**Preschool-Based Behavioral Intervention to Increase Fruit and Vegetable Consumption in a Sample of Preschoolers**

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A Dissertation

Presented to

The Faculty of the School of Education and Human

Development

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In Partial Fulfillment

Of the Requirement for the Degree

Doctor of Philosophy

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by

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# Dedication

This thesis is dedicated to my Mom, Basema; Dad, Omran; Sister, Hala; Brother, Fares; sister-in-law, Asli; and my Godparents: Barbara, Tim, Tom, and Linda. Your unconditional love and continued support throughout this journey were vital to my success. I am, and will always be, grateful for you all.

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This doctoral dissertation has been approved by the Graduate Faculty of the School of Education and Human Development in partial fulfillment for the degree of Doctor of Philosophy.

**Approved Title of Doctoral Dissertation:**

Preschool-Based Behavioral Intervention to Increase Fruit and Vegetable Consumption in a Sample of Preschoolers

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Abstract

*Background*: Diet quality in young children is low, with most children over-consuming refined grains, added sugars, sodium, and saturated fats, while simultaneously under-consuming whole grains, seafood, fatty acids, and fruits and vegetables (FVs). More specifically, in 2019, only 75% and 15% of preschoolers met the guidelines for fruits and vegetables, respectively. Despite the hundreds of studies conducted to address this low intake, several major gaps exist in this literature; there is a lack of studies conducted in a childcare setting that 1) objectively-measured dietary intake, 2) are informed by behavior change theory and the associated behavior change techniques, and 3) assess the known confounding variables that influence FV intake. Additionally, adult studies suggest a direct relationship exists between skin carotenoid levels and physical activity (PA) levels, although this has yet to be investigated in preschoolers. *Purpose*: As such, the purpose of this dissertation was to 1) evaluate the existing literature to identify the best approach, 2) design a study to assess the feasibility of an objectively-measured intervention informed by the best practices identified by the systematic review, and 3) explore the relationship between skin carotenoid scores (SCS) and PA in preschoolers. *Methods*: The best practices and existing gaps in this field was assessed using a systematic literature review (Manuscript I). This information was used to design a feasibility randomized-controlled trial within a local childcare center that assessed FV intake using the objective plate-waste method (Manuscript II). Additional factors included measurement of body mass index (BMI) percentile, socio-demographic factors, and at-home dietary intake. The relationship between skin carotenoids and PA was assessed objectively using skin carotenoid scores (SCS) and actigraphy (Actigraph GT3X) collected across three days (Manuscript III). *Results:* The results of the systematic review supported the use of an intervention informed by Social Cognitive Theory

with components pertaining to nutrition education and repeated exposure to increase FV intake in preschoolers. As such, our study assessed such an intervention and established the feasibility of the proposed study design within a childcare setting using objective measures of dietary intake. Although this study was not adequately powered to assess changes in FV intake, it highlighted significant inter- and intra-subject variability in day-to-day FV intake in preschoolers, which should be further examined in future studies. Thirdly, SCS and PA were significantly associated in this sample of preschoolers ($\beta = 3.448$, $p=0.032$). *Discussion*: This study design and intervention informed by the existing literature was well-accepted by the participating childcare center personnel and included children and may be used to inform future interventions that aim to improve diet in preschoolers. Further, the association between SCS and PA supports the use of skin carotenoids as biomarker of health status, as opposed to solely FV intake. *Conclusion*: The findings of this dissertation contribute to the existing literature on health-related behaviors in preschoolers.

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# CHAPTER 1: LITERATURE REVIEW AND DESCRIPTION OF AIMS

## 1.1 Introduction

We proposed to describe the role of fruit and vegetable (FV) intake in preschoolers, as well as the predictors of intake and how to measure and, subsequently, change FV intake in preschoolers. We will also discuss how physical activity levels, sedentary behavior, and screen time may influence FV intake in preschoolers.

## 1.2 Current FV intake

The Dietary Guidelines for Americans (DGA) recommends that children consume at least 1.5 cups each of FVs per day [for a 1400kcal diet](1). Further, these guidelines emphasize consuming a variety of fruits and vegetables in their whole form (i.e.. not in juice form). Despite the long-standing explicit USDA recommendation and evidence regarding the importance of FV intake, average FV consumption in the US remains low: only 40% of children meet the recommended intake for fruits, and 7% meet the recommended intake for vegetables (2). The current average intake for children is only 0.9 cup equivalents of each and consumption levels decline with age (1). Moreover, variety in the type of vegetable intake is limited relative to what is recommended in the guidelines; there is overrepresentation of less nutritious fried or excessively processed starchy vegetables and underrepresentation of highly nutritious dark green, red, and orange vegetables and beans, peas, and lentils (1). Thus, in support of public health, it is important to examine reasons for low intake to subsequently develop strategies to improve FV intake in children.

## 1.3 Predictors of FV intake in children

Several factors are shown to predict FV intake in preschoolers; the most prominent factors include socioeconomic status (SES), parental consumption, the presence of competing foods, and child eating characteristics.

Analyses of nationally-representative data using the National Health and Examination Survey (NHANES) of parent-reported food intake in young children and adolescents have found SES to be related to FV intake in children (3); children of low SES tend to have less access to, and therefore lower consumption of, FVs (4). However, smaller studies have shown equivocal findings regarding this relationship, with few studies showing that low SES is associated with higher FV intake in preschool-aged children who attend Head Start childcare center due to their participation in the Child and Adult Care Food Program (CACFP) (5-8). On the other hand, parental education is more consistently associated with FV intake in preschoolers; children of parents who have lower attained education consume fewer FVs than those of parents with higher education (9, 10). Parents who regularly consume fruits and vegetables have children who consume more fruits and vegetables, likely due to the effect of modeling behavior and ensuring adequate access to FVs within the home (9-11). Parent modeling of fruit and vegetable intake is also associated with higher FV intake in their 5–7-year-old children, while parent modeling of energy dense foods is associated with greater intake of sugar-sweetened beverages (12).

Similarly, parental feeding practices, such as ensuring early introduction to FVs and enforcing FV consumption, are both highly influential on FV intake in preschool-aged children (9, 10), although too much control has been found to have the opposite effect (11). Exposure to FVs may help promote early liking of FVs; liking of FVs at 2.5 years significantly predicted fruit intake 7 years of age ($r=0.27$, $p<0.001$) (13). In a logistic regression model, liking of fruits and vegetables at age 30mo significantly increased the odds of consuming greater than 1 portion of fruit (OR:

1.31, p=0.016) and vegetable (OR: 1.28, p<0.001) per day at age 7(13). The number of FVs tried during preschool age (2.5 years) significantly predicted FV consumption at 7 years old (r=0.15, p<0.001)(13). In a cross-sectional study amongst 5-year old girls and their parents, the researchers found that parent intake of FVs predicted their child's FV intake, and lower pressure in child feeding was associated with greater FV intake and greater micronutrient intake. Combining these two methods (parental FV intake and low-pressure in child feeding) was associated with the greatest FV intake in the children (14). Lastly, children's eating behaviors and genetic traits also predict their FV intake (15). A child with higher enjoyment of food is more likely to consume FVs, while a child with neophobia, the trait of disliking novel foods, is less likely to consume FVs (13). In summary, numerous internal and external factors contribute to FV intake in young children and the complexity of these influences continue to be examined in this field of research.

### 1.4 Importance of FV intake

Both fruits and vegetables are considered vital components of a healthy dietary pattern because they provide a host of nutrients that are important for overall health and development. More specifically, they are touted for their high fiber content, moderately low energy density, and wide variety of vitamins, minerals, and phytochemicals (16).

Fiber is a form of indigestible carbohydrate found in plant cell walls, resistant starch, and oligosaccharides. Because the human body is unable to digest them, they remain intact as they pass through the stomach and small intestines into the large intestines. Although they are not absorbed in the human digestive tract, they offer numerous benefits to human health. There are two types of dietary fiber: soluble and insoluble fiber. Soluble fibers, named so due to its high affinity for water (17), mainly consist of resistant oligosaccharides and viscous fiber. They are unique in that they are a probiotic?; the carbohydrates within them act as a food source to beneficial bacterial species present in the colon, allowing them to proliferate and improve the intestinal environment. The various species of the gut microbiome have different abilities to utilize the various forms of soluble dietary fibers. For example, *Bacteroides* are most efficient at degrading polysaccharides, and therefore the dominant bacteria in the gut microbiota. The benefit of proliferation of these beneficial bacteria lies in their ability to prevent overgrowth of potentially pathogenic bacterial species, such as *Clostridium histolyticum*, which is associated with colitis, inflammation of the colon (17). In addition to the benefits of soluble dietary fibers within the gut microbiota, they also have beneficial physiological effects on human health. First, these microorganisms ferment the carbohydrates into short chain fatty acids (SCFAs) such as butyrate, propionate, and acetate (17). Within the gut, SCFAs play an anti-inflammatory role in their inhibition of histone deacetylase, which is an enzyme that contributes to downstream intestinal inflammation and may contribute to colon cancer cell proliferation (17). Additionally, they also bind to specific G-Protein Coupled Receptors (GPCRs) that increase the production of interleukin-18 (IL-18), which functions to decrease inflammation and maintain mucosal integrity, thereby promoting healthy digestion and reducing the risk of irritable bowel syndrome (IBS) and irritable bowel diseases (IBD) (16, 17). Finally, due to their solubility and viscosity, soluble fibers form a gel-like substance in the small intestines that slows the digestion of foods, including starch and triglycerides, and signals the release of glucagon-like peptide-1 (GLP) in the terminal ileum that further inhibits gastric emptying (17). These effects functionally help to reduce appetite and, subsequently, weight, and promote regular glucose metabolism. Additionally, the gel-like substance formed from the fibers traps dietary cholesterol and prevents the body from reabsorbing it, effectively lowering serum cholesterol. This viscosity also slows the breakdown and absorption of other food matter that helps to soften stool and promote stool movement through the colon (18). On the other hand, insoluble fibers are named so due to their hydrophobic and crystalline structure that makes them resistant to hydrolysis (17). Thus, they not as readily digested by the gut microbiome. Rather, they impart their health benefits by increasing stool weight and consistency which then stimulates the intestinal mucosa to reduce transit time, lowering the risk of functional constipation (19). The properties of dietary fibers described above have been shown to contribute to improvements in digestive diseases, such as IBD, protect against metabolic dysfunction and obesity, and lower the risk of colorectal cancer and cardiovascular disease (16, 19). However, it is important to note that different types of dietary fiber (i.e.. soluble, insoluble, readily fermentable, poorly fermented or nonfermented) hold different health-promoting properties, and it is important to consume dietary fiber from a variety of sources to obtain the various benefits discussed above (18). Thus, fruits and vegetables remain an importance source of dietary fiber in the human diet (16).

Energy density is a measure of the amount of energy a food contains per unit of weight, usually grams, of food. Consumption of foods that are low in energy density is thought to be beneficial to enhance satiety and reduce hunger, thus promoting a reduction of total energy intake during a meal or throughout the day. In fact, the World Health Organization suggested that the increase in foods with higher energy density is partly responsible for the global increase in overweight and obesity (20). This is primarily because satiation, or the cessation of eating during a meal, is largely driven by a food's weight, as well as the portion size (21), rather than the energy consumed in the meal (21, 22). The mechanisms responsible for this pattern are complex and not entirely understood. However, theories include a slowing of gastric emptying rate, increased gastric filling, visual cues, including aeration of foods or altering the size of food particles, and a decline of the hedonic response to food that responds to the amount of food eaten rather than the energy content (22, 23). A recent meta-analysis of the impact of energy density on energy intake in both children and adults indicated a linear relationship between energy density and energy intake, and that this effect is greater when decreasing the energy density of an entire ad libitum meal rather than consuming a low energy dense preload (23, 24). This effect was seen in children as young as 3 years of age (25). Methods of lowering the energy content of a meal include preceding the meal with fruits, salads or soups, which are inherently low energy dense due to their high-water content (26). Reducing the fat content of a food also effectively reduces the energy density due to fat being the most energy-dense macronutrient (9 kilocalories (kcals) per gram of fat vs 4kcal per gram of carbohydrates and protein (26). Fruits and vegetables are known for being especially low in energy density due to their high water and fiber content and low fat content and they may therefore be added to meals to help reduce the overall energy density of the meal and, subsequently, help to reduce energy intake. In addition to being low in energy density, fruits and vegetables tend to be high in nutrient density.

Nutrient density refers to the nutrient content of a food relative to its weight and/or energy content. Fruits and vegetables tend to be high in nutrient density due to their high content of vitamins and minerals relative to their energy content (27, 28). Vitamins are organic compounds found primarily in plant foods that are essential for metabolic pathways necessary for cell function, growth, and development. There are 13 essential vitamins including the fat-soluble vitamins A, D, E, and K, and the water-soluble vitamins B1, B2, B3, B5, B6, B8, B9, B12, and C. Like vitamins, minerals are inorganic compounds that are also essential for normal human functioning and have functions including maintaining bone, muscle, brain, and heart health, cell protection and functionality, appropriate pH balance within the cells and the body, and aiding in the function of enzymes and hormones (29). Minerals are classified into two categories based on the amount that the body requires for functioning: macrominerals, which the body needs in large amounts and include calcium, phosphorus, magnesium, sodium, potassium, chloride, and sulfur, and trace minerals, which the body needs in small amounts and include iron, manganese, copper, iodine, zinc, cobalt, fluoride, and selenium. While all of these minerals are considered important, calcium, copper, iron, selenium, and zinc are considered especially vital to obtain in the diet due to their role in maintaining normal physiological and biological processes and their association with genetic diseases (30). In children, adequate consumption of calcium is especially important because of its critical role in mineralization of bone. In addition to this, it also plays a role in nerve impulse transmission, contraction of muscles, blood clotting and blood pressure regulation, and cellular metabolism. Significant dietary sources of calcium include dairy products, certain fish, and dark green leafy vegetables, cruciferous vegetables, legumes, and dried fruits (30). Thus, children should be able to achieve the recommended intake of calcium by adhering to the DGA set forth by the USDA. Although fruits and vegetables may not supply the diet with all essential micronutrients (vitamins and minerals), such as vitamin B-12 which is primarily sourced from meat and dairy products, consuming them is undoubtedly an efficient way to meet most of these recommendations in addition to the other benefits they offer (31). As a result of the low fruit and vegetable intake observed in children and adolescents in the United States, the USDA Dietary Guidelines for Americans 2020-2025 notes four nutrients of public health concern: calcium, vitamin D, potassium, and dietary fiber (32).

