EXAMINATION OF THE EFFECTIVENESS OF NUTRITION AND PHYSICAL ACTIVITY INTERVENTIONS ON BEHAVIOR CHANGE AND CARDIOVASCULAR RISK AMONG ADOLESCENT STUDENTS PARTICIPATING IN A SCHOOL-BASED HEALTH PROGRAM IN MICHIGAN

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ABSTRACT

IMPORTANCE: Overweight/obesity, dyslipidemia, and elevated blood pressure (BP) are increasingly common among U.S. adolescents and increase risk for development of cardiovascular disease (CVD) in adulthood. These risk factors are highly influenced by diet and activity levels, both of which are modifiable with appropriate intervention. School-based programs may be an ideal setting for nutrition intervention programs to improve health behaviors, however data examining the effectiveness of such programs is limited, and results have been inconsistent depending on the outcomes examined. **OBJECTIVE:** Among a sample of adolescents in Michigan participating in Project Healthy Schools (PHS), a multi-component school-based intervention program, the objective of this dissertation is to 1) examine the effectiveness of the PHS program at achieving favorable change in participating students' dietary consumption of foods and/or beverages associated with CVD risk; 2) examine if achievement of optimal consumption of fruits and vegetables, sugar sweetened beverages (SSB) and levels of physical activity (PA) is associated with improvement in blood lipid and BP levels and; 3) determine if change in dietary consumption of foods high in saturated fatty acids (SFA) and trans-fatty acids (TFA) is associated with change in low density lipoprotein cholesterol (LDL-C) concentrations.
DESIGN, SETTING, AND PARTICIPANTS: A non-randomized, quasi-experimental pre-post design evaluation of sixth grade students from 94 middle-schools across the state of Michigan enrolled in the first year of a school-based nutrition intervention program between 2005-2019. **MEASURES:** Measures of dietary intake and PA were collected from a validated health behavior questionnaire administered at baseline and following completion of the 10-week nutrition intervention program. Physiologic outcome measures of a non-

fasting lipid profile which included total cholesterol [TC], high-density lipoprotein cholesterol [HDL-C], triglycerides [TG] and calculated low-density lipoprotein cholesterol (LDL-C), as well as systolic and diastolic BP measurements were collected. **RESULTS:** Intake of fruit and vegetables significantly increased post-intervention, as did consumption of sugary beverages. Students who were more physically active following the PHS intervention had significantly higher post-intervention HDL-C and lower TG levels, whereas students with low SSB intake post-intervention experienced lower post-intervention HDL-C levels compared to students with higher SSB intake. Reduced consumption of high-risk, high fat foods was associated with a significant decrease in mean LDL-C when compared with high consumption at baseline and follow-up. **CONCLUSIONS AND FUTURE DIRECTIONS:** Significant increases in dietary intake of fruit and vegetable consumption following participation in the intervention program were modest. Improvement in cardiometabolic risk factors were also observed. Students who improved their intake of foods high in saturated and trans-fat experienced the largest decreases in LDL-C post-intervention. The results also suggest that PA in this age group may be an effective way to improve HDL-C and TG levels, especially among high-risk students. Future work should examine whether modest changes in dietary consumption and/or PA levels can promote a meaningful shift in physiological measures of cardiovascular risk over a longer period of time.

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TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION

Background/Overview

Cardiovascular disease (CVD) remains the leading cause of death among adults in the United States (U.S.).[1] Data from the 2019 Heart Disease and Stroke Statistics update of the American Heart Association (AHA) reported that 48 percent of persons 20 years of age and older in the U.S. have CVD.[2] Although the clinical manifestations of CVD occur in adulthood, evidence supports that atherosclerotic vascular changes often begin in childhood.[3] Furthermore, the presence of identifiable risk factors in some children, such as obesity, dyslipidemia, and hypertension, may accelerate the atherosclerotic process.[3] Data from longitudinal studies have demonstrated childhood measures of blood pressure (BP), serum lipid levels and body mass index (BMI) correlate strongly with corresponding physiological measures in adulthood.[4] Children and adolescents with CVD risk factors are more likely to be at risk in adulthood, creating opportunities during youth for preventative measures to be adopted through lifestyle behaviors.

The existence of CVD risk factors, specifically hypertension and dyslipidemia, are documented within the pediatric population. According to data from the United States National Health and Nutrition Examination Survey (NHANES), the prevalence of elevated BP and dyslipidemia among children and adolescents between the ages of 8 to 19 is estimated between 8 and 14 percent, and 20 to 30 percent, respectively.[5] The burden of CVD risk factors among adolescents is especially high among youth who are overweight or obese. NHANES study participants aged 12 to 19 years who were overweight or obese demonstrated increased prevalence of elevated BP and blood lipids compared to participants of normal weight.[6]

Dyslipidemias refer to abnormal blood lipid levels and are considered disorders of lipid metabolism that may result in elevated total cholesterol (TC), elevated low-density lipoprotein cholesterol (LDL-C), elevated triglyceride levels (TG), and/or low high-density lipoprotein cholesterol (HDL-C).[7] Long term studies to establish a direct link between abnormal blood lipids during childhood and adolescence with CVD events in adulthood have not been conducted. However, elevated blood levels of TC and LDL-C in youth is associated with the development of early atherosclerosis, and elevated levels in childhood have been shown to track into adulthood.[8-10] LDL-C is the lipoprotein most intimately linked with atherosclerotic process, and childhood LDL-C concentrations strongly predict abnormal blood lipid levels in adulthood.[8] In contrast, there is an inverse relationship between HDL-C concentrations and CVD risk in adulthood, largely due to several anti-atherogenic properties of this lipoprotein.[11] Some experts consider TG levels to be an independent risk factor for CVD in adults, but to a lesser degree compared to LDL-C, and TC to HDL-C ratios.[12,13] Similar to blood lipid levels, elevated BP is a well-established risk factor for CVD in adults, with limited data establishing the same direct link to disease outcomes with elevated BP during childhood.[14]

The Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents reports that dietary and physical activity (PA) interventions can result in modest improvements in abnormal lipid levels and BP measurements in children and adolescents.[7] In addition, lipid levels change with normal growth and maturation. During puberty, TC and LDL-C can decrease up to 20% or more, and HDL-C levels are also known to decrease during this timeframe, especially among male adolescents.[15]

Important components of a dietary pattern that is considered beneficial to heart health for children and adolescents includes one that is low in saturated fat, low in sugar-sweetened beverages (SSB) and foods, and high in fiber rich foods such as fruits, vegetables, whole grains, and legumes.[16] Dietary consumption of saturated fatty acids (SFA) has the potential to raise LDL-C, and to a lesser degree, raise HDL-C.[17] It is recommended that children and adolescents limit saturated fat intake to less than 10% of total calories in their diet and limit dietary cholesterol to less than 300 mg per day as a safe and effective way to reduce TC and LDL-C blood lipid levels in healthy children.[7] The predominant dietary sources of SFA in U.S. children and adults are dairy foods such as cheese, milk, ice cream, as well as red meat. Other sources include commercial baked goods and fast foods, including fried meats and French fries.[18,19] Trans-fatty acids (TFA) are particularly harmful as they raise LDL-C and lower HDL-C.[20,21] In contrast to the effect of trans-fat and saturated fat on blood lipid levels, consumption of fiber rich foods such as fruits, vegetables, whole grains, and legumes has been shown to modestly lower TC and LDL-C levels in adults.[22] Reduced intake of simple carbohydrates such as SSBs, refined grains in processed foods, snack foods, and candy coupled with reduced saturated fat intake and increased consumption of complex carbohydrates, is associated with decreased TG levels in children whose levels are elevated.[23] This process is likely mediated by weight loss that ensues with improvement in diet and reduction of sugar intake.[7]

Dietary habits during adolescence can impact BP, and the adoption of healthy eating habits is an effective means to control and/or improve BP among youth with elevated BP or hypertension.[24] The Dietary Approach to Stop Hypertension (DASH) dietary pattern, promotes the consumption of fruits, vegetables, lean dairy products, whole grains, fish, poultry, and nuts and encourages reduced consumption of red and processed meat and sugary drinks. This dietary pattern is an effective means to lower BP safely and significantly in adults, with similar benefits in adolescent populations.[24] The DASH dietary pattern is especially effective at lowering BP among youth with elevated BP or hypertension and among those that are overweight or obese.[25] Adherence to the DASH dietary pattern among adolescents is generally low.[24] Specific components of the DASH pattern may provide some benefit to BP health independently, however data on the effect of dietary changes upon BP in children is limited. Reducing consumption of foods high in sodium, increasing fruit and vegetable intake, and lowering intake of sugar containing foods and beverages each on their own may provide beneficial effects on BP, especially among youth with elevated BP or hypertension, which is likely mediated by weight status.[7,26,27]

HDL-C concentrations increase with regular PA and aerobic exercise in the adult population.[28] PA can also have a positive impact on blood lipid levels in adolescents. Evidence supports that increases in moderate-to-vigorous PA are associated with lower TC levels, lower LDL-C levels, lower TG, and higher HDL-C levels.[7] A sample of adolescents of diverse racial backgrounds ages 12 to 19 who participated in the 1999–2004 cycles of NHANES demonstrated that increased PA levels were positively associated with HDL-C in all groups studied and negatively associated with TC, TG, and LDL-C according to different race identification and sex.[29]

Additionally, PA is a well-documented means to improve BP in adolescents, which in turn can decrease the risk of atherosclerosis and CVD. This is especially true when PA interventions are combined with dietary intervention and weight loss measures when appropriate. Sustained regular PA in youth is most effective in lowering BP. In addition, more vigorous activity is most effective in reducing BP, whereas short-term PA can lead to small but statistically insignificant reductions of BP measures.[30-33]

Schools provide a unique setting for the promotion of healthy eating behaviors and PA, as they engage a large majority of children and adolescents in the U.S. most days of the week throughout a standard school year. There are many school-based nutrition and PA intervention programs that focus on obesity prevention, with fewer examining other health benefits that may arise from such programs.[34] School-based intervention programs focused on promoting healthy lifestyles differ in strategies utilized, target age, program length, and program evaluation outcomes. Additionally, programs that combine nutrition and PA interventions may be more effective at reducing obesity and CVD risk factors than just diet or PA interventions alone.[34,35]

Project Healthy Schools (PHS) is a middle school-based nutrition and physical activity intervention program implemented predominantly in the state of Michigan. The goal of PHS is to not only reduce childhood obesity but to also improve CVD risk factors primarily through an educational component which consists of 10 student learning modules, as well as through environmental changes within the schools. There are five primary goals of the PHS program: 1) eat more fruits and vegetables; 2) choose less sugary foods and beverages; 3) eat less fast and fatty foods; 4) be active every day, specifically by performing 150 minutes of exercise per week; and 5) spend less time in front of a screen.[36] Previous research examining the PHS program has shown that participation in the program correlates with significant improvement in physiological measures including blood lipid levels and BP. This assessment was done early in the program and did not specifically assess whether a correlation between dietary and/or PA behavior change was linked to the improvements noted in the outcome measures.[36,37]

Significance

Few studies have examined the association between changes in diet and PA behaviors targeted by a school-based intervention program and subsequent change in CVD risk factors. This association is crucial to examine to adequately assess the effectiveness of programs that focus on behavior change. As such, this research study is significant because it provides insight into the impact of a relatively short-term and scalable school-based intervention on dietary intake and PA patterns among a diverse population of sixth grade students in Michigan. Moreover, this research, specifically assesses whether changes in these behaviors is associated with improvement in CVD risk factors beyond weight, such as blood lipid levels and BP measurements.

The overall purpose of this dissertation work is to evaluate if favorable change in dietary intake is achievable following participation in a short-term school-based nutrition and PA intervention program. Additionally, this work will assess if favorable changes in specific components of diet and PA following completion the program is associated with favorable change in biomarkers associated with CVD risk.

Specific Aims and Hypotheses

Aim 1: To examine the effectiveness of PHS, at improving participating students' dietary consumption of foods and/or beverages associated with CVD risk.

Hypothesis: Students will exhibit a favorable change in dietary intake among foods and/or beverages targeted by the intervention program lessons after participation in the PHS program.

- Hypothesis a): Post-intervention consumption of fruits and vegetables will increase compared to baseline reported consumption.
- Hypothesis b): Post-intervention consumption of SSBs will decrease compared to baseline reported consumption.
- Hypothesis c): Post-intervention consumption of high fat, high sodium, and high sugar foods will decrease compared to baseline reported consumption.

Aim 2: To examine if favorable change in self-reported fruit and vegetable intake, SSB intake and PA is associated with favorable change in biomarkers of cardiovascular risk, specifically blood lipid levels and BP following participation in a school-based nutrition and PA intervention program.

Hypothesis: Students who achieve or maintain favorable consumption of fruits and vegetables, and SSBs and who achieve or maintain an adequate level of PA following participation in PHS will exhibit improvement in blood lipid and BP levels.

- Hypothesis a): Students who achieve or maintain consumption of fruits and vegetables at least 5 times per day of post-intervention will exhibit more favorable change in blood lipid and BP levels compared to students who consume fruits and vegetables less than 5 times per day post-intervention.
- Hypothesis b): Students who achieve or maintain consumption of SSBs 1 time per day or fewer post-intervention will exhibit more favorable change in blood lipid and BP levels compared to students who consume SSBs more than 1 time per day post-intervention.

- Hypothesis c): Students who achieve or maintain at least 5 sessions of moderate (30 min) and/or vigorous (20 min) activity per week post-intervention will exhibit more favorable change in blood lipid and BP levels compared to students who report less than 5 sessions of moderate (30 min) and/or vigorous (20 min) activity per week post-intervention.
- Hypothesis d): Students who exhibit optimal consumption of fruits and vegetables, and SSBs as well as optimal levels of PA post-intervention will exhibit more favorable change in blood lipid and BP levels than students who do not exhibit optimal consumption of fruits and vegetables, SSBs, and levels of PA.

Aim 3: To examine if change in consumption of foods high in saturated and trans-fat (red and processed meats, snacks foods, baked goods, and sweets) is associated with change in LDL-C following participation in a school-based health intervention program.

Hypothesis: Among students with high intake of foods deemed high in saturated fat and trans-fat at baseline, favorable change in reported consumption of these foods will lead to favorable change in LDL-C.

- Hypothesis a): Favorable change in combined consumption of foods high in saturated fat and trans-fat will result in favorable change in LDL-C post-intervention, with largest improvement in LDL-C among students with high consumption at baseline.
- Hypothesis b): Favorable change in consumption of specific foods and/or food groups high in saturated fat and trans-fat will result in favorable change in LDL-C post-intervention.

CHAPTER 2: REVIEW OF LITERATURE

Introduction

CVD is the leading cause of death for adult men and women in the U.S.[1] Coronary heart disease (CHD), specifically, is responsible for 43 percent of deaths attributable to CVD in the U.S.[38] Clinical disease is generally revealed in adulthood, yet evidence supports that the atherosclerotic process for some begins in childhood.[7,8,9,10] Detection and/or prevention of cardiovascular risk factors, both behavioral and biological in nature, early in life may be effective in mitigating the manifestation or progression of disease in adulthood.[7]

The purpose of this literature review is to 1) summarize the prevalence of CVD risk factors among children and adolescents, including obesity, hypertension, dyslipidemia, and altered blood glucose, 2) summarize lifestyle factors contributing to CVD risk in adolescence, including specific dietary factors and physical inactivity, 3) summarize nutrition recommendations for US children, focusing on those foods and/or beverages associated with CVD risk and 4) provide an overview of the effectiveness of school-based nutrition and PA intervention programs targeting adolescents.

CVD risk factors among children and adolescents

CHD is a type a CVD that is associated with atherosclerosis.[1] Established modifiable risk factors for CHD include overweight or obesity, hypertension, dyslipidemia, altered blood glucose levels (including insulin resistance, hyperinsulinemia, and elevated blood glucose), smoking, dietary factors, and lack of PA.[14] The atherosclerotic process, including changes to the vasculature, can begin early in childhood. Vascular changes due to the atherosclerotic process are subtle for most children. Consistent adherence to healthy lifestyle practices can prevent the development or minimize progression of these vascular changes.[39]

Atherosclerosis is a pathologic process that causes disease of the coronary, cerebral, and peripheral arteries, including the aorta due to the hardening and narrowing of these vessels. This pathologic process begins with the thickening of the innermost layer of endothelial cells which line a blood vessel which is referred to as the intima. This thickening occurs due to the accumulation of foam cells within the endothelium. The initial damage to the endothelium results in the recruitment of macrophages to the intima. These macrophages consume oxidized cholesterol and form foam cells, which over time accumulate into fatty streaks, and can progress to significant plaque formation which hardens and narrows the arteries. In addition, smooth muscle cells within the arterial wall multiply to populate the intima and move to the surface of plaque deposits and contribute to the formation of a fibrous cap that covers the plaque.[39] This process eventually reduces the diameter of the vessel, which reduces blood flow and oxygen delivery to vital organs, including the heart.[39]

Fatty streaks were found in nearly half of subjects aged 2 to 15 years. This is according to autopsy data on a population of ethnically diverse young persons from the Bogalusa Heart Study. The Bogalusa data also demonstrated that with increasing age, BMI, BP, and blood lipid levels, the prevalence and severity of atherosclerosis increases significantly.[8,40] Similarly, the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study used autopsy data and examined the aortas of nearly 2,900 subjects between the age of 15 and 34. Findings included evidence of advanced fatty streaks with raised lesions among 10 percent of coronary arteries examined and 30 percent of aortas examined in subjects aged 15-19 years.[9] This study provides more evidence that atherosclerosis can originate in childhood and adolescence, and that not only fatty streaks, but also the prevalence of fibrous plaques in the arterial wall can increase precipitously during the age span of 15-34 years.[9,41]

In addition to autopsy studies, imaging studies conducted in children have demonstrated vascular changes consistent with the early development of atherosclerosis, which is associated with the manifestation of CVD in adulthood.[10] Arterial imaging technologies and other simple tests can detect early anatomic, physiologic, mechanical, proinflammatory, and/or prothrombotic changes in a non-invasive manner, offering indirect evidence of the early stages of atherosclerosis in young persons. Groner and colleagues reviewed nearly 150 peer-reviewed papers investigating childhood antecedents of adult CVD published between 1980 and 2006. Their review found evidence that vascular alterations in anatomy, physiology, mechanical properties, and/or proinflammatory and prothrombotic changes occur as early as age 3 and are associated with CVD risk factors in adults. Notably, the research in their review also concluded that with appropriate and timely intervention, childhood vascular alterations can improve, which may modify subsequent CVD risk in adulthood.[10]

According to the National Heart Lung and Blood Institute (NHLBI) and the American Heart Association (AHA), the two primary goals of cardiovascular health promotion in children are 1) to prevent the development of risk factors associated with atherosclerosis with measures that focus on adherence to a healthy lifestyle; and 2) to identify and manage children and adolescents at risk for early atherosclerosis based on the presence of established risk factors including obesity, hypertension, dyslipidemia, and insulin resistance.[7,42]

The AHA defines ideal cardiovascular health as the absence of clinically evident CVD along with the simultaneous presence of optimal levels of the following 7 health metrics; not smoking, consuming a healthy diet, achieving adequate PA, normal body weight, normal levels of TC, BP and fasting blood glucose in the absence of medication management. From 2011-2012, approximately 5 percent of U.S. children aged 12-19 met only 0, 1, or 2 of the 7-health metrics, approximately 54 percent met 3 or 4 criteria for ideal cardiovascular health, 41 percent met 5 or 6 criteria, and <1 percent met all 7 criteria. Since it is rare for U.S. children to meet all 7 criteria for ideal cardiovascular health, the AHA broke down the criteria into ideal, intermediate, and poor adherence. Of all 7 criteria, children were least likely to achieve an ideal level of a healthy diet, PA, and BMI. In contrast, nearly 80 percent of children met the ideal health criteria for TC, BP diabetes, and smoking status.[2]

Obesity

Definition and Measurement

The World Health Organization (WHO) defines overweight and obesity as "abnormal or excessive fat accumulation that presents a risk to health."[43] Tools or methods that provide a direct measurement of body fat are not widely or readily available for clinical use. Therefore, anthropometric data that examines the relationship between weight and height, or BMI, is considered an accurate estimation of body fat for clinical purposes as well as for epidemiological research and is the universally accepted measurement of overweight and obesity in children starting at age 2.[44] BMI is calculated by taking body weight in kilograms divided by height in meters squared. There are other validated body composition measurement methods that can be used as indices of childhood obesity, including waist circumference and waist-to-hip ratio. These measurements of fat distribution are associated with weight-related health concerns in adult populations, however, the association is less clear in children and adolescents making BMI the preferred measurement in this population.[45]

BMI cut-offs for underweight, normal weight, overweight and obese status in children differ based on age and sex due to varying degrees of expected growth in height and weight over time. Many groups including the Centers for Disease Control and Prevention (CDC), American Academy of Pediatrics (AAP), Institute of Medicine (IOM), and International Obesity Task Force (IOTF), have agreed on similar recommendations regarding BMI terminology and cut-points. The definitions shown in **Table 1** are most often used to categorize weight status for children between 2 and 20 years of age:[46]

Table 1: Pediatric BMI Classification

Underweight	BMI <5th percentile for age and sex.
Normal Weight	BMI between the 5th and <85th percentile for age and sex.
Overweight	BMI between ≥85th and <95th percentile for age and sex.
Obese	BMI ≥95th percentile for age and sex.
Severe Obesity	Severe (class II) obesity: BMI ≥120% of the 95th percentile or a BMI ≥35 (whichever is lower).[47,48] Severe (class III) obesity: BMI ≥140% of the 95th percentile or a BMI ≥40 (whichever is lower).[48,49]

Prevalence and Trends of Overweight and Obesity in U.S. Children and Adolescents

According to data from NHANES, the prevalence of overweight and obesity among children and adolescents increased over the years 1999-2012, with rates becoming more stable in recent years.[48] Among U.S. adolescents aged 12 to 19 years in 2016, approximately 40 percent are classified as overweight or obese, meaning they have BMI greater than or equal to the 85th percentile for age and sex.[7,50] Skinner and colleagues analyzed more recent NHANES data between 1999 and 2016 and included all children aged 2-19 years to provide updated prevalence data on obesity trends among US children and adolescents. In their analysis, approximately one-third of children and adolescents in that age range were overweight or obese based on BMI, with an increasing proportion of subjects in higher weight categories with increasing age as shown in more detail below in **Table 2.**[49]

Table 2: Prevalence of Overweight and Obesity in US Children ages 2-19 years, 2016

	Preschool-aged children (2 – 5 years)	**School-aged children (6 – 11 years)**	**Adolescents (12 – 19 years)**
Overweight: BMI between ≥85th and 95th percentile for age and sex.	12.3 percent	15.4 percent	19.4 percent
Obese (Class I): BMI ≥95th percentile and <120 of the 95th percentile for age and sex, and <35 mg/kg^2.	11.7 percent	12.3 percent	8.9 percent
Severe Obesity **Class II:** Severe (class II) obesity: BMI ≥120% of the 95th percentile or a BMI ≥35. **Class III:** BMI ≥140% of the 95th percentile or a BMI ≥40	2 percent	6.4 percent	23.3 percent (10.1 percent of females) 13.2 percent of males)

In the United States, rates of overweight and obesity among children and adolescents differ by several demographic characteristics. The prevalence of obesity in youth aged 2- 19 years who identify as Hispanic is 25.8 percent based on NHANES data from 2015-2016. This compares to prevalence rates of 22.0 percent among non-Hispanic black youth, 14.1 percent among non-Hispanic whites, and 11.0 percent among non-Hispanic Asian youth.[51] A higher prevalence of childhood obesity is also observed in Native American populations when compared to non-Hispanic white children, namely, 30 percent of American Indian and Alaska Native youth are obese based on a non-nationally representative sample from 2015.[52]

Similarly, the prevalence of childhood obesity differs by household income, head of household education level, and urbanization. When analyzing income differences, NHANES data between 2011 and 2014 demonstrated that the prevalence of obesity among 2 to 19-year-olds was 18.9 percent among those in the lowest income group, 19.9 percent among those in the middle-income group, and 10.9 percent among those in the highest income group.[53] The same data showed that the prevalence of youth obesity decreased with increasing level of education of the head of household, specifically 21.6 percent among those with a high school education or less, 18.3 percent in those with some college, and 9.6 percent among those with a college degree. A similar pattern was seen overall and in females and males.[53] The prevalence of overweight or obesity among US youth aged 10 to 17 years living in areas identified as rural was 38 percent compared with 30 percent of youth in urban areas. This data was based on self-reported height and weight.[52]

Trends in obesity prevalence among children and adolescents have increased over time, with differences in time trends between age groups. Among children aged 2 to 5 years, obesity prevalence increased from 7.2 percent in the years 1988 through 1994 to 13.9 percent in 2003 - 2004, however, then decreased to 9.4 percent in 2013-2014. Obesity prevalence for children between the ages of 6 to 11 increased from 11.3 percent in 1988 - 1994, to 19.6 percent in 2007-2008, a prevalence rate which was stable in this age group in 2013-2014. Among adolescents aged 12 to 19 years, the prevalence of obesity increased from 10.5 percent in 1988-1994 to 20.6 percent in 2013-2014.[54]

Childhood Obesity and Risk for CVD

When compared with children of normal weight, children who are overweight or obese are more likely to have risk factors for CVD, including hypertension, insulin resistance, type 2 diabetes, and dyslipidemia, and are more likely to develop CVD in adulthood.[50] Analysis of data from the Harvard Growth Study demonstrated that risk of morbidity from CHD was increased among men and women who had been overweight in adolescence, independent of adult weight status.[55] Obesity status during adolescence is predictive of obesity status in adulthood. According to a study of middle and high school students in Minneapolis/St. Paul, two-thirds of obese adolescents remained obese as adults.[56] In addition, the severity of obesity during adolescent years can be considered an important predictor of the persistence of obesity into adulthood.[57,58] According to data from the Framingham offspring study, obesity in adulthood significantly and independently predicted the occurrence of CHD.[59]

The association between BMI in childhood and CHD in adults 25 years of age or older was investigated in a large prospective study of nearly 280,000 Danish children born between 1930 and 1976. Childhood BMI measurements were ascertained from data available due to mandatory school examinations on the children and the presence of adulthood ischemic coronary events were identified through a national registry. The researchers observed a positive linear association for risk of any CHD event in adulthood with BMI at age 7-13 for males and age 10-13 for females, with the risk increasing as the age of the child increased. This study demonstrated that even small increases in weight in childhood were associated with increased CHD risk in adulthood.[60]

Evidence of subclinical atherosclerosis, such as arterial stiffness, demonstrated on imaging studies is associated with CVD in adulthood. Multiple studies have shown that among adolescents and young adults a higher prevalence of CVD risk factors, including obesity, is associated with worsening arterial stiffness after adjusting for age and sex.[61] Urbina and colleagues studied differences in arterial stiffness in youth with obesity or type 2 diabetes mellitus (T2DM) compared with lean controls. They studied 670 youth who ranged in age from 10-24 years and were stratified based on BMI into groups "lean," "overweight," "obese," and "overweight/obese with T2DM." They reported a progressive increase in arterial stiffness from the lean group to the obese and obese/type 2 DM groups. Central obesity remained an independent predictor for increased arterial stiffness after adjusting for other CVD risk factors.[61]

Hypertension

Definition of hypertension

The AAP published revised guidelines for defining, screening, and managing elevated BP for children and adolescents in 2017. The updated definitions for pediatric blood pressure categories are provided in **Table 3**.[62] Elevated BP was previously referred to as prehypertension and is considered a category, whereas hypertension is considered a health condition. Elevated BP is predictive of hypertension in youth, so for consistency given updated guidelines, the term "elevated BP" will be used to describe children with either elevated BP or hypertension according to the 2017 guidelines, unless hypertension was the specific outcome measured.[62]

Table 3: Updated Pediatric Blood Pressure Cut-offs[62]

Children between 1 and 13 years of age:
• **Normal BP** – Both systolic BP (SBP) and diastolic BP (DBP) <90th percentile.
• **Elevated BP** (previously referred to as prehypertension) – SBP and/or DBP ≥90th percentile but <95th percentile, or 120/80 mmHg to <95th percentile (whichever is lower). Elevated BP is predictive of hypertension.
• **Stage 1 hypertension** – SBP and/or DBP ≥95th percentile to <95th percentile + 12 mmHg, or 130/80 to 139/89 mmHg (whichever is lower).
• **Stage 2 hypertension** – SBP and/or DBP ≥95th percentile + 12 mmHg, or ≥140/90 mmHg (whichever is lower).
For children ≥13 years of age:
• **Normal BP –** BP <120/80 mmHg.
• **Elevated BP (previously referred to as prehypertension) –** SBP between 120 and 129 with a DBP <80 mmHg.
• **Stage 1 hypertension –** BP between 130/80 to 139/89 mmHg.
• **Stage 2 hypertension –** BP ≥140/90 mmHg.

Epidemiology of hypertension

The presence of elevated BP during the child and adolescent years increases the risk for hypertension in adulthood and may contribute to premature atherosclerosis and subsequent early development of CVD.[63] Early identification of elevated BP in childhood along with early intervention in treating hypertension may positively impact long-term outcomes of CVD.[7,63]

The prevalence of hypertension, using the 2017 AAP definition, in U.S. adolescents aged 12-19 decreased from 7.7 percent to 4.2 percent from 2001 to 2016 based on data from NHANES. When using the older definition from The Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents, with the same data, there was a decrease over time from 3.2 percent to 1.5 percent, as the use of the 2017 AAP guidelines identifies more children with elevated BP and hypertension. Between 2001 and 2016, it was estimated that approximately 1 in 25 youth had elevated BP or hypertension based on the new cut points outlined in the 2017 guideline. Hypertension risk increased with increasing weight, with an approximate 14 percent prevalence among those who were classified as severely obese.[64]

Elevated BP among children and adolescents varies by modifiable risk factors such as BMI, diet, level of PA and non-modifiable risk factors such as sex, ethnicity, and family history, in addition to other factors as described in the sections below.

Selected risk factors for hypertension

Body Mass Index

BMI is the strongest risk factor for elevated BP in children and adolescents, with those classified as overweight or obese at the greatest risk. A 2007 report from National

Health Examination survey data showed that the risk of elevated BP doubled for every 1 unit of increase in the BMI Z-score unit among U.S. children aged 8-17 years.[65] When comparing outcomes between 2 NHANES survey populations of children aged 8-17 years, specifically NHANES III (1988-1994) and NHANES (1999–2008), increasing BMI and waist circumference were associated with an increased prevalence of elevated BP between the two-time points.[26] A retrospective cohort study involving over 100,000 youth whose data was ascertained from electronic medical records from Kaiser Permanente health systems showed that children and adolescents who were obese or became obese had the largest increases in BP percentile over approximately 3 years of follow-up.[66]

There is evidence to support that the association between elevated BP and BMI begins at a very young age. A retrospective study of over 18,000 children aged 2-19 years in the primary care setting demonstrated positive associations between BMI and both systolic blood pressure (SBP) and diastolic blood pressure (DBP) in all age groups, including children between two and five years of age.[67] In contrast, data from the Bogalusa Heart study did not show increases in mean SBP and DBP levels despite a rise in the prevalence of obesity over the study period.[68]

Sex, race/ethnicity, and family history

In the U.S, elevated BP in childhood is more common in males than females. NHANES data from 2013-2016 found that among children aged 12-19 years, 5.8 percent of males and 2.4 percent of females met criteria for hypertension.[64] Trends from earlier data using the old BP guidelines found similar results, with male children and adolescents having higher rates of elevated BP than females.[65] A Canadian cohort study including subjects aged 12-17 years, observed that males were more likely to have elevated SBP

(>90th percentile) compared with females. This difference was evident among 7th (OR=1.29, 95% CI=0.77-2.16), 9th (RD=1.98, 95% CI=1.35-2.93) and 11th (OR=2.74, 95% CI=1.52-4.94) graders.[69]

Risk of elevated BP varies by race/ethnicity, with greater risk seen in black and Hispanic children when compared to white and Asian children. Between 1999 and 2002, NHANES data demonstrated the prevalence of hypertension was 4.6 percent, 4.2 percent and 3.3 percent in Hispanic, Black and White children aged 8-17 years old, respectively.[65] Data from over 20,000 adolescents whose BP was screened in a school-based program between 2000 and 2015 in Texas also observed significant differences between rates of hypertension by race. Similarly, hypertension rates were highest among Hispanic children at 3.1 percent, followed by black children at 2.7 percent, white children at 2.6 percent and Asian children at 1.7 percent.[70] Additionally, NHANES data was used to examine trends in SBP and DBP among children and adolescents between 1988 and 2000. Mean SBP was significantly higher among non-Hispanic black males when compared with non-Hispanic white males, however these differences were attenuated with adjustment for BMI, which points to the role of obesity in hypertension risk.[71]

Offspring of parents who have hypertension are at greater risk for elevated BP during childhood. In a cross-sectional data sample of 70 children referred to a pediatric hypertension clinic, a family history of hypertension in a parent or grandparent was present in 86.5 percent of those for whom family history information was available.[72] Data from the Framingham Heart Study demonstrate that in second generation participants, early onset hypertension in parents (diagnosis under age 45), not late onset hypertension in parents (diagnosis at 65 years or older) was strongly associated with hypertension in offspring.[73]

Dyslipidemia

Definition of Dyslipidemia

Alterations in lipid metabolism can result in dyslipidemia, which can include the presence of one or more adverse lipid levels of TC, LDL-C, TG levels, and/or HDL-C based on normative values presented in **Table 4**. TC and HDL–C can be measured accurately in plasma from non-fasting patients.[7] Dyslipidemia is an established risk factor for CVD in adults. Correction of dyslipidemia through lifestyle and/or medication intervention is associated with a reduction in CVD risk. Long-term studies connecting dyslipidemia in childhood and CVD events in adulthood are lacking. There is a strong theoretical basis for addressing and reducing dyslipidemia in children to reduce CVD risk in adulthood, as this alteration in lipid metabolism often begins during childhood and adolescence.[23] According to the Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents, a clear correlation exists between lipoprotein disorders and onset and severity of atherosclerosis in children, adolescents, and young adults.[7]

Dyslipidemia in the pediatric population is based off normative values derived from population-based samples from the Lipid Research Clinical Prevalence Study and from NHANES data. From these normative values, cutoff points are used to define lipid values as "acceptable," "borderline," and "abnormal." These definitions are consistent with guidelines from the NHLBI, the AAP, and the AHA/ACC and are provided in **Table 4**.[7,74,75]

Table 4: Pediatric Normative and Abnormal Values of Blood Lipid Concentrations

Lipid Profile[1]	**Acceptable mg/dL**	**Borderline mg/dL**	**High mg/dL**
TC	<170	170-199	>/= 200
LDL-C	<110	110-129	>/= 130
TG			
0-9 years of age	<75	75-99	>/= 100
10-19 years of age	<90	90-129	>/= 130
Lipid Profile	**Acceptable mg/dL**	**Borderline mg/dL**	**Low mg/dL**
HDL-C	>45	40-45	<40

[1]TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol.

Epidemiology of dyslipidemia

Based on data through 2012, approximately 20 percent of U.S. children and adolescents aged 6 to 19 years have at least one abnormal blood lipid level. Specifically, 8 percent have elevated TC, 7 percent have elevated LDL-C, 12 percent have elevated TG levels, and 13 to 15 percent have low HDL-C.[5] Prevalence of dyslipidemia during adolescence increases with increasing weight. NHANES data from 1996-2006 observed the prevalence of dyslipidemia among normal weight subjects to be 14 percent, which increased to 22 and 43 percent among overweight and obese subjects, respectively.[76] Lipid profiles among children and adolescents appear to be improving over time according to NHANES data from 1999-2016 with decreases in TC and increases in HDL-C. These trends were maintained in various racial and ethnic groups and by BMI categories.[77]

Blood lipid levels during adolescence are modified by lifestyle behaviors including diet and exercise. Identification of children with dyslipidemia through screening, and implementation of lifestyle changes to improve lipid profiles, may reduce the risk of accelerated atherosclerosis and premature CVD.[7]

Altered Blood Glucose

Impaired glucose metabolism can lead to insulin resistance, hyperinsulinemia, and/or elevated blood glucose levels, which are associated with atherosclerotic CVD. Children with type 1 diabetes Mellitus (T1DM) or type 2 diabetes mellitus (T2DM) are at an increased risk for other CVD risk factors compared to children without DM.[78]

Type 1 Diabetes Mellitus

Studies have shown that adults with T1DM are at a significant increased risk of mortality from CVD compared to adults without T1DM.[79] A limited number of studies have demonstrated an increased prevalence CVD risk factors in children and adolescents with T1DM. Autopsy data has demonstrated that children with T1DM had increased aortic intima-medial thickness, which some consider a pre-clinical marker of atherosclerosis, compared to children without T1DM.[80] Another study has shown that children with T1DM had higher rates of endothelial dysfunction, a marker of early atherosclerotic disease process, when compared with children without diabetes.[81]

Type 2 Diabetes Mellitus

Prediabetes is a risk factor for the development of T2DM. Prediabetes is defined by presence of at least one of the following: hemoglobin A1c (A1C) 5.7 to 6.4 percent, fasting plasma glucose (FPG) 100 to 125 mg/dL or oral glucose tolerance test (OGTT) with plasma glucose 140 to 199 mg/dL two hours after a 75 gram glucose load.[82] According to the American Diabetes Association (ADA), diabetes mellitus (DM) is diagnosed based upon presence of any one of the following: A1C ≥6.5 percent, FPG ≥126 mg/dL, random plasma glucose ≥200 mg/dL in a patient with classic symptoms of hyperglycemia and/or OGTT with plasma glucose ≥200 mg/dL two hours after a 75 gram glucose load.[82]

Epidemiology of Diabetes Mellitus

According to NHANES data from 1999-2008, the prevalence of prediabetes or T2DM among adolescents aged 12-19 years was 15 percent, which significantly increased over time between the 2 survey periods. Specifically, prevalence of prediabetes or T2DM increased from 9 percent in 1999-2000 to 23 percent in 2008-2009 survey years. There was a consistent dose-response increase in burden of prediabetes or T2DM among adolescents with increasing BMI.[83] More recent data from NHANES 2005-2016, demonstrates a prevalence of prediabetes to be 18 percent among youth aged 12-18, with significantly higher rates among males compared to females, and among those defined as overweight compared to normal weight.[84]

Data from SEARCH for Diabetes in Youth study noted a similar trend and reported a substantial rise in T2DM from 9.0 cases per 100,000 in 2002-2003 to 13.8 cases per 100,000 in 2014-2015, with an adjusted annual increase of T2DM of 4.8 percent.[85] There is significant variation in the disease burden of T2DM by race/ethnicity in adolescents. The incidence of T2DM in youth aged 10 to 19 years in 2014-2015 was highest among non-Hispanic blacks at 37.8 cases per 100,000, followed by Native Americans at 32.8 per 100,000, with 20.9 cases per 100,000 among Hispanics, 11.9 cases per 100,000 among Asian and Pacific Islanders and 4.5 cases per 100,000 among white youth.[85]

Lifestyle Risk Factors for Cardiovascular Disease

Dietary Considerations

Epidemiological research has contributed significantly to understanding the role of diet in the pathogenesis of disease. Diet is thought to play a substantial role in the risk of developing CVD and cancer, the two leading causes of death for American adults.[86] The 2015 Dietary Guidelines Advisory Report, the Nutrition Evidence Library (NEL) Dietary Patterns Systematic Review Project, and the AHA/ACC Guideline on Lifestyle Management to Reduce Cardiovascular Disease Risk, agree that there is strong and consistent evidence demonstrating that a dietary pattern characterized by high intake of fruits, vegetables, whole grains, low-fat dairy, and seafood and low intake of red and processed meats, refined grains, and sugar-sweetened foods and beverages is associated with a decreased CVD risk.[87] In the Nurses' Health Study (NHS) II, information on dietary intake during high school was collected on nearly 50,000 women. It was found that a high-quality diet in adolescence, was associated with lower risk of developing CVD risk factors in middle age.[88]

The relationship between these dietary components and adverse health effects in children and adolescents is less clear, and most research has focused on the role of childhood diet with the genesis of obesity.[89,90] The atherosclerotic vascular changes that occur during childhood and adolescence are minimal for most and can be curtailed or even prevented with adherence to a healthy lifestyle, which includes a healthy diet. In some children, however, the atherosclerotic process is enhanced due to the existence of identifiable risk factors, especially obesity and hypertension, as well as dyslipidemia and DM.[3,7,8,9] Aspects of the diet have been evaluated for CVD risk, mainly through their role in the promoting obesity, contributing to hypertension, influencing blood lipid levels, and increasing insulin resistance.[78]

Fruit and Vegetable Intake

There is considerable evidence that fruit and vegetable consumption is associated with reduced risk of CHD.[91-94] The Nurses' Health Study and The Health Professionals Follow-up Study, both prospective cohort studies, evaluated the association of fruit and vegetable consumption with risk for CHD in adults and found that persons in the highest quintile of fruit and vegetable intake had a relative risk for CHD of 0.80 (95% CI, 0.69 to 0.93) compared with those in the lowest quintile of intake. It was also observed that for every daily serving increase of fruit or vegetable intake, there was a 4 percent lower risk of heart disease (RR =0.96m 95% CI, 0.94 to 0.99).[91] Data from the INTERHEART study, a case-control study of acute myocardial infarction (MI) in 52 countries, revealed that daily consumption of fruits and vegetables was associated with a 30 percent reduction in odds of MI when compared to lack of daily consumption (OR =0.70, 99% CI=0.64 to 0.77).[94]

Fruit and vegetables are generally high in vitamins, minerals, fiber, and water content and low in total energy. The nutrient-rich, energy poor qualities of fruits and vegetables are postulated to play a role in weight management, promotion of healthy blood lipid levels, and BP regulation. In children and adolescents, fruit and vegetable intake alone does not directly correlate with obesity risk.[95] Higher consumption of energy dense foods is associated with increased risk of overweight/obesity in childhood. NHANES data from 2000-2004 demonstrated that obese children had higher dietary energy density (defined as kcal per gram of food) than lean children. Diets high in energy density were also found to be associated with greater intakes of energy from added sugars, more energy from fat; and significantly lower intake of fruits and vegetables.[96]

Intake of foods high in fiber, especially soluble fiber which can be found in a variety of fruits and vegetables, is associated with minor reduction in LDL-C concentrations.[97] A study examining the associations between diet and CVD risk factors among teens aged 16–17 years found that consumption of green vegetables and legumes was associated with a significant reduction in total and LDL cholesterol levels.[98]

A dietary pattern that emphasizes high intake of fruits, vegetables, low fat dairy, whole grains and low intake of red and processed meat, saturated fat, and concentrated sweets, known as the DASH diet, has been shown to be highly effective in lowering BP in adults.[99] Additionally, pediatric data from observational studies and clinical trials in adolescents have shown that the DASH dietary pattern is associated with reduced BP among youth with elevated BP.[25] Results from the Framingham Children's Study revealed that children with higher intakes of fruits and vegetables had lower mean SBP compared with children with lower intakes of fruits and vegetables.[100]

Red and Processed Meat Intake

There is evidence to support an association between red and processed meat intake and various adverse health outcomes, including CVD mortality. A study looking at data from both the Health Professionals Follow-up Study and the Nurses' Health Study, found that higher intakes of red meat, both processed and unprocessed, was associated with increased risk of total mortality, as well as cause-specific mortality from CVD and cancer.[101] Similar conclusions were reached from the National Institutes of Health-American Association of Retired Persons (NIH-AARP) cohort.[102]

Results from meta-analyses have been less clear when considering processed and unprocessed red meat and risk of CVD. A systematic review and meta-analysis of 20 studies examining the relationship of unprocessed and processed red meat, as well as total meat consumption, with incident CHD, stroke and DM found no association between incident CHD with unprocessed red meat intake, whereas processed meat was associated with 42 percent higher risk of CHD.[103] Conversely, results from a meta-analysis of cohort studies examining risk estimates for the highest vs. the lowest consumption of total, red, white and processed meat and all cause and CVD mortality found that those who consumed the highest amount of processed meat had an 18 percent higher risk of CVD mortality compared to those with the lowest intake. Unprocessed red meat consumption was found to be associated with a 16 percent higher risk of CVD mortality.[104] A cohort study of nearly 30,000 US adults pooled from 6 prospective cohort studies found that intake of processed meat, unprocessed red meat, or poultry was significantly associated with incident CVD. In addition, intake of processed meat or unprocessed red meat was significantly associated with all-cause mortality.[105]

In the adolescent diet, red meat can provide a rich source of protein and minerals including iron but may also provide significant amounts of saturated fat and sodium. Data exploring the health effects of red and processed meat consumption in younger individuals are limited. Results from two German birth cohorts showed that children with the highest meat intake (from any animal source) were significantly more likely to be overweight at age 10 years compared with children consuming less total meat.[106]

