to-hip ratio, total BF%, android BF%, and gynoid BF%, with BC showing the strongest associations[109].

This relationship between adiposity and carotenoid concentrations led to further research on its association with BMI[61] and inflammation[110]. Andersen *et al* reported that obese subjects have 24-37% lower serum carotenoid concentrations compared to individuals with a BMI of <22 kg/m^2 ($p<0.0001$)[111]. Additionally, other studies have shown an inverse relationship between serum BC concentrations and BMI ($r=-0.22$, $p<0.0001$); for every 1 unit increase in serum BC, there is a 0.077 decrease in

BMI ($p<0.0001$)[16]. Similar results were obtained by Kabat *et al* when assessing the relationship between BMI and other serum carotenoids in women. Their findings showed a significant and inverse relationship between BMI and AC ($r=-0.24, p<0.0001$), BC ($r=-0.22$, $p<0.0001$), β-cryptoxanthin ($r=-0.17$, $p<0.0001$), lutein + zeaxanthin ($r=-0.22$, $p<0.0001$), and lycopene ($r=-0.10$, $p<0.0001$)[16]. This evidence helps solidify the inverse relationship between individual serum carotenoids and BMI.

Lower serum BC concentrations are also associated with HTN, dyslipidemia, and MetS[10,112]. A longitudinal study showed that individuals with low consumption of fruits and vegetables, suggesting low carotenoid consumption, and high intakes of sugar-sweetened beverages, meat, and fried potatoes are significantly more likely to have higher obesity prevalence ($p<0.001$)[12]. An inverse relationship was observed between serum BC concentrations and HTN ($p<0.01$)[113], dyslipidemia($p<0.029$)[8], waist circumference ($p<0.001$) and MetS ($p<0.001$)[16]. Beydoun *et al* analyzed the relationship between serum antioxidant statuses and MetS in adults, finding that participants with MetS had higher serum retinol, but lower total carotenoid concentrations than those without MetS ($p<0.05$)[18]. These data support a correlation between serum carotenoid concentrations and risk for chronic disease. Additionally, 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[114]. Therefore, conclusions can be

drawn that there is a correlation between serum carotenoid levels and risk for chronic disease and all-cause mortality.

Lastly, lower serum carotenoid concentrations are associated with smoking status due to its proposed increase in free radicals, which often deplete circulating antioxidants. Interestingly, Kabat *et al* found that women who were current smokers showed an average of 39% lower serum carotenoid concentrations than their non-smoking counterparts with similar anthropometric measurements[16]. Other research supports their findings showing significant reductions in serum BC concentrations for both men and women when an individual was either a former smoker or a current smoker ($p<0.0001$)[115]. Additionally, Wallstrom *et al* found similar findings, noting that male smokers had significantly lower concentrations of serum BC ($p<0.01$), but the reasoning is uncertain. It is possible that smokers may metabolize BC differently than non-smokers or generally consume less BC than their non-smoking counterparts[116]. It is important to assess the relationship between smoking and serum carotenoid concentrations as the pro-oxidant nature of chemicals in cigarettes disrupts antioxidant status.

c. Associations between diet and inflammation

Dietary antioxidants, more specifically carotenoids, vitamin E, vitamin C, magnesium, and selenium, are proposed to reduce oxidative stress related to low-grade inflammation[7,117]. To assess the relationship between diet, oxidative stress, and inflammation, Ford *et al* scored diet quality based on the Healthy Eating Index

(HEI) scale ranging from 0-100. They found that individuals with elevated CRP had reduced HEI scores in dietary variety, especially with regards to grains, fats, and dairy products[117].

There are noteworthy associations present between diet quality and CRP[117]. Of note, low intakes of vitamins and minerals were found to contribute to higher risk for chronic disease[7]. A longitudinal retrospective chart review study in which 43 patients followed the Low Inflammatory Foods Everyday diet, a dark green leafy vegetable-rich diet, for five years concluded that serum BC concentrations were inversely associated with CRP. Moreover, the degree in which CRP decreased was greater with increasing concentrations of BC in the serum[48].

Other research on diet quality and inflammation was conducted by Watzl *et al* in 63 middle-aged men following two 4-week dietary interventions consuming two, five, or eight servings of fruits and vegetables daily. The group consuming two servings of fruits and vegetables daily had no significant changes in serum carotenoid concentrations, whereas the groups consuming five and eight servings of fruits and vegetables daily had significant increases in serum carotenoid concentrations ($p<0.01$). After four weeks of eight servings of fruits and vegetables daily, an inverse relationship between serum BC concentrations and CRP ($r=-0.51$, $p=0.016$) was present. Significant reductions in CRP were not associated with any other carotenoids[118]. Therefore, research identifying specific dietary factors that influence inflammation is of high importance.

A longitudinal study by Mazidi *et al* assessing the relationship between serum antioxidants concentrations and inflammatory markers suggested that serum BC concentrations were negatively associated with CRP concentrations[119]. Epidemiological studies show that subgroups among populations with the highest serum carotenoid concentrations, especially BC, AC, and β-cryptoxanthin, are older[114], female[105,114,120], non-smoking individuals[111,114,120]. Interestingly and likely due to the fat-soluble nature of carotenoids, individuals surveyed in NHANES III with high total cholesterol and high-density lipoprotein (HDL) also had higher serum carotenoid concentrations than their counterparts with lower total cholesterol and HDL values ($p<0.001$, $p<0.05$)[114]. Hozawa *et al* obtained similar results in longitudinal analysis of trends in antioxidants assessing Coronary Artery Risk Development in Young Adults data[120]. Interestingly, serum BC, AC, lutein/zeaxanthin, and β-cryptoxanthin concentrations were significantly higher in individuals with undetectable inflammatory marker CRP ($p<0.01$)[114] and lower leukocyte concentrations($p<0.01$)[120].

Dietary associations between dietary fiber, total fat intakes, and dietary magnesium have been studied due to their proposed effects on inflammation. Utilizing NHANES data, individuals reporting higher intakes of fiber [121,122] and magnesium[123] were found to have lower CRP levels. However, higher intakes of saturated fats were found to moderately increase risk for elevated CRP[122]. Low-grade inflammation is related to an increase in oxidative stress, suggesting a need for increased dietary antioxidants[8,124]. The best sources of dietary antioxidants are

fruits, vegetables, nuts, and whole-grains. When comparing Mediterranean diet patterns, high in antioxidants, to the Westernized diet containing highly processed foods and an abundance of red meats, it is evident that the Westernized diet supports a proinflammatory state in the body[14].

Additionally, factors such as increasing age, the female sex, decreased physical activity levels, increased alcohol consumption, current status as a smoker, and disease states such as T2DM, cardiovascular disease, congestive heart failure, HTN, angina, cancer, and/or recent heart attack have been associated with increased CRP concentrations[41,121,122]. All aforementioned factors must be taken into account when assessing CRP and serum carotenoid concentrations, as many overlap with factors affecting serum carotenoid concentrations as well.

V. Summary and objectives

Obesity and its comorbidities continue to increase in the US, therefore, understanding preventative measures is increasingly important for population health. There is a well-researched relationship between obesity and serum carotenoid concentrations; however, it is not well understood how dietary and anthropometric factors affect this association in the US adult population. By examining data from the 2003-2004 and 2005-2006 NHANES collection and assessing the associations between dietary BC intakes, serum BC concentrations, and markers of adiposity in the US population, information regarding the relationship between dietary BC, serum BC concentrations, and the factors that

influence them will contribute to the knowledge base on BC. Moreover, investigating the associations between BC and obesity are pivotal in understanding obesity as a diet-related condition.

The specific aims of this project are 1) to test the hypothesis that serum BC concentrations are associated with BMI and the inflammatory marker high-sensitivity C-reactive protein (hsCRP) in the US population, 2) to assess the association between reported dietary BC intakes and serum BC concentrations in United States (US) adults and to examine the impact of body mass index (BMI) and region-specific body fat percentage on the association between reported BC intakes and serum concentrations of BC, and 3) to assess the association between serum BC concentrations and reported intake of specific fatty acid classes, utilizing data from the What We Eat in America (WWEIA) and National Health and Nutrition Examination Surveys (NHANES). This work is the first to assess multiple factors that might influence the association between dietary BC and serum BC concentrations utilizing population-based data collected in the US.

CHAPTER 2

Markers of inflammation are associated with serum β-carotene concentrations in United States adults

Ambria C. Crusan[1], Marla Reicks[1], Ryan T Demmer[2], David R. Jacobs, Jr[2], Susan K. Raatz[1]

[1] Department of Food Science and Nutrition, University of Minnesota, Minneapolis, MN

[2] Division of Epidemiology, School of Public Health, University of Minnesota, Minneapolis, MN

I. Abstract

Chronic low-grade inflammation associated with obesity may be attenuated by dietary β-carotene (BC) intake. The relationship between obesity, low-grade, systemic inflammation, and oxidative stress has been well established in longitudinal studies. However, how serum carotenoid concentrations are associated with obesity-related inflammation is not well understood. The primary objective was to test the hypothesis that serum BC concentrations are independently associated with body mass index (BMI) and the inflammatory marker high-sensitivity C-reactive protein (hsCRP) in the United States (US) population. Data from 3,886 male and non-pregnant female participants aged 18-85 years from the National Health and Nutrition Examination Surveys (NHANES) 2003-2006 nationally representative, cross-sectional survey were analyzed to estimate the relationships among BMI, hsCRP, and serum BC concentrations. Due to skewing, natural log (ln) transformation of serum BC concentrations and hsCRP was applied. Multiple linear regression estimated the relationship of ln(hsCRP) to ln(serum BC) adjusted for age, sex, and ethnicity**.** In this cross-sectional analysis, individuals with the highest concentrations of serum BC were more likely to be women, non-Hispanic white, older (70+), and have a normal BMI. Participants in the highest quintile for serum BC concentrations had the lowest hsCRP concentrations and a correlation between serum BC and hsCRP was present ($r=-0.12$, $p<0.0001$). Multivariable linear regression modeling hsCRP on serum BC concentrations adjusted for age, sex, race/ethnicity, and reported dietary BC intake, show a negative association ($p<0.0001$). The present negative association between hsCRP

and serum BC concentrations, which is partially mediated by BMI, may suggest a protective effect of increased serum BC concentrations against low-grade, systemic inflammation often associated with adipose tissue dysfunction present in obese individuals.

II. Introduction

The prevalence of obesity in the US in 2018 was 42.4%[5]. Multiple studies have demonstrated a positive relationship between obesity and cardiometabolic diseases, especially Type II Diabetes Mellitus (T2DM) and cardiovascular disease[16,18,111,112]. Moreover, the American Medical Association has officially recognized obesity as a disease[2] due to the association with chronic, systemic inflammation and increased oxidative stress, and exacerbation of the progression of other comorbidities.

Chronic low-grade inflammation associated with obesity may be attenuated by dietary BC intake[99,119]. Nutrition has been recognized as a modulating factor for low-grade inflammatory response resulting from adipose tissue dysfunction present in obesity[47]. Dietary intake of the antioxidant BC, has been known to reduce oxidative stress associated with low-grade inflammation[26,57–59,119]. Evidence supports an independent, inverse relationship between obesity, as assessed by BMI, and serum carotenoid concentrations[111], the best marker of dietary carotenoid intake[114]. Limited data on the relationship between serum BC concentrations and inflammation in population samples exists.

The present study aimed to evaluate the associations between serum BC concentrations, the inflammatory marker hsCRP, and BMI in US adults aged 18-85 years who participated in the NHANES collection between 2003-2006.

III. Methods

Design overview

Demographic, anthropometric, laboratory, and questionnaire data obtained from NHANES were compiled to create a dataset for cross-sectional analysis of the associations present between serum BC concentrations, hsCRP concentrations, and BMI. This data was assessed via multivariable linear modeling to examine how inflammatory marker hsCRP is affected by serum BC concentrations and BMI among US adults.

Participants and Dataset

The NHANES dataset utilized cross-sectional survey methods to obtain a unique collection of nationally-representative, health- and nutrition-related data on non-institutionalized civilians in the US. Sampling design of NHANES includes the selection of approximately 5,000 people per year representing fifteen locations across the US, enrolling a total of 20,470 individuals in the years 2003-2006 [125]. Data are released on a two-year cycle (e.g. 2003-2004) with the most current serum carotenoid collection conducted in 2003-2004 and 2005-2006. NHANES was designed to obtain data to assess the health and nutritional status of the US

population using interviews, clinical examinations and laboratory data[18]. The data collection process was well documented by NHANES[126].

This analysis includes males and non-pregnant females aged 18-85 years who participated in laboratory analysis for serum BC and hsCRP and had recorded information on race/ethnicity, sex, age, smoking status, and measured height and weight to calculate BMI. Exclusions were made for individuals who were missing serum BC concentrations (n=13,202), BMI (n=2,834), high-sensitivity CRP (hsCRP) (n=4,044), pregnancy status (n=4,968), and current smoking status (n=2,517). Participants were also excluded if: a) BMI was less than 14 kg/m^2 (n=181) due to metabolic implications of very low body weight for height, or greater than 70 kg/m^2 (n=3), as these were outliers in the data, b) if hsCRP was greater than 10 mg/dL (n=20), an indicator of acute versus chronic inflammation[55], c) if they were currently pregnant (n=566) or unsure of their pregnancy status (n=21) due to weight changes related to pregnancy, and d) if they are current smokers smoking every day (n= 1,844) or some days (n=376) due to the effects of smoking on serum carotenoid concentrations[111]. This resulted in 3,668 participants included in the current analysis to estimate the relationships among BMI, hsCRP, and serum BC concentrations.

Study variables

Sociodemographic covariates included age, sex, and race/ethnicity (non-Hispanic (NH) whites, NH blacks, Mexican Americans, other Hispanic, and other ethnicities). Anthropometric and laboratory data were collected via in-person analysis at mobile examination centers by trained individuals using protocols developed by NHANES[18,121,125]. The anthropometric measurement of BMI was used in this study as a marker of adiposity, categorized by World Health Organization (WHO) classifications: <18.5 kg/m^2= underweight, 18.6-24.99 kg/m^2= normal, 25-29.99 kg/m^2= overweight, 30-34.99= class I Obesity, 35-39.99 kg/m^2= class II obesity, and ≥ 40 kg/m^2 = class III obesity[23].

To obtain laboratory data for hsCRP and serum BC concentrations, standard phlebotomy procedures were used to obtain and process blood from non-fasted participants. Serum samples of 0.3-1.0 mL are stored in properly sealed vials and frozen at -70°C until analysis[127,128]. Serum trans-BC and cis-BC concentrations were determined via high performance liquid chromatography with multiwavelength photodiode-array absorbance detection[127]. The sum of cis- and trans-BC was used in this assessment (LBXBCC in μg/dL). hsCRP concentrations were determined using latex-enhaced nephelometry[128]. hsCRP, a cytokine-induced, positive acute phase protein, was used to determine inflammatory status, as it has been noted as a sensitive marker for systematic inflammation[129]. Low hsCRP was defined by the Center for Disease Control as <0.05 mg/dL, average concentrations

range from 0.05-0.30 mg/dL, and high hsCRP was above 0.30 mg/dL[130]. Dietary consumption of BC is highest of all the carotenoids[61]. BC is the primary carotenoid present in plant sources, has highest quantities in serum[82] (with normal serum BC concentrations range from 2.2- 122.7 mg/dL[89]), therefore, was the primary carotenoid assessed. There are no specified deficiency markers set for serum BC[131].

Statistical analysis

To accommodate the complex survey design, weighted variables were created in SAS statistical software (version 9.4, Cary, NC, USA) according to the guidelines for analysis published by the Center for Disease Control[125,132]. Sex, ethnicity, education level, age, and BMI data were normally distributed. Due to skewing, natural log transformation of serum BC concentrations and hsCRP was conducted. Mean and standard errors for continuous variables and percentages for categorical variables were used. Multiple linear regression estimated ln(hsCRP) based on ln(serum BC) adjusted for age, sex, and ethnicity. Associations between given variables were estimated using Pearson correlations coefficients. Adjustments of covariates were conducted by stratification for CRP, serum BC concentration, age, sex, smoking status, total caloric intake, alcohol consumption. Multivariable linear regression modeled serum BC concentrations (ln transformed) on BMI adjusted for hsCRP, 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.

IV. Results

Analysis included 3,668 participants, comprised of 1,782 men (48.6%) and 1,886 women (51.4%) from the nationally representative survey. The mean age was 46.9±0.6 years overall, with no difference between mean age of men and women (48.5 years versus 49.9 years, respectively; p=0.07). Table 2.1 shows other participant demographics. Mean and SE for serum BC is 13.87±0.01 mg/dL, hsCRP was 0.18±0.04 mg/dL, and BMI was 28.3±0.2 kg/m^2. Serum BC concentrations ranges from 0.58 μg/dL to 411.58 μg/dL with a greater than 6-fold difference in the mean concentration from the lowest to the highest quintiles (Table 2.2). Demographic characteristics across quintiles of serum BC concentrations are shown in Table 2.2. When comparing the participants with highest concentrations of serum BC to those with the lowest, the participants were more likely to be women, NH white, older (70+), and categorized as having a normal BMI. An increase in age was found from 40.1 years to 53.5 years by increase of quintiles of BC ($p<0.0001$). There were significant differences present in distribution of sex and BMI category across quintiles of BC. Race/ethnicity and education level did not show any significant differences in distribution by quintiles of serum BC concentrations. Participants in the highest quintile for serum BC concentrations had the lowest hsCRP concentrations. All quintiles of hsCRP concentrations were within the normal range (0.05-0.30 mg/dL).

