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Phytonutrient Intake and Body Composition Color Correlations

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PHYTONUTRIENT INTAKE AND BODY COMPOSITION: COLOR CORRELATIONS

by

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We hereby recommend that the thesis prepared under our supervision by Ryan Orgeron II entitled PHYTONUTRIENT INTAKE AND BODY COMPOSITION: COLOR CORRELATIONS be accepted in partial fulfillment of the requirements for the Degree of Master of Science—Nutrition and Dietetics.

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ABSTRACT

This study evaluated the role of phytonutrient intake on body composition. Both the quantity and types of phytonutrients in diets were analyzed. Fifty (50) subjects (15 males; 35 females) from Thibodaux, Louisiana, were randomly selected to participate. All participants completed three 24-hour food recalls to gather intake data (food and calorie intake). The phytonutrient index (PI) was an established formula used to calculate the percentage (%) of the diet comprised of phytonutrient rich foods. Three different body assessment tools were used to determine body adiposity (body mass index [BMI], waist circumference, and fat percentage).

Participants who met healthy standards for BMI, waist circumference, and fat percentage all averaged much higher PI scores compared to those who were classified as obese/overweight. The data showed a strong inverse relationship between PI and BMI ($r = -0.753$, $p = 0.00$), waist circumference ($r = -0.730$, $p = 0.00$), and body fat percentage ($r = -0.701$, $p = 0.00$).

Each participant was administered a food frequency questionnaire to assess the intakes of the specific phytonutrients based on color identity (green colored plants are chlorophyll-rich; reddish-purple foods are anthocyanin-rich; and orange-yellowish foods are carotenoid rich). Individuals with higher weekly intakes of chlorophyll, carotenoid, and anthocyanin rich foods had better body composition in comparison to those who consumed less ($p<0.05$).

These results suggest a strong correlation between higher phytonutrient intake and improved body composition, regardless of the type of phytonutrients consumed. Overall diet quality seems to make the most difference, but the phytonutrients in fruits/vegetables are thought to be a key reason for those benefits.

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CHAPTER 1

INTRODUCTION

Eating more fruits and vegetables has consistently been shown to elicit many health benefits (Oguntibeju, Truter, & Esterhuyse, 2013; Pem & Jeewon, 2015; Slavin & Lloyd, 2012). More specifically, increased fruit and vegetable intake has been correlated with reduced risk of diseases such as heart disease, stroke, and even some cancers (Aune, et al., 2017). Some studies have shown that increased fruit and vegetable intake is correlated with healthy body weight and composition (Dehghan, Akhtar-Danesh, & Merchant, 2011; Halkjaer, Tjonneland, Overvad, & Sorensen, 2009; Schwingshackl et al., 2015). However, other researchers have argued that recommending increased fruit and vegetable intake to treat or prevent obesity without efforts to reduce intake of other energy sources is not warranted (Kaiser et al., 2014).

Statement of Problem

Being overweight or obese is one of the most harmful risk factors to the health of a population. Many studies have shown the negative impact that having excess adipose tissue has on the risk of chronic diseases (Ahima & Lazar, 2013; Guh et al., 2009; Hruby et al., 2016; Williams et al., 2015). According to the World Health Organization, (WHO), being overweight or obese has been associated with many diseases such as cardiovascular disease, diabetes, and cancer (WHO, 2016).

The cause of obesity is complex and multifactorial, but the primary cause is poor nutrition (Mytton, Nnoaham, Eyles, Scarborough, & Mhurchu, 2017; Swinburn, Caterson, Seidell, & James, 2004). More specifically, poor nutrition is defined as eating large amounts of calorie-dense, but nutrient-poor foods such as sugar-sweetened beverages, processed meats, and snack foods (Swinburn et al., 2004). Research has shown that despite excess intake of calorie dense foods, obese individuals have high rates of micronutrient deficiencies **(**Kaidar-Person, Person, Szomstein, & Rosenthal, 2008a, 2008b). A common dietary deficiency among individuals with excess adiposity is the lack of phytonutrients (Carnauba et al., 2016). These phytonutrients are primarily sourced from fruits and vegetable intake (Carnauba et. al., 2016). Thus, excess calorie consumption and phytonutrient deficiencies can lead to excess adipose tissue (Bell & Rolls, 2008; Hill, Wyatt & Peters, 2012). Although phytonutrient intake can be a predictor of health, it is not the only a predictor of obesity, as normal weight individuals could also have deficiencies (Kwon & Hagedorn, 2016).

The Centers for Disease Control and Prevention (CDC) data indicate that over one-third of the United States population is obese (CDC, 2018). Data from the Behavioral Risk Factor Surveillance System (BRFSS) show that nearly 70% of Louisiana adults are overweight or obese (BRFSS, 2016).

Data from the CDC show that 46.7% of Louisiana's population eats less than one serving of fruit and 32.5% eats less than one serving of vegetables each day (CDC, 2016). Many Louisianans do not consume one serving per day, much less the five servings per day recommended by the United States Department of Agriculture (USDA). The data available depicts that a low fruit and vegetable intake, thus low phytonutrient intake, is correlated with excess adiposity (CDC, 2018; CDC, 2016; BRFSS, 2016).

Research has shown a strong correlation between low fruit and vegetable (F/V) intake and excess adipose tissue (Mytton et al., 2017). A low intake of these foods is associated with higher energy intake and reduced nutrient intake (Swinburn et al., 2004; Bell & Rolls, 2008). Thus, a lack of F/V consumption and/or phytonutrients (plant nutrients) could potentially be a major contributor to Louisiana's high obesity rates.

Purpose of Study

The primary aim of this study was to determine if phytonutrient intake, measured by the phytonutrient index (PI), is correlated with body composition in participants from Thibodaux Regional Wellness Center in Thibodaux, Louisiana. In addition, a second aim was to examine if intake of certain phytonutrient rich foods (based on green, reddish/purple, and yellow/orange colored fruits and vegetables) correlates with body fat percentage, waist circumference, and Body Mass Index (BMI).

Hypotheses

The following hypotheses were tested:

1. Higher phytonutrient intake (as measured by PI) will correlate with lower body fat and lower body mass index, and smaller waist circumference.
2. Higher intakes of specific colored foods (green, red/purple, and orange/yellow) will not correlate with lower body mass, body fat, and smaller waist circumference.

Justification

Most of the current literature shows that higher intakes of fruit and vegetables (F/V) are associated with improvements in anthropometric parameters and risk of adiposity (Dehghan et al., 2011; Halkjaer et al., 2009; Schwingshackl et al., 2015). Despite large evidential support, a recent analysis showed increased F/V consumption has not been proven to effectively improve adiposity measures without efforts to reduce intake of other energy sources (Kaiser et al., 2014). Although the literature has reported correlation between higher F/V intake and better body composition, it is inconclusive if low F/V intake is the primary cause of excess adiposity (Ledoux, Hingle, & Baranowski, 2010). However, national data also show a correlation between F/V intake and body composition, with states with the highest obesity rates also having the lowest intakes of fruits and vegetables (CDC, 2018). Interestingly, Louisiana has consistently been in the top five for both, which makes the state a notable location to recruit subjects.

Some research has shown varying body composition changes among subjects depending on which fruits and vegetables they consumed more often (Bertoia et al., 2016). This suggests characteristics of fruits and vegetables other than the foods themselves influence the degree of their association with body composition improvements. The diverse phytonutrients present in plants have been shown to elicit a variety of benefits including weight loss and body composition changes (Zhao, 2007).

The primary difference between various types of fruits and vegetables are the specific phytonutrients they contain. Therefore, F/V intake may not be the only factor that impacts body composition. Considering correlations between specific phytonutrients could help give a better understanding of which role (if any) these phytonutrients have in reducing body adiposity.

CHAPTER 2

REVIEW OF LITERATURE

Phytonutrients

Obesity is a major health issue for the United States and is believed to be primarily the result of burning less energy than the amount consumed (Ard, Miller, & Kahan, 2016; Bell & Rolls, 2008; Hill et al., 2012). However, there is no single cause of obesity and it involves many components such as genetics, medications, inflammation, stress, and other lifestyle factors (Mytton et al., 2017; Swinburn et al., 2012). Despite being a multifaceted problem, proper nutrition is the biggest tool available to combat obesity (Mytton et al., 2017; Swinburn et al., 2004).

Most of the current literature has shown higher intakes of fruit and vegetables (F/V) are associated with improvements in anthropometric parameters and risk of adiposity (Ledoux et al., 2010; Mytton et al., 2017; Schwingshackl et al., 2015). In addition, the Academy of Nutrition and Dietetics (AND) considers increased fruit and vegetable (F/V) intake to be an evidence-based intervention for obesity, especially when less energy is consumed from sugar-sweetened beverages and fast foods (Raynor & Champagne, 2016). However, some studies suggest that the phytonutrients in fruits and vegetables could also be playing a major role in the prevention/treatment of many diseases including overweight/obesity (Carnauba et al., 2016; Rodriguez-Casado, 2014).