Trans-fat and Saturated Fat

Industrial trans-fatty acids (TFA) result from the partial hydrogenation of unsaturated fatty acids in the commercial food supply.[107] Data from the Nurses' Health Study and the Health Professional Follow-up Study have demonstrated that TFA from industrial partial hydrogenation have been proven to cause harmful cardiovascular effects in adult populations.[108] Trans-fats are particularly harmful because they can increase LDL-C levels and lower HDL-C levels.[109,110] As of 2018, industrial trans-fats were effectively banned from the U.S. food supply. Prior to the ban, the main food sources of TFA in the diet of U.S. adults and children were fast foods, margarines, and commercial baked goods.[18] Although saturated fatty acids (SFA) are shown to increase LDL-C levels, they may also raise HDL-C levels, often equating to no net change in the total cholesterol to HDL ratio, making SFA less harmful compared to TFA. Guidance on saturated fat intake has started to shift based on conflicting findings in the literature with regard to cardiovascular risk.[18,111]

In U.S. adults, full fat dairy and red meat, are the leading contributors of saturated fat in the diet.[112] SFA can increase TC levels, including LDL-C, and may increase risk for CHD.[18,111] Still, an independent association between saturated fat intake and CVD risk in adults has not been consistently shown in prospective population-based studies, however some have provided evidence of an increased risk in young persons.[112] In children with dyslipidemia, dietary intervention which targets reduction of saturated fat intake as well as increasing consumption of fruits, vegetables and whole grains can promote modest improvement in blood lipid levels.[23] The Special Turku Coronary Risk Factor Intervention Project for Children (STRIP) trial found that adolescents aged 14 and young adults aged 19 whom received repeated dietary counseling for a low saturated fat diet had significantly lower saturated fat intake as well as lower serum LDL-C levels compared with controls. This study is the basis for the recommendation that saturated fat be limited to 7-10 percent of total calories for children, adolescents, and adults.[113,114] While research has supported the link between saturated fat intake and CVD risk in children and adults, more recent research on the role of specific SFA has clouded this picture and may be changing national guidance on saturated fat intake recommendations. When SFA are not grouped together, but studied individually, evidence has suggested that different SFA from different food sources, have different effects on cardiovascular health.[115] Some SFA will raise LDL-C as well as HDL-C levels, whereas others appear to have a smaller or neutral effect on LDL-C and HDL-C levels.[110] However, studies continue to demonstrate that higher intake of unsaturated fatty acids such as polyunsaturated fatty acids (PUFAs), improves blood cholesterol and lipoprotein ratios such as total to HDL cholesterol.[110] Studies have also demonstrated that lowering saturated fat intake by replacing with PUFAs decreases the risk of CHD events. This association is not consistent when saturated fat is replaced with refined carbohydrate, in fact, replacing saturated fat with refined carbohydrates may increase CHD risk.[116-118] Even though saturated fat is not considered to be as harmful for heart health as in the past, the 2020 Dietary Guidelines Advisory Committee (DGAC) concluded that there is strong evidence replacing that saturated fats with polyunsaturated fats reduces the risk of CHD events and CVD mortality. There is limited evidence available on whether replacing saturated fats with monounsaturated fats improves CVD endpoints, and there is strong evidence that replacing saturated fat with carbohydrate does not reduce risk.[119]

For adults, children and adolescents, dietary modification remains the cornerstone for initial management of dyslipidemias. Specifically, individuals with elevated LDL-C concentrations are advised to reduce saturated fat intake and in addition to total caloric intake.[111] For children with dyslipidemias, which may include elevated LDL-C, a diet high in fiber from fruits and vegetables, whole grains and legumes, high in poly- and monounsaturated fat, low in saturated fat and devoid of trans-fat is recommended. Those with persistent elevated cholesterol levels may require more rigid restrictions on saturated fat intake.[7,74,75]

Sodium Intake

In adults, a high sodium diet is considered one of several risk factors for hypertension. In U.S. adults, more than 40 percent of sodium in the diet comes from ten foods, which includes but is not limited to, processed meats and meat-based dishes.[120] In children and adolescents, data from observational studies have demonstrated that higher dietary sodium consumption is associated with higher BP levels. Rosner et al, evaluated a sample of children aged 8-17 years old from NHANES data in survey years 1988-1994, and 1999-2008. After controlling for central obesity and overall obesity, they found that children with high sodium intake, defined as consuming greater than 1.5 times the recommended daily intake, had 36 percent higher odds of having elevated SBP compared with children with lower sodium intakes, defined as consuming less than 1.5 times the recommended daily intake (OR=1.36, 95% CI= 1.04-1.77).[121] A meta-analysis from 2019 examined the association between sodium intake and BP in children with clinical conditions. They reported that among children with elevated BP, for every additional gram of sodium intake, SBP increased by 6.3 mmHg and DBP increased by 3.5 mmHg.[122]

Sugar Sweetened Beverage Intake

Data in U.S. adults have demonstrated an association between SSB intake and risk for CHD. In adult women participating in the Nurses' Health Study, daily consumption of SSBs was significantly associated with increased incidence of CHD.[123] Similarly, among adult men studied as part of the Health Professional's Follow-up Study, participants in the top quartile of SSB intake had a 20 percent higher relative risk of CHD than those in the bottom quartile (RR=1.20; 95% CI = 1.09–1.33).[124]

Studies have also linked SSB consumption with increased prevalence of known CVD risk factors including hypertension, dyslipidemia and most notably T2DM.[125] A meta-analysis of eight prospective cohort studies evaluating SSB intake and the risk of T2DM found that individuals in the highest category of SSB intake had a 26 percent (RR 1.26, 95% CI 1.12–1.41) greater risk of developing T2DM compared to those in the lowest category.[126] Clinical trial data has suggested that reduction in consumption of SSBs can significantly reduce BP, independent of weight change.[127] Data from the Framingham Heart Study has shown that individuals consuming 1 or more servings of regular soda per day had increased odds of impaired fasting glucose (OR, 1.25; 95% CI, 1.05 to 1.48), higher BP (OR, 1.18; 95% CI, 0.96 to 1.44), hypertriglyceridemia (OR, 1.25; 95% CI, 1.04 to 1.51), and low HDL-C (OR, 1.32; 95% CI 1.06 to 1.64) than those consuming < 1 drink per day.[128]

There is considerable evidence to indicate that consumption of SSBs increases risk of weight gain in both children and adults and plays a significant role in becoming overweight or obese.[129] Through the promotion of obesity, it is postulated that reducing consumption of such beverages would reduce the prevalence of obesity and obesity-related diseases, including CVD.[125] Data from 2 NHANES surveys, 1988-1994 and 1999-2004, demonstrated that 10 to 15 percent of daily caloric intake in children and adolescents was provided by SSBs.[130] A Systematic Review from 2013 to 2015 of 26 prospective cohort studies found a positive association between consumption of SSBs and weight/BMI in adults and children.[131]

A study published in the Lancet in 2001, showed that the risk of becoming obese among middle-school students over 2 school-year periods increased by 60 percent for every additional serving of SSB per day.[132] More recent studies have supported these findings and have demonstrated that greater SSB consumption in childhood or adolescence predicted weight gain into adulthood.[133] Overall, the literature suggests that SSB consumption increases obesity risk in children and adolescents. Obese children are more likely to become obese adults, which is associated with increased risk for CVD and its risk factors, including T2DM. Although direct evidence on the role of SSB consumption and adolescent onset of T2DM or risk of other cardiometabolic factors is needed, population-based approaches to reduce intake of SSBs within this population could prove beneficial.

Physical Activity Considerations

According to the US Department of Health and Human Services (USDHHS) 2008 physical activity guidelines for Americans, physical activity is defined as bodily movement produced by the contraction of skeletal muscle that increases energy expenditure above the basal level. Types of PA may include occupational, household, leisure time, and transportation and typically categorized by level of intensity. The terms physical activity and exercise are not interchangeable. Exercise refers to a form of PA that is planned, structured, repetitive, and purposeful with a main objective of improvement or maintenance of one or more components of physical fitness.[134] Regular PA and reduced sedentary behavior improve CVD risk factors and decreases the risk atherosclerosis and occurrence of CVD events. PA has beneficial effects on risk factors for CVD, including weight status, T2DM, BP, and blood lipid profiles.

Physical Activity and Atherosclerosis

In adults, there is substantial evidence, primarily from observational studies, that demonstrates a strong inverse relationship between PA and risk of CHD and mortality. This association is observed in both sexes, across different racial and age groups, and in different countries.[94,135-139] Studies have also investigated the effect of PA on atherosclerotic risk in children and adolescents. The Special Turku Coronary Risk Factor Intervention Project for Children (STRIP), a prospective longitudinal atherosclerosis prevention study, investigated the association of leisure-time PA with endothelial function (brachial artery flow-mediated dilatation; FMD) and aortic intima-media thickness (IMT) in adolescents. They found that PA was positively associated with preserved endothelial function and inversely associated with IMT in adolescents. In this group, even a moderate increase in PA was related to decreased progression of IMT. The study investigators concluded PA can act as a means to prevent development of subclinical atherosclerotic vascular changes in healthy adolescents.[140] The association of fitness with aortic and carotid artery IMT and elasticity in adolescents was examined in this same cohort and found that cardiorespiratory fitness was favorably associated with aortic IMT and elasticity in adolescents, with no associated found in terms of carotid IMT or elasticity.[141] The Cardiovascular Risk in Young Finns cohort involved 1809 subjects aged 3-18 who were followed for 27 years. In this group, low PA was associated with accelerated IMT progression over the 27 years of follow-up.[142]

Weight

Studies examining the role of PA in the prevention or treatment on obesity in adults have yielded mixed results. The randomized clinical trial, Studies of Targeted Risk Reduction Interventions through Define Exercise (STRRIDE), investigated the effects of exercise and exercise intensity on cardiovascular risk factors, including weight status among overweight and obese men and women with dyslipidemia. They found that without any significant changes in dietary intake, aerobic exercise and resistance training resulted in weight loss and a reduction in body fat in a dose-response manner.[143] Similarly, the Coronary Artery Risk Development in Young Adults (CARDIA) study, a prospective longitudinal study with 20 years of follow-up, found that maintenance of high levels of activity was associated with smaller gains in BMI and waist circumference compared with low activity levels after adjustment for race, baseline BMI, age, education, cigarette smoking status, alcohol use, and energy intake in both men and women, with differences in weight gain especially robust among women.[144] In contrast, a 15-year longitudinal cohort study aimed to examine the association of different amounts of PA with long-term weight changes among women consuming a usual diet. They found that found that PA was associated with less weight gain only among normal or underweight women, and that a minimum of 60 minutes a day of moderate intensity activity, sustained over the study period, was necessary to prevent weight gain.[145]

In children and adolescents, data from the 1999 Youth Risk Behavior Survey (YRBS) demonstrated that increased levels of PA are associated with a lower BMI among youth aged 14-18 years. However, within this group, sedentary behavior, measured by hours of tv watched per day, was a more important predictor of weight status, with those students watching more TV being significantly more likely to be classified as overweight or obese.[146] A randomized clinical trial in New Zealand examined the impact of high-intensity progressive resistance training (PRT) on measures of central adiposity in youth. They found significant, favorable changes in measures of waist circumference and BMI when comparing the intervention group compared with the control group.[147] A crossover study involving obese adolescents with lean controls randomized to eight weeks of exercise training or a non-training period found that the circuit training intervention decreased abdominal and trunk fat and significantly improved fitness and muscular strength.[148]

Type 2 Diabetes

PA may improve glycemic control and insulin sensitivity and even prevent the development of T2DM in high-risk adults. Among patients with diagnosed T2DM, PA has also been shown to improve other cardiovascular risk factors including BMI, BP and blood lipid levels.[149] Women with T2DM participating in the Nurses' Health Study who reported at least four hours of week of moderate or vigorous PA had a 40 percent lower risk of developing CVD than those who did not achieve that level of activity.[150] A systematic review investigating the effect of resistance training (RT) on glycemic control and insulin sensitivity in adults with T2DM concluded that supervised RT improved glycemic control and insulin sensitivity. Their investigation also noted that when supervision was removed, compliance with RT decreased glycemic control worsened.[151] A meta-analysis of clinical trials studied effect of exercise on HgbA1c and body mass in patients with T2DM found that when comparing exercise groups with control groups, exercise reduced HbgA1C but not body mass.[152]

Regarding the prevention of T2DM via PA, a systematic review and meta-analysis of prospective cohort studies found that compared with sedentary behavior, 150 min/week of moderate PA was associated with a 26 percent reduced risk of developing T2DM (RR 0.74, 95% CI 0.69-0.80).[153] Men participating in the Health Professionals Follow-up Study who were physically active through either weight training or aerobic exercise for at least 150 minutes per week compared with no PA had a 34 percent and 52 percent reduced risk for developing T2DM respectively.[154]

In children and adolescents, studies examining the effect of PA on fasting blood glucose levels and/or insulin resistance are limited. Pooled data from the International Children's Accelerometry Database of 14 studies collected between 1998 and 2009 comprising 20,871 children ages 4-18 years found that PA was inversely associated with fasting insulin, and sedentary behavior was significantly and positively associated with fasting insulin (a potential marker of insulin resistance).[155] There remains strong physiological evidence that being physically active can reduce the risk for T2DM in children due to its ability to improve insulin sensitivity and decrease insulin resistance.[156]

Blood Pressure

PA has been found to be an effective way to lower BP in adults and is considered a cornerstone therapy for the prevention, treatment, and control of hypertension.[157] Data from large, prospective cohort studies have shown an inverse relationship between self-reported levels of PA and hypertension. Results from the Aerobics Center Longitudinal Study (ACLS) found that PA and cardiorespiratory fitness were associated with lower risk of hypertension among men in a dose-response manner.[158] Similar results were noted in women from the Nurses' Health Study with a significant decline in hypertension noted when PA was combined with other healthy lifestyle behaviors.[159] A meta-analysis of nine randomized controlled trials revealed a significant reduction in BP associated with exercise.[157] A systematic review of clinical trials examining the effect of walking on BP found walking can be an effective intervention for BP control, especially among studies with a large sample size.[160] Scientific organizations have determined that implementation a regular exercise routine can lower BP levels by as much as 5 to 15 mmHg in patients with primary hypertension in as little as 4 weeks' time.[161]

There is data to support a positive impact of PA in children and adolescence and on BP. In a report from the STRIP study, girls who participated in leisure-time physical activity (LTPA) experienced significantly lower SBP compared with those who were sedentary.[162] Similar findings were noted in the Dietary Intervention Study in Childhood (DISC), a randomized clinical trial that aimed to assess PA patterns in boys and girls longitudinally from late childhood through puberty and to determine the association of level of PA on SBP, LDL-C, and BMI. They found that higher self-reported levels of PA were associated with significantly lower levels SBP.[163] Results from the Avon Longitudinal Study of Parents and Children observed small inverse associations between PA and BP among children aged 11-12.[164] A 3-month randomized controlled trial with a modified crossover design aiming to determine the effects of PA on systemic BP and early markers of atherosclerosis in pre-pubertal obese children found SBP was significantly reduced following the PA intervention program compared to controls in the study.[165] Research has demonstrated that elevated BP during childhood and adolescence can follow into adulthood.[164] Therefore, PA interventions to address childhood hypertension may provide important benefits that can persist later in life.

Blood Lipid Profiles

PA has been shown to reduce serum TG and increase HDL-C levels.[7] Evidence supporting the role of PA in the reduction of other blood lipid levels such as LDL-C and TC has not been clearly established. A meta-analysis of randomized controlled trials examining the effect of exercise training on HDL-C level found that regular aerobic exercise modestly increased HDL-C levels, especially among individuals with high TC levels or low BMI.[166] The STRRIDE trial investigated the effects of exercise on serum lipoproteins in overweight and obese adults with mild to moderate dyslipidemia. Those randomized to the high frequency of high-intensity exercise group had significantly higher HDL-C levels after 8 months compared to controls and those randomized to other lower amount and lower intensity exercise groups ($p=0.017$). There were significant improvements in TG levels in the high-amount–high-intensity, low-amount–high-intensity, and low-amount–moderate-intensity groups ($p=0.006$, $p=0.07$, and $p<0.001$, respectively). No differences in LDL-C and TC levels were observed between the groups.[167] A small clinical trial among patients with established CHD was conducted to determine the effect of exercise intervention on serum lipid levels. Thirty-eight patients with CHD were randomly assigned to a non-exercise group or exercise group. After 8 weeks, concentrations of TG were significantly lowered ($p<0.01$) and HDL-C levels were significantly increased ($p<0.01$) in the exercise group compared to baseline levels. There was no change in LDL-C.[168]

In children and adolescents, PA is associated with improved blood lipid levels, which may be mediated by weight loss.[23] Similar to results in adult populations, PA in children and adolescents seems to favorably impact TG and HDL-C levels with limited evidence showing an impact on LDL-C and TC levels.[169] In the STRIP study, LTPA was associated with increased levels of HDL-C in girls and boys.[162] Pooled data from the International Children's Accelerometry Database demonstrated that higher mean time spent in moderate to vigorous activity correlated with reduction in TG levels and increases in HDL-C.[155]

Physical Activity Recommendations in Children

A panel convened by the CDC recommends that school-age children participate in at least 60 minutes per day of moderate to vigorous physical activity (MVPA) that is age and developmentally appropriate and enjoyable. Included in the 60 minutes per day goal is a recommendation to participate in activities that strengthen bones and activities that build muscles – each to be done 3 days per week. According to the CDC, activity may occur in a variety of contexts, including play, games, sports, work, transportation, recreation, physical education, and/or planned exercise.[170,171] According to the 2018 Physical Activity Guidelines for Americans, examples of moderate PA include hiking, skateboarding, and brisk walking; examples of vigorous PA include jumping rope, running, and sports such as soccer, basketball, and ice or field hockey. Activities that strengthen bones include jumping, running, and skipping, and activities that build muscles include climbing, push-ups, pull-ups, and weightlifting.[172]

Nutrition Recommendations for U.S. Children

The 2011 NHLBI expert panel endorsed the USDA/HHS dietary recommendations for children aged two years and older as means to promote good nutrition and promote health.[7] Data from NHANES demonstrate that children and adolescents in the U.S. have poor adherence to the recommendations set forth by the U.S. dietary guidelines, suggesting poor diet quality and therefore, increased risk for chronic disease in adulthood.[173] Various national health organizations have issued dietary guidelines for children and adolescents including the AAP, the AHA, the Academy for Nutrition and Dietetics (AND), and the USDA/HHS. The recommendations from these organizations are comparable and are summarized below.[174]

Nutrient Dense Foods

The USDA Dietary Guidelines for Americans defines nutrient-dense foods and beverages as those that provide vitamins, minerals, and other health promoting components and have little added sugars, saturated fat, and sodium. Foods considered to be nutrient dense include fruits, vegetables, whole grains, seafood, eggs, beans, pea, lentils, unsalted nuts and seeds, fat-free and low-fat dairy, and lean meats and poultry prepared with none or with very little added sugar, saturated fat, and sodium. The recommended daily servings of food groups for children and adolescents aged 9-18 are based on average daily energy requirements and are described below.

Fruits (includes any fruit or 100% fruit juice)

For children and adolescents ages 9-13, at least 1 ½ cups of fruit is recommended per day. For adolescents ages 14-18, at least 1 ½ to 2 cups of fruit is recommended per day. 1 cup of fruit is equivalent to ½ cup of dried fruit, 1-piece small whole fruit, or ½ large whole fruit. 100% fruit juice should be limited.

Vegetables (includes any vegetable or 100% vegetable juice)

For children and adolescents ages 9-13, at least 2 to 2 ½ cups of vegetables is recommended per day. For adolescents ages 14-18, at least 2 ½ to 3 cups of vegetables is recommended per day. 1 cup of vegetables is equivalent to 1 cup raw or cooked vegetables or vegetable juice, 2 cups raw leafy greens.

Grains (includes any food made from wheat, rice, oats, cornmeal, barley, or other cereal grain)

For children and adolescents ages 9-13, 5 to 6 ounces of grains is recommended per day with half coming from whole grains. For adolescents ages 14-18, 6 to 7 ounces of grains is recommended per day with half coming from whole grains. 1 ounce of grains is equivalent to 1 slice whole grain bread, 1 6-inch tortilla, ½ cup cooked cereal, rice, or pasta, 1 cup dry cereal.

Dairy (includes all fluid milk products such as milk, yogurt, cheese, and calcium-fortified soy beverage and yogurts)

For children and adolescents ages 9-13 and those ages 14-18, 3 to 4 cups of dairy is recommended per day. 1 cup of dairy is equivalent to 1 cup milk or yogurt, 1 ½ ounces natural cheese, 2 ounces processed cheese, 1/3 cup shredded cheese.

Protein foods (includes meat, poultry, seafood, beans, peas, eggs, nuts, seeds, processed soy)

For children and adolescents ages 9-13, 5 ounces of protein foods is recommended per day. For adolescents ages 14-18, 5-6 ounces of protein foods is recommended per day. 1 ounce is equivalent to 1 ounce meat, poultry, or fish, ¼ cup cooked beans, 1 egg, 1 tablespoon nut or seed butter, ½ ounce nuts or seeds.

Fats and oils (includes oils, avocado, olives, nuts, seeds, soft margarine, and dressings)

For children and adolescents ages 9-13 years, 5 teaspoons of fat is recommended per day. For adolescents ages 14-18 years, 5-6 teaspoons of fat is recommended per day. 1 teaspoon is equivalent to 1 teaspoon oil, margarine, mayonnaise, nut butter, or 1 tablespoon dressing.

Energy dense, Nutrient Poor Foods

Foods and beverages high in added sugars, saturated fat, and sodium are considered energy dense, and nutrient poor. These foods and beverages are often termed "obesogenic" because they contribute significant calories to the diet and can promote excessive weight gain. SSB, fried and salty snack foods such as chips, baked goods, and sweets high in saturated fat and sugar such as cookies, cakes and other grain-based desserts, and fast food are considered energy dense, nutrient poor foods.[174-176] While definitions vary, ultra-processed foods (UPF) are industrially produced foods and beverages that generally have high fat, sugar, and sodium content and low micronutrient content.[177] The scientific community recommends limiting consumption of these foods, obesogenic and/or UPF to as little as possible, or more specifically, to provide less than 15 percent of daily calories.[174,178] The 2020 Dietary Guidelines for Americans recommends that processed meats such as sausage, bacon, and hot dogs are high in protein but also often contain high amounts of cholesterol, saturated fat and sodium and should be limited to as little as possible in the diet.[174]

Current Dietary Intakes of U.S. Children and Adolescents (2020 guidelines)

The USDA utilizes the Healthy Eating Index score (HEI) which is an overall measure of diet quality and is used to assess how well intakes align with key recommendations of the Dietary Guidelines. A higher total HEI score indicates a diet that aligns better with dietary recommendations, with a score of 100 considered ideal.[174,179] According NHANES data from 2015-2016 children and adolescents ages 9-13 years and 14-18 years had HEI scores of 52 and 51, respectively. Fruit and vegetable consumption among 9-13- and 14–18-year-olds was about half of the recommended range of intake for males and females. Notably, vegetable consumption was largely in the form of starchy vegetables, which are often fried or prepared with added fat and salt, as opposed to from non-starchy red and orange; dark green vegetables; or from beans, peas, and lentils vegetable subgroups.[174]

Grain consumption was largely within the recommended range of intake for males and females, however less than half of starches consumed were from whole grain sources, the majority from refined grains. Children and adolescents aged 9-13 years consumed adequate amounts of protein foods, but very low amounts of seafood. Adolescent males ages 14-18 years consumed an excessive amount of protein foods, which is consistent with data from the previous NHANES cycles. Females in this age group consumed adequate amounts of protein.[174] The 2015 dietary guidelines recommend that teen boys and adult men need to reduce overall intake of protein foods including meat, poultry, and eggs and therefore increase amounts of vegetables and/or other under-consumed foods. This recommendation was based on findings from NHANES 2007-2010 data showing that average intakes of meats, poultry, and eggs were high for teen boys and adult men compared to recommended amounts.[86]

According to data from the 2015-2020 Dietary Guidelines for Americans, children and adolescents ages 9-18 years significantly exceeded recommended consumption of added sugars, saturated fat and sodium. The percent of males ages 9-13 years who exceeded the recommended limit on added sugars, saturated fat and sodium was 79, 88, and 97 respectively. The percent of males ages 14-18 years who exceeded the recommended limit on added sugars, saturated fat and sodium was 72, 85, and 97 respectively. The percent of females ages 9-13 who exceeded the recommended limit on added sugars, saturated fat and sodium was 78, 86, and 96 respectively. Finally, the percent of females ages 14-18 years who exceeded the recommended limit on added sugars, saturated fat and sodium was 72, 78, and 77 respectively.[86]

School-Based Nutrition and Physical Activity Programs

According to the CDC and the AHA, schools are an ideal setting to help children and adolescents develop healthy behaviors, including those related to nutrition and PA, which in turn can influence CVD risk factors including BMI, BP, and blood lipid levels.[180,181] Project Healthy Schools (PHS) is a middle-school based wellness program established in collaboration with the University of Michigan Health System to address adolescent obesity and reduce cardiovascular risk factors. The program has been implemented in over 100 schools since 2004, with more than 80,000 students participating in the curriculum. PHS utilizes school-based environmental changes and health education implemented in grade 6, emphasizing the following 5 goals: 1) eat more fruits and vegetables; 2) choose less sugary foods and beverages; 3) eat less fast and fatty foods; 4) be active every day; and 5) spend less time in front of a screen. Studies have demonstrated that participation in the PHS program has resulted in significant improvements in students' physiologic measures, including blood lipid levels and BP measures.[36] Among a sample of 287 students participating in PHS, there were significant decreases in LDL-C levels following intervention, however HDL-C levels also decreased significantly. Within this sample, there were no significant changes in BMI or BP from baseline to follow-up.[182] Among a larger sample of 2118 students who participated in PHS, significant improvements in TC, LDL-C, TG and SBP were noted post-intervention, and appeared to correlate with self-reported increases in vegetable and fruit consumption, increases in PA, and less screen time, although correlation with improvement in these behaviors was not specifically analyzed.[37]

Another study by Bernardo et al, reported that following PHS intervention, students with abnormal BP at baseline demonstrated a greater reduction in systolic and diastolic BP than students with normal BP at baseline.[183]

There are a variety of other school-based intervention programs which have differed in strategies utilized, age ranges targeted, program length, and program evaluation outcomes when compared with PHS. The Child and Adolescent Trial for Cardiovascular Health (CATCH) was a randomized controlled field trial involving students from ethnically diverse backgrounds in public schools located in California, Louisiana, Minnesota, and Texas and was a study that PHS was modeled after. The objective of the CATCH trial was to assess the outcomes of health behavior interventions, focusing on the elementary school environment, classroom curricula, and home programs, for the primary prevention of CVD. They assessed school-level and individual-level outcomes. At the school level, the intervention was able to significantly decrease the fat content of school lunches and significantly increase the intensity of PA in physical education classes when compared to the control schools. On the individual level, students in the intervention reported significantly reduced energy intake from fat and a significant increase in reported daily vigorous activity than control students. There were no significant differences in BP, body size, and cholesterol measures.[184] A follow-up study found that these effects were maintained for three years without further intervention, suggesting sustainability of the intervention.[185] The CATCH trial was conducted in a younger population than PHS, it was also longer in duration, and included a non-intervention control group. Both CATCH and PHS did examine biomarkers of blood lipids and BP as outcomes, however CATCH did not detect any improvement in these measures among students participating in the program compared to the students in the control schools.[184,185]

In collaboration with the CDC, the CATCH program has expanded and evolved and is now referred to as Coordinated Approach to Child Health. CATCH is a school health program that focuses on coordinating the efforts of teachers, school staff, and communities to promote PA and healthy food choices for children from preschool through eighth grade. The effectiveness of the program resulted in Texas signing legislation requiring implementation of coordinated school health programs in the state.[186] CATCH programs are used in approximately 15,000 schools throughout the U.S and has been adopted in other countries.[187]

There are many school-based intervention programs targeting obesity that have generated mixed results regarding effectiveness. Often, these intervention programs involve both nutrition and PA components. A school-based health behavior intervention program called Planet Health is a randomized controlled field trial conducted in 5 intervention and 5 control schools in Massachusetts. The goal was to evaluate the impact of the intervention on measured obesity among a diverse group of middle schoolers. The intervention extended over 2 years and involved incorporating Plant Health sessions within existing curricula in the classroom. Sessions targeted decreasing screen time, decreasing consumption of high-fat foods, increasing fruit and vegetable intake, and increasing moderate and vigorous PA. The prevalence of obesity was reduced among females in the intervention program compared with controls, with no differences noticed among males. There was a significant reduction in television hours per day in males and females in the intervention group compared with controls. Females in the intervention group demonstrated increased fruit and vegetable consumption and a smaller increase in estimated energy intake per day over 2 years compared with controls. There were no differences in these outcomes among males, suggesting different types of interventions may be more effective in males versus females. Planet Health and PHS targeted a similar age group of students, but Planet Health did not assess biomarkers of CVD risk such as blood lipid and/or BP in their study.[188] The Middle-School Physical Activity and Nutrition (M-SPAN) study was a randomized field trial, with the school as the unit of intervention and analysis designed to evaluate the effects of environmental, policy, and social marketing interventions on PA and fat intake of middle school students. The PA interventions were designed to increase activity in physical education classes and throughout the school day, whereas the nutrition interventions offered and marketed low-fat foods within the school. Contrary to the PHS program, no classroom health education was involved. Their results showed a significant intervention effect for PA for the total population, and sex-specific analyses showed increased PA among males, but not females. There were no changes in fat consumption following the intervention.[189]

A randomized controlled trial involving approximately 1100 adolescents ages 12-14 years in the Netherlands was conducted to assess the effectiveness of a multi-component health promotion intervention in changing body composition, and dietary and PA behaviors at 8-, 12- and 20-months post-intervention. The Dutch Obesity Intervention in Teenagers (DOiT) trial randomly assigned schools to the intervention or control group. The intervention targeted behaviors associated with consumption of SSBs and high-energy snacks, as well as PA and screen-time behaviors. Similar to PHS, the DoIT trial involved an educational component covering 11 lessons focused on biology and PA, and an environmental component which encouraged schools to offer additional physical education class and advice for schools on changes in and around school cafeterias. There were no significant changes in BMI between intervention and control groups, but other measurements of body composition including skinfold thickness, did show favorable change. Consumption of SSBs was significantly lower in the intervention group at both 8-month and 12-month follow-up compared to controls, but not at 20 months. No significant intervention effect on consumption of snacks or PA behaviors were found.[190] Similar findings were reported in a PA intervention program with no nutrition intervention in North Carolina participating in the Cardiovascular Health in Children and Youth Study (CHIC II). CHIC II was designed to test 8-week PA interventions in middle school youth to reduce CVD risk factors, namely BP, blood lipids, obesity, and increase PA levels. They did not see any significant changes in BMI between intervention and control groups, but the intervention group did experience smaller increases in skinfold measurements compared to control groups. The control groups experienced significantly higher increases in SBP and DBP measurements compared to the intervention groups.[191]

The school-based Healthier Options for Public Schoolchildren (HOPS) is a 2-year, quasi-experimental randomized school-based obesity prevention intervention that aimed to measure the effects of interventions composed of dietary, education, and PA components on BMI percentiles and academic performance, but not on other measures of CVD risk, among low-income elementary school children ages 6-13 years in Osceola, Florida. The HOPS study reported that significantly more children within the intervention schools stayed within normal BMI percentile ranges both years compared to control students.[192] The School Nutrition Policy Initiative (SNPI) was designed to examine the effectiveness of a multicomponent school-based intervention on the on the prevention of overweight and obesity among 1349 students in grades 4 through 6 from 10 schools in the Mid-Atlantic region in the U.S. over a 2-year period. The intervention involved school self-assessment, nutrition education, nutrition policy, social marketing, and parent outreach. The intervention resulted in a 50 percent reduction in the incidence of overweight with 7.5 percent of children in the intervention schools becoming overweight after 2 years compared with 14.9 percent in control schools. The prevalence of overweight was lower in the intervention schools, however there were no differences observed in the incidence or prevalence of obesity between the groups after 2 years. There were no differences in secondary outcomes, specifically, no differences in self-reported consumption of energy, fat, fruits and vegetables over 2 years, and no differences in self-reported amounts of PA.[193]

A longitudinal school-based controlled evaluation study in Chile examined the impact of a 6-month nutrition education and PA intervention on children in primary school, grades 1 through 8, through changes in adiposity and physical fitness. The nutrition interventions involved classroom nutrition education, meetings with vending machine companies supplying snacks and beverages to the schools to encourage healthier options, meetings with parents directed at healthy eating, obesity prevention and to reinforce national food-based dietary guidelines. The PA interventions involved use of a behavioral resource by the physical education teacher designed to instill a healthy and active lifestyle for children aged 6–18 years, provision of an extra 90 minutes per week of PA, and implementation of an active recess program. The intervention was determined effective in improving physical fitness parameters in males and females, and improvement in adiposity measures in males only.[194]

A systematic review by Kropski et al, examined the effectiveness of school-based programs for reducing childhood overweight or obesity and found only a limited number of studies of acceptable quality and concluded that the quantity and strength of evidence for these intervention programs were insufficient to draw specific conclusions as to their effectiveness on weight outcomes.[195] Similarly, Gonzalez-Suarez et al, conducted a meta-analysis in order to evaluate the effectiveness of school-based programs in the prevention and management of childhood obesity and concluded that there was strong evidence that school-based interventions are effective in reducing the prevalence of childhood obesity in the short-term and that longer-running programs were more effective than shorter programs. However, intervention programs were not effective in decreasing BMI compared with controls.[196]

The Shaping Healthy Choices Program (SHCP) is a clustered, randomized, controlled intervention lasting 1 school year among fourth-graders (aged 9–10 years) at 2 control schools and 2 intervention schools in northern and central California. The goal of the SHCP was to improve dietary behaviors and prevent childhood obesity with interventions designed to 1) increase nutrition knowledge and use of science processing skills; 2) promote availability, consumption, and enjoyment of fruits and vegetables; 3) improve dietary patterns and encourage PA; 4) foster positive changes in the school environment; and 5) facilitate the development of an infrastructure to sustain the program. They assessed changes in BMI, nutrition knowledge, science process skills, and vegetable identification and preferences, and reported fruit and vegetable intake. Researchers found that SHCP resulted in improvements in nutrition knowledge, vegetable identification, and a significant decrease in BMI percentiles among students in the intervention schools compared with controls.[197]

A program similar to PHS referred to as The Healthy, Energetic, Ready, Outstanding, Enthusiastic, Schools or HEROES initiative is a grant-funded multilevel and multiple-year obesity prevention intervention in three U.S. states with high rates of childhood obesity. The HEROES Initiative aims to facilitate change within individual schools by implementing a coordinated school health (CSH) approach recommended by the CDC to help schools decrease childhood obesity and increase healthy lifestyle habits among students, their families, and school staff. School-level interventions within the HEROES framework were designed with the intention to increase opportunities for PA and healthy eating among students and staff, to integrate health and wellness education into the overall academic curriculum, to engage parents and community-based organizations in enhancing the healthfulness of the school environment, and to empower the school to develop and implement policies that support healthy lifestyles for students, their families, and the school staff. Assessment of student-level outcomes based on physiological measurements and self-reported behavioral data showed that the percentage of students who were overweight after 18 months of the intervention was smaller than the percentage of overweight students at the beginning of the intervention ($p=0.006$). They also found that a greater percentage of students were considered a normal weight following the intervention compared to baseline ($p<0.001$). In addition, improvements in PA, fruit and vegetable consumption were noted following intervention, as well as decreases in consumption of soda, suggesting small, but significant changes in student's weight and behaviors could be achieved with this type of program.[198]

As described, a variety of nutrition and/or PA intervention programs have been implemented in the school setting often varying in length, target audience, outcome measurements and intervention strategies utilized when compared with PHS. PHS is unique in that it is considered a relatively short-term intervention program, which assesses outcomes associated with CVD risk other than just weight-based measures.

Summary

In summary, the adolescent years present an important time for the development of lifestyle behaviors that may positively or negatively impact cardiovascular health. Although CVD manifests in adulthood, the atherosclerotic process can get underway in childhood. Many risk factors for CVD are present during childhood and adolescence, and there are lifestyle factors, namely diet and PA, that can modify risk factors or even prevent disease. The CDC, AHA and AND agree that schools provide an ideal environment to implement multi-component programs for adolescents that promote PA and provide nutrition education to prevent obesity in addition to other cardiometabolic risk factors for CVD. Few studies, however, have consistently shown school-based nutrition and PA intervention programs to be effective in reducing overweight and obesity among adolescents. Even fewer studies have examined the effectiveness of school-based nutrition and PA interventions at reducing other cardiometabolic risk factors, such as blood lipid level and BP. In addition, few studies have examined associations between these factors between positive changes in dietary intake and PA levels with changes in physiological measures associated with CVD risk during the adolescent years.

CHAPTER 3: ASSESSMENT OF CHANGE IN DIETARY INTAKE FOLLOWING PARTICIPATION IN A SCHOOL-BASED HEALTH INTERVENTION PROGRAM (MANUSCRIPT 1)

ABSTRACT

IMPORTANCE: Overweight and obesity are increasingly common among U.S. adolescents and remain a risk factor for development of cardiovascular disease in adulthood. Weight status in childhood is highly influenced by diet, which is modifiable with appropriate intervention. School based programs have been postulated to be an appropriate setting for nutrition intervention programs to effect meaningful change in health behaviors, however data examining the effectiveness of school-based nutrition intervention programs is limited, and results have been inconsistent depending on outcomes of interest. **OBJECTIVE:** To examine the effectiveness of Project Healthy Schools, a multi-component school-based intervention program utilizing interactive health education sessions in the homeroom setting, at achieving favorable change in participating students' dietary consumption of foods and/or beverages associated with cardiovascular disease risk (i.e., fruits, vegetables, sugar sweetened beverages, red and processed meat, fried and salty snacks, and baked goods and sweets). **DESIGN, SETTING, AND PARTICIPANTS:** A non-randomized, quasi-experimental pre-post design evaluation of 8,951 sixth grade students from 94 middle-schools across the state of Michigan enrolled in the first year of a school-based nutrition intervention program between 2005-2019. **MEASURES AND APPROACH TO ANALYSIS:** Measures of dietary intake were self-reported using a validated health behavior questionnaire administered to students at baseline and following completion of the 10-week intervention program. Change in dietary intake following the nutrition intervention was examined using Bowker's test of symmetry, from which, direction and magnitude of change was also established to examine effectiveness of the intervention. **RESULTS:** Intake of fruit and vegetables significantly increased at follow-up compared to baseline, $p=<0.0001$ and 0.0013, respectively. Unexpectedly, consumption of sugary beverages also increased following participation in the nutrition intervention program, $p=0.009$. There were no significant changes in consumption of regular soda, red and processed meat, fried and salty snacks, and baked goods and sweets post-intervention. **CONCLUSIONS AND FUTURE DIRECTIONS:** Changes in dietary intake for all variables of interest following participation in the intervention program were modest. Positive change in fruit and vegetable intake was achieved, however increased intake of sugary beverages was also noted. This study suggests that school-based nutritional programs may be effective at increasing fruit and vegetable consumption among middle school children. However, sugary beverage consumption increased post-intervention, and no changes were observed for other dietary behaviors examined. Future work should examine the sustainability of school-based nutrition interventions and investigate whether modest changes in dietary consumption can drive meaningful shift in physiological measures of cardiovascular risk.

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death for adult men and women in the United States.[1] Atherosclerotic CVD, also known as CHD, is the most common type of CVD. Manifestation of CVD in adolescence is rare, however evidence strongly supports that risk factors and behaviors associated with cardiovascular risk begin in childhood. Reducing or preventing the development of CVD risk factors during childhood and adolescence may delay or prevent progression of clinical disease in adulthood.[3,71]

According to the CDC and the AHA, schools are an ideal setting to help children and adolescents develop healthy behaviors, including those related to nutrition and PA, which in turn can positively influence CVD risk factors including BMI, BP and blood lipid levels. Project Healthy Schools (PHS) is a multicomponent school-based wellness program designed to improve nutritional and PA behaviors among middle-school children in grade six in Michigan. Early in the program implementation and among a small sample of students has been shown to be successful in improving risk factors associated with early atherosclerosis, including increased fruit intake and reduced blood lipid levels and BP measurements.[37] However, it was not assessed if behavioral change was the driving factor behind the changes in physiological measures noted. The program continues to expand each year into more middle schools across the state of Michigan and warrants continued research from the larger sample size now available.

The purpose of this study was to examine the effectiveness of PHS at achieving favorable change in participating students' dietary consumption of foods and/or beverages associated with cardiovascular disease risk. We hypothesize that students will improve dietary intake among foods and/or beverages targeted by the intervention program lessons after participation in the PHS program. For our first objective, we hypothesize that post-intervention consumption of fruits and post-intervention consumption of vegetables will increase compared to baseline reported consumption. For our second objective, we hypothesize that post-intervention consumption of sugar-sweetened beverages (SSB) will decrease compared to baseline reported consumption. Finally, for our third objective, we hypothesize that post-intervention of high fat, high sodium, and high sugar foods (red and processed meats, fried and salty snack foods and baked goods and sweets) will decrease compared to baseline reported consumption of these foods.

METHODS

Program Description

PHS is a middle-school-based multidisciplinary education program, implemented in grade 6, established in collaboration with the University of Michigan Health System, now known as Michigan Medicine, to address adolescent obesity and reduce cardiovascular risk factors.[36] PHS utilizes school-based environmental changes and health education emphasizing the following 5 goals: 1) eat more fruits and vegetables; 2) choose less sugary foods and beverages; 3) eat less fast and fatty foods; 4) be active every day, specifically performing 150 minutes of exercise per week; and 5) spend less time in front of a screen, specifically decreasing time spent with television and computer games while increasing time spent doing enriching activities such as music and reading. School-based environmental changes consisted of a variety of strategies to encourage healthier behaviors in students including but not limited to bulletin boards promoting healthy behaviors, physical activity events at the school, and/or increased access to healthier food and snack options at the school cafeteria and/or vending machines. The frequency and type of environmental changes were determined by school administrators and varied by participating schools. Over time, the educational lessons and environmental changes evolved based on feedback from participating students and staff, however, the goals of the PHS have remained the same since program inception. The educational component of the program consists of ten, 45-minute learning modules that address the five primary goals. The PHS lessons are evidence-based, derived from programs such as the Coordinated Approach to Child Health (CATCH), and developed by University of Michigan expert dietitians, physical activity experts, physicians, and registered nurses.[184] The teaching program emphasizes both the use of visual aids and student participation to engage the students in the process. The educational modules are administered during the students' advisory (homeroom) class period. The modules are taught by the advisory teachers or, in a few instances, by educators provided by the PHS team. Teachers are provided a standardized curriculum for each lesson; however, they are allowed some flexibility and autonomy regarding the presentation of the educational material. Educational lessons which targeted dietary habits are outlined in **Table 5:**

Table 5: Description of Educational Topics Addressing Dietary Habits in PHS

Educational Lessons	Lesson Objectives/Description
Lesson 3: My Plate! My Choice!	1. Describe and calculate energy in and energy out. 2. Use MyPlate to describe a balanced meal. 3. Define food groups and what they include. 4. Assess their personal dietary choices and reflect on ways they can include more nutritional variety.
Lesson 4: Sugar Shock	1. Understand that sugary food and beverages often do not provide beneficial nutrients. 2. Identify the ingredients and added sugar on a nutrition label. 3. Determine ways to reduce the amount of added sugar that they eat and drink.

Table 5 (cont'd)

Lesson 6: Rainbow of Color	1. Understand that a variety of fruits and vegetables provide health benefits. 2. Identify the roles of various vitamins and minerals within the body. 3. Assemble a salad that provides a variety of vitamins and minerals.
Lesson 9: Facts on Fats	1. Understand how fat intake plays a role in our health. 2. Understand there are health differences between fat sources and that research is evolving to help us determine which fat sources are considered healthy and unhealthy. 3. Distinguish between the following types of fat: unsaturated and saturated. 4. Evaluate a fast food meal and determine ways to improve it.

Study Design and Participants

This was a non-randomized, quasi-experimental study, with a pre-post design, where students served as their own controls. Students from 94 middle schools in Michigan participating in PHS during the first year the school was enrolled in the program between 2005-2019 were eligible for this study. Students participating in this study received an assent form to read prior to completing the health behavior questionnaire (Appendix A.1). The PHS protocol was approved by The University of Michigan Institutional Review Board. Secondary data analysis for this project was approved by the Michigan State University Institutional Review Board.