The proportion of obesity was significantly higher in women compared to men ($p<0.0001$) as seen in Table 2.4. In assessing the highest quintiles of serum BC

concentrations compared to the lowest, participants with the highest concentrations of BC have lower average BMI (25.7 kg/m^2) than those with the lowest concentrations (31.0 kg/m^2). Moreover, the mean serum hsCRP concentrations for individuals with a BMI categorized as Class II obesity and Class III obesity were defined as high (>0.30 mg/dL), whereas the mean serum hsCRP concentrations for all other BMI classifications (underweight, normal, overweight, and Class I obesity) were within the normal range (Figure 2.1). There was a 1.9-fold increase when comparing the mean hsCRP concentrations of individuals with a normal BMI compared to those that are overweight, a 2.9-fold increase between normal and Class I obesity, a 5.1-fold increase between normal and Class II obesity, and a 7.8-fold increase between normal and Class III obesity.

Table 2.1. Weighted participant demographics of NHANES 2003-2006 sample of adults ranging from 18-85 years (*n=3,668*).

Variable	*Number of Participants*	*Percent Sample*
Sex		
Men	1782	48.58%
Women	1886	51.42%
Ethnicity		
Mexican American	795	21.67%
Other Hispanic	111	3.03%
Non-Hispanic White	1848	50.38%
Non-Hispanic Black	759	20.69%
Other-Multiracial	155	4.23%
Age in years		
18-30	932	25.41%
31-50	926	25.25%
51-70	957	26.09%
70+	853	23.36%
Education		
Less than HS diploma	1094	29.83%
HS diploma or equivalent	901	24.56%
More than HS	1668	45.47%
Unknown/refused	5	0.14%
Income to Poverty Ratio		
Less than 1.0	653	17.80%
1.0-5.0	1388	37.84%
Greater than 5.0	578	15.76%
Missing	215	5.86%
BMI Category		
Underweight	61	1.66%
Normal	1148	31.30%
Overweight	1293	35.25%
Class I Obesity	707	19.27%
Class II Obesity	280	7.63%
Class III Obesity	179	4.88%

Table 2.2. Participant demographic characteristics by serum BC concentration in quintiles; NHANES 2003-2006 (*n=3,668*)

Variables	*All (n)*	*Q1 n=736 (% sample)*	*Q2 n=731 (% sample)*	*Q3 n=734 (% sample)*	*Q4 n=733 (% sample)*	*Q5 n=734 (% sample)*	*p-for trend**
Serum BC (µg/dL), mean±SE	13.9±0.1	4.5± 0.1	8.8± 0.1	13.3± 0.1	16.8± 0.1	27.4± 0.1	<0.0001
Sex							<0.0001
Men	1782	431 (11.8)	410 (11.2)	366 (10.0)	312 (8.5)	263 (6.4)	
Women	1886	305 (8.3)	321 (8.8)	368 (10.0)	421 (11.5)	471 (12.8)	
Race/ Ethnicity							0.27
Mexican American	795	163 (4.4)	172 (4.7)	179 (4.9)	170 (4.6)	111 (3.0)	
Other Hispanic	111	25 (0.7)	17 (0.5)	25 (0.7)	20 (0.5)	24 (0.7)	
NH White	1848	313 (8.5)	351 (9.6)	379 (10.3)	375 (10.2)	430 (11.7)	
NH Black	759	204 (5.6)	170 (4.6)	126 (3.4)	133 (3.6)	126 (3.4)	
Other-Multiracial	155	31 (0.8)	21 (0.6)	25 (0.7)	35 (1.0)	43 (1.2)	
Age (years), mean±SE	46.9±0.6	40.1±0.6	44.5±0.6	47.9±0.7	49.4± 0.7	53.5± 0.7	<0.0001
18-30	932	314 (8.6)	242 (6.6)	176 (4.8)	127 (3.5)	73 (2.0)	
31-50	926	189 (5.2)	181 (4.9)	192 (5.2)	194 (5.3)	170 (4.6)	
51-70	957	156 (4.3)	193 (5.3)	184 (5.0)	198 (5.4)	226 (6.2)	
70+	853	77 (2.1)	115 (3.1)	182 (5.0)	214 (5.8)	265 (7.2)	
Education							0.24
< HS diploma	1094	245 (6.7)	235 (6.4)	217 (5.9)	216 (5.9)	181 (4.9)	
HS diploma or =	901	15 (0.4)	8 (0.2)	12 (0.3)	12 (0.3)	13 (0.4)	
> than HS	1668	254 (6.9)	292 (8.0)	316 (8.6)	315 (8.6)	376 (10.3)	
Unknown/ refused	5	1 (<0.1)	0	2 (<0.1)	0	2(<0.1)	
Income: Poverty							
< 1.0	653	143 (3.9)	129 (3.5)	122 (3.3)	97 (2.6)	61 (1.7)	<0.0001
1.0-5.0	1388	347 (9.5)	372 (10.1)	376 (10.3)	384 (10.5)	377 (10.3)	
>5.0	578	58 (1.6)	74 (2.0)	80 (2.2)	104 (2.8)	145 (4.0)	

*variables were compared across quintiles of serum BC using ANOVA or Rao- Scott χ^2 analysis

Table 2.3. Participant health characteristics by serum BC concentration in quintiles; NHANES 2003-2006 (*n=3,668*)

Variables	*All (n)*	*Q1 n=736 (% sample)*	*Q2 n=731 (% sample)*	*Q3 n=734 (% sample)*	*Q4 n=733 (% sample)*	*Q5 n=734 (% sample)*	*p-for trend**
BMI (kg/m^2), mean±SE / BMI Category	28.3± 0.2	31.0± 0.3	29.6± 0.2	28.0± 0.2	27.4± 0.2	25.7± 0.2	<0.0001
Underweight	61	11 (0.3)	16 (0.4)	12 (0.3)	10 (0.3)	12 (0.3)	
Normal	1148	173 (4.7)	189 (5.2)	222 (6.1)	249 (6.8)	315 (8.6)	
Overweight	1293	226 (6.2)	229 (6.2)	273 (7.4)	288 (7.7)	277 (7.6)	
Class I Obesity	707	170 (4.6)	164 (4.5)	156 (4.3)	120 (3.3)	97 (2.6)	
Class II Obesity	280	80 (2.2)	78 (2.1)	45 (1.2)	48 (1.3)	29 (0.8)	
Class III Obesity	179	76 (2.1)	55 (1.5)	26 (0.7)	18 (0.5)	4 (0.1)	
hsCRP (mg/dL), mean±SE	0.18±0.04	0.28±0.05	0.21±0.05	0.17±0.04	0.15±0.05	0.12±0.04	<0.0001

*variables were compared across quintiles of serum BC using ANOVA or Rao- Scott χ^2 analysis

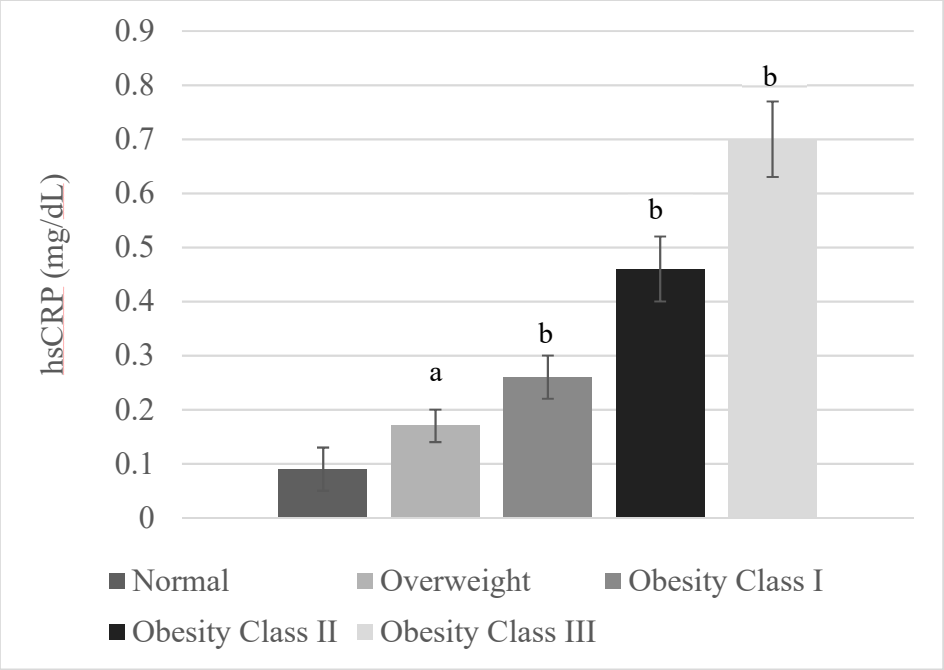
Pearson correlation suggests a moderate, negative correlation between serum BC and BMI, r=-0.22 (Figure 2.2a). When adjusted for age, sex, and race/ethnicity, a stronger negative association was present (r=-0.24, $p<0.001$). Serum BC concentrations were also moderately correlated with age and hsCRP (r=0.18 and -0.12, respectively). Multivariable linear regression modeled hsCRP on serum BC concentrations adjusted for age, sex, race/ethnicity, and reported dietary BC intake, showing a significant negative association (Table 2.5). There was also a significant inverse relationship present when modeling serum BC concentrations on BMI adjusted for age, sex, race/ethnicity, and reported dietary BC intake (β=-0.04±0.002, 95% CI (-0.05, -0.04)). Table 2.4 shows that when BMI was added to the model, the relationship between hsCRP and serum BC concentrations was attenuated toward the null compared to the crude model.

Table 2.4. Participant characteristics by WHO BMI classifications; NHANES 2003-2006 (*n=3,668*)

Variables	*All*	*Under-weight (n=61)*	*Normal (n=1,148)*	*Overweight (n=1,293)*	*Class I Obesity (n=707)*	*Class II Obesity (n=280)*	*Class III Obesity (n=179)*	*p-value**
Sex								<0.0001
Men	1782	29	512	717	353	117	54	
Women	1886	32	636	576	354	163	125	
Race/Ethnicity								<0.0001
Mexican American/ Other Hispanic	906	10	263	351	184	65	33	
Non-Hispanic White	1848	31	592	676	349	125	75	
Non-Hispanic Black	759	14	209	229	158	83	66	
Other-Multiracial	155	6	84	37	16	7	5	
Age (years), mean±SE		35.7± 0.6	43.6± 2.6	49.8 ±0.5	48.3± 0.6	49.8± 0.9	45.7± 1.1	<0.0001
18-30	932	42	451	234	114	51	40	
31-50	926	4	236	341	216	69	60	
51-70	957	4	204	358	219	114	58	
70+	853	11	257	360	158	46	21	
hsCRP (mg/dL), mean±SE		0.07± 0.17	0.09± 0.04	0.17± 0.03	0.26± 0.04	0.46± 0.06	0.70± 0.07	<0.0001

*variables were compared across quintiles of serum BC using ANOVA or Rao- Scott χ^2 analysis

Figure 2.1. Mean hsCRP concentrations (mg/dL) by WHO BMI Classifications from NHANES 2003-2006 sample (*n*= 3,668)



[a] indicates p<0.001 compared to normal BMI, [b] indicates p<0.0001 compared to normal BMI

Figure 2.2.a. Pearson correlations between ln(serum BC) concentrations and BMI; NHANES 2003-2006 (*n=3,668*)

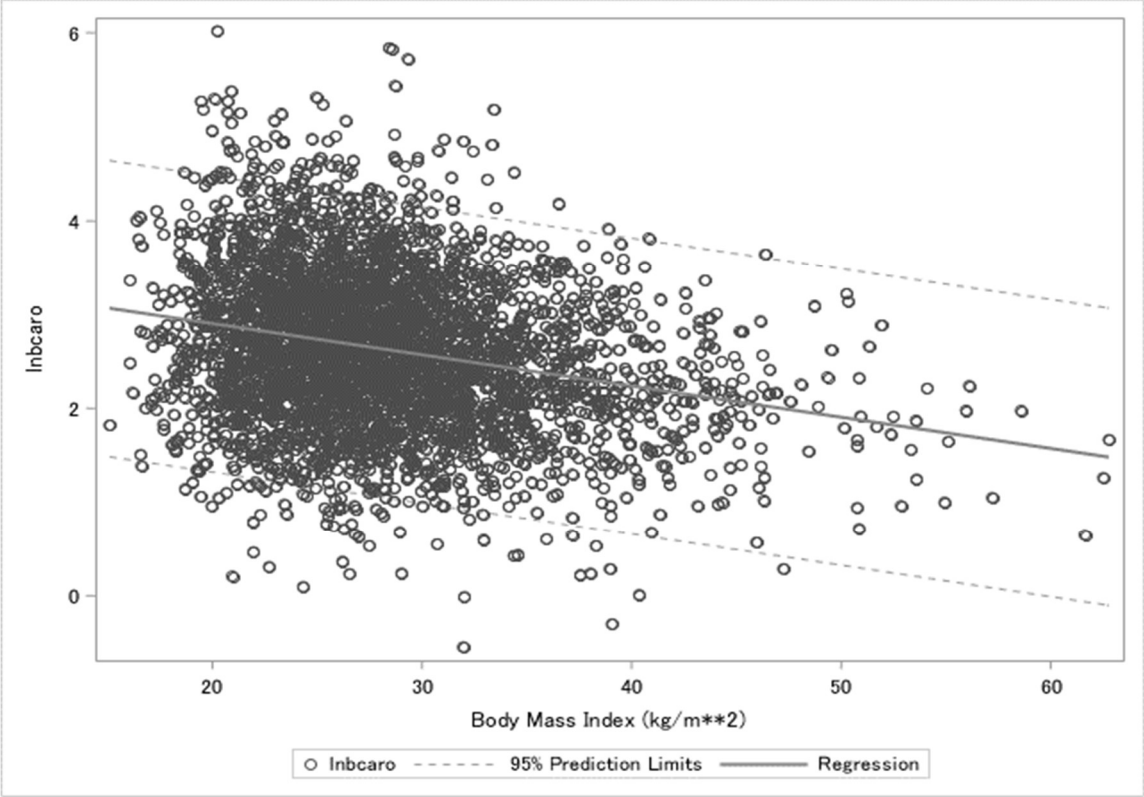


Figure 2.2.b. Pearson correlations between ln(hsCRP) and ln(serum BC) concentrations; NHANES 2003-2006 (*n=3,668*)

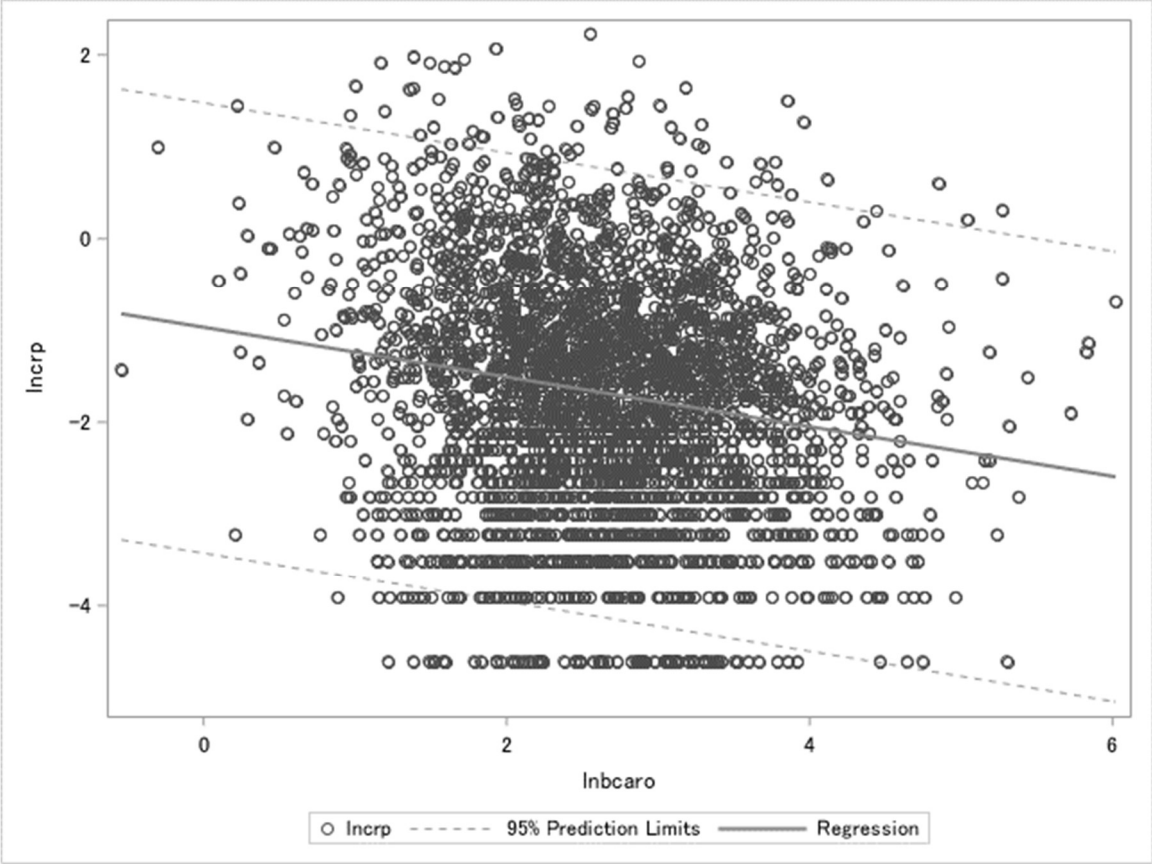


Table 2.5. Linear regression modeling for the association between ln(hsCRP) concentrations and ln(serum BC) concentrations; regression coefficients and SE for ln(serum BC); NHANES 2003-2006 (*n=3,668*)

models	***β***	***95% CI***	***P for trend***
1	-0.36± 0.03	(-0.43; -0.29)	<0.0001
2	-0.51± 0.03	(-0.57; -0.44)	<0.0001
3	-0.51± 0.04	(-0.58; -0.43)	<0.0001
4	-0.29± 0.04	(-0.37; -0.21)	<0.0001

1: crude; 2: age, sex, & race/ethnicity adjusted; 3: age, sex, race/ethnicity, & reported dietary BC intake adjusted; 4: age, sex, race/ethnicity, reported dietary BC & BMI adjusted

V. Discussion

There are significant inverse associations present among serum BC concentrations and BMI in this cross-sectional study. This observation is unaffected by adjustment for confounding factors, suggesting a potential anti-adipogenesis effect of BC. Additionally, individuals with the highest mean serum BC concentrations had the lowest mean hsCRP concentrations. The present association between hsCRP and serum BC concentrations, which is partially mediated by BMI, suggests a protective effect of increased serum BC concentrations against low-grade, systemic inflammation often associated with adipose tissue dysfunction present in obese individuals. These factors may also contribute to the increased risk of cardiometabolic diseases, particularly in obese individuals. However, this association may be due to the antioxidant capacity of BC itself, or as BC is a marker of fruit and vegetable intake, and the effect is a result of other unmeasured factors such as fiber or reduced caloric intake in a high-plant food diet.