In addition to the vitamins and minerals needed for optimal human functioning, fruits and vegetables also contain high amounts of bioactive phytochemicals. Phytochemicals are non-nutritive compounds found in plant foods that have been found to hold antioxidant properties and help to reduce the risk of chronic diseases. Unlike vitamins and minerals, phytochemicals are not considered essential for human functioning. Rather, they hold strong antioxidant and anti-cancer properties that are partially responsible for the role of fruit and vegetable intake in reducing inflammation, the risk of developing cardiovascular disease and cancer, their mortality, and all-cause mortality (28). Although there are over 5,000 known dietary phytochemicals, the most important and well-known ones are classified into 6 unique categories: phytosterols, alkaloids, nitrogen-containing compounds, organosulfur compounds, phenolics, and carotenoids, with the most well-studied being the latter two (28). Because of the wide variety and complexity of these compounds and their synergistic effect, the same phytochemicals isolated into dietary supplements are not as potent as consuming them in the form of fruits, vegetables, and whole grains (28). Additionally, one must consume a large variety of fruits, vegetables, and whole grains to obtain all the beneficial phytochemicals available in these foods. Hence, the DGA recommends consuming vegetables from each subgroup (dark green, red, orange, beans, peas, lentils, and starchy), whole fruits, and whole grains as part of a healthy diet (32).

Although the majority of the chronic diseases mentioned above occur in adulthood, the predisposing risk factors to these diseases can begin developing during childhood. In fact, children are now found to have vascular changes including hypertension, dysglycemia, dyslipidemia, atherosclerotic lesions, and systemic inflammation that put them at greater risk of developing cardiovascular diseases in adulthood (33-36). A significant risk factor for these vascular and metabolic disturbances in children is obesity (36, 37). This is important to note because the prevalence of childhood obesity is rapidly increasing globally (33). Therefore, measures *must* be taken to address these issues during childhood to create a healthier population of adults in the US; among measures may be to increase their fruit and vegetable intake to promote a healthy weight, support metabolic function, and increase anti-inflammatory antioxidant intake, as has been shown in adults (38).

Emphasizing fruit and vegetable intake during childhood and during preschool age is particularly important because this is when primary establishment of dietary habits occurs; dietary habits developed around preschool age tend to persist into adolescence and adulthood (39, 40).

### 1.5 Measuring FV intake in preschoolers

This field of research is limited by the accuracy of dietary intake measurement to assess intervention effects (41). Different ways to measure this include direct observation, parent-reported 24-hour food intake, and measuring skin carotenoids. Direct observation of food intake is the technique of either estimating food intake by directly watching people eat (following extensive researcher training) (42) or objectively measuring the weight of food before and after the meal is consumed using the plate-waste method. This method is associated with the least amount of error, assuming that the trained researchers remain attentive and ensure that all foods children spill or drop are accounted for (41). However, this method is only practical when measuring cross-sectional food intake at a given time; it is not feasible to use this method to measure dietary habits and patterns in children(42). Parent-reported 24-hour dietary recalls are considered the gold standard for measuring average, typical food intake in children(43). This tool involves asking parents to recall all foods and beverages that the child consumed the previous day using specific phrases and questions to prompt their memory and is typically done in across three days. However, a commonly-reported limitation of this method is that parents may not know everything that their child has eaten, particularly if the parents have multiple children or the child is fed by different people throughout the day (44). Additionally, validation studies have repeatedly shown misreporting of portion size estimates and energy intake by parents when using this method (45). Nonetheless, 24-hour dietary recalls are still considered the most accurate and practical method of recording children’s food intake (46).

Skin carotenoids are measured using reflection spectroscopy, which is the quantification of the color and intensity of reflected light in the skin after illumination with white light (45). When fruits and vegetables are consumed, certain molecules known as carotenoids are absorbed in the intestines and deposited in various tissues including the skin (47). While skin carotenoids have various benefits in themselves, they may also be used as a biomarker to objectively measure FV intake in preschoolers (48-50) and have been repeatedly validated as a proxy for the traditional 24-hour dietary recall method (51-53) to more efficiently measure the effectiveness of an intervention over time (49, 54).

There are several considerations when measuring food intake to determine intervention effectiveness, and these are largely influenced by the available resources. These will be discussed in the context of FV interventions.

The first consideration is the timing of food intake measurement. The most accurate measure of FV intake is measuring all food consumption throughout the entire day, including meals consumed at home and at the childcare center. One method of achieving this is by directly measuring food intake during snacks and meals at the childcare center and providing pre-weighed foods for the children to consume while not at the childcare center and asking them to return all uneaten foods back. However, this requires an immense amount of resources. Additionally, using these methods of measuring food intake may not provide an accurate reflection of normal eating behavior. For instance, the foods provided by the researchers may not be comparable to the foods consumed at home (41, 42, 55). Thus, a potential solution to these considerations is to directly measure food intake of regularly consumed foods while the children are at the childcare centers, provided these resources are available, and conducting parent-reported dietary recalls to record food intake while the children are at home.

### 1.6 FV intake and physical activity, sedentary behavior, and screentime

Physical activity during childhood is important because it promotes healthy cardiometabolic fitness, body composition, and brain (56), bone, and emotional health. Like diet, physical activity is a lifestyle pattern that tracks into adolescence and adulthood (57), where it provides further benefits for physical and emotional health (58). However, similar to FV intake, physical activity levels in the United States are also much lower than what is recommended.

Estimates of the number of preschoolers meeting the MVPA guidelines range from 14% to 34.6% (59-62). There seems to be a slight increase in 6-11 year old children with the rise of sports participation (63). However, after 12 years of age, physical activity rapidly decreases; only 7.5% of 12-15 year old children and 5.1% of 16-19 year old children meet MVPA guidelines (63). Further, these statistics were obtained prior to the COVID-19 pandemic; PA levels are suspected to have decreased significantly since the start of the pandemic (64). A longitudinal study in over 2300 European preschoolers found that a majority of the children did not meet the recommendations for adequate PA (87.6%) or limiting screentime (69.8%). After one year, they repeated the measurement and found that only 0.6% of the children met both recommendations of PA and screentime at both time points (baseline and after 1 year), while 50.4% did not meet either recommendation at either time point. Thus, efforts should be placed in ensuring that young children, as young as preschool age, build healthy PA habits that do not rely on sports participation, and children should be educated on the various benefits of PA (57, 65). Similar to diet, the predictors of physical activity in children are also multifactorial (57). Examining the predictors of and understanding the reason for the low levels of PA observed in preschoolers is an important factor to effectively develop ways to address the PA deficiency. A literature review by Hodges and colleagues (57) examined various predictors of PA levels in children and identified associations such as children's personality traits including shyness and anxiety, and their enjoyment of physical activity (57). Like diet, parents' PA levels also predict their child's PA levels, as does their sedentary hours per day, simply because active parents model this behavior to their children, and they are more likely to engage their children in activity along with them (12). Hence, the reasons for parents not being active, including being a single parent, lack of time, and lack of energy by the end of the day (57). Factors related to socioeconomic status (SES) and education level of parents and families also influence PA levels in children. Firstly, low SES in many instances is associated with neighborhood environments that are less safe and not conducive to children engaging in playing outdoors. Instead, they are more likely to engage in sedentary behavior, such as watching TV and, consequently, are more likely to be obese (57). Parents may also believe that their children meet the PA guidelines while at preschool or childcare and may not encourage their child to engage in additional activity when they are home. Other predictors include the PA opportunities provided at preschools and childcare centers that the children attend. In this case, children have little control over how active they are allowed to be, and factors including available space, equipment, and resources, safety concerns, inclement weather, and whether they incorporate and encourage periods of structured and unstructured PA throughout the school day (57, 66).

Studies show that meeting physical activity recommendations and consuming a healthy diet tend to present in tandem, in a phenomenon termed "clustering" (67, 68), and the presence of these behaviors are also associated with better mental health (67, 69). The directionality of the association between physical activity and diet has long perplexed scientists. This is particularly true in children, where engaging in physical activity and diet are not driven by the same motivations typically observed in adults, such as the desire to achieve mastery, to achieve a healthy weight, meet aesthetic standards, and to promote cardiac and emotional health (70). While this is partially driven by the parents' desires to promote a healthy lifestyle for their child (71, 72), there are also links between children's PA and healthy eating habits that seem to be independent of the parent's behaviors and remain to be explained by the literature (57).

The association between healthy eating habits and PA In children may have origins in the fact that sedentary behavior, including screen time, tends to elicit less healthy eating behaviors; they have both been implicated in unhealthy snacking patterns in children (73). A cross-sectional study by Knebel et al. (67) investigated clustering of physical activity, diet, and screentime, and subsequently categorized adolescents into one of three clusters of behaviors that tended to present together: the Phubbers, characterized by high cell phone usage and low FV intake and PA levels, the Gamers, characterized by high computer and gaming usage, low PA levels, and high processed food intake, and the Healthier cluster, characterized by low cell phone usage and gaming, and high levels of both PA and FV intake. The very fact that these clusters exist suggests that the behaviors within the clusters are likely interrelated with one-another, and that addressing them as such may be useful in developing methods of promoting health in both of those aspects of lifestyle (67). In a longitudinal study of European preschoolers, watching more than 1 hour of TV per day was associated with increased consumption of consuming carbonated drinks, sugar-sweetened beverages including juice and milk, sugar- and salt-laden processed snack foods, and processed meats, and decreased consumption of fruits, vegetables, and fish (73). Similarly, a longitudinal study in Hawaiian children also observed this clustering in children. In this study, researchers implemented a nutrition and physical activity-promoting program in 9-12 year old children and measured the children's physical activity and intake of fruits and vegetables at baseline, and at a

5- and 10-year follow-up. The findings of this research showed that MVPA at baseline and year 5 was significantly correlated with both fruit and vegetable intake at the respective year. MVPA at year 10 correlated with vegetable intake at year 10, but not with fruit intake at year 10. Overall, these findings support the notion of the clustering of PA engagement and FV intake in childhood and early adulthood, and that these various behaviors persist for at least 5 years (74). Importantly, this data was measured using self-reported questionnaires and may therefore be subject to self-report bias (75). Nonetheless, this highlights the importance of promoting healthy habits in children from an early age and supporting these behaviors as children get older to promote their maintenance and prevent the decline typically observed for all three of these behaviors, as discussed in section 1.

### 1.7 Measuring physical activity, sedentary behavior, and screentime in preschoolers

Reporting of physical activity in preschoolers is more difficult than in older children or adults (76, 77). This is because preschoolers tend to have several short bursts of activity as opposed to longer bouts of structured, planned activity in older children (76). There are both subjective and objective methods of measuring PA in children, and each of these has their benefits and limitations.

The subjective methods of measuring PA include using questionnaires, usually reported by parents or the childcare providers. These tools ask the parents or caregivers to quantify their child's behaviors related to how active they are, including making judgements of the intensity level of the activity, and can also include questions about sedentary activities and screen time. A benefit of these tools that the objective tools are unable to measure is their ability to capture children's motivations and feelings related to PA, their screen time, and quality of their sedentary behavior. This is particularly important in preschoolers because motivation and enjoyment of the activities influence how likely children will continue being active (65). In fact, this quality of PA is explicitly stated in recommendation for PA guidelines. Screentime is helpful to measure as well because it is associated with negative behaviors such as unhealthy snacking, as mentioned above. Finally, determining the quality of children's sedentary behavior, such as what they are doing when they are being sedentary, is also informative to determine correlates of PA and where to intervene to limit sedentary behavior in children (76). The limitations associated with using these questionnaires is that it is difficult to estimate the intensity level of the activity. Additionally, when the preschoolers move in short bouts activity, the recording may be prone to either under- or over-reporting. When it comes to parent-reported PA, misreporting usually presents as over-estimation of PA, likely due to the knowledge that increasing levels of PA are deemed positive, while sedentary behavior is deemed as negative (76).

There are numerous methods used to objectively measure physical activity. A relatively precise assessment of physical activity is the measurement of direct or indirect calorimetry (77). Direct calorimetry involves the measurement of all carbon dioxide and oxygen exchange within the body, measured with a closed-system calorimeter, such as an enclosed metabolic chamber (79). Indirect calorimetry is a similar technique that uses a canopy or breath-by-breath mask to measure inhaled and expired carbon dioxide and oxygen (79). This measure does not account for the release of water or gases from the body from areas other the airways (79). Additionally, while both calorimetry methods are precise methods of energy expenditure, they are very restrictive and require staying in a metabolic chamber or connected to a metabolic cart throughout the measurement. Thus, these are not practical methods of measuring PA in preschoolers in free-living conditions (79). Other objective measures of physical activity use accelerometry-based devices (80). These devices are typically placed around the child's wrist, arm, waist, hip, or leg, and they measure movement in one, two, or three planes of motion and measure movement in epochs that summarize movement within 10-, 30-, or 60-seconds periods that are translated into the various levels of physical activity based on the amount of movement. Other factors that are included in this algorithm include age, height, and sex (81, 82). Of these accelerometry devices, the gold standard devices for free-living preschoolers are the triaxial ActiGraph (GT3X), Actical, and ActivPal devices (77, 83). According to a recent meta-analysis comparing the effectiveness of the various measurement methods, combining these accelerometers with the proxy tools, such as questionnaires, may be an optimal way to qualify and quantify helpful physical activity data from preschoolers (77).

### 1.8 Changing FV intake in preschool age children

Efforts to improve FV intake in young children date back to 1991 with the 5-A-Day initiative initially developed by the National Cancer Institute and later adopted by the US Centers for Disease Control and Prevention (CDC) (84). This initiative aimed to raise awareness about the importance of daily consumption of fruits and vegetables and, in partnership with the Produce for Better Health Foundation, supported innovative projects and programs within communities (84, 85). Since then, there have been numerous interventions developed to address this low FV intake, with studies finding mixed results regarding intervention effectiveness (86). These will be further discussed in Chapter 2 of this manuscript.