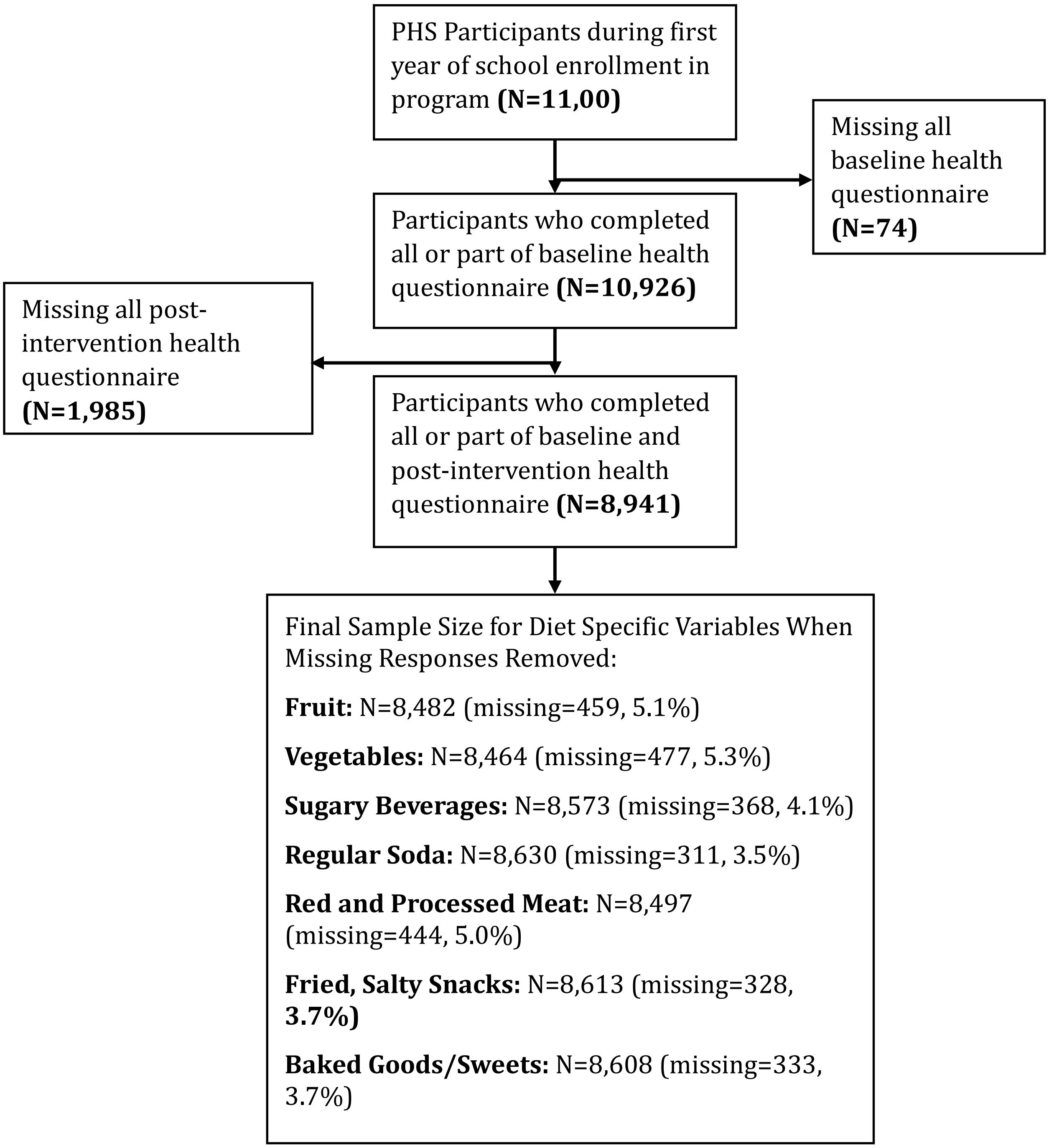
Study Sample

As shown in **Figure 1**, 23,394 students participated in the PHS program from 2004 to 2019, with 11,000 students participating in the first year the school-based program was implemented. Students who completed the behavior health questionnaire at baseline and follow-up were eligible to be included in the analysis. Among the 11,000 students participating in the first year of PHS implementation, 74 (0.67%) students did not complete a baseline health behavior questionnaire. Among the 10,926 students with partial or complete baseline health behavior questionnaire data, 1,985 (18.2%) students did not fill out any portion of the post-intervention health behavior questionnaire. After these exclusions, a total of 8,941 students, or 81.8% of eligible sample, who completed all or part of both the baseline and post-intervention health behavior questionnaire, were eligible to be included in this analysis **(Figure 1)**.

To assess for potential selection bias, demographic characteristics of the students excluded from the analysis due to missing response data were compared to those included in the analysis. In addition, random imputation was applied to our study sample and differences in associations were examined between the sample with complete cases and the sample with random imputation. Random imputation was conducted for each dietary variable of interest using PROC HPIMPUTE in SAS, which replaces missing values with a random value that is drawn between the minimum and maximum of the variable, and in this case, the imputed values were rounded to the nearest whole number/response. Based on this assessment and as reported below in the results, it was determined that complete case analysis was a reasonable approach for this study and therefore was utilized. As such, the study sample included students with no missing data on the dietary variables of interest at baseline or follow-up.

Of the seven dietary measures under evaluation, only three had 5% or more of students with a missing response to the question of interest, namely the question on fruit intake (5.1% missing), the question on vegetable intake (5.3% missing) and the question on red and processed meat intake (5.0% missing). A total sample size of 8,482 was available for assessment of fruit intake; 8,464 for vegetables; 8,573 for sugary beverages; 8,630 for regular soda; 8,497 for red and processed meat; 8,613 for fried and salty snacks; and 8,608 for baked goods and sweets **(Figure 1)**.

Figure 1: Inclusion of Study Participants Flowchart



Measures

Students completed a health behavior questionnaire, which is a modified version of the validated School Physical Activity and Nutrition (SPAN) Questionnaire developed by the University of Texas Health Science Center along with the CDC and US Department of Agriculture (USDA).[199] The questionnaire was filled out by the student at baseline and following completion of the PHS curriculum to allow for the comparison of health-related behaviors before and after taking part in the program. The amount of time between the baseline and post-intervention assessments was variable between schools and was at minimum, 10 weeks apart.

Dietary Variables of Interest

All dietary variables examined in this study represented foods and/or beverages that were targeted by educational lessons in the PHS intervention. From the questionnaire, the following 7 measures of self-reported dietary intake were ascertained: fruit, vegetables, sugary beverages (fruit punch, lemonade, sweet tea, sport's drinks), regular soda, red and processed meats, fried and salty snacks, and baked goods and sweets. All questions focused on dietary intakes from the previous 24 hours. Teachers were instructed to distribute the questionnaire to students on any school day other than Monday to avoid a weekend effect. Responses for the dietary intake questions of interest were on a 4-point Likert scale and included: "none of the time, " "1 time," "2 times," or "3 or more times."

Three specific nutritional areas were addressed with the questionnaire, using individual questions. Intake of non-obesogenic foods, namely fruit and vegetables, were measured using the following questions:

1. “Yesterday, how many times did you eat vegetables? Include all cooked and uncooked vegetables, salads, boiled, baked, and mashed potatoes. Do not count French fries or chips.”
2. “Yesterday, how many times did you eat fruit? Fruits are all fresh, frozen, canned or dried fruits. Do not count juice.”

Intake of obesogenic sugar-sweetened beverages were measured using the following questions:

1. “Yesterday, how many times did you drink any punch, Kool-Aid®, sports drinks, lemonade, sweet tea, or other fruit-flavored drinks? Do not count 100% fruit juice.”
2. “Yesterday, how many times did you drink any regular (not diet) sodas or soft drinks?”

Intake of obesogenic foods high in fat, sodium and/or sugar were measured using the following questions:

1. “Yesterday, how many times did you eat hamburger meat, hot dogs, sausage, steak, bacon, or ribs?”
2. “Yesterday, how many times did you eat French fries or regular chips? Include potato chips, tortilla chips, Cheetos®, corn chips, or other snack chips?”
3. “Yesterday, how many times did you eat sweet rolls, doughnuts, cookies, brownies, pies or cakes?”

For all dietary variables, we defined frequency of consumption of “0 times” as “very low consumers,” “1 time” as “low consumers,” “2 times” as “high consumers,” and “3 or more times” as “very high consumers.” For fruits and vegetables, being a high or very high consumer was considered a desirable level of intake. For all other variables, being a low or very low consumer was considered a desirable level of intake. These definitions are derived from the USDA dietary guidelines for healthy Americans.[174]

Direction of change was categorized and presented as "improved," "no change," and "worsened." Improved for fruit intake and vegetable intake, was defined as an increase in intake from baseline to follow-up by a frequency of at least 1, whereas worsened was defined as a decrease in intake by a frequency of at least 1 from baseline to follow-up. Improved for all other dietary variables was defined as a decrease in intake by a frequency of at least 1 from baseline to follow-up and worsened was defined as an increase in intake by a frequency of at least 1 from baseline to follow-up. For all dietary variables examined, no change was defined as a student reporting the same intake response at baseline and follow-up.

Magnitude of change was presented as the proportion of students who reported an increase or decrease in dietary intake of each measure of interest by a frequency of either 1, 2, or 3 or more. The proportion of students who would most benefit from dietary change, and therefore had the most potential to be impacted by the intervention, was examined. This was determined according to baseline responses. For fruit intake and vegetable intake, very low and low consumers at baseline accounted for the students who would most benefit from changing their intake in the desired direction, specifically, increasing their intake. For all other dietary variables examined, high and very high consumers at baseline accounted for the students who could most benefit from changing their intake in the desired direction, specifically, decreasing their intake of those foods and/or beverages.

Covariates

Demographic characteristics (age, sex, race) were assessed at baseline through self-report. Students could self-identify as "male" or "female" for sex, and as either "white," "black," "Asian," "Hispanic," "American Indian," or "other" for race. Median household income was not available for individual students, and therefore, socioeconomic status (SES) was determined based on the median household income for each school's zip code. Tertiles for SES classification were based on federal poverty levels established by the Department of Health and Human Services (HHS) and were categorized as low (median household income <\$36,180), middle (\$36,180-\$48,240) or high (>\$48,240).[200] Metropolitan status of "suburban," "urban" or "rural" community type was self-reported by each school on their application to join the program.

Analysis approach/statistics:

Descriptive statistics were used to evaluate the distribution of baseline characteristics, including sex, race, SES, and school metropolitan status. Change in dietary intake following the nutrition intervention was examined using Bowker's test of symmetry, from which, direction and magnitude of change was also established to examine effectiveness of the intervention.[201] The Bowker test of symmetry is an omnibus test, which provides a measure of symmetry overall, but does not reveal the direction of change. Mantel Haenszel chi-square tests were used to compare the change in dietary intake across different demographic characteristics. The statistical package SAS 9.4 (SAS Institute, Cary NC) was used for statistical analysis. The significance level was set at $P < 0.05$.

RESULTS

Demographic characteristics at baseline are presented in **Table 6**.

Table 6: Demographic Characteristics of Eligible Students

Characteristics	**Total**
Sample Size (n)	8,941
Sex (n, %)	
Male	4,414 (49.4%)
Female	4,447 (49.7%)
Missing	80 (0.9%)
Race (n, %)	
White	5,682 (63.5%)
Black	1,246 (14.0%)
Asian	242 (2.7%)
Hispanic	414 (4.6%)
American Indian	187 (2.1%)
Other	649 (7.3%)
Missing	521 (5.8%)
School-level socioeconomic status (n, %)	
Low SES	3,228 (36.1%)
Mid SES	3,058 (34.2%)
High SES	2,655 (29.7%)
Metropolitan status (n, %)	
Suburban	4,525 (50.6%)
Urban	993 (11.1%)
Rural	3,423 (38.3%)

Missing data

For most of the dietary variables, a higher proportion of students excluded from the study were from rural schools, low SES schools and were male when compared to students included in the analysis (Appendix B, Tables B.1-B.7). Among those included for analysis of fruit intake, there was a lower proportion of males (49.1% vs. 54.7%, p=0.019), and a higher proportion of black students (14.2% vs. 9.6%, p=0.006) compared to those excluded. Those included were also less likely to be from low SES schools (35.8% vs. 41.4%, p=0.015), and rural schools (37.8% vs. 47.1%, p=<0.0001), and were more likely to be from high SES schools (30.0% vs. 23.5%, p=0.003) and suburban schools (50.9% vs. 44.6%, p=0.009) compared to those excluded.

Similarly, among those included for analysis of vegetable intake, there was a lower proportion of males (49.1% vs. 54.3%, p=0.03), and a higher proportion of black students (14.2% vs. 9.4%, p=0.003) compared to those excluded. Those included were also less likely to be from low SES schools (35.8% vs. 41.7%, p=0.009), and rural schools (37.8% vs. 46.8%, p=<0.0001), and were more likely to be from high SES schools (30.0% vs. 24.3%, p=0.008) and suburban schools (51.0% vs. 44.0%, p=0.003) compared to those excluded.

Among those included for analysis of sugary beverage intake, there was a lower proportion of males (49.0% vs. 59.0%, p=0.0002), a higher proportion of Asian students (2.8% vs. 0%, p=0.001), and lower proportions of American Indian students (2.0% vs. 4.1%, p=0.007) compared to those excluded. Students included in the analysis were also less likely to be from low SES schools (35.8% vs. 43.5%, p=0.002), and rural schools (37.9% vs. 48.6%, p=<0.0001), and were more likely to be from high SES schools (30.1% vs. 19.3%, p=<0.0001) and suburban schools (51.0% vs. 41.3%, p=0.0003) compared to those excluded. Among those included for analysis of regular soda intake, there was a lower proportion of males (49.1% vs. 55.0%, p=0.001), higher proportions of white (63.9% vs. 55.0%, p=0.001) and Asian (2.8% vs. 0.3%%, p=0.008) students, and lower proportions of Hispanic (4.5% vs. 8.7%, p=0.0005) students compared to those excluded. Students included in the analysis were also less likely to be from low SES schools (35.9% vs. 42.4%, p=0.018), and rural schools (38.0% vs. 45.7%, p=0.006), and were more likely to be from high SES schools (29.9% vs. 22.5%, p=0.005) and suburban schools (51.0% vs. 40.5%, p=0.0003) compared to those excluded.

Among those included for analysis of red and processed meat intake, there was a lower proportion of males (49.0% vs. 57.2%, p=0.0007), and a higher proportion of females (50.0% vs. 42.8%, p=<0.0001), and black students (14.2% vs. 9.0%, p=0.002) compared to those excluded. Students included in the analysis were also less likely to be from rural schools (37.8% vs. 48.0%, p=0.006), and more likely to be from suburban (50.9% vs. 44.4%, p=0.007) and urban schools (11.3% vs. 8.3%, p=0.02) compared to those excluded. Among those included for analysis of fried and salty snack intake, there was a lower proportion of males (49.0% vs. 58.5%, p=0.0007), and a higher proportion of females (50.1% vs. 41.5%, p=<0.002), and Asian students (2.8% vs. 0%, p=0.006) compared to those excluded. Students included in the analysis were also less likely to be from low SES (35.8% vs. 42.4%, p=0.02), and rural schools (38.1% vs. 43.6%, p=0.04), and more likely to be from high SES (30.0% vs. 22.8%, p=0.006) and suburban schools (50.8% vs. 45.1%, p=0.04) compared to those excluded. Finally, among those included for analysis of baked goods and sweets intake, there a higher proportion of Asian students (2.8% vs. 0.6%, p=0.02) and a lower proportion of American Indian students (2.0% vs. 3.6%, p=0.05) compared to those excluded. Students included in the analysis were also less likely to be from low SES (35.7% vs. 46.0%, p=0.0001), and rural schools (38.1% vs. 43.5%, p=0.04), and more likely to be from high SES (30.0% vs. 24.0%, p=0.02) compared to those excluded. The full results are presented in Appendix B, Tables B.1-B.7.

Primary Results

Results for our primary objective evaluating the effectiveness of the PHS intervention program at achieving favorable change in participating students' dietary consumption of foods and/or beverages associated with CVD risk are presented in **Table 7**. There were significant differences in consumption of fruit, vegetables, and sugary beverages, before and after participation in the PHS program, ($p < 0.0001$), ($p = 0.0013$) and ($p=0.009$), respectively. There was no change in red and processed meat intake ($P=0.085$), fried and salty snack intake ($P=0.39$), regular soda intake ($P=0.43$), and baked goods and sweets intake ($P=0.06$) from baseline to follow-up. For reference, an example of the 4x4 contingency table for fruit intake is provided in **Table 8.**

Table 7: Change in Reported Dietary Intake from Baseline to Follow-up

Dietary Variable of interest (N)	**Worsened Intake (decreased) N (%)**	**No Change N (%)**	**Improved Intake (increased) N (%)**	**Bowker Test of Symmetry P-value (before/after comparison)**
Fruit Intake[1,3] (8,482)	2130 (25.1)	3437 (40.5)	2915 (34.4)	<0.0001
Vegetable Intake[1,3] (8,464)	2385 (28.2)	3451 (40.8)	2628 (31.0)	0.001
Dietary Variable of Interest (N)	**Improved intake (decreased) N (%)**	**No Change N (%)**	**Worsened Intake (increased) N (%)**	**Bowker Test of Symmetry P-value (before/after comparison)**
Sugar-Sweetened Juice, Sport's Drink Intake[2,4] (8,573)	2258 (26.3)	3992 (46.6)	2323 (27.1)	0.009
Regular Soda Intake[2] (8,630)	1962 (22.7)	4608 (53.4)	2060 (23.9)	0.43

Table 7 (cont'd)

Red and Processed Meat Intake[2] (8,497)	2275 (26.8)	3743 (44.1)	2479 (29.1)	0.08
High fat, High Sodium Snack Intake[2] (8,613)	2293 (26.6)	4006 (46.5)	2314 (26.9)	0.39
High fat, high Sugar Baked Goods Intake[2] (8,608)	2371 (27.5)	4090 (47.5)	2147 (25.0)	0.06

[1]Increased intake is desired outcome.
[2]Decreased intake is desired outcome.
[3]Significant change in desired direction detected.
[4]Significant change in undesired direction detected.

Table 8: Example of Bowker Test of Symmetry 4x4 Contingency Table (Fruit Consumption Example)

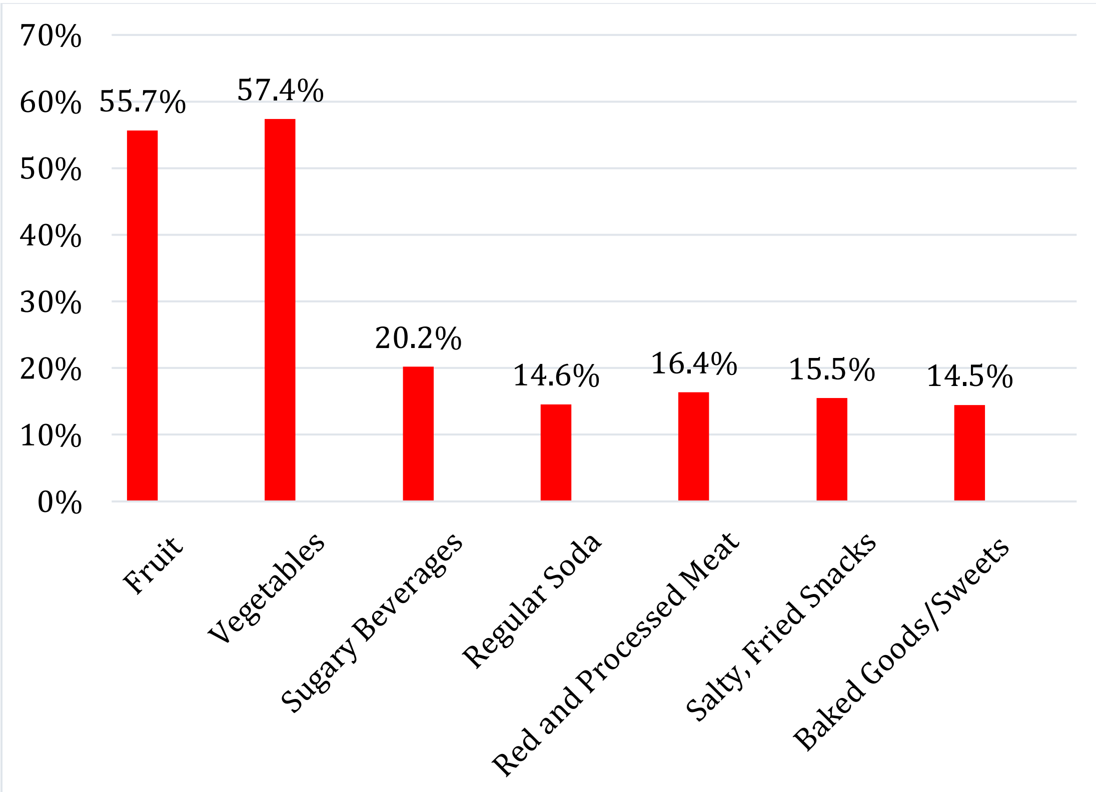
Baseline x Post-intervention Fruit Consumption N (row %)					
Baseline Level of Consumption	**Post-Intervention Level of Consumption**				
	0 portions (very low consumer)	**1 portion (low consumer)**	**2 portions (high consumer)**	**3 or more portions (very high consumer)**	**Total N (%)**
0 portions (very low consumer)	886 (43.2)	643 (31.4)	322 (15.7)	198 (9.7)	2049 (24.1)
1 portion (low consumer)	541 (20.2)	955 (35.7)	763 (28.5)	419 (15.6)	2678 (31.6)
2 portions (high consumer)	210 (9.8)	548 (25.6)	816 (38.1)	570 (26.5)	2144 (25.3)
3 or more portions (very high consumer)	129 (8.0)	261 (16.2)	441 (27.4)	780 (48.4)	1611 (19.0)
Total N (%)	1766 (20.8)	2407 (28.4)	2342 (27.6)	1967 (23.2)	**8482 (100)**

Overall, significantly more students improved their intake of fruit when compared to students who worsened their intake. Specifically, 34.4% or ((643 + 322 + 198 + 763 + 419 + 570) / 8482) of students increased their intake of fruit from baseline to follow-up, compared to 25.1% or ((541 + 210 + 548 + 129 + 261 + 441) / 8482) who decreased their intake from baseline to follow-up. No change was observed among 40.5% of students from baseline to follow-up. In addition, significantly more students improved their intake of vegetables when compared to students who worsened their intake. An increase in vegetable intake was observed in 31.0% of students from baseline to follow-up compared to 28.2% of students who decreased their vegetable intake. No change was observed in 40.8%. In contrast, significantly more students worsened their intake of sugary beverages when compared to students who improved their intake, which was not the desired outcome. Specifically, 27.1% of students increased their intake of sugary beverages like sport's drinks, lemonade, and juice, compared to 26.3% who decreased their intake, and 46.6% of students had no change in sugar-sweetened beverage intake from baseline to follow-up. More students improved their intake of baked goods and sweets from baseline to follow-up (27.5%) compared to those who worsened their intake (25.0%), with 47.5% exhibiting no change intake. This difference was notable, but not statistically significant (p=.06). In our secondary analysis using random imputation for missing values, there were no differences in the outcomes assessed or conclusions made (Appendix B, table B.8).

For each dietary variable of interest, several significant differences between baseline and post-intervention intake according to demographic characteristics were observed (Appendix B, tables B.9-B.15). For fruit intake, significantly more female students improved their intake from baseline to follow-up (35.6%) compared to male students (33.1%; p-value =0.05). There were more students from schools in urban communities and fewer from schools in suburban communities whose fruit intake decreased than was expected (p=0.03). There were no significant differences in change in fruit consumption by race, and school-level SES (Appendix B, table B.9). There was a similar trend regarding vegetable intake with significantly more female students improving their intake (32.1%) compared to males (29.8%; p-value=0.02). There were no significant differences in change in intake of vegetable intake by race, school level socio-economic status or metropolitan status (Appendix B, table B.10; all p-values $\geq$0.05). There were no significant differences in the change in sugary beverage consumption according to any demographic characteristics (Appendix B, table B.11). Significant differences for change in regular soda consumption by school-level SES was observed, driven largely by fewer than expected students from high SES schools and higher than expected students from low SES schools exhibiting an undesirable change (increase) in soda consumption (p=0.007) (Appendix B, table B.12). For change in red and processed meat consumption, there was a significant change in intake by metropolitan status with more students from schools in an urban setting exhibiting an undesirable change in their intake compared to students from schools in rural and suburban settings (p=0.008) (Appendix B, table B.13). A similar trend was noted when considering change in fried and salty snack consumption. The difference largely driven by a higher-than-expected number of students from schools in urban communities reporting an undesirable change in their intake, and fewer than expected reporting no change in intake (p=0.006) (Appendix B, table B.14). There were significant differences in the change in consumption of baked goods and sweets by metropolitan status with a higher proportion of students from schools in an urban setting exhibited a desirable change in consumption (31.8%) compared to students from schools in rural (25.8%) and suburban settings (27.9%), p=0.01. (Appendix B, table B.15).

The proportion of students in the study sample who were consuming an undesirable level of foods and/or beverages and thus could most benefit from improvement in intake of the various dietary components examined is presented in **Figure 2**.

Figure 2: Proportion of Students with an undesirable level of intake at baseline according to dietary variable of interest



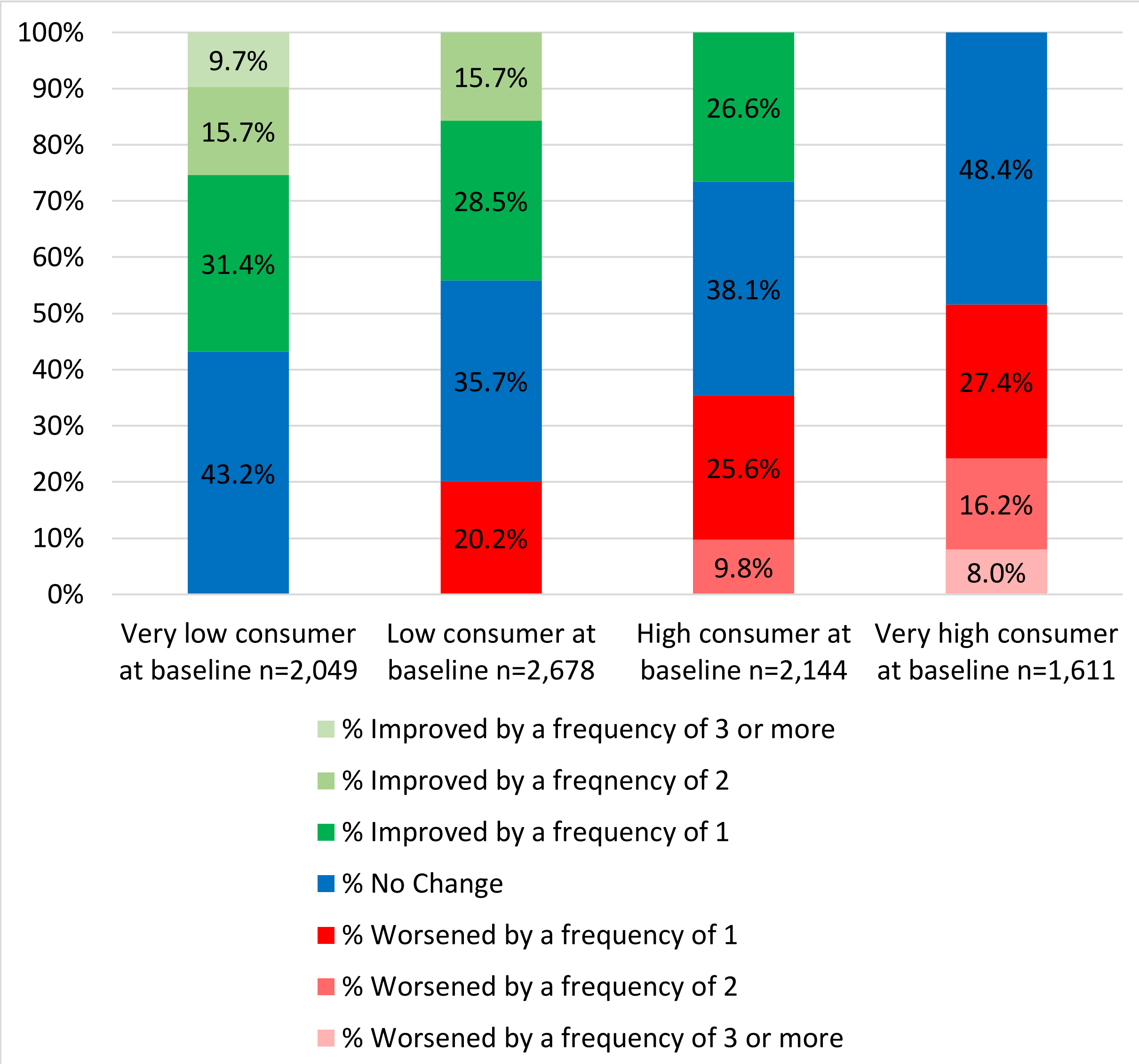
More than half of the students in our sample consumed inadequate amounts of fruits and vegetables at baseline and therefore could benefit from improving intake, that is 55.7% and 57.4%, for fruit and vegetables respectively. In contrast, a much smaller proportion of students could benefit from improving intake of sugary beverages (20.2%), regular soda (14.6%), red and processed meat (16.4%), salty, fried snacks (15.5%), and baked goods and sweets (14.5%), as most were already consuming a desirable amount of these foods and/or beverages at baseline.

When accounting for baseline intake, the direction of change and the magnitude of change of dietary intake were measured and are presented in **Figures 3-9** and described below.

Fruit Intake

At baseline, 24.2% (2049 / 8482) of students were very low consumers of fruit compared with 20.8% (1766 / 8482) post-intervention. In addition, 19.0% (1611 / 8482) of students were very high consumers of fruit at baseline, which increased to 23.2% (1967 / 8482) post-intervention. Among those students with very high consumption of fruit at baseline, 48.4% (780 / 1611) maintained that level of consumption post-intervention. Among high consumers, 26.6% (870 / 2144) improved to very high consumption post-intervention and 38.1% (816 / 2144) remained high consumers, whereas 35.4% (758 / 2144) decreased their intake to an undesirable level. Among those with an undesirable level of intake at baseline, 44.2% (1182 / 2678) of low consumers improved their intake to high or very high levels post-intervention. Similarly, among very low consumers of fruit at baseline, 25.4% (520 / 2049) increased their consumption to a desirable level of intake post-intervention as high or very high consumers, whereas 43.2% (886 / 2049) maintained a very low level of intake **(Figure 3)**.

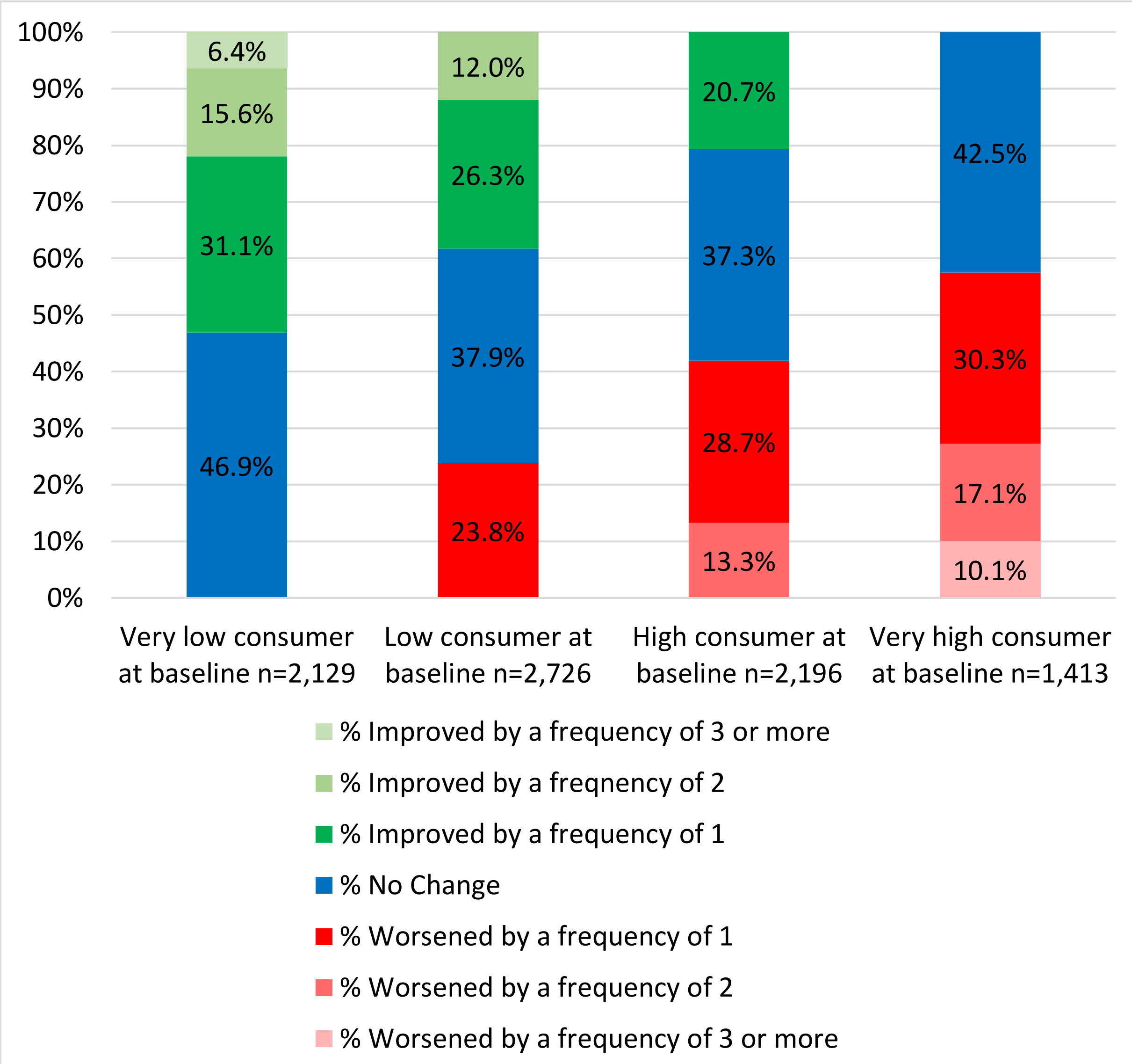
Figure 3: Direction and Magnitude of Change in Fruit Intake Baseline to Follow-up



Vegetable Intake

At baseline, 25.2% (2129 / 8464) of students were very low consumers of vegetables, compared with 24.6% (2084 / 8464) post-intervention. In addition, 16.7% (1413 / 8464) of students were very high consumers of vegetables at baseline, which increased to 18.0% (1519 / 8464) post-intervention. Among those students with very high consumption of vegetables at baseline, 42.5% (600 / 1413) maintained that level of consumption post-intervention. Among high consumers, 20.7% (455 / 2196) improved to very high consumption post-intervention and 37.3% (818 / 2196) remained high consumers, whereas 42.0% (923 / 2196) decreased their intake to an undesirable level. Among those with an undesirable level of intake at baseline, 38.3% (1043 / 2726) of low consumers improved their intake to high or very high levels post-intervention. Similarly, among very low consumers of vegetables at baseline, 22.0% (468 / 2129) increased their consumption to a desirable level of intake post-intervention as high or very high consumers, whereas 46.9% (1661 / 2129) maintained a very low level of intake **(Figure 4)**.

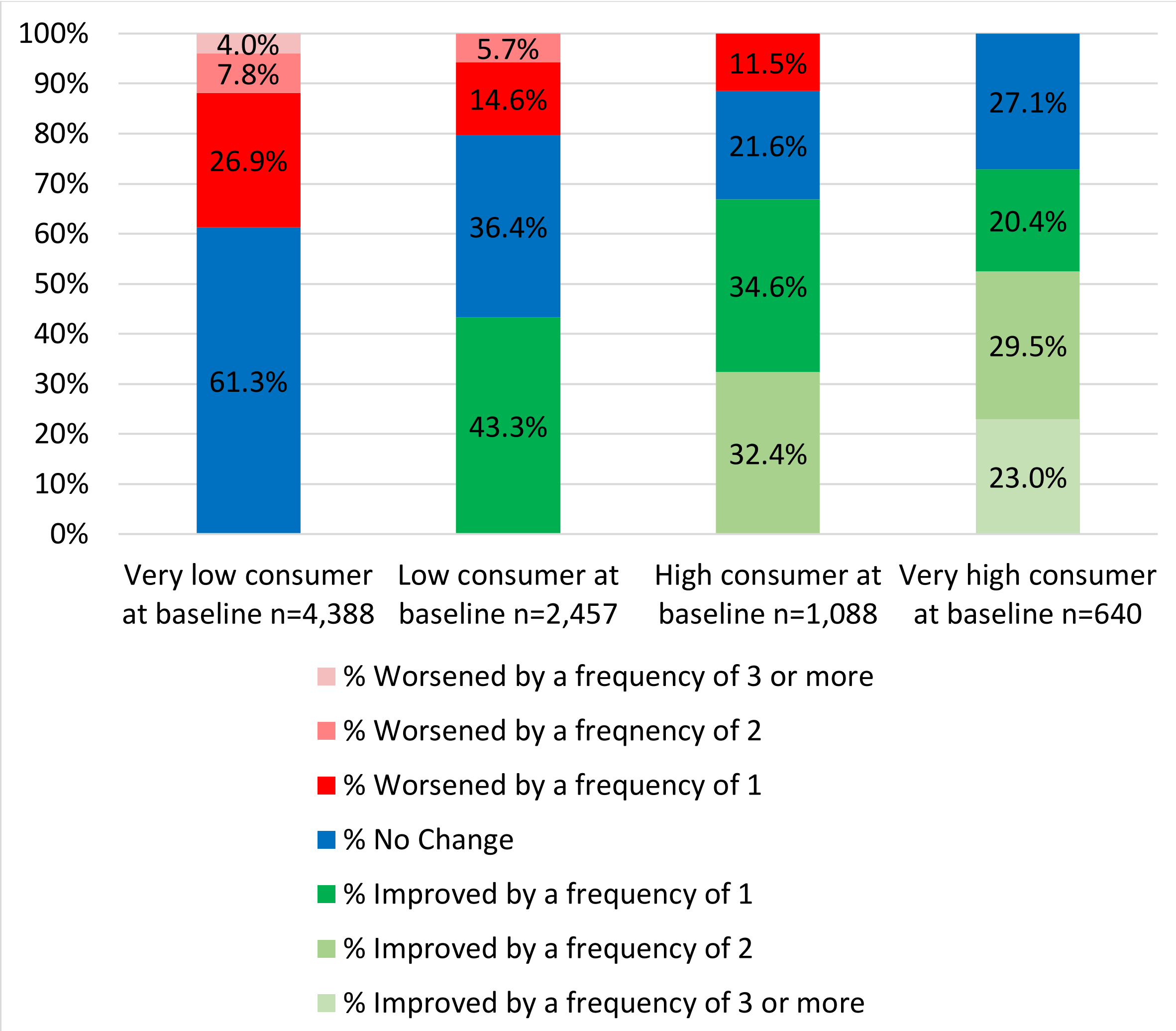
Figure 4: Direction and Magnitude of Change in Vegetable Intake Baseline to Follow-up



Sugary Beverage Intake

At baseline, 51.2% (4388 / 8573) of students were very low consumers of sugary beverages which decreased to 49.6% (4251 / 8573) post-intervention. In addition, 7.5% (640 / 8573) of students were very high consumers at baseline, which decreased to 7.2% (616 / 8573) post-intervention. Among very low consumers of sugary beverages at baseline, and 61.3% (2689 4388) of those students maintained that very low level of consumption post-intervention. Among low consumers, 43.3% (1063 / 2457) improved to very low consumption post-intervention and 36.4% (895 / 2457) remained low consumers, whereas 20.3% (499 / 2457) increased their intake to an undesirable level. Among those with an undesirable level of intake at baseline, 67.0% (728 / 1088) of high consumers decreased their intake to low or very low levels post-intervention. Similarly, among very high consumers of sugary beverages at baseline, 52.5% (336 / 640) decreased their consumption to a desirable level of intake post-intervention as low or very low consumers **(Figure 5)**.

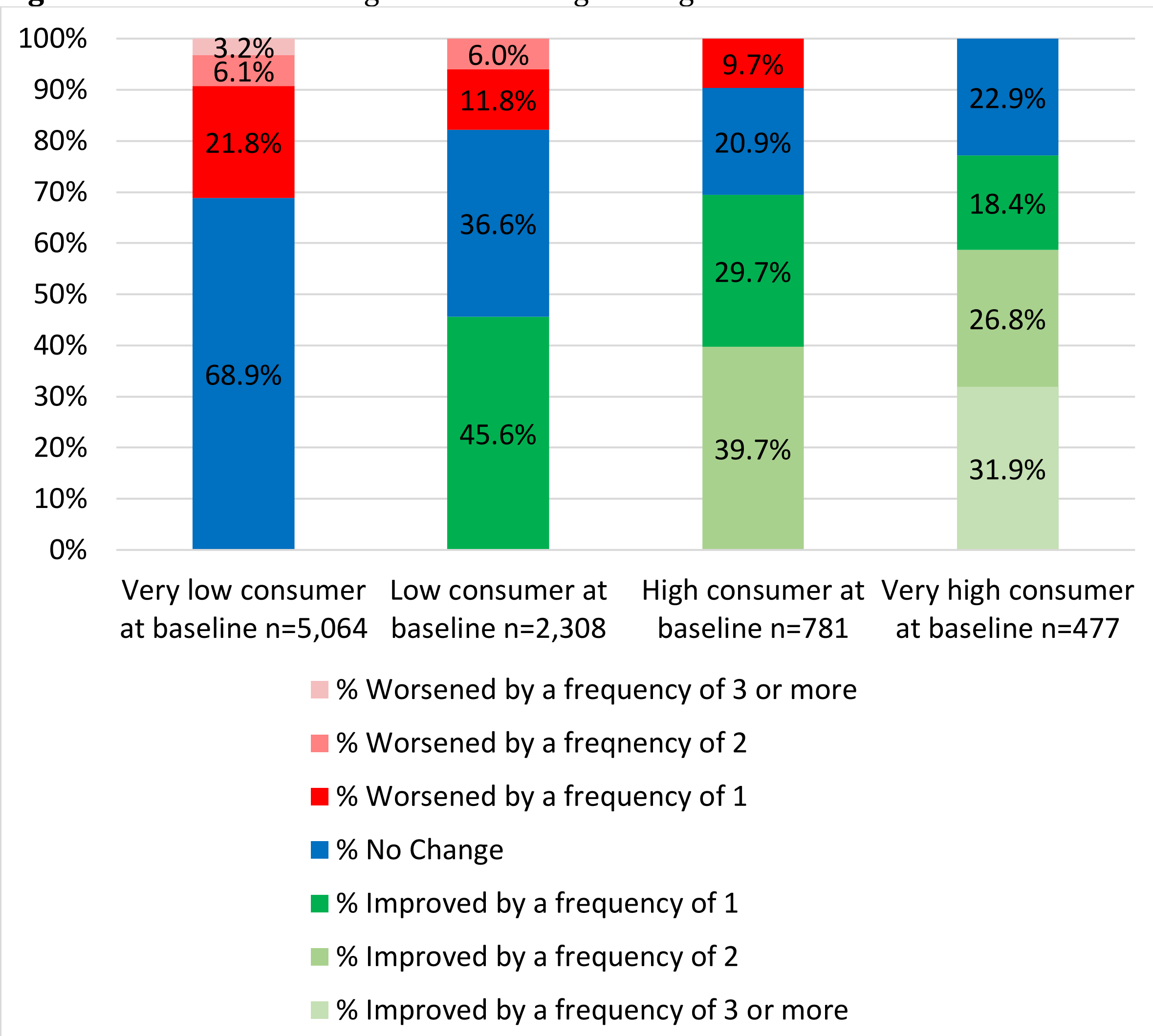
Figure 5: Direction and Magnitude of Change in Sugary Beverage Intake Baseline to Follow-up



Regular Soda Intake

At baseline, 58.7% (5064 / 8630) of students were very low consumers of regular soda which decreased to 58.0% (5005 / 8630) post-intervention. In addition, 5.5% (477 / 8630) of students were very high consumers at baseline compared to 5.6% (483 / 8630) post-intervention. Among very low consumers, 68.9% (3491 / 5064) maintained that level of consumption post-intervention, and 9.3% (471 / 5064) increased to an undesirable level of intake. Among low soda consumers, 45.6% (1052 / 2308) changed in the desired direction to very low consumption post-intervention and 36.6% (845 / 2308) remained low consumers, whereas 17.8% (411 / 2308) increased their intake to an undesirable level. Among those with an undesirable level of regular soda intake at baseline, 69.4% (542 / 781) and 58.7% (280 / 477) of high and very high consumers decreased their intake to low or very low levels post-intervention respectively **(Figure 6)**.

