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Association of Antioxidant Intake and Body Mass Index in Pre-to-Early Adolescent Children

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ABSTRACT

ASSOCIATION OF ANTIOXIDANT INTAKE AND BODY MASS INDEX IN PRE-TO-EARLY ADOLESCENT CHILDREN

by

E. Kelly Imboden

Background: The prevalence of overweight (Body Mass Index [BMI]85-<95th percentile) and obesity (BMI≥95thpercentile) for individuals aged 2-19 years in the United States in 2009-2010 was estimated to be 31.8%. Excessive body fat increases the risk for chronic conditions such as hypertension and type 2 diabetes mellitus. Studies have established an association between obesity and oxidative stress and inflammation in children and adolescents. Antioxidants have been shown to have protective effects against inflammation and oxidative stress. However, the effect of dietary antioxidant intake on obesity is not fully understood.

Objective: To examine dietary antioxidant intake by BMI classification in a population of normal, overweight and obese children.

Methods: The study population included 296 healthy pre-to-early adolescent (age 6-15 years) African American and Caucasian children residing in Pittsburgh, PA. Demographic characteristics, anthropometric measures and nutrient intake were assessed at baseline and six months. A food frequency questionnaire was used to assess antioxidant intake (vitamin C, carotene, total vitamin A, zinc and vitamin E). Frequency analysis was used to describe demographic, anthropometric and nutrient data. The Kruskal Wallis test was used to evaluate difference in median antioxidant and kilocalorie intake by BMI classification at baseline. A Kendall's tau correlation was performed to test for a linear relationship between BMI and antioxidant intake at baseline.

Results: The median age of the population was 10 years (range, 8 to 11 years). The majority of the population was male (53%) and African American (60%). Weight and BMI (p = 0.028 and 0.000, respectively) were the only demographic and anthropometric characteristics that differed by gender. For the total cohort, median nutrient intake by BMI classification was significantly different for vitamin C (p = 0.015), zinc (p = 0.019), vitamin E (p = 0.022) and kilocalories (p = 0.015). When divided by gender, zinc intake in males (p = 0.047) and kilocalorie intake in females (p = 0.017) were the only nutrients found to be statistically different by weight classification. No linear relationship was observed between antioxidant intake and BMI for the total cohort and for each gender.

Conclusion: Our results do not support a linear relationship between antioxidant intake and BMI. In contrast to our hypothesis, antioxidant intake was found to be highest in children who were overweight. Future studies should include a serum measure of inflammation and antioxidant levels in addition to antioxidant intake to better understand the impact, if any, of antioxidants in overweight and obese children and adolescents.

ASSOCIATION OF ANTIOXIDANT INTAKE AND BODY MASS INDEX IN PRE-TO-EARLY ADOLESCENT CHILDREN

by

E. Kelly Imboden

A Thesis

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ABBREVIATIONS

25 (OH) D	25-hydroxyvitamin D
AOPPs	Advanced Oxidation Protein Products
BMI	Body Mass Index
CAT	Catalase
CG	Plasma Carbonyl Groups
cm	centimeter
CRP	C Reactive Protein
CVD	Cardiovascular Disease
DM	Diabetes Mellitus
F_2IsoP	F_2Isoprostane
FFQ	Food Frequency Questionnaire
GPx	Glutathione Peroxidase
HDL	High Density Lipoprotein
high- VF	high vegetable-fruit diet
IL-6	Interleukin 6
IRB	Institutional Review Board
IU	International Units
kg	kilogram
LDL	Low Density Lipoprotein

low- VF	low vegetable-fruit diet
m^2	meters squared
MDA	Malondialdehyde
mg	milligram
NHANES	National Health and Nutrition Examination Survey
PA	Pennsylvania
ROS	Reactive Oxygen Species
SOD	Super Oxide Dismutase
TAC	Total Antioxidant Capacity
TNF	Tumor Necrosis Factor
UPMC	University of Pittsburgh Medical Center
WBC	White Blood Cells
YAQ	Youth/Adolescent Questionnaire

CHAPTER I

ASSOCIATION OF ANTIOXIDANT INTAKE AND BODY MASS INDEX IN PRE-TO-EARLY ADOLESCENT CHILDREN

Introduction

The number of children and adolescents in the United States that are overweight (Body Mass Index [BMI]85-<95th percentile) and obese (BMI≥95th percentile) remains elevated. However, a report based on National Health and Nutrition Examination Survey (NHANES) data published by the Centers for Disease Control and Prevention in 2013 indicates that the prevalence of obesity in children and adolescents did not increase between 2007-2008 and 2009-2010.[1] Although the prevalence of overweight and obesity has remained stable, in 2009-2010 16.9% of the population,aged 2-19 years, were obese and 31.8% were either overweight or obese.[2] Globally, childhood overweight and obesity rates are increasing in some countries while others have stabilized.[2] As a result, many researchers acknowledge the need for continued research focusing on excess weight and confounding health problems. Obese children are at risk for developing hypertension and type 2 diabetes mellitus.[4] Additionally, it is projected that nearly one-third of obese children are diagnosed with hypertension and or hyperinsulinemia.[4] Overweight and obesity are also of concern in adult populations. It is estimated that 69% of the US adult population (≥ 20 years of age) is either overweight or obese.[5] Obesity has been linked to many health related complications in adult populations. Some obesity related disease states include: type 2 diabetes mellitus, cardiovascular disease, hypertension and metabolic syndrome. While research conducted to date is not as extensive in children and adolescents, it is believed that cardiovascular and metabolic complications of increased adiposity are already present in some younger individuals who are overweight or obese.[6]

The increased presence of inflammation and oxidative stress in overweight and obese individuals has gained recent attention. Codoner-Franch et al. (2010) defines oxidative stress as an inequality between reactive oxygen species (ROS) and oxidative defense mechanisms, in which ROS ultimately overwhelms oxidative defense mechanisms.[7] Sfar et al. (2013) alludes to the need for further research to determine if any dietary components can reduce the production of ROS.[8] ROS can damage or alter cell function and is believed to contribute to conditions such as atherosclerosis, insulin resistance, diabetes mellitus (DM), and cardiovascular disease (CVD).[9] Researchers propose that many comorbidities associated with obesity, including DM, CVD and hypercholesterolemia, could in fact be attributed to or a result of oxidative stress.[7,10]

Research has shown that BMI is associated with oxidative stress in adults, adolescents, and children.[8,10] The relationship between obesity and oxidative stress is supported in a review by Savini et al. (2013) which states that although the exact sequence of their relationship is difficult to ascertain, oxidative stress and inflammation look to be connected to obesity.[10] This is explained by the cyclic relationship between proinflammatory cytokines and ROS. It is suggested that ROS generate the production and freeing of proinflammatorycytokines which in turn further stimulates an increase in ROS.[10] Furthermore, Sfar et al. (2013) suggests that because they found healthy, obese children to have heightened antioxidant enzyme defenses, oxidative stress may be attributable to excessive adipose tissue and not just obesity related diseases.[8]

Antioxidants such as vitamin E, vitamin C and carotenoids are substances naturally found in many foods including fruits, vegetables, nuts and grains.[11] Antioxidants have been shown to have protective effects on cells from damage caused by free radicals, a precursor to oxidative stress.[12] In addition, antioxidants have shown an ability to neutralize the negative effects of oxidative stress.[12] Due to the connection of oxidative stress and inflammation to obesity, antioxidants could possibly alter the link between adiposity and inflammation by its impact on proinflammatory cytokines.[6]

Although an association between obesity and oxidative stress and inflammation in children and adolescents has been established, the effect of dietary antioxidant consumption in this relationship is not yet fully understood.[4] Dietary antioxidants are believed to be able to influence the expression of proinflammatory cytokines and offset decreased vitamin levels and antioxidant capacity observed in obese children and adolescents.[4] Some research has suggested dietary antioxidant intake does play a role, but it has not been well established whether or not oxidative stress and inflammation associated with increased adiposity are the result of decreased dietary antioxidant intake or if antioxidant needs are greater in individuals who are overweight or obese.[10] The purpose of the proposed study is to determine if overweight and obese children and adolescents have a lower dietary antioxidant intake compared to their normal weight counterparts. Specifically, we wish to determine if children and adolescents with an elevated body mass index (BMI $\geq$85th percentile) consume a lower intake of antioxidants compared to children and adolescents with a normal body mass index (BMI 5-<85thpercentile). To do this, we will analyze reported dietary intake and anthropometric measures obtained in a population of children and adolescents in Pittsburgh, PA.

Null Hypothesis (Ho): There is no difference in dietary antioxidant intake between normal weight (BMI 5^{th} -<85^{th} percentile), overweight (BMI 85^{th} -<95^{th} percentile) and obese (BMI $\geq 95^{th}$ percentile) pre-to-early adolescent children.