The location of an intervention is an important consideration that may influence the efficacy of an intervention to improve FV intake; previous interventions have targeted children in home settings, in community settings, and during childcare or preschool (87). In 2019, nearly two-thirds (64%) of 3-5 year old children in the United States were enrolled in pre-primary (preschool and kindergarten) programs, with 64.7% of all enrollments being full-time (88). Thus, childcare services have a large influence on children's development in the United States, and this may be an efficient avenue to effectively influence children's behavior, namely dietary behavior(89-91). Specific correlates of children's dietary intake were found to be the child's age, sex, the type of foods being served and serving methods, book reading, teacher's feeding practices, peer influence, repeated exposure, and nutrition education (7), making these important considerations for childcare-based interventions.

The concern with home-based interventions is that they must overcome the obstacle that lack of access or ability to purchase FVs may pose to families (92). If parents are busy at work or preoccupied with other children, the intervention may not have the same effectiveness compared to parents who have fewer time-commitments and restrictions (93). Similarly, while lab-based interventions, such as an intervention by Staiano et al. (94), are effective, these have very limited external validity, as it is difficult to determine the effectiveness and feasibility of this study in a preschool setting, which is required to reach more children.

Use of theoretical frameworks and behavior change techniques that support the development of habits. Two theoretical frameworks will be discussed that are closely related to dietary behavior in young children. Social Cognitive Theory (SCT) was first developed by Bandura (95) and has been repeatedly used as a model to develop behavior change interventions, including those aimed at improving health and lifestyle (96). The theory highlights three agencies that interplay in one's life and are collectively termed reciprocal determinism: personal factors, behavior, and environmental facilitators (97). The main tenets of SCT include perceived self-efficacy, outcome expectations and expectancies, goal-setting, and self-regulation and evaluation of self-regulatory capabilities (96). Perceived self-efficacy refers to a person's belief that they can complete a specific action or to produce an effect (96). The three main components of this are mastery experiences, which reinforce a person's resilience and belief in their own abilities, vicarious experiences, which rely on a person watching others' efforts and success to increase their own perception of their capabilities, and social persuasion, which uses external motivation and positive evaluation of their abilities to encourage greater and sustained effort (96). Outcome expectations and expectancies are heavily influenced by the physical effects, social effects/sanctions, and self-evaluative reactions of a person (96). Goal setting is used to promote self-motivation and, ultimately, contribute to self-efficacy through evaluation of a person's personal, situational, and systemic impediments towards reaching a specific goal, and ideally finding solutions to overcome those obstacles (96). Lastly, self-regulatory capabilities, such as performance judgements, are also an important component of the SCT in that they reaffirm a person's abilities and ultimately contributes to their self-efficacy (96). Evidently, there are several SCT tenets that behavioral interventions may draw on to improve health outcomes (96).

The Mere Exposure hypothesis, first developed by Zajonc in 1968, describes the phenomenon that repeated exposed to a stimulus increases attitude towards that stimulus (98). This hypothesis has been applied in context of development of food preferences, especially with regards to healthy foods (99). Dr. Leann Birch, a pioneer and major contributor in field of child nutrition and eating behavior, repeatedly exemplified this effect in her work. In 1982, Birch and Marlin observed that preschoolers who were repeatedly exposed to novel foods, including cheeses and fruits, were more likely to report liking the foods. In fact, the more often they were exposed to these novel foods, the more likely they were to accept them (100). Studies have also shown that babies that are exposed to FVs early in life tend to like FVs more as they grow older (99), but, as many studies have repeatedly observed, liking of foods does not equate to increased consumption over time (101). Similarly, as mentioned earlier in this review, dietary behaviors are multifaceted; they are affected by both internal and external contexts. As such, some children seem to be "resistant" to the effects of repeated exposure to increase food acceptability (102, 103). Since these cornerstone findings, the use of repeated exposure to and flavor learning has been widely examined in children in different situations, contexts, and with different backgrounds to identify other mediators of this relationship (99, 102, 104-107). It is certainly progress, but there are many additional factors that may contribute more to the establishment of dietary habits. Therefore, the next step for this field of research is to answer the question of how long the effects will last, and then develop and implement strategies to improve the efficacy of the interventions on preschoolers FV intake. The long-term goal of this approach is to increase, or at least prevent the decline of, the number of children that meet the USDA recommendations for FV intake and eventually mitigate the ailments attributed to low FV intake in adulthood. It is well established that dietary patterns are established early in childhood, and therefore great effort should be put in prioritizing the development of healthy habits.

**1.9 Conclusion**

It is evident that health-promoting behaviors, namely FV consumption and adequate physical activity, in young children is complex. The plethora of factors that contribute to low FV intake and PA levels in young children make it difficult to develop interventions to effectively target the behaviors. As such, there is a need for research to address this deficiency. The gaps in the literature pertain to the low availability of evidence-driven and theory-informed interventions that objectively assess intervention effectiveness. Thus, the feasibility of such an intervention should be assessed to more successfully target this low FV intake. These findings may have important implications and have the potential to develop more effective approaches to improve dietary behaviors in preschoolers and address this significant public health concern.

**1.10 Aims**

To address these gaps in the literature, we examined the current literature to identify and evaluate previous interventions that aimed to improve fruit and vegetable intake (Manuscript I). These findings were then used to inform the development of a study to assess the feasibility of a behavior change theory-based intervention that aimed to increase FV intake in preschoolers (Manuscript II). Secondarily, the relationships between FV intake, physical activity, and screentime were examined in this sample (Manuscript III). Consequently, three manuscripts were developed.

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# CHAPTER 2: MANUSCRIPT I

**Preschool- and childcare center-based interventions to increase fruit and vegetable intake in preschool children in the United States: A systematic review of effectiveness and behavior change techniques.**


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**Introduction**

Promoting fruit and vegetable (FV) intake in children is critical to support proper brain and body development (40, 108, 109) and to establish healthy dietary habits that persist over the life course (110, 111). Despite the long-standing explicit guidelines and evidence regarding the importance of FV intake, average FV consumption amongst all US children (2-18 years old) remains low: only 40% and 7% meet the recommended intake for fruits and vegetables, respectively (112). The current average intake for children is only 0.9 cup equivalents of each (60% of recommended), with consumption levels that decline with age and are restricted in variety relative to recommended guidelines (1). Thus, in support of public health, it is important to develop strategies to improve FV intake in children.

Preschool age children begin developing their own dietary habits by gaining autonomy over their food choice (110, 111), evident in the decline in FV intake as children transition from preschool age to school age (113-115). Thus, preschool age (2-5 years old) may be an optimal time for a dietary intervention to promote FV intake throughout the lifespan. However, there are key gaps and inconsistencies present in diet research that will be discussed below.

Consideration of the measurement tool in diet research is particularly important in young children because they are unable to accurately report their own intake (55, 116). Parent-reported measures, while most frequently used in research (43, 46, 117), have been repeatedly shown to be subjective and prone to recall and reporting bias (44, 45); this is especially problematic when parents are asked to report on periods of time for which they are not directly responsible for child feeding, such as during childcare hours (118, 119). As such, use of objective observation measures is critical with this age group (120, 121). An additional objective measure specific to FV intake is the skin carotenoid level (49, 51-54). When FVs are consumed, the carotenoids in the FVs are absorbed and then deposited in various tissues including the skin (47), which can be quantified using reflection spectroscopy (45) to objectively measure FV intake within the previous two to four weeks (48-50). Due to the discrepancies associated with the different techniques used to measure dietary intake, reviews should distinguish between studies with subjective and objective measures to adequately evaluate the validity of a large proportion of this body of work.

The setting of dietary interventions in children is another significant consideration. In 2019, nearly two-thirds (64%) of 3-5 year old children in the US were enrolled in childcare or preschool with 64.7% of all enrollments being full-time(88). Thus, childcare services have a large influence on children's development in the US(7), and this may be an efficient avenue to effectively influence children's behavior, namely dietary behavior(89-91). In evaluating intervention effectiveness, it is therefore vital to consider the setting of the interventions to avoid extrapolating evidence for intervention effectiveness to other contexts.

Behavior change techniques (BCTs) are the intervention components regarded as the "active ingredients" within behavior change interventions. It is often helpful to examine the use of BCTs within interventions aimed at changing dietary behaviors to assess the mechanism by which interventions may be effective at causing the behavior change(122). Given the heterogeneity in intervention techniques used in dietary interventions, evaluation of BCTs in reviews and meta-analyses may provide important insight on the underlying intervention components that may be at play (122-124).

Use of theory in developing an intervention is another metric that can be examined within interventions that target behavior change. This metric evaluates how exactly a specific theory and its concepts are utilized to tailor intervention techniques and components and allows for a better understanding of why an intervention is effective or ineffective. This knowledge may then be evaluated in systematic reviews and applied to the refinement of an interventions to better target the tenets of the theory (125, 126).

A recent systematic literature review by Hodder et al. (127) of FV intake in children five years and younger identified 80 trials reporting a large variety of interventions to promote FV intake in preschool-aged children globally. This review included interventions conducted in all settings and using various intervention methods, though it did not distinguish between subjective and objective measurements of food intake or evaluate BCTs and use of theory in the included studies. The main implications drawn from these findings is that more pragmatic studies are needed to test the interventions, and that these interventions should be based on BCTs and theoretical frameworks that may explain the mechanism by which these interventions may change children's dietary behaviors related to FV intake (128).

In 2012, Hendrie et al. (129)published a systematic review to explore the use of BCTs in home- and school-based interventions for the prevention of childhood obesity involving children and parents. More specifically, they compared the number and type of BCTs used in effective and ineffective studies and reported that effective studies incorporated more BCTs than ineffective studies (median of 10 versus 6.5). However, this review was not specific to interventions aimed at improving FV intake, so we are unable to determine whether these findings are applicable to FV interventions specifically.

In 2017, Hendrie et al. (130) used a similar technique to evaluate interventions to improve vegetable intake in children and found that the BCTs "Repeated exposure", "Provision of staff training," and "Planning for social support or change," were associated with effective behavior change. This review only examined interventions conducted in home or community settings and, therefore, these results may not be extrapolated to interventions conducted in other settings, such as childcare centers (53). Similar to the review by Hodder et al., these two reviews are limited by their inclusion of subjective measures of dietary change, and lack of evaluation of use of theory within the included studies.

To our knowledge, there are currently no published reviews that evaluate both the effectiveness of FV interventions specifically in childcare- or preschool-based settings in the US, and the use of theoretical frameworks and BCTs within these studies. This is a critical gap as comparing different types of interventions, exploring whether use of theory and BCTs moderate effectiveness, and summarizing the level of evidence is critically needed to develop effective interventions in the future. Therefore, the purpose of this review was to systematically identify published randomized controlled trials (RCTs) evaluating childcare- or preschool-based interventions designed to increase objectively measured intake of fruits, vegetables, or both, in preschool children (aged 2-5 years) in the US and to summarize their methods and results. This review also aimed to identify the use of theoretical models and BCTs in each study and to assess their effectiveness in improving FV intake.

**Methods**

**Search Strategy**. This systematic review utilized the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and was pre-registered with PROSPERO International Prospective Register of Systematic Reviews (ID: CRD42022350953). Studies were identified using CINAHL, PubMed, Web of Science, and MEDLINE (Ovid) between August 15 and September 4, 2022. The following search terms, using Boolean operators and MeSH terms (PubMed), were used: (fruit OR vegetable) AND (intake OR consumption) AND ("young children" OR preschool OR pre-kindergarten) AND (preschool OR center* OR childcare* OR daycare*) AND (intervention* OR program OR "nutrition education"). Additional search hedges included in the search were designed to include only randomized-controlled trials and studies conducted within the US. The search was restricted to articles published in English between January 2012 and September 2022. The reference list of included articles were reviewed to identify additional relevant articles. Search strategies were reviewed by a university librarian.

**Grey Literature**. The risk of publication bias was evaluated by examining preprints and unpublished studies from clinical trial registries and dissertation/theses, using a google search tool to limit results to ".gov," ".org", and ".edu" sources, ClinicalTrials.gov, ProQuest Dissertations and Theses Global database (unpublished theses), and MedRxiv (pre-prints of relevant studies). The search terms used for the grey literature search were "fruit and vegetable interventions in preschoolers in the United States." The search using the ProQuest Dissertations and Theses database was limited to scholarly journals and dissertations and theses, only English and in the US, and within the last 10 years. The search using the MedRxiv database used the previously stated Boolean search terms and was also limited to the last 10 years.

The risk of outcome reporting bias was assessed by comparing planned outcomes in the Methods section with reported outcomes to identify any missing outcomes. Two researchers (FH and AVN) independently screened the titles and abstracts of all articles based on the inclusion and exclusion criteria stated below. Any discrepancies between reviewers were resolved by discussion until consensus was reached. If necessary, a third reviewer (SK) was consulted.

**Eligibility Criteria.** Eligible studies were randomized controlled trials (RCTs) conducted in the US that utilized an intervention to improve fruit and/or vegetable intake in preschoolers (children 2-5 years old), were conducted in preschool or childcare settings, and examined the magnitude of change from baseline (between an intervention and control group) of the number of servings, portions, or grams of FVs consumed or changes in skin carotenoid levels used as a proxy for FV intake. We restricted our inclusion criteria study reports from interventions in the US to allow for comparison between studies that are based on the uniform national recommendations/guidelines, similar food supply, similar access to federal nutrition programs (WIC and SNAP) and known cultural differences in dietary patterns. For instance, the United Kingdom National Health Services (NHS) dietary guidelines for two- to five-year old children are: "should gradually move to eating the same foods as the rest of the family in the proportions shown in the Eatwell Guide(131)," with no specific consumption amount or type of FV specified during childhood. The Eatwell Guide specification for which FV to consume is different from the US guidance, for example, potatoes are not counted as vegetable. Only studies published within the previous 10 years were considered for inclusion to ensure recency of data and to reflect changes in early childhood education approaches. For example, the Mere Exposure hypothesis, first developed in 1968 and later applied to the context of children's dietary behavior, was considered the cornerstone method of improving children's diet until relatively recent studies found that it may not be applicable to all children (102) and results may not be sustainable (103) and researchers modified their approach. Only studies in which FV intake was measured objectively were included, and FV or diet must have been measured as one of the primary aims. Studies that reported only subjective measures of FV intake were excluded due to the lower validity of subjective dietary measures. Hence, those that used self-reported measures, such as dietary recalls or caregiver questionnaires, were excluded. Interventions that aimed to improve overall diet quality with fruit and/or vegetables as a component were also included; interventions whose primary aim were not diet related, such as those aiming to produce weight loss, were excluded due to the potential for confounding.