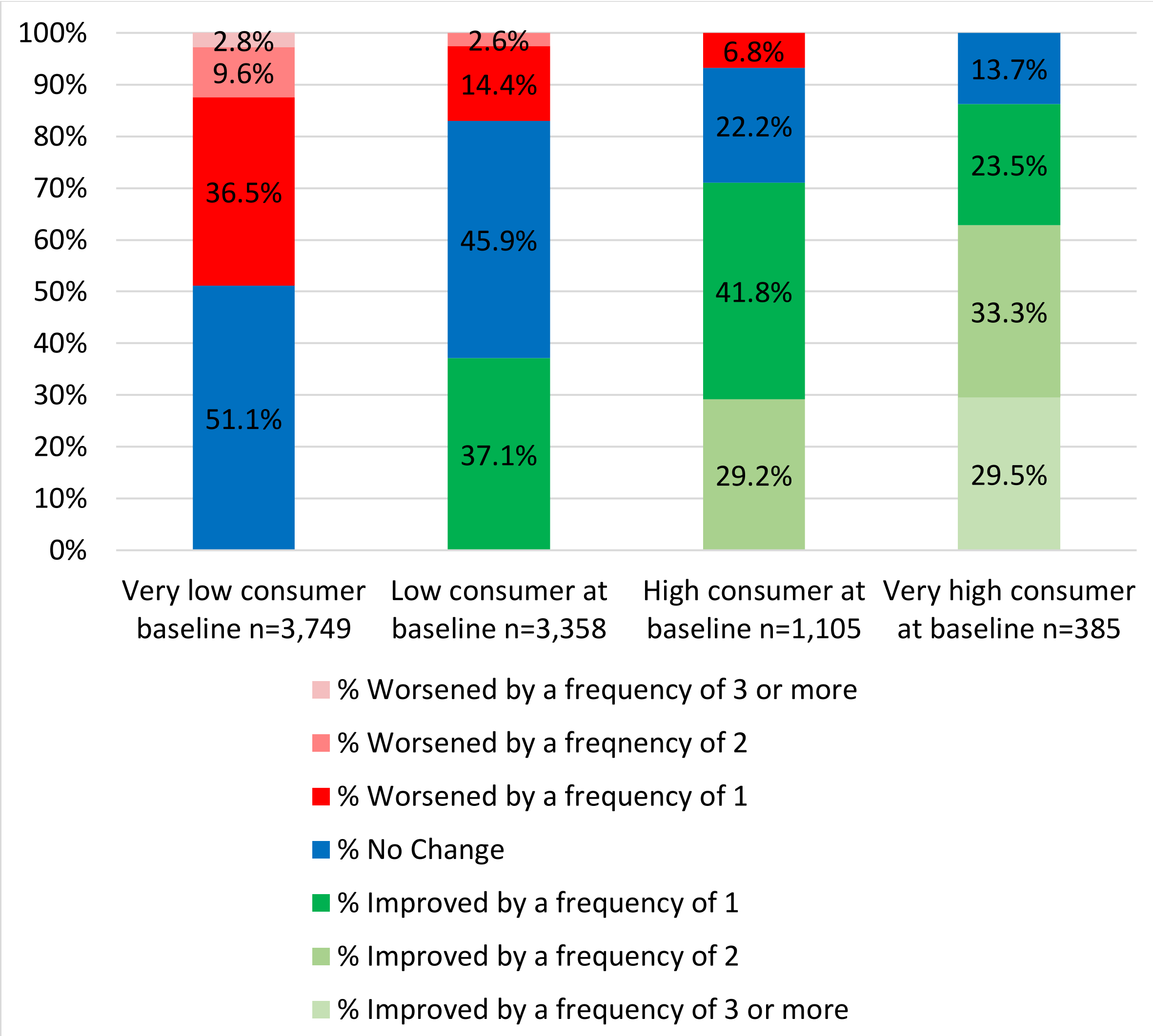
Figure 6: Direction and Magnitude of Change in Regular Soda Intake Baseline to Follow-up



Red and Processed Meat Intake

At baseline, 44.1% (3749 / 8497) of students were very low consumers of red and processed meat which decreased to 42.0% (3569 / 8497) post-intervention. In addition, 3.4% (285 / 8497) of students were very high consumers at baseline compared to 3.6% (305 / 8497) post-intervention. Among very low consumers, 51.1% (1918 / 3749) maintained that level of consumption post-intervention, and 12.4% (464 / 3749) increased to an undesirable level of intake. Among low consumers of red and processed meat, 37.1% (1244 / 3358) changed in the desired direction to very low consumption post-intervention and 45.9% (1541 / 3358) remained low consumers, whereas 17.0% (573 / 3358) increased their intake to an undesirable level. Among those with an undesirable level of red and processed meat intake at baseline, 71.0% (785 / 1105) and 62.8% (179 / 285) of high and very high consumers decreased their intake to low or very low levels post-intervention respectively **(Figure 7)**.

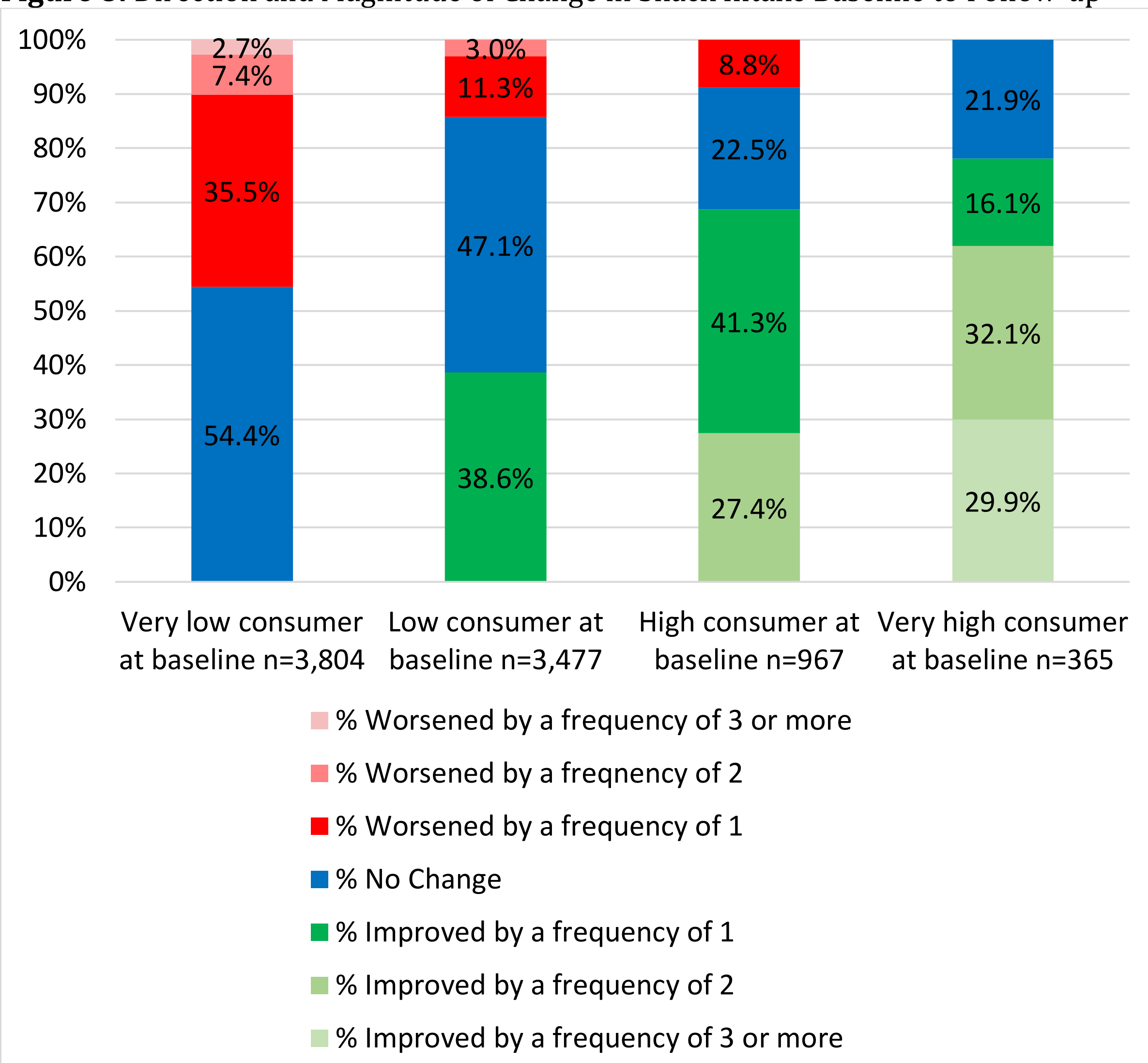
Figure 7: Direction and Magnitude of Change in Red and Processed Meat Intake Baseline to Follow-up



Fried and Salty Snack Intake

At baseline, 44.1% (3804 / 8613) of students were very low consumers of fried and salty snacks compared to 44.0% (3789 / 8613) post-intervention. In addition, 4.2% (365 / 8613) of students were very high consumers at baseline compared to 4.3% (370 / 8613) post-intervention. Among very low consumers, 54.4% (2071 / 3804) maintained that level of consumption post-intervention, and 10.1% (383 / 3804) increased to an undesirable level of intake. Among low consumers of fried and salty snacks, 38.6% (1344 / 3477) changed in the desired direction to very low consumption post-intervention and 47.1% (1637 / 3477) remained low consumers, whereas 14.3% (496 / 3477) increased their intake to an undesirable level. Among those with an undesirable level of fried and salty snack intake at baseline, 68.7% (664 / 967) and 62.0% (226 / 365) of high and very high consumers decreased their intake to low or very low levels post-intervention respectively **(Figure 8)**.

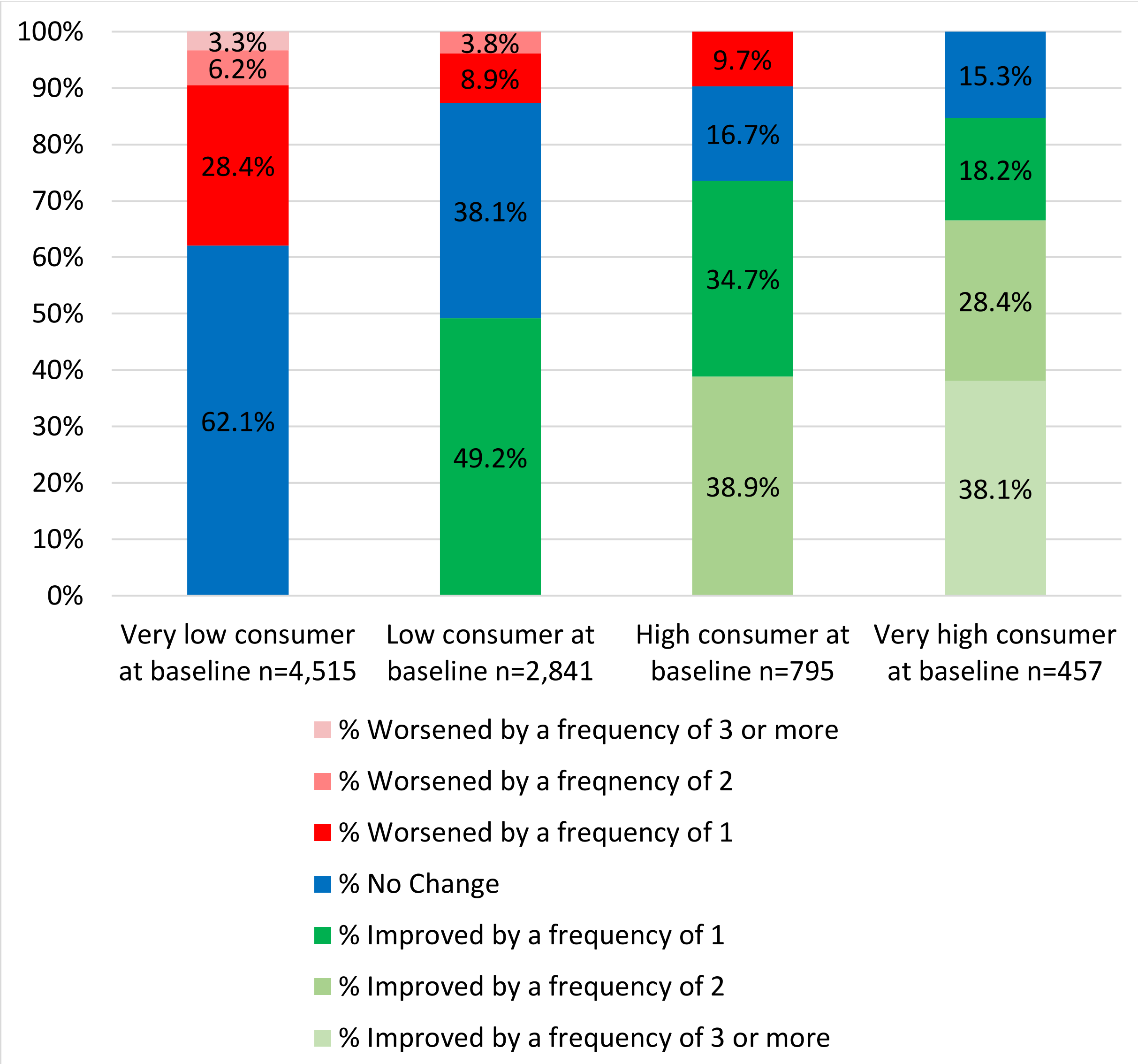
Figure 8: Direction and Magnitude of Change in Snack Intake Baseline to Follow-up



Baked Goods/sweets Intake

At baseline, 52.5% (4515 / 8608) of students were very low consumers of baked goods and sweets compared to 54.5% (4687 / 8608) post-intervention. In addition, 5.3% (457 / 8608) of students were very high consumers at baseline compared to 4.7% (402/ 8608) post-intervention. Among very low consumers, 62.1% (2805 / 4515) maintained that level of consumption post-intervention, and 9.5% (426 / 4515) increased to an undesirable level of intake. Among low consumers of baked goods and sweets, 49.2% (1399 / 2841) changed in the desired direction to very low consumption post-intervention and 38.1% (1082 / 2841) remained low consumers, whereas 12.7% (360 / 2841) increased their intake to an undesirable level. Among those with an undesirable level of baked goods and sweets at baseline, 73.6% (585 / 795) and 66.5% (304 / 457) of high and very high consumers decreased their intake to low or very low levels post-intervention respectively **(Figure 9)**.

Figure 9: Direction and Magnitude of Change in Baked Goods/Sweets Intake Baseline to Follow-up



DISCUSSION

The results of this study demonstrate improvements in healthy eating behaviors (consumption of fruits and vegetables) following completion of the PHS intervention, but also showed an increase in some unhealthy eating behaviors (consumption of sugary beverages). Significantly more students improved their intake of fruit following the PHS intervention than those that worsened their intake. This finding was consistent with other studies examining effectiveness of school-based nutrition intervention programs, as well as national trends. A systematic review by Black et al, assessed the impact of family-based and school/preschool nutrition programs on the dietary intake and health of children aged 12 or younger. Their review provided evidence that both school and family interventions, had a positive impact on both fruit and vegetable intake, but especially on fruit consumption.[202] Comparing NHANES data from 2003-2004 to surveys from 2009-2010, children ages 2-18 years consumed more fruit over this time period, whereas vegetable intake did not change.[203]

Vegetable intake also improved significantly more than it worsened in this sample of students. Fewer students were very low consumers of vegetables following the PHS intervention compared to baseline. Similarly, more students were very high consumers of vegetables post-intervention than at baseline. These changes were small, but significant. Similar to fruit consumption, among those who could most benefit from improvement in intake, very low consumers at baseline, nearly a quarter of the sample improved to become high or very consumers post-intervention. However, more than half of very high and high consumers of vegetables at baseline changed to low or very low consumers post-intervention. These findings were unexpected. Similar findings have been reported by King et al, when examining changes in dietary intake among school-aged children who participated in a school-based wellness program using a health behavior questionnaire similar to the one utilized in PHS. Their findings demonstrated an increase of one to three servings of vegetables following the intervention, but also a significant decrease in the proportion of students who reported eating vegetables four or more times over the same time period.[198]

Concerning our second objective, significantly more students worsened their intake of sugary drinks like fruit punch, lemonade, sport's drinks and sweet tea post-intervention compared to those who improved their intake. This could be attributed to the large proportion of the sample who were already low or very low consumers at baseline, but also could indicate that theory-based education along with environmental changes in the school setting might not be sufficient to reduce children's intake of sugary beverages. Despite this overall finding, the majority of students with high or very high intake of sugary beverages at baseline improved their intake to low over very low levels post-intervention. This finding is encouraging and suggests that students with higher risk dietary consumption did seem to benefit from the intervention program.

Research on the effectiveness of interventions designed to reduce SSB intake among youth have yielded mixed results. A longitudinal study in Canada examined the association between change in beverage availability in school vending machines over time with SSB consumption among students. They found no association between sugary beverage consumption and availability of such beverages.[204] Researchers from the University of Chicago examined associations between school vending machine access and soda consumption in students. Contrary to their hypothesis, they observed that students who had access to vending machines containing SSBs, consumed fewer servings of soda per week than students with less access to vending machines containing SSBs at school.[205] A study examining NHANES data showed that among a typical weekday, 55% to 70% of all SSB calories consumed by children and adolescents, were consumed in the home environment, with 7% to 15% consumed in schools.[130] These findings suggest that interventions targeting consumption within the home setting or involvement of parents and/or broader population-level efforts to reduce SSB consumption are necessary.

For our third objective, we did not observe statistically significant post-intervention improvements in the consumption of high fat, high sodium foods (red and processed meats and fried and salty snacks), although a trend ($p=0.06$) towards statistical significance in the direction of improved intake (decrease) was observed. Also, we observed that students who consumed large amounts of red and processed meat at baseline, the vast majority reduced their intake to a desired level post-intervention. A similar trend was noted for intake of fried and salty snacks. Much of our sample were low or very low consumers at baseline, and among very high consumers, more than half reduced their consumption to low or very low levels post-intervention. Change in consumption of baked good and sweets was in the desired direction, however, did not reach statistical significance. More than half of the students in our sample were very low consumers at baseline, and few of these students increased their intake to high or very high levels post-intervention. Among very high consumers at baseline, the majority reduced their intake to low or very low levels post-intervention. Again, this suggests that students in this study who could most benefit from reducing their consumption of these foods did so to low or very low levels. Baked goods and sweets are major contributors of low-nutrient, energy dense foods in the diets of

U.S. adolescents. Data from the 2004-2005 third School Nutrition Dietary Assessment Study found that the largest proportion of total daily energy from low-nutrient, energy-dense foods, including baked goods, were consumed at home.[206] The students in our study may not have significant autonomy over food selections or available options in the home setting. An educational component that involves parents/guardians may be helpful in addressing food options and/or eating behaviors at home.

Many of the findings from this study suggest a modest change in dietary intake is possible following completion of a school-based intervention program. Small changes can facilitate a meaningful impact on children's health at the population level, with benefits possibly extending into adulthood. Past studies have indicated that poor dietary behaviors developed in childhood may persist later into life and is a major contributor of rapid weight gain seen in early adulthood, as well as increases in various other cardiometabolic risk factors.[207,208] A report of the Joint Task Force of the American Society for Nutrition, Institute of Food Technologists, and International Food Information Council suggests that small changes in specific components of the diet could produce minor but important changes in energy intake, and therefore protect against incremental weight gain over time.[209] This joint task force suggests that small changes in dietary fat specifically, would be more sustainable than larger ones. This could be especially true among children and adolescents, who are still growing and in whom large changes to overall energy intake may not be ideal. A similar approach was echoed in a review article by Hills et al. The authors suggest that small dietary changes are more realistic, and feasible to achieve and maintain compared to large changes.[210]

Strengths

The major strength of this study is that it provides data on a heterogeneous population, namely students from 94 schools across the state of Michigan. Study participants include population groups often underrepresented in randomized trials, including children of different racial and ethnic groups, different geographic locations (rural/urban/suburban) and of various socioeconomic backgrounds. The large sample size of this study provided adequate power in order to detect meaningful differences in dietary intake from baseline to follow-up that could possibly be attributed to the PHS intervention. Finally, PHS has proven to be a feasible and effective program that can be easily implemented in the school setting. Through collaboration with multiple health systems, communities, schools, and stake holders, PHS has been implemented in over 140 schools primarily across the state of Michigan in addition to several international initiatives.[36,211]

Limitations

This study is subject to inherent limitations and potential biases, and any interpretation of these findings should consider the limitations to the data collection strategies utilized. Bias due to the self-reported nature of the health behavior questionnaire cannot be ruled out. The research team attempted to mitigate the bias by instructing teachers to avoid administering the health behavior questionnaires on a Monday. It remains possible that some students completed the health questionnaire on a Monday, which would therefore reflect their intake on Sunday. Analysis of NHANES data on adult dietary consumption has demonstrated that the weekend diet is less healthful than weekday, with diet on Saturday being the worst.[212] A study out of Brazil looked at data from a one-day food log from the National Food Survey, a representative sample of the

Brazilian population aged 10 years or older. They found that this population had increased energy intake and unhealthy food markers, notably increased consumption of SSBs, on weekends compared to weekdays.[213] Although self-reported dietary intake using a 24-hour recall approach is generally considered an acceptable method to obtain dietary intake data, the health behavior questionnaire used in this study can capture components of diet but cannot identify a dietary pattern. Regarding assessment of vegetable intake specifically, the PHS health behavior questionnaire does not differentiate between starchy and non-starchy vegetables. This distinction could be helpful, as these groups of vegetables may provide different health benefits. Seasonal availability of fruits and vegetables likely differed when the baseline and post-intervention questionnaires were completed. This could impact responses for the students, although frozen fruits and vegetables are an affordable option available year-round. Other non-obesogenic foods such as lean proteins (poultry, fish, shellfish, legumes), and heart healthy fats from consumption of nuts and seeds are not captured on this questionnaire. This questionnaire does not measure volume of food and/or beverages consumed, instead it only measures frequency of consumption. These types of questions are likely feasible for students to answer with some accuracy but may result in widely different volumes of food intake being captured in the same response category. Eating a food "1 time" could equate to a single bite or an entire plate of said food. With the assessment of change in dietary consumption, it is valuable to know what food or foods replace those that were decreased in the diet, and in the same way, when a food is increased, what food or foods were replaced is an important consideration. This was not evaluated in this study. This is important when examining diet and cardiovascular risk, as evidence suggests replacing foods high in saturated fat with foods high in sugar does not lower risk of heart disease. This study only evaluates diet at 2 time points, which may not be representative of a student's usual diet at that time. It could be helpful to have more than 1 post-intervention survey done to help provide a clearer interpretation of the effectiveness and sustainability of the intervention. While this study demonstrated several improvements in nutritional consumption post-intervention, it is important to note that 6th grade students in this study likely have minimal autonomy over food selections available in the home setting. Studies have shown that parental dietary intake greatly influences dietary intake and habits in young children.[214,215] The World Health Organization (WHO) School Health Promotion Framework advocates parental involvement in school-based intervention programs.[216] The CDC advocates that the most successful school-based nutrition education interventions are intensive, comprehensive, whole school interventions, that last longer than a year and include changes to promote a healthy school food environment as well as efforts to increase parental or family support, especially in young children. In older youth, parental involvement has not been as clearly linked to improvement in eating behaviors.[217] However, there is evidence to support that school intervention programs that include a parental education component have been effective in improving dietary intake and potentially reducing obesity among youth of all ages.[218] Future work in PHS could involve a pilot program among a sample of schools that includes an intervention that involves a familial educational component to determine if this can provide an effective means to improve outcomes associated with the program.

CONCLUSIONS AND FUTURE DIRECTIONS

This study provides valuable insight into dietary behaviors of a large cohort of middle school aged children in Michigan before and after participation in a school-based health intervention program. We observed significant improvement in fruit and vegetable intake following the PHS intervention. However, a significant increase in sugary beverage consumption following PHS intervention was also observed, which was not the expected or desired outcome. Although not statistically significant, the intervention appeared to be most effective in lowering intake of red and processed meat, fried, salty snacks, and baked goods and sweets among the highest consumers. Further research to assess whether this decrease in consumption of obesogenic foods among high or very high consumers is associated with any health benefit could prove useful. This study included post-intervention dietary assessment at one point in time. Additional assessment points to capture longitudinal data would be helpful in determining if the changes were sustained over time. Dietary behaviors during adolescence can influence dietary behaviors in adulthood. In the same way, small changes to diet during adolescence can promote lasting health benefits. School-based programs like PHS may provide a catalyst for the development of healthy dietary habits during the highly formative school-aged years in a way that is easily implemented and highly transferrable in the school environment.

CHAPTER 4: IMPROVEMENT IN CVD RISK FACTORS FOLLOWING BEHAVIORAL CHANGE AMONG A POPULATION OF MIDDLE SCHOOL STUDENTS PARTICIPATING IN A SCHOOL-BASED HEALTH INTERVENTION PROGRAM (MANUSCRIPT 2)

ABSTRACT

IMPORTANCE: CVD risk factors, specifically dyslipidemia and elevated BP/hypertension are common in the pediatric population. Presence of these factors during child and adolescent years track into adulthood, and it is reasonable to postulate that the prevention or reduction of these risk factors early in life may reduce risk of CVD in adulthood. Dyslipidemia and hypertension are both modifiable through diet and lifestyle measures. **OBJECTIVE:** To examine if achievement of optimal consumption of fruits and vegetables, SSBs and levels of PA among students participating in PHS is associated with improvement in blood lipid and BP levels. **DESIGN, SETTING, AND PARTICIPANTS:** A non-randomized, quasi-experimental pre-post design evaluation of 1,442 eligible students from 26 middle-schools across the state of Michigan enrolled in the first year of a school-based nutrition intervention program between 2005-2019. **MEASURES AND APPROACH TO ANALYSIS:** Measures of fruit, vegetable, and SSB consumption and PA were collected from a self-reported health behavior questionnaire which was completed by participating students at baseline and following completion of a 10-week health intervention program. Physiologic outcome measures of a non-fasting lipid profile which included total cholesterol [TC], high-density lipoprotein cholesterol [HDL-C], triglycerides [TG] and calculated low-density lipoprotein cholesterol (LDL-C), as well as three systolic blood pressure (SBP) and diastolic blood pressure (DBP) measurements from which the final two were analyzed, were taken before and after participation in the PHS intervention. Associations between the health

behaviors and post-intervention physiological measurements was assessed using multivariable regression models, adjusted for potential confounding factors. Additionally, associations between dietary components and PA and mean change in each outcome measures according to baseline status of the physiological measure of interest (normal or abnormal), as well as baseline BMI status was assessed with T-tests. **RESULTS:** We observed significant reductions in all blood lipid levels from baseline to follow-up and minimal and insignificant changes in SBP and DBP measurements. Multivariable analyses demonstrated that PA was associated with significantly higher post-intervention HDL-C and lower TG, whereas low SSB intake was associated with lower post-intervention HDL-C levels. Increased fruit and vegetable consumption and low SSB intake was associated with larger reductions in LDL-C levels among students with abnormal LDL-C at baseline, and increased PA was associated with greater decreases in SBP among students with abnormal SBP at baseline. **CONCLUSIONS AND FUTURE DIRECTIONS:** These results suggest that the changes in the observed physiological measures were not largely driven by changes in health risk behaviors targeted by the PHS intervention. However, PA in this age group may be an effective way to improve HDL-C and TG levels. High risk students, based on their baseline physiological measurements, seemed to benefit the most from the intervention program. Future work should consider including additional assessment points to help determine if behavior change and improvement in physiological measures following the intervention is sustained over a longer period of time.

INTRODUCTION

The early stages of atherosclerotic disease process and progression in children and adolescents are influenced by risk factors such as dyslipidemia, hypertension, and obesity, which can track into adulthood and thereby impact CVD risk in later in life.[78] These risk factors are largely modifiable by lifestyle, specifically diet and PA. Prevention, identification, and management of risk factors early in life may provide a means to lower risk of CVD later in life.

In the U.S, over 95 percent of children and adolescents generally attend school 5 days per week for an average of 6 hours per day throughout most of the calendar year.[219] Schools are in communities of every socioeconomic, racial, and ethnic group and not only teach academic skills, but also provide an environment where cultural expectations and social norms are learned, that in turn can strongly influence health behaviors. The school setting provides an opportunity for implementation of interventions to potentially play an important role in detection and reduction of CVD risk factors in youth.[220]

It is broadly accepted that overweight and obesity, as well as dietary factors and physical inactivity are associated with dyslipidemia and abnormal BP in adolescents.[7] Whether school-based wellness programs are associated with improvement in these risk factors among adolescents is less understood. During the period of growth and maturation that embodies adolescence, often, the primary focus when promoting healthy lifestyle and healthy weight goals involves encouraging increased intake of fruits and vegetables, limiting intake of SSBs and increasing PA.[174,221] These specific behaviors are also 3 of the 5 principal goals of the PHS program, a middle school-based intervention program that promotes healthy lifestyle behaviors, targeting diet and activity levels. To explore these associations further, we used data collected through PHS. We hypothesized that students who achieved or maintained favorable consumption of fruits and vegetables, and SSBs and who achieved or maintained an adequate level of PA following participation in PHS would exhibit improvement in blood lipid and BP levels.

METHODS

Program Description

PHS is a middle-school-based multidisciplinary education program established in collaboration with the University of Michigan Health System, now known as Michigan Medicine, to address adolescent obesity and reduce cardiovascular risk factors.[36] PHS utilizes school-based environmental changes and health education implemented in grade 6, emphasizing the following 5 goals: 1) eat more fruits and vegetables; 2) choose less sugary foods and beverages; 3) eat less fast and fatty foods; 4) be active every day, specifically performing 150 minutes of exercise per week; and 5) spend less time in front of a screen, specifically decreasing time spent with television and computer games while increasing time spent doing enriching activities such as music and reading. The educational component of the program consists of ten, 45-minute learning modules that address the five primary goals. The PHS lessons are evidence-based, derived from programs such as Coordinated Approach to Child Health (CATCH), and developed by University of Michigan expert dietitians, physical activity experts, physicians, and registered nurses.[185] Physiologic measurements which included a non-fasting lipid panel collected via capillary blood specimen, as well as height, weight and blood pressure were collected by trained staff before and after the PHS curriculum through optional health screenings. Each school that participated in PHS was allowed to choose whether to offer the health screening option before and after the PHS curriculum or to complete the curriculum only. In most cases, the cost to implement the health screening portion of PHS was at the responsibility of the participating school.

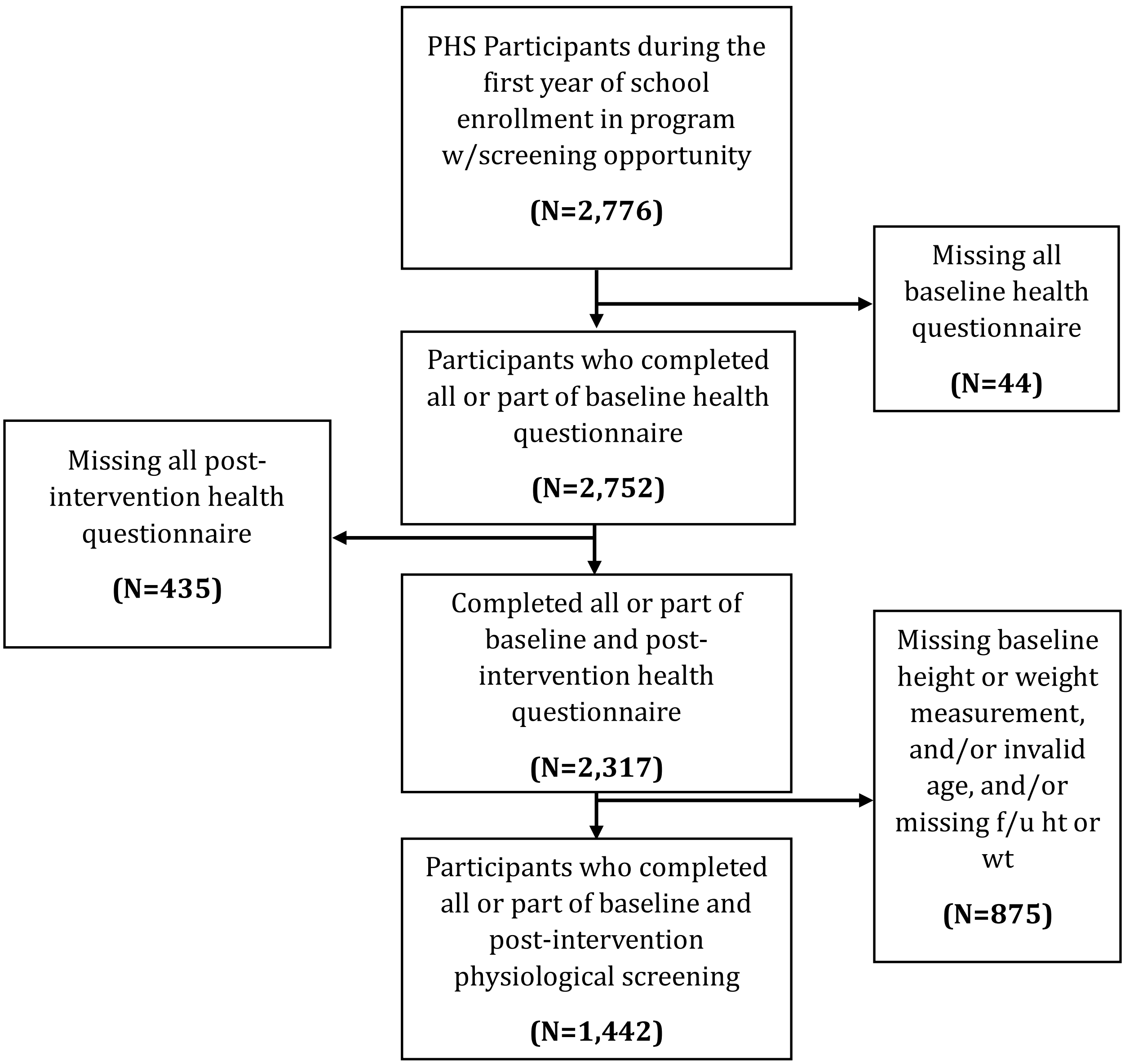
Study Design and Participants

This was a non-randomized, quasi-experimental study, with a pre-post design, where students served as their own controls. Students from 26 middle schools in Michigan participating in PHS and whose school agreed to participate in screening of physiological measures during the first year the school was enrolled in the program between 2005-2019 were eligible for this study. Students participating in this study received an assent form to read prior to completing the health behavior questionnaire (Appendix A.1). Parental and student consent was required to participate in the physiological screening (Appendix A.2, A.3). The PHS protocol was approved by The University of Michigan Institutional Review Board. Secondary data analysis for this project was approved by the Michigan State University Institutional Review Board.

Study Sample

As shown in Figure 10, there were 2,776 students who participated in PHS within its first year of implementation where there was also an opportunity to take part in the health screening of physiological measures. From this population, students who completed the health behavior questionnaire at baseline and follow-up and who also completed all or part of the baseline and post-intervention health screening were eligible to be included in the analysis. Of the 2,776 students included in the sample, 479 (17.2%) were missing all of either the baseline or post-intervention health behavior questionnaire. Among the 2,317 students with partial or complete baseline and post-intervention health behavior questionnaire data, 875 (37.8%) students chose not to participate in the health screening or had missing baseline or post-intervention height or weight measurements, and/or had an invalid or missing age entry. After these exclusions, a total of 1,442 students were eligible to be included in the analysis **(Figure 10)**.

Figure 10: Inclusion of Study Participants Flowchart



Measures:

Health Behavior Measures

Measures of fruit, vegetable, and SSB consumption and PA were collected from a self-reported health behavior questionnaire that was completed before and after implementation of the 10-week PHS curriculum. Teachers were instructed to distribute the questionnaire to students on any school day other than Monday to avoid a weekend effect. The intake of fruits and vegetables was estimated by combining reported intake of the following two questions:

1. "Yesterday, how many times did you eat vegetables? Include all cooked and uncooked vegetables, salads, boiled, baked, and mashed potatoes. Do not count French fries or chips."
2. "Yesterday, how many times did you eat fruit? Fruits are all fresh, frozen, canned or dried fruits. Do not count juice."

SSB intake was estimated by combining reported intake of the following two questions:

1. "Yesterday, how many times did you drink any punch, Kool-Aid®, sports drinks, lemonade, sweet tea, or other fruit-flavored drinks? Do not count 100% fruit juice."
2. "Yesterday, how many times did you drink any regular (not diet) sodas or soft drinks?"

All questions focused on dietary intakes from the previous 24 hours. Responses for the dietary intake questions of interest were on a 4-point Likert scale and included: "none of the time, " "1 time," "2 times," or "3 or more times". PA questions assessed the number of days during the previous week that the student had performed moderate PA for at least 30 or more minutes per day or vigorous PA for at least 20 more minutes per day. From the questionnaire, frequency of PA was ascertained by combing responses to the following questions:

1. "On how many of the past 7 days did you exercise or take part in physical activity that made your heart beat fast and made you breathe hard for at least 20 minutes. (For example: basketball, soccer, running or jogging, fast dancing, swimming laps, tennis, fast bicycling, or similar aerobic activities).
2. "On how many of the past 7 days did you take part in physical activity or exercise for at least 30 minutes where your heart did not beat fast or you did not breathe hard, such as fast walking, slow bicycling, skating, pushing a lawn mower, or household chores?"

Responses for the PA questions of interest included: "0 days, " "1 day," "2 days," "3 days," "4 days," "5 days," "6 days," or "7 days." The responses from both PA questions were combined to provide a reported total number of sessions of PA per week for each student, the highest possible being 14 sessions per week if the student answered "7 days" for both PA questions.

For each behavioral measure, a categorical variable of "met" or "not met" was established based on the goals of the PHS program, national guidelines, and/or previously published research. For fruit and vegetable consumption, students were categorized to the "met" group if they achieved or maintained consumption of fruits and vegetables ≥ 5 times per day, post-intervention or to the "not met" group if they reported consumption of fruits and vegetables < 5 times per day, post-intervention. For SSB consumption, students were categorized to the "met" group if they achieved or maintained consumption of ≤ 1 sugar SSB per day post-intervention or to the "not met" group if they consumed > 1 SSB per day post-intervention. For PA, students were categorized to the "met" group if they achieved or maintained ≥ 5 sessions of moderate (30 min) and/or vigorous (20 min) PA per week, post-intervention or to the "not met" group if they did not achieve or maintain at least 5 sessions of moderate (30 min) and/or vigorous (20 min) PA per week, post-intervention. A variable was also established that combined all 3 goals as "met" or "not met." For all 3 behavioral measures, students were categorized to "met" if they met the goal for fruit and vegetable intake, met the goal for SSB intake, and met the goal for PA, otherwise the student was categorized to "not met."

Physiological Measures

Physiologic measures were collected by trained staff before and after the PHS curriculum. A capillary blood test (finger poke) was used to directly measure a non-fasting lipid profile (total cholesterol [TC], high-density lipoprotein cholesterol [HDL-C], and triglycerides [TG]) in mg/dL using a Cholestech LDX machine. Low density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation (total cholesterol - HDL cholesterol -[triglycerides/5]).[222] A valid LDL-C level was not obtainable on students who measured TG levels of 45 mg/dL or lower, or for levels ≥400 mg/dL. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured in mmHg using a standardized protocol with an automated BP monitor (Mabis Model 04-244-001, Mabis Health Care, Waukegan, IL, USA). After the student had been sitting quietly for 3 to 5 minutes, three measurements were taken, with 2 minutes between each measurement. The final two values for both SBP and DBP were averaged and recorded. Height was measured using a stadiometer and recorded in centimeters (cm) and weight with a standing scale recorded in kilograms (kg). Body mass index (BMI) was calculated for each student. Baseline BMI status was categorized as “underweight/normal” or “overweight/obese” for each student based on cut-points established by the CDC, American Academy of Pediatrics (AAP), Institute of Medicine (IOM), and International Obesity Task Force (IOTF). Underweight, BMI <5th percentile for age and sex; Normal, BMI between the 5th and <85th percentile for age and sex; Overweight, BMI between >85Th and 95th percentile for age and sex; Obese, BMI ≥95th percentile for age and sex.[46] From these established cut-points, students meeting criteria for underweight or normal weight were combined into 1 category, “underweight/normal,” and students meeting criteria for overweight or obese were combined in 1 category, “overweight/obese.”

The physiological measures of interest were also categorized as either “normal” or “abnormal.” Normal and abnormal blood lipid levels were based on cut-points of "acceptable," "borderline," and "abnormal,” which are consistent with guidelines from the National Heart, Lung, and Blood Institute (NHLBI), the American Academy of Pediatrics (AAP), and the American Heart Association/American College of Cardiology (AHA/ACC).[7,74,75] Any student whose lipid level fell in the “acceptable” range was categorized to “normal” and any student whose lipid level was consistent with “borderline” or “abnormal” ranges were categorized as “abnormal.” TC levels <170 mg/dL were considered “normal,” and levels ≥170mg/dL were considered “abnormal.” LDL-C levels <110 mg/dL were considered “normal,” and levels ≥110 mg/dL were considered “abnormal.” HDL-C levels >45 mg/dL were considered “normal,” and levels ≤45 mg/dL were considered “abnormal.” TG levels <90 mg/dL were considered “normal,” and levels

≥90 mg/dL were considered "abnormal." SBP and DBP were categorized as "normal" or "abnormal" based on the 2017 AAP updated definitions for pediatric BP categories, and considered both SBP and DBP together, when determining normal or abnormal status. For students under 13 years of age, BP status for both SBP and DBP was considered "normal" if both systolic and diastolic levels were <90th percentile on the basis of age, sex, and height percentiles. For children ≥13 years of age, a blood pressure of <120/80 mmHg was considered normal. Children under 13 years of age who met criteria for elevated BP (SBP and/or DBP ≥90th percentile but <95th percentile, or 120/80 mmHg to <95th percentile), stage 1 hypertension (SBP and/or DBP ≥95th percentile to <95th percentile + 12 mmHg, or 130/80 to 139/89 mmHg) or stage 2 hypertension (SBP and/or DBP ≥95th percentile + 12 mmHg, or ≥140/90 mmHg), were categorized as "abnormal." Children ≥13 years of age who met criteria for elevated BP (SBP between 120 and 129 with a DBP <80 mmHg), stage 1 hypertension (BP between 130/80 to 139/89 mmHg), or stage 2 hypertension (BP ≥140/90 mmHg), were categorized as "abnormal."[62]

Covariates:

Demographic characteristics (age, sex, race) were assessed at baseline through self-report. Students could self-identify as "male" or "female" for sex, and as either "white," "black," "Asian," "Hispanic," "American Indian," or "other" for race. Median household income was not available for individual students, and therefore, socioeconomic status (SES) was determined based on the median household income for each school's zip code. Tertiles for SES classification were based on federal poverty levels established by the Department of Health and Human Services (HHS) and were categorized as low (median household income <$36,180), middle ($36,180-$48,240) or high (>$48,240).[200] Metropolitan status of "suburban," "urban" or "rural" community type was self-reported by each school on their application to join the program.

Analysis Approach:

Descriptive statistics were used to evaluate the distribution of baseline characteristics, including sex, race, SES, and geographic region for the overall sample of eligible students. Mantel Haenszel chi-square tests were used to compare differences in proportions of demographic characteristics (sex, race, SES, geographic region) and baseline BMI status (underweight/normal, overweight/obese) among students who met or did not meet the established goal for each behavioral measure of interest. In addition, chi-square tests were used to compare differences in proportions of demographic characteristics (sex, race, SES, geographic region) among students who consented to participate in the health screening and those that did not.

The mean difference in frequency of fruits and vegetable consumption, SSB consumption, and mean difference in number of weekly sessions of PA from baseline to follow-up according to status of meeting or not meeting the established goal for each behavioral measure was assessed. The proportion of students who exhibited change or maintenance in normal or abnormal status for each physiological measure from baseline to follow-up was assessed.

Mean change in each physiological measure from baseline to follow-up was examined by t-tests. T-tests were also used to compared mean change from baseline to follow-up in each physiological measure of interest according to baseline status of "normal" or "abnormal." T-tests were used to compare differences in mean change in each physiological measure according to fruit and vegetable goal status, SSB goal status, PA goal status, and all 3 behavioral goals combined (met, not met). Associations between behavioral factors (fruit and vegetable frequency, SSB frequency, PA frequency and all 3 combined) and post-intervention physiological measurements (TC, LDL-C, HDL-C, TG, SBP, DBP) was assessed using multivariable regression models, adjusted for potential confounding factors based on a priori knowledge that were determined to be associated with both exposure and outcome in the study sample through univariate analysis. From this, secondary analyses were conducted in effort to provide further examination and explanation of results from the multivariable analysis. T-tests were used to compare differences in mean change in each physiological measure between the groups of students who met or did not meet each behavioral goal (fruit and vegetable intake, SSB intake, PA, and all 3 behavioral goals combined) according to baseline status of the physiological measure of interest (normal or abnormal), as well as according to baseline BMI status (underweight/normal or overweight/obese). In addition, the change in categorical status from baseline to follow-up (normal, abnormal) according to meeting or not meeting each behavioral goal of interest was assessed using chi-square tests. The statistical package SAS 9.4 (SAS Institute, Cary NC) was used for all statistical analyses and the significance level was set at $P < 0.05$.