According to the United States Department of Agriculture (USDA), phytonutrients are non-nutritive substances found in plants (primarily in F/V) that are beneficial to human health. Plant foods like fruits and vegetables are comprised of many phytonutrients. There are over 5,000 types of phytonutrients that have been identified, but many still remain unknown (Liu, 2003). In order to completely understand these phytonutrients, they need to be isolated and identified or categorized in a manner that they can be observed (Liu, 2003). However, isolating phytonutrients outside of their synergistic combinations (as found in whole foods) is believed to deter some of the benefits (Liu, 2012). The Institute of Functional Medicine (IFM) also recommends not isolating any of the phytonutrients, but rather observing them in their actual form (Pizzorno, 2014). According to the IFM, categorizing the phytonutrients based on colors is the ideal way to consume and study them. This concept is derived from the knowledge that specific phytonutrients give off specific colors if there is an abundance in the food (Pizzorno, 2014). For example, foods rich in carotenoids are typically orange-yellow in color while anthocyanin rich foods are typically red-purple. This is primarily why carrots are orange and blueberries are blue-purple (Slavin & Lloyd, 2012). The consumption of various foods dense in specific phytonutrients has been identified to be beneficial in improving anthropometric measures of adiposity (Carnauba et al., 2016; Gonzalez-Castejon & Rodriguez-Casado, 2011).

Chlorophyll

Chlorophyll is a phytonutrient found in a variety of plants that commonly creates a greenish pigment (İnanç, 2011). Many therapeutic benefits have been established for chlorophyll intake, such as increasing immunity, purifying the blood, preventing or treating cancer, cleaning the intestines, normalizing blood pressure, and combating bad bodily odors (Ferruzzi, & Blakeslee, 2007). Chlorophyll intake also has been shown to have a role in decreasing body adiposity. A review of the effects of various phytonutrients specifically indicated that dark green vegetable intake was associated with lower visceral fat and improved insulin sensitivity (Cook et al., 2014).

Montelius et al. (2014) observed the effects of consuming a chlorophyll-rich green supplement once per day over a period of three months. Thirty-eight subjects were randomized to receive a placebo or a green supplement before breakfast every day for 12 weeks. After the 90-day duration, data indicated the addition of the chlorophyll rich dietary supplement resulted in significant weight loss in the intervention group. More precisely, the intervention group had 43% greater weight loss. Interestingly, the intervention group also had improved glucagon-like peptide 1 (GLP-1), which was hypothesized to be the mechanism that improves satiety and adiposity (Montelius et al., 2014). In a similar study, Stenblom et al. (2014) observed the effects of a supplemental source of chlorophyll on overweight subjects over a period of two months. They also observed significantly more fat loss in the intervention group when compared to placebo. Interestingly, both trials observed improved satiety and fewer cravings in the chlorophyll groups (Montelius et al., 2014; Stenblom et al., 2014). In a current review, many of the actions in which chlorophyll and its derivatives improve adiposity and suppress excess hunger were confirmed (Stenblom et al., 2016). Increased chlorophyll intake promotes satiety by influencing not only GLP-1, but also other appetite hormones such as ghrelin and cholecystokinin (CCK). All of these effects are connected to slower gastric emptying and decreased intestinal motility (Stenblom et al., 2016).

Food sources of chlorophyll have also been shown to improve satiety and hunger (Rebello et al., 2015). In a double blind, randomized crossover study, 60 overweight and obese subjects consumed either spinach or placebo before lunch. Subjects were between 18 and 65 years old, had a body mass index between 25 kg/m^2 and 35 kg/m^2, and had a waist circumference greater than 35 inches. Individuals were excluded if they had pre-existing medical conditions or were taking medications that could affect their appetite, food absorption, and/or body weight. They also were excluded if they had followed a low-calorie diet within two months prior to the study. Consistent with previous research using supplements, a single meal supplemented with spinach improved satiety for two hours after consumption (Rebello et al., 2015).

Another rich source of chlorophyll is spirulina, which has also been shown to improve body adiposity. In a recent randomized, double blind placebo controlled trial, spirulina was shown to significantly decrease body mass index (BMI), and waist circumference after three months of daily intake (Miczke et al., 2016). In a similar double-blind trial, researchers demonstrated three months of regular consumption of spirulina not only improved BMI and weight but also insulin sensitivity, blood pressure, and inflammation and oxidative stress biomarkers in overweight patients (Szulinska et al., 2017).

Although the research on chlorophyll is young and has limitations, many clinical trials demonstrate the benefits of chlorophyll on adiposity measures. More studies are needed to confirm, but current research shows potential for chlorophyll as a therapeutic phytonutrient that can help treat and/or prevent adiposity (Cook et al., 2014; Stenblom et al., 2016; Szulinska et al., 2017).

Carotenoids

Of all the known phytonutrients, the type that has been studied the most is carotenoids (Liu, 2013). More than 700 types of carotenoids have been identified in nature (Lu & Li, 2008). However, only about 50 of these carotenoids have been found in human tissues (Fiedor & Burda, 2014). Carotenoids play an essential role in promoting human health and preventing disease. The beneficial effects of carotenoids have been shown in diseases such as various types of cancer, cardiovascular disorders, and several eye diseases (Fiedor & Burda, 2014; Rao & Rao, 2007). These phytonutrients most likely contribute benefits due to their antioxidant capacity as well as being provitamin-A precursors (since humans cannot produce carotenoids) (Rao & Rao, 2007). The carotenoids most studied with regards to human health are beta-carotene, lycopene, lutein, and zeaxanthin. Beta-carotene may have added benefits due its enhanced ability to be converted to vitamin A (Rao & Rao, 2007; Tang, 2012). The synthesis of carotenoids by plants typically gives off an orange-yellow pigment as seen in the flesh of carrots and tomatoes (Wang et al., 2014). Unlike many other phytonutrients, carotenoid levels and intake can be predicted by the amount present in serum. In fact, serum carotenoid concentrations are shown to be a biomarker of vegetable intake, which can be more reliable than self-reported intake (Bogers et al., 2003).

Increased intake and increased serum carotenoids have been correlated with reduced adiposity (Bonet, Canas, Ribot, & Palou, 2015; Whigham et al., 2012). In a trial that observed 60 obese subjects for three months, researchers found those who had the highest serum carotenoid levels lost more weight and fat mass (Whigham et al., 2012). Even when compared to a group with reduced total calories, the participants who lost the most fat during the study also had the greatest increases in serum carotenoids (Whigham et al., 2012).

A similar correlation was noted in another study that aimed to validate parent reported vegetable intake in overweight children by using plasma carotenoids (Burrows, Warren, Colyvas, Garg, & Collins, 2009). Overweight and obese children had significantly lower levels of plasma carotenoids in comparison to healthy weight children. Researchers suggested further investigation of this correlation (Burrows et al., 2009). Ismail et al. (2014) added support to the correlation between carotenoids and weight by comparing serum carotenoid levels in 30 obese children to those of 30 normal weight children. Carotenoid and vitamin A insufficiency were greater in obese children in comparison to children with healthy weights. These researchers recommended that nutrition education about vitamin A become a part of obesity prevention (Ismail et al., 2014).

A recent randomized, double blind intervention trial aimed to see the effects supplemental carotenoids had on obese children. After a six-month intervention, researchers concluded an increase in carotenoid intake (via supplement) was able to decrease adipose tissue measures in overweight children. The intervention group showed improvements in body mass index (BMI), waist-to-height ratio, and subcutaneous adipose tissue (Canas et al., 2017). Research on an older adult population indicated similar results (Sluijs, Beulens, Grobbee, & Van Der Schouw, 2009). In a cross-sectional, single-center study of 400 independently living adults (40-80 years old), data also showed increased carotenoid intake was associated with better adiposity measures. More specifically, higher carotenoid intake was associated with lower visceral fat, subcutaneous fat mass, and waist circumference (Sluijs et al., 2009).

Researchers have established multiple pathways in which increased intake of carotenoids (and carotenoid derivatives) prevent adiposity (Bonet et al., 2015). More specifically, emerging pathways such as increasing production of adipocytes (adipogenesis), enhancing metabolic capacities of adipocytes, and reducing inflammation can all have a role in the anti-obesity effects of carotenoids (Bonet et al., 2015).

Although still sparse, human trials indicate a beneficial effect of increased carotenoid intake on adiposity. Studies support a role of carotenoids in the prevention of excess adiposity (Bonet et al., 2015). However, human intervention studies are limited and some contradicting (Bonet, Canas, Ribot, & Palou, 2016). Therefore, more human intervention trials are needed to verify the anti-obesity effects for specific carotenoids. In addition, more consistent and functional studies are needed to fully interpret the role and pathways of carotenoids in preventing adiposity (Bonet et al., 2016).

Anthocyanins

Anthocyanins combat obesity by suppressing fat absorption from the gut, increasing glucose uptake by skeletal muscles, stimulating catabolism in adipose tissues, inhibiting differentiation of pre-adipocytes to adipocytes, stimulating apoptosis of mature adipocytes, and reducing chronic inflammation associated with adiposity (Azzini, Giacometti, & Russo, 2017; Meydani & Hasan, 2010; Williams et al., 2013). Anthocyanins are phytonutrients responsible for most of the red, blue, and purple pigments of various fruits and vegetables. The chemical structure of anthocyanins seems to promote many health benefits, mainly antioxidant and anti-inflammatory actions

(Tsuda, 2011). In a review of many phytonutrients on preventing and treating obesity, researchers noted that anthocyanins in red-purple fruits and vegetables were found to be the best sources of potential anti-obesity factors (Williams et al., 2013).

One of the richest sources of anthocyanins is a blueberry, which has been shown to be a source of over 20 different types of anthocyanins (Schinner, 2009). In a review on the effects of blueberries on adiposity, Stull noted that researchers have reported evidence in animals, but little has been confirmed in human trials (Stull, 2016). Observations from the few human clinical trials have indicated some anti-obesity effects in the form of improving insulin sensitivity and reducing the chronic inflammation associated with obesity (Stull, 2016). Despite the current evidence looking promising, strong conclusions cannot be drawn due to the limited number of clinical trials available. There is a need for more long-term and properly designed trials to conclusively determine the role of blueberries in preventing or treating excess adiposity (Tsuda, 2016).