Alternate Hypothesis (H_1): Overweight (BMI: 85^{th}-<95^{th} percentile) and obese (BMI $\geq 95^{th}$ percentile) pre-to-early adolescent children will have a lower dietary antioxidant intake than normal weight (BMI 5^{th}-<85^{th} percentile) pre-to-early adolescent children.

CHAPTER II

LITERATURE REVIEW

Oxidative Stress and Body Composition

An inflammatory state is thought to be closely related to oxidative stress. In an article by Piva et al.(2013), researchers explain that oxidative stress could significantly increase the expression of proinflammatory cytokines.[13] A range of proinflammatory and oxidative stress markers have been looked at in various research studies. Some of the more commonly examined proinflammatory markers include: tumor necrosis factor (TNF), interleukin 6 (IL-6) and/or C reactive protein (CRP; produced from IL-6).[6,10,14,15] Markers used to assess oxidative stress include the following antioxidant enzymes: super oxide dismutase (SOD), glutathione peroxidase (GPx) and/or catalase CAT).[7,8] Malondialdehyde (MDA), plasma carbonyl groups (CG), F_2 isoprostane (F_2IsoP), and advanced oxidation protein products (AOPPs) are other markers that have been used by researchers to assess oxidative stress.[7,14,16] Proinflammatory markers appear to be a beneficial approach for determining levels of oxidative stress in individuals.

Several studies have shown consistently strong associations between IL-6 and BMI while other inflammatory markers have produced greater variation among studies.[6,13,15] Aerbeli et al. (2006) concluded that CRP and IL-6 were both significantly increased with increasing adiposity in overweight children; however, TNF was not found to be significantly increased.[6] In this study, adiposity encompassed BMI, percent body fat and waist to hip ratio.[6] Zimmerman et al. (2008) suggests that circulating TNF may not appear to be elevated in obese individuals when in fact it is due to the manner in which it functions.[4] TNF is thought to largely function in its transmembrane form in an autocrine/paracrine manner within adipocytes.[4] In addition to IL-6, Oliver et al. (2010) also found F_2IsoPs to be significantly increased in obese children compared to their normal weight counterparts.[15] Alternatively, a study by Krzystek-Korpackaet al. (2008) investigated AOPP status in overweight and obese children and the potential impact of weight reduction on AOPP status.[16] AOPPs are produced in response to oxidative stress and are suggested to play a role in the development of oxidative stress and inflammation through their ability to activate immune cells. Results concluded that AOPPs were significantly higher in overweight and obese children compared to their lean, normal weight counterparts, and even greater in overweight and obese children with diagnosed metabolic syndrome.[16] Moreover, with the obesity intervention (a bran-enriched diet) utilized in this study, a change in AOPP status was found to be correlated with a change in BMI; decreased body mass resulted in decreased AOPPs and oxidative stress.[16] The results of this study prompted authors to suggest that obesity interventions focus on reducing oxidative stress in order to reduce the risk of secondary obesity related disorders.[16]

Oxidative stress and inflammation in individuals with an increased BMI have been associated with adverse outcomes. Specifically, oxidative stress is thought to be a contributing factor in the pathophysiology of cardiovascular and metabolic outcomes of obesity, including the development of metabolic syndrome in adult populations.[7] The American Heart Association's definition of metabolic syndrome includes the following set of seven risk factors: 1) abdominal obesity; 2) triglyceride $\geq$ 150 mg/dL; 3) high density lipoprotein (HDL)<40 mg/dL for men and <50 mg/dL for women; 4) systolic blood pressure $\geq$ 130 mmHg; 5) diastolic blood pressure $\geq$ 85 mmHg; 6) fasting blood glucose of$\geq$ 100 mg/dL; and 7) insulin resistance.[17] A diagnosis of metabolic syndrome can be made if an individual meets three or more of the seven risk factors. Metabolic syndrome further increases one's risk of developing cardiovascular disease and type 2 diabetes mellitus.[17] While the majority of the existing research has focused on adult populations, there have also been some studies focused on inflammation and oxidative stress in children and adolescents. Many of these studies on overweight and obese children and adolescents have produced similar, adverse results. A study by Oliver et al.(2010) also found obese children to have elevated markers of inflammation (IL-6, white blood cells (WBC), and neutrophils) and oxidative stress (F_2-IsoP).[15] As a result of these findings, the authors believe that these children are at an even greater risk for the future development of CVD.[15] One accepted connection between obesity and oxidative stress (and inflammation) is that obese individuals experience compromised antioxidant defenses and or reduced antioxidant levels compared to normal weight individuals.[4,10]

Some studies have concluded that the presence of oxidative stress in overweight and obese individuals depends on whether or not an individual meets the criteria for metabolic syndrome. When looking at oxidative stress and inflammation, some studies have taken overweight and obese individuals and separated them based on whether or not they meet the criteria for metabolic syndrome. As previously stated, metabolic syndrome is characterized by a set of risk factors that increases an individual's risk for developing CVD and type 2 diabetes mellitus.[17] A study conducted by Venturini et al. (2012)

examined healthy and overweight (BMI 25-29.9 kg/m^2) adults with and without a diagnosis of metabolic syndrome.[18] Adults were considered healthy if they had a normal BMI (20-24.9 kg/m^2) and no diagnosis of metabolic syndrome. The study concluded that healthy and overweight individuals without metabolic syndrome did not have different measurements of oxidative stress or total antioxidant capacity.[18] However, overweight individuals with metabolic syndrome showed signs of increased oxidative stress and plasma oxidation and reduced antioxidant capacity.[18] However, authors of this study note that given the study's cross sectional design, one cannot conclude whether or not the increased oxidative stress found is a cause or consequence of metabolic syndrome.[18] Similarly, a study by Molnar et al. (2004), concluded that obese children diagnosed with metabolic syndrome had greater oxidative stress compared to obese children without a diagnosis of metabolic syndrome.[19] There have also been findings of a redox imbalance, favoring oxidative stress, in obese children and adolescents with partial or full metabolic syndrome.[20] Children and adolescents were categorized as having partial metabolic syndrome if they met at least one of the following factors: 1) systolic blood pressure $\geq$135 mmHg and diastolic blood pressure $\geq$85 mmHg; 2) fasting triglyceride $\geq$150 mg/dL; 3) fasting HDL $\leq$40 mg/dL; 4) fasting glucose $\geq$ 100 mg/dL or previous diagnosis of type 2 diabetes mellitus. Furthermore, the study also found oxidative stress in obese children and adolescents increased as the scale of obesity and metabolic abnormalities increased.[20]

In contrast, not all studies fully support these findings. A study by Codoner-Franch et al. (2010) did not find the presence of metabolic syndrome to be a factor in oxidative stress in obese children.[7] This study specifically looked for the presence of oxidative stress in obese children who did not have metabolic syndrome or other co-morbidities. The study determined non-obese vs. obese groups by standard deviation score of BMI, with the degree of obesity determined by Cole's least mean square method. Authors found that increased BMI was related to increased markers of oxidative stress in children without metabolic syndrome.[7] Specifically, two markers of free radical activity, MDA and CG, were found to be greater in the group of severely obese children compared to normal weight children.[7] GPx, an endogenous erythrocyte antioxidant enzyme, was also found to be greater in obese and severely obese children. Authors propose that this finding is in response to combating the increased oxidative stress found in obese children.[7] It was also found that obese children had lower high density lipoprotein (HDL) levels and higher low density lipoprotein (LDL) and triglyceride levels compared to normal weight children. Although it should be noted that even these levels still fell within a normal range.[7] No differences in concentrations of alpha tocopherol or beta carotene were found in obese children compared to normal weight children. Furthermore, BMI was found to be positively correlated with MDA but not the other markers of oxidative stress. As a result, the study concluded that BMI may be a factor in predisposing an individual to oxidative damage.[7] Sfar et al. (2013) also demonstrated the presence of increased oxidative stress in healthy obese children compared to normal weight children.[8] In this study, the effects of obesity on children were determined by looking at the activity of three antioxidant enzymes: SOD, GPx, and CAT. These are biomarkers that can be used to evaluate oxidative stress. Findings demonstrated that SOD activity was significantly higher in obese versus normal weight children, however, GPx and CAT showed no difference. Based on findings presented in this study, it can be concluded that oxidative stress is a characteristic of otherwise healthy obese children.[8] Lopez- Legarrea et al. (2013) states that oxidative stress is likely associated with the development of metabolic syndrome among other obesity-related metabolic disorders.[21] This is also supported by findings that obesity is correlated with the presence of inflammation before other conditions, like metabolic syndrome, develop or are diagnosed.[22]