RESULTS

Participant Characteristics

Of the students eligible for this study, 54.2% were female, and 45.8% male. Students who self-identified as white made up 56.9% of the eligible sample, with 22.1% identifying as black, 15.6% identifying as a race other than white or black, and 5.4% with missing race data. The socioeconomic status of the schools in this sample were relatively evenly distributed with 31.7% of students from low SES schools, 39.3% from mid SES schools and 29.0% from high SES schools. Students from schools that identified as being in a rural community made up 46.2% of the sample, with 43.4% from suburban communities and 10.4% from urban communities. Within the eligible population of students, 1,126 completed baseline height and weight measurements for BMI calculation. Among the 1,126 students with BMI measurements at baseline, 74.2% were underweight or normal weight and 25.8% were overweight or obese according to the definitions used to categorize BMI **(Table 9)**.

Table 9: Demographic Characteristics including BMI status at Baseline for Overall Sample

Demographic Characteristics	**Overall Eligible Sample N=1446 N (%)**
Sex	
Male	662 (45.8)
Female	783 (54.2)
Missing	1 (<0.01)
Race	
White	823 (56.9)
Black	319 (22.1)
Other	225 (15.6)
Missing	79 (5.4)
School SES	
Low	459 (31.7)
Mid	568 (39.3)
High	419 (29.0)

Table 9 (cont'd)

Community Type	
Suburban	628 (43.4)
Urban	150 (10.4)
Rural	668 (46.2)
Baseline BMI Status	**Available N = 1,126**
Underweight/Normal	836 (74.2)
Overweight/Obese	290 (25.8)

There were significant differences between the students who consented to participate the health screening and those that did not according to demographic characteristics. Females were more likely to consent to participate, as were white students. Students from schools of low SES and urban populations were more likely not to consent to participate in the screening (Appendix C, Table C.1).

Characteristics according to meeting nutritional and PA goals

There were significant differences in the racial and SES characteristics of students who met the fruit and vegetable goal compared to those that did not. Differences in race were largely drive by a larger proportion of students who identified as black who did not meet the fruit and vegetable goal compared to the sample that did meet the goal, 25.1% vs. 17.0% respectively. In addition, more students who identified as "other" race met the fruit and vegetable goal compared to those that did, 21.2% vs. 15.9%. Differences noted between the groups by school SES were largely driven by fewer students from low SES schools meeting the goal, 22.8%, compared with 34.2% of students from low SES schools who did not meet the fruit and vegetable goal **(Table 10)**.

Table 10: Demographic Characteristics of Students According to Status of Meeting or Not Meeting Established Fruit and Vegetable Intake Goal

Demographic Characteristics	Fruit and vegetable goal=Met[1] N(%)	Fruit and vegetable goal=Not Met[2] N(%)	P-value[3]
Sex	**(n=224)**	**(n=1173)**	0.54
Male	107 (47.8)	534 (45.5)	
Female	117 (52.2)	639 (54.5)	
Race	**(n=212)**	**(n=1108)**	0.02
White	131 (61.8)	654 (59.0)	
Black	36 (17.0)	278 (25.1)	
Other	45 (21.2)	176 (15.9)	
School SES	**(n=224)**	**(n=1174)**	0.0005
Low	51 (22.8)	402 (34.2)	
Mid	87 (38.8)	449 (38.3)	
High	86 (38.4)	323 (27.5)	
Community Type	**(n=224)**	**(n=1174)**	0.18
Suburban	110 (49.1)	507 (43.2)	
Urban	18 (8.0)	129 (11.0)	
Rural	96 (42.9)	538 (45.8)	
Baseline BMI Status[4]	**(n=177)**	**(n=909)**	0.63
Underweight/Normal	129 (72.9)	678 (74.6)	
Overweight/Obese	48 (27.1)	231 (25.4)	

[1]"Met" defined as student achieved or maintained consumption of fruits and vegetables ≥ 5 times per day, post-intervention.
[2]"Not met" defined as student reported consuming fruits and vegetables < 5 times per day, post-intervention.
[3]P-values from chi-square tests comparing differences in proportions of demographic characteristics among students who achieved or did not achieve established goal for fruit and vegetable consumption.
[4]BMI, Body Mass Index. Categories based on cut-points established by The Centers for Disease Control and Prevention (CDC), American Academy of Pediatrics (AAP), Institute of Medicine (IOM), and International Obesity Task Force (IOTF). Underweight, BMI <5th percentile for age and sex; Normal, BMI between the 5th and <85th percentile for age and sex; Overweight, BMI between >85Th and 95th percentile for age and sex; Obese, BMI ≥95th percentile for age and sex.

When considering frequency of SSB consumption, there were significant differences between students who met or did not meet the goal according to sex, race and school SES. A greater proportion of female students met the SSB goal, 57.4% compared to 47.0% of those that did not meet the SSB goal. Alternatively, male students made up 42.6% of the population that met the SSB goal and 53.0% of the population that did not meet the SSB goal. The racial differences noted between the groups were largely driven by the distribution of black students. Fewer black students met the SSB goal than expected, 20.6% compared with a higher proportion of black students not meeting the goal than was expected at 30.0%. The proportion of students who met vs. did not meet the SSB goal from low SES schools was 34.6% compared to 43.6%, with 38.7% vs. 37.0% for mid SES and 26.7% vs. 19.4% for high SES. There were no differences in the distribution of community type or baseline BMI status between the groups **(Table 11)**.

Table 11: Demographic Characteristics of Students According to Status of Meeting or Not Meeting Established SSB Intake Goal

Demographic Characteristics	**SSB goal=Met[1] N(%)**	**SSB goal=Not Met[2] N(%)**	**P-value[3]**
Sex	**(n=942)**	**(n=468)**	0.0002
Male	401 (42.6)	248 (53.0)	
Female	541 (57.4)	220 (47.0)	
Race	**(n=882)**	**(n=450)**	0.0005
White	553 (62.7)	240 (53.3)	
Black	182 (20.6)	135 (30.0)	
Other	147 (16.7)	75 (16.7)	

Table 11 (cont'd)

School SES	**(n=943)**	**(n=468)**	<0.0001
Low	252 (34.6)	204 (43.6)	
Mid	365 (38.7)	173 (37.0)	
High	326 (26.7)	91 (19.4)	
Community Type	**(n=943)**	**(n=468)**	0.30
Suburban	428 (45.4)	198 (42.3)	
Urban	92 (9.8)	57 (12.2)	
Rural	423 (44.8)	213 (45.5)	
Baseline BMI Status[4]	**(n=725)**	**(n=376)**	0.47
Underweight/Normal	535 (73.8)	285 (75.8)	
Overweight/Obese	190 (26.2)	91 (24.2)	

[1] "Met" defined as student achieved or maintained consumption of SSBs ≤1 times per day, post-intervention.
[2] "Not Met" defined as student reported consuming SSBs >1 time per day, post-intervention.
[3]P-values from chi-square tests comparing differences in proportions of demographic characteristics among students who achieved or did not achieve established goal for sugar sweetened beverage (SSB) consumption.
[4]BMI, Body Mass Index. Categories based on cut-points established by The Centers for Disease Control and Prevention (CDC), American Academy of Pediatrics (AAP), Institute of Medicine (IOM), and International Obesity Task Force (IOTF). Underweight, BMI <5th percentile for age and sex; Normal, BMI between the 5th and <85th percentile for age and sex; Overweight, BMI between >85Th and 95th percentile for age and sex; Obese, BMI ≥95th percentile for age and sex.

There were significant differences in the racial, socioeconomic, community type, and baseline BMI status distributions between students who met or did not meet the established goal for PA. The racial differences were largely driven by the distribution of black students between the groups with 22.5% among those who met the goal compared with 30.8% among those who did not meet the goal. The differences in school SES between the groups were largely driven by a more students from low SES schools not meeting the PA goal vs. meeting the PA goal, 45.1% vs. 30.0%, and more students from high SES schools meeting the goal vs. not meeting the goal, 32.3% vs. 16.7%.The distribution of students from urban schools explained the majority of the differences noted by school community type with only 8.9% and 18.5% meeting and not meeting the PA goal, respectively. A greater percentage of students who met the PA goal were underweight or normal weight at baseline compared to students who did not meet the PA goal, 75.5% vs. 66.8%. Consequently, a higher proportion of students who were overweight or obese at baseline did not meet the PA goal compared to those that did meet the PA goal, 33.2% vs. 24.5%, respectively. There were no differences in the sex distribution between the groups **(Table 12)**.

Table 12: Demographic Characteristics of Students According to Status of Meeting or Not Meeting Established Physical Activity Goal

Demographic Characteristics	**Physical Activity Goal=Met[1] N(%)**	**Physical Activity Goal=Not Met[2] N(%)**	**P-value[3]**
Sex	**(n=1122)**	**(n=264)**	0.57
Male	523 (46.6)	118 (44.7)	
Female	599 (53.4)	146 (55.3)	
Race	**(n=1058)**	**(n=250)**	0.01
White	645 (61.0)	130 (52.0)	
Black	238 (22.5)	77 (30.8)	
Other	175 (16.5)	43 (17.2)	
School SES	**(n=1123)**	**(n=264)**	<0.0001
Low	337 (30.0)	119 (45.1)	
Mid	423 (37.7)	101 (38.2)	
High	363 (32.3)	44 (16.7)	
Community Type	**(n=1123)**	**(n=264)**	<0.0001
Suburban	520 (46.3)	96 (36.4)	
Urban	100 (8.9)	49 (18.5)	
Rural	503 (44.8)	119 (45.1)	

Table 12 (cont'd)

Baseline BMI Status[4]	**(n=877)**	**(n=205)**	0.01
Underweight/Normal	662 (75.5)	137 (66.8)	
Overweight/Obese	190 (24.5)	91 (33.2)	

[1] "Met" "Met" defined as student achieved or maintained ≥ 5 Sessions of moderate (30 min) and/or vigorous (20 min) PA per week, post-intervention.
[2] "Not met" defined as student report of participating in < 5 Sessions of moderate (30 min) and/or vigorous (20 min) PA per week, post-intervention.
[3]P-values from chi-square tests comparing differences in proportions of demographic characteristics among students who achieved or did not achieve established goal for physical activity.
[4]BMI, Body Mass Index. Categories based on cut-points established by The Centers for Disease Control and Prevention (CDC), American Academy of Pediatrics (AAP), Institute of Medicine (IOM), and International Obesity Task Force (IOTF). Underweight, BMI <5th percentile for age and sex; Normal, BMI between the 5th and <85th percentile for age and sex; Overweight, BMI between >85Th and 95th percentile for age and sex; Obese, BMI ≥95th percentile for age and sex.

Considering all 3 behavioral goals, there were significant differences in the racial and socioeconomic characteristics of students between the groups. Specifically, fewer black students and more students of "other" race met the goal for all 3 behaviors than expected. Black students made up 14.7% of the students who met the goal, compared with 25.9% of students who did not meet the goal. Students of "other" race made up 24.3% of students who met the goal, compared with 14.7% of students who did not meet the goal. Fewer students from low SES and more from high SES schools met all 3 goals compared to those that did not, 20.0% vs. 35.2% and 45.5% vs, 27.9%, respectively. There were no other significant differences between the groups according to sex, community type or baseline BMI status **(Table 13)**.

Table 13: Demographic Characteristics of Students According to Status of Meeting or Not Meeting Established Goal for All 3 Behaviors

Demographic Characteristics	**All 3 Behavioral Goals=Met[1] N(%)**	**All 3 Behavioral Goals=Not Met[2] N%)**	**P-value[3]**
Sex	**(n=145)**	**(n=1185)**	0.38
Male	62 (42.8)	552 (46.6)	
Female	83 (57.2)	633 (53.4)	
Race	**(n=136)**	**(n=1117)**	0.004
White	83 (61.0)	647 (57.9)	
Black	20 (14.7)	289 (25.9)	
Other	33 (24.3)	181 (16.2)	
School SES	**(n=145)**	**(n=1186)**	<0.0001
Low	29 (20.0)	418 (35.2)	
Mid	50 (34.5)	438 (36.9)	
High	66 (45.5)	330 (27.9)	
Community Type	**(n=145)**	**(n=1186)**	0.17
Suburban	76 (52.4)	528 (44.5)	
Urban	12 (8.3)	133 (11.2)	
Rural	57 (39.3)	525 (44.3)	
Baseline BMI Status[4]	**(n=117)**	**(n=919)**	0.28
Underweight/Normal	82 (70.1)	687 (74.8)	
Overweight/Obese	35 (29.9)	232 (25.2)	

[1] "Met" defined as student achieved fruit and vegetable consumption goal, SSB consumption goal and PA goal, post-intervention.
[2] "Not met" defined as student did not achieve fruit and vegetable consumption goal, SSB consumption goal and PA goal, post-intervention.
[3]P-values from chi-square tests comparing differences in proportions of demographic characteristics among students who achieved or did not achieve established goal for all 3 behaviors of interest.
[4]BMI, Body Mass Index. Categories based on cut-points established by The Centers for Disease Control and Prevention (CDC), American Academy of Pediatrics (AAP), Institute of Medicine (IOM), and International Obesity Task Force (IOTF). Underweight, BMI <5th percentile for age and sex; Normal, BMI between the 5th and <85th percentile for age and sex; Overweight, BMI between >85Th and 95th percentile for age and sex; Obese, BMI ≥95th percentile for age and sex.

Average change in frequency of dietary intake and PA measures according to behavioral goal status

The mean difference in frequency of fruit and vegetable consumption, SSB consumption, and mean difference in number of weekly sessions of PA from baseline to follow-up differed significantly according to status of meeting or not meeting the established goal for each behavioral measure assessed, $p<0.0001$ for all 3 measures **(Table 14)**. Among students who met the fruit and vegetable intake goal, the mean frequency of consumption at baseline and follow-up was 3.9 and 5.3 times respectively, for a mean difference of 1.4. This was significantly different from students who did not meet the fruit and vegetable intake goal, where the mean reported frequency of consumption at baseline and follow-up was 2.3 and 2.2 times, respectively, for a mean difference of -0.15. Among students who met the SSB intake goal, the mean frequency of consumption at baseline and follow-up was 0.94 and 0.50 times respectively, for a mean difference of -0.44. This was significantly different from students who did not meet the SSB intake goal, where the mean frequency of consumption at baseline and follow-up was 1.9 and 2.8 times, respectively, for a mean difference of 0.85. Among students who met the PA goal, the mean number of weekly sessions at baseline and follow-up was 7.7 and 9.1, respectively for a mean difference of 1.5 sessions. This was significantly different compared to students who did not meet the PA goal, where the mean number of weekly sessions at baseline and follow-up was 5.0 and 2.6, respectively, for a mean difference of -2.3 sessions **(Table 14)**.

Table 14: Mean difference in frequency of fruit and vegetable consumption, SSB consumption, weekly sessions of PA from according to behavioral goal status

Behavioral Goal of Interest	Pre-Intervention Mean	Post-Intervention Mean	Average Change	P-value[7]
Fruits and Vegetables				
Met[1] (n=224)	3.9 (1.6)	5.3 (0.5)	1.4 (1.6)	
Did not meet[2] (n=1174)	2.3 (1.6)	2.2 (1.3)	-0.15 (1.6)	<0.0001
Sugar-Sweetened Beverages				
Met[3] (n=943)	0.94 (1.1)	0.50 (0.5)	-0.44 (1.1)	
Did not meet[4] (n=468)	1.9 (1.5)	2.8 (1.1)	0.85 (1.7)	<0.0001
Physical Activity				
Met[5] (n=1123)	7.7 (3.6)	9.1 (2.9)	1.5 (4.1)	
Did not meet[6] (n=264)	5.0 (3.6)	2.6 (1.3)	-2.3 (3.6)	<0.0001

[1]"Met" defined as student achieved or maintained consumption of fruits and vegetables ≥ 5 times per day, post-intervention.
[2]"Not met" defined as student reported consuming fruits and vegetables < 5 times per day, post-intervention.
[3] "Met" defined as student achieved or maintained consumption of SSBs ≤1 times per day, post-intervention.
[4] "Not Met" defined as student reported consuming SSBs >1 time per day, post-intervention.
[5]"Met" defined as student achieved or maintained ≥ 5 Sessions of moderate (30 min) and/or vigorous (20 min) PA per week, post-intervention.
[6] "Not met" defined as student report of participating in < 5 Sessions of moderate (30 min) and/or vigorous (20 min) PA per week, post-intervention.
[7]P-values from t-tests comparing differences in reported mean behavioral measure according to status of meeting or not meeting established goal for that particular behavioral measure.

The proportion of students whose physiological measure was normal at both the baseline and post-intervention measurement was 63.7% for TC, 77.4% for LDL-C, 53.0% for HDL-C, 39.2% for TG and 60.4% for BP. The proportion of students who whose physiological measure was abnormal at both the baseline and post-intervention measurement was 17.6% for TC, 9.8% for LDL-C, 26.3% for HDL-C, 29.9% for TG and 14.1% for BP. The proportion of students who exhibited a desired change, specifically changing from abnormal status at baseline to normal status post-intervention was 14.0%

for TC, 8.1% for LDL-C, 6.7% for HDL-C, 18.7% for TG and 14.8% for BP. Finally, the proportion of students who exhibited an undesired change, specifically changing from normal status at baseline to abnormal status at follow-up was 4.7% for TC and LDL-C, 14.0% for HDL-C, 12.2% for TG and 10.7% for BP **(Table 15)**.

Table 15: Maintenance or Change in Normal or Abnormal Status in Physiological Measurements from Baseline to Follow-up

	TC (N=1111) N (%)	**LDL-C** (N=823) N (%)	**HDL-C** (N=1111) N (%)	**TG** (N=1083) N (%)	**BP** (N=1263) N (%)
Remained Normal (no change)	707 (63.7)	637 (77.4)	589 (53.0)	425 (39.2)	763 (60.4)
Remained Abnormal (no change)	196 (17.6)	81 (9.8)	292 (26.3)	324 (29.9)	178 (14.1)
Positive change (abnormal to normal)	156 (14.0)	66 (8.1)	74 (6.7)	202 (18.7)	187 (14.8)
Negative change (normal to abnormal)	52 (4.7)	39 (4.7)	156 (14.0)	132 (12.2)	135 (10.7)

Primary Results:

Overall, we observed significant decreases in TC, LDL-C, HDL-C, and TG levels from baseline to follow-up, $p<0.0001$ for all measures. Minimal and insignificant changes were noted in SBP and DBP measurements from baseline to follow-up, $p=0.20$, and 0.07 respectively. Students whose physiological measure met criteria to be categorized as abnormal at baseline experienced significantly larger decreases for all measures other than HDL-C compared to students whose physiological measure met criteria to be categorized as normal at baseline, $p<0.0001$ for TC, LDL-C, TG, SBP, DBP. Students with abnormal HDL-C levels at baseline experienced an average increase of 1.6 mg/dL at follow-up compared to students with normal HDL-C levels at baseline who experienced an average decrease in

HDL-C of 3.6 mg/dl, this difference between the groups according to baseline status of normal or abnormal was statistically significant, p<0.0001 **(Table 16)**.

There were no significant differences in the mean change in TC, LDL-C, TG, SBP, or DBP measures according to fruit and vegetable intake status. Students who met the fruit and vegetable intake goal had a smaller average reduction in HDL-C compared to students who did not meet the fruit and vegetable intake goal, -0.6 mg/dL compared to -2.1 mg/dL, respectively, p=0.04. Similarly, were no significant differences in the mean change in TC, LDL-C, TG, SBP, or DBP measures according to SSB intake status. However, students who met the SSB intake goal had a significantly larger average reduction in HDL-C compared to students who did not meet the SSB intake goal, -2.4 mg/dL compared to -1.1 mg/dL, respectively, p=0.02. Students who met the PA goal experienced a significantly larger average decrease in TG levels compared to students who did not meet the PA goal (-11.8 mg/dL compared 0.2 mg/dL, respectively, p=0.02). There were no other significant differences in mean change in TC, LDL-C, HDL-C, SBP, or DBP measures according to PA status. There were no significant differences in mean change of any physiological measure when comparing students who met all 3 behavioral goals and students who did not **(Table 17)**.

Table 16: Mean Change in Physiological Measures Overall and According to Baseline Status

Physiological Measure[1]	Change baseline to follow-up N, mean change (SD)	P-value[2]	Change according to baseline status N, mean change (SD)		P-value[3]
			Baseline Normal[4,5]	Baseline Abnormal[4,5]	
TC (mg/dL)	N=1111 -6.5 (19.3)	<0.0001	N=759 -1.9 (17.1)	N=352 -16.5 (19.9)	<0.0001
LDL-C (mg/dL)	N=810 -2.8 (18.3)	<0.0001	N=664 -0.4 (16.9)	N=146 -13.9 (18.9)	<0.0001
HDL-C (mg/dL)	N=1111 -1.9 (9.4)	<0.0001	N=745 -3.6 (9.6)	N=366 1.6 (7.9)	<0.0001
TG (mg/dL)	N=1083 -9.4 (63.3)	<0.0001	N=557 13.9 (38.3)	N=526 -34.6 (74.3)	<0.0001
SBP (mmHg)	N=1435 -0.35 (10.5)	0.20	N=933 1.5 (9.1)	N=390 -5.5 (11.9)	<0.0001
DBP (mmHg)	N=1434 -0.40 (8.5)	0.07	N=932 1.1 (7.5)	N=390 -4.7 (9.6)	<0.0001

[1]TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; SBP, systolic blood pressure; DBP, diastolic blood pressure.
[2]P-values from t-tests comparing mean change in mg/dL in physiological measures from baseline to follow-up.
[3]P-values from t-tests comparing differences in mean change in mg/dL according to baseline grouping.
[4]Cutpoints based on guidelines set by NHLBI, National Heart, Lung, and Blood Institute; AAP, American Academy of Pediatrics; and AHA/ACC, American Heart Association/American College of Cardiology. "Abnormal" defined as students with values consistent with "borderline" or "abnormal" readings based on these guidelines.
[5]Blood pressure cut points based on 2017 AAP definitions. "Abnormal" defined as students with values consistent with "elevated," "stage I hypertension," or "stage 2 hypertension" from these guidelines.

Table 17: Mean Change in Physiological Measures from Baseline to Follow-up According to Behavioral Goal Status

Physiological Measure[2]	Mean Change in Physiological Measures Baseline to Follow-up According to Behavioral Goal Status[1] (N, mean change (sd))											
	Fruit and Veg Goal = Met	Fruit and Veg Goal = Not Met	P[3]	SSB Goal = Met	SSB Goal = Not Met	P[3]	PA Goal = Met	PA Goal = Not Met	P[3]	All 3 Goals = Met	All 3 Goals = Not Met	P[3]
TC (mg/dL)	N=167 -6.0 (20.5)	N=906 -6.5 (19.1)	0.78	N=712 -7.4 (19.1)	N=371 -5.1 (19.4)	0.78	N=888 -6.2 (19.1)	N=223 -7.9 (19.9)	0.25	N=107 -5.9 (20.7)	N=919 -6.6 (19.1)	0.76
LDL-C (mg/dL)	N=129 -3.6 (20.1)	N=652 -2.7 (17.9)	0.62	N=534 -3.5 (17.4)	N=257 -1.7 (19.8)	0.20	N=657 -2.4 (17.6)	N=153 -4.8 (20.9)	0.18	N=88 -2.8 (20.7)	N=660 -2.9 (18.1)	0.94
HDL-C (mg/dL)	N=169 -0.6 (8.8)	N=904 -2.1 (9.5)	0.04	N=712 -2.4 (9.2)	N=371 -1.1 (9.6)	0.02	N=891 -1.7 (9.3)	N=220 -2.8 (9.7)	0.13	N=109 -1.0 (9.2)	N=917 -2.1 (9.4)	0.25
TG (mg/dL)	N=166 -15.1 (59.7)	N=880 -8.2 (63.7)	0.18	N=700 -11.5 (59.4)	N=356 -5.9 (70.8)	0.20	N=869 -11.8 (60.9)	N=211 0.2 (17.7)	0.02	N=107 -20.2 (62.7)	N=894 -8.3 (64.0)	0.07
SBP (mmHg)	N=223 -0.4 (10.0)	N=1164 -0.4 (10.6)	0.98	N=939 -0.5 (10.3)	N=461 -0.03 (11.1)	0.40	N=1141 -0.5 (10.8)	N=294 0.4 (9.5)	0.14	N=145 -1.0 (10.3)	N=1177 -0.4 (10.7)	0.51
DBP (mmHg)	N=223 -0.5 (8.5)	N=1163 -0.4 (8.5)	0.90	N=938 -0.4 (8.7)	N=461 -0.4 (8.3)	0.98	N=1140 -0.4 (8.6)	N=294 -0.4 (8.0)	0.96	N=145 -0.8 (8.9)	N=1176 -0.3 (8.5)	0.52

[1]Fruit and Vegetable Goal: "Met" defined as student achieved or maintained consumption of fruits and vegetables ≥ 5 times per day, post-intervention. "Not Met" defined as student reported consuming fruits and vegetables < 5 times per day, post-intervention. SSB Goal: "Met" defined as student achieved or maintained consumption of SSBs ≤1 times per day, post-intervention. "Not Met" defined as student reported consuming SSBs >1 time per day, post-intervention. PA Goal: "Met" defined as student achieved or maintained ≥ 5 Sessions of moderate (30 min) and/or vigorous (20 min) PA per week, post-intervention. "Not met" defined as student report of participating in < 5 Sessions of moderate (30 min) and/or vigorous (20 min) PA per week, post-intervention. All 3 Goals: "Met" defined as student achieved fruit and vegetable consumption goal, SSB consumption goal and PA goal, post-intervention. "Not met" defined as student did not achieve fruit and vegetable consumption goal, SSB consumption goal and PA goal, post-intervention.

Table 17 (cont'd)

[2]TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; SBP, systolic blood pressure; DBP, diastolic blood pressure.
[3]P-values from t-tests comparing differences in mean change in physiological measure according to status of meeting or not meeting behavioral goal.

Multivariable results:

There were no significant differences in any post-intervention physiological measures between students who met the post-intervention fruit and vegetable goal and those that did not meet the goal. This was consistent in the minimally adjusted models as well as the multivariable adjusted models for all outcomes of interest. Students who met the post-intervention SSB consumption goal experienced lower post-intervention HDL-C levels compared to students who did not meet the SSB goal consumption when adjusted for baseline HDL-C levels, p=0.04. No other differences were noted in post-intervention TC, LDL-C, TG, SBP, and DBP levels by SSB consumption status in either the minimally adjusted models or multivariable adjusted models. Meeting the post-intervention PA goal of 5 or more sessions of moderate and/or vigorous PA per week was associated with a significantly higher post-intervention TC level compared to not meeting the PA goal in the minimally adjusted model, but the association became nonsignificant after adjusting for covariates (baseline TC, race, SES). Similarly, meeting the post-intervention PA goal was associated with a significantly higher post-intervention LDL-C level compared to not meeting the PA goal in the minimally adjusted model, but the association became nonsignificant after adjusting for covariates (baseline LDL-C, SES). Meeting the post-intervention PA goal was associated with a significantly higher post-intervention HDL-C levels compared to not meeting the PA goal when adjusting for baseline HDL-C level, p=0.04. Meeting the post-intervention PA goal was associated with significantly lower post-intervention TG levels compared to not meeting the PA goal in both the minimally adjusted model and multivariable adjusted model, p=0.006, p=0.01 respectively. There were no significant differences in any post-intervention physiological measure between students who met the post-intervention goal for all 3 behaviors of interest and students who did not meet the post-intervention goal for all 3 behaviors of interest. This was consistent in the minimally adjusted models as well as the multivariable adjusted models for all outcomes of interest. Only the multivariable adjusted results are provided in **Table 18**, as there were no significant differences to report compared to the minimally adjusted models.

Table 18: Multivariable Adjusted Results of Post-Intervention Physiological Measures According to Behavioral Goal Status

Behavioral Goal of Interest[1]	**Met Fruit and Vegetable Goal**	**Met Sugar Sweetened Beverage Goal**	**Met Physical Activity Goal**	**Met goal for all 3 Behaviors**
Post-intervention Physiological Measure[2]	**Multivariable Adjusted β (SE) P-value**	**Multivariable Adjusted β (SE) P-value**	**Multivariable Adjusted β (SE) P-value**	**Multivariable Adjusted β (SE) P-value**
TC[3] (md/dL)	N = 1017 0.44 (1.46) NS	N=1027 -1.62 (1.11) NS	N=1015 2.54 (1.35) NS	N=970 2.35 (1.79) NS
LDL-C[4] (md/dL)	N=781 -0.59 (1.55) NS	N=791 -1.61 (1.24) NS	N=784 2.33 (1.52) NS	N=748 1.43 (1.84) NS
HDL-C[5] (md/dL)	N=1073 0.96 (0.73) NS	N=1083 -1.13 (0.55) 0.04	N=1071 1.36 (0.69) 0.04	N=1026 0.79 (0.88) NS
TG[3] (md/dL)	N=990 -3.16 (4.61) NS	N=1000 -0.30 (3.54) NS	N=988 -10.86 (4.38) 0.01	N=945 -4.16 (5.62) NS
SBP[4] (mmHg)	N=1387 -0.0008(.67) NS	N=1400 -0.48 (0.53) NS	N=1378 -0.61 (0.63) NS	N=1322 -0.18 (0.81) NS
DBP[5] (mmHg)	N=1386 -0.38 (0.52) NS	N=1398 0.03[6] (0.41) NS	N=1377 -0.26 (0.49) NS	N=1321 -0.23 (0.63) NS

[1]"Met" Fruit and vegetable goal: "Met" defined as student achieved or maintained consumption of fruits and vegetables ≥ 5 times per day, post-intervention., reference category "not met." SSB Goal: "Met" defined as student achieved or maintained consumption of SSBs ≤1 times per day, post-intervention, reference "not met." PA Goal: Met" defined as student achieved or maintained ≥ 5 Sessions of moderate (30 min) and/or vigorous (20 min) PA per week, post-intervention, reference category "not met." All 3 goals: "Met" defined as student achieved fruit and vegetable consumption goal, SSB

Table 18 (cont'd)

consumption goal and PA goal, post-intervention, reference category "not met" (achieved 2 or fewer behavioral goals post-intervention).
[2] TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; SBP, systolic blood pressure; DBP, diastolic blood pressure. Sample sizes differed for each outcome of interest due to missing values.
[3] Multivariable model adjusted for baseline outcome measure, race, SES.
[4] Multivariable model adjusted for baseline outcome measure, SES.
[5]Adjusted for baseline outcome measure. No other covariates considered in multivariable analysis based on our criteria for selection.
[6] Multivariable model adjusted for baseline outcome measure, sex.

Secondary analyses results:

Among students with normal HDL-C levels at baseline, those that met the fruit and vegetable goal experienced a smaller average reduction in HDL-C levels compared to students who did not meet the fruit and vegetable goal, (p=0.04), however no differences in HDL-C between the groups were noted when HDL-C level was abnormal at baseline, p=0.80. Among students with abnormal LDL-C levels at baseline, there were significantly larger reductions in LDL-C levels noted among students who met the fruit and vegetable goal compared to students who did not meet the goal, p=0.03. There were no other differences in physiological outcomes according to fruit and vegetable intake status when considering baseline normal or abnormal status of the physiological measure of interest **(Table 19)**.

Table 19: Mean Change in Outcome Measures According to Fruit and Vegetables Goal Status and Baseline Status of each Physiological Measure of Interest

	Baseline Normal[7,8] N, mean change (sd)			**Baseline Abnormal**[7,8] N, mean change (sd)		
Physiological Measure[1]	Fruit and Veg Goal = Met[2]	Fruit and Veg Goal = Not Met[3]	P[5]	Fruit and Veg Goal = Met[2]	Fruit and Veg Goal = Not Met[3]	P[6]
TC (mg/dL)	N=117 -0.9 (17.3)	N=614 -1.8 (17.1)	0.80	N=50 -17.9 (20.5)	N=292 -16.3 (19.5)	0.60
LDL-C (mg/dL)	N=102 -1.4 (16.6)	N=536 -0.5 (16.9)	0.30	N=27 -22.2 (21.3)	N=116 -12.4 (19.1)	0.03
HDL-C (mg/dL)	N=109 -1.8 (9.1)	N=609 -3.8 (9.7)	0.04	N=60 1.8 (7.9)	N=295 1.6 (7.9)	0.80
TG (mg/dL)	N=80 15.1 (38.4)	N=460 13.7 (37.6)	0.80	N=86 -43.1 (62.4)	N=420 -32.1 (76.7)	0.15
SBP (mmHg)	N=160 1.0 (8.6)	N=741 1.6 (9.3)	0.50	N=49 -5.6 (12.7)	N=328 -5.5 (12.0)	0.99
DBP (mmHg)	N=160 0.9 (6.6)	N=740 1.2 (7.7)	0.60	N=49 -5.5 (11.7)	N=328 -4.6 (9.4)	0.60

[1]TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; SBP, systolic blood pressure; DBP, diastolic blood pressure.
[2]"Met" defined as student achieved or maintained consumption of fruits and vegetables ≥ 5 times per day,

Table 19 (cont'd)

post-intervention.
[3]"Not met" defined as student reported consuming fruits and vegetables < 5 times per day, post-intervention.
[4]P-values from t-tests comparing differences in mean change in physiological measure according to fruit and vegetable consumption status.
[5]P-values from t-tests comparing differences in mean change in physiological measure according to fruit and vegetable consumption status among those with normal baseline status of physiological measure of interest.
[6]P-values from t-tests comparing differences in mean change in physiological measure according to fruit and vegetable consumption status among those with abnormal baseline status of physiological measure of interest.
[7]Cutpoints based on guidelines set by NHLBI, National Heart, Lung, and Blood Institute; AAP, American Academy of Pediatrics; and AHA/ACC, American Heart Association/American College of Cardiology. "Abnormal" defined as students with values consistent with "borderline" or "abnormal" readings based on these guidelines.
[8]Blood pressure cut points based on 2017 AAP definitions. "Abnormal" defined as students with values consistent with "elevated," "stage I hypertension," or "stage 2 hypertension" from these guidelines.

Among students with abnormal LDL-C levels at baseline, there were significantly larger reductions in LDL-C levels noted among students who met the SSB goal compared to students who did not, p=0.04. There were no other differences in physiological outcomes according to SSB intake status when considering baseline normal or abnormal status of the physiological measure of interest **(Table 20)**.

Table 20: Mean Change in Outcome Measures According to SSB Goal Status and Baseline Status of each Physiological Measure of Interest

	Baseline Normal[7,8] N, mean change (sd)			**Baseline Abnormal**[7,8] N, mean change (sd)		
Physiological Measure[1]	SSB Goal = Met[2]	SSB Goal = Not Met[3]	P[5]	SSB Goal = Met[2]	SSB Goal = Not Met[3]	P[6]
TC (mg/dL)	N=476 -2.1 (16.8)	N=260 -1.4 (17.2)	0.60	N=236 -18.0 (19.2)	N=111 -13.8 (21.4)	0.08
LDL-C (mg/dL)	N=433 -0.5 (15.5)	N=212 -0.1 (19.4)	0.80	N=101 -16.2 (19.3)	N=45 -8.9 (20.3)	0.04
HDL-C (mg/dL)	N=485 -4.1 (9.4)	N=243 -2.8 (9.9)	0.09	N=227 1.0 (7.6)	N=128 2.2 (8.1)	0.17
TG (mg/dL)	N=354 11.7 (31.6)	N=191 17.6 (46.9)	0.12	N=346 -35.1 (70.7)	N=165 -33.1 (83.1)	0.80
SBP (mmHg)	N=613 1.5 (8.8)	N=298 1.6 (9.7)	0.84	N=260 -5.7 (11.8)	N=118 -5.5 (12.6)	0.98
DBP (mmHg)	N=612 1.2 (7.5)	N=298 1.0 (7.6)	0.65	N=260 -4.7 (10.0)	N=118 -4.8 (9.2)	0.92

Table 20 (cont'd)

[1]TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; SBP, systolic blood pressure; DBP, diastolic blood pressure.
[2] "Met" defined as student achieved or maintained consumption of SSBs ≤1 times per day, post-intervention.
[3] "Not met" defined as student reported consuming SSBs >1 time per day, post-intervention.
[4]P-values from t-tests comparing differences in mean change in physiological measure according to SSB consumption status.
[5]P-values from t-tests comparing differences in mean change in physiological measure according to SSB consumption status among those with normal baseline status of physiological measure of interest.
[6]P-values from t-tests comparing differences in mean change in physiological measure according to SSB consumption status among those with abnormal baseline status of physiological measure of interest.
[7]Cutpoints based on guidelines set by NHLBI, National Heart, Lung, and Blood Institute; AAP, American Academy of Pediatrics; and AHA/ACC, American Heart Association/American College of Cardiology. "Abnormal" defined as students with values consistent with "borderline" or "abnormal" readings based on these guidelines.
[8]Blood pressure cut points based on 2017 AAP definitions. "Abnormal" defined as students with values consistent with "elevated," "stage I hypertension," or "stage 2 hypertension" from these guidelines.

Among students with a normal TG level at baseline, those who met the PA goal experienced larger reductions in TG levels compared with students who did not meet the PA goal, p=0.03. This trend was not present when TG levels were abnormal at baseline. Among students with abnormal SBP at baseline, those who met the PA goal experienced significantly larger reductions in SBP from baseline to follow-up compared to students who did not meet the PA goal, p=0.04. No other differences between students who met and did not meet the PA goal were noted in any physiological measure when considering baseline status **(Table 21)**.

Table 21: Mean Change in Outcome Measures According to PA Goal Status and Baseline Status of each Physiological Measure of Interest

	Baseline Normal[7,8] N, mean change (sd)			**Baseline Abnormal**[7,8] N, mean change (sd)		
Physiological Measure[1]	PA Goal = Met[2]	PA Goal = Not Met[3]	P[5]	PA Goal = Met[2]	PA Goal = Not Met[3]	P[6]
TC (mg/dL)	N=597 -1.5 (16.9)	N=162 -3.4 (17.9)	0.23	N=291 -15.9 (19.9)	N=61 -19.9 (20.1)	0.16
LDL-C (mg/dL)	N=540 -0.2 (16.1)	N=124 -1.3 (20.1)	0.55	N=117 -12.6 (20.2)	N=29 -19.7 (17.5)	0.06
HDL-C (mg/dL)	N=598 -3.3 (9.4)	N=147 -4.7 (10.1)	0.13	N=293 1.7 (8.0)	N=73 1.1 (7.6)	0.60

Table 21 (cont'd)

TG (mg/dL)	N=447 11.9 (36.2)	N=110 21.8 (45.0)	0.03	N=422 -36.9 (70.9)	N=104 -22.6 (86.4)	0.12
SBP (mmHg)	N=742 1.6 (9.2)	N=191 1.3 (8.9)	0.75	N=314 -6.0 (12.3)	N=76 -3.2 (10.3)	0.04
DBP (mmHg)	N=741 1.2 (7.6)	N=191 0.9 (7.2)	0.61	N=314 -4.6 (9.9)	N=76 -5.1 (8.3)	0.65

[1]TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; SBP, systolic blood pressure; DBP, diastolic blood pressure.

[2] "Met" defined as student participated in ≥ 5 Sessions of moderate and/or vigorous PA per week, post-intervention.

[3] "Not met" defined as student participated in < 5 Sessions of moderate and/or vigorous PA per week, post-intervention..

[4]P-values from t-tests comparing differences in mean change in physiological measure according to status of meeting or not meeting all 3 behavioral goals.

[5]P-values from t-tests comparing differences in mean change in physiological measure according to status of meeting or not meeting all 3 behavioral goals among those with normal baseline status of physiological measure of interest.

[6]P-values from t-tests comparing differences in mean change in physiological measure according to status of meeting or not meeting all 3 behavioral goals among those with abnormal baseline status of physiological measure of interest.

[7]Cutpoints based on guidelines set by NHLBI, National Heart, Lung, and Blood Institute; AAP, American Academy of Pediatrics; and AHA/ACC, American Heart Association/American College of Cardiology. "Abnormal" defined as students with values consistent with "borderline" or "abnormal" readings based on these guidelines.

[8]Blood pressure cut points based on 2017 AAP definitions. "Abnormal" defined as students with values consistent with "elevated," "stage I hypertension," or "stage 2 hypertension" from these guidelines.

Among students with abnormal LDL-C levels at baseline, those who met all three combined behavioral goals post-intervention (consumed fruits and vegetables 5 or more times/day, consumed 1 or fewer SSB/day, participated in ≥ 5 sessions of moderate and/or vigorous PA per week) experienced significantly larger reductions in LDL-C levels from baseline to follow-up compared to students who did not meet all 3 combined behavioral goals, p=0.05. Similarly, among students with abnormal TG levels at baseline, those who met all three combined behavioral goals post-intervention experienced significantly larger reductions in TG levels from baseline to follow-up compared to students who did not meet all 3 combined behavioral goals, p=0.04. No other significant differences were noted between the groups **(Table 22)**.

Table 22: Mean Change in Outcome Measures According to Combined Behavioral Goal Status and Baseline Status of each Physiological Measure of Interest

	Baseline Normal[7,8] N, mean change (sd)			**Baseline Abnormal**[7,8] N, mean change (sd)		
Physiological Measure[1]	All 3 Goals = Met[2]	All 3 Goals = Not Met[3]	P[5]	All 3 Goals = Met[2]	All 3 Goals = Not Met[3]	P[6]
TC (mg/dL)	N=69 1.1 (15.6)	N=628 -1.9 (17.1)	0.13	N=38 -18.7 (22.9)	N=291 -16.6 (19.8)	0.60
LDL-C (mg/dL)	N=68 3.3 (15.7)	N=542 -0.7 (17.1)	0.06	N=20 -23.2 (22.6)	N=118 -13.2 (19.1)	0.08
HDL-C (mg/dL)	N=73 -2.0 (9.5)	N=610 -3.8 (9.6)	0.13	N=36 1.1 (8.2)	N=307 1.5 (7.7)	0.81
TG (mg/dL)	N=49 14.9 (31.9)	N=463 13.9 (38.6)	0.84	N=58 -49.8 (67.1)	N=431 -32.2 (76.2)	0.07
SBP (mmHg)	N=99 0.7 (8.8)	N=757 1.7 (9.2)	0.31	N=38 -5.9 (12.5)	N=321 -5.9 (12.1)	0.74
DBP (mmHg)	N=99 0.8 (6.8)	N=756 1.2 (7.7)	0.60	N=38 -5.8 (11.9)	N=321 -4.6 (9.4)	0.56

[1]TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; SBP, systolic blood pressure; DBP, diastolic blood pressure.
[2] "Met" defined as student achieved fruit and vegetable consumption goal, SSB consumption goal and PA goal, post-intervention.
[3] "Not met" defined as student did not achieve fruit and vegetable consumption goal, SSB consumption goal and PA goal, post-intervention.
[4]P-values from t-tests comparing differences in mean change in physiological measure according to status of meeting or not meeting all 3 behavioral goals.
[5]P-values from t-tests comparing differences in mean change in physiological measure according to status of meeting or not meeting all 3 behavioral goals among those with normal baseline status of physiological measure of interest.
[6]P-values from t-tests comparing differences in mean change in physiological measure according to status of meeting or not meeting all 3 behavioral goals among those with abnormal baseline status of physiological measure of interest.
[7]Cutpoints based on guidelines set by NHLBI, National Heart, Lung, and Blood Institute; AAP, American Academy of Pediatrics; and AHA/ACC, American Heart Association/American College of Cardiology. "Abnormal" defined as students with values consistent with "borderline" or "abnormal" readings based on these guidelines.
[8]Blood pressure cut points based on 2017 AAP definitions. "Abnormal" defined as students with values consistent with "elevated," "stage I hypertension," or "stage 2 hypertension" from these guidelines.

We observed some differences in the change in certain physiological outcomes in analysis stratified by baseline BMI status (underweight/normal, overweight/obese). Among students categorized as overweight or obese at baseline, those who met the fruit and vegetable goal experienced significant decreases in LDL-C levels, and less of an increase in DBP compared to students who did not meet the fruit or vegetable goal. Among students categorized as underweight or normal BMI at baseline, all students experienced a decrease in HDL-C levels, however students who met the fruit and vegetable goal experienced a smaller decrease in HDL-C compared to students who did not meet the fruit and vegetable goal (Appendix C, Table C.2). We did not observe any differences in the outcomes of interest between the groups who met or did not meet the SSB goal or the PA goal when stratifying by baseline BMI status (Appendix C, Table C.3 and Table C.4). Considering students who met all 3 behavioral goals and were underweight or normal BMI at baseline, we observed a greater increase in TC levels compared with students who did not meet all 3 behavioral goals. However, among students who were overweight or obese at baseline, those that met all 3 behavioral goals experienced significantly larger decreases in LDL-C, SBP, and DBP levels compared to students who did not meet all 3 behavioral goals (Appendix C, Table C.5).

There were no significant differences between the groups (met vs. not met) when considering overall categorial status change (normal, abnormal) of each physiological measure for behavioral measures of interest (Appendix C, Tables C.6-Table C.9).