Several studies have assessed the relationship between serum BC concentrations and BMI, reporting an inverse relationship between obesity and serum BC concentrations[16,35,100,111,119]. The direct relationship between BC and adiposity was still unclear, however it has been hypothesized that BC has a direct effect on the function of adipocytes. Interestingly, research by Harari *et al* found that BC was the carotenoid with the highest concentrations in both serum and adipose tissue; BC was 40% of the proportion of carotenoids present in the serum, and 26% in adipose

tissue[83]. Amengual *et al* found that supplementing BC in vivo in mice models showed a 28% decrease in body fat, down regulation of gene expression in WAT, and reduction in adipocyte size ($p<0.05$)[100]. Moreover, Mercader *et al* discovered a dose-dependent decrease in body weight ($p<0.001$) and WAT size ($p<0.005$) independent of energy intake in mice[35]. These rodent studies provide evidence in support of BC playing a role in adipocyte differentiation, lipid metabolism, and gene modulation of adipocytes. Östh *et al* also found that there was a significant, inverse relationship between BC concentrations in isolated human adipocytes and BMI of donor subjects ($p<0.02$); those with higher adiposity (defined as BMI ≥ 28) had a significantly lower concentration of BC stored in their isolated adipocytes compared to lean counterparts after adjusting for total body fat[107].

A complicated relationship has been presented between serum carotenoid concentrations, BMI, and inflammation. Our study supports the findings of other research as there is an inverse association between CRP and serum BC concentrations, and CRP was lowest in the participants with the highest serum BC concentrations. A longitudinal study by Mazidi *et al* assessing the relationship between serum antioxidants concentrations and inflammatory markers suggested that serum BC was negatively associated with CRP concentrations[119]. A study assessing diet quality and inflammation found an inverse relationship between serum BC concentrations and CRP ($r=-0.51$, $p=0.016$). Significant reductions in CRP were not associated with any other carotenoids[118]. In assessing the relationship

between BMI and CRP, Visser *et al* found a positive correlation between BMI and CRP concentrations with obese men and women being 2.13 and 6.21 times, respectively, more likely to have elevated CRP compared to the normal-weight respondents[52]. Similar results were ascertained in a study by Ishii *et al* assessing the relationship of sex, obesity, and CRP; CRP was more likely to be elevated in women than in men, and in individuals that were categorized as obese (BMI >30 kg/m^2)[55].

Strengths of this study include using the NHANES dataset, a large and nationally representative dataset, organized by a stratified, multistage, probability sampling, and designed to be representative of the US population[125]. For these reasons, these findings can be generalized to the US population. Data were combined from two 2-year cycles to obtain statistically significant estimates for the subgroups of interest. However, the NHANES dataset was collected using cross-sectional methods, which presented a limitation of temporality. The current analysis was only able to use hsCRP as a marker of inflammation, which may not capture the full scope of systemic inflammation associated with obesity. Additionally, this study accounted for several demographic and lifestyle factors, but potential for effects of other confounding factors may not have been captured.

In summary, substantial negative associations were found among serum BC concentrations and hsCRP concentrations. These factors may contribute to the

increased risk of cardiometabolic diseases, particularly in obese individuals in the US population. Our data is the first to identify BMI as a partial mediator in the association between inflammation and serum BC in population samples.

CHAPTER 3

Self-eported dietary β-carotene intakes are moderately associated with serum β-carotene concentrations in adults

Ambria C. Crusan[1], Marla Reicks[1], Ryan T Demmer[2], David R. Jacobs, Jr[2], Susan K. Raatz[1]

[1] Department of Food Science and Nutrition, University of Minnesota, Minneapolis, MN

[2] Division of Epidemiology, School of Public Health, University of Minnesota, Minneapolis, MN

I. Abstract

Serum β-carotene (BC) concentrations are not reflective of dietary BC status as serum concentrations can be variable as a result of intake, lifestyle factors, and physiological factors related to digestion and absorption. However, longitudinal studies have shown a positive relationship between serum carotenoids and dietary BC intake, but the relationship between BC concentrations and reported dietary BC intake in population samples is not well understood. The primary objective was to determine the association between reported dietary BC intakes and serum BC concentrations, controlling for body mass index (BMI), age, sex, and ethnicity. The relationships between serum BC concentrations and reported dietary intake of BC were estimated using data from National Health and Nutrition Examination Surveys (NHANES)/What We Eat in America (WWEIA) survey 2003-2006 for 2,580 male and non-pregnant female participants aged 20-85 years in the United States (US). The distributions of reported dietary and serum BC concentrations were skewed, therefore natural log (ln) was used to transform the data. Multivariable linear regression estimated serum BC concentrations based on reported dietary intake of BC adjusted for age, sex, race/ethnicity, BMI and body fat percentage. Mean serum BC concentrations were 14.59± 0.1 μg/dL, BMI was 27.80± 0.1kg/m^2, android body fat percentage (AF%) was 38.0± 0.2%, and gynoid body fat percentage (GF%) was 37.4± 0.3%. A moderate association was present between serum BC and reported dietary BC intake, $r= 0.30$, $p<0.0001$; a weak negative correlation was seen between other dietary factors, whereas a moderate inverse association was seen between age and anthropometric factors BMI and AF%. GF% showed no

significant correlation. The multivariable linear model predicts that for every 10µg increase in reported dietary BC intake, serum BC concentrations increased by 1.7µg/mL ($p<0.0001$). There was a moderate association present between reported dietary BC intakes and serum BC concentrations. Additionally, a moderate association was present between serum BC and anthropometric factors related to higher adiposity, suggesting individuals with an increased BMI and/or body fat percentage may have a greater risk of low serum BC concentrations despite dietary BC intake.

II. Introduction

Serum BC concentrations may not be reflective of dietary BC status. BC is the primary carotenoid in the diet[61,133] and has highest quantities in serum[81,82] out of over 700 species of these fat soluble, plant-derived pigments[64,65]. Serum carotenoid concentrations are the best biological indicator of fruit and vegetable consumption in the diet[89,131]. However, serum concentrations of carotenoids can be variable as a result of intake, lifestyle factors, and physiological factors related to digestion and absorption of BC[66,85,89]. A positive relationship between serum carotenoids and dietary intake are evident in longitudinal studies[102,105,111,115,116,134,135]. However, the relationship between dietary intakes of BC and serum BC concentrations in population samples is not well understood.

Carotenoids have been well studied for a proposed role in adiposity because adipose tissue is the main storage site for BC[16,34]. Wallstrom *et al* hypothesized that serum BC concentrations are attenuated due to BC distribution between plasma and adipose sites[116]. Moreover, El-Sohemy *et al* concluded that long-term intakes of carotenoids determined by food frequency questionnaire had higher correlation with serum carotenoid concentrations than with adipose tissue concentrations. However, serum carotenoid concentrations were consistent despite reported dietary intakes in comparison to adipose tissue concentrations, which were variable. This suggested that post-consumption factors may affect adipose carotenoid concentrations more than serum concentrations[135].

The primary objective of this study was to assess the association between reported dietary BC intakes and serum BC concentrations in US adults who took part in the NHANES/ WWEIA survey between 2003 and 2006. Our secondary objective was to examine the impact of BMI and region-specific body fat percentage on the association between reported BC intakes and serum concentrations of BC.

III. Methods

Design overview

A cross-sectional analysis of the associations present between reported dietary intake of BC and serum concentrations of BC were assessed using NHANES datasets. Pearson correlation coefficients and multivariable linear modeling was

used to examine how reported dietary BC intakes would affect serum BC concentrations among US adults.

Participants and Dataset

The NHANES dataset utilizes a cross-sectional surveying method to obtain health- and nutrition-related data via selection of approximately 10,000 people in the US every two years[126]. The aforementioned data collection process is well documented by the Center for Disease Control in their laboratory manuals[131], interview information[136], and clinical examination manual[25]. For this study, variables from the NHANES datasets were compiled from demographic data, examination data, dietary data, laboratory data, and questionnaire data. The most recent serum carotenoid collection was reported in the 2003-2004 and 2005-2006 waves. Sampling includes fifteen locations across the US[125,126] and ages of participants range from 0 to 85+ years old. The data obtained on reported BC consumption and total energy intake was acquired from the WWEIA survey, where individuals complete a 24-hour dietary recall administered by a trained interviewer in the mobile examination center (MEC). The data obtained by the WWEIA interview provides self-reported food intake data, which is analyzed to determine the individual dietary constituents, such as BC and total energy intake[136]. Serum was collected from non-fasted participants by standard phlebotomy procedures in which 0.3-1.0 mL serum samples were obtained and stored in properly sealed vials and frozen at -70°C until analysis[127]. Serum trans-BC and cis-BC concentrations were determined using high performance liquid chromatography with multiwavelength

photodiode-array absorbance detection[127]. This study used the sum of cis- and trans-BC (LBXBCC in μg/dL). Multistage probability sampling was used to estimate the relationship between BMI, reported dietary BC consumption, and serum BC concentrations using data from 2,580 participants participating in the NHANES waves 2003-2004 and 2005-2006.

There were 20,470 individuals enrolled in the NHANES survey between 2003-2006. This analysis includes males and non-pregnant females aged 20-85 years who a) had recorded demographic and questionnaire data on race/ethnicity, sex, age, smoking status, b) reliably reported day 1 dietary intakes in the WWEIA survey interview, and c) participated at the mobile examination center to obtain a blood draw for laboratory analysis of serum carotenoids, anthropometric measurements such as BMI, and participated in a dual-energy x-ray absorptiometry (DXA) scan for body fat percentage. Exclusions were made for individuals who were younger than 18 years of age (n= 10,450), missing serum BC (n=13,202), BMI (n=2,834), body fat percentages via DXA (n=15,539), reported dietary intake of BC (n=9,300), pregnancy status (n= 4,968), and current smoking status (n=2,517). Participants were also excluded if they indicated they were currently pregnant (n=566) or unsure of their pregnancy status (n=21) due to weight changes during pregnancy. If BMI was less than 14 kg/m^2 (n=181) or greater than 70 kg/m^2 (n=3) participants were removed as outliers in the data, and if they were current every day smokers (n= 1,844) or smoked some days (n=376) participants were removed due to the effects

of smoking on serum carotenoid concentrations[111]. After all exclusions, 2,580 participants were included in the current analysis.

Study Variables

Age, sex (male or female), race/ethnicity (non-Hispanic (NH) whites, NH blacks, Mexican Americans, other Hispanic, and other ethnicities), and education level (less than high school (HS) diploma, HS diploma or equivalent, more than HS, and unknown/refused) were sociodemographic characteristics utilized in this study. Reported dietary BC quantities are reported on a continuous scale as there is no identified Dietary Reference Intake (DRI) specified for BC to categorize intakes as adequate or deficient[89]. The sum of cis- and trans-BC was used for serum BC concentrations in this assessment (LBXBCC in μg/dL). Although normal serum BC concentrations are recorded to range from 2.2- 122.7 mg/dL[89], assessment of serum BC concentrations are done on a continuous scale or in quintiles, as there are not specified deficiency markers[131]. BMI, android body fat percent (AF%), and gynoid body fat percentage (GF%) were used as markers of adiposity. BMI is categorized by the World Health Organization (WHO) classifications: <18.5 kg/m^2= underweight, 18.6-24.99 kg/m^2= normal, 25-29.99 kg/m^2= overweight, 30-34.99= class I Obesity, 35-39.99 kg/m^2= class II obesity, and ≥ 40 kg/m^2 = class III obesity[1]. Obesity is defined as a total body fat percentage of greater than 25% for men and greater than 30% for women[17].

Statistical Analysis

Weighted variables were created in SAS statistical software (version 9.4, Cary, NC, USA), per the Analytical Guidelines by the Center for Disease Control[125,132] to accommodate the complex survey design. There is a normal distribution across sex, ethnicity, age, BMI, however, we natural log transformed serum BC concentrations, reported dietary BC, and reported dietary lycopene (LYC) due to skewing. Multivariable linear regression estimated ln(serum BC) based on BMI and ln(dietary BC) adjusted for age, sex, and ethnicity. Adjustments of covariates were conducted by stratification of serum BC concentrations, age, sex, total energy intake, and any other variable if found to confound, such as reported dietary LYC intake and reported total energy intake. Additional models were run adjusting for other confounding variables. The use of standardized p-values, betas, odds ratios, and 95% confidence intervals were computed using a mixed linear model with statistical significance at α <0.05. The Institutional Review Board at the University of Minnesota determined this study exempt due to secondary analysis of a de-identified dataset.

IV. Results

Of the 2,580 participants from NHANES who met all criteria for this study, 49% (n=1263) were men and 51% (n=1317) were women. Mean age of the participants was 47.7± 0.5 years, with the highest percentage of participants in the 51-70 year old age group. Other participant demographics are reported in Table 3.1. Mean BMI was 27.8± 0.1 kg/m^2 and mean region-specific body fat percentages were 38.0±

0.2% for AF% and 37.4± 0.3% for GF%. Mean serum BC concentrations were 14.59± 0.1 μg/dL, ranging from 0.58 μg/dL to 411.58 μg/dL with a greater than 7.8-fold difference in the mean concentration from the lowest (5.37 μg/dL) to the highest (42.10 μg/dL) quartiles. Additionally, mean reported dietary intakes of BC, total reported fat intake, and total reported energy intakes were 828.82± 0.06 μg, 82.6± 1.3 g and 2,167± 26 kcals, respectively. Reported dietary BC intakes were ranging from 0 μg to 44,993 μg with a 40-fold difference in the mean concentration from the lowest (126.5 μg) to the highest (5064.5 μg) quartiles.

Table 3.2a shows mean total reported energy intake, total reported fat intake, reported BC intake, AF%, GF%, BMI, and age by quartiles of serum BC concentrations. Interestingly, the means of dietary factors, total reported energy intake and total reported fat intake, both decreased slightly across quartiles of serum BC concentrations, whereas reported BC intakes increased 3-fold. AF% decreased by 3.8% from the highest and lowest quartiles, whereas GF% increased by 1%. BMI also decreased from a mean of 29.75± 0.2 in the lowest quartile to 25.8± 0.2 in the highest.

Additionally, average age increased by 10 years from the lowest quartile of serum BC concentrations to the highest, but not for reported dietary BC intakes (as shown in Table 3.2b). In Table 3.2b, total reported energy and fat intakes increased across quartiles of reported BC intake, however, total reported fat intake showed a

consistent increase from quartile 1 to 3, then decreased from quartile 3 to 4. For anthropometric measures by quartiles of reported BC intake, AF%, GF% and BMI showed similar patterns to serum BC, however, were not as consistent. When comparing mean intakes of reported BC across BMI categories, there were no significant differences in mean reported intake of BC between normal weighted group and the overweight, class II obesity, and Class III obesity groups (Figure 3.1). However, there were significant differences present between serum concentrations of individuals with a normal BMI versus individuals with a BMI categorized as overweight or obese (Figure 3.2).