Recent reviews on the anti-obesity properties of anthocyanins showed that further interventional studies are needed to establish the optimal dose and ideal food matrix for the best anthocyanin supplementation (Amiot, Riva, & Vinet, 2016; Azzini et al., 2017). In doing so, trials can become more consistent and less limited in the methodology (Amiot et al., 2016). Moreover, observational studies are more likely to suggest a role of anthocyanins in preventing and treating obesity (Azzini et al., 2017). Considering the observation trials available, most of them strongly suggest that anthocyanins are associated with improved adiposity (Azzini et al., 2017). A recent observational study analyzed food frequency questionnaires from over 124,000 participants from three different cohort studies (Health Professionals Follow-up Study, Nurses' Health Study, and Nurses' Health Study II) (Bertoia et al., 2016). An increased intake of anthocyanins was associated with weight loss in men and women (aged 27–65) in a follow-up covering 24 years. Researchers determined that increasing the daily consumption of blueberries (anthocyanin-rich food) by one half cup for four years could result in a weight loss of about 1.03 kg per year (Bertoia et al., 2016). Despite observational and correlational studies clearly associating anthocyanin intake with improved adiposity measures, there is still a need for more intervention trials (Amiot et al., 2016; Azzini et al., 2017).

Limitations of Specific Phytonutrient Studies

The correlational data reported above suggests that specific phytonutrients are associated with anti-obesity effects. However, correlation does not mean causation. One of the biggest limitations is the lack of well-designed and implemented clinical trials. A major issue with most clinical trials is the varied dosages, which can influence results (Probst, Guan, & Kent, 2017). In addition, the complex biological pathways in which phytochemicals function is also subject to the possibility that different compositions (such as supplements, isolated extracts, and whole foods) interact differently (Azzini et al., 2017). Another issue that may contribute to limitations in the field of study is the differences between therapy and prevention. Therapy is associated with higher doses for a shorter time, and prevention implies low doses for a long duration (Azzini et al., 2017; Gonzalez-Castejon & Rodriguez-Casado, 2011). Many researchers believe the field must consider establishing new and sufficient models that would allow the design to be more consistent and more efficient for clinical studies (Gonzalez-Castejon & Rodriguez-Casado, 2011; Probst et al., 2017).

The Phytonutrient Index

The study of phytonutrients consumed in foods is an expensive and difficult process. The laborious and expensive process can become unrealistic, especially in large subject pools (Vincent, Bourguignon, & Taylor, 2010). In an effort to streamline consistency of phytochemical intake studies, a concept called the "phytochemical index" (PI) score was proposed (McCarty, 2004). PI was calculated as the energy supplied by phytonutrient rich foods divided by the total energy consumed from all foods (PI = [phytochemical calories/total calories] X 100). Phytonutrient rich foods primarily include fruits and vegetables, but also legumes, whole grains, seeds, nuts, olive oil, soy sources, and wine (McCarty, 2004).

Although the PI is the most cost effective and efficient method to examine the effects of phytonutrients, it is not without limitations (Probst et al., 2017; Vincent et al., 2010). The most common limitation is the methods of acquiring dietary data (Probst et al., 2017). Traditional methods of gathering dietary data require food recalls, food diaries, and/or food frequency questionnaires. All of these dietary assessment methods involve subjective interpretations, which rely on the ability of the subject to not only recall food intake, but to quantify it precisely. The lack of an objective measure of intake leaves room for error (Probst et al., 2017). In addition, estimating dietary phytochemical intake through dietary assessment methods is unable to account for the phytochemical metabolism and absorption, which can be highly variable between individuals (Azzini et al., 2017; Probst et al., 2017). The assimilation of phytonutrients is influenced by other factors such as bioavailability and genetics. The individual variations in metabolism remain unaccounted for with little knowledge about the bioavailability of all phytochemicals (Azzini et al., 2017; Probst et al., 2017). This limitation gives researchers little option but to rely solely on phytochemical intake regardless of individual metabolism.

While there are known limitations when using the PI index, there are many favorable practical and clinical uses of the PI. Clinical nutritionists can estimate the diet quality without high-priced equipment or trained specialists (McCarty, 2004; Vincent et al., 2010). Although the PI would provide only a rough estimation of the quantity and/or quality of phytonutrient intake, it could help researchers in studying the health correlations of diets high and/or low in phytonutrient-rich foods (McCarty, 2004; Vincent et al., 2010).

Phytochemical Index and Adiposity

Since the proposal of the Phytochemical Index (PI) in 2004, researchers have used the tool to assess the quality of diets (Bahadoran, Golzarand, Mirmiran, Saaadati, & Azizi, 2013; Mirmiran, Bahadoran, Golzarand, Shiva, & Azizi, 2012; Vincent et al., 2010). The longest study that observed correlations between the PI and adiposity was published in 2012 (Mirmiran et al., 2012). The study ran for three years and observed almost 2,000 subjects. Registered dietitians collected dietary data through a food frequency questionnaire (measuring intake on a daily, weekly, and monthly basis). Anthropometric measures such as waist circumference, weight, and body mass index (BMI) were gathered at baseline and again every year. A significant inverse relationship was found between high dietary PI and low body adiposity. Moreover, as little as 37% of energy from phytochemical-rich foods decreased body adiposity in adults. The researchers concluded a high PI also correlated with weight gain prevention, as those with a lower PI gained significantly more weight (Mirmiran et al., 2012).

Another study aimed to correlate the PI with weight gain, inflammation, and oxidative stress (Vincent et al., 2010). The cross-sectional study included 54 adults divided into a normal weight group and an overweight group based on body mass index (< 25 kg/m^2 and ≥ 25 kg/m^2, respectively). Researchers administered a three-day food diary sheet for subjects to complete. In an attempt to confirm diet consistency throughout, another three-day food diary was administered after the eight-week study and compared to baseline. Food records were analyzed for macronutrient and caloric intake as well. A higher PI score was associated with significantly lower BMI, waist circumference, and plasma oxidative stress. In addition, researchers were able to control for caloric and carbohydrate intake because intakes were not significantly different between groups. Despite having similar caloric intakes, the overweight group consumed notably fewer green vegetables and fresh fruit than the normal weight group (Vincent et al., 2010). These findings indicate there could be much more involved in body adiposity than "calories in versus calories out" and phytonutrients could be a fundamental component. A current literature review on the PI and adiposity measures suggests that higher PI is associated with lower body mass index, waist circumference and body fat (Carnauba et al., 2016). More specifically, emerging research suggests an increase in PI reduces adipose tissue growth in humans (Rupasinghe, Sekhon-Loodu, Mantso, & Panayiotidis, 2016; Tucci, 2010). Though not fully understood, many mechanisms in which phytonutrients combat obesity have been identified. One way in which increasing PI improves adiposity is through differentiation of pre-adipocytes and appetite.

Phytonutrients can also promote lipolysis and fatty acid oxidation, which enables weight loss (Rupasinghe et al., 2016; Tucci, 2010). In addition, phytochemicals have anti-obesity properties through indirect pathways as well. These benefits include antioxidant, anti-inflammatory, anti-aging, anti-proliferative, and anti-carcinogenic properties (Szic, Declerck, Vidakovic, & Berghe, 2015. Along with many potential anti-obesity properties, increased intake of phytonutrients (and higher PI) has been shown to be safe to consume in all quantities. Phytonutrients have been established to cause no adverse health issues, even in higher doses through supplements (Kim & Park, 2016).

Body Mass Index

The evaluation of adiposity and designations of overweight and obesity use a variety of approaches. When assessing adults, the body mass index (BMI) is the most commonly used. It is a popular adiposity measure due to it being convenient, safe, and low cost (Gallagher et al., 1996; Shah & Braverman, 2012; Nuttall, 2015). According to the Centers for Disease Control and Prevention (CDC), BMI is calculated by the formula: weight (pounds) / [height (inches)]^2 x 703. The resulting BMI formula is then converted into set weight groups that estimate the amount of adiposity the individual has. The four categories are: underweight (below 18.5 kg/m^2), normal (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2), and obese (30 kg/m^2 and above) (CDC, 2017). Although the correlation between BMI and body adiposity is fairly strong, it is possible for people to have the same BMI, but different levels of body adiposity (Cornier et al., 2009; Flegal et al., 2009; Gurunathan & Myles, 2016). A common problem with BMI as a measurement of adiposity is that it does not differentiate between lean body mass (like muscle) and body fat mass (Gurunathan & Myles, 2016; Nuttell, 2015). For example, a person can have a

BMI categorized as overweight or obese, but still have a very low fat mass. This is commonly seen in athletes with more muscle mass (Gurunathan & Myles, 2016; Nuttell, 2015). Another disguised factor in a higher BMI could be bone density. Two people who are the same height and weight would be calculated to have the same BMI, but if one has denser/bigger bones, fat free mass would be higher (Gurunathan & Myles, 2016; Morin & Leslie, 2008). Despite having some limitations, BMI remains a consistent measure of overweight/obesity in clinical use. However, using BMI along with waist circumference measurements has been shown to be an even better measurement of body adiposity, as the combination can offset some of the limitations of BMI alone (Borruel et al., 2014; Yu et al., 2015).