DISCUSSION

In this quasi-experimental study, we compared the effects of school-based nutrition and PA interventions on CVD risk factors, namely blood lipid concentrations and BP measures. With respect to our primary objective and hypothesis that students who achieved or maintained favorable consumption of fruits and vegetables, and SSBs and who achieved or maintained an adequate level of PA following participation in PHS would exhibit improvement in blood lipid and BP measurements, our results demonstrate that PA was effective at raising HDL and lowering TG in this population, whereas larger decreases in HDL were noted among students with lowest SSB intake. Greater improvements in some physiological measurements were noted among students with abnormal levels at baseline who also engaged in healthier behaviors targeted by the PHS intervention. These findings suggest that behaviors targeted by the PHS intervention may reduce some CVD risk factors among higher-risk students

Among students whose physiological measurements were categorized as abnormal at baseline, we observed significant improvement in all measurements of interest compared to those students whose physiological measurements were normal at baseline. Additionally, greater improvements in some physiological measurements were noted among students with abnormal levels at baseline who also engaged in healthier behaviors targeted by the PHS intervention. Specifically, we observed significantly greater reductions in LDL-C in students who met the fruit and vegetable intake goal post-intervention compared to those that did not when baseline LDL-C levels were abnormal. This finding was also apparent for students who met the SSB goal compared to those that did not. We also observed significantly larger reductions in SBP measurements among students who met the PA goal compared to those that did not, when SBP was considered abnormal at baseline. These findings suggest that behaviors targeted by the PHS intervention may reduce some CVD risk factors, particularly among higher risk students.

Association of fruit and vegetable intake with CVD risk factors

In our study, consuming fruits, and vegetables 5 or more times per day (across the whole study population) was not associated with significant improvement in blood lipid levels or BP measurements compared to students who consumed fruits and vegetables less than 5 times per day. This finding was not unexpected given that previous studies assessing associations between fruit and vegetable consumption and cardiovascular risk indicators in adolescents have been inconsistent, remain limited, and differ greatly from our study with regard to methodology.[223] A cross-sectional analysis of data from the Penn State Young Women's Health Study, reported a significant inverse association between fruit and fruit juice consumption and the ratio of TC to HDL-C, but no differences according to vegetable intake.[224] A study of NHANES data which examined the relationship of metabolic syndrome (clustering of 3 or more risk factors such as waist circumference ≥90th percentile for age/sex, fasting blood glucose ≥100 mg/dL (5.6 mmol/L), blood triglycerides ≥110 mg/dL (≥1.2 mmol/L), HDL cholesterol ≤35 mg/dL (0.9 mmol/L), and systolic/diastolic BP ≥90th percentile for height or taking antihypertensive drugs) with diet and PA in adolescents, found a significant inverse association between a healthy eating index (HEI) score for fruits and the risk of developing metabolic syndrome after adjustment for covariates.[225] There are many dissimilarities between these studies and ours, as these studies found associations with fruit intake alone, not fruit and vegetable consumption, and diet and nutrient intakes were measured via prospective 3-day diet record or FFQ.

Conversely, a cross-sectional study that assessed 120 adolescents from 10 to 13 years of age in Brazil found no association between fruit and vegetable intake estimated from a FFQ, and blood lipid levels of TC, LDL-C, HDL-C and TG.[226] A randomized controlled trial of adults aged 25-64 years that assessed the effect of an intervention to increase fruit and vegetable consumption found no significant effect between increased fruit and vegetable consumption and improved blood TC.[227] A small study investigated associations between diet and CVD risk factors, including blood lipids, in 163 16–17 year old adolescents and found that green vegetables and bean consumption, but not total vegetable intake, was inversely associated with TC and LDL-C concentrations.[98]

Our results showing larger decreases in LDL-C levels among those with higher fruit and vegetable intake when LDL-C was elevated at baseline can be considered concordant with previous research when considering the role of fiber in LDL-C reduction. Research has shown that high dietary intake of fiber, including the fiber found in fruits and vegetables, can produce a modest reduction in TC and LDL-C levels in adults. This is especially true when foods rich in fiber replace foods high in saturated fat.[11] There are few studies available which examine the relationship between single nutrients, clusters of nutrients, and/or foods and food groups and blood lipid levels among adolescents. Results from The CARDIA cohort study involving 3031 young (age 18–30 years) black and white adults who were followed up with repeated dietary assessment, found that in white men and women ages 18-30 years, fiber intake was associated with lower serum TG, lower LDL-C, and higher HDL-C.[228] The population of this study was older than the subjects in our study, included a more expansive and sensitive measure of diet via repeated FFQ and had a 10-year follow-up period.

Our study did not find any difference in BP measurements according to fruit and vegetable intake. Previous studies have demonstrated inconsistent results. A cross-sectional study involving a random sample of 794 adolescents from private schools in Brazil reported lower SBP levels in adolescents who consumed fruits and vegetables at least twice per day than those who did not.[229] A 10-year cohort study found a significant inverse association between fruit and vegetable consumption and BP levels in prepubertal children but not in pubertal adolescents.[230] A population-based study out of Norway examined how PA, smoking status and dietary habits were related to overweight, obesity and BP in a population of adolescents, and found that fruit and vegetable intake did not have an independent effect on BP measurements.[231]

The DASH dietary pattern, which advocates the consumption of fruits, vegetables, lean dairy products, whole grains, fish, poultry, and nuts and encourages reduced consumption of red and processed meat and sugary drinks has been shown to be beneficial at improving BP levels in adults, as well as in adolescents.[24,232] The studies assessing the effect of the DASH dietary pattern on BP may not provide valid comparisons to our study, as we are not able to derive a dietary pattern from the health behavior questionnaire used in PHS.

Associations of sugar sweetened beverage intake with CVD risk factors

In our study, we did not find any differences in any physiological measurements of interest between students consuming ≤1 SSB per day and students consuming more than 1 SSB per day, the exception being HDL-C. Our results demonstrated that both groups of students saw a reduction in HDL-C from baseline to follow-up, however the students with lowest SSB consumption had significantly larger decreases in HDL-C. This finding was not expected and is potentially discordant from previous research findings, although findings have been inconsistent. A cross-sectional analysis of 4,880 individuals aged 3 to 11 years from nationally representative sample of US children participating in NAHNES during 1999-2004 examined the relationship between SSB intake and cardiometabolic markers, including concentrations of TC, HDL-C, LDL-C, and TG. This study reported that increased

SSB intake was independently associated with decreased HDL-C concentrations (P<0.001), but not with other blood lipid levels.[233]

A study involving an ethnically diverse cohort of children aged 8–15 years, examined cross-sectional associations between baseline SSB intake and blood lipid concentrations and longitudinal associations between mean SSB intake, changes in SSB intake, and lipid changes over a 12-month period. In the longitudinal analysis, mean SSB intake over 12 months was not associated with lipid changes; however, the 12-month increase in plasma HDL-C concentration was greater among children who decreased their intake by ≥1 serving/week compared with children whose intake stayed the same or increased (SSB HDL). In the cross-sectional analysis, greater SSB intake across tertiles was associated with higher plasma TG concentrations, but no differences in HDL-C.[234]

Sport's drinks were included in our assessment of total SSB consumption, which previous studies suggest may be associated with increased PA, among young adults. Cross-sectional analysis of survey data from the third wave of a cohort study (Project EAT-III: Eating and Activity in Teens and Young Adults) demonstrated that among males and females ages 20-34, weekly sports drink consumption was significantly associated with higher total and moderate-to-vigorous PA.[235] PA can increase HDL-C levels in children and adults. It is unknown if this would help explain the results in our study, a post-hoc analysis of PA between the SSB groups could be helpful. In addition, examination of the total to HDL-C ratios between the SSB groups would be helpful to provide further interpretation with regard to the clinical significance of this finding.

It is also important to note that blood lipid concentrations undergo considerable age and sex-specific changes during physical growth and sexual maturation and may differ significantly between pubertal stages. Specifically, TC, LDL-C, and HDL-C have all been shown to decrease during puberty.[7,236] The hormonal changes associated with pubertal growth spurt and progressive maturation lead to a significantly increased cholesterol requirement which subsequently leads to decreased blood lipid levels.[237] HDL-C levels have been shown to especially decrease during puberty in males, however our study saw an overall decline in HDL-C in both sexes. The pubertal status of students in our sample was not measured, however the average age of our population at baseline was 11.6 years, which is within the average age range of pubertal onset.[238]

In our study, we did not observe any differences in SBP or DBP between the SSB groups. Given the short follow-up period and limited data on a link between SSB consumption and BP, this finding was not unexpected. Findings from other studies have been mixed. A recent study of PHS data compared baseline health behaviors of PHS students with and without abnormal BP and found students with abnormal BP at baseline drank more regular and diet soda than students with normal BP at baseline.[183] A systematic review and meta-analysis summarized studies that evaluated the effects of SSB intake on BP among children and adolescents and found that high SSB consumption was associated with a significant increase in SBP, but not DBP in children and adolescents. However, the authors acknowledged there was significant between-study heterogeneity observed for studies that had evaluated SBP.[239] There are hypothesized mechanisms regarding the role of SSBs and BP regulation independent of weight status, but these require further investigation.[127] Most studies suggest that increased consumption of SSBs leads to weight gain which can in turn raise BP.[129] We did not see any differences in SBP or DBP between the SSB groups according to weight status.

Association of physical activity with CVD risk factors

The summary report prepared by the Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents concludes that there is strong evidence that increases in moderate-to-vigorous PA are associated with lower SBP and DBP, decreased BMI, lower TC levels, lower LDL-C levels, lower TG levels, and higher HDL-C levels in childhood and adolescence.[7] Evidence supporting these recommendations largely come from adult studies demonstrating that daily vigorous activity decreases the risk of CVD, T2DM, reduces BP, and improves fasting lipid profiles.

Literature demonstrating associations between PA and lipid levels among pediatric populations have provided inconsistent results. In our study, participating in at least 5 sessions of moderate or vigorous PA sessions per week was associated with higher HDL-C levels and lower TG levels, with no other differences noted in blood lipid levels or BP measurements. Similar findings were noted in the Atherosclerosis Risk in Communities (ARIC) Study, a cohort study of adults aged 45-65 years who were followed for 9 years and the association between increases in baseline PA on mean change in HDL, LDL, TC, and TG levels was assessed. The ARIC study found increases in the level of activity were associated with increases in HDL and decreases in TG among white participants.[240] This study was done in the adult population and studies within the pediatric population remain limited. Pooled analyses from a meta-analysis examining the effect of school-based PA interventions on cardiometabolic risk factors in children found that school-based PA interventions did not improve lipid profiles for TC, LDL-C, HDL-C, or TG and did not improve SBP. However, improvements in DBP, waist circumference and fasting insulin levels were noted.[241]

We observed larger improvements in SBP levels among students with increased moderate and/or vigorous PA when the baseline level of SBP was abnormal. At the same time, students who did not participate in at least 5 sessions of moderate to vigorous PA per week had larger reductions in LDL-C levels compared to students who participated in at least 5 weekly sessions of PA when their baseline LDL-C was abnormal. This difference did not meet statistical significance but was an unexpected trend. PA intervention studies on lowering BP measurements in adolescents have produced mixed results. In the DISC study, higher self-reported levels of PA were associated with lower SBP measurements.[163] A prospective study in Canada examined the relationships between PA intensity and frequency and the likelihood of having high BP in a population-based cohort of adolescents. They found that adolescents engaging in PA more intense than light during the previous year was associated with a lower odds of having BP in the hypertensive range and the association was not altered after adjusting for BMI.[242] A study of NHANES data sought to examine the dose-response relationship between PA and BP in a sample of 1170 children and adolescents aged 8-17 years and found an inverse dose-response relationship between total and moderate-to-vigorous PA with SBP and DBP. The slopes of the curves were modest indicating a minimal influence of PA on mean BP values, however PA strongly predicted hypertensive values.[243] These studies suggest that increased PA may be most helpful in reducing BP among youth who are high risk with either elevated BP or hypertension.

The majority of students in our sample (80.9%) did meet the PA goal of at least 5 sessions of moderate and/or vigorous activity per week, suggesting that students of this age group are largely participating in PA only at school. Assessment of sedentary behavior in addition to PA may have been a more helpful assessment within this group, as guidelines often recommend increased or sustained levels of PA along with reduction of sedentary behavior.[7] Data from a PA intervention study has shown that short-term PA leads to small but not significant reduction in BP among obese children specifically.[33] Yet, studies suggest that sustained, regular PA is effective in lowering BP in children and adolescents, with evidence supporting a greater reduction with more vigorous activity levels.[32,163,243,244] Longer term follow-up within our study would be helpful to assess if students who sustain optimal amounts of self-reported PA achieve beneficial changes in BP.

Association of all 3 behavioral goals with CVD risk factors

We did not observe any differences in any outcomes of interest when comparing students who met the goal for all 3 behaviors (fruit and vegetable intake, SSB intake and PA) and those that did not. It may be that many students were participating in healthy behaviors, just not all 3. It could be interesting to compare meeting 3 out of 3 behaviors to meeting 0 out of 3, but this may represent only a small sample of students, in fact, in our sample there were zero students who met 0 out of the 3 behavioral goals.

Strengths

A major strength of this is study is that it provides data on a heterogenous population of students from diverse income and demographic backgrounds in Michigan. Our study examines the relationship between healthy behaviors which are targeted by the school-based intervention program and CVD risk factors other than BMI and/or weight changes. Most school-based intervention programs focus on obesity prevention, however there are disadvantages to targeting obesity as a primary outcome measure in adolescents. The Planet Health Study, a randomized controlled trial in Massachusetts involving a similar population to that in PHS, namely, 1295 sixth- and seventh-grade students, saw a reduction in obesity among girls but not among boys. Planet Health did not include laboratory measures of lipids or glucose.[188] During a period of potentially rapid changes in growth, outcome measures other than BMI, such as blood lipids and BP, may be more helpful to examine when assessing the effectiveness of PHS, which our study effectively examined. In addition, the students in our sample were likely in the early stages of puberty and maturation. This is a period when accelerated linear growth may follow weight gain, making BMI changes over a short time period difficult to interpret. There is also some consideration for current school-based programs to decline assessment of weight status among students. The CDC reports that there is not enough evidence for scientists to conclude whether school-based BMI measurement programs are effective at preventing or reducing childhood obesity or whether they cause harm, by either increasing the stigma attached to obesity or increasing pressures to engage in unsafe weight control behaviors.[245] If BMI screening is implemented in the school, the CDC and others recommend consideration of the costs involved, potential negative consequences for students, and existing school-based strategies to support healthy weight-related behaviors and prevent weight-based bullying as well as implementation of safeguards that address the primary concerns raised about such programs.[245,246] The percentage of students in our sample that were categorized as underweight or normal weight based on BMI or overweight/obese were similar for all behavioral exposures other than PA.

Previous research involving PHS data has shown improvement in measures of cardiovascular risk factors, specifically statistically significant decreases in TC, LDL-C, TG and small but significant reduction in BP following participation in the intervention.[37]

However, no studies thus far have examined whether changes in the behaviors that are targeted by the PHS intervention are correlated with improvement in the physiological outcomes. To our knowledge, this is also the first study to investigate if change in physiological measures following participation in a school-based nutrition and PA intervention is different between groups based on meeting or not meeting specified behavioral goals while also considering baseline risk status of the physiological measures. Our study highlights the intervention was successful at affecting healthy behavior change which led to improvement in some physiological measures among students who were classified as at-risk at baseline based on their blood lipid and BP measurements.

Limitations

This study is subject to inherent limitations and potential biases, and any interpretation of these findings should consider the limitations to the data collection strategies utilized. It is possible that participation bias occurred, as the rate of participation in the health screening was just over 60%. Students and parents who consented to participate in the health screening did vary according to certain demographic characteristics as noted in the results above. It is possible these parents and students were more interested in the program and more motivated by the goals of PHS. Similar studies such as the Young FINNS study reported a participation rate of 83% in the initial sample whereas participation rates in the follow-up studies varied between 60 and 80%. There was 79% participation in the CATCH trial, however this involved a younger population of students.[184,247]

A systematic review and meta-analysis investigating the effectiveness of school-based interventions to prevent obesity among children aged 4 to 12 years old in middle-income countries reported characteristics of effective interventions included combined diet and PA interventions, schoolteacher-delivery, duration of >8 months, parental involvement, education sessions and school food modifications.[248] PHS does employ several of these characteristics, namely, a combined diet and PA intervention focus, schoolteacher-delivery of content, education sessions and potential school food modifications. PHS is a 10-week intervention program, which can be considered short term. Studies with longer-term follow-up and with multiple assessments between baseline and the end of the intervention would be an important next step in assessment of the sustainability of the positive achievements gained following the intervention. PHS health screenings were conducted based on the schools' schedules with limited time, and thus, it was not possible to complete repeated measures for each student. After the screening, each student was provided with written documentation of all values, which provided students and parents data to review with the child's pediatrician. It is possible that students with abnormal physiological measurements identified at the baseline screening, implemented lifestyle or other interventions as encouraged by their pediatrician that cannot be accounted for in this study.

Limitations of self-reported information on dietary and PA behaviors are subject to measurement error and recall biases, however these measures are considered to offer a valid assessment of lifestyle behaviors in children and adolescents and are commonly used in similar studies.[188,198] Another limitation of this study is that there is no control group. Given that lipid levels change with normal growth and maturation, a non-intervention control group would be helpful to assess the degree to which the decrease in blood lipid levels could be associated with the intervention vs normal physiological activity. In the same way, pubertal development should be considered when determining screening criteria to identify youths with adverse blood lipid levels. There was no assessment of pubertal development in the PHS sample, which could be very helpful given PHS is implemented predominately in sixth graders.

CONCLUSIONS AND FUTURE DIRECTIONS

The school environment provides an opportunity to reach a large and captive audience of students during highly formative years. School-based nutrition and PA intervention programs offer a valuable tool to encourage young people to adopt healthy behaviors which may carry into adulthood. Likewise, atherosclerotic disease processes can begin early in childhood, making the prevention and detection of CVD risk factors a potentially effective way to predict and prevent adult CVD. This stems from evidence from longitudinal studies that has demonstrated that childhood BP, serum lipid levels, and BMI correlate strongly with values measured in middle age.[4,249] In our study, consumption of fruits and vegetables 5 or more times per day following participation in PHS was not associated with improvement in blood lipid levels or BP measurements, nor was meeting the established goal for all 3 behaviors combined. Contrary to our hypothesis, consuming 1 or fewer SSBs post-intervention was associated with greater decreases in HDL-C levels. In concordance with our hypothesis, participation in 5 or more weekly sessions of moderate and/or vigorous PA was associated with higher post-intervention HDL-C levels and lower TG levels.

Our secondary analyses provided some interesting insights. First, among students with an abnormal (elevated) LDL-C level at baseline, consuming fruits, and vegetables 5 or more times per day was associated with larger reductions in LDL-C post-intervention compared with students who consumed fruits or vegetables 4 or fewer times per day. This finding was also noted among students who consumed 1 or fewer SSBs per day compared with those who consume more than 1 SSB per day. LDL-C is the blood lipid level most strongly associated with atherosclerosis and these changes among students with high-risk LDL-C levels at baseline are promising but require further evaluation. Additional assessment points to the need to capture longitudinal data to determine whether the students maintained these behaviors and if the physiological changes were sustained over a longer period of time. If PHS continues to gather physiological screening data in future cohorts of students, a non-intervention control group could prove to be essential in the evaluation of the effectiveness of the program, especially given the natural decline in blood lipid levels that is observed during adolescence.

CHAPTER 5: IMPACT OF DIETARY CHANGES ON LDL CHOLESTEROL AMONG A POPULATION OF MIDDLE SCHOOL STUDENTS PARTICIPATING IN A SCHOOL-BASED HEALTH INTERVENTION PROGRAM (MANUSCRIPT 3)

ABSTRACT

IMPORTANCE: LDL-C is an important risk factor for atherosclerotic cardiovascular disease and is modifiable by diet. Dietary saturated fatty acids (SFA) and trans-fatty acids (TFA) are strongly linked with LDL-C levels in adults, and reduced consumption of these fatty acids may correlate with a reduced risk of CVD. Dyslipidemia in adulthood is best predicted by childhood LDL-C concentrations and therefore reduction of LDL-C during adolescence via dietary modification may correspond with a reduction of CVD risk factors in adulthood. **OBJECTIVE:** To examine if reported change in overall consumption of high risk, high fat foods and in specific high risk, high fat food groups correspond with change in LDL-C concentrations among a population of 6th grade students participating in a school-based health program in Michigan. **DESIGN, SETTING, AND PARTICIPANTS:** A non-randomized, quasi-experimental pre-post design evaluation of 1,446 eligible students from 26 middle-schools across the state of Michigan enrolled in the first year of a school-based nutrition intervention program between 2005-2019 who completed a behavioral health questionnaire and participated in a physiological health screening. **PRIMARY MEASURES AND APPROACH TO ANALYSIS:** Measures of foods known to be high in SFA and TFA were collected from a self-reported health behavior questionnaire which was completed by participating students at baseline and following completion of a 10-week health intervention program. A non-fasting lipid profile was obtained from which LDL-C concentrations were calculated before and after participation in the PHS intervention. Associations between change in dietary consumption of foods high in SFA and TFA and associated change in LDL-C concentrations was assessed using multivariable generalized linear regression models, adjusted for potential confounding factors. Pairwise comparisons of the differences in means for LDL-C change between the exposure groups for combined intake of high risk, high fat foods were also conducted. **RESULTS:** There was a significant decrease in mean change in LDL-C between students whose intake of foods high in SFA and TFA was high at baseline and low at follow-up compared with students whose intake was high at baseline and high at follow-up, p=0.02. There were no significant differences in mean change in LDL-C according to the specific high risk food groups. The mean LDL-C concentrations at baseline and post-intervention for all exposure groups were within the normal range. These results suggest that changes in mean LDL-C concentrations were predicted by changes in reported dietary consumption of foods high in SFA and TFA, with students whose intake changed from "high" to "low" status experiencing the largest decrease in mean LDL-C. **CONCLUSIONS AND FUTURE DIRECTIONS:** This is first study to demonstrate that favorable change in dietary consumption of foods targeted by a school-based nutrition intervention program correlated with a reduction in LDL-C concentrations among adolescents. It is not known if modest reduction of LDL-C when maintained within normal ranges is beneficial. This lends to the potential impact that school-based programs may have on CVD risk factors during adolescence, at the level of the population.

INTRODUCTION

In adult populations, studies have demonstrated that elevated levels of LDL-C and its components are directly associated with risk for atherosclerotic cardiovascular events.[250] In the U.S, it is estimated that 6.4 percent of children aged 6-19 years have elevated LDL-C concentrations, and the likelihood of adverse lipid levels, including LDL-C, is higher among adolescents with higher BMI.[76,77] Dyslipidemia during childhood and adolescence, especially elevated LDL-C, contributes to early atherosclerosis, which is a risk factor for premature CVD.[7] Dyslipidemias, including adverse concentrations of LDL-C, in childhood often tracks into adulthood with studies demonstrating approximately 50 percent of children with abnormal serum lipoprotein levels will continue to have elevated lipid levels as adults.[7,251-253] Data from the Bogalusa Heart Study demonstrated that adult dyslipidemia was best predicted by childhood LDL-C concentrations. In addition, children with elevated LDL-C experienced higher levels of elevated TC, TG, lower HDL-C, as well as increased prevalence of obesity and hypertension.[253]

Certain dietary nutrients can negatively influence LDL-C levels, specifically saturated fatty acids (SFA) and trans-fatty acids (TFA). Data from the Nurses' Health Study and Health Professional's Follow-up Study have shown that isocaloric replacement of carbohydrate with TFA and/or SFA can increase LDL-C concentrations in adults.[108] Industrial TFA are particularly problematic in that they raise LDL-C concentrations and lower HDL-C concentrations and have proven to induce harmful cardiovascular effects in adult populations.[109,110] Intake of TFA in childhood and adolescence is likely to provoke the atherosclerotic processes that can lead to disease in adulthood. In 2018, industrial TFA were effectively removed from the U.S. food supply following years of encouragement by the scientific community, and after the FDA determined trans-fats were no longer "generally regarded as safe" for consumption.[86,254] Levels of plasma TFA among youth in the U.S. significantly decreased between two time points, 1999-2000 and 2009-2010 according to NHANES data, suggesting dietary intake also declined.[255] This corresponds with a rapid decline of industrial trans-fats in the U.S. food supply between 2005 and 2010.[256] The current study examines intakes of adolescents between 2005 and 2019, with much of the study population sampled between 2006-2014, prior to the trans-fat ban. Prior to the FDA ban on TFA, the chief food sources of TFA in the diets of U.S. adults were stick and full-fat margarine, commercial baked goods, and deep-fried foods.[18]

SFA consumption has historically been linked with increased risk for CVD, however this conclusion is now considered controversial as research has demonstrated divergent associations of different individual SFA with blood lipid levels and clinical endpoints.[115] Certain SFA, such as those found in dairy and red meat, increase LDL-C levels as well HDL-C levels.[110] Studies in adults have consistently shown that higher intakes of processed red meats high in saturated fats is associated with higher cardiovascular risk.[257] A systematic review and meta-analysis that examined the health effects of reducing saturated fat in children, adolescents and young adults aged 2-19, found that reduction of saturated fat intake was associated with a significant reduction in TC, LDL-C and DBP.[21] Older studies have demonstrated similar findings. The DISC study, a randomized controlled clinical trial, assessed the efficacy and safety of lowering dietary intake of total fat, saturated fat and cholesterol to decrease LDL-C levels in children aged 8-10 years. This study found that children randomized to the intervention group experienced significantly lower dietary intake of total fat, saturated fat and cholesterol, which corresponded with a significant decrease in LDL-C, but not with other blood lipid levels, compared with controls.[258] Data from the STRIP trial found that repeated dietary counseling was effective in reducing saturated fat intake and serum LDL-C concentrations from infancy until 19 years of age in both genders.[114] Less is known whether a population-based nutrition intervention in the school setting is effective at achieving dietary change that corresponds with favorable reduction in LDL-C levels.

PHS is a middle-school based nutrition and physical activity intervention program that aims to encourage healthy behaviors as a means to improve cardiovascular risk factors among adolescents. Previous PHS research has demonstrated that students participating in the program experience significantly reduced LCL-C concentrations post-intervention.[37] What is not clear is if dietary behaviors targeted by the intervention were associated with decreases in LDL-C. To explore these associations further, we used data collected through PHS. We hypothesized that favorable change in consumption of foods known to be high in SFA and/or TFA, namely, high risk, high fat foods, among students with high consumption at baseline will result in favorable change in LDL-C levels post-intervention.

METHODS

Program Description:

PHS is a middle-school-based multidisciplinary education program established in collaboration with the University of Michigan Health System, now known as Michigan Medicine, to address adolescent obesity and reduce cardiovascular risk factors.[36] PHS utilizes school-based environmental changes and health education implemented in grade 6, emphasizing the following 5 goals: 1) eat more fruits and vegetables; 2) choose less sugary foods and beverages; 3) eat less fast and fatty foods; 4) be active every day, specifically performing 150 minutes of exercise per week; and 5) spend less time in front of a screen, specifically decreasing time spent with television and computer games while increasing time spent doing enriching activities such as music and reading. The educational component of the program consists of 10, 45-minute learning modules that address the five primary goals. The PHS lessons are evidence-based, derived from programs such as CATCH, and developed by University of Michigan expert dietitians, physical activity experts, physicians, and registered nurses.[36,185] Physiologic measurements were collected by trained staff before and after the PHS curriculum through optional health screenings. Each school that participated in PHS was allowed to choose whether to offer the health screening option before and after the PHS curriculum or to complete the curriculum only. In most cases, the cost to implement the health screening portion of PHS was at the responsibility of the participating school.

Study Design and Participants:

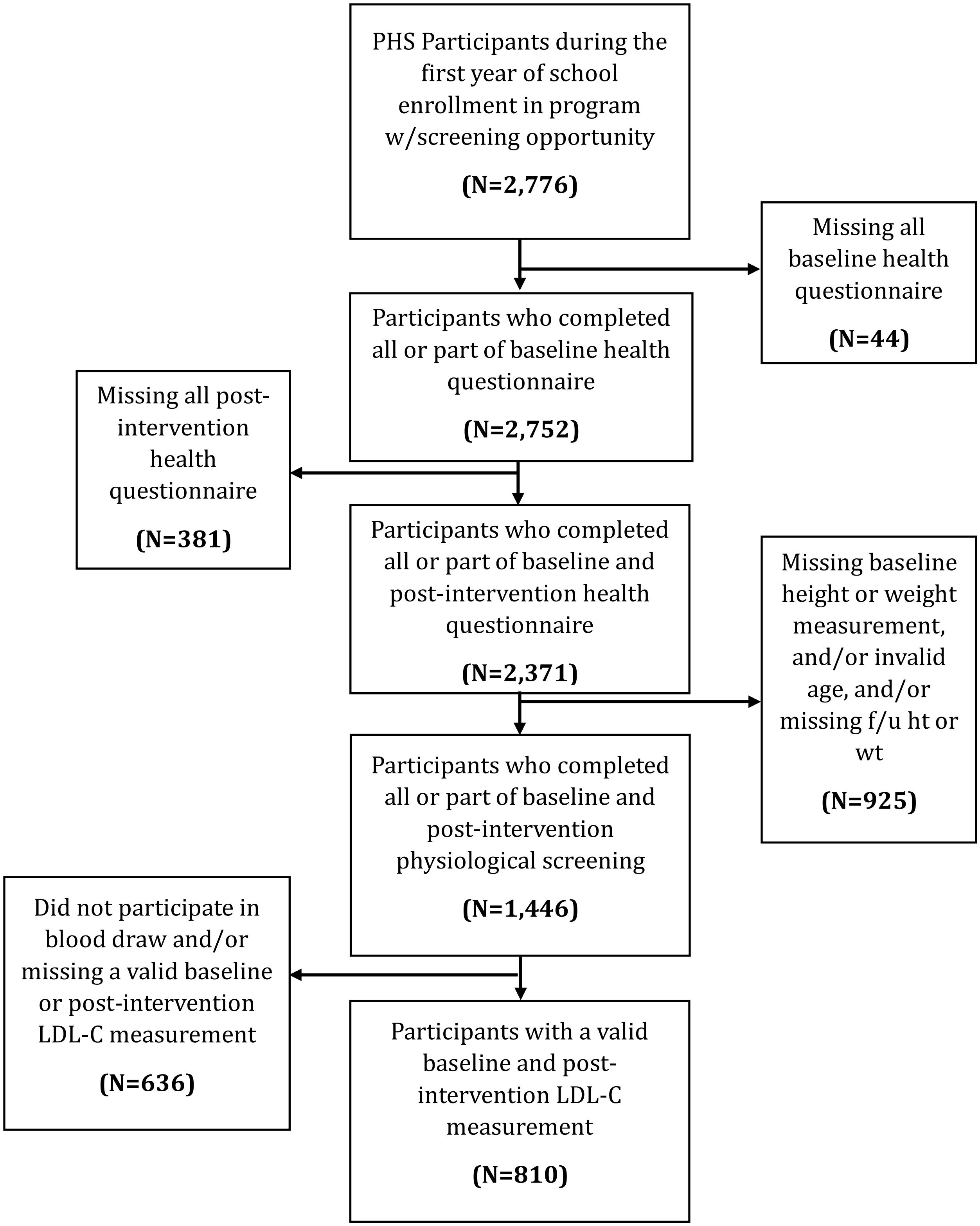
This was a non-randomized, quasi-experimental study, with a pre-post design, where students served as their own controls. Students from 26 middle schools in Michigan participating in PHS and whose school agreed to participate in screening of physiological measures during the first year the school was enrolled in the program between 2005-2019 were eligible for this study. Students participating in this study received an assent form to read prior to completing the health behavior questionnaire (Appendix A.1). Parental and student consent was required to participate in the physiological screening (Appendix A.1, A.2). The PHS protocol was approved by The University of Michigan Institutional Review Board. Secondary data analysis for this project was approved by the Michigan State

University Institutional Review Board.

Study Sample

As shown in figure (flowchart), there were 2,776 students who participated in PHS within its first year of implementation where there was also an opportunity to take part in the health screening of physiological measures. From this population, students who completed the health behavior questionnaire at baseline and follow-up and who also completed all or part of the baseline and post-intervention health screening were eligible to be included in the analysis. Of the 2,776 students included in the sample, 405 (14.6%) were missing all of either the baseline or post-intervention health behavior questionnaire. Among the 2,371 students with partial or complete baseline and post-intervention health behavior questionnaire data, 925 (39.0%) students chose not to participate in the health screening or had missing baseline or post-intervention height or weight measurements, and/or had an invalid or missing age entry. From there, 636 out of the 1,446 eligible chose not to participate in the blood draw, and/or did not have a valid LDL-C measurement. After these exclusions, 810 students had a valid baseline and post-intervention LDL-C measurement and were eligible to be included in the analysis **(Figure 11)**.

Figure 11: Inclusion of Study Participants Flowchart



Measures of high risk, high fat foods known to be high in SFA and/or TFA were collected from a self-reported health behavior questionnaire that was completed before and after implementation of the 10-week PHS curriculum. Teachers were instructed to distribute the questionnaire to students on any school day other than Monday to avoid a weekend effect. The intake of high fat red and processed and fried meats was estimated by combining reported intake of the following two questions:

3. "Yesterday, how many times did you eat hamburger meat, hot dogs, sausage, steak, bacon, or ribs?"
4. "Yesterday, how many times did you eat any fried meat?"

Intake of high fat fried, salty snacks was estimated by the following question:

3. "Yesterday, how many times did you eat French fries or regular chips? Include potato chips, tortilla chips, Cheetos®, corn chips, or other snack chips?"

Intake of high fat baked goods and sweets was estimated by the following question:

1. "Yesterday, how many times did you eat sweet rolls, doughnuts, cookies, brownies, pies or cakes?"

All questions focused on dietary intakes from the previous 24 hours. Responses for the dietary intake questions of interest were on a 4-point Likert scale and included: "none of the time, " "1 time," "2 times," or "3 or more times". All dietary variables of interest (high fat red and processed meat, fried meat, fried and salty snacks and baked goods and sweets) were combined to create a composite variable designed to assess combined exposure to high risk, high fat foods. Students were categorized as a "low" consumer if they reported a combined previous day consumption of the high risk, high fat foods, 3 or fewer times, or a "high" consumer if they reported previous day consumption of 4 or more times. In addition, a dichotomous categorial variable of "low" consumer or "high" consumer was established for each individual dietary variable of interest. For the high fat meat variable, the fried and salty snack variable and the baked goods and sweets variable, students were categorized as a "low" consumer if they reported previous day consumption or 1 or fewer times, or a "high" consumer if they reported previous day consumption of 2 or more times. From there, a 4-level exposure variable was created to account for change in intake from baseline to post-intervention with each student categorized as either of the following: "low to low," if baseline and post-intervention intake of the dietary variable of interest was "low;" "low to high" if baseline intake of the dietary variable of interest was "low" and post-intervention intake was "high;" "high to low" if baseline intake of the dietary variable of interest was "high" and post-intervention intake was "low;" and "high to high" if baseline and post-intervention intake of the dietary variable interest was "high."

Physiological Measures:

LDL-C measures were collected by trained staff before and after the PHS curriculum. A finger poke was used to directly measure a non-fasting lipid profile (total cholesterol [TC], high-density lipoprotein cholesterol [HDL-C], and triglycerides [TG]) in mg/dL using a Cholestech LDX machine. LDL-C was calculated using the Friedewald equation (total cholesterol - HDL cholesterol -[triglycerides/5]).[222] A valid LDL-C level was not obtainable on students who measured triglyceride levels of 45 mg/dL or lower, or for levels ≥400 mg/dL. Height was measured using a stadiometer and recorded in centimeters (cm) and weight with a standing scale recorded in kilograms (kg). Body mass index (BMI) was calculated for each student. Baseline BMI status was categorized as “underweight/normal” or “overweight/obese” for each student as well. BMI categories were based on cut-points established by the CDC, American Academy of Pediatrics (AAP), Institute of Medicine (IOM), and International Obesity Task Force (IOTF). Underweight, BMI <5th percentile for age and sex; Normal, BMI between the 5th and <85th percentile for age and sex; Overweight, BMI between >85Th and 95th percentile for age and sex; Obese, BMI ≥95th percentile for age and sex.[41] From these established cut-points, students meeting criteria for underweight or normal weight were combined into 1 category, “underweight/normal,” and students meeting criteria for overweight or obese were combined in 1 category, “overweight/obese.” LDL-C measures were also categorized as either “normal” or “abnormal.” Normal and abnormal blood lipid levels were based on cut-points of "acceptable," "borderline," and "abnormal,” which are consistent with guidelines from the National Heart, Lung, and Blood Institute (NHLBI), the AAP, and the American Heart Association/American College of Cardiology (AHA/ACC). Any student whose lipid level fell in the “acceptable” range was categorized to “normal” and any student whose lipid level was consistent with “borderline” or “abnormal” ranges were categorized as “abnormal.” LDL-C levels <110 mg/dL were considered “normal,” and levels ≥110 mg/dL were considered “abnormal.”[7,74,75]

Covariates:

Demographic characteristics (age, sex, race) were assessed at baseline through self-report. Students could self-identify as “male” or “female” for sex, and as either “white,” “black,” “Asian,” “Hispanic,” “American Indian,” or “other” for race. Median household income was not available for individual students, and therefore, socioeconomic status (SES) was determined based on the median household income for each school's zip code. Tertiles for SES classification were based on federal poverty levels established by the Department of Health and Human Services (HHS) and were categorized as low (median household income <$36,180), middle ($36,180-$48,240) or high (>$48,240).[200] Metropolitan status of "suburban," "urban" or "rural" community type was self-reported by each school on their application to join the program.

Analysis Approach:

Descriptive statistics and Mantel Haenszel chi-square tests were used to compare differences in proportions of demographic characteristics (sex, race, school SES, metropolitan status) baseline BMI status (underweight/normal, overweight/obese), as well as baseline LDL-C status (normal, abnormal) according to exposure group for the primary exposure variable of change in combined high risk, high fat consumption.

Mean change in LDL-C from baseline to post-intervention according to change in the primary exposure variable of combined intake of high risk, high fat foods were analyzed with one-way analysis of variance (ANOVA) overall, as well as according to baseline LDL-C status (normal, abnormal). Associations between mean change in LDL-C according to change in combined exposure of high risk, high fat foods, as well as the specific high risk, high fat food groups (high fat red and processed meats, and fried meats, high fat snack foods, high fat baked goods and sweets) was assessed using multivariable regression models, adjusted for baseline-LDL status and potential confounding factors based on a priori knowledge that were determined to be associated with both exposure and outcome in the study sample through univariate analysis. In addition, a linear combination of the estimated effects from the generalized linear model was used to estimate the expected change in LDL-C compared to no change, as well as compared to the specific reference group of "low to low." Standard error, 95% confidence intervals, and p-values were reported for all comparisons. Finally, for our primary exposure variable of change in combined intake of high risk, high fat foods, pairwise comparisons of the differences in means for LDL-C change between each group was conducted. From this, descriptive statistics detailing the mean baseline and post-intervention LDL-C levels for each exposure group, as well the proportion of students categorized as having either "normal" or "abnormal" LDL-C levels at baseline and post-intervention according to exposure group were calculated in effort to provide further examination and explanation of results from the multivariable analysis. The statistical package SAS 9.4 (SAS Institute, Cary NC) was used for all statistical analyses and the significance level was set at P <0.05.

RESULTS

Study subject characteristics according to exposure group

We observed significant differences in the racial, school SES, and metropolitan status characteristics of the study participants according to high risk, high fat exposure group, with all p<0.001 **(Table 23)**. Regarding race, we saw a higher number of students than expected who identified as black, in the "low to low" exposure group and in the "high to low" exposure group. We also saw a higher number of students than expected who identified as "other" race in the "low to high" exposure group. Regarding school SES, we saw a higher number of students than expected from high SES schools in the "low to low" exposure group, and a lower number than expected in the "low to high," "high to low," and "high to high" exposure groups. In addition, a higher number of students from mid SES schools were reported in the "low to high" exposure group than was expected, and a higher number of students from low SES schools was reported in the "high to high" exposure group than was expected. Regarding the metropolitan status of the school community, there were more students from schools in rural communities, and fewer students from schools in suburban communities in the "low to high," exposure group than were expected. In addition, there were more students from schools in urban communities in the "high to low," and "high to high" exposure groups than expected. We did not see any differences between the exposure groups according to sex.

We observed significant differences between the exposure groups of our primary exposure variable according to baseline BMI status (underweight/normal, overweight/obese) and baseline LDL-C status (normal, abnormal), p=0.02, and p=0.03, respectively. We observed fewer number of students than expected who were overweight or obese at baseline, and a higher number of students than expected who were underweight or normal weight in the "low to high" exposure group. Finally, we observed a higher number of students than expected with elevated LDL-C at baseline in the "low to low" exposure group, and a lower number of students with elevated LDL-C at baseline in the "low to high," and "high to low" exposure groups than expected.

Table 23: Demographic Characteristics According to Change in Consumption of High-risk, high-fat Foods

Demographic Characteristics	**Change in consumption frequency of high-risk, high fat foods baseline to post-intervention N (%)**				
	Low to Low	**Low to High**	**High to Low**	**High to High**	P-value
Sex					0.3
Male	420 (46.8)	86 (48.6)	79 (40.3)	53 (49.5)	
Female	478 (53.2)	91 (51.4)	117 (59.7)	54 (50.5)	

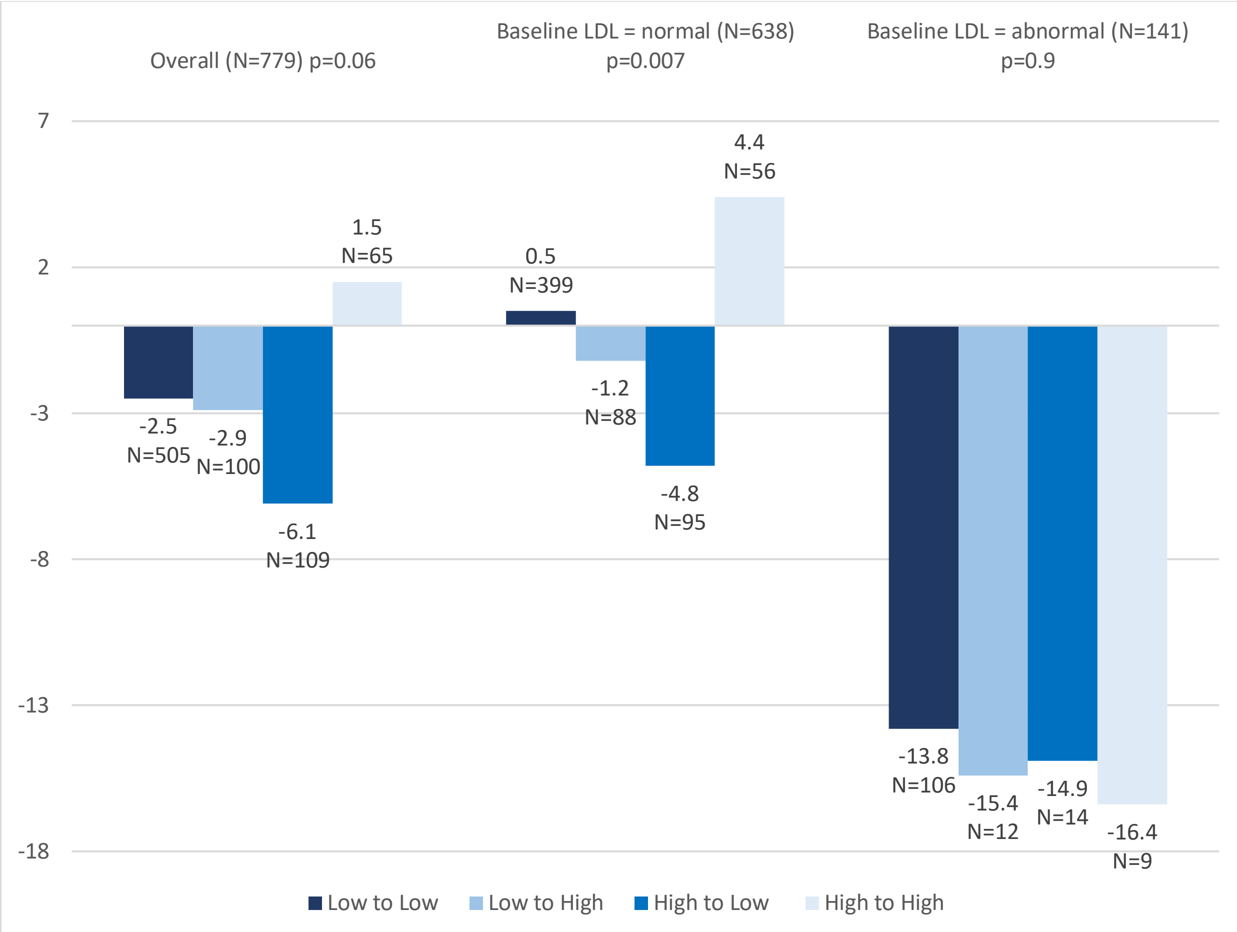
Table 23 (cont'd)

Race					<0.001
White	518 (61.3)	103 (62.8)	97 (51.6)	55 (52.4)	
Black	162 (19.2)	49 (29.9)	64 (34.0)	34 (32.4)	
Other	165 (19.5)	12 (7.3)	27 (14.4)	16 (15.2)	
School SES					<0.001
Low	268 (29.8)	60 (33.7)	75 (38.3)	46 (43.0)	
Mid	307 (34.2)	86 (48.3)	83 (42.3)	41 (38.3)	
High	323 (36.0)	32 (18.0)	38 (19.4)	20 (18.7)	
Community Type					<0.001
Suburban	439 (48.9)	61 (34.3)	76 (38.8)	42 (39.2)	
Urban	78 (8.7)	19 (10.7)	30 (15.3)	21 (19.6)	
Rural	381 (42.4)	98 (55.0)	90 (45.9)	44 (41.1)	
Baseline BMI					0.02
Underweight/Normal	516 (57.9)	122 (68.9)	125 (64.1)	69 (64.5)	
Overweight/Obese	375 (42.1)	55 (31.1)	70 (35.9)	38 (35.5)	
Baseline LDL-C					0.03
Normal	480 (80.9)	104 (89.7)	115 (89.2)	64 (85.3)	
Abnormal	113 (19.1)	12 (10.3)	14 (10.8)	11 (14.7)	

Primary results

Mean differences in LDL-C according to change in intake of high risk, high fat foods overall and according to baseline LDL-C status are presented in **Figure 12**. Mean differences in LDL-C according to change in specific high fat food groups are presented in Appendix D, Figure D.1.