Nutrition and Oxidative Stress

Studies have shown various dietary components to have an influence on markers of inflammation and oxidative stress in children and adolescents.[6,14] Holt et al. (2009) looked at the effects of select dietary components on markers of inflammation and oxidative stress in an adolescent population.[14] The study focused on the following dietary components: fruits and vegetables, antioxidants (vitamin C, beta carotene) , folate, and flavonoids.[14] Dietary components were assessed by a food frequency questionnaire that included supplements. The study concluded the following: 1) F_2isoprostane was inversely associated with fruits and vegetables, vitamin C, beta carotene, and flavonoids; 2) CRP was inversely associated with fruit, vitamin C, and folate; 3)IL-6 was inversely associated with vegetables, beta carotene, and vitamin C; 4) TNF was inversely associated with lutein and beta carotene.[14] Consequently, from these findings, the study demonstrated that diets rich in antioxidants, folate, and flavonoids are coupled with a decline in inflammation and oxidative stress in adolescents.[14] In addition, Aeberli et al. (2007) conducted a study looking at the relationship between dietary fat and antioxidant vitamins and inflammation in overweight children.[6] Researchers found that BMI, percent body fat and waist to hip ratio was significantly associated with CRP, IL-6 and leptin.[6] Total fat intake was one of the dietary factors that affected CRP and vitamin C, vitamin E, and beta carotene negatively forecasted leptin concentrations.[6] This study demonstrated that overweight children had increased concentrations of inflammatory markers.[6] Results from these studies were largely supported in a review by Zimmerman et al.(2008) that supports the impact of dietary antioxidants on the expression of proinflammatory cytokines in obese children.[4] Dietary free fatty acids and total fat intake were also indicated as having a role in altering the inflammatory response. In high amounts, some free fatty acids have the ability to stimulate the expression of proinflammatory cytokines in adipocytes.[4] However, it was noted that not all fatty acids have this stimulatory effect. For example, diets high in alpha linolenic acid have been shown to reduce inflammatory markers over the course of several months.[4]

Oxidative stress is believed to have a connection between obesity and obesity-related conditions.[10] The exact mechanism contributing to the relationship between oxidative stress and inflammation and obesity is not fully understood, however, various mechanistic links have been proposed. A review by Savini et al.(2013) proposes two different possibilities as to why obesity results in oxidative stress and reduced antioxidant defenses compared to normal weight individuals.[10] The first proposed reason is that obese individuals decrease their antioxidant defenses as their level of adiposity increases and secondly, that these individuals have increased levels of reactive oxygen and/or reactive nitrogen species.[10] Codoner-Franch et al.(2010) found no relationship between energy, total fat or cholesterol and markers of oxidation.[7] As a result, they proposed adipose tissue as being a causative agent for increased oxidative stress. The study also proposed that lipid metabolism may be altered with an increased BMI, causing lipid peroxidation which is likely a contributing factor for increased oxidative stress.[7] Piva et al.(2013)

proposed adipose tissue as being directly related to inflammation due to the fact that adipose tissue releases unesterfied fatty acids, cytokines, and adiponectin, all of which contribute to a proinflammatory state.[13] Therefore, as the amount of adiposity increases, one could expect it to result in increased inflammation.

In addition to the role of adipocytes in producing proinflammatory markers, it has also been proposed that overweight and obese individuals may have compromised antioxidant levels and antioxidant capacity. A review published by Zimmerman et al. (2008) concluded that obese children tend to have decreased antioxidant vitamin levels as well as reduced antioxidant capacity compared to normal weight children.[4] This was supported by results fromNHANES III. This survey found that children, 6-19 years, had reduced serum alpha tocopherol and beta carotene even though no significant differences were found in the intakes of these two vitamins or of fruits and vegetables between overweight and normal weight children.[4] Puchau et al. (2010) compared dietary intakes of obese children and adolescents to a control group and concluded that obese children and adolescents had a lower consumption of dietary antioxidants, including vitamins E and C, compared to the controls.[23] The authors also noted an inverse relationship between BMI and dietary TAC, used as a measurement of antioxidant intake.Similarly, Ford et al. (2003) examined antioxidant concentrations of adults with and without metabolic syndrome based off of NHANES III data.[24] The concentration of some antioxidants including: retinyl esters, vitamin C, vitamin E and some carotenoids were found to be lower among individuals with metabolic syndrome. Additionally, those with metabolic syndrome consumed fewer fruits and vegetables but supplement use was comparable between the two groups.[24] Even when these factors were adjusted for, the concentration of some antioxidants were still found to be lower among those with metabolic syndrome compared to those without metabolic syndrome. Concentrations of retinol, lycopene and selenium however, were not lower among individuals with metabolic syndrome. Authors suggest that these results provide evidence that these individuals may have greater antioxidant utilization or decreased intake.[24] However, it should be noted that NHANES III data provided no measure of oxidative stress.

In response to the largely accepted knowledge that inflammation and oxidative stress is elevated in overweight and obese individuals and the negative health impacts that can result, some studies are looking at potential ways to decrease inflammation and oxidative stress. Weight reduction, physical activity and an antioxidant rich diet are all implicated in being able to reduce oxidative stress in obesity.[10] Yeon et al. (2012) assessed the effects of two dietary interventions on obesity related markers on a population of overweight women utilizing a cross over study design.[25] The two interventions included: 1) A diet high in fruit and vegetable intake; 2) A diet low in fruit and vegetable intake. The high fruit and vegetable diet (high-VF) consisted of six servings of vegetables and six servings of fruit. Specifically, the high-VF diet consisted of two servings of each of the following: dark green, dark orange, dark red, other (vegetables), vitamin C rich fruits/juices, and other (fruits). The low-VF diet included two servings; one vegetable and one fruit, but did not specify type. Average energy intake and the proportion of macronutrients were comparable between the two intervention groups. Although, to reduce any potential confounders, each dietary intervention was adjusted for total calories and macronutrients. They found that the body fat of adult females was positively associated with IL-6, CRP ,and leptin and those overweight women who had high fruit and vegetable intakes had decreased obesity-related metabolic complications or risk factors.[25] Chae et al. (2013) looked at the effect of weight reduction on levels of inflammation and oxidative stress in overweight and obese individuals.[26] Findings from this study demonstrated that long term (3 years) mild weight loss, defined as a 5.4% weight decrease, reduced inflammatory cytokine levels and oxidative stress.[26] Weight was assessed at baseline and at the end of the three year intervention. Authors suggest that most of the weight loss in the study population occurred in the first two years. Although the health benefits of consuming high antioxidant foods, especially in regards to the effects of antioxidant combinations from the overall diet, is not fully understood, a positive dietary change largely follows the inclusion of these foods.[27] The main reason for this is because antioxidant rich foods most commonly come from plant sources. It is believed that polyphenolic compounds containing antioxidant activities have many benefits for humans.[27] As a result, studies support that antioxidants contained within fruits and vegetables can enhance health and decrease future disease risk. Furthermore, antioxidants are believed to have protective effects against oxidative damage and resulting complications.[28]

Assessing Dietary Intake in Children and Adolescents

There are various methods of assessing dietary intake including: 24 hour dietary recalls, food frequency questionnaires (FFQs), diet histories and food records.[29,30] Researchers acknowledge the faults and limitations associated with assessing dietary intake, especially in children and adolescents; however, they also recognize the importance of doing so in these populations. Accepted obstacles in assessing dietary intake in child and adolescent populations are age, cognition, estimation of portion sizes, and frequent eating outside of the home.[29,31,32] Another limitation associated with dietary assessment methods include inaccurate reporting.[29,32,33]

Given the adverse obstacles associated with assessing dietary intake in children and adolescents, researchers generally accept that children older than roughly 9 to 10 years of age have developed the cognitive ability to effectively complete a dietary assessment without parental guidance.[29,32,34] The use of parent proxies has been discussed in relation to the use of dietary assessment methods in children and adolescents.[29] However, there is a lack of consensus as to whether or not they help improve the validity of an assessment.[29] Regardless, in studies that have looked at the impact on parental involvement when assessing dietary intake in child and adolescent populations, there has still been some degree of over estimating intake.[29,30] McPherson et al. (2000) concluded that some studies had improved FFQ results with parent involvement in children (11-12 years old) with an over estimation of energy intake of 21% versus 36% without parental involvement.[30]

Many researchers have examined various dietary assessment methods and validated instruments suitable for younger populations. A review by Burrows et al. (2011) concluded that the best method for assessing estimated energy intake varied by age.[29] The review utilized the doubly labeled water gold standard as a comparison for assessing the best method for estimating energy intake.[29] Multiple 24-hour recalls (over a three day period) with use of parents as a proxy was the best method for children (4-11 years of age) while diet history was most effective for adolescents $\geq$ 16 years. Crawford et al. (1994) found that a three day food record had the least significant difference in observed and reported intakes compared to a 24-hour recall and 5-day food frequency in 9-10 year old girls.[35]