The population being studied included preschool children, parents, guardians, caregivers, and professionals responsible for the care of preschool children. Studies were included if they combined both child- and parent-targeted interventions. Studies were excluded if the interventions *only* targeted parents or caregivers of preschoolers, such as only parent-focused nutrition education interventions. Studies focused on populations with special developmental considerations, such as autism spectrum disorder, were also excluded because these children have been shown to respond differently to dietary interventions(132-134).

**Data Extraction and Synthesis.** Data selection and extraction were conducted with the use of Covidence, a program developed for conducting systematic reviews and meta-analyses. Studies were independently reviewed by two reviewers (FH and AVN) and selected for inclusion using a pre-specified form with the inclusion and exclusion criteria explicitly stated and reviewed by a professional expert in childhood nutrition (SK). Disagreements about article classifications between reviewers were resolved by discussion and consensus between the two reviewers (FH and AVN). A third reviewer (SK) was consulted, if necessary. A data extraction form was developed under supervision of an expert in the topic (WY) and piloted on five randomly selected studies that met inclusion criteria. Data was then extracted in duplicate by the first author (FH) and two additional authors (AVN and AR) to ensure duplicity.

Outcome data were extracted using mean difference in fruit and/or vegetable intake, where outcomes were reported in grams, and standardized mean difference, where outcomes were reported using a different method (grams per kilogram of body weight, grams per total energy intake, servings). The results of statistical analyses, including but not limited to t-tests, analysis of variance, analysis of covariance, and linear and mixed-model regressions. For studies that reported multiple timepoints for fruit and/or vegetable intake, the data for each timepoint was extracted separately. For studies that did not report effect size, effect size estimates (Cohen's *d*) were calculated by the first author using either means/standard deviations of each group or reported t-test values. We were unable to estimate effect size for two interventions due to lack of reported standard deviations(135). We also coded for additional study characteristics, if applicable to the study, including: 1) information pertaining to the study design, setting, length, frequency, description and length of interventions, outcomes measured and measurement tools used to obtain these measurements, 2) sample size, mean age of participants or proportion of children within provided age groups, sex of participants, 3) identification and classification of behavioral or cognitive theories and/or models used, 4) identification and classification of BCTs used, 5) predictors and/or confounders of response to the FV intervention, and 6) cost-effectiveness of the intervention, if applicable. The additional variables were evaluated on a case-by-case basis and compared to the usual care and/or comparator(s) defined within the intervention.

The use of BCTs were identified within studies and coded accordingly using a standardized taxonomy of behavior change techniques(123). This taxonomy consists of 93 unique BCTs grouped into 16 domains. BCTs used within studies were coded in duplicate by the first author (FH) and two independent reviewers (AVN and AR). Interclass correlation coefficient was used to establish intercoder reliability of BCTs present and absent, as well as number of BCTs used in each study. Any discrepancies were then resolved by consensus amongst all three coders.

The use of theory within studies was also evaluated by the first author (FH) using a Theory Coding Scheme developed by Michie and Prestwich in 2010 (125). This taxonomy consists of 19 items; items 1-6 evaluate if theory is mentioned, whether it was used to select participants, and whether it was tailored to participants, items 7-11 evaluate whether the relevant theoretical constructs were explicitly targeted and all intervention techniques are linked to a specific construct, and items 12-19 assess whether the theory was adequately measured within the intervention and explains the changes observed, and whether the theory was refined based on the intervention's outcomes. This review aimed to identify the use of theory in the development of interventions, rather than how well the specific theories were able to yield results. Hence, items 12-19 were not evaluated in this review (126). Items 1-11 were scored based on whether they were present within the paper and were summed to yield a Use of Theory score ranging from 0 to 11, with higher scores indicating greater use of theory (126). For studies for which a separate publication was available to describe the intervention development or methodology, we used that publication to assess the intervention's Use of Theory score.

The included studies were organized into subgroups by type of intervention (e.g. repeated exposure, nutrition education). Although intervention types were not directly compared to one another due to heterogeneity between studies, each subgroup was evaluated as a group based on the level of evidence, patterns in the measured effect (mean difference in fruit and/or vegetable intake), and any adverse effects unique to that intervention type. Information regarding the maintenance or sustainability of the behavior change was evaluated by assessing data of a post-intervention follow-up period, if applicable, within each included study. Following narrative synthesis of the results, the additional outcomes were evaluated in the context of the intervention and its overall desired effect.

**Quality Assessment.** Risk of bias within studies was examined using the Cochrane risk of bias assessment tool (136). This tool evaluates randomization, deviations from the intended interventions, outcome data, measurement of outcome, and selection of the reported results. Two researchers (FH and AVN) individually assessed the included studies for risk of bias using this tool. The risk of bias was judged within each domain and overall risk-of-bias as 'low risk', 'some concerns', 'high risk', or 'unclear risk'. Any discrepancies between reviewers were resolved by consensus between the two reviewers.

## Results

**Search Results.** A total of 70 studies was identified during the initial search. After removing duplicates, 53 remained and were screened by title and abstract using the inclusion and exclusion criteria. No additional studies were identified using backward and forward snowballing. No studies were identified in the grey literature. After title and abstract screening, 14 studies were screened as full text, and eight were excluded for various reasons, such as not measuring FV intake objectively (ie. using self-reported measures of dietary intake), not being conducted in the US, and not RCTs. The final review included six papers that reported on nine unique interventions from 2012 to 2022 (Figure 1). Studies that reported on multiple intervention groups were analyzed as separate interventions.

**Study Characteristics**. Study designs included cluster- (six interventions), crossover- (two interventions) and individual- (one intervention) RCTs. Interventions ranged from a single day to 12 weeks, with a frequency of two to five days per week. The mean total intervention days across all interventions was 20.33 days, with a range of 1 to 40 days. Only two studies (two interventions) included a follow-up period, which included seven days (94) and three months (137) post-intervention. The remaining four studies (seven interventions) only assessed FV intake immediately post-intervention (135, 138-140). All interventions were conducted in preschool settings; three studies (five interventions) specified the use of Head Start programs for the intervention (135, 139, 140). Only four of nine studies reported the mean age of the participants, which ranged from 4.1 to 4.9 years. The remaining studies reported the proportion of 2-3 and 4-5 year olds (135, 140) or were not permitted to record the children's ages(137).

Intervention components used across studies were nutrition education (137-140), changing the feeding environment (135, 140), peer modeling(94), and repeated exposure to FVs (137, 140). Two interventions were classified as multi-component as they included more than one of the intervention components: Witt et al. (137) included nutrition education and repeated exposure and Smith et al (140) included nutrition education, changing the food environment, and repeated exposure. Additionally, three interventions elicited parent involvement by sending home newsletters (140) or education materials (137) to families of participating children. No included study reported cost-effectiveness of the intervention.

Seven studies reported on directly measured fruit and/or vegetable consumption using direct observation methods of visual observation (135, 138), photo-assisted(139), or plate-waste method (94, 137) during either snack (94, 137, 138) or lunch (135, 139). The remaining two, both by Smith and colleagues (140), estimated FV intake by measuring skin carotenoid levels using resonance Raman spectroscopy. Four interventions measured only vegetables (94, 138, 139), three interventions measured both fruits and vegetables separately (135, 137), and two interventions measured combined fruits and vegetable intake (140).

**Intervention effectiveness and study quality assessment.** Overall, six out of nine interventions were able to significantly increase fruit and/or vegetable intake. Of these six, half of them were conducted in Head Start programs. Two (out of three) interventions observed an increase in fruit consumption. Four (out of seven) interventions observed an increase in vegetable consumption. One of the two interventions that measured skin carotenoids was effective at improving FV consumption. Two of the studies with no FV improvement used changing the feeding environment (135, 140) and one used peer modeling (94) as their intervention method. Unexpectedly, one intervention led to a significant decrease in FV consumption (135). Two of these three ineffective studies were conducted in Head Start programs. The characteristics of all included studies are described in **Supplementary Table 1**.

[Insert Additional file 1. Summary of Randomized Controlled Trials Included in this Systematic Review.]

We were unable to estimate effect size for two interventions due to lack of reported standard deviations (135). Amongst the studies with statistically significant effects, two studies (138, 139) had small effect sizes ($d<0.50$), one (138) had a medium effect size ($d$ is 0.50 to <0.80), and the remaining two (94, 137, 140) had large effect sizes ($d>0.80$). Amongst the studies with no statistically significant effects, both had large effect sizes ($d>0.80$)(94, 140).

Three of the six studies (which reported on five of nine interventions) were rated as low risk of bias and the remaining four were rated as having some concerns for bias. The reasons for were no mention of pre-specified analysis plan (138) and no information regarding concealment of allocation sequence (to both researchers and subjects) prior to assignment of the intervention (137, 139). A table reporting the risk of bias assessments is provided in Additional file 2.

**Behavior-change techniques.** Intercoder reliability determined by inter-rater intraclass correlation coefficient (ICC) was “moderate” for BCTs present and absent (ICC=0.70) and “good” for the number of BCTs used in each study (ICC=0.77). Overall, 23 of the 93 BCTs were used in at least one intervention. Interventions used between one and ten BCTs, spanning between one and seven domains, with an average of 4.44 techniques used.

The most commonly used BCTs were "Adding objects to the environment" and "Framing/reframing", which were both used in five of the nine interventions. The most commonly used domains (ie. used at least once within an intervention) were "Antecedents" and "Identity", both of which were used by five interventions. The most frequently used domains (ie. total number of BCTs within this domain used within studies) were "Antecedents" and "Repetition and substitution", whose BCTs were used nine and seven times across interventions, respectively. Two domains, "Feedback and monitoring" and "Regulation" were not used by any studies.

The BCTs and domains used in studies that did and did not effectively increase FV intake are described in **Table 1**. Effective studies used at least three BCTs and covered at least two domains. Two of the ineffective interventions used only the "Antecedents" domain (135, 140) and the remaining used only "Covert learning" domain (94). No domains or BCTs were exclusive to effective or ineffective interventions.

**Theoretical frameworks.** Two studies (54, 56) cited separate methodology publications that were used for the Use of Theory score (141, 142). Overall, the average Use of Theory score across interventions was 2.33 (out of 11) and ranged from 0 to 8. Four interventions (94, 137, 139, 140) mentioned a theory or model of behavior and only two of these (137, 139) linked the theory or its constructs to their intervention techniques. While the limited number of studies prohibits an empirical investigation, a narrative comparison reveals no pattern in the Use of Theory score and intervention effectiveness; the average score amongst effective and ineffective interventions was 2.17 and 2.67, respectively. In fact, the intervention with the highest Use of Theory score was ineffective (94). The Use of Theory scoring of included interventions is described in **Supplementary Table 2.**

## Discussion

We identified nine childcare- or preschool-based RCTs, reported in six different publications, that objectively measured FV intake in 2-5 year old children. Six of the nine achieved their goal of improving FV intake using the three following intervention methods: nutrition education, repeated exposure, and change in feeding environment. Two of the nine interventions had large effect sizes (55, 57), one had a moderate effect size (53), and two had small effect sizes (52, 53).

Even beyond the effects size heterogeneity, some studies reported substantial imprecision in effect size estimates. For example, in two of the studies reporting large effect sizes ($d > 2.5$), the standard errors were large and the differences did not reach the threshold for statistical significance at $p<.05$ (55, 56). Therefore, the nonsignificant findings in these two studies may be due to small sample sizes rather than an ineffective intervention.

Five of the six effective interventions incorporated the use of nutrition education. Importantly, all nutrition education interventions were interactive for children and improved FV intake even if children were not instructed to consume FVs; merely the knowledge of the importance of FVs led to increases in consumption. These findings are in line with similar reviews that found that interventions related to “experiential learning” of nutritional concepts and healthy eating were highly effective at improving FV preference and intake in young children, compared to those relying on parental involvement or contingent reinforcement (143), and especially if they contain multiple components or strategies (144, 145).

Repeated exposure was used alongside nutrition education in two interventions, both of which were effective at improving FV intake. The use of repeated exposure to achieve behavior change stems from the Theory of Mere Exposure (146), which predicts that repeatedly exposing children to certain things, including eating healthy foods, will make them more likely to engage in that behavior in the future (99, 147). What this theory fails to account for is that dietary behaviors are not dictated solely by liking of foods. Rather, they are driven by the complex interaction of food preferences, appetite, and external influences including, but not limited to, peer influence and pressure from a caregiver (148). Unfortunately, as with nutrition education, no intervention in this review solely used repeated exposure, so it cannot be determined whether this intervention method is able to increase FV intake when utilized without additional components.

Based on our narrative synthesis, changing the feeding environment was not consistently effective. The only manipulation of the feeding environment that was effective at improving fruit, but not vegetable, intake was serving FV five minutes before the rest of the meal (135). Conversely, neither providing pre-portioned meals (135), nor providing FVs for children to take home were able to improve FV intake (140). This suggests that the low FV intake amongst preschoolers is likely not improved solely by making them more available; rather, as suggested by these findings, an additional component, such as nutrition education, may be required to improve intake (140). In summary, these findings support the notion that many different forms of FV interventions may be effective at improving intake in preschoolers, although additional research is needed to confirm the findings.

The observation that studies with no significant improvement in FV intake used fewer BCTs is similar to the findings of other reviews evaluating BCTs and obesity-related behaviors in children (129, 149). The BCTs used in the studies that effectively improved FV intake are similar with those reported in the review by Hendrie et al. (129). We also observed the use of the "Framing/reframing" BCT within effective studies in this review, possibly due to different age groups (preschoolers versus all children) or the use of different versions of the BCT taxonomy. Finally, while "Restructuring the physical environment" and "Adding objects to the environment" were used in studies that both did and did not observe improvements in FV intake, the studies that exclusively used one or both of these BCTs observed no increase in FV intake, suggesting that using these two BCTs alone or in tandem is insufficient and that solely manipulating the food environment should be used with caution. Overall, our findings suggest both that no single BCT or domain must be included and no single BCT identified will certainly lead to observed improvements in FV intake in preschoolers.