Figure 12: Change in LDL-C in mg/dL according to change in frequency of intake of high risk, high fat foods



We observed differences in mean change in LDL-C between the combined high risk, high fat intake exposure groups, but this difference did not meet statistical significance, p=0.06. Students whose intake changed from "high to low" experienced the largest decrease in LDL-C, an average change of -6.1 mg/dL, whereas students whose intake was "high to high" experienced an average increase in LDL-C of 1.5 mg/dL. Significant differences were observed in mean change in LDL-C between the exposure groups when LDL-C was normal at baseline, p=0.007, but not when LDL-C was abnormal at baseline, p=0.9, where each exposure group experienced large decrease in LDL-C **(Figure 12)**.

Multivariable adjusted generalized linear regression models demonstrated a significant difference in mean change in LDL-C according to change in intake of high risk, high fats foods adjusted for baseline LDL-C status, race and SES, p=0.05. There were no significant differences in mean change in LDL-C according to the specific high risk food groups (high fat red and processed and fried meats, high fat snack foods, high fat baked goods and sweets) **(Table 24)**.

Table 24: Multivariable Adjusted Results of Change in LDL-C According to Change in Consumption Frequency of High-risk, High-fat Foods and Specific High-risk, High-fat Foods Groups

	Change in LDL-C in mg/DL Baseline to Post-intervention[1,2]			
Explanatory Variables	Multivariable Adjusted β (SE) P-value	Multivariable Adjusted β (SE) P-value	Multivariable Adjusted β (SE) P-value	Multivariable Adjusted β (SE) P-value
Change in consumption frequency	High Risk, High Fat Foods[3]	High Fat Meats[4]	High Fat Snacks[4]	High Fat Baked Goods[4]
Low to Low	Ref.	Ref.	Ref.	Ref.
Low to High	-0.8 (2.0)	-0.3 (1.8)	-1.1 (1.3)	0.6 (2.5)
High to Low	-3.9 (1.9)	-1.7 (1.8)	-2.3 (3.3)	1.4 (2.1)
High to High	3.4 (2.3)	1.8 (2.1)	1.5 (2.5)	-1.9 (3.2)
	0.05	NS	NS	NS

[1]LDL-C, low-density lipoprotein cholesterol
[2]Multivariable models adjusted for baseline LDL-C status, race, SES.
[3]"Low" defined as consuming all foods high in fat 3 or fewer times per day. "High" defined as consuming all foods high in fat 4 or more times per day.
[4]"Low" defined as consuming the specific high fat foods 1 or fewer times per day. "High" defined as consuming the specific high fat foods 2 or more times per day.

There were significant changes in mean LDL-C levels across exposure groups "low to low," p=0.0001, and "high to low," p=0.0004, for high risk, high fat consumption when compared to no change in LDL-C, but not for exposure groups "low to high," and "high to high," p=0.16 and p=0.8, respectively. There were significant changes in mean LDL-C levels across exposure groups "low to low," p=0.0002, "low to high," p=0.03, and "high to low," p=0.02, for change in high fat red and processed meat and fried meat consumption when compared to no change in LDL-C, but not for the exposure group “high to high,” p=0.6. We observed significant changes in mean LDL-C levels across exposure groups “low to low,” p=0.009, “low to high,” p=0.0001, and “high to low,” p=0.03, for change in high fat snack food consumption when compared to no change in LDL-C, but not for the exposure group “high to high,” p=0.6. There were significant changes in mean LDL-C levels across exposure groups “low to low,” p=0.0001, for change in high fat baked goods and sweets consumption when compared to no change in LDL-C, but not for the exposure groups “low to high,” “high to low,” or “high to high,” p=0.5, p=0.3, and p=0.08, respectively. There were no significant differences in mean change in LDL-C between exposure groups compared to mean change in LDL-C in the referent “low to low” group for all dietary change variables of interest **(Table 25)**.

Table 25: Least Square Mean Change in LDL-C Across Exposure Groups for Combined and Specific High-risk, High-fat Foods

Explanatory variable	**LDL Change** lsmean (SE)	**95% CI**	**P-value** (A)[1]	**Difference against referent**	**95% CI** (for difference)	**P-value** (B)[2]
High Risk High Fat Foods						
Low to Low	-3.0 (0.8)	-4.6, -1.5	0.0001	-------		
Low to High	-2.5 (1.8)	-5.9, 1.0	0.16	0.5	-3.2, 4.4	0.8
High to Low	-5.9 (1.7)	-9.2, -2.7	0.0004	-2.9	-6.5, 0.7	0.1
High to High	0.5 (2.2)	-3.7, 4.7	0.8	3.5	-0.9, 8.1	0.1
High Fat Meats						
Low to Low	-3.1 (0.8)	-4.8, 2.8	0.0002	-------		
Low to High	-3.4 (1.6)	-6.4, -0.3	0.03	-0.3	-3.7, 3.2	0.9
High to Low	-3.8 (1.6)	-6.9, -0.7	0.02	-0.7	-4.2, 2.8	0.7
High to High	-1.0 (1.9)	-4.8, 2.8	0.6	2.1	-2.0, 6.2	0.3

Table 25 (cont'd)

High Fat Snacks						
Low to Low	-2.5 (0.9)	-4.3, -0.6	0.009	-------		
Low to High	-3.9 (0.9)	-5.7, -2.1	<0.0001	-1.4	-4.0, 1.2	0.3
High to Low	-6.6 (3.1)	-12.8, -0.4	0.03	-4.1	-10.5, 2.4	0.2
High to High	-1.1 (2.2)	-5.5, 3.3	0.6	1.3	-3.4, 6.1	0.6
High Fat Sweets						
Low to Low	-3.3 (0.7)	-4.7, -1.9	<0.0001	-------		
Low to High	-1.7 (2.4)	-6.3, 2.9	0.5	1.7	-3.2, 6.5	0.5
High to Low	-2.0 (2.0)	-5.9, 1.8	0.3	1.3	-2.8, 5.4	0.5
High to High	-5.5 (3.1)	-11.7, 0.7	0.08	-2.2	-8.5, 4.2	0.5

[1]P-values for the test E(Y)=0 in each exposure group at alpha=0.05.
[2]P-values for null difference between exposure group and referent group at alpha=0.05.

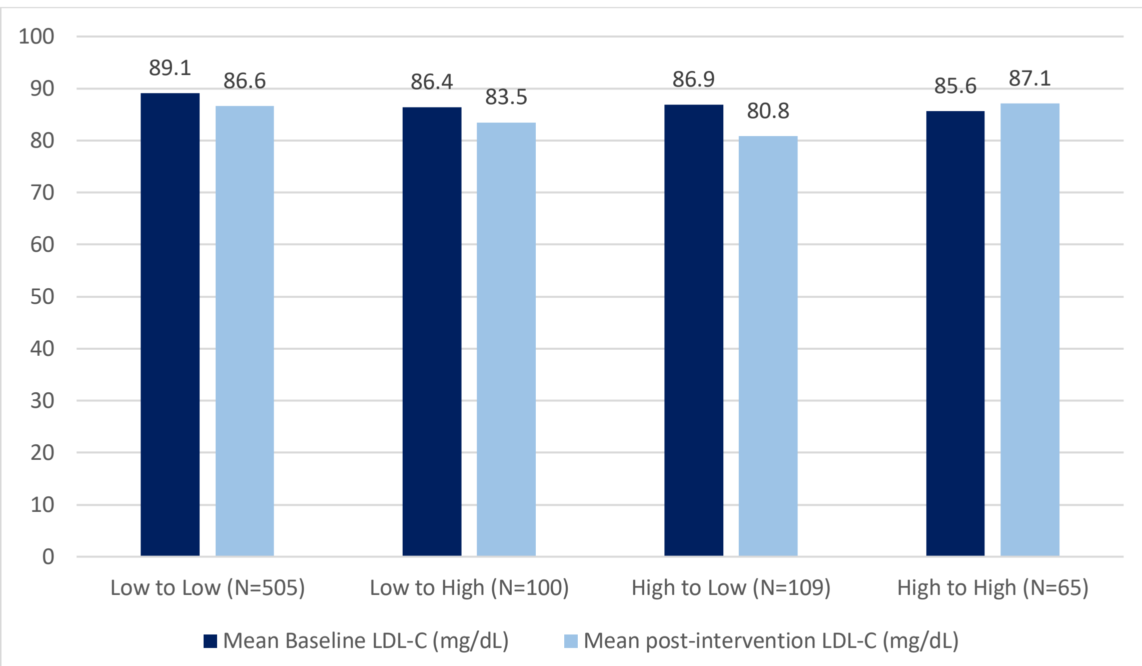
Pairwise comparisons of the difference between means of LDL-C change between the exposure groups of the primary exposure of interest, change in combined high risk, high fat consumption, demonstrated a significant difference in mean change in LDL-C of 6.5 mg/dL between the "high to high" exposure group and the "high to low" exposure group. This difference was statistically significant, p=0.02 (95% C.I. 1.12, 11.8). No other pairwise comparisons were statistically significant **(Table 26)**.

Table 26: Pairwise Comparisons of mean change in LDL-C according to change in High Risk, High Fat Intake

Exposure Group (i)	Exposure Group (j)	Difference Between Means	P-value	95% Confidence Limits for LSMean(i)-LSMean(j)	
High to High	High To low	6.5	0.02	1.1	11.8
High to High	Low to High	2.9	0.3	-2.5	8.5
High to High	Low to Low	3.5	0.1	-0.9	8.1
High to Low	Low to High	-3.5	0.2	-8.2	1.3
High to Low	Low to Low	-2.9	0.1	-6.5	0.7
Low to High	Low to Low	0.6	0.8	-3.2	4.4

For the primary exposure of interest, change in combined intake of high risk, high fat foods, the mean LDL-C levels at baseline and post-intervention for each exposure group are presented in **Figure 13**. The proportion of students with normal and abnormal LDL-C levels at baseline and post-intervention for each exposure group are presented in **Table 27**. Baseline mean LDL-C was highest in the "low to low" exposure group (89.1 mg/dL) and lowest in the "high to high" exposure group (85.6 mg/dL), whereas post-intervention mean LDL-C was highest in the "high to high" exposure group (87.1 mg/dL) and lowest in the "high to low" exposure group (80.8 mg/dL). Mean values for LDL-C at baseline and post-intervention for all exposure groups were in the normal range **(Figure 13)**.

Figure 13: Mean LDL-C in mg/dL at Baseline and Post-Intervention According to Change in Combined High-risk, High-fat Food Consumption



Students in the "low to low" exposure group had the highest percentage of students with abnormal LDL-C at baseline at 21.0%, which decreased to 14.8% post-intervention. Despite experiencing the largest mean decrease in LDL-C, students in the "high to low" exposure group saw a slight increase in the percentage of students with abnormal LDL-C from baseline to post-intervention, 12.8% to 14.7%. The largest increase in the percentage students with an abnormal LDL-C from baseline to post-intervention was among the "high to high" exposure group, namely 13.8% of students had an abnormal LDL-C level at baseline, which increased to 18.5% of students, post-intervention **(Table 27)**.

Table 27: Proportion of students with normal or abnormal LDL-C concentrations at baseline and post-intervention according to change in high risk, high fat food consumption

	Baseline LDL-C Status N (%)		**Post-Intervention LDL-C Status N (%)**	
Exposure Group	**Normal**	**Abnormal**	**Normal**	**Abnormal**
Low to Low	399 (79.0)	106 (21.0)	430 (85.2)	75 (14.8)
Low to High	88 (88.0)	12 (12.0)	88 (88.0)	12 (12.0)
High to Low	95 (87.2)	14 (12.8)	93 (85.3)	16 (14.7)
High to High	56 (86.2)	9 (13.8)	53 (81.5)	12 (18.5)

DISCUSSION

In this quasi-experimental study, we analyzed the effects of change in dietary consumption of foods high in saturated and trans-fat on change in LDL-C concentrations. With respect to our primary objective and hypothesis that students who demonstrated favorable change in reported frequency of intake of high risk, high fat foods following participation in the PHS intervention program would also demonstrate favorable change in LDL-C levels, our results were confirmatory. More specifically, our results showed that reduction in the frequency of consumption of foods known to be high in saturated fat and trans-fat in adolescents with high intake at baseline, was associated with statistically significant reductions in LDL-C, when compared with students whose frequency of intake remained high post-intervention. This association was not present when examining change in consumption of specific high fat food groups. Specifically, change in frequency of intake of high fat red and processed meat and fried meat, high fat snack foods, or high fat baked goods and sweets was not associated with significant change in LDL-C baseline to post-intervention. This study contributes to a body of research which demonstrates advantages of school-based educational health programs, however, is unique in that it offers examination of the effect of targeted interventions on behavior change and cholesterol values in adolescents, which has not been previously reported.

Fat consumption and LDL-C in adolescents

These findings suggest that improvement in combined intake of foods high in SFA and/or TFA, a dietary behavior targeted by the PHS intervention, may be effective at reducing LDL-C concentrations among students with high intake at baseline, but dietary change in one high risk, high fat food group alone is not an effective means to lower LDL-C. Although there are very few studies that have examined the impact of a school-based intervention programs on LDL-C levels in adolescents, studies utilizing different methodologies within different populations do exist to support our findings, as does strong clinical and theoretical evidence. The CATCH trial was a randomized controlled field trial involving 56 intervention and 40 control elementary schools where the objective was to assess the outcomes of health behavior interventions for the primary prevention of cardiovascular disease. Results from the CATCH study demonstrated significant reductions in self-reported daily energy intake from total fat, and saturated fat, among students in the intervention schools compared with control schools, however there were no differences in cholesterol measures.[184]

Previous research has shown that reducing SFA and TFA intake is beneficial in lowering LDL-C, primarily in persons with elevated LDL-C. The DISC study, a six-center randomized controlled clinical trial which examined the safety and efficacy of lowering dietary intake of total fat, saturated fat, and cholesterol on lowering LDL-C levels over 3 years in children aged 8-10 years found that LDL-C levels decreased significantly in children randomized to the intervention group compared to the usual care group (p=0.02).[258] The students in the DISC study had elevated LDL-C levels at baseline, specifically, greater than or equal to the 80th and less than the 98th percentiles for age and sex, therefore the results may not be generalizable to children with normal LDL-C levels. The average LDL-C concentrations in all exposure groups for our primary exposure were considered normal, both at baseline and post-intervention. The DISC study was also conducted over a longer time period, namely 3 years, compared to PHS which is a 10-week intervention program and can be considered short term. The intervention in the DISC study promoted adherence to a diet providing less than 8% of calories from saturated fat and did not assess or focus on specific fat containing foods.[258]

A study examining the effects of a home-based, parent-child autotutorial (PCAT) dietary education program on dietary knowledge, lipid consumption, and LDL-C concentrations of 4- to 10-year-old children with elevated plasma LDL-C found significant reductions in LDL-C levels in the intervention group compared with the at-risk control group ($p<0.05$). This study was relatively small, with 256 children randomized to one of three groups; PCAT program, dietary counseling group, or an at-risk control group, and was 3 months in duration. Mean change in LDL-C levels after program completion did not differ significantly between PCAT intervention group and the dietary counseling group (p=0.07).[259]

In adult populations with elevated LDL-C, diet modification through reduction of saturated fat, trans fat and total calories is advised.[111] Dietary patterns that encourage reduced intake of saturated fat, avoidance of trans fat, but also increased consumption of fiber rich fruits, vegetables, whole grains and legumes, and increased intake of mono- and polyunsaturated fatty acids can improve LDL-C levels by as much as 30 percent, especially in adults with very poor diets at baseline.[260]

In children and adolescents with lipid abnormalities, lifestyle counseling is beneficial for lowering LDL-C.[12,261] Evidence-based recommendations for dietary changes to reduce cardiovascular risk in the pediatric population have been established. Regarding SFA and TFA consumption, when TC and/or LDL-C levels are assessed and determined to be elevated on screening, the Cardiovascular Health Integrated Lifestyle Diet -1 (CHILD-1) supported by the NHLBI recommends that adolescents limit saturated fat intake to 8-10% of daily calories and to avoid trans-fat as much as possible.[261] Our results demonstrate that students who decreased their intake of foods high in saturated and trans-fat following participation in PHS experienced a significant reduction in LDL-C levels compared with students whose intake of these foods remained high. We were not able to ascertain energy intake or percentage of energy consumed by saturated and/or trans-fat from our study, and our sample was not restricted to students with elevated LDL-C.

We did not observe any significant differences in LDL-C according to change in specific high fat foods or food groups. Studies examining the impact of single nutrients or foods on the risk of CVD have been inconclusive. Most studies have demonstrated that dietary modification as a method to reduce LDL-C involves changes to, or adoption of a dietary pattern, with very limited evidence to support that changes in any one food or food group is powerful enough to demonstrate measurable change in lipid levels.[18] A meta-analyses of 36 randomized controlled trials evaluating the effect of red meat on cardiovascular risk factors in adults found that a reduction in dietary intake of red meat alone did not lower LDL-C, however a reduction of red meat intake combined with dietary replacement with high-quality plant-based protein (soy, nuts, legumes) did lead to improved LDL-C.[262]

Data from the Young FINNS study, a longitudinal prospective cohort study Finland, investigated the associations between two major dietary patterns and several risk factors for CVD, including LDL-C. A dietary pattern traditional to Finland was independently associated with LDL-C concentrations, whereas a dietary pattern reflecting more healthful food choices was inversely, but less strongly associated with cardiovascular risk factors, but not with LDL-C.[249]

Findings in Context

It is a potentially important finding that a school-based intervention applied at the level of the population may be an effective means to lower LDL-C concentrations regardless of whether LDL-C is normal or elevated. However, whether reduction of LDL-C in adolescents with normal levels is clinically meaningful, especially in the short term, is not known. Most studies have utilized a risk-based approach when examining the effect of dietary saturated and trans-fat modification on cholesterol levels in youth. The results of this study suggest there may be place for a school-based strategy using a low-risk intervention to try and shift the distribution of a risk factor, even modestly, utilizing measures implemented at the population level rather than targeting only those at high risk.

It should also be noted that the impact of school-based interventions on CVD incidence in adulthood is uncertain and would require well designed, long-term follow-up studies.

Strengths

A major strength of this is study is that it provides data on a heterogenous population of students from diverse income and demographic backgrounds in Michigan. There are limited studies that evaluate the effect of school-based intervention programs and effects of school food environments on metabolic risk factors. Generally, the outcomes assessed in school-based intervention programs involve dietary habits, measures of adiposity, BMI, and/or PA, but often no metabolic risk factors.[263] To our knowledge, this is the first study to examine the effect of change in dietary consumption of foods known to be high in SFA and/or TFA following participation in a school-based nutrition intervention, on change in LDL-C concentrations in adolescents. Modification to LDL-C by means of modest reduced intake of foods known to be high in SFA and/or TFA, but also high in refined carbohydrate, and low in nutrients, throughout a population may produce considerable public health effects.

Limitations

This study is subject to inherent limitations and potential biases, and any interpretation of these findings should consider the limitations to the data collection strategies utilized. PHS health screenings were conducted based on the schools' schedules with limited time, and thus, it was not possible to complete repeated measures for each student. After the screening, each student was provided with written documentation of all values, which provided students and parents data to review with the child's pediatrician. It is possible that students with abnormal LDL-C concentrations at baseline, implemented lifestyle or other interventions as encouraged by their pediatrician that cannot be accounted for in this study. Limitations of self-reported information on dietary behaviors are subject to measurement error and recall biases, including seasonal biases in data collection, which may influence responses related to dietary consumption. This study measured intake of foods often associated as being "unhealthy," and therefore students may have been compelled to misrepresent their intake of these foods in attempt demonstrate a healthier diet. Under-reporting total energy intake among a population of US children and adolescents aged 2-19 who participated in NHANES between 1999-2012 was more common in older adolescents, those who identified as non-Hispanic black and those who were overweight or obese.[264] In our study, under-reporting consumption of foods high in SFA and/or TFA could result in some students who were exposed to high intakes of high risk, high fat foods being misclassified as low consumers. If this misclassification was present, it could be predicted that this would underestimate the association between consumption of high risk, high fat foods and change in LDL-C. Although a validated health questionnaire was utilized to assess dietary intake, it can be considered a relatively crude assessment of dietary consumption which may have been easier for students to complete but precluded the ability to calculate total calories and/or nutrients consumed and dietary patterns overall.

Another limitation of this study is that there is no control group. There are potential factors that can contribute to variability in LDL-C that may not have been accounted for in our study. It has been shown that blood lipids, including LDL-C concentrations, do decrease during periods of growth and maturation. A non-intervention control group would be helpful to assess the degree to which the decrease in LDL-C concentrations could be associated with the intervention vs normal physiological activity. In the same way, pubertal development should be considered when determining screening criteria to identify youths with adverse blood lipid levels. There was no assessment of pubertal development in the PHS sample, which could be very helpful given PHS is implemented predominately in sixth graders. In addition, some variation in an individuals' total, HDL-C and LDL-C is normal and can be expected. Studies in adults have documented that individual cholesterol levels can vary dramatically from week to week while others may remain relatively constant. Changes in the consumption of dietary fat can raise or lower LDL-C as previously described, however individuals can respond somewhat differently to dietary changes. It has been reported that the individual response to a cholesterol-lowering diet can depend on many factors, including but not limited to genetics and BMI, with evidence suggesting that BMI is associated with less response to dietary change in adult populations.[265] Furthermore, differences in the way blood specimens are collected and handled can impact results. Past studies have reported that capillary (finger-stick) samples are more variable than venous samples. Blood lipid levels in our study were collected via capillary (finger-stick) collection. These factors, however, would likely affect the study population in a non-differential manner, although that is not known for.[266]

CONCLUSIONS AND FUTURE DIRECTIONS

School-based nutrition intervention programs, such as PHS, offer an opportunity to encourage healthy behaviors to populations of students during the highly formative adolescent years. Positive behavior change during childhood and adolescence, even modest in nature, may carry into adulthood and provide considerable health benefits. Past studies have suggested it may be more important for this age group to demonstrate the ability of a school-based program to modify nutrition behaviors in ways that lead to habits that are adopted for the lifetime, rather than achieve acute reductions in cardiometabolic outcomes in the short term.[184]

In our study, the primary outcome demonstrated LDL-C concentrations changed significantly across exposure groups of high risk, high fat food consumption. When compared with students whose intake of high risk, high fat foods was high at baseline and post-intervention, students who reported a change in intake of high to low consumption of these foods experienced significant reductions in LDL-C concentrations. Notably, mean LDL-C concentrations were normal for all exposure groups at baseline and post-intervention measurements. As a result, although there were significant differences between the exposure groups in mean change in LDL-C concentrations, the clinical significance may be inconsequential.

Future research should consider what foods replaced the high risk, high fat foods in the students who reduced their consumption. Examination of what replaced those foods is necessary to better understand the impact of diet on LDL-C change. Research has demonstrated that replacement of saturated fat with monounsaturated and/or polyunsaturated fats can lower LDL-C, whereas replacement of saturated fatty acids with polyunsaturated fatty acids alone can lower LDL-C but also is associated with decreased risk for CHD. Replacing foods high in saturated fat with foods high in refined carbohydrates and/or sugar, may still result in lower LDL-C concentrations, but does not improve CVD outcomes, in fact it may worsen outcomes.[112] Further exploration into the patterns of intake of other foods among students who lowered their consumption of high risk, high fat foods would add valuable insights into the results displayed in this study.

CHAPTER 6: SUMMARY

Summary of Findings

Prevention or reduction of CVD risk factors during adolescence is an important and understudied topic, as mitigation of risk factors during this time may reduce CVD risk in adulthood. It is widely recognized that the genesis of obesity and atherosclerotic risk factors are complex and result from poor dietary behaviors and physical inactivity, in addition to a multitude of other factors. Much of the research examining the effectiveness of school-based nutrition and/or physical activity programs has focused on weight-based outcomes such as weight change, BMI, and measures of adiposity. Few studies have compared changes in nutrition and physical activity behaviors following participation in a school-based nutrition and physical activity intervention program and cardiometabolic risk factors in adolescents.

The purpose of this dissertation research was to 1) examine the effectiveness of PHS at achieving favorable change in participating students' dietary consumption of foods and/or beverages associated with cardiovascular disease risk; 2) assess the effectiveness of 3 of the 5 principal goals of PHS on select CVD risk factors among participating students; and 3) determine if change in dietary consumption of foods high in SFA and TFA is associated with change in LDL-C concentrations among students participating in PHS.

With respect to aim 1, we hypothesized that following participation in the PHS program, dietary behaviors that were targeted by the interventions would improve. Our analyses indicated that following participation in the PHS intervention, student's reported intake of fruit and vegetables significantly increased at follow-up compared to baseline. Alternatively, consumption of sugary beverages increased following participation in the nutrition intervention program, and we did not observe any significant changes in consumption of regular soda, red and processed meat, fried and salty snacks, and baked goods and sweets post-intervention. Our results concerning the significant increase noted in fruit consumption and vegetable consumption has been supported by previous research involving a smaller sample of PHS students, as well as in a similar school-based initiative with comparable methodology known as HEROES (Healthy, Energetic, Ready, Outstanding, Enthusiastic Schools).[37,198] All of these studies demonstrated significant, yet very modest increases in fruit and vegetable intake following participation in the school-based wellness program. During a time of significant growth and development, large or drastic changes to dietary intake may not be feasible or even warranted. Members of the scientific community, including the Joint Task Force of the American Society for Nutrition, Institute of Food Technologists, and International Food Information Council, suggest that small changes in specific components of the diet could produce minor but important changes in energy intake, and therefore protect against incremental weight gain over time.[209]

Our results also demonstrated that students in our sample significantly increased their consumption of sugary beverages such as sweet tea, sport's drinks, lemonade and fruit punch, baseline to post-intervention, with no significant change in regular soda consumption noted. The increase in sugary beverage consumption was not expected, but we did note a large proportion of our study sample who were already low or very low consumers at baseline, with the majority reporting increased intake by a frequency of 1. In addition, among the students with very high consumption of sugary beverages at baseline, 73.0% reported a decreased frequency of consumption post-intervention suggesting the intervention may have been most effective in the highest consumers. These findings could also indicate that theory-based education along with environmental changes in the school setting might not be sufficient to reduce children's intake of sugary beverages, which has been supported by previous research.[204,205]

For our second aim, we evaluated the effectiveness of 3 of the 5 principal goals of PHS on CVD risk factors in our study population. Specifically, we examined if favorable change in self-reported fruit and vegetable intake, SSB intake and PA was associated with favorable change in measures of cardiovascular risk, precisely blood lipid levels and BP measurements following participation PHS. Our analyses showed significant decreases in all blood lipid levels from baseline to follow-up and minimal and insignificant changes in SBP and DBP measurements, which is consistent with previously published PHS data.[182] Our primary analyses indicated that students who were more physically active following the PHS intervention had significantly higher post-intervention HDL-C and lower TG levels than students who were less physically active. This finding was especially interesting given that overall, HDL-C concentrations decreased in our sample and considering the expected trends in HDL-C among adolescents. The impact of PA on HDL-C in adults has been supported by a plethora of research, as well as by the known and hypothesized physiological mechanisms by which HDL functions are influenced by PA and/or exercise.[7] Less is known as to whether an increase in HDL-C concentrations due to PA results in decreased CVD risk in adults, let alone in adolescents.[267]

Additionally, our results demonstrated that students with low SSB intake experienced lower post-intervention HDL-C levels compared to students with high SSB intake when adjusted for baseline HDL-C levels. This finding was not expected and is potentially discordant from previous research findings, although findings have been inconsistent.[233-235] Whether this decrease is clinically significant considering the natural decline in HDL-C concentrations that occur during adolescence is not known and would require further exploration.

We also considered the baseline status of students' physiological measurements and found that increased fruit and vegetable consumption and low SSB intake was associated with larger reductions in LDL-C levels among students with abnormal LDL-C at baseline. Correspondingly, increased PA was associated with greater decreases in SBP among students with abnormal SBP at baseline. These results suggest that students considered high risk at baseline may benefit the most from behavioral modification targeted by the PHS intervention. To our knowledge, we are the first study examining the PHS program that has considered the status of the student's physiological measurements in terms of being normal or abnormal. This distinction is useful in establishing the clinical significance of the findings.

For our third and final aim, we sought to examine if favorable change in consumption of high risk, high fat foods and in specific high risk, high fat food groups, following participation in the PHS intervention program corresponded with favorable change in LDL-C levels. We found evidence to support that change in reported intake of high risk, high fat foods was associated with change in LDL-C concentrations. Most notably, our analyses indicated there was a significant difference in mean change in LDL-C between students whose intake of foods high in SFA and TFA was high at baseline and low at follow-up compared with students whose intake was high at baseline and high at follow-up. Previous studies involving PHS students have reported decreases in LDL-C concentrations, however none have examined if LDL-C change was linked with change in dietary consumption of foods known to influence LDL-C levels.[37,182] We did not observe any significant differences in mean change in LDL-C according to the specific high risk food groups, namely, high fat red and processed and fried meats, high fat snack foods, high fat baked goods and sweets. Of particular clinical importance, the mean LDL-C concentrations at baseline and post-intervention for all exposure groups of our primary exposure were within the normal range. It is not known if reduction of LDL-C concentration when levels are normal to begin with offers much clinical benefit on the individual level. However, it could be hypothesized that shifting the distribution of a risk factor such as LDL-C, even modestly, within a population, could offer potential important public health implications.

Strengths

There are a number of strengths of these studies that should be highlighted. First, to our knowledge this one of few studies that examines the relationship between behavior modification following participation in a school-based intervention program and CVD risk factors other than measures obesity such as BMI, weight changes, and/or measures of adiposity. In addition, no studies thus far have examined whether changes in the behaviors that are targeted by the PHS intervention are correlated with improvement in the physiological outcomes assessed. Most school-based intervention programs are funded to focus on obesity prevention, however, given adolescence is a period when weight gain often precedes accelerated linear growth, there are disadvantages to targeting obesity as a primary outcome measure in this age group. Assessment of BMI changes over a short time period may not provide valuable insight in terms of program effectiveness.

To our knowledge, this is also the first study to investigate if change in physiological measures following participation in a school-based nutrition and PA intervention is different across behavioral change exposure groups while also considering baseline risk status of the physiological measures. This assessment provides clearer context from which the clinical significance of the results can be interpreted.

Limitations

This study is subject to inherent limitations and potential biases, and any interpretation of these findings should consider the limitations to the data collection strategies utilized. Bias due to the self-report nature of the health behavior questionnaire cannot be ruled out. PHS did utilize a valid instrument to assess dietary intake behaviors, however, the health behavior questionnaire used in this study can capture components of diet but cannot identify a dietary pattern. In addition, a number of heart-healthy and non-obesogenic foods such as lean proteins (poultry, fish, shellfish, legumes), and healthy fats from consumption of nuts and seeds were not captured on the questionnaire used in this study. The questionnaire also does not measure volume of food and/or beverages consumed, instead it only measures frequency of consumption. These types of questions are likely feasible for students to answer with some accuracy but may result in widely different volumes of food intake being captured in the same response category.

With the assessment of change in dietary consumption, it is valuable to know what food or foods replace those that were decreased in the diet, and in the same way, when a food is increased, what food or foods were replaced is an important consideration. This was not evaluated in this study. This is important when examining diet and cardiovascular risk, as evidence suggests replacing foods high in saturated fat with foods high in sugar does not lower risk of heart disease. This study only evaluates the behavioral exposures and physiological outcomes at 2 time points. It could be helpful to have more than 1 post-intervention survey and screening done to help provide a clearer interpretation of the effectiveness and sustainability of the intervention.

Another limitation of this study is that there is no control group. A non-intervention control group could be very helpful in establishing if dietary and PA behaviors are truly linked to the intervention. In addition, it has been shown that blood lipid levels change with normal growth and maturation, namely, measures often decrease to some degree during puberty. A non-intervention control group would be helpful to assess the degree to which the decrease in blood lipid levels could be associated with the intervention vs normal physiological activity.

Future Directions

For future research, a longer follow-up period with repeated measurements of dietary and PA and cardiovascular outcomes is recommended to determine if the health benefits identified are sustained for a longer term. In addition, a non-intervention control group would be highly valuable given that lipid levels change with normal growth and maturation. In addition, there may be normal variations in dietary intake, as well as physiological measures. A control group would allow for better assessment of the degree to which health behaviors and physiological measures can be attributed to the PHS intervention. Given adolescence is a period of potential rapid growth and development, self-assessment of pubertal stage could be considered as part of the PHS health behavior questionnaire using the Tanner criteria, although this has limitations and gathers information of a sensitive nature and may not be feasible in this setting.

Students in this study were highly physically active, therefore, it may have been more valuable to explore sedentary behaviors and subsequent health outcomes in addition to or instead of just measures of PA. A more sensitive and specific dietary questionnaire would be useful in to help determine the dietary patterns of students participating in PHS. A more extensive and detailed dietary questionnaire such as a FFQ could be considered in a pilot group of students for potential better acquisition of food and nutrient consumption. Similarly, this could potentially allow for better assessment of what foods, and/or nutrients replaced those that changed following the intervention.

Our data suggest that widespread implementation of a school-based nutrition and PA intervention program in middle school children could be an effective way to improve healthy lifestyle behaviors and affect CVD risk factors. Well designed, long-term follow-up studies are necessary to determine if school-based interventions occurring in adolescence are associated with reduced incidence of CVD in adulthood.

Public Health Implications

Healthy dietary and PA behaviors in adolescence can promote optimal health during youth, including positively affecting growth, intellectual development, and preventing a multitude of immediate health problems. Healthy eating patterns and optimal levels of PA during this time may also prevent long-term health problems including cardiovascular diseases, which remain the number one cause of death of U.S. adults. Applying health interventions in the school setting provides an optimal environment for adolescents to learn and adopt long-term healthy eating and activity behaviors, as diet and PA factors provide a substantial contribution to the burden of chronic disease in adults. Positive changes in these behaviors, even if modest in nature, across an entire school-based population, has the potential to confer long-term benefits to cardiovascular health.

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APPENDIX A: STUDENT AND PARENTERAL CONSENT LETTERS

A.1: Student Assent Form; Health Behavior Questionnaire

Dear Student,

The attached questions are designed to help us learn more about your eating and activity habits and how you feel about some other topics. This information will be helpful for a study to figure out if ongoing programs at your school are working. Your answers may help us know how to make programs at your school better.

The research from the answers to this questionnaire may be published so that other people can learn from this work. **No information that is published related to this research will include names or other personal information**; that is, it is unlikely that your answers could ever be linked to you.
You are not required to complete this questionnaire and nothing will happen to you if you do not. Of course, if you have any questions, please do not hesitate to ask.

Thank you so much for your time and assistance with this work. **We really appreciate your efforts.**

Most Sincerely,

Kim A. Eagle, M.D.
Founding Director, MCORRP
Michigan Medicine

A.2: Student Assent Form; Health Screening

For Students ages ~10 to 14

University of Michigan
Assent for Project Healthy Schools

Project Healthy Schools is the name of a program being offered in your school. The program teaches you about healthy eating and exercise habits in 10 fun lessons and activities that are geared just for kids.

There is a research part of the program that is optional. We want to see if you want to take part in it. Research is a way to learn more about something.

The researchers are: Kim Eagle, MD, Elizabeth Jackson, MD, Melissa Boguslawski PhD, MPH, Eva Kline-Rogers, MS, RN

It is **okay** to ask questions about what we are telling you. You can circle or highlight things on this paper you want to know more about. If you don't understand something, just ask us. We want you to ask questions.

If you decide to be in the research part, and you parent or guardian says yes, this is what will happen:

- We will **measure your blood pressure, height, weight and heart rate**
- We will **poke your finger to get a small drop of blood to measure the level of glucose and fats such as cholesterol in your blood**
- We will have you do a ***3* minute step test to test to see how fit you are**
- We will have you complete a **survey** about health behaviors
- We will take these measurements **before the lessons start, after they are completed and then once/year for several years**
- Doing the research part will only take about ½ hour

Some of the things that happen in the research may hurt or could be scary. Some of these things could be:

- Feeling pain or getting a bruise from the finger prick
- Feeling embarrassed about your height or weight measurements
- Feeling tired after the step test

We don't know if you will be helped by this study. We may learn something that will help other children someday. Some ways you could be helped are:

- Feeling smarter about diet and exercise
- Feeling good about helping other kids
- Feeling like you have more energy

You don't have you be in this study if you don't want to. Nobody will be mad at you if you don't want to try it. You can say okay now and you can change your mind later. Just tell the teacher or your parent if you want to stop at any time.

A.3: Parent/Guardian Assent Form; Health Screening

PARENT OR GUARDIAN

CONSENT TO BE PART OF A RESEARCH STUDY

UNIVERSITY OF MICHIGAN

NAME OF STUDY AND REASEARCHERS:

Title of Project: Project Healthy Schools

Principal Investigator: Kim Eagle, MD

Co-Investigators: Elizabeth Jackson, MD, Melissa Boguslawski PhD, MPH, Eva Kline-Rogers, MS, RN

GENERAL INFORMATION

Project Healthy Schools aims to improve the health of middle school students by teaching students about healthy eating and exercise habits. This program is being offered in your child's school. It involves 10 fun and interactive lessons geared to kids. We will also be working with the cafeteria to offer healthier meals and more fresh and local fruits and vegetables. It is important to know if this program is successful. If it is successful, then Project Healthy Schools can be offered at other schools in Michigan and around the country.

Students are also given the opportunity to participate in the research part of the program which includes a free health screening. The screening includes blood pressure, heart rate, height, weight and a 3-minute fitness test. In addition, for those who agree we will measure their cholesterol and blood glucose by taking a tiny bit of blood from a finger poke. The results from these tests will be sent to you. If you choose, you may share the results with your child's doctor. The screening will be given before the program starts and after the lessons are completed. It may also be repeated once a year for several years so we can see what the longer-term impact of the program is.

Students that agree to be part of the research portion of the PHS will have the results of these measurements entered into our confidential database. Once in the database the students will be identified by a code number, not by their name. This will allow us to evaluate the program and see if it's helping students become more healthy. We may also write about the program for possible publication. However, your student's name will never appear in any publication.

The risks to your child are minimal. For those that agree to the finger poke there may be some discomfort from pricking the finger. Sometimes people feel faint or dizzy when they have their finger poked. There is a very low risk of bleeding, bruising or infection. We minimize these risks by having trained personnel performing the finger poke and thoroughly cleaning the finger ahead of time. Sometimes we get an error on the cholesterol reading and need to repeat it.

Most importantly, you and your child could benefit from the discovery of elevated blood pressure or cholesterol by participating in this study. Additionally, students at other schools could benefit from this research if it is shown to be successful at your child's school.

Participation in the research portion of Project Healthy Schools is optional. If your child does not participate in the research portion of this program, they will still receive the educational lessons. If you or your child wishes to discontinue participation in the study, this will be done at any time without penalty to you or your child. If you ever have questions about the study or want your child to leave the study, please tell someone in the "Contact Info" section.

There is no charge to you or your health insurance for the testing in this program and you will not be paid for your child's participation. We have taken steps to minimize risks. But if you believe that your child has been harmed as a result of this study, please, notify the researchers listed on this form in the Contact Section below.

All information collected for this study will be kept confidential. Your child's data is identified in our database only by code number not by name. Results of the measurements will be sent to you and you may choose to share these results with your child's physician. Your child's name will never be used in any publication.