Table 3.1. Weighted participant demographics for US adults 20+ from NHANES 2003-2006 (*n*=2580)

Variable	Number of Participants	Percent Sample
Sex		
Men	1263	48.95%
Women	1317	51.05%
Ethnicity		
Mexican American	545	21.12%
Other Hispanic	85	3.29%
Non-Hispanic White	1385	53.68%
Non-Hispanic Black	459	17.80%
Other-Multiracial	106	4.11%
Age in years		
20-30	383	14.85%
31-50	777	30.11%
51-70	779	30.19%
70+	641	24.85%
Education		
Less than HS diploma	693	26.86%
HS diploma or equivalent	605	23.45%
More than HS	1278	49.3%
Unknown/refused	4	0.16%

Table 3.2. Mean and standard error of multiple factors across quartiles of a) serum BC concentrations and b) reported dietary intake of BC from NHANES 2003-2006 (*n*=2,580)

a)

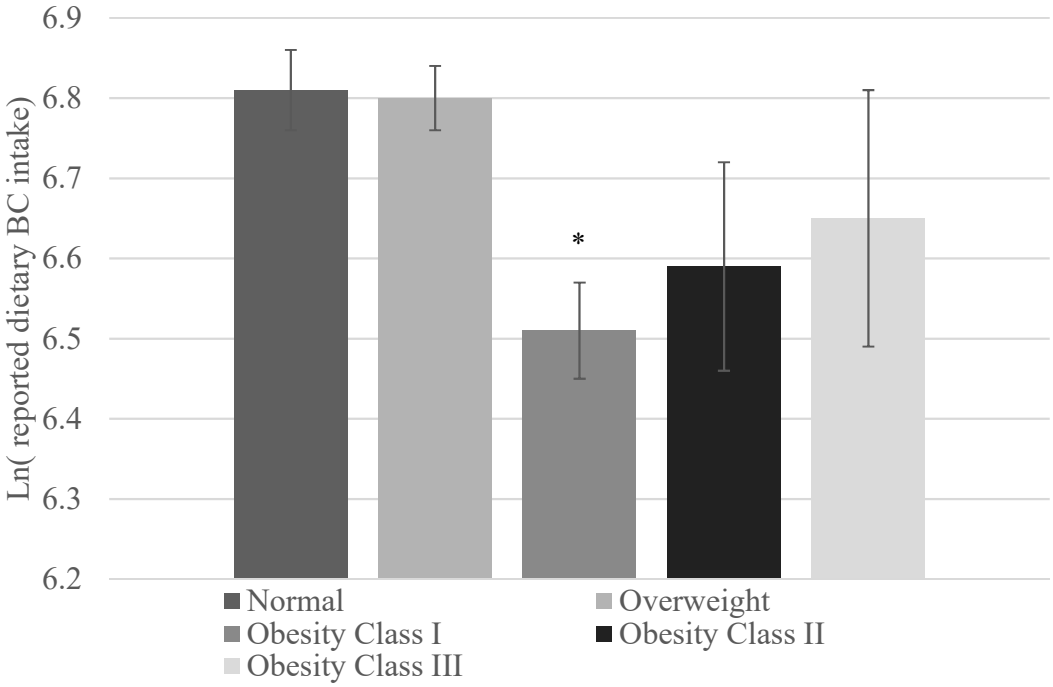
Variable	***Quartiles of serum BC concentrations (µg/dL)***				
	Q1 (n=645)	Q2 (n=645)	Q3 (n=645)	Q4 (n=645)	p for trend*
Serum BC (µg/dL), mean±SE	5.4± 0.1	11.4± 0.1	19.1± 0.1	42.1± 0.1	<0.0001
Total reported Energy intake (kcals)	2,365± 45	2,222± 39	2,138± 39	2,065± 34	0.001
Total reported Fat intake (g)	90.8± 2.0	86.0± 1.7	79.8± 1.7	77.6± 1.6	<0.0001
Reported BC intake (µg)	497.7± 0.1	720.5± 0.1	943.9± 0.1	1,571.8± 0.1	<0.0001
Android BF%	39.7± 0.3	38.9± 0.3	37.1± 0.4	35.9± 0.3	<0.0001
Gynoid BF%	37.0± 0.4	37.1± 0.3	37.5± 0.4	38.0± 0.3	0.02
BMI (kg/m^2)	29.8± 0.2	28.1± 0.2	27.3± 0.2	25.8± 0.2	<0.0001
Age (years)	42.9± 0.6	47.5± 0.6	48.8± 0.7	52.9± 0.7	<0.0001

b)

Variable	***Quartiles of Reported Dietary BC intake (µg)***				
	Q1 (n=645)	Q2 (n=645)	Q3 (n=645)	Q4 (n=645)	p for trend*
Serum BC (µg/dL), mean±SE	126.5± 0.1	492.8± 0.1	1,236.5± 0.1	5,064.5± 0.1	<0.0001
Total reported Energy intake (kcals)	1,762± 32	2,259± 38	2,410± 42	2,324± 41	<0.0001
Total reported Fat intake (g)	67.5± 1.5	87.3± 1.7	93.2± 1.9	85.9± 1.8	<0.0001
Android BF%	39.8± 0.3	37.5± 0.3	37.9± 0.3	37.0± 0.3	0.008
Gynoid BF%	36.99± 0.4	37.13± 0.3	37.51± 0.4	38.01± 0.3	0.005
BMI (kg/m^2)	28.25± 0.2	27.73± 0.2	27.81± 0.2	27.49± 0.2	0.08
Age (years)	48.0± 0.7	45.4± 0.6	48.4± 0.7	49.9± 0.7	0.007

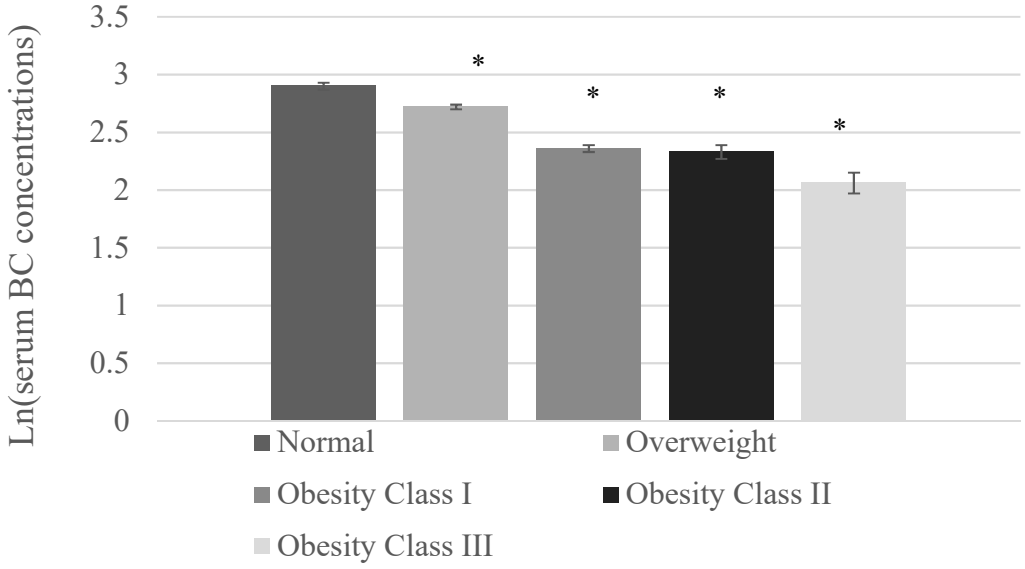
*variables were compared across quartiles of serum BC or reported dietary BC using ANOVA or Rao- Scott χ^2 analysis

Figure 3.1. Ln(reported dietary BC intake) by WHO BMI Classifications; from NHANES 2003-2006 (*n*=2,580)



*indicates p<0.01

Figure 3.2. Ln(serum BC concentrations) by WHO BMI Classifications; from NHANES 2003-2006 (*n*=2,580)



*indicates p<0.0001

A moderate association was observed between serum BC and reported dietary BC intake, r= 0.30, p<0.0001 (Figure 3.3a), however, a weak negative correlation was seen between serum BC and reported dietary fat and energy intakes, r=-0.11 and r=-0.10, respectively. BMI was the anthropometric factor showing the strongest correlation with serum BC using Pearson correlation (Figure 3.3b). GF% showed no significant correlation (Figure 3.3d). However, when adjusted for age, sex, and race/ethnicity, a stronger negative association was present between serum BC and AF% (r=-0.32, p<0.0001) and a significant negative correlation was present with GF% (r=-0.21, p<0.0001) as shown in Table 3.3. Serum BC is also moderately correlated with age (r=0.21, p<0.0001).

Table 3.3. Pearson correlations and partial correlations between serum BC concentration and dietary and anthropometric factors possibly influencing serum status from NHANES 2003-2006 (*n*= 2,580)

	Dietary Factors			***Anthropometric Factors***		
	Reported dietary BC intake (μg)	Reported total fat intake (g)	Reported total energy intake (kcal)	Body Mass index (kg/m^2)	Android body fat percentage	Gynoid body fat percentage
Crude	0.30 [a]	-0.11 [a]	-0.10 [a]	-0.29 [a]	-0.18 [a]	0.02 (p=0.18)
Partial*	0.31 [a]	-0.03 (p=0.12)	0.005 (p=0.81)	-0.31 [a]	-0.32 [a]	-0.21 [a]

*adjusted for age, race/ethnicity, and sex; [a] p<0.0001

Figure 3.3.a. Pearson correlation between reported BC intakes and serum BC concentrations from NHANES 2003-2006 (*n*=2,580)

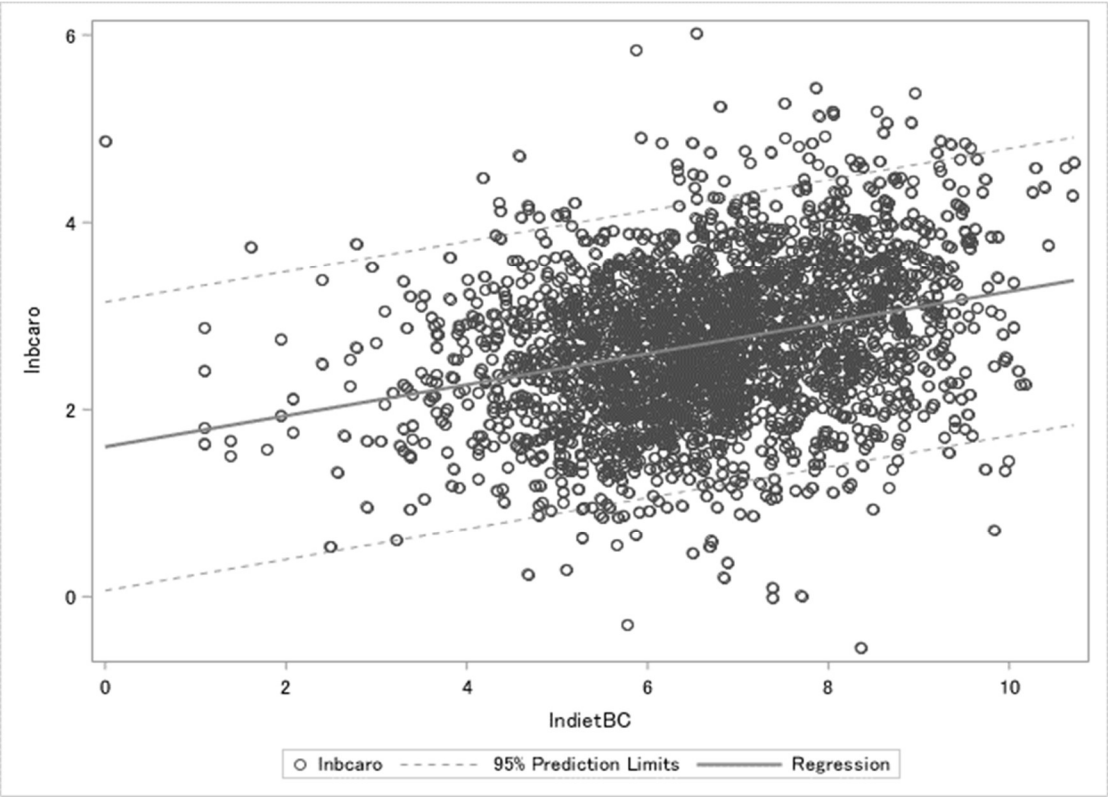


Figure 3.3.b. Pearson correlation between BMI and serum BC concentrations from NHANES 2003-2006 (*n*=2,580)

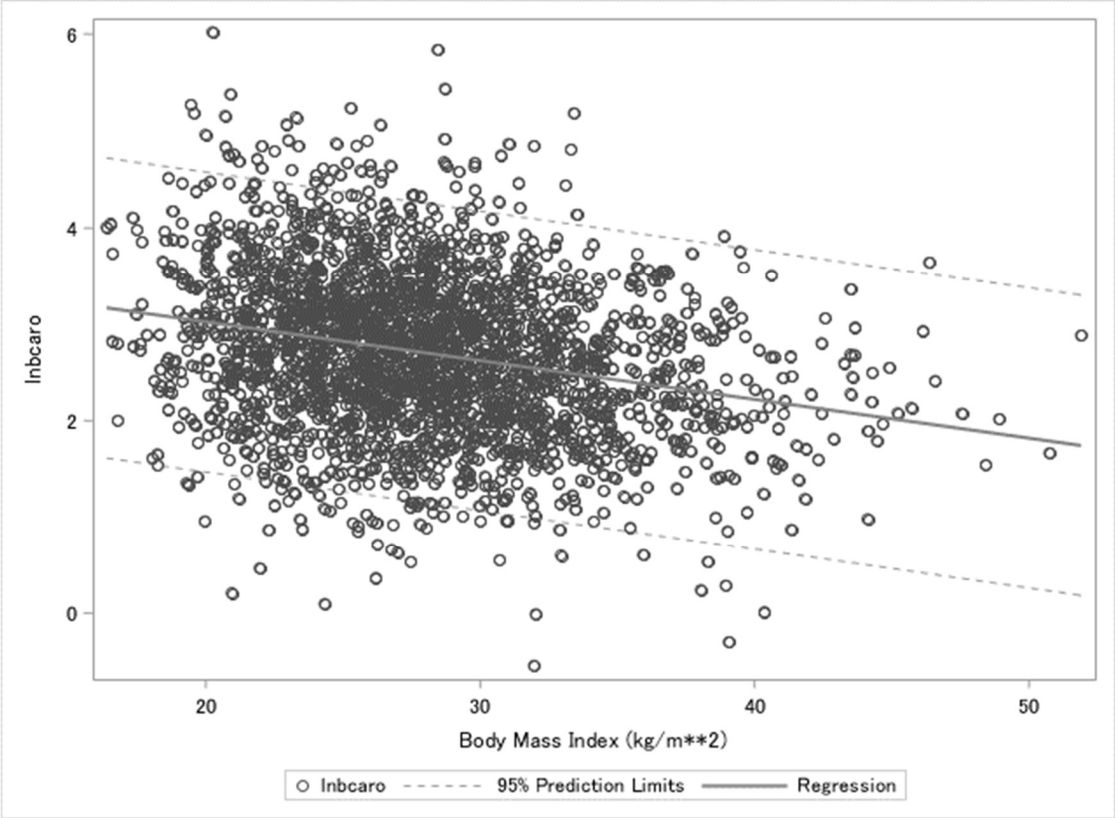


Figure 3.3.c. Pearson correlation between AF% and serum BC concentrations from NHANES 2003-2006 (*n*=2,580)

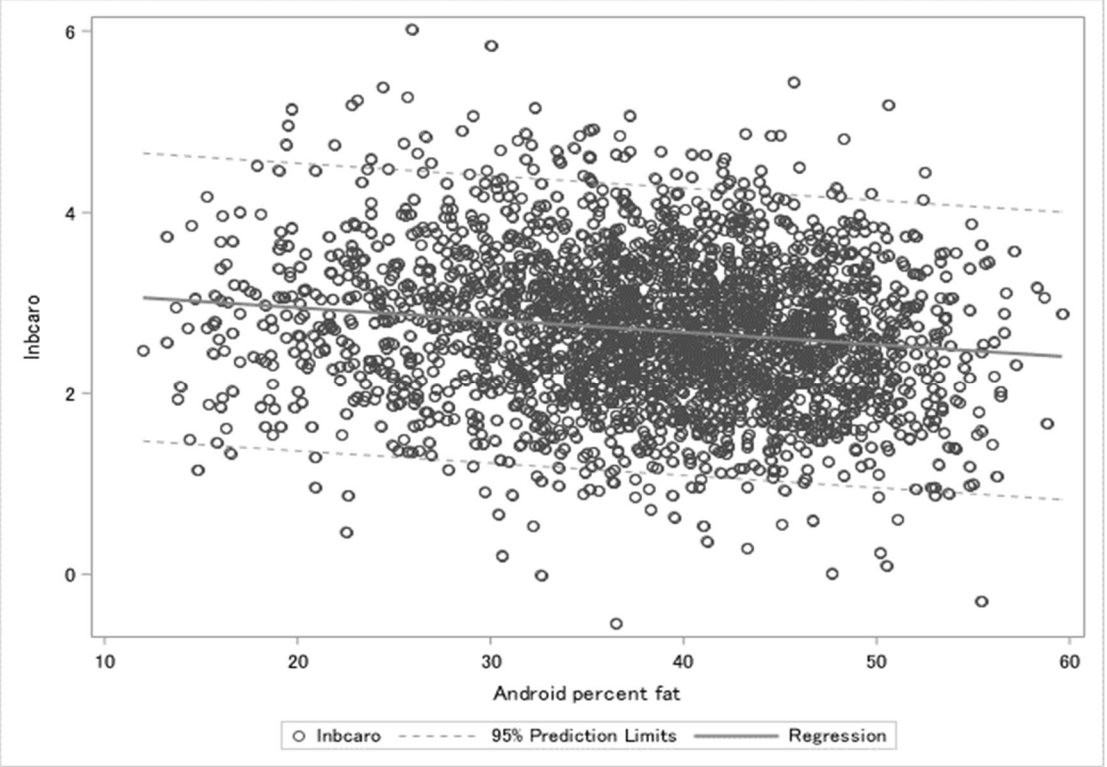


Figure 3.3.d. Pearson correlation between GF% and serum BC concentrations from NHANES 2003-2006 (*n*=2,580)