Waist Circumference

Waist circumference is a simple and cost efficient anthropometric measure to assess body adiposity. However, in comparison to other measures, research has shown waist circumference to be a better stand-alone predictor of adiposity than others (Borruel et al., 2015; Janssen, Katzmarzyk, & Ross, 2004).

The American Heart Association (AHA) and the National Heart, Lung, and Blood Institute (NHLBI) have established the methods of measuring waist circumference (Grundy, 2005). In the past, discrepancies in the proper technique, such as placement of tape, tightness of tape, and subjects' posture, have contributed to inconsistencies in research findings (Grundy, 2005; WHO 2008). The World Health Organization (WHO) created the WHO STEPS protocol for measuring waist circumference, which instructs the measurement be made at the approximate midpoint between the lower margin of the last palpable rib and the top of the iliac crest (NHLBI, 2013; WHO, 2008). For simplicity of measures, some studies have shown measuring at the point of minimal waist is just as efficient at assessing adiposity (Harrington, Staiano, Broyles, Gupta, & Katzmarzyk, 2012; Willis et al., 2007). In addition to the placement in relation to the body, it is also important to ensure that the tape is held parallel to the floor. Any deviation to a parallel measure can increase the circumference of the measurement (NHLBI, 2013; WHO, 2008)

Another component of waist measurements that is important to perform consistently is the tightness of the measuring tape (WHO, 2008). According to the WHO STEPS protocol, the measuring tape should be snug around the body, but not so tight that it is constricting. The tape should not be pulled so tight that it compresses the skin (NHLBI, 2013; WHO, 2008).

In order to get the most accurate measure in waist circumference, posture also must be considered (Higgins & Comuzzie, 2012; WHO, 2008). The WHO, along with the United States National Health and Nutrition Examination Survey (NHANES), recommend the subject stand upright, feet close together, and with body weight evenly distributed across feet. This positioning allows for the most accurate measure of the actual waist without other postural alterations (Higgins & Comuzzie, 2012; WHO, 2008). In addition, the subject should remain relaxed during the measurement to relieve any abdominal tension (Higgins & Comuzzie, 2012; NHANES, 2007; WHO, 2008). Many individuals naturally “suck in” the abdominal wall, which increases abdominal tension. Increasing the tension (by sucking in) falsely reduces the waist circumference. Moreover, decreasing tension in the abdominal wall increases waist circumference to its normal and actual size. Since many individuals unconsciously tense, the measurements should be taken at the end of normal expirations to prevent excess inward abdominal pull (Higgins

& Comuzzie, 2012; NHANES, 2007; WHO, 2008).

Despite having established protocols for measuring waist circumference, there can still be variability in the measurements, especially if a variety of personnel are involved (Mason & Katzmarzyk, 2009; WHO, 2008). In order to log the most efficient measurements and avoid discrepancies, measurements should be taken twice. If there is a one centimeter or less difference between measurements then the average of the two should be calculated. If the difference between the two measurements is more than one centimeter, two additional measurements should be taken (Mason & Katzmarzyk, 2009; WHO, 2008).

Waist circumference has been shown to be the most efficient anthropometric measure for body adiposity, especially when used in combination with the body mass index (BMI) (Borruel et al., 2014; Dijk, Takken, Prinsen, & Wittink, 2012; Yu et al., 2015). Not only are these measurements simple and cost effective but when combined, they can offset some of the limitations observed when they are used individually (Borruel et al., 2014; Dijk et al., 2012; Yu et al., 2015). However, BMI and waist circumference are only predictors of adiposity present and do not actually measure body composition.

Bioelectrical Impedance Analysis

Bioelectrical impedance analysis (BIA) is a method widely used in studies assessing body composition (Kyle, Piccoli, & Pichard, 2003; Mialich, Maria, Sicchieri, Afonso, & Junior, 2014). BIA provides a convenient, non-invasive, and relatively inexpensive method that estimates the distribution of body fluids in the intra- and intercellular spaces in addition to the body components (Mialich et al., 2014). Performance of the BIA is based on the passage of low amplitude electrical currents directed through cables connected to conducting surfaces placed in contact with the skin. In this respect, BIA estimates body compartments such as fat mass, lean mass, and total body water (Mialich et al., 2014). According to the National Institutes of Health Technology Assessment Conference Statement on BIA in body composition measurement, BIA is more accurate than body mass index and most other anthropometric measures at estimating fat mass in adults (Kyle et al., 2003). Although BIA is accurate in measuring fat adiposity in all body types, it is less accurate when used to assess weight loss over periods of time in comparison to methods like the Dual-Energy X-ray Absorptiometry (Kettaneh et al., 2005; Li et al., 2013; Miyatani, Yang, Thomas, Craven, & Oh, 2012). However, when compared to more advanced body composition analysis methods, BIA has demonstrated to be a valid and accurate measure of general body composition (Bedogni et al., 2013; Miyatani et al., 2012). In addition, BIA is more cost effective, non-invasive, and easier to use than more advanced options (Kyle et al., 2003; Mialich et al., 2014; Miyatani et al., 2012). Many studies have observed different models of Tanita™ BIA scales and have deemed them as an effective and efficient tool for estimating body composition in small and large clinical studies (Dehghan & Merchant, 2008; Beeson et al., 2010; Wang et al., 2013; Wang & Hui, 2015).

Summary

Obesity is a major issue for the United States and is believed to be a root cause of many prominent diseases (Ard et al., 2016; Bell & Rolls, 2008; Hill et al., 2012). An abundance of research leads to the conclusion that increased intakes of fruit and vegetables (F/V) are correlated with improvements in anthropometric parameters and preventing/treating adiposity (Ledoux et al., 2010; Mytton et al., 2017; Schwingshackl et al., 2015). Research also has indicated there is more to fruits and vegetables than fiber and vitamins, but that phytonutrients could also be a primary component in improving excess body adiposity (Carnauba et al., 2016; Rodriguez-Casado, 2014).

Despite researchers isolating phytonutrients in studies, these nutrients elicit benefits best when left in whole foods (Liu, 2012; Pizzorno, 2014). Moreover, instead of isolating phytonutrients, studying them in their whole form based on color could be a more efficient method (Pizzorno, 2014). This is due to phytonutrients producing specific pigments when abundant in the plant (Slavin & Lloyd, 2012).

Phytonutrients of particular interest are chlorophyll, carotenoids, and anthocyanins. The current research shows potential for chlorophyll as a therapeutic phytonutrient that can help treat and/or prevent adiposity (Cook et al., 2014; Stenblom et al., 2016; Szulinska et al., 2017). Anthocyanins may play a role in combatting obesity by stimulating decreases in adipose tissue through various pathways (Azzini et al., 2017; Meydani & Hasan, 2010; Williams et al., 2013). Carotenoids were found in a variety of studies to play a role in the prevention of excess adiposity. Although human trials are scarce, they have indicated a beneficial effect of increased carotenoid intake on adiposity (Bonet et al., 2015; Whigham et al., 2012).

Very few clinical intervention trials have been properly designed and executed. This is primarily due to these studies being difficult to conduct (Probst et al., 2017). The data from observational studies are more likely to suggest a role for phytonutrients in the prevention and treatment of obesity (Azzini et al., 2017). In the future, new and sufficient models that would allow the design to be more consistent and more efficient for clinical studies are needed (Gonzalez-Castejon & Rodriguez-Casado, 2011; Probst et al., 2017).

In an effort to study phytonutrients in an easily accessible and inexpensive way, a method called the "Phytochemical Index" (PI) was proposed (McCarty, 2004). Although it is not the most precise method available, it can provide a valid estimation of the of phytonutrient intake which can aid in studying the health correlations of diets high and/or low in phytonutrient-rich foods (Bahadoran et al., 2013; McCarty, 2004; Mirmiran et al., 2012; Vincent et al., 2010). The current literature suggests a higher PI is associated with lower body mass index, waist circumference and body fat percentages (Carnauba et al., 2016; Kim & Park, 2016; Rupasinghe et al., 2016; Tucci, 2010).

There are many methods in which adiposity and obesity can be measured. The most commonly used method is the body mass index (BMI) (Gallagher et al., 1996; Shah & Braverman, 2012; Nuttall, 2015). Another common anthropometric measure of adiposity is waist circumference, which is often shown to be more efficient than BMI alone. Interestingly, using BMI and waist circumference in tandem has been shown to offset limitations of each (Borruel et al., 2014; Dijk et al., 2012; Yu et al., 2015). Despite being simple and effective methods, BMI and waist circumference are only predictors of adiposity but they do not actually measure body composition. BIA is more accurate than body mass index and most other anthropometric measures in assessing body composition (Kyle et al., 2003). When compared to more advanced measures, BIA is less expensive, less invasive, and easier to use (Kyle et al., 2003; Mialich et al., 2014; Miyatani et al., 2012). Studies have shown the Tanita™ BIA scales to be an effective and efficient tool for estimating body composition in clinical studies (Beeson et al., 2010; Dehghan & Merchant, 2008; Wang et al., 2013; Wang & Hui, 2015)

CHAPTER 3

METHODS

This study investigated if a higher phytonutrient index (PI) correlated with a lower body mass index (BMI), less body fat, and smaller waist circumference. In addition, a second aim was to examine if any correlations existed between certain colors of fruits and vegetables (green, red/purple, and yellow/orange) and fat percentage, waist circumference, and Body Mass Index (BMI). The study design was a cross-sectional design. Institutional Review Board approval was obtained from the Thibodaux Regional Wellness Center and the Louisiana Tech University Human Use Committee (Appendix A).