Recognizing the need for effective dietary assessments in child and adolescent populations, researchers have developed assessments to validate in younger populations.[34,36] Rockett et al. (1995) developed and assessed the reproducibility of a semi quantitative FFQ for children and adolescents, referred to as the Youth/Adolescent Questionnaire (YAQ).[34] The YAQ was modeled after a validated FFQ used in the Nurses Health Study and it assessed one year test-retest reproducibility in children and adolescents (9-18 years of age). Results showed the reproducibility of some nutrients to be 0.26 for protein and iron and 0.58 for calcium. It also concluded that girls reproducibility overall was higher than that for boys.[34] In response to the belief that children and adolescents have a shorter attention span, Rockett et al. (2007) designed and compared a short FFQ and a long FFQ (YAQ) for use in children and adolescents.[36] Study findings showed that nutrient means were higher from the longer FFQ compared to the shorter FFQ. However, there was a mean correlation of 0.9 for all nutrients between the short and long FFQ. Therefore, authors concluded a similar ranking of participants when analyzing both the short and long FFQs.[36]

FFQs are widely used in epidemiological studies as they are relatively easy to administer, economical and provide information on an individual's usual intake.[30] However, a FFQ also has its limitations. A FFQ frequently results in over or under reporting of dietary intakes among children and adolescents. In addition, a review by Burrows et al. (2011) included two studies that investigated the impact of weight on misreporting information that concluded overweight obese individuals were more likely to under-report energy intake compared to normal weight individuals.[29] Additionally, Champagne et al. (1996) evaluated the degree of under reporting of energy expenditure in African American and Caucasian children.[37] Underreporting was assessed by calculating the difference between total energy expenditure, determined by the doubly labeled water method, and reported energy intake. The authors reported that African American children underreported energy intake by approximately 37% compared to approximately 13% in Caucasian children.[37] Studies assessing FFQ's in minority and lower socioeconomic children and adolescent populations are lacking.

In light of the limitations surrounding FFQs, researchers acknowledge that the effectiveness of a FFQ is largely related to its design.[30,33,36] It is suggested that the actual development of a FFQ is the most significant part.[30] In addition, a FFQ ought to be specific to the population of study, meaning the provided list of foods on a FFQ should be aligned with the targeted population's dietary habits.[33] Furthermore, there is a recognized need for FFQ's to be validated and calibrated in order to gain knowledge on the degree of error associated with the FFQ.

CHAPTER III

METHODS

Study Design

The proposed study is an exploratory, secondary analysis of data collected as part of Dr. Kumaravel Rajakumar's National Institutes of Health funded (R03-K23 grants) vitamin D clinical research protocols. The objectives were to assess the seasonal variation and racial differences in African American and Caucasian children and refine the serum 25(OH) D thresholds for defining vitamin D insufficiency in children. The study occurred in two phases: Phase I monitored the subjects for sunlight exposure and vitamin D intake and was conducted in 2006-2008 and Phase II randomly assigned a vitamin D supplement (1000 IU D3) or placebo to the subjects and was conducted between 2008 and 2011. Subjects enrolled in the randomized control trial were either not taking a multivitamin or were willing to stop taking multivitamins for at least one month for a washout period prior to entry into the study. Vitamin D research protocols were approved by the University of Pittsburgh Institutional Review Board (IRB) and signed parental informed consent and participants' assent were obtained prior to enrollment. Approval for the secondary analysis was obtained by the Georgia State University IRB. Demographic characteristics, anthropometric measures and nutrient intake were assessed at baseline and six months later. All study participants completed the YAQ FFQ (Appendix A) designed and validated by Rockett et al. (2007; Harvard Medical School, ©

1995 Brigham and Women's Hospital) at the baseline and 6 month follow-up visits. Parents were permitted to assist in the completion of the FFQ. The FFQs were analyzed at Brigham and Women's Hospital and reported daily nutrient intake for macro- and micronutrients for each participant at each time point was provided to the researchers. This study evaluated the relationship between dietary antioxidant and kilocalorie intake and body composition in this population. Antioxidants available through analysis of the FFQ included: vitamin C, carotene, total vitamin A, zinc and vitamin E.

Study Population

Our study population consists of 296 healthy pre-to-early adolescent (aged 6-15 years) African American and Caucasian children residing in Pittsburgh, PA. Participants were recruited from the Primary Care Center of the Children's Hospital of Pittsburgh of the University of Pittsburgh Medical Center (UPMC). Exclusion criteria included use of anticonvulsants or systemic glucocorticoids, diagnosed hepatic renal disease, malabsorptive disorders, cancer, disorders in vitamin D or calcium metabolism, and use of oral contraceptives or depot medoroxyprogesterone.

Data Analysis

Demographic, anthropometric and nutrient data were described using frequency analysis. Data were determined to be non-normally distributed even after log transformation. The nonparametric KruskalWallis test was used to evaluate difference in median antioxidant and kilocalorie intake by BMI classification (normal weight, overweight, obese) using baseline data. A Kendall's tau correlation was used to test for a linear relationship between BMI and antioxidant intake using baseline data. All statistical analyses were conducted for the total cohort and by gender using SPSS (version 20.0, SPSS, Inc., Chicago, IL). A p-value of<0.05 was considered significant.

CHAPTER IV

RESULTS

The demographic characteristics of the population are shown in Table 1. The median age of the population was 10 years (range, 8 to 11 years). The majority of the population was male (53%) and Black (60%). Normality testing determined that continuous variables were not normally distributed even after log transformation. Therefore, non-parametric statistical tests were used for the remaining analyses. Weight and BMI differed by gender ($p = 0.028$ and <0.001, respectively) but no other demographic or anthropometric differences were observed.

Table 1: Demographic and anthropometric characteristics of the population and by gender

	Total (n= 296)	**Males (n=158)**	**Females (n=138)**	**P-value**
Age in years (range)	10 (8 - 11)	10 (8 - 11)	10 (8 - 11)	0.946
Race (%)				
Caucasian	39.9	42.4	37.0	0.340
Black	60.1	57.6	63.0	
Weight (kg)*	38.2 (31.1, 49.6)	36.8 (30.2, 48.2)	41.0 (31.5, 55.4)	**0.028**
Height (cm)*	140.6 (131.9, 152.7)	140.8 (132.5, 150.4)	140 (131.0, 154.1)	0.933
BMI (kg/m^2)*	19.2 (16.9, 22.6)	18.1 (16.5, 21.0)	19.9 (17.7, 24.125)	**<0.001**

*Median (25%, 75%); BMI - body mass index

Median nutrient intake by BMI classification (normal weight, overweight, obese) is shown in Table 2. For the total cohort, a significant difference between weight classification was observed for vitamin C (p = 0.015), zinc (p = 0.019), vitamin E (p = 0.022) and kilocalories (p = 0.015). Post hoc testing determined pairwise differences. Vitamin C, vitamin E and kilocalorie intake significantly differed between the normal weight and overweight groups and the overweight and obese groups. For zinc, significant differences were found between overweight and obese as well as normal weight and obese. After division by gender, zinc intake in males and kilocalorie intake in females were the only nutrients found to be statistically different (p = 0.047 and p = 0.017, respectively) by weight classification (Tables 3 and 4). The difference in zinc among males occurred between normal weight and obese as well as between overweight and obese participants. In females, difference in kilocalorie intake was observed between the overweight and obese groups. Correlations between antioxidant intake (vitamin C, carotene, vitamin A, zinc, vitamin E) and BMI were performed for the total cohort and for each gender. No significant relationships were observed.