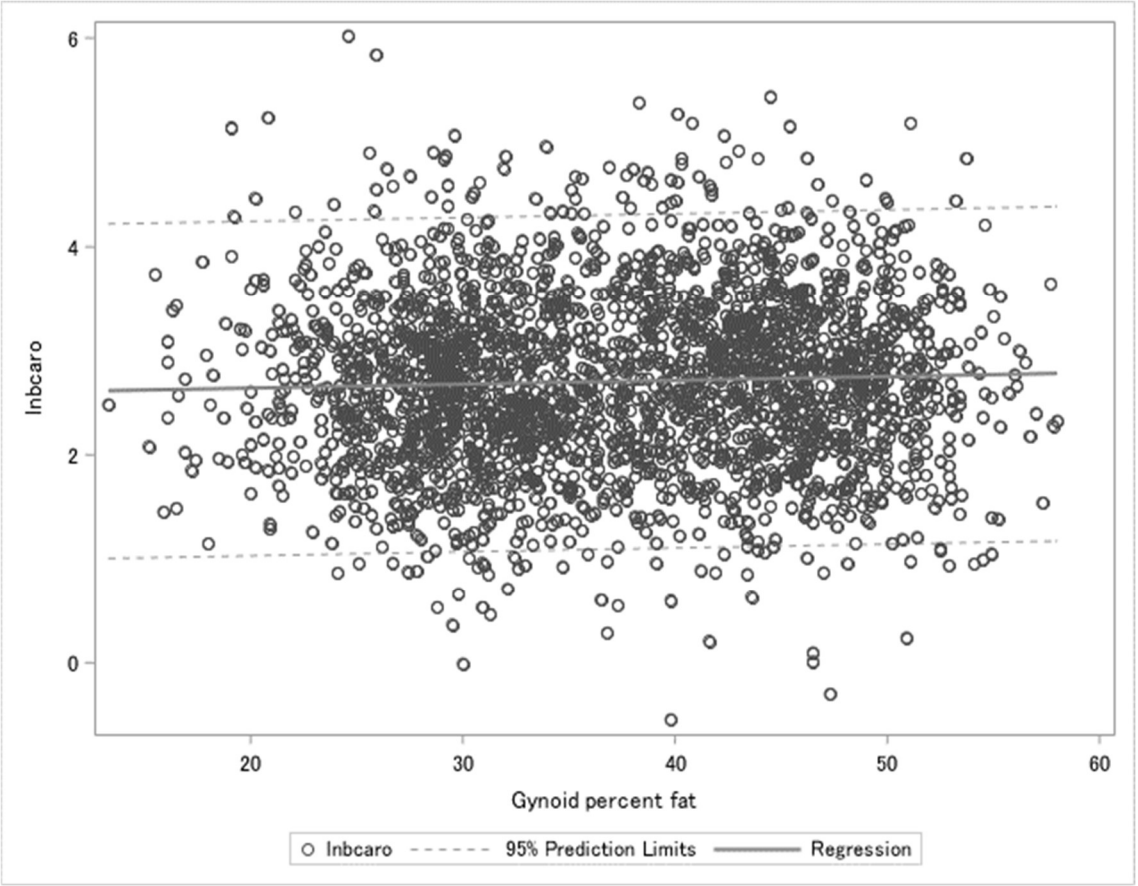


Figure 3.3.e. Pearson correlations between reported BC intakes and BMI from NHANES 2003-2006 (*n*=2,580)

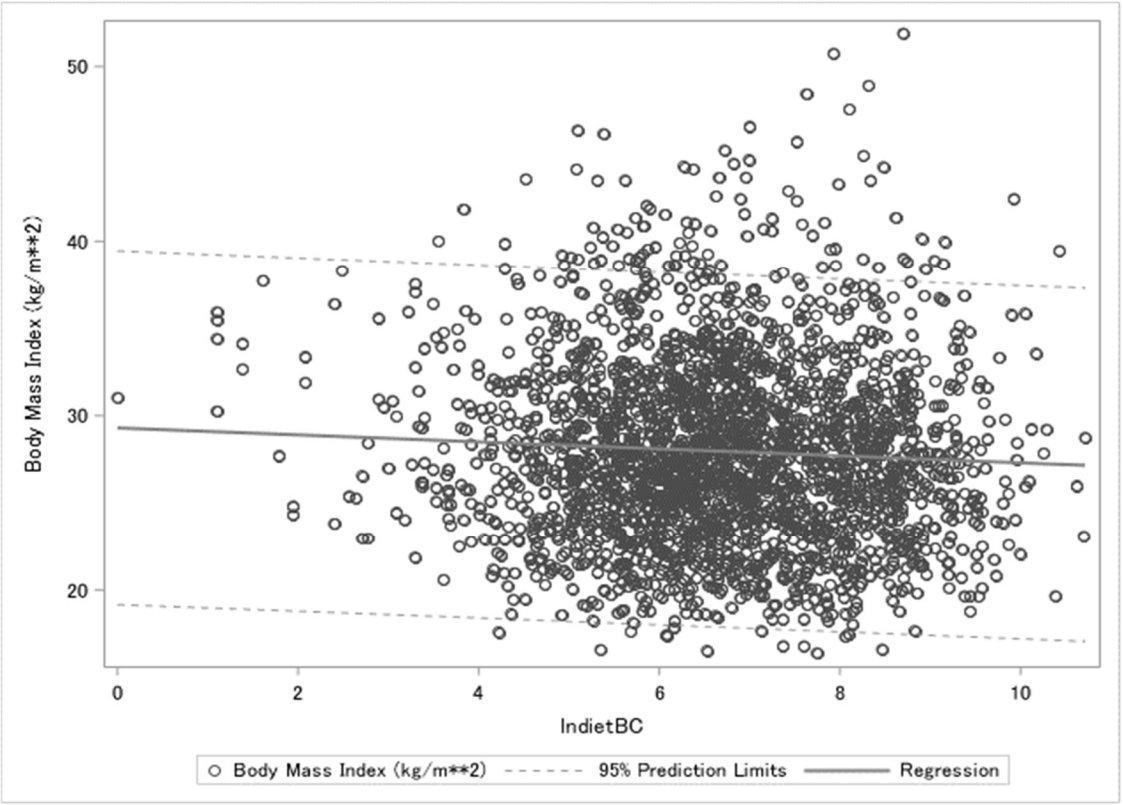


Figure 3.3.f. Pearson correlations between reported BC intakes and AF% from NHANES 2003-2006 (*n*=2,580)

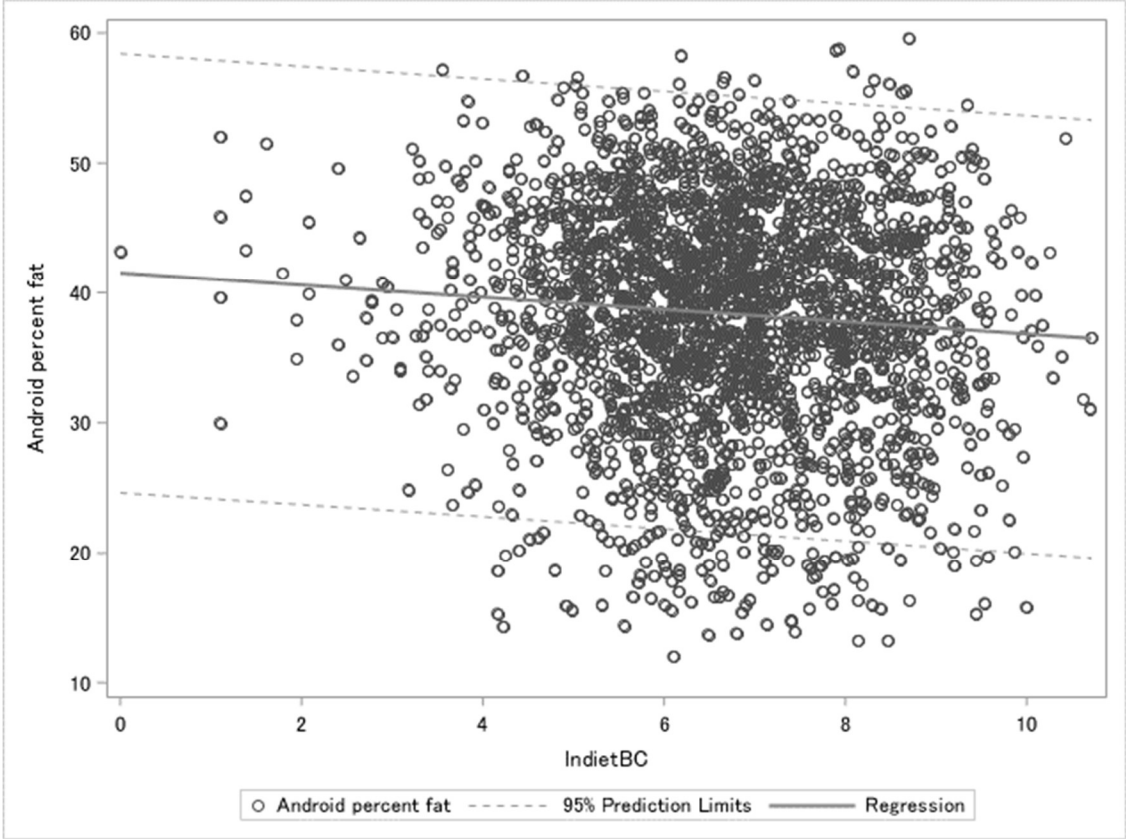


Table 3.4 shows the multivariable linear regression modeling serum BC on reported dietary BC intake, with adjustments made for age, sex, race/ethnicity, BMI, AF%, and reported dietary LYC intakes. Associations for all comparisons are shown in Table 3.4. The relationship between natural log-transformed serum BC concentrations and natural log-transformed reported dietary BC intakes remained significant despite adjustments made to the model. The model predicts that for every 10 µg increase in reported dietary BC intake, serum BC concentrations increased by 1.7 µg/mL ($p<0.0001$). Table 3.5 shows the multivariable linear regression modeling serum BC on reported dietary BC intake by BMI strata.

Table 3.4. Linear regression modeling for the association between ln(serum BC) concentrations and ln(reported dietary BC intakes); regression coefficients and SE for ln(reported dietary BC intakes); NHANES 2003-2006 (*n=2,580*)

model	***β***	***95% CI***	***p for trend***
1	0.17± 0.02	(0.14; 0.21)	<0.0001
2	0.17± 0.02	(0.13; 0.20)	<0.0001
3	0.17± 0.01	(0.14; 0.21)	<0.0001
4	0.17± 0.02	(0.14; 0.21)	<0.0001
5	0.16± 0.02	(0.13; 0.20)	<0.0001
6	0.16± 0.01	(0.12; 0.19)	<0.0001
7	0.19± 0.02	(0.16; 0.23)	<0.0001

1: crude; 2: age adjusted; 3: age & sex adjusted; 4: age, sex, & race/ethnicity adjusted; 5: age, sex, race/ethnicity, & BMI adjusted; 6: age, sex, race/ethnicity, BMI & AF% adjusted; 7: age, sex, race/ethnicity, BMI, AF% adjusted, and reported dietary LYC intakes

Table 3.5. Linear regression modeling for the association between ln(serum BC) concentrations and ln(reported dietary BC intakes) adjusted for age, sex, race/ethnicity and stratified by BMI; regression coefficients and SE for ln(serum BC); NHANES 2003-2006 (*n=2,580*)

Model by BMI Classification	***β***	***95% CI***	***p for trend***
Normal	0.21± 0.03	(0.15; 0.27)	<0.0001
Overweight	0.16± 0.02	(0.11; 0.20)	<0.0001
Class I Obesity	0.12± 0.03	(0.06; 0.18)	0.0005
Class II Obesity	0.12± 0.05	(0.01; 0.24)	0.04
Class III Obesity	0.13± 0.03	(0.05; 0.22)	0.006

V. Discussion

The current cross-sectional study shows a moderate, but positive association between serum BC concentrations and reported dietary BC intake. Since the association is not strong, it suggests that post-consumption factors may be attenuating serum carotenoid concentrations. Kabat *et al* obtained similar results when comparing serum carotenoid concentrations with dietary intake of carotenoids reported via a food frequency questionnaire in women ($r=0.19$, $p<0.001$)[16].

This moderate correlation between dietary BC intakes and serum BC concentration indicates that there may be other factors associated with the outcomes. One possible mechanism is adiposity, as Vioque *et al* obtained opposing results when they investigated the relationship between carotenoid digestion and absorption and BMI. Their research found that serum BC was positively associated with dietary intakes of BC for those with a BMI categorized as normal ($r=0.35$, $p<0.05$), overweight ($r=0.15$, $p<0.05$), and obese ($r=0.20$, $p<0.01$)[105]. Wang *et al* assessed the associations between serum BC concentrations and markers of adiposity and found significant, inverse associations with BMI, waist circumference (WC), waist-to-hip ratio, total body fat percentage (BF%), AF%, and GF%[109].

Our data suggest a moderate inverse relationship exists between serum BC and the anthropometric factors BMI and AF%, suggesting individuals with an increased android adiposity may have a greater risk of low serum BC concentrations. Harari

et al. analyzed serum and adipose tissue in subjects with BMI ranging between 30.1-48.5 kg/m^2. Serum BC concentrations were inversely correlated with weight, BMI, WC, total BF%, body fat-free mass, and central body fat. Interestingly, adipose BC concentrations did not significantly correlate with the abdominal subcutaneous or visceral fat depots, but did with total BF%. Moreover, adipose tissue BC was the only carotenoid that did not show any correlation with the aforementioned anthropometric measurements[83]. Additionally, Östh *et al* found that those with higher adiposity also have significantly lower concentrations of BC stored in their adipose tissue compared to lean counterparts ($p<0.02$)[107].

Other dietary factors such as energy intake and total reported fat intake showed weak correlation with serum BC concentrations. Carotenoids are a fat-soluble, provitamin A pigment. Therefore, the relationship between BC and dietary fat intakes is important to understand bioavailability of BC in the body. However, the low association we observed with total fat is likely due to adequate carotenoid absorption even at lowest levels of reported fat consumption. Additionally, most foods containing BC also contain other carotenoids[72]. Positive relationships between co-consumption of BC and other carotenoids have been noted, however, other studies have found that the carotenoid LYC can reduce the absorption of BC[81]. Our findings show that reported dietary LYC did not have any substantial effect on the association between reported dietary BC and serum BC concentrations.

Another factor that may be influencing serum BC concentrations is age. There was a moderate correlation between age and serum BC concentrations in this study. Although it is not a post-consumption factor, epidemiological studies show that subgroups among populations with the highest serum carotenoid concentrations, especially BC, are older[114], female[105,114,120], and/or non-smoking individuals[111,114,120]. Additionally, BC concentrations in adipose of the abdomen and buttock were positively correlated with age ($p<0.05$) [102]. This significant relationship with age may be due to the association of higher BC intakes with age[137].

There are multiple strengths identified in this study, including the use of a large, nationally representative dataset, NHANES[125]. This data was collected using multistage, probability sampling to be reflective of the representation in the US, allowing generalization of the findings to the US population. Additionally, trained professionals collected objective data points in obtaining the demographic, laboratory, and anthropometric data; the self-reported data collected through WWEIA was conducted through approved protocol. Limitations to a cross-sectional dataset also apply, as temporality is not able to be determined. Additionally, we only had access to self-reported dietary intakes of BC, fat, and energy through a 24-recall from WWEIA, which may not be as accurate as monitored or directly measured intakes. Moreover, only one carotenoid was assessed, where most foods containing BC also contain other carotenoids[72]. Several post-consumption factors

were accounted for in this study, however, there is still potential for effects of other confounding factors or unmeasured variables that may not have been assessed. Other limitations to this study include possible misclassification of participants due to self-reporting, random error, within-person variability of nutrients assessed, and bias (recall, social desirability, etc.) related to dietary recall collections.

In summary, an association was found between reported dietary BC intakes and serum BC concentrations, however the association was not as strong as expected, suggesting post-consumption factors are attenuating serum concentrations of BC. Additionally, a moderate association was present between serum BC and anthropometric factors related to higher adiposity, suggesting individuals with an increased BMI and/or body fat percentage may have a greater risk of lower serum BC concentrations despite dietary BC intake.

CHAPTER 4

Serum β-carotene concentrations are inversely associated with self-reported fat intake in United States adults

Ambria C. Crusan[1], Marla Reicks[1], Ryan T Demmer[2], David R. Jacobs, Jr[2], Susan K. Raatz[1]

[1] Department of Food Science and Nutrition, University of Minnesota, Minneapolis, MN

[2] Division of Epidemiology, School of Public Health, University of Minnesota, Minneapolis, MN

I. Abstract

The main source of dietary β-carotene (BC) is fruits and vegetables. The bioavailability of BC is dependent on dose, quantity and dispersion, and presence of other nutrients in the diet, specifically fat. However, there is a gap in research on whether specific fatty acid classes affect serum BC concentrations. The primary objective was to assess the association between serum BC concentrations and reported intake of specific fatty acid classes, utilizing data from the What We Eat in America (WWEIA) and National Health and Nutrition Examination Surveys (NHANES). Data from 3,278 male and female participants 20-85 years of age in the NHANES 2003-2006 nationally representative, cross-sectional survey were analyzed to estimate the relationships between serum BC concentrations and reported saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acid intakes. Due to skewing, we natural log transformed serum BC and reported dietary BC. 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) was 14.31±0.05μg/dL for serum BC concentrations. Means and SEs for total fat, SFA, MUFA, and PUFA were 85.73±1.3g, 26.88±0.4g, 31.14±0.5, and 17.8±0.4g, respectively. After adjusting for age, sex, and race/ethnicity, serum BC concentrations were associated with fatty acid classes: SFA ($r= -0.07$, $p<0.0001$), MUFA ($r=-0.05$, $p<0.006$), and PUFA ($r=0.05$, $p<0.005$). There was a trend for association between serum BC and reported total fat intakes ($r=-0.03$, $p=0.06$) when adjusted for age, sex, and race/ethnicity. Multiple linear regression modeling showed positive, but weak

associations between serum BC concentrations and increased reported dietary PUFA consumption. PUFA intakes are positively associated with serum BC concentrations. An inverse association between specific fatty acid classes suggests there may be multiple post-digestion factors affecting serum carotenoid concentrations.

II. Introduction

The dietary carotenoid, β-carotene (BC), is a fat-soluble antioxidant found in fruits and vegetables. The 2015-2020 Dietary Guidelines for Americans recommend most adults consume approximately 2 cups of fruits and 2.5 cups of vegetables daily, as research has tied consumption to a reduced risk for many chronic diseases[138]. Low serum BC status is associated with increased cardiometabolic disease (CMD) risk[10,112]. An inverse relationship was observed between serum BC and hypertension ($p<0.01$)[113], dyslipidemia($p<0.029$)[8], waist circumference ($p<0.001$) and Metabolic Syndrome ($p<0.001$)[16]. Moreover, research on mortality in United States (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[114]. Therefore, understanding the relationship between BC and dietary fat intakes regarding bioavailability in the body is important.

The bioavailability of carotenoids is complex and dependent on dose[73,74], quantity and dispersion throughout the day[65], and presence of other nutrients in the diet,

specifically fat[65,75–77,85]. 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 28g of fat versus 0g[78]. Additionally, the intestinal absorption of carotenoids varies by the chemical structure of the carotenoid[66], release of the carotenoid from the food matrix[79] and intestinal cleavage of BC to retinol[65,79]. BC still bound to its food matrix and not solubilized in a micelle limits absorption, ultimately affecting circulating concentrations of BC[80]. 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[84].