Participants

Fifty (50) participants were randomly selected from Thibodaux Regional Wellness Center in Thibodaux, Louisiana through computerized simple random sampling. Inclusion criteria required the participants to be between the ages of 18 and 55. Due to uncertain validity of body fat measures in morbidly obese subjects (>40 kg/m^2), subjects with a BMI of >40 kg/m^2 were excluded (Stahn, Terblanche & Gunga, 2012). Due to the use of electrical currents in the bioelectrical impedance analysis (BIA), pregnant subjects were also excluded. Participants were required to give informed consent prior to being enrolled in the study (see Appendix A-2).

Body Adiposity Assessments

The primary instrument used to assess body adiposity was a Tanita™ BF-350 bioelectrical impedance analysis (BIA) scale. The scale was linked to a desktop computer with the program Polar Body Age™ in order to assess body composition. The linked Tanita™ BF-350 and the Polar Body Age™ program calculated body mass index (BMI) and body fat percentage. Waist circumference was measured with a flexible tape, and a mounted wall measuring tape was used to measure height. The BIA scale accurately weighs up to 440 pounds. However, body fat percentage could be overestimated in patients who are dehydrated due to BIA scales assuming normal hydration (Stahn et al., 2012). Participants were given a protocol sheet when scheduled for assessment to promote proper hydration the day before and the day of their assessment.

Nutrition Assessment

All subjects participated in three pass 24-hour food recalls which was used to estimate the phytonutrient index score. Each participant had recalls from two weekdays and one weekend day to acquire an estimate of their daily intake (Vincent et al., 2010). All food recalls were administered face-to-face and one-on-one with each participant by the primary investigator. Each food recall was done on separate days and within 24 hours of the day being recalled. A Microsoft Excel data log was used to record food, meals, and beverages recalled by each subject. In order to estimate serving sizes of foods consumed, standard measuring tools were used to give visual amounts to better predict intake. The primary investigator (a registered dietitian), probed for details in individual consumption to get as accurate estimations as possible. For example, if a participant recalled eating a sandwich, the dietitian researcher asked follow up questions to recall what smaller components made up the sandwich such as tomatoes, mustard, lettuce, etc.

In order to determine different sources of phytochemicals by color, each participant was also administered a Food Frequency Questionnaire (FFQ) to determine the intake of the specific color classifications (green, purple, and orange) (Pierce et al., 2004). Example foods were presented to participants to allow a more accurate measure of frequency. Although there is no gold standard to FFQ categories, the categories used by the National Health and Nutrition Examination Survey were reflected (NHANES, 2017; Thompson, Burley, & Warm, 2002). The categories include less than once per month, 1-3 per month, once per week, 1-3 per week, 4-6 per week, once per day, and 2-3 per day. These servings were averaged for data analysis so that all were reported as weekly intakes without ranges. Less than once per month and 1-3 per month were recorded as 0.5 servings per week; 1-3/week was reported as 2/week; 4-6/week were reported as 5/week, 1/day was reported as 7/week; and 2-3/day was reported as 17.5/week. In order to control for seasonal intake (i.e. fruits and vegetables), the FFQ represented intake on an annual basis and that was explained to each participant.

Procedures

Subjects' were screened in the member computer system in order to identify those meeting age and BMI inclusion criteria. Subject selection was done through computerized simple random sampling. After selecting subjects, their health questionnaire and doctor notes were pulled to confirm that they were not on any obesity-related medications. Once eligibility was determined for the study, individuals were contacted for verbal and written informed consent and participation acceptance. Once participation was accepted, subjects were scheduled to come into the clinic for their body composition and nutrition assessment.

The first part of the assessment was measuring the subject's height for body mass index (BMI). Afterwards, waist circumference was measured following the World Health Organization (WHO) approved protocol (WHO, 2008). The Tanita™ BF-350 bioelectrical impedance analysis (BIA) scale was used to provide weight (in pounds), BMI, and body fat composition (fat mass and lean mass).

The nutrition assessment consisted of three 24-hour recalls by a Registered Dietitian in a one-on-one setting with each subject. The dietitian recorded all of the recalled data (meals, beverages, snacks, etc.). To increase accuracy of food quantity, household measures were used as visuals during the food recall. Each recall was done on separate days and within 24 hours of the day being recalled.

The total energy intake (estimated with Elizabeth Stewart Hands and Associates (ESHA) software) was used for the phytonutrient index (PI) calculation. The PI estimated how much of the subject's energy intake was accounted for by phytonutrient rich foods. The formula for the PI is [(phytochemical-rich foods kcal/total daily energy intake kcal) × 100]. Phytonutrient rich foods primarily include fruits and vegetables, but also legumes, whole grains, seeds, nuts, olive oil, soy sources, and wine (McCarty, 2004). In addition to calculating the PI, the dietitian also identified intakes of the specific colors (green, orange, and purple) through a FFQ. More specifically green colored plants are chlorophyll-rich (such as spinach, broccoli, celery, etc.), reddish-purple foods are anthocyanin-rich (such as purple cabbage, grapes, blueberries, etc.) , and orange-yellowish foods are carotenoid rich (such as carrots, sweet potatoes, mangoes, etc) (Pizzorno, 2014; Slavin & Lloyd, 2012).

Data Analysis

IBM SPSS Statistics version 24 was used to analyze the data. Background data on participants was summarized including age, sex, health status, and race Pearson correlation was used to assess correlations between phytonutrient index and body adiposity measures. Analysis of variance (ANOVA) with post-hoc tests was used to determine differences in PI and phytochemical intakes among the BMI, waist circumference and percent body fat groups. Results were considered statistically significant if p-value was less than or equal to 0.05.

CHAPTER 4

RESULTS

Fifty (50) participants were recruited to participate in the study and none of them declined. All 50 participants remained until the completion of the study. Table 1 provides a description of the subjects in the study including sex, ethnicity, and current health diagnosis. The average age was 36.8 $\pm$ 11.4 years with a range of 18 to 54 years. Despite having a variety of ethnicities, almost 75% of the population identified as White/Caucasian and 70% of the population was female. Nearly half of the sample had some form of health condition (most notably diabetes and hypertension) Demographic characteristics of the group are summarized in Table 1.

Table 1

Demographics of Participants (*n*=50)

Demographic	(*n*)	%
Gender		
Male	15	30%
Female	35	70%
Ethnicity		
White/Caucasian	37	74%
African American	9	18%
Latino	4	8%
Health Diagnosis		
Hypertension	8	16%
Type 2 Diabetes	9	18%
Hyperlipidemia	3	6%
Osteoarthritis	3	6%
Cystic Fibrosis	1	2%

The mean PI score and average caloric intake of the entire group was 38.1 ± 21.1 and 1271 ± 393, respectively. Participants with various health conditions had average phytonutrient index (PI) lower than the population average This was true of participants with type 2 diabetes (22 ± 17.7), hypertension (17.4 ± 11.6), hyperlipidemia (21.6 ± 19.4), and osteoarthritis (24.3 ± 27.4). Waist circumference and body fat percentage categorized nearly half of the participants as having healthy body composition (waist circumference = 27/50; body fat = 24/50), while the body mass index (BMI) categorized 17 out of 50 as healthy. Table 2 summarizes the average phytonutrient index (PI) of the participants in each category of body composition measures as well as significant differences between categories based on Analysis of Variance (ANOVA).

Table 2

Average Phytonutrient Index of Participants (*n*=50) [1]

Body Composition Measures	(*n*)	Mean ± SD
Body Mass Index (kg/m^2)		
Healthy (18.5-24.9)	17	53 ± 12.4 [a]
Overweight (25-29.9)	9	57 ± 10.8 [a]
Obese (30-34.9)	12	20 ± 14.4 [b]
Severe Obese (35-39.9)	11	18 ± 7.7 [b]
Waist Circumference[2]		
Healthy – meets standard	27	54 ± 11.9 [a]
Overweight – over standard	23	20 ± 12.9 [b]
Body Fat Percentage[3]		
Healthy	24	56 ± 10.5 [a]
Overweight	11	28 ± 10.5 [b]
Obese	15	16 ± 11.3 [c]

[1] Phytonutrient index represents the percentage of diet that comes from phytonutrient-rich foods

[2] Meets standard in waist circumference is 40 inches or less for males and 35 inches or less for females.

[3] World Health Organization guidelines for body fat percentage are based on gender and age of individual.

[a,b,c] different superscripts indicate values are significantly different ($p < .05$)

Statistically significant correlations were found between the PI ratings and body composition ranges. The only measure that did not follow the trend for positive correlations between PI and lower body adiposity was body mass index. Participants with an overweight BMI had a higher PI average than those with a healthy BMI. Overall, the data analysis showed a strong inverse correlation between PI and BMI ($r = -0.753$, $p = 0.00$), waist circumference ($r = -0.730$, $p = 0.00$), and body fat percentage ($r = -0.701$, $p = 0.00$). Figures 1, 2, and 3 show the correlation graphs.