Table 2. Median nutrient intake by weight classification for the total cohort

Nutrient[a]	Normal Weight	Overweight	Obese	P-value
Vitamin C (mg)	131.5 (78.1, 196.2)*	180.3 (108.6, 230.3)***	122.8 (73.1, 205.8)	**0.015**
Carotene (IU)	4290.9 (2429.1, 6120.3)	3832.3 (2381.8, 5553.8)	3585.9 (2441.9, 5614.3)	0.419
Vitamin A (IU)	6904.2 (4974.1, 10055.6)	6882.2 (5149.4, 9984.4)	6593.2 (5246.3, 9320.6)	0.952
Zinc (mg)	14.4 (10.6, 18.0)**	15.0 (11.6, 20.0)***	11.6 (8.8, 16.3)	**0.019**
Vitamin E (mg)	7.3 (5.4, 10.0)*	9.1 (6.6, 11.1)***	6.8 (4.7, 9.4)	**0.022**
Kilocalories	2196.4 (1790.7, 2904.1)*	2650.9 (1996.8, 3390.4)***	2099.8 (1579.1, 2736.6)	**0.015**

[a]Median (25%, 75%); mg – milligrams, IU – international units

*Significant difference between normal weight and overweight groups

**Significant difference between normal weight and obese groups

***Significant difference between overweight and obese groups

Table 3. Median nutrient intake by weight classification (males)

Nutrient[a]	**Normal Weight**	**Overweight**	**Obese**	**P-value**
Vitamin C (mg)	121.6 (79.5, 187.7)	178.0 (107.5, 232.5)	134.4 (65.8, 211.7)	0.155
Carotene (IU)	3979.7 (2371.0, 6699.0)	2990.9 (2344.5, 5067.3)	3585.9 (2323.8, 5722.1)	0.403
Vitamin A (IU)	6730.3 (4590.3, 10051.2)	6716.9 (5737.6, 9294.7)	6087.3 (4710.5, 8530.2)	0.811
Zinc (mg)	14.4 (10.6, 18.6)**	15.2 (12.0, 19.0)***	10.7 (8.1, 15.1)	**0.047**
Vitamin E (mg)	7.4 (5.5, 10.0)	8.7 (6.6, 10.9)	7.3 (4.2, 9.6)	0.188
Kilocalories	2222.8 (1864.7, 2981.0)	2498.3 (1721.1, 3206.3)	2069.9 (1424.2, 2696.6)	0.278

[a]Median (25%, 75%); mg – milligrams, IU – international units

**Significant difference between normal weight and obese groups

***Significant difference between overweight and obese groups

Table 4. Median nutrient intake by weight classification (females)

Nutrient[a]	Normal Weight	Overweight	Obese	P-value
Vitamin C (mg)	141.8 (73.1, 198.2)	180.5 (105.8, 235.8)	115.5 (75.8, 202.8)	0.117
Carotene (IU)	4340.5 (2636.5, 5751.7)	4324.2 (2267.5, 5917.4)	3544.1 (2590.1, 5573.3)	0.738
Vitamin A (IU)	7259.4 (5266.9, 10064.2)	7508.8 (4963.9, 11180.5)	7254.8 (5944.4, 9731.7)	0.984
Zinc (mg)	14.4 (10.6, 17.2)	14.8 (11.5, 21.0)	11.7 (9.8, 17.8)	0.32
Vitamin E (mg)	7.3 (5.3, 9.9)	9.2 (6.6, 11.4)	6.7 (5.0, 9.0)	0.133
Kilocalories	2164.3 (1722.1, 2813.8)	2793.6 (2150.5, 4177.3)***	2139.9 (1702.2, 2774.5)	**0.017**

[a]Median (25%, 75%); mg – milligrams, IU – international units

***Significant difference between overweight and obese groups

CHAPTER V

DISCUSSION

We observed significant differences in the dietary intake of vitamin C, zinc, vitamin E and kilocalories by weight classification. For the total cohort, median intake of each antioxidant and kilocalories were highest in the overweight weight class and lowest in the obese weight class. However, with the exception of zinc, antioxidant intake was not statistically different between normal weight and obese children in our population. Few differences in antioxidant intake were observed after the cohort was subdivided by gender. These results do not support our research hypothesis that dietary antioxidant intake would be highest among normal weight pre-to-early adolescent children compared to their overweight and obese counterparts. The children in our population who were overweight consumed significantly more kilocalories than children of normal weight. We speculate that this higher energy intake may have contributed to the increased antioxidant intake also observed in this group.

Relatively few studies have compared dietary antioxidant intake in children and adolescents of different weight classes (normal weight, overweight and obese). Pachau et al. (2010) conducted one study that did evaluate antioxidant intake in children who were obese compared to a control group.[23] In contrast to our findings, this study observed overall antioxidant intake to be greater among the control group compared to the obese group, with vitamins C and E intake being significantly higher.[23] The population of the two studies, however, were not identical. Pachau only looked at obese vs. a control group of children whereas our study included normal weight, overweight and obese children and adolescents. Also in contrast to Pachau et al. (2010) which concluded a negative linear relationship between BMI and antioxidant intake, we observed no relationship between the two. Our findings are more comparable to results from NHANES III data. However, there are slight differences in the population ages and NHANES III data looked only at normal weight and overweight children, 6-19 years.[4] Both studies observed no significant difference in carotene intake among weight classes. However, unlike NHANES III data, we did find vitamin E intake to be significantly higher in the overweight group compared to both the normal weight and obese groups.

Savini et al. (2013) proposed that more research is required to determine if oxidative stress and inflammation associated with increased adiposity are the result of decreased dietary intake or if antioxidant needs are greater among individuals who are overweight or obese.[10] Our results support the latter possibility. Future research should include measurements of serum markers of inflammation and antioxidant levels to determine if in fact there are increased antioxidant requirements in overweight or obese children. Tumor necrosis factor (TNF), interleukin 6 (IL-6), and C reactive protein (CRP) are proinflammatory markers that have been used in previous studies to assess the presence of inflammation and have proven to be beneficial measures for determining levels of oxidative stress.[10,14,15,38] Having measures of proinflammatory markers in addition to antioxidant levels would be beneficial to compare to dietary antioxidant intake to better understand whether or not individuals with increased adiposity have greater antioxidant needs.

Our study has several limitations. As a secondary analysis, we were only able to assess antioxidant intake rather than also obtaining serum antioxidant levels and inflammatory markers. Previous studies have shown dietary antioxidants to have an impact on inflammatory markers. However, many researchers have not compared levels of antioxidant intake in individuals of different weight classes. Holt et al. (2009) did find an inverse association between markers of inflammation and oxidative stress and dietary intake.[14] Furthermore, Oliver et al. (2010), Krrzystek-Korpacka et al. (2008) and Codoner-Franch et al. (2010) suggest that oxidative stress and inflammation is increased in overweight and obese children compared to normal weight counterparts.[15,16,39] Given that previous research found increased inflammation in overweight and obese individuals and our results showed that overall dietary antioxidant intake was not lower in overweight and obese children, it would be important to assess serum antioxidant levels and inflammation in future research. By addressing both antioxidant intake and measures of inflammation and antioxidant levels, one could better establish the impact, if any, of antioxidants in overweight and obese pre-to-early adolescent children.

Another limitation to our study is that we utilized a FFQ to assess dietary antioxidant intake. Although the dietary assessment tool used in our study has been validated in this population, it relies on self-reported information and introduces room for over or under reporting intake.[36,40] Previous researchers have found tools for assessing dietary intake to produce some inaccurate data.[29,32,33] Some studies have shown that even with parental involvement in reporting dietary intake in child and adolescent populations, there is still some degree of over estimating intake.[29,30] McPherson et al. (2000) found that parental involvement on FFQ results yielded a 21% over estimation of energy intake versus 36% without parental involvement.[30] A review by Burrows et al. (2011) found that overweight and obese individuals were more likely to under-report energy intake compared to normal weight individuals.[29] Champagne et al. (1996) evaluated the degree of under reporting of energy expenditure in African American and Caucasian children and found that African American children underreported energy intake by approximately 37% compared to approximately 13% in Caucasian children.[37] Another limitation related to the FFQ is that not all foods that contain antioxidants were included on the questionnaire. As a result, it is possible that intakes could have been higher for some participants. Finally, the results of our study may not apply to the general population as the sample was recruited from an urban setting and did not include children who live in suburban or rural areas.

In conclusion, we observed a significant difference between the intake of several dietary antioxidants and BMI in an urban population of pre-to-early adolescents. Children who were obese consumed higher amounts of these nutrients vs. children who were overweight, which does not support our research hypothesis. Additional research is needed to investigate the relationship between inflammation and inflammatory markers, adiposity and dietary antioxidant intake in children from both urban and rural areas. Understanding the relationship between antioxidant intake and body composition would assist with the design of clinical trials aimed at reducing or preventing oxidative damage and the risks associated with the development of chronic illnesses in adulthood.

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APPENDIX A

PAGE ONE EATING SURVEY K-95-1 HARVARD MEDICAL SCHOOL

MARKING INSTRUCTIONS

- Use a **NO. 2 PENCIL** only.
- Do not use ink or ballpoint pen.
- Darken in the circle completely.
- Erase cleanly any marks you wish to change.
- Do not make any stray marks on this form.

The RIGHT way to mark your answer! ●

The WRONG way to mark your answers! ⊘ ⊗ ◒ ⊙

USE NO. 2 PENCIL ONLY

1. What is your AGE?

- ○ Less than 9
- ○ 9
- ○ 10
- ○ 11
- ○ 12
- ○ 13
- ○ 14
- ○ 15
- ○ 16
- ○ 17
- ○ 18 or older

2. Are you:

- ○ Male
- ○ Female

3. Your Height

FEET | INCHES

4. Your Weight (lbs)

Questionnaire refers to what you ate over the past year.

5. Do you now take vitamins (like Flintstones, One-A-Day, etc.)?

○ No ○ Yes → **If yes)**

a) How many vitamin pills do you take a week?