The lack of association between the use of theory within interventions and their effectiveness is in line with the findings of some reviews (150-152), but opposed the findings of other reviews (153, 154). The differences observed in the Use of Theory scores between studies likely lie in the expertise of the researchers publishing the data; the researchers with psychological backgrounds will likely be more inclined to base interventions on theory, whereas those primarily in the dietetics or physiology field may not. Regardless, most intervention studies inherently target constructs of behavioral theories, even if they are not explicitly linked, evidenced by the use of similar methods. Hence, although interventions may have been informed by theory, the authors may not have explicitly reported the theory used, and therefore received a lower Use of Theory score. Amongst the included studies, Social Cognitive Theory was the most commonly mentioned theory, in line with a similar review of obesity-preventing interventions in children(155). Nonetheless, although rarely practiced, designing a behavior change intervention that is comprehensively informed by behavior change theory is agreed on to be a valuable factor in designing interventions aimed at changing behavior (125, 150, 151, 153).

This review differs from similar reviews in the number of included studies. This is primarily due to its narrow inclusion criteria for studies; we only included RCTs that objectively measured FV intake and were conducted in the US. This distinction was intentional to highlight the need for objectively measured outcomes in evaluating dietary interventions (118, 119). Importantly, the findings of this review pertain only to studies in the US and may not be generalizable to other countries. The small number of studies fulfilling the inclusion criteria, and the heterogeneity in measured outcomes, precluded this review from meta-analysis. Heterogeneity in effect sizes was likely partially due to the difference in the selected outcome measures; the two largest effect sizes were observed in studies that measured skin carotenoid levels (55), and the third study measured grams of vegetable intake of a snack of bell peppers and cheerios (56). The remainder of the studies measured pieces of FV (53) or grams (54) of FVs consumed during lunch or percent of FVs consumed (of total amount served) during lunch (57). Hence, comparison of the effect sizes in these studies should be conducted with caution. Another consequence of a limited number of studies is the lack of inferential testing of the role of theory and BCT. As observed in larger reviews that were able to empirically test the role of theory and BCTs, this information is valuable to understanding children's eating behavior in the US. Additionally, the evaluation of theory and BCT use is limited by the use of only published materials; authors were not contacted for additional information regarding their use of theoretical frameworks and the relevant constructs. Finally, while this review aimed to examine the cost-effectiveness of included studies, no studies provided this information. These limitations should be considered within the strengths of this systematic review, including the inclusion of only study designs that objectively measure the effects of interventions within a very specific setting and population. Importantly, this review highlights the need for further research in this field to allow for a more pragmatic evaluation in future reviews.

**Implications for future research.**

In addition to the need for future studies to address the limitations of this systematic review, the reviewed FV interventions and studies also highlight several opportunities for future research. Firstly, although it was not one of our primary outcomes, we found that only one study included an acclimation period to familiarize children with the research staff, methods, and changes to the eating environment. The lack of run-in time in the remaining studies may have increased the risk of the Hawthorne and placebo effects, which suggest that behavior may be altered due to the knowledge of being observed (156), particularly in studies that were implemented by unfamiliar researchers. To overcome this limitation, future studies should include an acclimation period to introduce researchers to the children and establish familiarity with equipment and methods, unless FV intake data is collected covertly. This suggestion may also mitigate any changes in eating behavior caused by the novelty of certain FVs or just novelty of being part of an intervention. Similar to studies in adults, children may be more inclined to consume FVs immediately following a nutrition education intervention on the benefits of FVs or have experienced associative conditioning from characters or books (157). There is also need for studies with longer-term outcome measurements (ie. follow-up) to evaluate whether an intervention effect is sustainable beyond the time of direct exposure (137, 158), a critical component of all public health improvement (159). Only two of the identified studies in this review included a follow-up period (one week and three months)(137), thus, it is unknown whether the reported short-term changes in eating behavior continued (159). This is an important aspect because only behavior changes that are maintained over time and after the children have returned to their usual environment are meaningful contributors to the effort to improve children’s diet quality. Future studies should also consider different levels of FV influence across the socioecological model (e.g. peers, family members) and potential moderators of FV (e.g. race, SES) to provide a more comprehensive understanding of FV intake behaviors (4, 39, 86, 127). It is also important to consider study feasibility, acceptability, and risk of unintended impacts on children, staff, or families (160, 161), as only four of the included studies reported these effects (139, 162-164). Evaluation of these effects in future studies may elucidate the factors associated with these undesirable or unintended effects to mitigate their impact.

Future research may also benefit from using a factorial study design that compares both different intervention components and different BCTs. These BCTs should also be placed in various contexts, such as within different intervention components, to ascertain whether the BCT or the intervention component is at play, and in heterogenous populations to explore whether different populations, or different characteristics, may respond differently to BCTs or intervention components.

**Conclusion**

In conclusion, this systematic review highlights the existing evidence on RCT interventions implemented in preschools and childcare centers to improve FV intake in preschool-age children in the United States. Although only nine interventions were identified, the most consistent evidence observed is that inclusion of nutrition education components were consistently effective at improving FV intake. Studies that manipulated the feeding environment, by providing pre-portioned meals at preschool or sent FVs for children to take home, but did not directly educate children, produced inconsistent results including decreases in FV intake. Further, there was no observable pattern between the use of theoretical frameworks or BCTs and effectiveness of the studies. While several studies have shown promising results, this review highlights key gaps in this field: there is a need for more studies to test FV interventions in US childcare settings that 1) use robust designs, such as RCTs that use objective measures of dietary intake, 2) directly compare intervention components and BCTs using a factorial model, 3) explicitly report their use of theoretical frameworks, and 4) include follow-up measures to assess long-term behavior change, to determine the most effective methods to reduce the deficiency in FV intake amongst young children in the United States.

Abbreviations

FV: Fruit and vegetable

US: United States

BCT: Behavior change technique

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

RCT: Randomized controlled trial

ICC: Intraclass correlation coefficient

SCT: Social Cognitive Theory

CACFP: Child and Adult Care Food Program

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Table 1. Behavior change techniques observed in included RCTs.

| Domain | BCT | Increased FV intake (6 interventions) | Did not increase FV intake (3 interventions) | Total (9 interventions) |
|---|---|---|---|---|
| Goals and planning | Action planning | 1 | 0 | 1 |
| Social support | Social support (unspecified) | 1 | 0 | 1 |
| Shaping knowledge | Instruction on how to perform a behavior | 0 | 0 | 0 |
| | Information about antecedents | 1 | 0 | 1 |
| Natural consequences | Information about health consequences | 4 | 0 | 4 |
| | Salience of consequences | 3 | 0 | 3 |
| Comparison of behavior | Demonstration of the behavior | 1 | 0 | 1 |
| Associations | Prompts/cues | 1 | 0 | 1 |
| | Exposure | 1 | 0 | 1 |
| Repetition and substitution | Behavioral practice/rehearsal | 2 | 0 | 2 |
| | Habit formation | 2 | 0 | 2 |
| | Graded tasks | 1 | 0 | 1 |
| Comparison of outcomes | Comparative imagining of future outcomes | 2 | 0 | 2 |
| Reward and threat | Material incentive (behavior) | 1 | 0 | 1 |
| | Material reward (behavior) | 1 | 0 | 1 |
| Antecedents | Restructuring the physical environment | 2 | 1 | 3 |
| | Restructuring the social environment | 1 | 0 | 1 |
| | Adding objects to the environment | 3 | 2 | 5 |
| Identity | Framing/reframing | 5 | 0 | 5 |
| Scheduled consequences | Reward approximation | 1 | 0 | 1 |
| Self-belief | Mental rehearsal of successful performance | 1 | 0 | 1 |
| Covert learning | Vicarious consequences | 1 | 1 | 2 |

Figure 1. PRISMA flow diagram of literature search

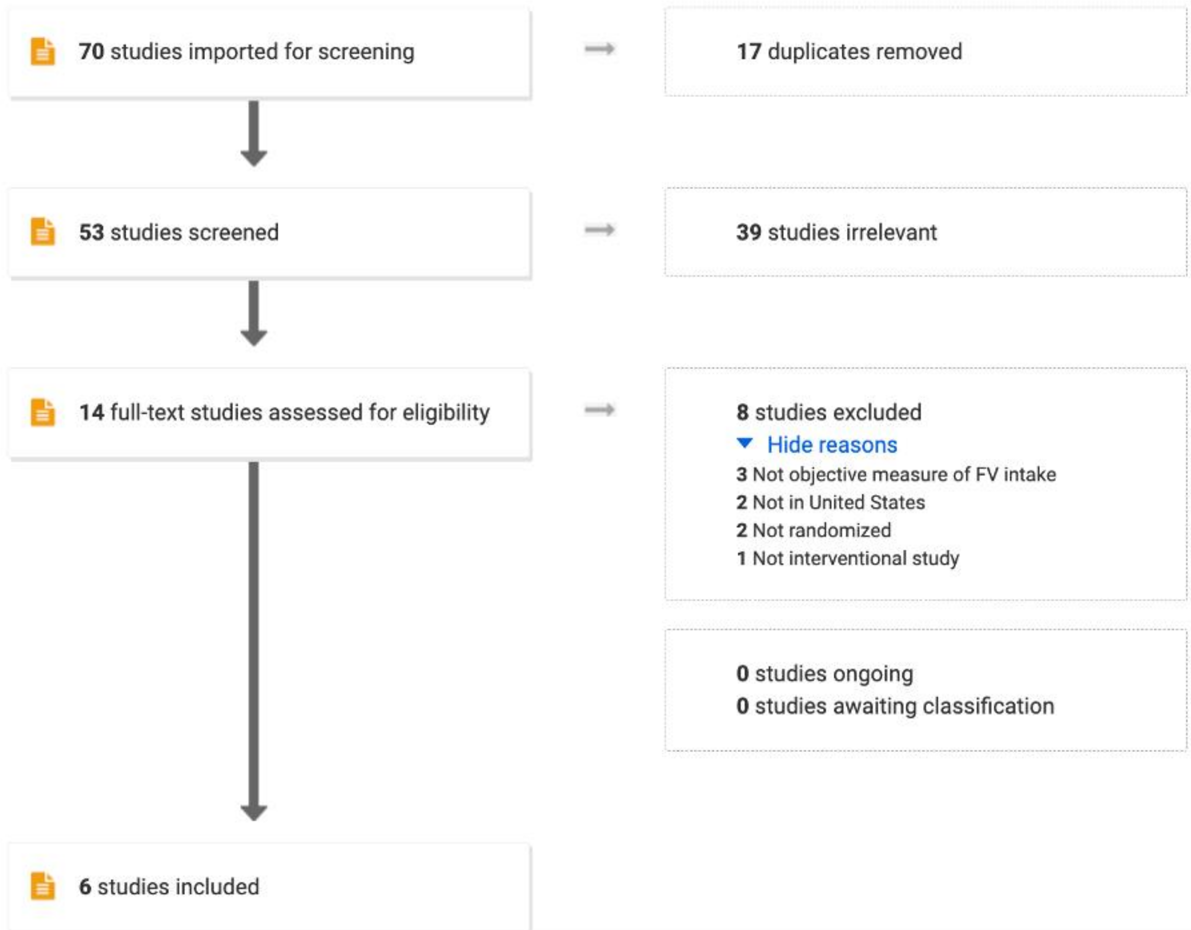

Table 1. Summary of Randomized Controlled trials Included in this Systematic Review.