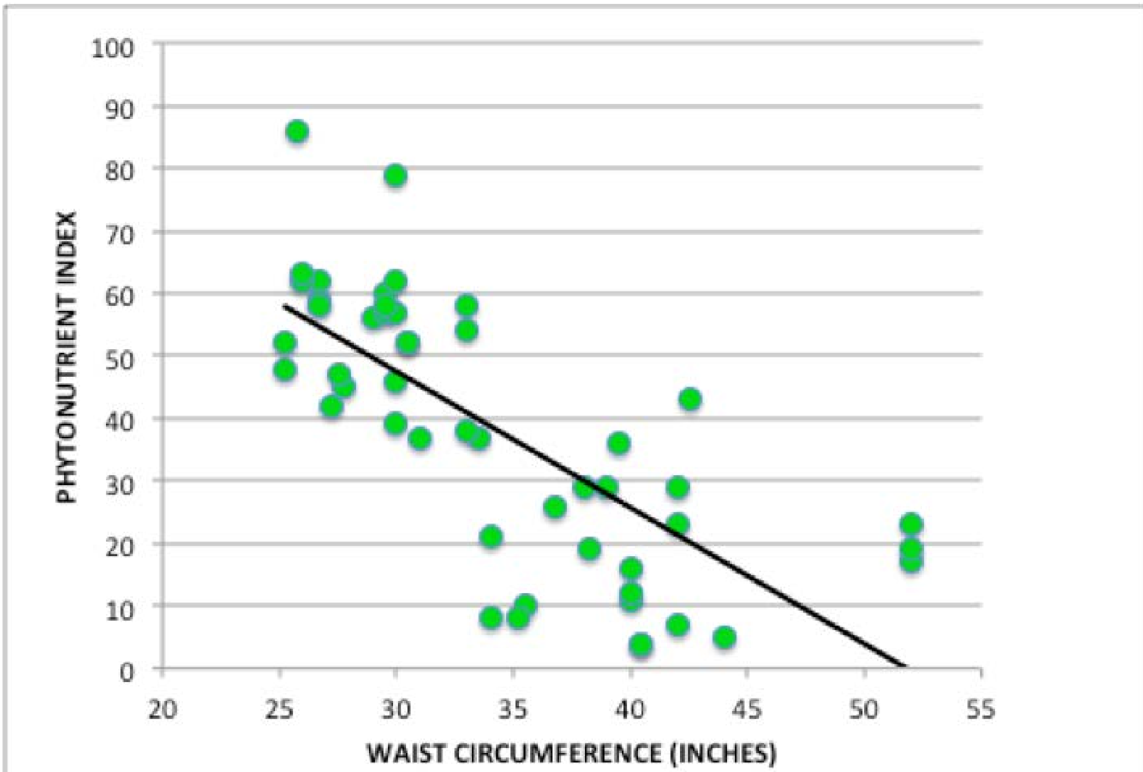

Figure 1: Phytonutrient Intake and Waist Circumference

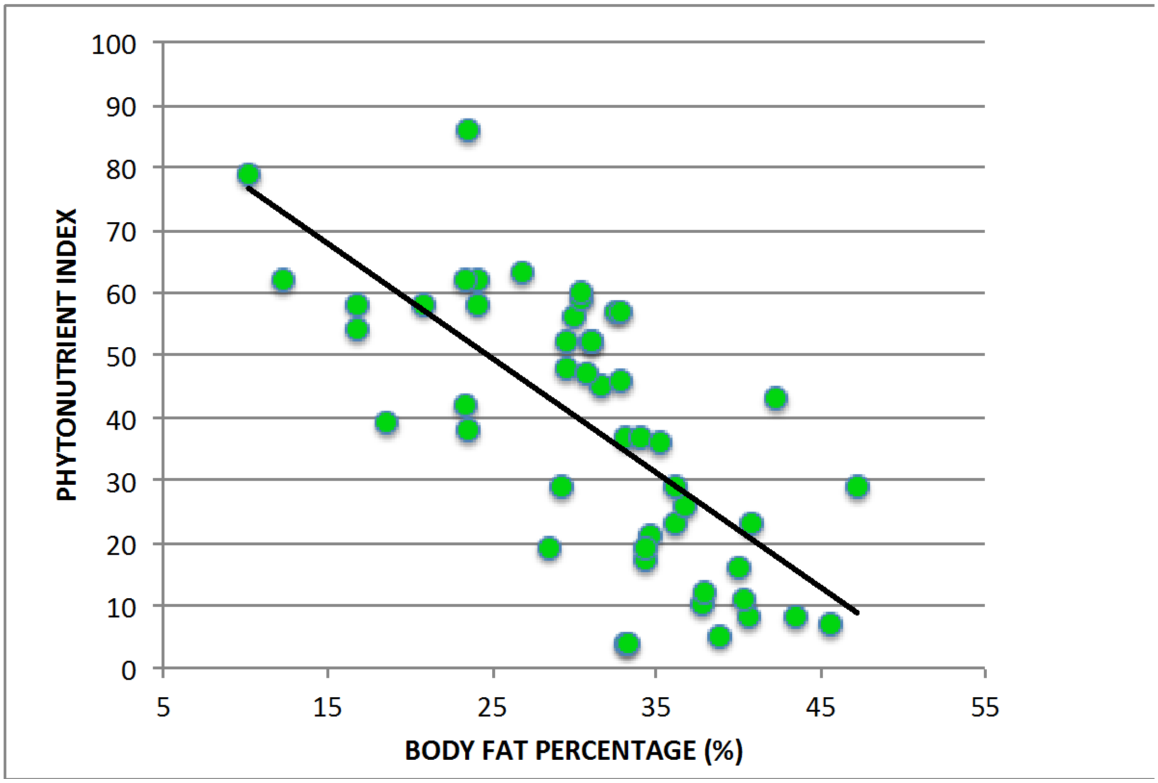

Figure 2: Phytonutrient Intake and Body Fat Percentage

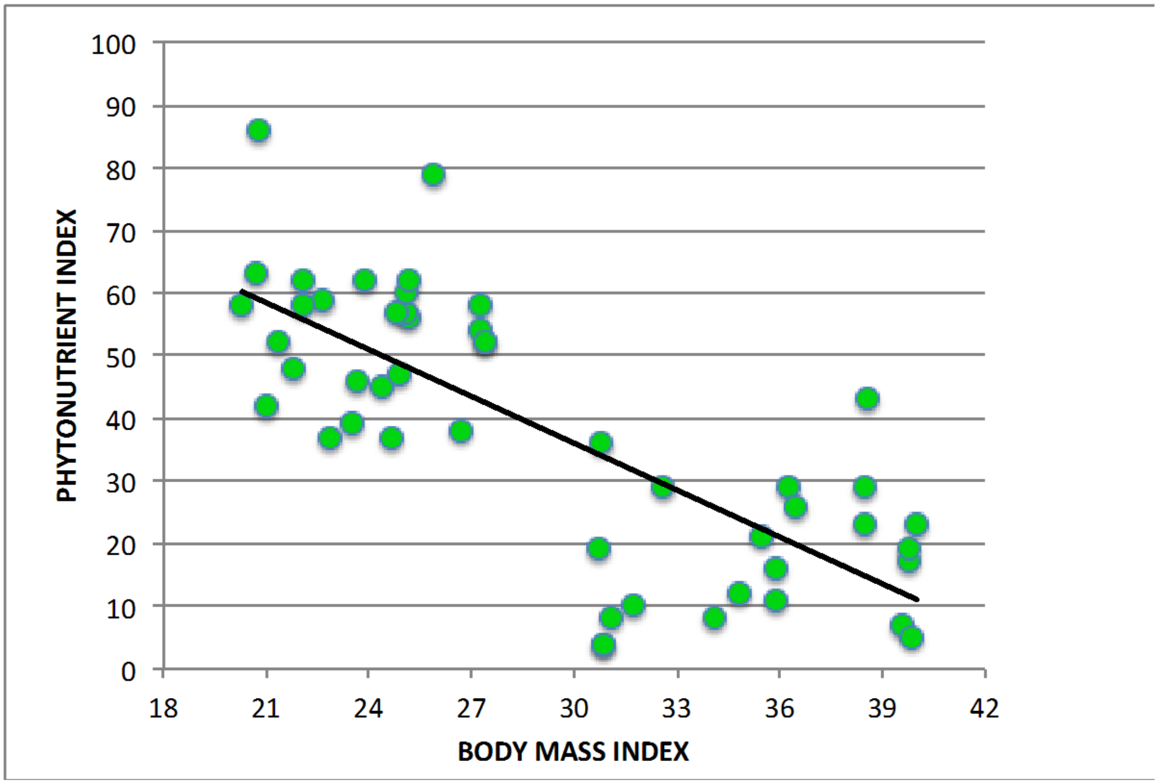

Figure 3: Phytonutrient Intake and Body Mass Index

ANOVA testing was used to analyze significant differences ($p < .05$) in the intake of foods containing specific types of phytonutrients (chlorophyll, carotenoids and anthocyanin) based on body composition measures (shown in Tables 3, 4, and 5). All the standards of body composition showed significant differences ($p < .05$) except for the "healthy" and "overweight" standards measured by BMI.

Individuals with "healthy" body fat percentages had significantly greater intakes of all phytonutrient types than those with excess body fat (overweight and obese). However, there was not a significant difference in intakes between "overweight" and "obese" body fat percentages. The waist circumference standards followed the same trend as those with "healthy" waist sizes had significantly greater intakes of all phytonutrients in comparison to those that exceeded healthy waist sizes.

Considering BMI measurements, individuals that were within “healthy” standards had significantly greater intakes of all phytonutrient types when compared to those within “obese” and “severe obese” standards. However, there was no significant difference between the intakes of those with “healthy” and “overweight” standards.