However, there is a gap in research on whether total fat intakes and specific fatty acid 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 fatty acid is important to better understand the bioavailability of carotenoids in foods. Our primary objective was to assess the association between serum BC concentrations and reported intake of total fat and specific fatty acid classes in US adults, utilizing the What We Eat in America (WWEIA) and National Health/Nutrition Examination Surveys (NHANES) data.

III. Methods

Design overview

Cross-sectional evaluation of data from the demographic, anthropometric, laboratory, dietary, and questionnaire components of NHANES were analyzed to determine associations present between serum BC, total reported fat intakes and reported intake of specific fatty acid classes. Multivariable linear modeling 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 5,000 people per year were selected from fifteen locations across the US[125] with data released on a two-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[126]. NHANES used trained personnel to conduct dietary interviews using the WWEIA survey in partnership with the U.S. Department of Agriculture and the U.S. Department of Health and Human Services[126], perform clinical examinations, and obtain laboratory measurements in Mobile Examination Centers (MEC) to compile the data collected[18]. The reported dietary intakes for BC, total fat, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) was acquired from WWEIA survey questions, which required participants

to complete a 24-hour dietary recall administered by a trained interviewer in the MEC[136].

There were 20,470 individuals enrolled in the NHANES survey between 2003-2006. 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 intakes of total fat, SFA, MUFA, PUFA, and dietary BC in the WWEIA survey, 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 missing serum BC (n=5,567), reported dietary intake of total fat (n=1,052), SFA (n=1,052), MUFA (n=1,052), PUFA (n=1,052), dietary BC (n=1,052), and current smoking status (n=5,176). Due to the effects of smoking on serum carotenoid concentrations[111], participants were also excluded if they indicated they were current every day smokers (n= 1,844) or smoked some days (n=376). There were 3,278 participants included in the current analysis after all exclusions.

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 [18,121,125]. 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[127]. Serum trans-BC and cis-BC concentrations were determined via high performance liquid chromatography with multiwavelength photodiode-array absorbance detection[127]. 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 collected via WWEIA survey questions collected in the MEC[139]. There is no identified Dietary Reference Intake (DRI) for BC, therefore, reported dietary BC intake was noted on a continuous scale[89]. BC was the carotenoid of focus as it has both highest quantities in serum[82], with normal serum BC concentrations range from 2.2- 122.7 mg/dL[89], and has the highest consumption in the diet in comparison to other carotenoids[61].

Reported dietary fat, SFA, MUFA, and PUFA was assessed on a continuous scale, as the DRIs vary. The DRI's Acceptable Macronutrient Distribution Range (AMDR) was set as a percentage of total caloric intake. The AMDR for total fat is 20-35% of calories from fat for adults greater than 18 years[140]. The 2015-2020 Dietary Guidelines for Americans recommend SFA make up less than 10% of total calories per day[138]. Specific guidelines for PUFA set by the AMDR recommend that

5-10% of total calories are derived from Omega-6 PUFA and 0.6-1.2% of total calories from Omega-3 PUFA, however, no AMDR for MUFA has been established[140].

Statistical analysis

Weighted variables were created in SAS statistical software (version 9.4, Cary, NC, USA) according to the guidelines for analysis published by the Center for Disease Control[125,132] to accommodate the complex survey design. Distribution was assessed, finding sex, ethnicity, education level, age, and reported fat intakes (total, SFA, MUFA, PUFA) were normally distributed. Natural log transformation was used for serum BC concentrations and reported dietary BC due to skewing. Using the SURVEYMEANS procedure, the mean and standard errors were used for continuous variables and percentages for categorical variables. Multiple linear regression was used via SURVEYREG to estimate ln(serum BC) based on total fat or specific fatty acid 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 given variables, 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.

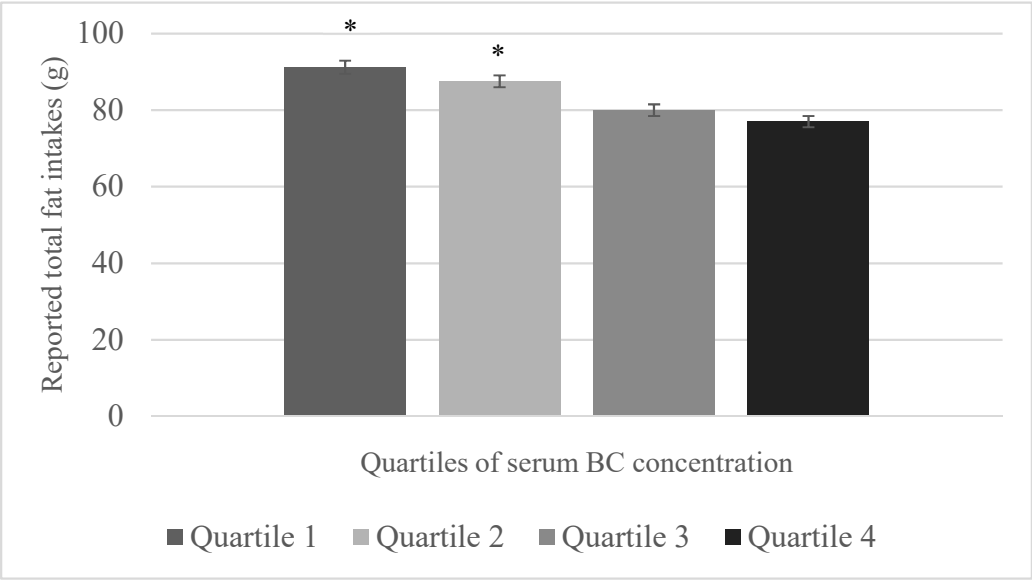
IV. Results

Of the 3,278 participants in this analysis, there were 1,493 men (45.55%) and 1,785 women (54.55%) with a mean age of 48.2±0.5 years. Other participant demographics are shown in Table 4.1. Mean and standard error (SE) was 14.31±0.05µg/dL for serum BC, with concentrations ranging from 0.43µg/dL to 422.59µg/dL. 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 shown in Table 4.2. Mean and SE for dietary factors such as reported total fat intake was 85.73±1.3g, reported SFA intake was 26.88±0.4g, reported MUFA intake was 31.14±0.5g, reported PUFA intake was 17.8±0.4g and reported dietary BC intake was 827.0±1.1µg. The first and second quartiles of serum BC concentrations showed significantly higher reported total fat consumption in comparison to individuals in the highest quartile of serum BC concentrations (Figure 4.1). Additionally, intakes for mean SFA were significantly higher in Quartile 1 and 2 versus quartile 4 of serum BC concentrations. Similar trends were observed in MUFA, however, there were no significant differences between PUFA intakes between the quartiles of serum BC concentrations (Figure 4.2).

Table 4.1. Weighted demographic characteristics for the 3,278 US adults 20+ from NHANES 2003-2006

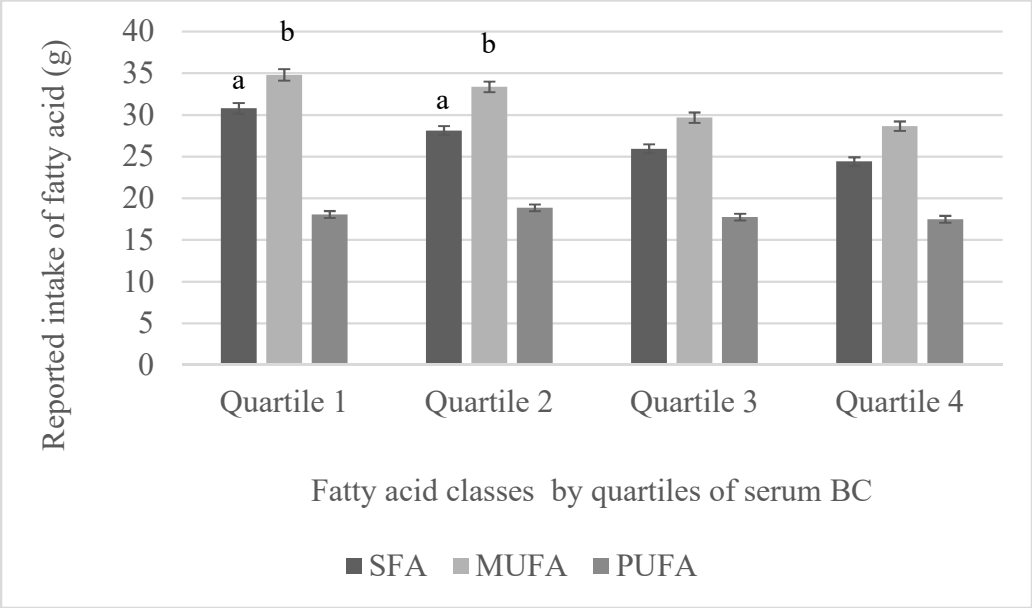
Variable	*Number of Participants*	*Percent Sample*
Sex		
Men	1493	45.6%
Women	1785	54.4%
Ethnicity		
Mexican American	684	20.9%
Other Hispanic	101	3.1%
Non-Hispanic White	1768	53.9%
Non-Hispanic Black	595	18.1%
Other-Multiracial	130	4.0%
Age in years		
20-30	539	16.44%
31-50	960	29.29%
51-70	930	28.37%
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 to 5	2035	65.56%
>5	579	18.65%

Figure 4.1 Reported total fat intakes (g) by quartiles of serum BC from NHANES 2003-2006 (*n*= 3,278)



*indicates p<0.05 from highest quartile of intake (Q4)

Figure 4.2. Reported intake of specific fatty acid classes by quartiles of serum BC concentrations from NHANES 2003-2006 (n=3,278)



[a] indicates p<0.0001 from highest quartile of intake (Q4),
[b] indicates p<0.001 from highest quartile of intake (Q4)

Table 4.2. Weighted demographic characteristics by BC concentration in quartiles from NHANES 2003-2006 (*n*=3,278)

Variables	*All (n=3,278)*	*Q1 (n=819)/ (% sample)*	*Q2 (n=820)/ (% sample)*	*Q3 (n=820)/ (% sample)*	*Q4 (n=819)/ (% sample)*	*p-value*
Mean±SE Serum BC (µg/dL)	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 to 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)	

*variables were compared across quartiles of serum BC using ANOVA and Rao- Scott χ^2 analysis

Serum BC concentrations were weakly and inversely associated with total fat (r=-0.12, p<0.0001), SFA (r= -0.15, p<0.0001), MUFA (r=-0.13, p<0.0001). There was no association between serum BC and PUFA (r=-0.02, p=0.30). However, when adjusted for age, sex, and race/ethnicity, serum BC showed no significant association with total fat (r=-0.03, p=0.06) and weaker associations with SFA (r= -0.07, p<0.0001), MUFA (r=-0.05, p=0.006). Figure 4.1 shows the correlation with PUFA was positive and weak, but significant (r=0.05, p=0.005). After adjusting for demographic characteristics, the strongest association was between serum BC concentrations and SFA. There was a significant, positive association between reported dietary BC and reported total fat intakes after adjusting for age, sex, and race/ethnicity (r=0.18, p<0.0001).

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 4.3), although the negative association was weak. For each 10g increase in reported total fat intake, serum BC concentrations decreased by 0.02µg/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.

Table 4.3. Linear regression modeling for the association between serum BC concentrations and total fat; regression coefficients and SE for log-transformed serum BC from NHANES 2003-2006

models	β	95% CI	P for trend
1	-0.002± 0.0003	(-0.003; -0.002)	<0.0001
2	-0.001± 0.0003	(-0.002; -0.001)	0.0003
3	-0.001± 0.0003	(-0.001; -0.0001)	0.03
4	-0.001± 0.0003	(-0.001; -0.0001)	0.03
5	-0.002± 0.0002	(-0.002; -0.001)	<0.0001

1: crude; 2: age adjusted; 3: age & sex adjusted; 4: age, sex, & race/ethnicity adjusted; 5: age, sex, race/ethnicity, & reported dietary BC intake adjusted.

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 4.4). For each 1g increase in reported SFA intake, which is 3.72% of the mean intake of SFA, serum BC concentrations decreased by 0.006μg/mL ($p<0.0001$). Additionally, the associations between serum BC concentrations and specific fatty 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 5. Mean reported dietary intake was highest for long chain SFA, palmitic acid (14.62±0.2g), followed by long chain SFA, stearic acid (6.96±0.1g), which were reflective of typical intakes[141,142].

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 1g increase in reported MUFA intake, serum BC concentrations decreased by 0.005μg/mL ($p<0.0001$). A 1g increase in MUFA is 3.21% of the mean MUFA intake. Moreover, Table 5 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±0.5g versus 0.24±0.01g and 0.04±0.002 g). Mean reported intakes of oleic acid were also over 22 times higher than palmitoleic acid (1.31±0.03g), however, palmitoleic acid showed a stronger association to serum BC concentrations.

For reported PUFA intakes, results were inconsistent, although PUFA was the only fatty acid class to show positive associations with serum BC concentrations after adjusting for demographic factors using Pearson partial correlation. 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. However, when adjusted for only age, sex, and race/ethnicity, the multilinear model suggests for each 1g increase in

reported PUFA intake, serum BC concentrations increased by 0.004 μg/mL (p=0.03). The association between specific PUFA and serum BC concentrations determined by Pearson partial correlation are presented in Table 4.5. Means and SEs for Omega-3 fatty acid alpha-linolenic acid (ALA) (18:3) and Omega-6 fatty acid linoleic acid (LA) (18:2) were 1.55±0.04g and 15.58±0.40g, respectively.

Table 4.4. Linear regression modeling for the association between serum BC concentrations and reported saturated, monounsaturated, and polyunsaturated fatty acids; regression coefficients and SE for log-transformed serum BC from NHANES 2003-2006 (*n*=3,278)

	SFA		***MUFA***		***PUFA***	
models	β	95% CI	β	95% CI	β	95% CI
1	-0.008± 0.001	(-0.01; -0.006) [d]	-0.006± 0.001	(-0.008; -0.004)[d]	-0.001± 0.002	(2.57; 2.80)
2	-0.006± 0.001	(-0.008; -0.003) [d]	-0.004± 0.001	(-0.006; -0.002) [d]	0.001± 0.001	(-0.002; 0.004)
3	-0.004± 0.001	(-0.006; -0.002) [c]	-0.002± 0.001	(-0.004; -0.001) [b]	0.004± 0.001	(0.0003; 0.007)[a]
4	-0.004± 0.001	(-0.006; -0.002) [c]	-0.002± 0.001	(-0.004; -0.001) [b]	0.004± 0.001	(0.0003; 0.007) [a]
5	-0.006± 0.001	(-0.008; -0.004) [d]	-0.005± 0.001	(-0.006; -0.003) [d]	-0.002± 0.002	(0.006; -0.002)

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

Table 4.5. 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*= 3,278)

SFA	r	MUFA	r	PUFA	r
Butyric acid (4:0)	-0.01	Palmitoleic acid (16:1)	-0.09 [c]	Linoleic acid (18:2)	0.04 [a]
Caproic acid (6:0)	-0.01	Oleic acid (18:1)	-0.04 [a]	Alpha-linolenic acid (18:3)	0.09 [c]
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 [b]
Myristic acid (14:0)	-0.05 [b]			Docosapentaenoic acid (22:5)	0.05 [b]
Palmitic acid (16:0)	-0.07 [c]			Docosahexaenoic acid (22:6)	0.05 [b]
Stearic acid (18:0)	-0.09 [c]				

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

V. 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[84]. The findings of this study indicate that there are significant associations between serum BC concentrations and reported dietary fat intakes. However, the results of this study suggest that reported quantity of total fat consumed is not a factor dictating serum BC concentrations in population samples, as a significant, inverse relationship was present when adjusted for participant demographics and unaffected by confounding factors. Research indicates that the addition of fat to a carotenoid-containing meal improves intestinal absorption of BC[65,75–78,85]. Goltz *et al* determined that adding 20g of lipids to a meal containing BC significantly affected the absorption rates of BC, independent of the type of lipid consumed ($p<0.01$)[10]. White *et al* found similar results when adding 0, 2, 4, 8, 16 and 32g of soybean oil to a salad containing 11.54±0.5mg BC. There was a positive linear relationship between BC and total grams of soybean oil between 0-8g, with highest BC absorption with 32g of oil[88]. 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 3 eggs (150g) versus 1.5 eggs (75g) significantly increased BC absorption 10 hours post

consumption ($p<0.001$)[86]. 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 hours after consumption of 300g salsa with 150g avocado ($p<0.003$) and 200g salad with 75g avocado ($p<0.01$), 150g avocado ($p<0.01$), or 24g avocado oil ($p<0.01$)[87].

This study indicates that the SFAs with the highest mean concentrations in the diet, such as stearic acid and palmitic acid, also showed 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 ALA, but ALA had a stronger positive association to BC concentrations. This suggests that even though ALA is consumed in small quantities, it may have stronger biologic effects with regards to BC absorption. However, we don't know if this is a biologic effect via absorption or is a result of intake of foods that contain both ALA and BC, such as leafy greens.

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 + ALA), 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 ($p<0.05$)[85]. BC uptake was dependent on the type of fat, suggesting the food matrix, BC polarity, and type of dietary fat determine BC bioavailability[85]. 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) ($p<0.05$)[84].

The strongest association between specific fatty acids and serum BC concentrations in this study was ALA (omega-3), 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 CMD[142].

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[125]. 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, reported dietary intakes of foods containing fat and BC were self-reported through the WWEIA survey questions, which may increase both social desirability and recall biases in comparison to intakes being monitored or directly measured intakes. 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 was 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.

This study suggests that reported PUFA intake, especially ALA is associated with increased BC in circulation, whereas, reported SFA stearic acid and MUFA palmitoleic acid is 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 is not associated with serum BC concentrations likely due to adequate absorption even at lowest levels of reported fat consumption.