| Study; design; intervention name and classification | Risk of Bias | Setting | Duration of intervention | Total sessions | Follow-up | Participants | F, V, or FV | Delivery of intervention | Intervention | Comparator | Use of theory score | Outcomes measured | Results |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gripshover, 2013a; RCT; New Theory for Nutrition; nutrition education | Some concerns | Preschool | 10-12 weeks | 22 | None | T: n=30, Mean age=4.8 years, 51% girls<br>C: n=29, Mean age=4.6 years, 51% girls | V | Researchers | Conceptual framework "new theory for nutrition" for understanding nutrition using 5 storybooks. | No treatment | 0 | Pieces of vegetables consumed during snack time, measured using visual observation | Children significantly increased their vegetable intake after the intervention (5.27 pieces) compared to no change in the control group. Mean difference in change from baseline between groups is 5.37 pieces; t(38)=2.28, p=0.03 Cohen’s d=0.74 |
| Gripshover, 2013b; RCT; New Theory for Nutrition; nutrition education | Some concerns | Preschool | 10-12 weeks | 22 | None | T: n=53, Mean age=4.9 years, 52% girls<br>C: n=50, Mean age=4.7 years, 52% girls | V | Researchers | Conceptual framework "new theory for nutrition" for understanding nutrition using 5 storybooks. | USDA’s Team Nutrition materials | 0 | Pieces of vegetables consumed during snack time, measured using visual observation | Children significantly increased their vegetable intake after the intervention (6.15 pieces) compared to no change in the control group. Mean difference in change from baseline between groups is 4.07 pieces; t(88)=2.06, p=0.04 Cohen’s d=0.44 |
| Harnack, 2012a; cross-over RCT; feeding environment | Low | Head Start | 2 weeks | 10 | None | N=53, 27 2-3 year olds; 26 4-5 year olds | FV | Classroom teachers | FVs were served 5 minutes prior to the rest of the meal, with the remaining portions of the meal served traditional family-style. | Typical family-style meals | 0 | Grams of FVs consumed during lunch, measured using visual observation and common household measuring tools. | Fruit: Children significantly increased their fruit intake after the intervention (0.08 cup-eq) compared to no change in the control condition; mean difference in change between groups: p<0.01 Vegetable: No difference in change in vegetable intake between groups; p>0.05 |
| Harnack, 2012b; cross-over RCT; feeding environment | Low | Head Start | 2 weeks | 10 | None | | FV | Classroom teachers | Children were served provider-portioned meals consistent with the CACFP guidelines | Typical family-style meals | 0 | Grams of FVs consumed during lunch, measured using visual observation and common household measuring tools. | Fruit: Children significantly decreased their fruit intake after the intervention (-0.07 cup-eq) compared to no change in the control condition; mean difference in change between groups: p<0.001 Vegetable: Children significantly decreased their vegetable intake after the intervention (-0.03 cup-eq) compared to no change in the control condition; mean difference in change between groups: p<0.01 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Nicklas, 2017; RCT; nutrition education | Some concerns | Head Start | 4 weeks | 20 | None | T: n=128, mean age 4.4 years, 51% girls<br>C: n=125, mean age 4.4 years, 51% girls | V | Classroom teachers and parents | Before lunch each day, children watched 1 of 4 DVDs theater-based puppet shows that incorporated encouragement, rationale, reinforcement, and role modeling. Children were also sent home with a copy of the DVDs, pamphlet (with positive feeding practices), and ingredients to prepare a vegetable snack. | No treatment | 7 | Vegetable consumption during normal lunch meals, measured using digital photography-assisted direct observation and plate-waste method. | Children significantly increased their vegetable intake after the intervention (13.8g) compared to no change in the control group. Mean difference in change from baseline between groups is 11.9g; p=0.022 Cohen's d=0.28 |
| Smith, 2020a; RCT; Harvest for Healthy Kids; feeding environment | Low | Head Start | 8 weeks | 40 | None | T: n=61, 56% girls<br>C: n=66, 55% girls | FV | Parents | Children received high-carotenoid FVs to take home | No treatment | 0 | Skin carotenoid levels measured using Resonance Ramen Spectroscopy | No difference in change in skin carotenoids from baseline between the intervention (4887) and control (2623) groups. Mean difference in change from baseline between groups is 2264, p=0.10 Cohen's d=2.89 |
| Smith, 2020b; RCT; Harvest for Healthy Kids; nutrition education, change in feeding environment, and repeated exposure | Low | Head Start | 8 weeks | 40 | None | T: n=82, 45% girls<br>C: n=66, 55% girls | FV | SNAP-Ed staff members | 30-min daily SNAP-Ed classroom curriculum (Harvest for Healthy Kids) which included a story and picture cards (with FV characters), hands-on food preparation activity, and taste-testing, *plus* children received high carotenoid FVs and parent nutrition education materials to take home. | No treatment | 4 | Skin carotenoid levels measured using Resonance Ramen Spectroscopy | Skin carotenoid levels increased significantly more in the intervention (7834) versus the control (2623) group. Mean difference in change from baseline between groups is 5211; F(2,206)=12.961, p<0.001 Cohen's d=7.14 |
| Staiano, 2020; RCT; Copy-Kids Eat Fruits and Vegetables; peer modeling | Low | Preschool | 1 day | 1 | 7 days | T: n=14, mean age 4.5 years, 50% girls<br>C: n=14, mean age 4.1 years, 50% girls | V | Researchers | On day 1, children were presented with a snack while watching a 7.5min "Copy-Kids" DVD clip designed to encourage positive eating habits, where similarly-aged toddlers happily ate and vocally interacted with the foods (bell peppers). The snack procedure was repeated on days 2 and 7, without any video. | "Brush Teeth" DVD segment | 8 | Consumption of bell peppers during snack time, measured using plate-waste method | No difference in vegetable intake following the intervention video (11.01g), compared to the control video (-3.75g); p>0.05 Cohen's d=3.37 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Witt, 2012a; RCT; Color Me Healthy; nutrition education and repeated exposure | Some concerns | Childcare center | 6 weeks | 18 | None | T: n=165, majority 4-5 years old<br>C: n=98, majority 4-5 years old | FV | Classroom teachers | 2 circle-time lessons and 1 imaginary trip (15-30min each) consisting of interactive learning opportunities on physical activity and healthy eating. Toolkit includes picture cards, posters, music CD, mostly focusing on FVs of different colors, and interactive taste-tests. Interactive take-home activities were also provided. | No treatment | 2 | Percentage of FV consumed during snack time (Fs and Vs served on separate days and Vs served with fat-free ranch), measured using plate-waste method | Fruits: Children consumed a greater percentage of fruits following the intervention (31.20%) compared to the control group (-8%). Mean difference in change between groups is 39.2%; F(1,149)=62.26, p<0.001 Cohen's d=1.29 Vegetables: Children consumed a greater percentage of vegetables following the intervention (24.20%) compared to the control group (-2.4%). Mean difference in change between groups is 26.6%; F(1,120)=24.14, p<0.001 Cohen's d=0.90 |
| Witt, 2012b; RCT; Color Me Healthy; nutrition education and repeated exposure | Some concerns | Childcare center | 6 weeks | 18 | 3 months | T: n=165, majority 4-5 years old<br>C: n=98, majority 4-5 years old | FV | Classroom teachers | 2 circle-time lessons and 1 imaginary trip (15-30min each) consisting of interactive learning opportunities on physical activity and healthy eating. Toolkit includes picture cards, posters, music CD, mostly focusing on FVs of different colors, and interactive taste-tests. Interactive take-home activities were also provided. | No treatment | 2 | Percentage of FV consumed during snack time (Fs and Vs served on separate days and Vs served with fat-free ranch), measured using plate-waste method | Fruits: Children consumed a greater percentage of fruits following the intervention (20.80%) compared to the control group (-1.4%). Mean difference in change between groups is 22.2%; F(1,149)=17.41, p<0.001 Cohen's d=0.68 Vegetables: Children consumed a greater percentage of vegetables following the intervention (33.10%) compared to the control group (-1.6%). Mean difference in change between groups is 34.5%; F(1,120)=43.41, p<0.001 Cohen's d=1.20 |

Supplementary file 2. Risk of Bias Assessment of Studies Included in this Systematic Review

| Study; design; intervention name and classification | Domain 1a. Randomization process | Domain 1b: Risk of bias arising from the timing of identification or recruitment of participants | Domain 2. Deviations from intended interventions | Domain 3. Missing outcome data | Domain 4. Measurement of the outcome | Domain 5. Selection of the reported result | Domain 6. Overall Bias |
|---|---|---|---|---|---|---|---|
| Gripshover, 2013; RCT; New Theory for Nutrition | Low | Low | Low | Low | Low | Some concerns | Some concerns |
| Harnack, 2012; cross-over RCT | Low | Low | Low | Low | Low | Low | Low |
| Nicklas, 2017; RCT | Some concerns | Low | Low | Low | Low | Low | Some concerns |
| Smith, 2020; RCT; Harvest for Healthy Kids | Low | Low | Low | Low | Low | Low | Low |
| Staiano, 2020; RCT; Copy-Kids Eat Fruits and Vegetables | Low | Low | Low | Low | Low | Low | Low |
| Witt, 2012; RCT; Color Me Healthy | Some concerns | Low | Low | Low | Low | Low | Some concerns |

Supplementary file 3. Use of Theory Scoring of Studies Included in this Systematic Review

| Study; design; intervention name and classification | Use of Theory coding scheme | | | | | | | | | | | Overall Use of Theory Score |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Theory/ model of behavior mentioned | Targeted construct mentioned as predictor of behavior | Intervention based on single theory | Theory/ predictors used to select recipients for the intervention | Theory/ predictors used to select/develop intervention techniques | Theory/ predictors used to tailor intervention techniques to recipients | ALL intervention techniques are explicitly linked to at least one theory-relevant construct/predictor | At least one of the intervention technique is explicitly linked to at least one theory-relevant construct/predictor | Group of techniques are linked to a group of constructs/ predictors | All theory-relevant constructs/ predictors are explicitly linked to at least one intervention technique | At least one theory-relevant constructs/ predictors is explicitly linked to at least one intervention technique | |
| Gripshover, 2013a; RCT; New Theory for Nutrition; nutrition education | | | | | | | | | | | | 0 |
| Gripshover, 2013b; RCT; New Theory for Nutrition; nutrition education | | | | | | | | | | | | 0 |
| Harnack, 2012a; cross-over RCT; feeding environment | | | | | | | | | | | | 0 |
| Harnack, 2012b; cross-over RCT; feeding environment | | | | | | | | | | | | 0 |
| Nicklas, 2017; RCT; nutrition education | x | x | x | | x | x | | x | x | | | 7 |
| Smith, 2020a; RCT; Harvest for Healthy Kids; feeding environment | | | | | | | | | | | | 0 |
| Smith, 2020b; RCT; Harvest for Healthy Kids; nutrition education, change in feeding environment, and repeated exposure | x | x | x | | x | | | | | | | 4 |
| Staiano, 2020; RCT; Copy-Kids Eat Fruits and Vegetables; peer modeling | x | x | x | | x | | x | x | x | | x | 8 |
| Witt, 2012; RCT; Color Me Healthy; nutrition education and repeated exposure | x | x | | | | | | | | | | 2 |

# CHAPTER 3: MANUSCRIPT II

## Preschool-Based Behavioral Intervention to Increase Fruit and Vegetable Consumption in a Sample of Preschoolers: A Pilot Randomized Controlled Study

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## Introduction

Fruit and vegetable (FV) consumption among preschoolers in the United States (US) is very low, with the majority of children not meeting the national intake recommendations (165). Factors that may play a role in FV consumption include parental FV consumption(9, 10), socioeconomic status (SES)(9, 166), parental education(9, 10), child eating characteristics (e.g., picky eating or neophobia)(10), and more palatable or desirable foods served with the FVs (167). FVs are considered vital components of a healthy dietary pattern because they provide a large variety and amount of nutrients, including dietary fiber, vitamins, minerals, and phytochemicals that are critical to support health and development (16, 40, 109) and promote tracking of healthy dietary habits into adolescence and adulthood (110, 111).

Preschool-age is a formative period for the development of children's eating habits, and the patterns established during this time tend to persist (110, 111). In fact, children who have early liking and exposure of FVs were found to have 28% greater odds of consuming more than a serving of FVs per day compared to those who did not; thus, it is critical to support healthy dietary behaviors during this age (13). Most preschool-aged children in the US attend daycare (88), making this an opportune setting to conduct interventions. Furthermore, several studies have demonstrated the promise of interventions implemented in childcare centers to improve children's FV intake in the short term (86, 87, 89, 90). Hence, we sought to develop an intervention to improve FV intake within a childcare setting.

Within the last decade, there have been over 80 published studies aimed at improving FV intake in young children (127). However, a major limitation of the existing literature is the use of subjective measures of dietary intake that are likely affected by bias and may not accurately capture changes in dietary patterns (168). In contrast, objective measures of food intake often include measuring plate-weight during an ad libitum meal or the use of the FV-related biomarker of skin carotenoids. These measurements provide a more accurate reflection of dietary intake (168). A recent systematic review (168) identified only six studies published in the US within the past decade that objectively measured changes in FV intake among preschool samples. Further, the measurements of FV intake varied considerably and included measures of just fruits, just vegetables, or both, using plate-weight, visual observation, percentage of FVs consumed, or skin carotenoid biomarkers, assessed at either lunch or snack (168). Additionally, most of the included studies assessed FV intake on a single day at each timepoint, despite ample evidence suggesting

FV intake in children is highly variable and depends on a variety of factors including, but not limited to, the liking and preference of the FV being served, competing foods at the meal/snack, and unintended external influence (55, 168). As such, measurement of multiple days of intake may provide a more accurate representation of FV intake pattern.

There are numerous reasons that contribute to the paucity of published studies that objectively assess dietary interventions within a childcare setting. Firstly, childcare centers differ greatly in structural characteristics, such as quality, availability of resources, and degree of director and teacher support. These factors may influence the willingness and ability for many centers to support research interventions and programs (169). Research within childcare centers may also be unpredictable due to factors including child and teacher absences and changes in day-to-day schedules. Additionally, it is difficult to accurately measure foods consumed within childcare settings due to the intrusive nature of direct measures and imprecise nature of indirect measures (168). Additionally, these measures may be particularly difficult if the children provide their own foods from home, as opposed to being provided meals by the center (168). Lastly, if randomized controlled trials in this setting are conducted by randomizing classrooms, researchers must be careful to avoid the intervention effects to bleed between classrooms. These difficulties within childcare centers likely contribute to the lack of studies available that objectively assess dietary interventions in preschoolers (168).

Another limitation in the available literature is the lack of studies using and reporting on the underlying theoretical frameworks (behavior change models) and associated behavior change techniques (BCTs) employed in the studies (168). Experts suggest the use of theory and reporting of BCTs to provide a basis for the proposed behavior change mechanisms by which these interventions may induce change (128). Similarly, incorporating outcome measures that are directly and uniquely associated with the theory's constructs may allow for a better understanding of *why* and *how* an intervention elicited behavior change (125, 126). Presently, there is inconclusive evidence about which BCTs may be optimal due to lack of reporting within other studies (168). As such, together, the use and reporting of these components may advance knowledge and aid researchers in improving intervention approaches (125).

Given these gaps in the literature, there is a need for 1) better use of behavior change theory in intervention development; and 2) studies that employ more objective measures of primary outcomes. Therefore, we developed an intervention informed by behavioral change theory that leveraged key elements from other evidence-based interventions to optimize impact. We then designed a pilot study to evaluate intervention feasibility and preliminary evidence for efficacy in a childcare setting in preparation for a fully powered randomized controlled trial. Importantly, this study assessed changes in objectively measured FV, and key intermediate variables including feasibility of the behavioral intervention and children's willingness to try FVs. We hypothesized that the intervention and study would be well received by teachers and children, and that successful implementation would result in measurably increased FV intake in children relative to a non-intervention control group.

**Methods**

Study design

This feasibility study was a parallel randomized controlled feeding trial that examined the immediate and sustained (six weeks) effects of an intervention to improve FV intake in preschoolers (two to five years old). Two preschool classrooms from a single childcare center were enrolled in the study. Parents of eligible children (no developmental delays, food allergies, or medications) were invited to enroll their child. All parents provided written informed consent and participating children were asked for verbal assent before each study procedure. Parents were monetarily compensated with a $20 gift card for their participation.