- ○ 2 or less
- ○ 3 - 5
- ○ 6 - 9
- ○ 10 or more

b) For how many years have you been taking them?

- ○ 0 - 1 years
- ○ 2 - 4
- ○ 5 - 9
- ○ 10+ years

6. How many teaspoons of sugar do you ADD to your beverages or food each day?

- ○ None/less than 1 teaspoon per day
- ○ 1 - 2 teaspoons per day
- ○ 3 - 4 teaspoons per day
- ○ 5 or more teaspoons per day

7. Which cold breakfast cereal do you usually eat?

- ○ Never eat cold breakfast cereal

8. Where do you usually eat breakfast?

- ○ At home
- ○ At school
- ○ Don't eat breakfast
- ○ Other

9. How many times each week (including weekdays and weekends) do you usually eat breakfast prepared away from home?

- ○ Never or almost never
- ○ 1 - 2 times per week
- ○ 3 - 4 times per week
- ○ 5 or more times per week

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10. How many times each week (including weekdays and weekends) do you usually eat lunch prepared away from home?

- ◯ Never or almost never
- ◯ 1 - 2 times per week
- ◯ 3 - 4 times per week
- ◯ 5 or more times per week

11. How many times each week do you usually eat after-school snacks or foods prepared away from home?

- ◯ Never or almost never
- ◯ 1 - 2 times per week
- ◯ 3 - 4 times per week
- ◯ 5 or more times per week

12. How many times each week (weekdays and weekends) do you usually eat dinner prepared away from home?

- ◯ Never or almost never
- ◯ 1 - 2 times per week
- ◯ 3 - 4 times per week
- ◯ 5 or more times per week

13. How many times per week do you prepare dinner for yourself (and/or others in your house)?

- ◯ Never or almost never
- ◯ Less than once per week
- ◯ 1 - 2 times per week
- ◯ 3 - 4 times per week
- ◯ 5 or more times per week

14. How often do you have dinner that is ready made, like frozen dinners, Spaghetti-O's, microwave meals, etc.

- ◯ Never/less than once per month
- ◯ 1 - 2 times per week
- ◯ 3 - 4 times per week
- ◯ 5 or more times per week

15. How many times each week (including weekdays and weekends) do you eat late night snacks prepared away from home?

- ◯ Never/less than once per month
- ◯ 1 - 2 times per week
- ◯ 3 - 4 times per week
- ◯ 5 or more times per week

16. How often do you eat food that is fried at home, like fried chicken?

- ◯ Never/less than once per week
- ◯ 1 - 3 times per week
- ◯ 4 - 6 times per week
- ◯ Daily

17. How often do you eat fried food away from home (like french fries, chicken nuggets)?

- ◯ Never/less than once per week
- ◯ 1 - 3 times per week
- ◯ 4 - 6 times per week
- ◯ Daily

DIETARY INTAKE

How often do you eat the following foods:

Example If you drink one can of diet soda 2 - 3 times per week, then your answer should look like this:

E1. Diet soda (1 can or glass)

- ◯ Never
- ◯ 1 - 3 cans per month
- ◯ 1 can per week
- ● 2 - 6 cans per week
- ◯ 1 can per day
- ◯ 2 or more cans per day

BEVERAGES

FILL OUT ONE BUBBLE FOR EACH FOOD ITEM

18. Diet soda (1 can or glass)
- ○ Never/less than 1 per month
- ○ 1 - 3 cans per month
- ○ 1 can per week
- ○ 2 - 6 cans per week
- ○ 1 can per day
- ○ 2 or more cans per day

19. Soda - not diet (1 can or glass)
- ○ Never/less than 1 per month
- ○ 1 - 3 cans per month
- ○ 1 can per week
- ○ 2 - 6 cans per week
- ○ 1 can per day
- ○ 2 or more cans per day

20. Hawaiian Punch, lemonade, Koolaid or other non-carbonated fruit drink (1 glass)
- ○ Never/less than 1 per month
- ○ 1 - 3 glasses per month
- ○ 1 glass per week
- ○ 2 - 4 glasses per week
- ○ 5 - 6 glasses per week
- ○ 1 glass per day
- ○ 2 or more glasses per day

21. Iced Tea - sweetened (1 glass, can or bottle)
- ○ Never/less than 1 per month
- ○ 1 - 3 glasses per month
- ○ 1 - 4 glasses per week
- ○ 5 - 6 glasses per week
- ○ 1 or more glasses per day

22. Tea (1 cup)
- ○ Never/less than 1 per month
- ○ 1 - 3 cups per month
- ○ 1 - 2 cups per week
- ○ 3 - 6 cups per week
- ○ 1 or more cups per day

23. Coffee - not decaf. (1 cup)
- ○ Never/less than 1 per month
- ○ 1 - 3 cups per month
- ○ 1 - 2 cups per week
- ○ 3 - 6 cups per week
- ○ 1 or more cups per day

24. Beer (1 glass, bottle or can)
- ○ Never/less than 1 per month
- ○ 1 - 3 cans per month
- ○ 1 can per week
- ○ 2 or more cans per week

N/A

25. Wine or wine coolers (1 glass)
- ○ Never/less than 1 per month
- ○ 1 - 3 glasses per month
- ○ 1 glass per week
- ○ 2 or more glasses per week

N/A

26. Liquor, like vodka or rum (1 drink or shot)
- ○ Never/less than 1 per month
- ○ 1 - 3 drinks per month
- ○ 1 drink per week
- ○ 2 or more drinks per week

N/A

Example If you eat:

3 pats of margarine on toast
1 - 2 pats of margarine on sandwich
1 pat of margarine on vegetables

5 - 6 pats total all day

then answer this way →

E2. Margarine (1 pat) - not butter
- ○ Never
- ○ 1 - 3 pats per month
- ○ 1 pat per week
- ○ 2 - 6 pats per week
- ○ 1 pat per day
- ○ 2 - 4 pats per day
- ● 5 or more pats per day

DAIRY PRODUCTS

27. What TYPE of milk do you usually drink?
- ○ Whole milk
- ○ 2% milk
- ○ 1% milk
- ○ Skim/nonfat milk
- ○ Don't know
- ○ Don't drink milk

28. Milk (glass or with cereal)
- ○ Never/less than 1 per month
- ○ 1 glass per week or less
- ○ 2 - 6 glasses per week
- ○ 1 glass per day
- ○ 2 - 3 glasses per day
- ○ 4+ glasses per day

29. Chocolate milk (glass)
- ○ Never/less than 1 per month
- ○ 1 - 3 glasses per month
- ○ 1 glass per week
- ○ 2 - 6 glasses per week
- ○ 1 - 2 glasses per day
- ○ 3 or more glasses per day

30. Instant Breakfast Drink (1 packet)

- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 4 times per week
- 5 or more times per week

31. Whipped cream

- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 4 times per week
- 5 or more times per week

32. Yogurt (1 cup) - Not frozen

- Never/less than 1 per month
- 1 - 3 cups per month
- 1 cup per week
- 2 - 6 cups per week
- 1 cup per day
- 2 or more cups per day

33. Cottage or ricotta cheese

- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 or more times per week

34. Cheese (1 slice)

- Never/less than 1 per month
- 1 - 3 slices per month
- 1 slice per week
- 2 - 6 slices per week
- 1 slice per day
- 2 or more slices per day

35. Cream cheese

- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 or more times per week

36. What TYPE of yogurt, cottage cheese & dairy products (besides milk) do you use mostly?

- Nonfat
- Lowfat
- Regular
- Don't know

37. Butter (1 pat) - NOT margarine

- Never/less than 1 per month
- 1 - 3 pats per month
- 1 pat per week
- 2 - 6 pats per week
- 1 pat per day
- 2 - 4 pats per day
- 5 or more pats per day

38. Margarine (1 pat) - NOT butter

- Never/less than 1 per month
- 1 - 3 pats per month
- 1 pat per week
- 2 - 6 pats per week
- 1 pat per day
- 2 - 4 pats per day
- 5 or more pats per day

39. What FORM and BRAND of margarine does your family usually use?

- None
- Stick
- Tub
- Squeeze (liquid)

WHAT SPECIFIC BRAND AND TYPE (LIKE "PARKAY CORN OIL SPREAD")?

Leave blank if you don't know.