REFERENCES

1. Overweight and obesity. World Health Organization. http://www.who.int/mediacentre/factsheets/fs311/en/. Published 2018.

2. Kyle TK, Dhurandhar EJ, Allison DB. Regarding Obesity as a Disease: Evolving Policies and Their Implications. *Endocrinol Metab Clin North Am*. 2016;45(3):511-520. doi:10.1016/j.ecl.2016.04.004

3. Ng M, Fleming T, Robinson M, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: A systematic analysis for the Global Burden of Disease Study 2013. *Lancet*. 2014;384(9945):766-781. doi:10.1016/S0140-6736(14)60460-8

4. Ritchie H, Roser M. Obesity. https://ourworldindata.org/obesity. Published 2017.

5. Centers for Disease Control and Prevention. Adult obesity facts. https://www.cdc.gov/obesity/data/adult.html. Published 2020.

6. Ogden CL, Carroll MD, Kit BK, Flegal M. Prevalence of Childhood and Adult Obesity in the United States, 2011–2012. *Jama*. 2016;311(8):806-814. doi:10.1001/jama.2014.732.Prevalence

7. de Souza Valente da Silva L, Valeria da Veiga G, Ramalho RA. Association of serum concentrations of retinol and carotenoids with overweight in children and adolescents. *Nutrition*. 2007;23(5):392-397. doi:10.1016/j.nut.2007.02.009

8. Guerendiain M, Mayneris-Perxachs J, Montes R, et al. Relation between plasma antioxidant vitamin levels, adiposity and cardio-metabolic profile in adolescents: Effects of a multidisciplinary obesity programme. *Clin Nutr*. 2015:1-9. doi:10.1016/j.clnu.2015.11.001

9. Sarni ROS, Suano de Souza FI, Ramalho RA, et al. Serum retinol and total carotene concentrations in obese pre-school children. *Med Sci Monit*. 2005;11(11):510-514.

10. Beydoun MA, Canas JA, Beydoun HA, Chen X, Shroff MR, Zonderman AB. Serum Antioxidant Concentrations and Metabolic Syndrome Are Associated among U.S. Adolescents in Recent National Surveys. *J Nutr*. 2012;142:1693-1704. doi:10.3945/jn.112.160416.of

11. Doak CM, Adair LS, Bentley M, Monteiro C, Popkin BM. The dual burden household and the nutrition transition paradox. *Int J Obes*. 2005;29(1):129-136. doi:10.1038/sj.ijo.0802824

12. Shaikh RA, Siahpush M, Singh GK, Tibbits M. Socioeconomic Status, smoking, alcohol use, physical activity, and dietary behavior as determinants of obesity and body mass index in the United States: Findings from the National Health Interview Survey. *Int J MCH AIDS*. 2015;4(1):22-34.

13. Dietary recommendations / nutritional requirements. *World Heal Organ*. 2017. http://www.who.int/nutrition/topics/nutrecomm/en/.

14. Cao Y, Wittert G, Taylor AW, Adams R, Appleton S, Shi Z. Nutrient patterns and chronic inflammation in a cohort of community dwelling middle-aged men. *Clin Nutr*. 2016:1-8. doi:10.1016/j.clnu.2016.06.018

15. Baek SH, Kim M, Kim M, et al. Metabolites distinguishing visceral fat obesity and atherogenic traits in individuals with overweight. *Obesity*. 2016;00(00):1-9. doi:10.1002/oby.21724

16. Kabat GC, Heo M, Ochs-Balcom HM, et al. Longitudinal association of measures of adiposity with serum antioxidant concentrations in postmenopausal women. *Eur J Clin Nutr*. 2015;70:1-7. doi:10.1038/ejcn.2015.74

17. Gray DS. Diagnosis and prevalence of obesity. *Obesity*. 1989;73(1):1-13. doi:10.1016/S0025-7125(16)30688-5

18. Beydoun MA, Shroff MR, Chen X, Beydoun HA, Wang Y, Zonderman AB. Serum antioxidant status is associated with metabolic syndrome among U. S. adults in recent national surveys. *J Nutr*. 2011;141(12):903-913. doi:10.3945/jn.110.136580

19. Centers for Disease Control and Prevention. Adult Obesity Causes & Consequences.

20. Cawley J, Meyerhoefer C. The medical care costs of obesity: An instrumental variables approach. *J Health Econ*. 2012;31(1):219-230. doi:10.1016/j.jhealeco.2011.10.003

21. Cawley J, Meyerhoefer C, Biener A, Hammer M, Wintfeld N. Savings in Medical Expenditures Associated with Reductions in Body Mass Index Among US Adults with Obesity, by Diabetes Status. *Pharmacoeconomics*. 2015;33(7):707-722. doi:10.1007/s40273-014-0230-2

22. Deurenberg P, Weststrate JA, Seidell JC. Body mass index as a measure of body fatness: age- and sex- specific prediction formulas. *Br J Nutr*. 1991;65:105-114.

23. BMI Classification. World Health Organization. http://www.assessmentpsychology.com/icbmi.htm. Published 2006. Accessed November 4, 2017.

24. National Health and Nutrition Examination Survey. Dual Energy X-ray Absorptiometry (DXA) Procedures Manual. 2006;(January). http://www.cdc.gov/nchs/data/nhanes/nhanes_07_08/manual_dexa.pdf.

25. Anthropometry Procedures Manual. National Health and Nutrition Examination Survey. https://wwwn.cdc.gov/nchs/data/nhanes/2003-2004/manuals/BM.pdf. Published 2004. Accessed November 4, 2017.

26. Cinkajzlová A, Mráz M, Haluzík M. Lymphocytes and macrophages in adipose

tissue in obesity: markers or makers of subclinical inflammation? *Protoplasma*. 2017:1-14. doi:10.1007/s00709-017-1082-3

27. Power ML, Schulkin J. Sex differences in fat storage, fat metabolism, and the health risks from obesity: possible evolutionary origins. *Br J Nutr*. 2008;99(05):931-940. doi:10.1017/S0007114507853347

28. Xia Q, Grant SFA. The genetics of human obesity. *Ann N Y Acad Sci*. 2013;1281(1):178-190. doi:10.1111/nyas.12020

29. Fernández-Sánchez A, Madrigal-Santillán E, Bautista M, et al. Inflammation, oxidative stress, and obesity. *Int J Mol Sci*. 2011;12(5):3117-3132. doi:10.3390/ijms12053117

30. Viroonudomphol D, Pongpaew P, Tungtrongchitr R, et al. The relationships between anthropometric measurements, serum vitamin A and E concentrations and lipid profiles in overweight and obese subjects. *Asia Pac J Clin Nutr*. 2003;12(1):73-79.

31. Alexander CM, Landsman PB, Grundy SM. The influence of age and body mass index on the metabolic syndrome and its components. *Diabetes, Obes Metab*. 2008;10(3):246-250. doi:10.1111/j.1463-1326.2006.00695.x

32. Frey SK, Vogel S. Vitamin A metabolism and adipose tissue biology. *Nutrients*. 2011;3(1):27-39. doi:10.3390/nu3010027

33. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in Inflammation and Metabolic Disease. *Nat Rev Immunol*. 2011;11(2):85-97. doi:10.1038/nri2921.Adipokines

34. Bonet ML, Ribot J, Palou A. Lipid metabolism in mammalian tissues and its control by retinoic acid. *Mol Cell Biol Lipids*. 2012;1821(1):177-189. doi:10.1016/j.bbalip.2011.06.001

35. Mercader J, Ribot J, Murano I, et al. Remodeling of white adipose tissue after retinoic acid administration in mice. *Endocrinology*. 2006;147(11):5325-5332. doi:10.1210/en.2006-0760

36. Villarroya F, Iglesias R, Giralt M. Retinoids and retinoid receptors in the control of energy balance: novel pharmacological strategies in obesity and diabetes. *Curr Med Chem*. 2004;11(6):795-805. doi:10.2174/0929867043455747

37. Moro C, Klimcakova E, Lolmède K, et al. Atrial natriuretic peptide inhibits the production of adipokines and cytokines linked to inflammation and insulin resistance in human subcutaneous adipose tissue. *Diabetologia*. 2007;50(5):1038-1047. doi:10.1007/s00125-007-0614-3

38. Luisa Bonet M, Canas JA, Ribot J, Palou A. Carotenoids and their conversion products in the control of adipocyte function, adiposity and obesity. *Arch Biochem Biophys*. 2015;572:112-125. doi:10.1016/j.abb.2015.02.022

39. Achten J, Jeukendrup AE. Optimizing fat oxidation through exercise and diet. *Nutrition*. 2004;20(7-8):716-727. doi:10.1016/j.nut.2004.04.005

40. Schutz Y, Tremblay A, Nelson KM, Weinsier RL. Role of fat oxidation in the long-term of body weight in obese women. *Am J Clin Nutr*. 1992;55(August):670-674.

41. Wood AD, Strachan A a, Thies F, et al. Patterns of dietary intake and serum carotenoid and tocopherol status are associated with biomarkers of chronic low-grade systemic inflammation and cardiovascular risk. *Br J Nutr*. 2014;112(8):1341-1352. doi:10.1017/S0007114514001962

42. Thurnham DI, Northrop-Clewes CA. Inflammation and biomarkers of micronutrient status. *Curr Opin Clin Nutr Metab Care*. 2016;19(6):458-463. doi:10.1097/MCO.0000000000000323

43. Marques-Vidal P, Bastardot F, Von Känel R, et al. Association between circulating cytokine levels, diabetes and insulin resistance in a population-based sample (CoLaus study). *Clin Endocrinol (Oxf)*. 2013;78(2):232-241. doi:10.1111/j.1365-2265.2012.04384.x

44. Kaulmann A, Bohn T. Carotenoids, inflammation, and oxidative stress-implications of cellular signaling pathways and relation to chronic disease prevention. *Nutr Res*. 2014;34(11):907-929. doi:10.1016/j.nutres.2014.07.010

45. Navarro P, de Dios O, Gavela-Pérez T, Jois A, Garcés C, Soriano-Guillén L. High-sensitivity C-reactive protein and leptin levels related to body mass index changes throughout childhood. *J Pediatr*. 2016;178:2541-2546. doi:10.1016/j.jpeds.2016.08.020

46. C-Reactive protein concentrations as a marker of inflammation or infection for interpreting biomarkers of micronutrient status. World Health Organization; Vitamin and Mineral Nutrition Information System. http://apps.who.int/iris/bitstream/10665/133708/1/WHO_NMH_NHD_EPG_14.7_eng.pdf. Published 2014.

47. Amara BN, Tourniaire F, Maraninchi M, et al. Independent positive association of plasma B-carotene concentrations with adiponectin among non-diabetic obese subjects. *Eur J Nutr*. 2015;54(3):447-454. doi:10.1007/s00394-014-0728-6

48. Schultz H, Ying G-S, Dunaief J, Dunaief DM. Rising plasma beta-carotene is associated with diminishing C-reactive protein in patients consuming a dark green leafy vegetable-rich, low inflammatory foods everyday (LIFE) diet. *Am J Lifestyle Med*. 2019. doi:10.1177/1559827619894954

49. Bode JG, Albrecht U, Häussinger D, Heinrich PC, Schaper F. Hepatic acute phase proteins - Regulation by IL-6- and IL-1-type cytokines involving STAT3 and its crosstalk with NF-κB-dependent signaling. *Eur J Cell Biol*. 2012;91(6-7):496-505. doi:10.1016/j.ejcb.2011.09.008

50. Jain S, Gautam V, Naseem S. Acute-phase proteins: As diagnostic tool. *J Pharm Bioallied Sci*. 2011;3(1):118. doi:10.4103/0975-7406.76489

51. Kramer F, Torzewski J, Kamenz J, et al. Interleukin-1β stimulates acute phase response and C-reactive protein synthesis by inducing an NFκB- and C/EBPβ-dependent autocrine interleukin-6 loop. *Mol Immunol*. 2008;45(9):2678-2689. doi:10.1016/j.molimm.2007.12.017

52. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Elevated C-reactive protein levels in overweight and obese adults. *J Am Med Assoc*. 1999;282(22):2131-2135.

53. Paepegaey AC, Genser L, Bouillot JL, Oppert JM, Clément K, Poitou C. High levels of CRP in morbid obesity: The central role of adipose tissue and lessons for clinical practice before and after bariatric surgery. *Surg Obes Relat Dis*. 2015;11(1):148-154. doi:10.1016/j.soard.2014.06.010

54. Ford ES, Galuska DA, Gillespie C, Will JC, Giles WH, Dietz WH. C-reactive protein and body mass index in children: Findings from the Third National Health and Nutrition Examination Survey, 1988-1994. *J Pediatr*. 1994;138(4):486-492. doi:10.1067/mpd.2001.112898

55. Ishii S, Karlamangla AS, Bote M, et al. Gender, obesity and repeated elevation of C-reactive protein: Data from the CARDIA cohort. *PLoS One*. 2012;7(4). doi:10.1371/journal.pone.0036062

56. Vincent HK, Taylor AG. Biomarkers and potential mechanisms of obesity-induced oxidant stress in humans. *Int J Obes*. 2006;30(3):400-418. doi:10.1038/sj.ijo.0803177

57. Manna P, Jain SK. Obesity, oxidative stress, adipose tissue dysfunction, and the associated health risks: Causes and therapeutic strategies. *Metab Syndr Relat Disord*. 2015;13(10):423-444. doi:10.1089/met.2015.0095

58. Savini I, Catani MV, Evangelista D, Gasperi V, Avigliano L. Obesity-associated oxidative stress: Strategies finalized to improve redox state. *Int J Mol Sci*. 2013;14(5):10497-10538. doi:10.3390/ijms140510497

59. Dandona P, Mohanty P, Ghanim H, et al. The suppressive effect of dietary restriction and weight loss in the obese on the generation of reactive oxygen species by leukocytes, lipid peroxidation, and protein carbonylation. *J Clin Endocrinol Metab*. 2001;86(1):355-362. doi:10.1210/jcem.86.1.7150

60. Goodwin K, Abrahamowicz M, Leonard G, et al. Dietary vitamin A and visceral adiposity: A Modulating role of the retinol-binding protein 4 gene. *J Nutrigenet Nutrigenomics*. 2015;8:164-173. doi:10.1159/000442090

61. Tourniaire F, Gouranton E, Von Lintig J, et al. B-Carotene conversion products and their effects on adipose tissue. *Genes Nutr*. 2009;4(3):179-187. doi:10.1007/s12263-009-0128-3

62. Thatcher JE, Isoherranen N. The roe of CYP26 enzymes in retinoic acid clearance. *Expert Opin Drug Metab Toxicol*. 2009;5(8):875-886. doi:10.1517/17425250903032681

63. Muenzner M, Tuvia N, Deutschmann C, et al. Retinol-Binding Protein 4 and its membrane receptor STRA6 control adipogenesis by regulating cellular retinoid homeostasis and retinoic acid receptor-a activity. *Mol Cell Biol*. 2013;33(20):4068-4082. doi:10.1128/MCB.00221-13

64. Moore T. Vitamin A and carotene: the conversion of carotene to vitamin A in vivo. *Biochem J*. 1930;24(3):692-702.

65. Goltz SR, Sapper TN, Failla ML, Campbell WW, Ferruzzi MG. Carotenoid bioavailability from raw vegetables and a moderate amount of oil in human subjects is greatest when the majority of daily vegetables are consumed at one meal. *Nutr Res*. 2013;33(5):358-366. doi:10.1016/j.nutres.2013.02.010

66. Courraud J, Berger J, Cristol J, Avallone S. Stability and bioaccessibility of different forms of carotenoids and vitamin A during in vitro digestion. *Food Chem*. 2013;136(2):871-877. doi:10.1016/j.foodchem.2012.08.076

67. Evans M, Beck M, Elliott J, Schalch W. Effects of formulation on the bioavailability of lutein and zeaxanthin : a randomized , double-blind , cross-over , comparative , single-dose study in healthy subjects. *Eur J Nutr*. 2013;52:1381-1391. doi:10.1007/s00394-012-0447-9

68. Couillard C, Lemieux S, Vohl M-C, Couture P, Lamarche B. Carotenoids as biomarkers of fruit and vegetable intake in men and women. *Br J Nutr*. 2016;116(07):1206-1215. doi:10.1017/S0007114516003056

69. Jansen MCJF, Van Kappel AL, Ocké MC, et al. Plasma carotenoid levels in Dutch men and women, and the relation with vegetable and fruit consumption. *Eur J Clin Nutr*. 2004;58(10):1386-1395. doi:10.1038/sj.ejcn.1601981

70. Pennant M, Steur M, Moore C, Butterworth A, Johnson L. Comparative validity of vitamin C and carotenoids as indicators of fruit and vegetable intake: a systematic review and meta-analysis of randomised controlled trials. *Br J Nutr*. 2015;114(09):1331-1340. doi:10.1017/S0007114515003165

71. Souverein OW, de Vries JHM, Freese R, et al. Prediction of fruit and vegetable intake from biomarkers using individual participant data of diet-controlled intervention studies. *Br J Nutr*. 2015;113(09):1396-1409. doi:10.1017/S0007114515000355

72. Holden JM, Eldridge AL, Beecher GR, et al. Carotenoid content of U. S. foods: An update of the database. *J Food Compos Anal*. 1999;12:169-196.

73. Tang G, Qin J, Dolnikowski GG, Russell RM. Short-term (intestinal) and long-term (postintestinal) conversion of B-carotene to retinol in adults as assessed by a stable-isotope reference method. *Am J Clin Nutr*. 2003;78:259-266.

74. Novotny JA, Harrison DJ, Pawlosky R, Flanagan VP, Harrison EH, Kurilich AC. B-carotene conversion to vitamin A decreases as the dietary dose increases in humans. *J Nutr*. 2010:915-918. doi:10.3945/jn.109.116947.Bloomberg

75. van Vliet T, Schreurs WH, van den Berg H. Intestinal B-carotene absorption and cleavage in men: response of B-carotene and retinyl esters in the triglyceride-rich lipoprotein fraction after a single oral dose of B-carotene. *Am J Clin Nutr*. 1995;62:110-116.