The study design is reflected in Figure 1. The children in both classrooms underwent a one-week run-in period where they were acclimated to the researchers and the various measurements and testing devices (170, 171). This week mimicked the baseline week testing schedule, but no data were collected during this period. The run-in period was also useful for researchers to acclimate to the classroom dynamics and refine outcome measurements and techniques to best fit the classroom environment. To optimize the approach, the lead researcher (FH) met with each classroom teacher at the end of each day of the run-in period to discuss feedback and solicit suggestions for improvement unique to the classroom environment.

Following the run-in period, baseline data collection was completed across three consecutive days (Tuesday through Thursday) in both classrooms. Following baseline data collection, one classroom was randomly allocated to the intervention condition (IC) and the other allocated to the control condition (CC). This was completed by the lead researcher and a witness using a computer-generated random number generator. Randomization was completed after the baseline measurements had been completed to limit potential researcher bias in the selection. The IC received 18 total curriculum sessions over a span of six weeks (three per week), while the CC had no contact with researchers during this time. In the week immediately following the six-week curriculum, all baseline measurements, except height and weight, were repeated in both classrooms to assess the immediate effects of the intervention. Six-weeks after the post-intervention measurements, dietary measurements at lunch were repeated in both classrooms to assess sustained effects of the intervention on lunch intake.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving research study participants were approved by the University of Virginia Social and Behavioral Sciences Institutional Review Board (UVA IRB-SBS #5304). Written informed consent was obtained from all parents and verbal consent was obtained from all children. This trial was registered with ClinicalTrials.gov (NCT05730530).

### Setting

The participating childcare center provided all meals and snacks to all children, and actively participated in the Child and Adult Care Food Program (CACFP). This ensured that children were served FVs at each meal as required by the CACFP (172). The classrooms chosen to participate in the study included 12 (IC) and 13 (CC) children. Nineteen children (IC: 10; CC: 9) met the inclusion criteria (no parent-reported developmental delays, food allergies, or appetite-affecting medications) and were invited to participate. At baseline, the parents of 17 children provided consent to enroll their child in the study (IC=8, CC=9).

### Intervention design

The FV intervention was informed by Social Cognitive Theory (SCT) and consisted of two weekly lessons and a weekly taste-test activity (18 total sessions), each lasting 15-20 minutes. The curriculum components were developed based on the tenets of SCT using the process of intervention mapping (173) led by the primary author (FH) with consultation from two additional authors (SK and CD). A diagram linking each tenet to the corresponding intervention component, the BCT domains present in the intervention, and measured outcomes, is provided in Figure 2. A detailed description of all activities and the difference between IC and CC are described in

Supplementary Table 1. More detailed descriptions of our use of theory and included BCTs in this context are provided in Supplementary Tables 2 and 3, respectively.

All materials used in this adapted curriculum were informed by the prior systematic review (168). The lessons were adapted from three programs to improve FV intake in preschoolers: 1) *Color Me Healthy (CMH)*, a multi-component curriculum published by Carolyn Dunn (CD) and colleagues (137, 142), 2) *Team Nutrition*, a program developed by the USDA Food and Nutrition Services (USDA Team Nutrition; http://teamnutrition.usda.gov/), and 3) principles from Theory of Nutrition, a curriculum published by Gripshover and Markman (138). All materials used in this study were publicly available; and we collaborated with the founder of the CMH program (CD) to obtain guidance and ensure proper execution. The CMH program includes 12 lessons, nine of which pertain to FVs and three to physical activity, and six weekly taste-test activity sessions. The CMH program was adapted for our study purposes and informed by our systematic review. Specifically, our intervention included the nine FV lessons and six weekly taste-test activities from CMH. A detailed description of the CMH lessons is available in a separate publication (137, 142) and materials are available online for purchase (https://www.colormehealthy.com/). Of the remaining three sessions, two sessions were adapted from Theory of Nutrition lessons including 1) a lesson of the process of digestion and absorption and 2) an activity designed to educate children on the concept of nutrients using sugar dissolved in water (138). The final session was a Team Nutrition lesson "The Two Bite Club", which teaches children about tasting novel foods (174).

In this study, one day per week (Tuesday) was dedicated to teaching children about various nutrition concepts, a second day (Wednesday) focused on a specific color of FV and its associated nutrients and benefits. On the third day, taste-test activities (Thursday) were conducted to highlight a FV corresponding to the color of focus. This template was followed during the six-week intervention period. A more detailed description of each lesson is described in Supplementary Table 1.

Data collection

Data were collected at the childcare center in February 2023 (baseline), April 2023 (post-intervention), and May/June 2023 (follow-up). Researcher schedules ensured that the researchers who administered the curriculum were not present in the IC for the post-intervention or follow-up data collection days to limit any potential researcher bias and a possible influence on the children's FV intake due to familiarity with the researcher. All data were entered directly into a secure RedCap database (175).

Parent-reported measures

*Demographic characteristics.* Immediately following parental consent, parents were asked to complete a series of questionaries regarding their own and their child's sociodemographic characteristics. This included their own and their child's race, their highest education level (<9$^{th}$ grade, some high school, high school degree, some college, 2-year college, 4-year college, graduate work, and graduate degree), and whether they participate in Special Supplemental Nutrition Program for Women, Infant, and Children (WIC) or Supplemental Nutrition Assistance Program (SNAP). Highest parental education was used as a proxy for socioeconomic status (176). Parents were also asked to report their child's age and sex and confirm that their child does not have any food allergies or dietary restrictions.

*Food intake outside of the preschool.* Food intake outside the childcare center was ascertained by researcher-administered parent-reported 24-hour dietary recalls at baseline and post-intervention. Parents received a telephone call and were asked to report their child's food intake on the previous day using a validated multiple-pass method (177) on the Nutrient Data System for Research (NDSR) software (version 2021, Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN). This was completed on two days at baseline and post-intervention (Wednesday and Thursday to reflect intake on Tuesday and Wednesday) to minimize parental burden.

Dietary measures

*Food intake at lunch.* To assess effects of the intervention, we examined FV intake during lunch using plate weight method on three consecutive days at each time point. This was measured by weighing all food components (each plated separately) served and leftover using transportable food scales (Mettler-Toledo, GmbH, Switzerland); the difference of the two was calculated to yield the amount consumed. The transportable scales were set up on a table in the room; a researcher carried each child's plates and cups (labeled with participant ID) to the scale, recorded the weight of the plate with food, and then served the food to the child. After the child verbally stated that they were finished eating, a researcher weighed and recorded the weight of each plate with leftover food. FV intake on each day was averaged to yield a more accurate reflection of children's "typical" food intake (178). All food consumed was entered into the NDSR database for energy and nutrient analysis. Due to high variability in total caloric intake at lunch, we adjusted FV intake (grams) for total lunch energy intake (kilocalories; method described below) to yield a measure of mass of FVs consumed (grams) per 100kcal (g/100kcal).

*Food intake at snack.* Morning and afternoon snack intake were measured on each of the three days (Tuesday through Thursday) using the validated visual observation technique developed by Ball and colleagues (42). This measure involved trained researchers visually estimating amount of food served and leftover to yield the estimated amount consumed. This method was used in place of plate weighing to minimize burden and disruption within the childcare center classroom.

Food intake at each of these periods (lunch, snacks, outside of preschool) on each day was separately entered into NDSR database to calculate energy, nutrient, and food group intake at each meal. Total daily food intake on each day was assessed by combining these three periods and used to assess energy, nutrient, and food group intake for each day. These were then averaged to yield mean intake at each time point (baseline, post-intervention, follow-up). If any child was present on only one measurement day, food intake on the single day was used.

### Anthropometric measures

Height and weight were measured in each classroom on the first day of baseline measurements using transportable stadiometer (SECA, GmbH & Co, Hamburg, Germany). Height, weight, and age were used to calculate body mass index percentile (BMIp) for each participant according to the CDC growth charts (179), which was then used to categorize children based on weight status (healthy weight: 5 - <85%, overweight: 85 - <95 %, obese: ≥95%)(180).

### Physical activity measures

PA was measured using triaxial Actigraph GT3X accelerometers secured to each child's hip using a colorful belt. Prior to baseline measures at T1, all children underwent a run-in period where they were acclimated to the *ActiGraph GT3X+* (ActiGraph, Pensacola, Florida) devices. ActiGraph devices were programmed and given to children to measure PA from Tuesday 8:30am until Thursday 4:30pm at baseline and post-intervention. Parents were instructed to ensure children wore the device for three consecutive days (except while sleeping or bathing) and were provided with instructions to remove the device during the child's bath time and bedtime. Actigraphy data for each day was converted to time spent in each activity level (sedentary, light, moderate, vigorous) using the Butte Preschoolers VM cut points (181, 182) on the ActiLife (ActiGraph, Pensacola, FL) (181) software program (82, 83).

### Behavioral intervention measures (IC only)

Key intermediate variables directly related to the intervention and considered mediators of change were also measured: i) curriculum attendance, ii) FV recipe consumption; iii) FV willingness to try (183), and iv) taste-test recipe liking (183). Although the children's knowledge was not directly measured, we asked all children to exhibit their grasp of the concepts by responding to questions throughout the sessions. As a proxy for knowledge, we summed the number of days present during the sessions to yield a curriculum attendance score. Consumption of the taste-test recipes was assessed by weighing the amount of the recipe each child consumed, measured using plate-weight at the laboratory. Children's self-reported willingness to try was directly measured by asking the children about their willingness to try the various FVs, with questions such as how likely they are to consume the provided FVs if provided with them at school and at home, and whether they are likely to ask for the FVs again at home (183). Taste-test recipe liking was assessed by asking children if they like the taste, texture, appearance, smell of the recipe, as well as their overall liking of the recipe (183, 184).

### Sample size

Sample size calculations were based on a similar intervention by Nicklas and colleagues (139), who reported a small effect size of 0.28 with 153 total participants (intervention group n = 128, control group n = 125). We assumed a slightly larger effect size for the following reasons: a.) longer study duration (seven weeks vs. four weeks); b.) more intense intervention (18 unique sessions including a combination of both interactive nutrition education and repeated exposure vs four videotaped shows each shown on five consecutive days (20 sessions total); c.) FV intake outcome measurement (intake of both fruits and vegetables vs only vegetables). Accordingly, we aimed to recruit a total of 24 participants accounting for 50% attrition (ie. children traveling during data collection days, sickness, absences, or withdrawal from the study for other reasons) at the end of the seventh week to result in an expected total of 12 participants for final data analysis.

Statistical analysis

Children included in analysis must have been present for at least one day at each measurement timepoint (baseline, post-intervention, follow-up). Student's t-test was used to assess baseline differences in age, BMIp, and daily energy intake, and chi-square test was used to assess baseline differences in the sex, parental education, and weight status between the two classrooms.

To examine intake across the three timepoints, grams of FV intake were first adjusted for total energy intake at lunch (grams FV per 100kcal). To test if there were any changes in FV intake at post-intervention and follow-up, we conducted a two-way RM-ANOVA (for parametric data) or Friedman test (for non-parametric data) with treatment (IC vs CC) and time (baseline, post-intervention, follow-up) as the two factors. If global differences were observed, post hoc analysis with Tukey HSD (for parametric data) or Wilcoxon signed rank test (for non-parametric data) was used to examine differences in the main effect.

Due to the nature of conducting research in childcare settings, unscheduled absences resulted in missing data. In these cases, the Fully Conditional Specification Method of multiple imputation was used to complete the data set (185, 186). Data was tested for normality using the Shapiro-Wilk test as appropriate for the small sample size (187).

All data was analyzed using IBM SPSS software (IBM Corp. Released 2021. IBM SPSS for Macintosh, Version 28.0.1.0, Armonk, NY: IBM Corp). A significance level of $p<0.05$ was defined for all analyses.

**Results**

Participants and baseline characteristics

In total, 17 children were enrolled to participate in this study (IC=8, CC=9). Three children were excluded from analysis due to being absent for all three days of measurement at post-intervention (n=1) or follow-up (n=2) resulting in 14 children included in analysis (Figure 3).

Mean age, BMIp, parental education, and daily energy intake are presented in Table 1. Parents were predominantly non-Hispanic White (12/14), over 90% (13/14) held at least a bachelor's degree, and no parents reported participating in WIC or SNAP. Although children in the CC were significantly younger than the IC (t=4.24, p<0.001), all other demographic characteristics were similar between classrooms. The majority of children (10/14) were male in both the IC and CC, most children (12/14) were non-Hispanic White, and most (13/14) had a healthy weight status. Due to the homogeneity of this sample, we were unable to control for age, BMIp, parental education, or physical activity levels in our primary analyses.

At baseline, prior to adjusting for total lunch energy intake, children consumed, on average, 63±14g and 42±14g of fruit and 26±20g and 19±15g of veg, in the IC and CC, respectively. Total FV consumption at baseline was greater in the IC compared to the CC (MD: 20g, SE: 8g, p=0.024). Based on the combined servings of FV consumed at snack, lunch, and out-of-school food intake measurements, 71% of children met the daily recommendation of two servings of fruit, while only 6% (one child) met the daily recommendation of three servings of vegetables. Preschoolers engaged in 137±18 minutes of total PA at baseline and 111±34 minutes at post-intervention; 79% (n=11) met the guidelines for daily PA (≥ 120 minutes total PA) at baseline, while only 43% (n=6) met the guidelines at post-intervention.

All children who participated in the intervention (IC) willingly participated in all intervention components. Overall, children attended between 78% (14/18 sessions) and 100% of total sessions, with children, on average, attending 90% (16 sessions). Five of eight (62%) children attended all 12 nutrition lessons, two children were present for 11/12 lessons (92%) and one child was present for 10/12 (83%) lessons. Children attended, on average, 79% (4.74) of the six weekly taste-test activities (range between 33% and 100% of sessions). Overall, there was a large variation in FV consumption during the taste-test activity; children consumed an average of 44.4±44.9g of the FVs within the recipes. The two most-consumed recipes were both fruit-based: melon salad (90.1±39.0) and berry parfait (123.5±23.6). Results of the willingness to try FVs are presented in Table 2.