40. What TYPE of oil does your family use at home?

- Canola oil
- Corn oil
- Safflower oil
- Olive oil
- Vegetable oil
- Don't know

MAIN DISHES

41. Cheeseburger (1)

- Never/less than 1 per month
- 1 - 3 per month
- One per week
- 2 - 4 per week
- 5 or more per week

42. Hamburger (1)

- Never/less than 1 per month
- 1 - 3 per month
- One per week
- 2 - 4 per week
- 5 or more per week

43. Pizza (2 slices)

- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 4 times per week
- 5 or more times per week

44. Tacos/burritos (1)

- Never/less than 1 per month
- 1 - 3 per month
- One per week
- 2 - 4 per week
- 5 or more per week

45. Which taco filling do you usually have:

- Beef & beans
- Beef
- Chicken
- Beans

46. Chicken nuggets (6)

- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 4 times per week
- 5 or more times per week

47. Hot dogs (1)
- Never/less than 1 per month
- 1 - 3 per month
- One per week
- 2 - 4 per week
- 5 or more per week

48. Peanut butter sandwich (1) (plain or with jelly, fluff, etc.)
- Never/less than 1 per month
- 1 - 3 per month
- One per week
- 2 - 4 per week
- 5 or more per week

49. Chicken or turkey sandwich (1)
- Never/less than 1 per month
- 1 - 3 per month
- One per week
- 2 or more per week

50. Roast beef or ham sandwich (1)
- Never/less than 1 per month
- 1 - 3 per month
- One per week
- 2 or more per week

51. Salami, bologna, or other deli meat sandwich (1)
- Never/less than 1 per month
- 1 - 3 per month
- One per week
- 2 or more per week

52. Tuna sandwich (1)
- Never/less than 1 per month
- 1 - 3 per month
- One per week
- 2 or more per week

53. Chicken or turkey as main dish (1 serving)
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 4 times per week
- 5 or more times per week

54. Fish sticks, fish cakes or fish sandwich (1 serving)
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 or more times per week

55. Fresh fish as main dish (1 serving)
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 4 times per week
- 5 or more times per week

56. Beef (steak, roast) or lamb as main dish (1 serving)
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 4 times per week
- 5 or more times per week

57. Pork or ham as main dish (1 serving)
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 4 times per week
- 5 or more times per week

58. Meatballs or meatloaf (1 serving)
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 4 times per week
- 5 or more times per week

59. Lasagna/baked ziti (1 serving)
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 or more times per week

60. Macaroni and cheese (1 serving)
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 or more times per week

61. Spaghetti with tomato sauce (1 serving)
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 4 times per week
- 5 or more times per week

62. Eggs (1)
- Never/less than 1 per month
- 1 - 3 eggs per month
- One egg per week
- 2 - 4 eggs per week
- 5 or more eggs per week

63. Liver: beef, calf, chicken or pork (1 serving)
- Never/less than 1 per month
- Less than once per month
- Once per month
- 2 - 3 times per month
- Once per week or more

64. Shrimp, lobster, scallops (1 serving)
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 or more times per week

65. French toast (2 slices)
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 or more times per week

66. Grilled cheese (1)
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 or more times per week

67. Eggrolls (1)
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 or more times per week

MISCELLANEOUS FOODS

68. Brown gravy
- Never/less than 1 per month
- Once per week or less
- 2 - 6 times per week
- Once per day
- 2 or more times per day

69. Ketchup
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 4 times per week
- 5 or more times per week

70. Clear soup (with rice, noodles, vegetables) 1 bowl
- Never/less than 1 per month
- 1 - 3 bowls per month
- 1 bowl per week
- 2 or more bowls per week

71. Cream (milk) soups or chowder (1 bowl)
- Never/less than 1 per month
- 1 - 3 bowls per month
- 1 bowl per week
- 2 - 6 bowls per week
- 1 or more bowls per day

72. Mayonnaise
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 6 times per week
- Once per day

73. Low calorie/fat salad dressing
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 6 times per week
- Once or more per day

74. Salad dressing (not low calorie)
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 6 times per week
- Once or more per day

75. Salsa
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 6 times per week
- Once or more per day

76. How much fat on your beef, pork, or lamb do you eat?
- Eat all
- Eat some
- Eat none
- Don't eat meat

77. When you have chicken or turkey, do you eat the skin?
- Yes
- No
- Sometimes

BREADS & CEREALS

78. Cold breakfast cereal (1 bowl)
- ◯ Never/less than 1 per month
- ◯ 1 - 3 bowls per month
- ◯ 1 bowl per week
- ◯ 2 - 4 bowls per week
- ◯ 5 - 7 bowls per week
- ◯ 2 or more bowls per day

79. Hot breakfast cereal, like oatmeal, grits (1 bowl)
- ◯ Never/less than 1 per month
- ◯ 1 - 3 bowls per month
- ◯ 1 bowl per week
- ◯ 2 - 4 bowls per week
- ◯ 5 - 7 bowls per week
- ◯ 2 or more bowls per day

80. White bread, pita bread, or toast (1 slice)
- ◯ Never/less than 1 per month
- ◯ 1 slice per week or less
- ◯ 2 - 4 slices per week
- ◯ 5 - 7 slices per week
- ◯ 2 - 3 slices per day
- ◯ 4+ slices per day

81. Dark bread (1 slice)
- ◯ Never/less than 1 per month
- ◯ 1 slice per week or less
- ◯ 2 - 4 slices per week
- ◯ 5 - 7 slices per week
- ◯ 2 - 3 slices per day
- ◯ 4+ slices per day

82. English muffins or bagels (1)
- ◯ Never/less than 1 per month
- ◯ 1 - 3 per month
- ◯ 1 per week
- ◯ 2 - 4 per week
- ◯ 5 or more per week

83. Muffin (1)
- ◯ Never/less than 1 per month
- ◯ 1 - 3 muffins per month
- ◯ 1 muffin per week
- ◯ 2 - 4 muffins per week
- ◯ 5 or more muffins per week

84. Cornbread (1 square)
- ◯ Never/less than 1 per month
- ◯ 1 - 3 times per month
- ◯ Once per week
- ◯ 2 - 4 times per week
- ◯ 5 or more per week

85. Biscuit/roll (1)
- ◯ Never/less than 1 per month
- ◯ 1 - 3 per month
- ◯ 1 per week
- ◯ 2 - 4 per week
- ◯ 5 or more per week

86. Rice
- ◯ Never/less than 1 per month
- ◯ 1 - 3 times per month
- ◯ Once per week
- ◯ 2 - 4 times per week
- ◯ 5 or more times per week

87. Noodles, pasta
- ◯ Never/less than 1 per month
- ◯ 1 - 3 times per month
- ◯ Once per week
- ◯ 2 - 4 times per week
- ◯ 5 or more times per week

88. Tortilla - no filling (1)
- ◯ Never/less than 1 per month
- ◯ 1 - 3 per month
- ◯ 1 per week
- ◯ 2 - 4 per week
- ◯ 5 or more per week

89. Other grains, like kasha, couscous, bulgur
- ◯ Never/less than 1 per month
- ◯ 1 - 3 times per month
- ◯ Once per week
- ◯ 2 or more times per week

90. Pancakes (2) or waffles (1)
- ◯ Never/less than 1 per month
- ◯ 1 - 3 times per month
- ◯ Once per week
- ◯ 2 or more times per week

91. French fries (large order)
- ◯ Never/less than 1 per month
- ◯ 1 - 3 orders per month
- ◯ 1 order per week
- ◯ 2 - 4 orders per week
- ◯ 5 or more orders per week

92. Potatoes - baked, boiled, mashed
- ◯ Never/less than 1 per month
- ◯ 1 - 3 times per month
- ◯ Once per week
- ◯ 2 - 4 times per week
- ◯ 5 or more times per week

FRUITS & VEGETABLES

93. Raisins (small pack)
- Never/less than 1 per month
- 1 - 3 times per month
- 1 per week
- 2 - 4 times per week
- 5 or more times per week

94. Grapes (bunch)
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 4 times per week
- 5 or more times per week

95. Bananas (1)
- Never/less than 1 per month
- 1 - 3 per month
- 1 per week
- 2 - 4 per week
- 5 or more per week

96. Cantaloupe, melons (1/4 melon)
- Never/less than 1 per month
- 1 - 3 times per month
- 1 per week
- 2 or more times per week

97. Apples (1) or applesauce
- Never/less than 1 per month
- 1 - 3 per month
- 1 per week
- 2 - 6 per week
- 1 or more per day

98. Pears (1)
- Never/less than 1 per month
- 1 - 3 per month
- 1 per week
- 2 - 6 per week
- 1 or more per day

99. Oranges (1), grapefruit (1/2)
- Never/less than 1 per month
- 1 - 3 per month
- 1 per week
- 2 - 6 per week
- 1 or more per day

100. Strawberries
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 or more times per week

101. Peaches, plums, apricots (1)
- Never/less than 1 per month
- 1 - 3 per month
- 1 per week
- 2 or more per week

102. Orange juice (1 glass)
- Never/less than 1 per month
- 1 - 3 glasses per month
- 1 glass per week
- 2 - 6 glasses per week
- 1 glass per day
- 2 or more glasses per day

103. Apple juice and other fruit juices (1 glass)
- Never/less than 1 per month
- 1 - 3 glasses per month
- 1 glass per week
- 2 - 6 glasses per week
- 1 glass per day
- 2 or more glasses per day