76. Granado-Lorencio F, Olmedilla-Alonso B, Herrero-barbudo C, Blanco-Navarro I, Perez-Sacristan B, Blazquez-Garcia S. In vitro bioaccessibility of carotenoids and tocopherols from fruits and vegetables. *Food Chem*. 2007;102:641-648. doi:10.1016/j.foodchem.2006.05.043

77. Goltz SR, Campbell WW, Chitchumroonchokchai C, Failla ML, Ferruzzi MG. Meal triacylglycerol profile modulates postprandial absorption of carotenoids in humans. *Mol Nutr Food Res*. 2012;56:866-877. doi:10.1002/mnfr.201100687

78. Brown MJ, Ferruzzi MG, Nguyen ML, et al. Carotenoid bioavailability is higher from salads ingested with full-fat than with fat-reduced salad dressings as measured with electrochemical detection. *Am J Clin Nutr*. 2004;80(2):396-403. doi:10.1093/ajcn/80.2.396

79. Fleshman MK, Riedl KM, Novotny JA, Schwartz SJ, Harrison EH. An LC/MS method for d8-B-carotene and d4-retinyl esters: B-carotene absorption and its conversion to vitamin A in humans. *J Lipid Res*. 2012;53:820-827. doi:10.1194/jlr.D021139

80. Tyssandier V, Reboul E, Dumas J-F, et al. Processing of vegetable-borne carotenoids in the human stomach and duodenum. *Am J Physiol Gastrointest Liver*. 2003;284:913-923.

81. O'Neill ME, Thurnham DI. Intestinal absorption of β-carotene, lycopene and lutein in men and women following a standard meal: response curves in the triacylglycerol-rich lipoprotein fraction. *Br J Nutr*. 1998;79(02):149-159. doi:10.1079/BJN19980026

82. Prince R, Frisoli K. Beta-carotene accumulation in serum and skin. *Am J Clin Nutr*. 1993;57:175-181.

83. Harari A, Coster ACF, Jenkins A, et al. Obesity and Insulin Resistance Are Inversely Associated with Serum and Adipose Tissue Carotenoid Concentrations in Adults. *J Nutr*. 2020;150(1):38-46. doi:10.1093/jn/nxz184

84. Failla ML, Chitchumronchokchia C., Ferruzzi MG., Goltz, S. R.; Campbell WW. Unsaturated fatty aids promote bioaccessibility and basolateral secretion of carotenoids and a -tocopherol by Caco-2 cells. *Food Funct*. 2014;5:1101-1112. doi:10.1039/c3fo60599j

85. Mashurabad PC, Ravindranadh P, Jyrwa YW, Bhaskarachary K., Pullakhandam R.

Dietary fat composition, food matrix and relative polarity modulate the micellarization and intestinal uptake of carotenoids from vegetables and fruits. *J Food Sci Technol*. 2017;54(2):333-341. doi:10.1007/s13197-016-2466-7

86. Kim JE, Gordon SL, Ferruzzi MG, Campbell WW. Effects of egg consumption on carotenoid absorption from. *Am J Clin Nutr*. 2015;102:75-83. doi:10.3945/ajcn.115.111062.INTRODUCTION

87. Unlu NZ, Bohn T, Clinton SK, Schwartz SJ. Carotenoid Absorption from Salad and Salsa by Humans Is Enhanced by the Addition of Avocado or Avocado Oil. *J Nutr*. 2005;135(3):431-436. doi:10.1093/jn/135.3.431

88. White WS, Zhou Y, Crane A, Dixon P, Quadt F, Flendrig LM. Modeling the dose effects of soybean oil in salad dressing on carotenoid and fat-soluble vitamin bioavailability in salad vegetables. *Am J Clin Nutr*. 2017;106(4):1041-1051. doi:10.3945/ajcn.117.153635

89. Institute of Medicine. *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids: A Report of the Panel on Dietary Antioxidants and Related Compounds, Subcommittees on Upper Reference Levels of Nutrients and of Interpretation and Use of Dietary Reference In*.; 2000.

90. Johnson EJ, Russell RM. Distribution of orally administered B-carotene among lipoproteins in healthy men. *Am J Clin Nutr*. 1992;56:128-135.

91. Mathews-Roth MM. Plasma concentrations of carotenoids after large doses of beta-carotene. *Am J Clin Nutr*. 1990;52(3):500-501. doi:https://doi.org/10.1093/ajcn/52.3.500

92. Kopec RE, Gleize B, Borel P, Desmarchelier C, Caris-Veyrat C. Are lutein, lycopene, and β-carotene lost through the digestive process? *Food Funct*. 2017;8(4):1494-1503. doi:10.1039/C7FO00021A

93. Parker RS. Absorption, metabolism, and transport of carotenoids. *FASEB J*. 2017;10(5):542-551.

94. Goodman DS, Blomstrand R, Werner B, Huang HS, Shiratori T. The intestinal absorption and metabolism of vitamin A and B-carotene in man. *J Clin Invest*. 1966;45(10):1615-1623.

95. Cooperstone JL, Goetz HJ, Riedl KM, Harrison EH, Schwartz SJ, Kopec RE. Relative contribution of a-carotene to postprandial vitamin A concentrations in healthy humans after carrot consumption. *Am J Clin Nutr*. 2017;106(March):59-66. doi:10.3945/ajcn

96. During A, Harrison EH. Mechanisms of provitamin A (carotenoid) and vitamin A (retinol) transport into and out of intestinal Caco-2 cells. *J Lipid Res*. 2007;48:2283-2294. doi:10.1194/jlr.M700263-JLR200

97. Cardinault N, Tyssandier V, Grolier P, et al. Comparison of the postprandial chylomicron carotenoid responses in young and older subjects. *Eur J Nutr*.

2003;42(6):315-323. doi:10.1007/s00394-003-0426-2

98. Kawada T, Aoki N, Kamei Y, Maeshige K, Nishiu S, Sugimoto E. Comparative investigation of vitamins and their analogues on terminal differentiation, from preadipocytes to adipocytes, of 3T3-L1 cells. *Comp Biochem Physiol -- Part A Physiol*. 1990;96(2):323-326. doi:10.1016/0300-9629(90)90699-S

99. Kameji H, Mochizuki K, Miyoshi N, Goda T. B-Carotene accumulation in 3T3-L1 adipocytes inhibits the elevation of reactive oxygen species and the suppression of genes related to insulin sensitivity induced by tumor necrosis factor-a. *Nutrition*. 2010;26:1151-1156. doi:10.1016/j.nut.2009.09.006

100. Amengual J, Gouranton E, van Helden YGJ, et al. Beta-carotene reduces body adiposity of mice via BCMO1. *PLoS One*. 2011;6(6):1-13. doi:10.1371/journal.pone.0020644

101. Ribot J, Felipe F, Bonet ML, Palou A. Changes of adiposity in response to vitamin A status correlate with changes of PPAR gamma 2 expression. *Obes Res*. 2001;9(8):500-509. doi:10.1038/oby.2001.65

102. Chung HY, Ferreira ALA, Epstein S, Paiva SAR, Castaneda-Sceppa C, Johnson EJ. Site-specific concentrations of carotenoids in adipose tissue: Relations with dietary and serum carotenoid concentrations in healthy adults. *Am J Clin Nutr*. 2009;90(3):533-539. doi:10.3945/ajcn.2009.27712

103. Felipe F, Bonet ML, Ribot J, Palou A. Modulation of resistin expression by retinoic acid and vitamin A status. *Diabetes*. 2004;53(4):882-889. doi:10.2337/diabetes.53.4.882

104. Bonet ML, Oliver J, Pico C, et al. Opposite effects of feeding a vitamin A-deficient diet and retinoic acid treatment on brown adipose tissue uncoupling protein 1 (UCP1), UCP2 and leptin expression. *J Endocrinol*. 2000;166(3):511-517. doi:10.1677/joe.0.1660511

105. Vioque J, Weinbrenner T, Asensio L, Castelló A, Young IS, Fletcher A. Plasma concentrations of carotenoids and vitamin C are better correlated with dietary intake in normal weight than overweight and obese elderly subjects. *Br J Nutr*. 2007;97(5):977-986. doi:10.1017/S0007114507659017

106. Burrows TL, Hutchesson MJ, Rollo, M.E. B, M.M., Guest M, Collins CE. Fruit and vegetable intake assessed by food frequency questionnaire and plasma carotenoids: a validation study. *Nutrients*. 2015;7:3240-3251.

107. Osth M, Ost A, Kjolhede P, Stralfors P. The concentration of B-carotene in human adipocytes, but not the whole-body adipocyte stores, is reduced in obesity. *PLoS One*. 2014;9(1):1-5. doi:10.1371/journal.pone.0085610

108. Bovier ER, Lewis RD, Hammond Jr BR. The relationship between lutein and zeaxanthin status and body fat. *Nutrients*. 2013;5(3):750-757. doi:10.3390/nu5030750

109. Wang C, Ling C, Ding D, et al. Associations of Serum Carotenoids with DXA-Derived Body Fat and Fat Distribution in Chinese Adults: A Prospective Study. *J Acad Nutr Diet*. 2020;120(6):985-1001.

110. Rubin LP, Ross AC, Stephensen CB, Bohn T, Tanumihardjo SA. Metabolic effects of inflammation on vitamin A and carotenoids in humans and animal models. *Adv Nutr*. 2017;8(2):197-212. doi:10.3945/an.116.014167

111. Andersen LF, Jacobs Jr DR, Gross MD, Schreiner PJ, Williams OD, Lee DH. Longitudinal associations betweeen body mass index and serum carotenoids: The CARDIA study. *Br J Nutr*. 2006;95(2):358-365. doi:10.1079/bjn20051638

112. Liu J, Shi W-Q, Cao Y, et al. Higher serum carotenoid concentrations associated with a lower prevalence of the metabolic syndrome in middle-aged and elderly Chinese adults. *Br J Nutr*. 2014;112:2041-2048. doi:10.1017/S000711451400316X

113. Hozawa A, Jacobs DR, Steffes MW, Gross MD, Steffen LM, Lee D-H. Circulating carotenoid concentrations and incident hypertension: the Coronary Artery Risk Development in Young Adults (CARDIA) study. *J Hypertens*. 2009;27(2):237-242. doi:10.1097/HJH.0b013e32832258c9

114. Shardell MD, Alley DE, Hicks GE, et al. Low-serum carotenoid concentrations and carotenoid interactions predict mortality in US adults: the Third National Health and Nutrition Examination Survey. *Nutr Res*. 2011;31(3):178-189. doi:10.1016/j.nutres.2011.03.003

115. Galan P, Viteri FE, Bertrais S, et al. Serum concentrations of beta-carotene, vitamins C and E, zinc and selenium are influenced by sex, age, diet, smoking status, alcohol consumption and corpulence in a general French adult population. *Eur J Clin Nutr*. 2005;59(10):1181-1190. doi:10.1038/sj.ejcn.1602230

116. Wallstrom P, Wirfalt E, Lahmann PH, Gullberg B, Janzon L, Berglund G. Serum concentrations of beta-carotene and alpha-tocopherol are associated with diet, smoking, and general and central adiposity. *Am J Clin Nutr*. 2001;73(4):777-785. http://www.ncbi.nlm.nih.gov/pubmed/11273853.

117. Ford ES, Mokdad AH, Liu S. Healthy Eating Index and C-reactive protein concentration: findings from the National Health and Nutrition Examination Survey III, 1988 – 1994. *Eur J Clin Nutr*. 2005;59:278-283. doi:10.1038/sj.ejcn.1602070

118. Watzl B, Kulling SE, Möseneder J, Barth SW, Bub A. A 4-wk intervention with high intake of carotenoid-rich vegetables and fruit reduces plasma C-reactive protein in healthy, nonsmoking men. *Am J Clin Nutr*. 2005;82(5):1052-1058. doi:10.1093/ajcn/82.5.1052

119. Mazidi M, Kengne AP, Katsiki N, Mikhailidis DP, Banach M. Inverse association between serum antioxidant levels and inflammatory markers is moderated by adiposity: A report based on a large representative population sample of American

adults. *Br J Nutr*. 2018;120(11):1272-1278. doi:10.1017/S0007114518002581

120. Hozawa A, Jacobs Jr DR, Steffes MW, Gross MD, Steffen LM, Lee D-H. Relationships of circulating carotenoid concentrations with several markers of inflammation, oxidative stress, and endothelial dysfunction: the Coronary Artery Risk Development in Young Adults (CARDIA)/Young Adult Longituninal Trends in Antioxidants (YALT. *Clin Chem*. 2007;53(3):447-455. doi:10.1109/TMI.2012.2196707.Separate

121. Ajani UA, Ford ES, Mokdad AH. Dietary fiber and C-reactive protein: Findings from National Health and Nutrition Examination Survey data. *J Nutr*. 2004;134(5):1181-1185.

122. King DE, Egan BM, Geesey ME. Relation of dietary fat and fiber to elevation of C-reactive protein. *Am J Cardiol*. 2003;92(December):1335-1339. doi:10.1016/j.amjcard.2003.08.020

123. King DE, Mainous III AG, Geesey ME, Woolson RF. Dietary magnesium and C-reactive protein levels. *J Am Coll Nutr*. 2005;24(3):166-171. doi:10.1080/07315724.2005.10719461

124. Gammone MA, D'Orazio N. Anti-obesity activity of the marine carotenoid fucoxanthin. *Mar Drugs*. 2015;13(4):2196-2214. doi:10.3390/md13042196

125. Ahluwalia N, Dwyer J, Terry A, Moshfegh A, Johnson C. Update on NHANES dietary data: Focus on collection, celease, analytical considerations, and uses to inform public policy. *Adv Nutr*. 2016;7(1):121-134. doi:10.3945/an.115.009258

126. Sondik EJ, Madans JH, Johnson CL. National Health and Nutrition Examination Survey: Sample Design, 1999-2006. Center for Disease Control. https://wwwn.cdc.gov/nchs/data/series/sr02_155.pdf. Published 2012.

127. Laboratory Procedure Manual: Fat Souble Micronutrients. *Natl Heal Nutr Exam Surv*. 2008:1-45.

128. Laboratory Procedure Manual: C-Reactive Protein. National Health and Nutrition Examination Survey.

129. Administration F and D. Review Criteria for Assessment of C Reactive Protein (CRP), High Sensitivity C-Reactive Protein (hsCRP) and Cardiac C-Reactive Protein (cCRP) Assays - Guidance for Industry and FDA Staff. 2005. https://www.fda.gov/MedicalDevices/ucm077167.htm#6.

130. Chemistry AA of C. High-sensitivity C-reactive protein (hs-CRP). https://labtestsonline.org/tests/high-sensitivity-c-reactive-protein-hs-crp. Published 2018.

131. Center for Disease Control. Fat Soluble Vitamins & Micronutrients: Vitamins A and E and Carotenoids. https://www.cdc.gov/nutritionreport/99-02/pdf/nr_ch2a.pdf. Accessed November 4, 2017.

132. Rothwell CJ, Madans JH, Porter JS. National Health and Nutrition Examination Survey: Analytic Guidelines, 1999-2010. Vital and Health Statistics. https://stacks.cdc.gov/view/cdc/21305. Published 2013. Accessed November 4, 2017.

133. Mueller L, Boehm V. Antioxidant activity of β-carotene compounds in different in vitro assays. *Molecules*. 2011;16(2):1055-1069. doi:10.3390/molecules16021055

134. Ribaya-Mercado JD, Maramag CC, Tengco LW, Blumberg JB, Solon FS. Relationships of body mass index with serum carotenoids, tocopherols and retinol at steady-state and in response to a carotenoid-rich vegetable diet intervention in Filipino schoolchildren. *Biosci Rep*. 2008;28(2):97-106. doi:10.1042/BSR20070045

135. El-Sohemy A, Baylin A, Kabagambe E, Ascherio A, Spiegelman D, Campos H. Individual carotenoid concentrations in adipose tissue and plasma as biomarkers of dietary intake. *Am J Clin Nutr*. 2002;76(1):172-179.

136. Center for Disease Control. Measuring Guides for the Dietary Recall Interview. https://www.cdc.gov/nchs/nhanes/measuring_guides_dri/measuringguides.htm. Published 2015. Accessed November 4, 2017.

137. Carroll YL, Corridan BM, Morrissey PA. Carotenoids in young and elderly healthy humans: Dietary intakes, biochemical status and diet-plasma relationships. *Eur J Clin Nutr*. 1999;53(8):644-653. doi:10.1038/sj.ejcn.1600827

138. USDA. Dietary Guidelines for Americans 2015-2020. https://www.dietaryguidelines.gov/sites/default/files/2019-05/2015-2020_Dietary_Guidelines.pdf. Published 2015.

139. Center for Disease Control. National Health and Nutrition Examination Survey 2003-2004 Data Documentation, Codebook, and Frequencies Dietary Interview - Total Nutrient Intakes, First Day. https://wwwn.cdc.gov/Nchs/Nhanes/2003-2004/DR1TOT_C.htm. Published 2007.

140. Food and Nutrition Board, Institute of Medicine NA. Dietary Reference Intakes (DRIs): Acceptable Macronutrient Distribution Ranges. https://www.ncbi.nlm.nih.gov/books/NBK56068/table/summarytables.t5/?report=objectonly.

141. Raatz SK, Conrad Z, Johnson LAK, Picklo MJ, Jahns L. Relationship of the reported intakes of fat and fatty acids to body weight in US adults. *Nutrients*. 2017;9(5):1-13. doi:10.3390/nu9050438

142. Iggman D, Risérus U. Role of different dietary saturated fatty acids for cardiometabolic risk. *Clin Lipidol*. 2011;6(2):209-223. doi:10.2217/clp.11.7