CONTACT INFORMATION

If you have additional questions about this study, want to leave the study before it is finished or express a concern about the study you may contact:

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Institutional Review Board for Human Subjects Research (IRBMED),
2800 Plymouth Road, Building 520, Room 3214
Ann Arbor, MI 48108-2800
Ph: (734)763-4768
Email: irbmed@umich.edu

University of Michigan Compliance Help Line at 1-888-296-2484

APPENDIX B: SUPPLEMENTAL TABLES CHAPTER 3

Table B.1: Characteristics of Students Included and Excluded from Analysis of Fruit Intake

Characteristics	**Complete Case Sample** N (%)	**Excluded Sample** N (%)	**P-value**
Sample Size	8,482	459	
Sex			
Male	4,163 (49.1)	251 (54.7)	0.02
Female	4,239 (50.0)	208 (45.3)	0.05
Missing sex info	80 (0.9)	0 (0%)	0.04
Race			
White	5,392 (63.6)	290 (63.2)	0.87
Black	1,202 (14.2)	44 (9.6)	0.006
Asian	233 (2.7)	9 (1.9)	0.31
Hispanic	386 (4.6)	28 (6.1)	0.12
American Indian	173 (2.0)	14 (3.1)	0.14
Other	619 (7.3)	30 (6.5)	0.54
Missing race info	477 (5.6)	44 (9.6)	0.0004
School level SES			
Low	3,038 (35.8)	190 (41.4)	0.01
Mid	2,897 (34.2)	161 (35.1)	0.68
High	2,547 (30.0)	108 (23.5)	0.003
Metropolitan status			
Suburban	4,320 (50.9)	205 (44.6)	0.009
Urban	955 (11.3)	38 (8.3)	0.05
Rural	3,207 (37.8)	216 (47.1)	<0.0001

Table B.2: Characteristics of Students Included and Excluded from Vegetable Intake

Characteristics	**Complete Case Sample** N (%)	**Excluded Sample** N (%)	**P-value**
Sample Size	8,464	477	
Sex			
Male	4,155 (49.1)	259 (54.3)	0.03
Female	4,229 (50.0)	218 (45.7)	0.07
Missing sex info	80 (0.9)	0	0.03
Race			
White	5,374 (63.5)	308 (64.6)	0.63
Black	1,201 (14.2)	45 (9.4)	0.004
Asian	235 (2.8)	7 (1.5)	0.09

Table B.2 (cont'd)

Hispanic	382 (4.5)	32 (6.7)	0.03
American Indian	175 (2.0)	12 (2.5)	0.51
Other	616 (7.3)	33 (6.9)	0.77
Missing race info	481 (5.7)	40 (8.4)	0.01
School level SES			
Low	3,029 (35.8)	199 (41.7)	0.009
Mid	2,896 (34.2)	162 (34.0)	0.91
High	2,539 (30.0)	116 (24.3)	0.008
Metropolitan status			
Suburban	4,315 (51.0)	210 (44.0)	0.003
Urban	949 (11.2)	44 (9.2)	0.18
Rural	3,200 (37.8)	223 (46.8)	<.0001

Table B.3: Characteristics of Students Included and Excluded from Analysis of Sugar-sweetened Juice and Beverages Intake

Characteristics	**Complete Case Sample** N (%)	**Excluded Sample** N (%)	**P-value**
Sample Size	8573	368	
Sex			
Male	4,197 (49.0)	217 (59.0)	0.0002
Female	4,296 (50.1)	151 (41.0)	0.0006
Missing sex info	80 (0.9)	0 (0)	0.06
Race			
White	5,453 (63.6)	229 (62.2)	0.59
Black	1,191 (13.9)	55 (14.9)	0.57
Asian	242 (2.8)	0 (0)	0.001
Hispanic	391 (4.6)	23 (6.3)	0.13
American Indian	172 (2.0)	15 (4.1)	0.007
Other	625 (7.3)	24 (6.5)	0.58
Missing race info	499 (5.8)	22 (6.0)	0.90
School level SES			
Low	3,068 (35.8)	160 (43.5)	0.002
Mid	2,921 (34.1)	137 (37.2)	0.21
High	2,584 (30.1)	71 (19.3)	<0.0001
Metropolitan status			
Suburban	4,373 (51.0)	152 (41.3)	0.0003
Urban	956 (11.1)	37 (10.1)	0.51
Rural	3,244 (37.9)	179 (48.6)	<0.0001

Table B.4: Characteristics of Students Included and Excluded from Analysis of Regular Soda Intake

Characteristics	Complete Case Sample N (%)	Excluded Sample N (%)	P-value
Sample Size	8,630	311	
Sex			
Male	4,241 (49.1)	173 (55.0)	0.02
Female	4,309 (50.0)	138 (45.0)	0.05
Missing sex info	80 (0.9)	0 (0)	0.09
Race			
White	5,511 (63.9)	171 (55.0)	0.001
Black	1,200 (13.9)	46 (14.8)	0.66
Asian	241 (2.8)	1 (0.3)	0.008
Hispanic	387 (4.5)	27 (8.7)	0.0005
American Indian	176 (2.0)	11 (3.5)	0.07
Other	618 (7.2)	31 (10.0)	0.06
Missing race info	497 (5.7)	24 (7.7)	0.15
School level SES			
Low	3,096 (35.9)	132 (42.4)	0.02
Mid	2,949 (34.2)	109 (35.0)	0.75
High	2,585 (29.9)	70 (22.5)	0.005
Metropolitan status			
Suburban	4,399 (51.0)	126 (40.5)	0.0003
Urban	950 (11.0)	43 (13.8)	0.12
Rural	3,281 (38.0)	142 (45.7)	0.006

Table B.5: Characteristics of Students Included and Excluded from Analysis of Red and Processed Meat Intake

Characteristics	Complete Case Sample N (%)	Excluded Sample N (%)	P-value
Sample Size	8497	444	
Sex			
Male	4,160 (49.0)	254 (57.2%)	0.0007
Female	4,257 (50.0)	190 (42.8%)	0.003
Missing sex info	80 (1.0)	0 (0%)	0.04
Race			
White	5,396 (63.5)	286 (64.4%)	0.70
Black	1,206 (14.2)	40 (9.0)	0.002
Asian	236 (2.8)	6 (1.4)	0.07
Hispanic	389 (4.6)	25 (5.6)	0.30

Table B.5 (cont'd)

American Indian	172 (2.0)	15 (3.4)	0.05
Other	612 (7.2)	37 (8.3)	0.37
Missing race info	486 (5.7)	35 (7.9)	0.05
School level SES			
Low	3,052 (35.9)	176 (39.6)	0.11
Mid	2,904 (34.2)	154 (34.7)	0.83
High	2,541 (29.9)	114 (25.7)	0.05
Metropolitan status			
Suburban	4,328 (50.9)	197 (44.4)	0.007
Urban	959 (11.3)	34 (8.3)	0.02
Rural	3,210 (37.8)	213 (48.0)	<0.0001

Table B.6: Characteristics of Students Included and Excluded from Analysis of Fried and Salty Snack Intake

Characteristics	**Complete Case Sample** N (%)	**Excluded Sample** N (%)	**P-value**
Sample Size	8613	328	
Sex			
Male	4,222 (49.0)	192 (58.5)	0.0007
Female	4,311 (50.1)	136 (41.5)	0.002
Missing sex info	80 (0.9)	0 (0)	0.08
Race			
White	5,485 (63.7)	197 (60.1)	0.18
Black	1,198 (13.9)	48 (14.6)	0.71
Asian	241 (2.8)	1 (0.3)	0.00
Hispanic	394 (4.6)	20 (6.1)	0.20
American Indian	177 (2.1)	10 (3.1)	0.22
Other	624 (7.2)	25 (7.6)	0.80
Missing race info	494 (5.7)	27 (8.2)	0.06
School level SES			
Low	3,089 (35.8)	139 (42.4)	0.01
Mid	2,944 (34.2)	114 (34.8)	0.83
High	2,580 (30.0)	75 (22.8)	0.005
Metropolitan status			
Suburban	4,377 (50.8)	148 (45.1)	0.04
Urban	956 (11.1)	37 (11.3)	0.92
Rural	3,280 (38.1)	143 (43.6)	0.04

Table B.7: Characteristics of Students Included and Excluded from Analysis of Baked Goods and Sweets Intake

Characteristics	**Complete Case Sample** N (%)	**Excluded Sample** N (%)	**P-value**
Sample Size	8,608	333	
Sex			
Male	4,237 (49.2)	177 (53.2)	0.16
Female	4,292 (49.9)	155 (46.5)	0.24
Missing sex info	79 (0.9)	1 (0.3)	0.24
Race			
White	5,487 (63.7)	195 (58.6)	0.05
Black	1,193 (13.9)	53 (15.9)	0.29
Asian	240 (2.8)	2 (0.6)	0.01
Hispanic	395 (4.6)	19 (5.7)	0.34
American Indian	175 (2.0)	12 (3.6)	0.04
Other	627 (7.3)	22 (6.6)	0.64
Missing race info	491 (5.7)	30 (9.0)	0.01
School level SES			
Low	3,075 (35.7)	153 (46.0)	0.0001
Mid	2,958 (34.3)	100 (30.0)	0.10
High	2,575 (30.0)	80 (24.0)	0.02
Metropolitan status			
Suburban	4,374 (50.8)	151 (45.4)	0.05
Urban	956 (11.1)	37 (11.1)	0.99
Rural	3,278 (38.1)	145 (43.5)	0.04

Table B.8: Pre-post Comparison of Dietary Intake with random Imputation Applied

Dietary Variable of interest (N)	**Decreased Intake** N (%)	**No Change** N (%)	**Increased Intake** N (%)	**Bowker Test of Symmetry P-value**
Fruit Intake[1,3] (8,941)	2,284 (25.6)	3,553 (39.7)	3,104 (34.7)	<0.0001
Vegetable Intake[1,3] (8,941)	2,571 (28.7)	3,575 (40.0)	2,795 (31.3)	0.002
Sugar-Sweetened Juice, Sport's Drink Intake[2,4] (8,941)	2,393 (26.8)	4,080 (45.6)	2,468 (27.6)	0.03
Regular Soda Intake[2] (8,941)	2,086 (23.3)	4,674 (52.3)	2,181 (24.4)	0.38

Table B.8 (cont'd)

Red and Processed Meat Intake[2] (8,941)	2,433 (27.2)	3,865 (43.2)	2,643 (29.6)	0.10
High fat, High Sodium Snack Intake[2] (8,941)	2,416 (27.0)	4,096 (45.8)	2,429 (27.2)	0.30
High fat, High Sugar Baked Goods Intake[2] (8,941)	2,501 (28.0)	4,161 (46.5)	2,279 (25.5)	0.09

[1]Increased intake is desired outcome.
[2]Decreased intake is desired outcome.
[3]Significant change in desired direction detected.
[4]Significant change in undesired direction detected.

Table B.9: Demographic Characteristics According to Change in Fruit Intake

Characteristics	**Increased Intake** N (%)	**No Change** N (%)	**Decreased Intake** N (%)	**Mantel-Haenszel Chi-square p-value**
Sex				0.05
Male	1378 (33.1)	1,721 (41.3)	1064 (25.6)	
Female	1510 (35.6)	1,680 (39.6)	1049 (24.8)	
Race				0.12
White	1908 (35.4)	2,191 (40.6)	1293 (24.0)	
Black	392 (32.6)	454 (37.8)	356 (29.6)	
Asian	78 (33.5)	112 (48.1)	43 (18.4)	
Hispanic	122 (31.6)	170 (44.0)	94 (24.4)	
American Indian	62 (35.8)	63 (36.4)	48 (27.8)	
Other	207 (33.4)	247 (39.9)	165 (26.7)	
School-level SES				0.55
High	873 (34.3)	1,071 (42.0)	603 (23.7)	
Mid	992 (34.2)	1,147 (39.6)	758 (26.2)	
Low	1050 (34.6)	1,219 (40.1)	769 (25.3)	
Metropolitan status				0.03
Suburban	1516 (35.1)	1,780 (41.2)	1024 (23.7)	
Urban	318 (33.3)	366 (38.3)	271 (28.4)	
Rural	1081 (33.7)	1,291 (40.3)	835 (26.0)	

Table B.10: Demographic Characteristics According to Change in Vegetable Intake

Characteristics	**Increased Intake** N (%)	**No Change** N (%)	**Decreased Intake** N (%)	**Mantel-Haenszel Chi-square p-value**
Sex				0.02
Male	1239 (29.8)	,715 (41.3)	1201 (28.9)	
Female	1358 (32.1)	1712 (40.5)	1159 (27.4)	
Race				0.18
White	1700 (31.6)	2188 (40.7)	1486 (27.7)	
Black	377 (31.4)	456 (38.0)	368 (30.6)	
Asian	68 (29.0)	111 (47.2)	56 (23.8)	
Hispanic	90 (23.6)	175 (45.8)	117 (30.6)	
American Indian	41 (23.4)	87 (50.3)	47 (26.9)	
Other	181 (29.4)	266 (43.2)	169 (27.4)	
School-level SES				0.47
High	804 (31.7)	1,036 (40.8)	699 (27.5)	
Mid	897 (31.0)	1,162 (40.1)	837 (28.9)	
Low	927 (30.6)	1,253 (41.4)	849 (28.0)	
Metropolitan status				0.33
Suburban	1323 (30.7)	1765 (40.9)	1227 (28.4)	
Urban	300 (31.6)	368 (38.8)	281 (29.6)	
Rural	1005 (31.4)	1318 (41.2)	877 (27.4)	

Table B.11: Demographic Characteristics According to Change in Sugary Beverage Intake

Characteristics	**Increased Intake** N (%)	**No Change** N (%)	**Decreased Intake** N (%)	**Mantel-Haenszel Chi-square p-value**
Sex				0.32
Male	1133 (27.0)	1868 (44.5)	1196 (28.5)	
Female	1109 (25.8)	2081 (48.4)	1106 (25.8)	
Race				0.63
White	1359 (24.9)	2679 (49.1)	1415 (26.0)	
Black	383 (32.1)	439 (36.9)	369 (31.0)	
Asian	52 (21.5)	133 (55.0)	57 (23.5)	
Hispanic	101 (25.8)	158 (40.4)	132 (33.8)	
American Indian	57 (33.1)	70 (40.7)	45 (26.2)	
Other	176 (28.2)	279 (44.6)	170 (27.2)	
School-level SES				0.72
High	612 (23.7)	1331 (51.5)	641 (24.8)	
Mid	817 (28.0)	1303 (44.6)	801 (27.4)	
Low	829 (27.0)	1358 (44.3)	881 (28.7)	

Table B.11 (cont'd)

Metropolitan status				0.37
Suburban	1133 (25.9)	2,044 (46.7)	1196 (27.4)	
Urban	282 (29.5)	388 (40.6)	286 (29.9)	
Rural	843 (26.0)	1,560 (48.1)	841 (25.9)	

Table B.12: Demographic Characteristics According to Change in Regular Soda Intake

Characteristics	**Increased Intake** N (%)	**No Change** N (%)	**Decreased Intake** N (%)	**Mantel-Haenszel Chi-square p-value**
Sex				0.18
Male	988 (23.3)	2174 (51.3)	1079 (25.4)	
Female	958 (22.2)	2386 (55.4)	965 (22.4)	
Race				0.63
White	1190 (21.6)	3,077 (55.8)	1244 (22.6)	
Black	321 (26.7)	554 (46.2)	325 (27.1)	
Asian	41 (17.0)	154 (63.9)	46 (19.1)	
Hispanic	88 (22.7)	179 (46.3)	120 (31.0)	
American Indian	55 (31.2)	76 (43.2)	45 (25.6)	
Other	146 (23.6)	314 (50.8)	158 (25.6)	
School-level SES				0.007
High	530 (20.5)	1566 (60.6)	489 (18.9)	
Mid	686 (23.3)	1540 (52.2)	723 (24.5)	
Low	746 (24.1)	1502 (48.5)	848 (27.4)	
Metropolitan status				0.08
Suburban	1000 (22.7)	2404 (54.7)	995 (22.6)	
Urban	247 (26.0)	440 (46.3)	263 (27.7)	
Rural	715 (21.8)	1,764 (53.8)	802 (24.4)	

Table B.13: Demographic Characteristics According to Change in Red and Processed Meat Intake

Characteristics	**Increased Intake** N (%)	**No Change** N (%)	**Decreased Intake** N (%)	**Mantel-Haenszel Chi-square p-value**
Sex				0.52
Male	1131 (27.2)	1776 (42.7)	1253 (30.1)	
Female	1127 (26.5)	1923 (45.2)	1207 (28.3)	
Race				0.39
White	1419 (26.3)	2420 (44.8)	1557 (28.9)	

Table B.13 (cont'd)

Black	362 (30.0)	451 (37.4)	393 (32.6)	
Asian	42 (17.8)	145 (61.4)	49 (20.8)	
Hispanic	111 (28.5)	157 (40.4)	121 (31.1)	
American Indian	56 (32.6)	78 (45.3)	38 (22.1)	
Other	167 (27.3)	271 (44.3)	174 (28.4)	
School-level SES				0.50
High	635 (25.0)	1239 (48.8)	667 (26.2)	
Mid	786 (27.1)	1242 (42.8)	876 (30.1)	
Low	854 (28.0)	1262 (41.3)	936 (30.7)	
Metropolitan status				0.008
Suburban	1195 (27.6)	1931 (44.6)	,202 (27.8)	
Urban	262 (27.3)	390 (40.7)	307 (32.0)	
Rural	818 (25.5)	1422 (44.3)	970 (30.2)	

Table B.14: Demographic Characteristics According to Change in Salty Snack Intake

Characteristics	**Increased Intake** N (%)	**No Change** N (%)	**Decreased Intake** N (%)	**Mantel-Haenszel Chi-square p-value**
Sex				0.51
Male	1121 (26.5)	1945 (46.1)	1156 (27.4)	
Female	1152 (26.7)	2,016 (46.8)	1143 (26.5)	
Race				0.73
White	1384 (25.2)	2613 (47.7)	1488 (27.1)	
Black	375 (31.3)	501 (41.8)	322 (26.9)	
Asian	53 (22.0)	137 (56.8)	51 (21.2)	
Hispanic	118 (30.0)	178 (45.2)	98 (24.8)	
American Indian	45 (25.4)	87 (49.2)	45 (25.4)	
Other	171 (27.4)	268 (43.0)	185 (29.6)	
School-level SES				0.11
High SES	691 (26.8)	1,243 (48.2)	646 (25.0)	
Mid	786 (26.7)	1,349 (45.8)	809 (27.5)	
Low	816 (26.4)	1,414 (45.8)	859 (27.8)	
Metropolitan status				0.006
Suburban	1201 (27.5)	2,063 (47.1)	1113 (25.4)	
Urban	270 (28.2)	391 (40.9)	295 (30.9)	
Rural	822 (25.1)	1,552 (47.3)	906 (27.6)	

Table B.15: Demographic Characteristics According to Change in Baked Goods and Sweets Intake

Characteristics	Increased Intake N (%)	No Change N (%)	Decreased Intake N (%)	Mantel-Haenszel Chi-square p-value
Sex				0.52
Male	1173 (27.7)	2026 (47.8)	1038 (24.5)	
Female	1178 (27.4)	2,029 (47.3)	1085 (25.3)	
Race				0.07
White	1440 (26.2)	2661 (48.5)	1386 (25.3)	
Black	375 (31.4)	511 (42.8)	307 (25.8)	
Asian	54 (22.5)	135 (56.3)	51 (21.2)	
Hispanic	118 (29.9)	197 (49.9)	80 (20.2)	
American Indian	55 (31.4)	70 (40.0)	50 (28.6)	
Other	179 (28.5)	302 (48.2)	146 (23.3)	
School-level SES				0.98
High	674 (26.2)	1,317 (51.1)	584 (22.7)	
Mid	818 (27.7)	1,352 (45.7)	788 (26.6)	
Low	879 (28.6)	1,421 (46.2)	775 (25.2)	
Metropolitan status				0.01
Suburban	1222 (27.9)	2098 (48.0)	1054 (24.1)	
Urban	304 (31.8)	422 (44.1)	230 (24.1)	
Rural	845 (25.8)	1,570 (47.9)	863 (26.3)	

APPENDIX C: SUPPLEMENTAL TABLES CHAPTER 4

Table C.1: Characteristics of Students According to Participation in the Physiological Health Screenings

Characteristics	Consented to Participate in Health Screening	Did Not Consent to Participate in Health Screening	P-value
Sample Size (n)	1442	875	
Sex (n, %)			
Male	660 (45.6)	370 (42.5)	
Female	781 (54.0)	331 (38.0)	
Missing sex info	1 (0.4)	174 (19.5)	0.002
Race (n, %)			
White	821 (56.8)	316 (36.3)	
Black	319 (22.0)	255 (29.2)	
Other	223 (15.4)	94 (10.8)	
Missing race info	79 (5.7)	210 (23.7)	<0.0001
School-level socioeconomic status (n, %)			
Low SES	459 (31.8)	470 (53.5)	
Mid SES	565 (39.2)	231 (26.5)	
High SES	418 (29.0)	174 (20.0)	<0.0001
Metropolitan status (n, %)			
Suburban	627 (43.5)	350 (40.0)	
Urban	150 (10.4)	152 (17.4)	
Rural	665 (46.1)	373 (42.6)	<0.0001

Table C.2: Mean Change in Physiological Outcomes According to Baseline, Fruit and Vegetable Goal, and Baseline BMI Status

	Underweight/Normal BMI at Baseline[1] N, mean change (sd)			**Overweight/Obese BMI at Baseline**[1] N, mean change (sd)		
Physiological Measure[2,3,4]	Fruit and Veg Goal = Met[5]	Fruit and Veg Goal = Not Met[6]	P[7]	Fruit and Veg Goal = Met[5]	Fruit and Veg Goal = Not Met[6]	P[7]
TC						
Baseline normal	N=69, 0.4 (16.1)	N=377, -1.7 (16.5)	0.33	N=45, -2.6 (19.2)	N=235, -1.9 (17.9)	0.83
Baseline abnormal	N=29, -12.8 (19.6)	N=163, -15.4 (19.1)	0.52	N=21, -24.9 (24.9)	N=128, -17.4 (20.2)	0.21
LDL-C						
Baseline normal	N=59, 1.1 (14.8)	N=292, -0.9 (16.8)	0.35	N=41, 2.4 (19.0)	N=242, -0.1 (17.0)	0.43
Baseline abnormal	N=13, -13.2 (19.9)	N=56, -12.2 (17.1)	0.87	N=14, -30.6 (19.5)	N=60, -12.6 (21.0)	0.006
HDL-C						
Baseline normal	N=78, -0.9 (8.9)	N=417, -3.3 (9.8)	0.03	N=28, -3.7 (9.5)	N=190, -5.1 (9.3)	0.48
Baseline abnormal	N=22, 1.8 (8.0)	N=124, 2.8 (8.5)	0.58	N=38, 1.8 (7.9)	N=170, 0.7 (7.4)	0.46
TG						
Baseline normal	N=56, 11.1 (36.1)	N=327, 13.2 (39.3)	0.70	N=21, 16.1 (35.0)	N=131, 14.8 (33.3)	0.88
Baseline abnormal	N=41, -51.3 (51.2)	N=191, -40.0 (82.8)	0.26	N=45, -35.7 (70.7)	N=228, -25.7 (70.8)	0.39
SBP						
Baseline normal	N=108, 0.8 (9.0)	N=522, 1.3 (9.6)	0.62	N=49, 1.2 (7.7)	N=215, 2.2 (8.5)	0.45
Baseline abnormal	N=17, -7.0 (10.2)	N=146, -7.6 (12.6)	0.83	N=32, -4.8 (13.9)	N=181, -4.0 (11.1)	0.74
DBP						
Baseline normal	N=108, 1.14 (6.7)	N=521, 0.6 (7.6)	0.45	N=49, 0.4 (6.6)	N=215, 2.7 (7.8)	0.04
Baseline abnormal	N=17, -9.8 (13.0)	N=146, -6.1 (8.3)	0.26	N=32, -3.3 (10.4)	N=181, -3.5 (10.0)	0.91

Table C.2 (cont'd)

[1] BMI categories based on cut-points established by the CDC, American Academy of Pediatrics (AAP), Institute of Medicine (IOM), and International Obesity Task Force (IOTF). Underweight, BMI <5th percentile for age and sex; Normal, BMI between the 5th and <85th percentile for age and sex; Overweight, BMI between >85Th and 95th percentile for age and sex; Obese, BMI ≥95th percentile for age and sex.
[2]TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; SBP, systolic blood pressure; DBP, diastolic blood pressure.
[3]Baseline normal and abnormal cut points for blood lipids based on guidelines set by NHLBI, National Heart, Lung, and Blood Institute; AAP, American Academy of Pediatrics; and AHA/ACC, American Heart Association/American College of Cardiology. "Abnormal" defined as students with values consistent with "borderline" or "abnormal" readings based on these guidelines.
[4]Baseline normal and abnormal cut points for blood pressure based on 2017 AAP definitions. "Abnormal" defined as students with values consistent with "elevated," "stage I hypertension," or "stage 2 hypertension" from these guidelines.
[5] "Met" defined as student achieved or maintained consumption of fruits and vegetables ≥ 5 times per day, post-intervention.
[6] "Not Met" defined as student reported consuming fruits and vegetables < 5 times per day, post-intervention.
[7]P-values from t-tests comparing differences in mean change in physiological measure between students who met and did not meet the fruit and vegetable goal according to baseline measurement status and baseline BMI status.

Table C.3: Mean Change in Physiological Outcomes According to Baseline, SSB Goal, and Baseline BMI Status

	Underweight/Normal at Baseline[1] N, mean change (sd)			**Overweight/Obese at Baseline**[1] N, mean change (sd)		
Physiological Measure[2,3,4]	SSB Goal = Met[5]	SSB Goal = Not Met[6]	P[7]	SSB Goal = Met[5]	SSB Goal = Not Met[6]	P[7]
TC						
Baseline normal	N=285, -1.7 (15.5)	N=170, -1.0 (17.6)	0.67	N=187, -2.7 (18.7)	N=89, -2.1 (16.5)	0.79
Baseline abnormal	N=134, -16.1 (18.4)	N=61, -12.5 (20.8)	0.24	N=101, -20.4 (20.1)	N=50, -15.4 (22.3)	0.19
LDL-C						
Baseline normal	N=242, -0.9 (15.2)	N=117, 0.6 (18.8)	0.47	N=188, -0.02 (16.0)	N=94, -0.7 (20.0)	0.76
Baseline abnormal	N=50, -13.6 (18.2)	N=21, -7.5 (15.7)	0.16	N=51, -18.8 (20.2)	N=24, -10.2 (23.9)	0.32
HDL-C						
Baseline normal	N=326, -3.4 (9.5)	N=177, -2.1 (9.9)	0.18	N=155, -5.3 (9.1)	N=65, -4.5 (9.9)	0.56
Baseline abnormal	N=95, 2.3 (7.9)	N=55, 2.8 (8.8)	0.72	N=131, 0.1 (7.3)	N=73, 1.8 (7.5)	0.14

Table C.3 (cont'd)

TG Baseline normal	N=250, 9.7 (29.7)	N=140, 18.4 (50.8)	0.06	N=100, 14.6 (33.2)	N=50, 15.0 (34.6)	0.95
Baseline abnormal	N=159, -43.6 (72.3)	N=78, -42.9 (88.6)	0.95	N=186, -28.0 (68.9)	N=87, -24.3 (77.2)	0.70
SBP Baseline normal	N=420, 1.4 (9.2)	N=224, 1.2 (9.9)	0.84	N=188, 1.7 (8.1)	N=72, 2.9 (9.1)	0.35
Baseline abnormal	N=109, -7.3 (12.6)	N=53, -8.6 (12.2)	0.54	N=150, -4.6 (11.1)	N=65, -3.3 (12.5)	0.47
DBP Baseline normal	N=419, 0.8 (7.5)	N=224, 0.6 (7.3)	0.70	N=188, 2.2 (7.4)	N=72, 2.3 (8.4)	0.90
Baseline abnormal	N=109, -6.8 (9.7)	N=53, -5.9 (7.2)	0.47	N=150, -3.3 (9.9)	N=65, -4.0 (10.5)	0.65

[1] BMI categories based on cut-points established by the CDC, American Academy of Pediatrics (AAP), Institute of Medicine (IOM), and International Obesity Task Force (IOTF). Underweight, BMI <5th percentile for age and sex; Normal, BMI between the 5th and <85th percentile for age and sex; Overweight, BMI between >85Th and 95th percentile for age and sex; Obese, BMI ≥95th percentile for age and sex.

[2]TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; SBP, systolic blood pressure; DBP, diastolic blood pressure.

[3]Baseline normal and abnormal cut points for blood lipids based on guidelines set by NHLBI, National Heart, Lung, and Blood Institute; AAP, American Academy of Pediatrics; and AHA/ACC, American Heart Association/American College of Cardiology. "Abnormal" defined as students with values consistent with "borderline" or "abnormal" readings based on these guidelines.

[4]Baseline normal and abnormal cut points for blood pressure based on 2017 AAP definitions. "Abnormal" defined as students with values consistent with "elevated," "stage I hypertension," or "stage 2 hypertension" from these guidelines.

[5] "Met" defined as student achieved or maintained consumption of SSBs ≤1 times per day, post-intervention.

[6] "Not Met" defined as student reported consuming SSBs >1 time per day, post-intervention.

[7]P-values from t-tests comparing differences in mean change in physiological measure between students who met and did not meet the SSB goal according to baseline measurement status and baseline BMI status.

Table C.4: Mean Change in Physiological Outcomes According to Baseline, PA goal, and Baseline BMI Status

	Underweight/Normal at Baseline[1] N, mean change (sd)			**Overweight/Obese at Baseline**[1] N, mean change (sd)		
Physiological Measure[2,3,4]	PA Goal = Met[5]	PA Goal = Not Met[6]	P[7]	PA Goal = Met[5]	PA Goal = Not Met[6]	P[7]
TC						
Baseline normal	N=366, 0.9 (16.2)	N=83, -2.7 (17.2)	0.40	N=216, -2.1 (18.1)	N=61, -2.6 (18.2)	0.90
Baseline abnormal	N=163, -14.6 (19.7)	N=25, -19.3 (16.9)	0.20	N=119, -17.8 (20.6)	N=32, -22.8 (22.1)	0.30
LDL-C						
Baseline normal	N=300, -0.1 (16.1)	N=54, -1.3 (18.4)	0.70	N=227, 0.04 (16.4)	N=58, -1.3 (21.2)	0.60
Baseline abnormal	N=57, -11.5 (18.0)	N=11, -17.8 (13.4)	0.20	N=56, -14.1 (22.7)	N=17, -23.0 (18.0)	0.10
HDL-C						
Baseline normal	N=410, -2.7 (9.7)	N=81, -3.9 (10.0)	0.40	N=170, -4.6 (8.9)	N=48, -6.6 (10.9)	0.20
Baseline abnormal	N=122, 2.5 (8.5)	N=27, 2.8 (7.8)	0.90	N=165, 0.9 (7.5)	N=42, -0.1 (7.0)	0.40
TG						
Baseline normal	N=319, 11.3 (37.5)	N=61, 21.6 (45.1)	0.09	N=110, 11.9 (29.1)	N=37, 20.0 (41.9)	0.30
Baseline abnormal	N=194, -46.8 (72.6)	N=41, -23.5 (100.9)	0.20	N=223, -28.2 (68.9)	N=53, -17.5 (81.4)	0.40
SBP						
Baseline normal	N=515, 1.4 (9.5)	N=113, 1.2 (9.4)	0.80	N=202, 1.9 (8.4)	N=54, 2.3 (8.6)	0.80
Baseline abnormal	N=136, -8.4 (12.5)	N=21, -5.6 (10.9)	0.30	N=171, -4.3 (11.9)	N=48, -3.1 (9.7)	0.40
DBP						
Baseline normal	N=514, 0.9 (7.5)	N=113, 0.4 (7.3)	0.50	N=202, 2.2 (7.9)	N=54, 2.6 (6.9)	0.70
Baseline abnormal	N=136, -6.9 (9.3)	N=21, -4.9 (6.1)	0.20	N=171, -2.7 (10.0)	N=48, -5.1 (9.3)	0.10

[1] BMI categories based on cut-points established by the CDC, American Academy of Pediatrics (AAP), Institute of Medicine (IOM), and International Obesity Task Force (IOTF). Underweight, BMI <5th percentile for age and sex; Normal, BMI between the 5th and <85th percentile for age and sex;

Table C.4 (cont'd)

Overweight, BMI between >85Th and 95th percentile for age and sex; Obese, BMI ≥95th percentile for age and sex.
[2]TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; SBP, systolic blood pressure; DBP, diastolic blood pressure.
[3]Baseline normal and abnormal cut points for blood lipids based on guidelines set by NHLBI, National Heart, Lung, and Blood Institute; AAP, American Academy of Pediatrics; and AHA/ACC, American Heart Association/American College of Cardiology. "Abnormal" defined as students with values consistent with "borderline" or "abnormal" readings based on these guidelines.
[4]Baseline normal and abnormal cut points for blood pressure based on 2017 AAP definitions. "Abnormal" defined as students with values consistent with "elevated," "stage I hypertension," or "stage 2 hypertension" from these guidelines.
[5] "Met" defined as student participated in ≥ 5 Sessions of moderate and/or vigorous PA per week, post-intervention.
[6] "Not Met" defined as student participated in < 5 Sessions of moderate and/or vigorous PA per week, post-intervention..
[7]P-values from t-tests comparing differences in mean change in physiological measure between students who met and did not meet the PA goal according to baseline measurement status and baseline BMI status.

Table C.5: Mean Change in Physiological Outcomes According to Baseline, Combined Behavioral Goal, and Baseline BMI Status

	Underweight/Normal at Baseline[1] N, mean change (sd)			**Overweight/Obese at Baseline**[1] N, mean change (sd)		
Physiological Measure[2,3,4]	3 Goals = Met[5]	3 Goals = Not Met[6]	P[7]	3 Goals = Met[5]	3 Goals = Not Met[6]	P[7]
TC						
Normal at baseline	N=41, 3.3 (13.7)	N=386, -1.6 (16.7)	0.03	N=26, -2.5 (18.3)	N=239, -2.3 (17.8)	0.90
Abnormal at baseline	N=23, -14.1 (20.7)	N=160, -15.7 (19.0)	0.70	N=15, -25.7 (25.1)	N=130, -17.8 (20.7)	0.30
LDL-C						
Normal at baseline	N=37, 2.5 (13.0)	N=298, -0.8 (17.0)	0.20	N=30, 4.4 (19.0)	N=241, 0.4 (17.1)	0.20
Abnormal at baseline	N=12, -15.1 (19.4)	N=54, -12.7 (16.8)	0.70	N=8, -35.4 (22.6)	N=64, -13.7 (21.0)	0.03
HDL-C						
Normal at baseline	N=50, -0.8 (8.9)	N=419, -3.3 (9.9)	0.07	N=21, -4.0 (10.5)	N=188, -5.1 (9.1)	0.60
Abnormal at baseline	N=16, 0.9 (8.4)	N=128, 2.7 (8.2)	0.40	N=20, 1.4 (8.2)	N=178, 0.7 (7.2)	0.70

Table C.5 (cont'd)

TG						
Normal at baseline	N=38, 8.8 (24.0)	N=326, 13.8 (40.7)	0.30	N=9, 20.4 (29.6)	N=134, 13.8 (33.4)	0.50
Abnormal at baseline	N=26, -52.2 (59.1)	N=198, -40.6 (81.6)	0.40	N=32, -47.8 (73.9)	N=232, -25.2 (70.7)	0.10
SBP						
Normal at baseline	N=68, 1.3 (9.6)	N=533, 1.4 (9.6)	0.90	N=29, -0.9 (6.5)	N=219, 2.3 (8.6)	0.02
Abnormal at baseline	N=13, -6.8 (11.6)	N=140, -8.4 (12.4)	0.70	N=25, -5.4 (13.1)	N=180, -4.0 (11.5)	0.60
DBP						
Normal at baseline	N=68, 1.3 (7.0)	N=532, 0.7 (7.6)	0.50	N=29, -0.3 (6.6)	N=219, 2.6 (7.9)	0.04
Abnormal at baseline	N=13, -9.5 (14.8)	N=140, -6.4 (8.3)	0.50	N=25, -3.8 (9.8)	N=180, -3.3 (10.0)	0.80

[1] BMI categories based on cut-points established by the CDC, American Academy of Pediatrics (AAP), Institute of Medicine (IOM), and International Obesity Task Force (IOTF). Underweight, BMI <5th percentile for age and sex; Normal, BMI between the 5th and <85th percentile for age and sex; Overweight, BMI between >85Th and 95th percentile for age and sex; Obese, BMI ≥95th percentile for age and sex.

[2]TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; SBP, systolic blood pressure; DBP, diastolic blood pressure.

[3]Baseline normal and abnormal cut points for blood lipids based on guidelines set by NHLBI, National Heart, Lung, and Blood Institute; AAP, American Academy of Pediatrics; and AHA/ACC, American Heart Association/American College of Cardiology. "Abnormal" defined as students with values consistent with "borderline" or "abnormal" readings based on these guidelines.

[4]Baseline normal and abnormal cut points for blood pressure based on 2017 AAP definitions. "Abnormal" defined as students with values consistent with "elevated," "stage I hypertension," or "stage 2 hypertension" from these guidelines.

[5] "Met" defined as student achieved fruit and vegetable consumption goal, SSB consumption goal and PA goal, post-intervention.

[6] "Not Met" defined as student did not achieve fruit and vegetable consumption goal, SSB consumption goal and PA goal, post-intervention.

[7]P-values from t-tests comparing differences in mean change in physiological measure between students who met and did not meet the goal for all 3 behaviors according to baseline measurement status and baseline BMI status.

Table C.6: Change in Categorial Status of Physiological Outcomes According to Fruit and Vegetable Goal Status

Baseline and Post-intervention Status of Physiological Measure[1]	Fruit and Vegetable Goal=Met[2] N (%)	Fruit and Vegetable Goal=Not met[3] N (%)	P-value[4]
TC	(n=167)	(n=906)	0.91
Normal→ Normal	108 (64.6)	572 (63.1)	
Abnormal→ Abnormal	27 (16.2)	164 (18.1)	
Abnormal → Normal	23 (13.8)	128 (14.1)	
Normal→ Abnormal	9 (5.4)	42 (4.7)	
LDL-C	(n=129)	(n=665)	0.30
Normal→ Normal	95 (73.6)	516 (77.6)	
Abnormal→ Abnormal	11 (8.6)	67 (10.1)	
Abnormal → Normal	16 (12.4)	50 (7.5)	
Normal→ Abnormal	7 (5.4)	32 (4.8)	
HDL-C	(n=169)	(n=904)	0.39
Normal→ Normal	93 (55.0)	480 (53.1)	
Abnormal→ Abnormal	49 (29.0)	235 (26.0)	
Abnormal → Normal	11 (6.5)	60 (6.6)	
Normal→ Abnormal	16 (9.5)	129 (14.3)	
TG	(n=166)	(n=880)	0.78
Normal→ Normal	61 (36.7)	350 (39.8)	
Abnormal→ Abnormal	55 (33.1)	259 (29.4)	
Abnormal → Normal	31 (18.7)	161 (18.3)	
Normal→ Abnormal	19 (11.5)	110 (12.5)	

Table C.6 (cont'd)

BP	(n=202)	(n=1017)	0.26
Normal→ Normal	133 (65.8)	603 (59.3)	
Abnormal→ Abnormal	24 (11.9)	146 (14.4)	
Abnormal → Normal	23 (11.4)	159 (15.6)	
Normal→ Abnormal	22 (10.9)	109 (10.7)	

[1]Cutpoints based on guidelines set by NHLBI, National Heart, Lung, and Blood Institute; AAP, American Academy of Pediatrics; and AHA/ACC, American Heart Association/American College of Cardiology. "Abnormal" defined as students with values consistent with "borderline" or "abnormal" readings based on these guidelines. Blood pressure cut points based on 2017 AAP definitions. "Abnormal" defined as students with values consistent with "elevated," "stage I hypertension," or "stage 2 hypertension" from these guidelines. TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; SBP, systolic blood pressure; DBP, diastolic blood pressure.
[2]"Met" defined as student achieved or maintained consumption of fruits and vegetables ≥ 5 times per day, post-intervention.
[3]"Not met" defined as student reported consuming fruits and vegetables < 5 times per day, post-intervention.
[4] P-values from chi-square tests of overall differences in the change in status of physiological measure according to meeting or not meeting fruit and vegetable consumption goal.

Table C.7: Change in Categorial Status of Physiological Outcomes According to SSB Goal Status

Baseline and Post-intervention Status of Physiological Measure[1]	**SSB goal=Met**[2] N (%)	**SSB goal=Not met**[3] N (%)	**P-value**[4]
TC	(n=712)	(n=371)	0.71
Normal→ Normal	445 (62.5)	241 (65.0)	
Abnormal→ Abnormal	132 (18.5)	61 (16.4)	
Abnormal → Normal	104 (14.6)	50 (13.5)	
Normal→ Abnormal	31 (4.4)	19 (5.1)	
LDL-C	(n=541)	(n=263)	0.44
Normal→ Normal	412 (76.2)	206 (78.3)	
Abnormal→ Abnormal	52 (9.6)	29 (11.0)	
Abnormal → Normal	50 (9.2)	16 (6.1)	
Normal→ Abnormal	27 (5.0)	12 (4.6)	

Table C.7 (cont'd)

HDL-C	(n=712)	(n=371)	0.67
Normal→ Normal	382 (53.6)	197 (53.1)	
Abnormal→ Abnormal	181 (25.4)	105 (28.3)	
Abnormal → Normal	46 (6.5)	23 (6.2)	
Normal→ Abnormal	103 (14.5)	46 (12.4)	
TG	(n=700)	(n=356)	0.80
Normal→ Normal	270 (38.6)	146 (41.0)	
Abnormal→ Abnormal	215 (30.7)	100 (28.1)	
Abnormal → Normal	131 (18.7)	65 (18.3)	
Normal→ Abnormal	84 (12.0)	45 (12.6)	
BP	(n=832)	(n=398)	0.74
Normal→ Normal	501 (60.2)	242 (60.8)	
Abnormal→ Abnormal	121 (14.5)	49 (12.3)	
Abnormal → Normal	122 (14.7)	62 (15.6)	
Normal→ Abnormal	88 (10.6)	45 (11.3)	

[1]Cutpoints based on guidelines set by NHLBI, National Heart, Lung, and Blood Institute; AAP, American Academy of Pediatrics; and AHA/ACC, American Heart Association/American College of Cardiology. "Abnormal" defined as students with values consistent with "borderline" or "abnormal" readings based on these guidelines. Blood pressure cut points based on 2017 AAP definitions. "Abnormal" defined as students with values consistent with "elevated," "stage I hypertension," or "stage 2 hypertension" from these guidelines. TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; SBP, systolic blood pressure; DBP, diastolic blood pressure.
[2] "Met" defined as student achieved or maintained consumption of SSB ≤1 times per day, post-intervention.
[3] "Not met" defined as student reported consuming SSBs >1 time per day, post-intervention.
[4]P-values from chi-square tests of overall differences in the change in status of physiological measure according to meeting or not meeting SSB consumption goal.

Table C.8: Change in Categorial Status of Physiological Outcomes According to PA Goal Status

Baseline and Post-intervention Status of Physiological Measure[1]	**PA Goal=Met**[2] N (%)	**PA Goal=Not met**[3] N (%)	**P-value**[4]
TC	(n=869)	(n=202)	0.54
Normal→ Normal	543 (62.5)	136 (67.3)	
Abnormal→ Abnormal	159 (18.3)	29 (14.3)	
Abnormal → Normal	124 (14.3)	28 (13.9)	
Normal→ Abnormal	43 (4.9)	9 (4.5)	
LDL-C	(n=653)	(n=144)	0.91
Normal→ Normal	508 (77.8)	109 (75.7)	
Abnormal→ Abnormal	60 (9.2)	16 (11.1)	
Abnormal → Normal	54 (8.3)	12 (8.3)	
Normal→ Abnormal	31 (4.7)	7 (4.9)	
HDL-C	(n=872)	(n=199)	0.43
Normal→ Normal	463 (53.1)	101 (50.8)	
Abnormal→ Abnormal	227 (26.0)	60 (30.1)	
Abnormal → Normal	61 (7.0)	9 (4.5)	
Normal→ Abnormal	121 (13.9)	29 (14.6)	
TG	(n=851)	(n=193)	0.46
Normal→ Normal	338 (39.7)	70 (36.3)	
Abnormal→ Abnormal	258 (30.3)	60 (31.1)	
Abnormal → Normal	160 (18.8)	34 (17.4)	
Normal→ Abnormal	95 (11.2)	29 (15.0)	

Table C.8 (cont'd)

BP	(n=985)	(n=225)	0.85
Normal→ Normal	592 (60.1)	133 (59.1)	
Abnormal→ Abnormal	142 (14.4)	30 (13.3)	
Abnormal → Normal	147 (14.9)	34 (15.1)	
Normal→ Abnormal	104 (10.6)	28 (12.5)	

[1]Cutpoints based on guidelines set by NHLBI, National Heart, Lung, and Blood Institute; AAP, American Academy of Pediatrics; and AHA/ACC, American Heart Association/American College of Cardiology. "Abnormal" defined as students with values consistent with "borderline" or "abnormal" readings based on these guidelines. Blood pressure cut points based on 2017 AAP definitions. "Abnormal" defined as students with values consistent with "elevated," "stage I hypertension," or "stage 2 hypertension" from these guidelines. TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; SBP, systolic blood pressure; DBP, diastolic blood pressure.
[2] "Met" defined as student achieved or maintained ≥ 5 Sessions of moderate (30 min) and/or vigorous (20 min) PA per week, post-intervention.
[3] "Not met" defined as student participated in <5 Sessions of moderate (30 min) and/or vigorous (20 min) PA per week, post-intervention.
[4]P-values from chi-square tests of overall differences in the change in status of physiological measure according to meeting or not meeting PA goal.

Table C.9: Change in Categorial Status of Physiological Outcomes According to Combined Behavioral Goal Status

Baseline and Post-intervention Status of Physiological Measure[1]	**All 3 Behavioral Goals=Met**[2] N (%)	**All 3 Behavioral Goals=Not Met**[3] N (%)	**P-value**[4]
TC	(n=107)	(n=1004)	0.73
Normal→ Normal	63 (58.9)	644 (64.1)	
Abnormal→ Abnormal	22 (20.6)	174 (17.3)	
Abnormal → Normal	16 (14.9)	140 (14.0)	
Normal→ Abnormal	6 (5.6)	46 (4.6)	
LDL-C	(n=88)	(n=735)	0.21
Normal→ Normal	63 (71.6)	574 (78.1)	
Abnormal→ Abnormal	8 (9.1)	73 (9.9)	
Abnormal → Normal	12 (13.6)	54 (7.4)	
Normal→ Abnormal	5 (5.7)	34 (4.6)	

Table C.9 (cont'd)

HDL-C	(n=109)	(n=1002)	0.45
Normal→ Normal	63 (57.8)	526 (52.5)	
Abnormal→ Abnormal	28 (25.7)	264 (26.3)	
Abnormal → Normal	8 (7.3)	66 (6.6)	
Normal→ Abnormal	10 (9.2)	146 (14.6)	
TG	(n=107)	(n=976)	0.46
Normal→ Normal	35 (32.7)	390 (40.0)	
Abnormal→ Abnormal	38 (35.5)	286 (29.3)	
Abnormal → Normal	20 (18.7)	182 (18.6)	
Normal→ Abnormal	14 (13.1)	118 (12.1)	
BP	(n=132)	(n=1131)	0.78
Normal→ Normal	83 (62.9)	680 (60.1)	
Abnormal→ Abnormal	20 (15.2)	158 (14.0)	
Abnormal → Normal	16 (12.1)	171 (15.1)	
Normal→ Abnormal	13 (9.8)	122 (10.8)	

[1]Cutpoints based on guidelines set by NHLBI, National Heart, Lung, and Blood Institute; AAP, American Academy of Pediatrics; and AHA/ACC, American Heart Association/American College of Cardiology. "Abnormal" defined as students with values consistent with "borderline" or "abnormal" readings based on these guidelines. Blood pressure cut points based on 2017 AAP definitions. "Abnormal" defined as students with values consistent with "elevated," "stage I hypertension," or "stage 2 hypertension" from these guidelines. TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; SBP, systolic blood pressure; DBP, diastolic blood pressure.
[2] "Met" defined as student achieved fruit and vegetable consumption goal, SSB consumption goal and PA goal, post-intervention.
[3] "Not met" defined as student did not achieve fruit and vegetable consumption goal, SSB consumption goal and PA goal, post-intervention.
[4] P-values from chi-square tests of overall differences in the change in status of physiological measure according to meeting or not meeting all 3 behavioral goals.

APPENDIX D: SUPPLEMENTAL FIGURES CHAPTER 5

Figure D.1: Change in LDL-C in mg/dL according to change in frequency of intake of specific high fat food groups