Table 3

Mean Daily Servings of Chlorophyll Foods Based on BMI, Waist Circumference, and Percent Body Fat

Body Composition		Mean ± SD
Body Mass Index (kg/m^2)		
Healthy	(18.5-24.9)	7.0 ± 5.4 [a]
Overweight	(25-29.9)	8.9 ± 6.1 [a]
Obese	(30-34.9)	2.5 ± 2.5 [b]
Severe Obese	(35-39.9)	1.8 ± 1.8 [b]
Waist Circumference		
Healthy – meets standard		7.5 ± 5.7 [a]
Overweight – over standard		2.3 ± 2.3 [b]
Body Fat Percentage		
Healthy		8.5 ± 5.5 [a]
Overweight		2.6 ± 2.0 [b]
Obese		1.7 ± 1.8 [b]

[1] The intake means are based off of how many servings per week were consumed
[a,b,c] different superscripts indicate values are significantly different ($p < .05$)

Table 4

Mean Daily Servings of Carotenoid Foods Based on BMI, Waist Circumference, and Percent Body Fat

Body Composition		Mean ± SD
Body Mass Index (kg/m^2)		
Healthy	(18.5-24.9)	4.1 ± 4.1 [a]
Overweight	(25-29.9)	2.5 ± 1.9 [ab]
Obese	(30-34.9)	1.0 ± 0.6 [b]
Severe Obese	(35-39.9)	0.7 ± 0.3 [b]
Waist Circumference		
Healthy – meets standard		3.5 ± 3.5 [a]
Overweight – over standard		2.3 ± 2.3 [b]
Body Fat Percentage		
Healthy		3.8 ± 3.6 [a]
Overweight		0.8 ± 0.5 [b]
Obese		1.0 ± 0.6 [b]

[1] The intake means are based off of how many servings per week were consumed

[a,b,c] different superscripts indicate values are significantly different ($p < .05$)

Table 5

Mean Daily Servings of Anthocyanin Foods Based on BMI, Waist Circumference, and Percent Body Fat

Body Composition		Mean ± SD
Body Mass Index (kg/m^2)		
Healthy	(18.5-24.9)	4.0 ± 2.9 [a]
Overweight	(25-29.9)	3.5 ± 2.5 [ab]
Obese	(30-34.9)	0.9 ± 0.5 [bc]
Severe Obese	(35-39.9)	0.7 ± 0.5 [c]
Waist Circumference		
Healthy – meets standard		3.7 ± 2.8 [a]
Overweight – over standard		1.0 ± 0.6 [b]
Body Fat Percentage		
Healthy		4.1 ± 2.7 [a]
Overweight		1.1 ± 0.7 [b]
Obese		0.7 ± 0.4 [b]

[1] The intake means are based off of how many servings per week were consumed
[a,b,c] different superscripts indicate values are significantly different ($p < .05$)

CHAPTER 5

DISCUSSION

An abundance of nutrition and scientific research leads to the conclusion that higher intakes of fruit and vegetables (F/V) are correlated with improvements in anthropometric parameters and preventing/treating adiposity (Ledoux et al., 2010; Mytton et al., 2017; Schwingshackl et al., 2015). However, some research has indicated there is more to fruits and vegetables than fiber and vitamins, and that phytonutrients could also be a primary component of food contributing to the improvement in body composition (Carnauba et al., 2016; Rodriguez-Casado, 2014). The purpose of this study was to determine if phytonutrient intake measured by the phytonutrient index (PI) is correlated with body composition. Data were collected on 50 individuals from Thibodaux Regional Wellness Center in Thibodaux, Louisiana. The data showed that higher intakes of phytonutrients significantly correlate with better body composition in all three measures (body mass index, waist circumference and body fat percentage). BMI categorized the least amount of participants as healthy (17/50). It is a possibility that individuals with more muscle/bone dense had BMIs classifying them in the overweight category despite having low body-fat and waist circumference. The population in Louisiana is particular interesting because nearly 70% of Louisiana adults are overweight or obese, and reportedly consume low amounts of fruits and vegetables (BRFSS, 2016, CDC, 2018). According to the National Health and Nutrition Examination Survey

(NHANES), American males consume an average of 2,640 calories/day and females consume an average of 1785 calories/day (average of 2213 for both genders). The data on this population sample averaged a much lower intake of daily calories at 1271 ± 393. Many of the participants (21/50) reported eating only once or twice per day, consuming as little as 565 calories per day. This data further suggests that calorie consumption is not the only factor influencing obesity. However, the United States Department of Agriculture states that many respondents under report intake and that studies show actual intakes may be considerably higher.

Every participant with a health condition had a lower PI score than the population average (38.1 ± 21.1) except for one who had cystic fibrosis (PI = 57). This exception is likely related to cystic fibrosis being a genetic condition and not one developed by an individual's lifestyle factors (e.g., diet). These data support previous studies showing that increased fruit and vegetable intake has been correlated with reduced risk of diseases (Aune et al., 2017; Oguntibeju et al., 2013; Pem & Jeewon, 2015; Slavin & Lloyd, 2012). Although a small population, these data are some of the first to show any relationship between PI and disease prevalence.

In an effort to determine differences in the intakes of specific phytonutrients (chlorophyll, carotenoids, and anthocyanin) based on body composition, one-way ANOVA testing was used. Phytonutrient intake was not precisely measured, but rather identified by categorizing foods by color as some research supports due to evidence that whole food is more applicable and health promoting than precisely isolated phytonutrients (Liu, 2012; Pizzorno, 2014).

Individuals with “healthy” body fat percentages had significantly greater intakes of all three phytonutrient types than those with excess body fat (overweight and obese). However, there was not a significant difference in intakes between “overweight” and “obese” body fat percentages. The waist circumference standards followed the same trend as those with “healthy” waist sizes had significantly greater intakes of all phytonutrients in comparison to those that exceeded healthy waist sizes.

Considering BMI measurements, individuals that were within “healthy” standards had significantly greater intakes of all phytonutrient types when compared to those within “obese” and “severe obese” standards. However, there was no significant difference between the intakes of those with “healthy” and “overweight” standards.

Interestingly, each body composition measure had a different phytonutrient that showed the most significant difference. For BMI measurements, the intake of carotenoids between “healthy” and “severely obese” individuals showed the most significance ($p < 0.00$). The intake of anthocyanin showed the most significant difference between standard and excess waist sizes ($p < 0.00$). Chlorophyll intakes between those with “healthy” and “obese” body fat percentages showed most significant differences ($p < 0.00$). Further analysis showed that participants who had higher intakes of one phytonutrient, typically had higher intakes of others which skews any evidence that one has any more of an effect than others.

The cause of obesity is very complex, but poor nutrition seems to be a primary cause (Mytton et al., 2017; Swinburn et al., 2004). Fruit and vegetable intake has long been shown to have a positive effect on health and body composition (Aune et al., 2017; Dehghan et al, 2011; Halkjaer et al., 2009; Schwingshackl et al., 2015). Researchers are not clear as to why fruits and vegetables have this effect, but some studies have shown varying body composition changes among subjects depending on which fruits and vegetables they consumed more often (Bertoia et al., 2016; Zhao, 2007). In considering the differences between various plant foods, a major difference is the specific phytonutrients they contain.

Research has shown that despite excess intake of calorie dense foods, obese individuals lack phytonutrient intake **(**Carnauba et al., 2016; Kaidar-Person et al., 2008a, 2008b). These phytonutrients are primarily sourced from fruit and vegetable intake (Carnauba et. al., 2016).

In a population like Louisiana, data shows that less than half of the population eats at least one serving of fruits/vegetables each day, which is much less the five servings per day recommended by the United States Department of Agriculture (USDA, 2016). The data available indicates that low fruit and vegetable intake, thus low phytonutrient intake, is correlated with excess adiposity.

The data in this study demonstrate a strong correlation between higher phytonutrient intake and improved body composition (and disease prevalence). However this correlation remained consistent despite the type of phytonutrients consumed. These findings imply that overall diet quality seems to make the most difference, but the phytonutrients in fruits/vegetables are thought to be a key reason for those benefits.

The study of phytonutrients is difficult and expensive, which is why the PI is used in research studying health and intake of phytonutrient-rich foods (McCarty, 2004; Vincent et al., 2010). A major strength of the study was the ability to have a registered dietitian meet with each participant face-to-face to conduct the dietary recalls. In comparison to a voice call or participant filled record, the face-to-face recalls allowed for more follow up questions and visual aids to more precisely determine food intake. Another strength of the study was the use of three different measures of body composition. The study used body mass index (BMI), waist circumference, and body fat percentage that in combination help more accurately determine body adiposity by offsetting any limitations that each individual measure may have (Borruel et al., 2014; Wang & Hui, 2015; Yu et al., 2015).

The most notable limitation of this study was the method of gathering dietary data through food recalls and food frequency questionnaires. These methods are subjective interpretations that rely on the ability of each participant to recall food and quantify food intake, which leaves room for error (Probst et al., 2017). In addition, the food recalls could not quantify for very small intakes of phytonutrient rich foods (such as sesame seeds on a hamburger bun) that were part of an individual's diet. Another limitation is the inability to rule out of other factors that can be contributing to better body composition such as higher fiber intake and lower calorie intake which are both related to diet high in phytonutrients (Kaiser et al., 2014; Ledoux, et al., 2010; Schwingshackl et al., 2015). Another factor that could have improved this study would be to increase the sample size and vary the source of the population instead of selecting all from one facility. This would not only allow for a larger data set, but potentially more diverse data as well.