104. Tomatoes (1)
- Never/less than 1 per month
- 1 - 3 per month
- 1 per week
- 2 - 6 per week
- 1 or more per day

105. Tomato/spaghetti sauce
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 4 times per week
- 5 or more times per week

106. Tofu
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 4 times per week
- 5 or more times per week

107. String beans
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 4 times per week
- 5 or more times per week

108. Beans/lentils/soybeans
- ◯ Never/less than 1 per month
- ◯ Once per week or less
- ◯ 2 - 6 times per week
- ◯ Once per day

109. Broccoli
- ◯ Never/less than 1 per month
- ◯ 1 - 3 times per month
- ◯ Once per week
- ◯ 2 - 4 times per week
- ◯ 5 or more times per week

110. Beets (not greens)
- ◯ Never/less than 1 per month
- ◯ Once per week or less
- ◯ 2 or more times per week

111. Corn
- ◯ Never/less than 1 per month
- ◯ 1 - 3 times per month
- ◯ Once per week
- ◯ 2 - 4 times per week
- ◯ 5 or more times per week

112. Peas or lima beans
- ◯ Never/less than 1 per month
- ◯ 1 - 3 times per month
- ◯ Once per week
- ◯ 2 - 4 times per week
- ◯ 5 or more times per week

113. Mixed vegetables
- ◯ Never/less than 1 per month
- ◯ 1 - 3 times per month
- ◯ Once per week
- ◯ 2 - 4 times per week
- ◯ 5 or more times per week

114. Spinach
- ◯ Never/less than 1 per month
- ◯ 1 - 3 times per month
- ◯ Once a week
- ◯ 2 - 4 times per week
- ◯ 5 or more times per week

115. Greens/kale
- ◯ Never/less than 1 per month
- ◯ 1 - 3 times per month
- ◯ Once per week
- ◯ 2 - 4 times per week
- ◯ 5 or more times per week

116. Green/red peppers
- ◯ Never/less than 1 per month
- ◯ 1 - 3 times per month
- ◯ Once a week
- ◯ 2 - 4 times per week
- ◯ 5 or more times per week

117. Yams/sweet potatoes (1)
- ◯ Never/less than 1 per month
- ◯ 1 - 3 times per month
- ◯ Once a week
- ◯ 2 - 4 times per week
- ◯ 5 or more times per week

118. Zucchini, summer squash, eggplant
- ◯ Never/less than 1 per month
- ◯ 1 - 3 times per month
- ◯ Once per week
- ◯ 2 - 4 times per week
- ◯ 5 or more times per week

119. Carrots, cooked
- ◯ Never/less than 1 per month
- ◯ 1 - 3 times per month
- ◯ Once per week
- ◯ 2 - 4 times per week
- ◯ 5 or more times per week

120. Carrots, raw
- ◯ Never/less than 1 per month
- ◯ 1 - 3 times per month
- ◯ Once per week
- ◯ 2 - 4 times per week
- ◯ 5 or more times per week

121. Celery
- ◯ Never/less than 1 per month
- ◯ 1 - 3 times per month
- ◯ Once per week
- ◯ 2 - 4 times per week
- ◯ 5 or more times per week

122. Lettuce/tossed salad
- ◯ Never/less than 1 per month
- ◯ 1 - 3 times per month
- ◯ Once per week
- ◯ 2 - 6 times per week
- ◯ One or more per day

123. Coleslaw
- ◯ Never/less than 1 per month
- ◯ 1 - 3 times per month
- ◯ Once per week
- ◯ 2 or more times per week

124. Potato salad
- ◯ Never/less than 1 per month
- ◯ 1 - 3 times per month
- ◯ Once per week
- ◯ 2 or more times per week

Think about your usual snacks. How often do you eat each type of snack food.

Example If you eat poptarts rarely (about 6 per year) then your answer should look like this:

E3. Poptarts (1)
- ● Never/less than 1 per month
- ○ 1 - 3 per month
- ○ 1 - 6 per week
- ○ 1 or more per day

SNACK FOODS/DESSERTS

125. Fill in the number of snacks (food or drinks) eaten on school days and weekends/vacation days.

Snacks	School Days					Vacation/Weekend Days				
	NONE	1	2	3	4 OR MORE	NONE	1	2	3	4 OR MORE
Between breakfast and lunch	○	○	○	○	○	○	○	○	○	○
After lunch, before dinner	○	○	○	○	○	○	○	○	○	○
After dinner	○	○	○	○	○	○	○	○	○	○

126. Potato chips (1 small bag)
- ○ Never/less than 1 per month
- ○ 1 - 3 small bags per month
- ○ One small bag per week
- ○ 2 - 6 small bags per week
- ○ 1 or more small bags per day

127. Corn chips/Doritos (small bag)
- ○ Never/less than 1 per month
- ○ 1 - 3 small bags per month
- ○ One small bag per week
- ○ 2 - 6 small bags per week
- ○ 1 or more small bags per day

128. Nachos with cheese (1 serving)
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 or more times per week

129. Popcorn (1 small bag)
- ○ Never/less than 1 per month
- ○ 1 - 3 small bags per month
- ○ 1 - 4 small bags per week
- ○ 5 or more small bags per week

130. Pretzels (1 small bag)
- ○ Never/less than 1 per month
- ○ 1 - 3 small bags per month
- ○ 1 small bags per week
- ○ 2 or more small bags per week

131. Peanuts, nuts (1 small bag)
- ○ Never/less than 1 per month
- ○ 1 - 3 small bags per month
- ○ 1 - 4 small bags per week
- ○ 5 or more small bags per week

132. Fun fruit or fruit rollups (1 pack)
- ○ Never/less than 1 per month
- ○ 1 - 3 packs per month
- ○ 1 - 4 packs per week
- ○ 5 or more packs per week

133. Graham crackers
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ 1 - 4 times per week
- ○ 5 or more times per week

134. Crackers, like saltines or wheat thins
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ 1 - 4 times per week
- ○ 5 or more times per week

135. Poptarts (1)
- Never/less than 1 per month
- 1 - 3 poptarts per month
- 1 - 6 poptarts per week
- 1 or more poptarts per day

136. Cake (1 slice)
- Never/less than 1 per month
- 1 - 3 slices per month
- 1 slice per week
- 2 or more slices per week

137. Snack cakes, Twinkies (1 package)
- Never/less than 1 per month
- 1 - 3 per month
- Once per week
- 2 - 6 per week
- 1 or more per day

138. Danish, sweetrolls, pastry (1)
- Never/less than 1 per month
- 1 - 3 per month
- 1 per week
- 2 - 4 per week
- 5 or more per week

139. Donuts (1)
- Never/less than 1 per month
- 1 - 3 donuts per month
- 1 donut per week
- 2 - 6 donuts per week
- 1 or more donuts per day

140. Cookies (1)
- Never/less than 1 per month
- 1 - 3 cookies per month
- 1 cookie per week
- 2 - 6 cookies per week
- 1 - 3 cookies per day
- 4 or more cookies per day

141. Brownies (1)
- Never/less than 1 per month
- 1 - 3 per month
- 1 per week
- 2 - 4 per week
- 5 or more per week

142. Pie (1 slice)
- Never/less than 1 per month
- 1 - 3 slices per month
- 1 slice per week
- 2 or more slices per week

143. Chocolate (1 bar or packet) like Hershey's or M & M's
- Never/less than 1 per month
- 1 - 3 per month
- 1 per week
- 2 - 6 per week
- 1 or more per day

144. Other candy bars (Milky Way, Snickers)
- Never/less than 1 per month
- 1 - 3 candy bars per month
- 1 candy bar per week
- 2 - 4 candy bars per week
- 5 or more candy bars per week

145. Other candy without chocolate (Skittles) (1 pack)
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 4 times per week
- 5 or more times per week

146. Jello
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 4 times per week
- 5 or more times per week

147. Pudding
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 4 times per week
- 5 or more times per week

148. Frozen yogurt
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 4 times per week
- 5 or more times per week

149. Ice cream
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 4 times per week
- 5 or more times per week

150. Milkshake or frappe (1)
- Never/less than 1 per month
- 1 - 3 per month
- 1 per week
- 2 or more per week

151. Popsicles
- Never/less than 1 per month
- 1 - 3 popsicles per month
- 1 popsicle per week
- 2 - 4 popsicles per week
- 5 or more popsicles per week

152. Please list any other foods that you usually eat at least once per week that are not listed (for example, coconut, hummus, falafel, chili, plantains, mangoes, etc. . .)

FOODS	HOW OFTEN?
a) ______	a) ______
b) ______	b) ______
c) ______	c) ______
d) ______	d) ______

THANK YOU FOR COMPLETING THIS SURVEY!

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