There have been very few clinical intervention trials on phytonutrient intake that have been properly designed and executed due to these studies being difficult to conduct and expensive (Azzini et al., 2017; Probst et al., 2017). However, data from correlational studies like this do suggest a role for phytonutrients in the prevention/treatment of obesity. Future studies should adopt an observational design that monitors the intakes and body composition of individuals over time to see if changes in either or both support the role of these nutrients. In addition, future studies should also observe the health conditions in participants to see if these phytonutrients have a role in prevention/treatment of diseases. In the future, new and more sufficient models are likely to be developed that would allow the design to be more consistent and accurate in the way data can be more objectively measured. Until those tools are developed, researchers should continue to use nutrition experts to recall intakes with participants in person to determine nutrition intake as accurately as possible (Gonzalez-Castejon & Rodriguez-Casado, 2011; Probst et al., 2017).

APPENDIX A

A-1 HUMAN USE COMMITTEE APPROVAL FORMS

A-2 HUMAN SUBJECTS CONSENT FORM

A-3 CLINIC APPROVAL FORM

A1 - HUMAN USE COMMITTEE APPROVAL FORMS

LOUISIANA TECH UNIVERSITY

MEMORANDUM

OFFICE OF SPONSORED PROJECTS

TO:	Dr. Katherene Anguah and Mr. Ryan Orgeron
FROM:	Dr. Richard Kordal, Director of Intellectual Property & Commercialization (OIPC) rkordal@latech.edu
SUBJECT:	HUMAN USE COMMITTEE REVIEW
DATE:	February 27, 2018

In order to facilitate your project, an EXPEDITED REVIEW has been done for your proposed study entitled:

"Fruit/Vegetable Intake & Body Composition: Phytonutrient Connections"

HUC 18-098

The proposed study's revised procedures were found to provide reasonable and adequate safeguards against possible risks involving human subjects. The information to be collected may be personal in nature or implication. Therefore, diligent care needs to be taken to protect the privacy of the participants and to assure that the data are kept confidential. Informed consent is a critical part of the research process. The subjects must be informed that their participation is voluntary. It is important that consent materials be presented in a language understandable to every participant. If you have participants in your study whose first language is not English, be sure that informed consent materials are adequately explained or translated. Since your reviewed project appears to do no damage to the participants, the Human Use Committee grants approval of the involvement of human subjects as outlined.

Projects should be renewed annually. ***This approval was finalized on February 27, 2018 and this project will need to receive a continuation review by the IRB if the project continues beyond February 27, 2019.*** **ANY CHANGES** to your protocol procedures, including minor changes, should be reported immediately to the IRB for approval before implementation. Projects involving NIH funds require annual education training to be documented. For more information regarding this, contact the Office of Sponsored Projects.

You are requested to maintain written records of your procedures, data collected, and subjects involved. These records will need to be available upon request during the conduct of the study and retained by the university for three years after the conclusion of the study. If changes occur in recruiting of subjects, informed consent process or in your research protocol, or if unanticipated problems should arise it is the Researchers responsibility to notify the Office of Sponsored Projects or IRB in writing. The project should be discontinued until modifications can be reviewed and approved.

Please be aware that you are responsible for reporting any adverse events or unanticipated problems.

A MEMBER OF THE UNIVERSITY OF LOUISIANA SYSTEM

P.O. BOX 3092 • RUSTON, LA 71272 • TEL: (318) 257-5075 • FAX: (318) 257-5079

AN EQUAL OPPORTUNITY UNIVERSITY

A2 - HUMAN SUBJECTS CONSENT FORM

Note: Use the Human Subjects Consent form to briefly summarize information about the study/project to participants and obtain their permission to participate. Assure that subjects in protected classes (e.g. prisoners, pregnant women or human fetuses or neonates, children) are provided complete information about the risks and benefits.

The following is a brief summary of the project in which you are asked to participate. Please read this information before signing the statement below. You must be of legal age or must be co-signed by parent or guardian to participate in this study.

TITLE OF PROJECT: Fruit/Vegetable Intake & Body Composition: Phytonutrient Correlations

PURPOSE OF STUDY/PROJECT: The primary aim of this study is to determine if phytonutrient intake from fruits and vegetables has any correlations with body composition in a sample of subjects from Thibodaux Regional Wellness Center in Thibodaux, Louisiana. In addition, a second aim is to investigate if there are any correlations between specific phytonutrient colors (green, reddish/purple, and yellow/orange) and fat percentage, waist circumference, and Body Mass Index (BMI).

SUBJECTS: Fifty(50) male and females between ages of 18-55 years. Subjects that have a body mass index greater than $40 kg/m^2$ and/or that been administered any form of obesity-related medications in the past 3 months will be excluded. Due to the use of electrical currents in the bioelectrical impedance analysis (BIA), pregnant subjects will also be excluded.

PROCEDURE: Full body assessment utilizing bioelectrical impedance analysis scale to gather data on weight, body mass index, and body composition. A waist circumference measure will taken using a flex tape. Three 24-hour food recalls and a food frequency questionnaire (FFQ) will also be administered. An initial food recall will be done during physical assessment with two follow up food recalls will be scheduled that day for future dates. Following the food recalls, a FFQ will be administered to collect data on if correlations exist between foods of specific colors and body composition.

BENEFITS/COMPENSATION: none

RISKS, DISCOMFORTS, ALTERNATIVE TREATMENTS: The participant understands that Louisiana Tech is not able to offer financial compensation nor to absorb the costs of medical treatment should you be injured as a result of participating in this research.

The following disclosure applies to all participants using online survey tools: This server may collect information and your IP address indirectly and automatically via "cookies".

I, ___________________, attest with my signature that I have read and understood the following description of the study, "Fruit/Vegetable Intake & Body Composition: Phytonutrient Correlations", and its purposes and methods. I understand that my participation in this research is strictly voluntary and my participation or refusal to participate in this study will not affect my relationship with Louisiana Tech University or my grades in any way. Further, I understand that I may withdraw at any time or refuse to answer any questions without penalty. Upon completion of the study, I understand that the results will be freely available to me upon request. I understand that the results of my survey will be confidential, accessible only to the principal investigators, myself, or a legally appointed representative. I have not been requested to waive nor do I waive any of my rights related to participating in this study.

________________________________ _____________
Signature of Participant or Guardian Date

CONTACT INFORMATION: The principal experimenters listed below may be reached to answer questions about the research, subjects' rights, or related matters.

Principal Investigator: Ryan Orgeron II RD, Katherene Anguah PhD

Members of the Human Use Committee of Louisiana Tech University may also be contacted if a problem cannot be discussed with the experimenters:

Dr. Richard Kordal, Director of Intellectual Properties
(318) 257-2484 rkordal@latech.edu

A3 – CLINIC APPROVAL FORM

FITNESS CENTER
OF THIBODAUX REGIONAL

Fitness Center of Thibodaux Regional
726 North Acadia Rd
Thibodaux, Louisiana 70301
(985)493-4950

January 17, 2018

To whom it may concern:

I have received the proposed study "Fruit/Vegetable Consumption: Phytonutrient Correlations" from Ryan Orgeron. I have determined that the following study will not violate any rights of our clientele or corporation policies. The Fitness Center of Thibodaux Regional is willing to allow Ryan Orgeron to assess and use data from the facility for research purposes.

Sincerely,

Jamie Semien
Fitness Center Manager

APPENDIX B

B1 – 24 HOUR FOOD RECALL SHEET

B2 - DATA COLLECTION SHEETS

B3 – FOOD FREQUENCY QUESTIONNAIRE

B1 - 24 HOUR RECALL

24-Hour Recall Form

1. Are you on any kind of specific diet?

2. Over the past three (3) months, have you done anything to purposely alter your diet?

3. Over the past three (3) months, have you done anything to alter your body weight?

4. Do you take any dietary supplements / vitamins ?

Meal Type	Food Item / Beverage	Portion Size	How was it prepared / Where obtained	Was anything added?

B2 – DATA LOG SHEET

Participants	Age	Sex	Race	Health Conditions	Height	Weight	BMI	Waist Circumference	Body Fat %	Calorie Intake
1										
2										
3										
4										
5										
6										
7										
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50										

B3 – FOOD FREQUENCY QUESTIONNAIRE

FOOD FREQUENCY QUESTIONNAIRE

Servings Equivalent		
Vegetable Group	Fruit Group	Beverages
1 cup of raw leafy vegetables	1 medium apple, banana, orange, pear	3/4 cup fruit/vegetable juice
1/2 cup of other vegetables cooked or raw	1/2 cup of chopped, cooked, or canned fruit	5 oz wine

Phytonutrient Groups	Average Use Last Year						
CHLOROPHYLL (GREEN)	<Once per Month	1-3 per month	Once per week	1-3 per week	4-6 per week	Once per day	2-3 per day
Dark leafy greens							
Green cabbage							
Green peas							
Green bell pepper							
Brussel sprouts							
Broccoli							
Asparagus							
Cucumber							
Zucchini							
Herbs (basil, oregano, parsley, etc)							
Green Tea							
Other:							
CAROTENOIDS (YELLOW/ORANGE)	<Once per Month	1-3 per month	Once per week	1-3 per week	4-6 per week	Once per day	2-3 per day
Carrots							
Sweet Potatoes							
Pumpkin							
Tomatoes							
Cantaloupe							
Mango							
Peach							
Oranges (satsumas, navel, etc.)							
Butternut Squash							
Other:							
ANTHOCYANINS (RED/PURPLE)	<Once per Month	1-3 per month	Once per week	1-3 per week	4-6 per week	Once per day	2-3 per day
Blackberries							
Blueberries							
Raspberries							
Strawberries							
Cranberries							
Purple Grapes							
Purple Cabbage							
Red Onion							
Eggplant							
Cherries							
Red Wine							
Other:							

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