Overall, the RM-ANOVA indicated no effect of condition or time-by-condition interaction. However, there was a significant effect of time (F(2,24) = 23.4, p<0.001, partial eta squared = 0.661); both classrooms observed a similar decrease in FV intake at post-intervention (MD: 22.6±3.5g, p<0.001) and subsequent increase in FV intake (MD: 16.7±2.8g, p<0.001) at follow-up (Figure 4). Despite differences in lunch FV intake, there were no changes in total daily servings of FVs consumed at baseline compared to post-intervention (MD: -0.17 servings; F(1,12)=0.09, p=0.77).

We observed high intra- and inter-subject variability in FV intake on each day, as depicted in Figure 5. The five foods with the highest between-subject variability were fruit cocktail (61±32g), apple (48±33), pear (48±33), mashed potatoes (28±44g), corn (56±38g), and vegetable soup (63±62). There were also significant within-subject differences in the IC fruit intake at baseline ($F(1,10)= 11.95$, $P=0.002$) and postintervention ($F(1,10) = 4.58$, $p=0.04$), and in the CC fruit intake at baseline (only days 2 and 3) ($F(1,6) = 6.41$, $p=0.045$).

**Discussion**

Despite the large number of studies conducted to address this topic, low FV intake in young children remains a large public health concern. While previous studies have shown promising short-term results, there is a lack of studies that evaluate the effects of a behavior change theory-informed intervention using objective measures (168). Hence, we conducted a pilot study of an adapted intervention to evaluate and assess the feasibility and initial indicators of efficacy the intervention, as well as the study approach. In summary, although we observed no effect of the intervention on FV intake, this study established the feasibility of conducting such an intervention.

Although our intervention used methods adapted from previously published studies that found success in improving FV intake in this age group (137, 138), we observed no impact of the intervention compared to the control classroom. We postulate that the lack of improved measured FV intake is likely due to the unexpectedly large individual variation in FV intake observed in our sample. To our knowledge, variability in objectively measured lunch FV intake within a childcare-based intervention setting has not been previously assessed. In 2011, Erkkola and colleagues (188) examined energy and nutrient intake variability in Finnish preschoolers using parent-reported food records to determine the number of intake days required to accurately depict habitual nutrient intake based on hypothetical *r* analysis. Although they did not specifically assess FV intake, they found that three to four days of food record are required to yield an *r* of 0.8 and eight days are required to yield an *r* of 0.9 in energy intake of three-year-old children. Although these findings are not directly translatable to our use of direct observation (as opposed to food records), they do emphasize the importance of considering intra- and inter-individual food intake variability in young children. A very similar intervention by Witt et al. (137) observed high variability in FVs *served* at each time point they assessed. To account for this in their analysis, they calculated and compared the percentage of FVs consumed from what was served at each time point. A similar study by Nicklas and colleagues (139) measured vegetable consumption at lunch two days before and after administering their intervention. Although they did not examine or explicitly report variability in vegetable intake at each timepoint, their standard deviation in average intake at each timepoint was between five and eight grams, much lower individual variability that we observed.

Overall, both the study approach and intervention components were successfully implemented to yield our outcome measures and intervention delivery and were well-received by the participating children. We observed lower attrition than we anticipated, with 82% of enrolled children being present for at least one measurement day at each timepoint. Parents willingly participated in all questionnaires and dietary recalls for a compensation of a $20 gift card.

The childcare director and teachers were generally accepting of the various study procedures. During the first two days of the baseline measurements, one of the CC teachers was absent and replaced by a substitute teacher who was unaware of the study procedures. Consequently, the substitute teacher instructed the children to dispose of the meals prior to post-meal weighing resulting in only two days of lunch intake measured in the CC. Additionally, one CC teacher suggested that in the future we should include at least two additional days of simulating the full lunch measurement procedures during the run-in period to acclimate children to not dispose of their foods after they finish eating.

Among the children included in the analysis, 13 of 14 children were present for all days of baseline, 13 of 14 present for all days of post-intervention, and 12 of 14 present for all days of follow-up measurements. Children willingly participated in the measurements of height and weight on day one of baseline measurements. With the exception of day one of baseline measurements in CC (discussed above), food intake measurements at lunch were successful at all time points. All children willingly participated in hunger/satiety and food liking measures before and after each lunch meal.

The intervention components were well received by all children within the IC, and children attended an average of 90% of the 18 sessions. Despite relatively high overall attendance, most of the absences observed were from the taste-test activity sessions, which occurred every Thursday. Consequently, this low attendance likely inhibited our ability to comprehensively target SCT, as the taste-test activities were an integral part of our intervention and the sole methods used to target the vicarious learning and mastery experiences (personal/cognitive factors) and social support/social norms (environmental factors) tenets of SCT. Although this pattern may have been due to random chance, staggering of intervention days for studies in a childcare center may be warranted in future research.

The majority of the children receiving the intervention had seen the taste-test activity FV before and, with the exception of purple cabbage, had also tried the FV previously. Most children at least tried the FV recipes, and the vast majority reported liking the recipes; at least half of the children reported that they would try the food again either at school or at home, although, with the exception of the melon salad, less than 2/3 of the children claimed that they would ask for the food at home. As observed in similar studies (183), children liked and consumed substantially more of the fruit recipes (melon salad and berry parfait) compared to the vegetable recipes.

First and foremost, children seem to have high individual variability in FV intake, which makes it difficult to compare the effects of an intervention in a small sample. Similarly, we also observed high variability in intake of different FVs; a study aligned with a childcare center's menu rotation to allow assessment of the same FVs at each timepoint may mitigate this unintended variability. Additionally, more strategic, less intrusive methods to measure plate-weight during may be helpful to minimize the effects on children's food intake behaviors, such as completing the weighing outside of the classroom or packaging the leftovers and weighing them later. Given that parents willingly participated in surveys and diet recalls, parents may also be willing to provide more precise measures of food intake using measures such as at-home plate-weight method or photo-assisted dietary recall. With regards to increasing the efficacy of a behavioral intervention, potential strategies include planning "make-up" sessions to address child absences during both the outcome measures and the intervention sessions. Additionally, incorporating additional components such as parental education and involvement within an intervention or a longer intervention with more sessions may translate to a greater increase in FV intake, as have been proposed in other studies (189).

This study has several limitations that should be considered. Most notably, while the findings of this study provide us with expected variability in FV intake in this population, the analyses discussed above were underpowered to examine changes in FV intake between classrooms. Similarly, we were unable to account for factors known to influence dietary intake within our analysis, including sex, age, BMIp, SES, and physical activity levels. Further, we were only able to objectively examine FV intake during a single meal each day, while intake in the remaining meals was assessed using subjective measures. This limitation is not unique to this study; the advantages and disadvantages of using various assessment methods for FV intake in young children has long been a topic of debate and requires careful consideration of the internal and external validity, feasibility, and practicality desired within the study (41, 55, 168, 190, 191). For example, while the plate-weight measurement is considered the most accurate and precise measures, it is limited by the intrusive and resource-intensive nature of the measure, while dietary recalls or food records are less intrusive and more convenient for larger samples but are more subject to bias. Additionally, our study included a homogenous sample of children that is not reflective of the US population; therefore, our findings, and our high variability in FV intake in particular, may not be generalizable to other populations of preschool-aged children. Nonetheless, mean baseline fruit and vegetable intake in this sample of preschoolers was comparable to the national average (fruit: 2.8 vs 2.4 servings/day; vegetable: 1.3 vs 1.4 servings/day) (32). Additionally, as discussed above, the effects of the intervention are contingent on children's attendance during the intervention curriculum, so we may have not targeted each SCT construct and tenet in most of the children. The tenets that were more consistently targeted (nutrition education lessons), including knowledge, short-term and long-term outcome expectancies likely were not sufficient to lead to significant changes.

Overall, these limitations should be considered within the strengths of this study: 1) the plate-weight method we utilized is considered the gold standard of objective measures and allows a very precise measure of intakes at one meal (121, 177), 2) we assessed intake of several FVs on separate days that allowed us to examine variability in FV intake; thus, external validity was high (188), and 3) the homogeneity of our population minimized the likelihood of confounding variables known to influence FV intake.

**Conclusion**

To the extent of our knowledge, this was the first study to systematically assess the feasibility of a behavioral intervention in preschoolers with repeated direct measurement of FV intake at lunch. Although we did not observe an intervention effect, we established the feasibility of conducting intervention studies using our methods in preschoolers attending childcare. As such, the findings within this study may be used to inform future studies that employ similar methodology to objectively assess efficacy of dietary interventions in this age group and setting.

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Table 1. Baseline characteristics of included participants

| | Age (months)* | | Male (n) | BMIp (%) | | Highest parental education | | | Daily energy intake (kcal) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | | Mean | SD | High school (n) | 4-year college (n) | Graduate degree (n) | Mean | SD |
| IC (n=7) | 49 | 5 | 5 | 52 | 29 | 0 | 3 | 4 | 1254 | 290 |
| CC (n=7) | 38 | 4 | 5 | 36 | 29 | 1 | 1 | 5 | 1193 | 262 |
| Overall (n=14) | 44 | 7 | 5 | 44 | 29 | 1 | 4 | 9 | 1224 | 268 |

BMIp, body mass index percentile. *Significant difference between classrooms, $p<0.05$

Table 2. Willingness to try, liking, and consumption of FV taste-test recipe; highlight FV [recipe name]

| | Have you ever seen this food before? (% “yes”) | Have you ever tried this food before? (% “yes”) | Did you try this food today? (% “yes”) | Would you try this food again at school? (% “yes”) | Would you try this food again at home? (% “yes”) | Would you ask for this food at home? (% “yes”) | Overall, do you like the food? (% “yes”) | Mean fruit and vegetable consumption (grams) | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Mean | SD |
| Cabbage [rainbow slaw] | 50 | 38 | 75 | 50 | 75 | 38 | 86 | 12.9 | 13.7 |
| Tomato [mango salsa] | 71 | 71 | 100 | 86 | 86 | 67 | 86 | 15.7 | 12.3 |
| Cantaloupe [melon salad] | 100 | 100 | 100 | 86 | 100 | 100 | 100 | 90.1 | 39.0 |
| Bell pepper [bell pepper people with ranch] | 100 | 67 | 83 | 83 | 50 | 50 | 83 | 26.0 | 22.9 |
| Broccoli [cruciferous forests] | 100 | 100 | 83 | 100 | 67 | 67 | 100 | 32.2 | 23.1 |
| Blueberries [mixed berry parfait] | 100 | 100 | 100 | 100 | 100 | 50 | 100 | 123.5 | 23.6 |

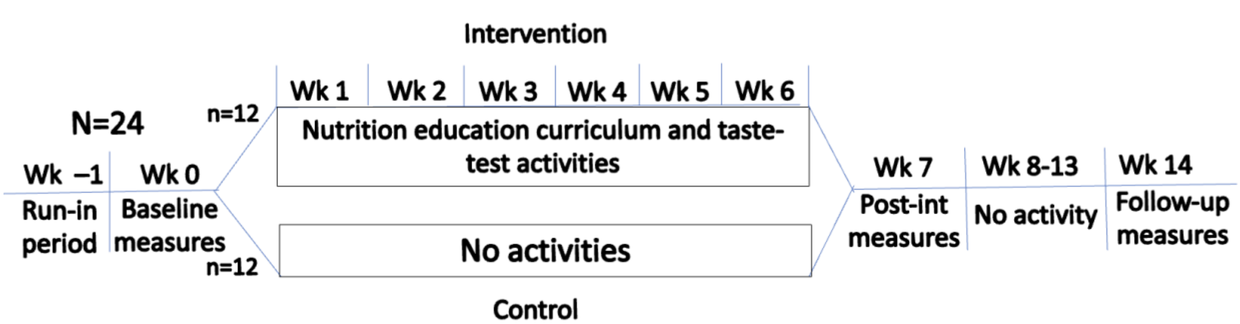


Figure 1. Flow diagram of study timeline.

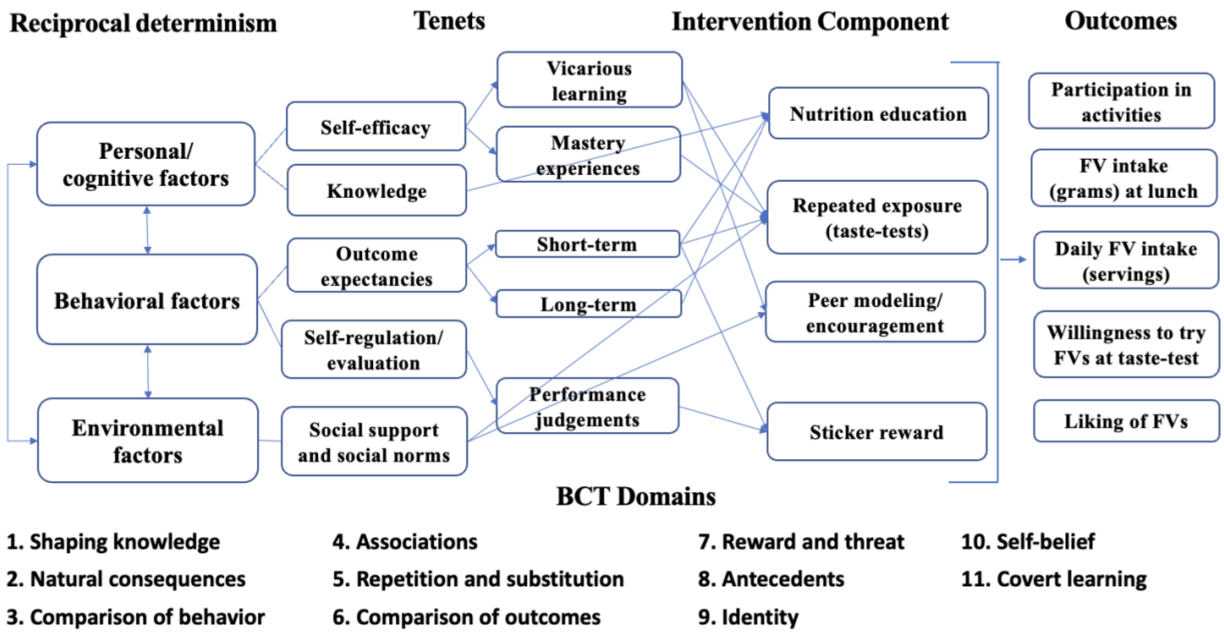


Figure 2. Intervention map to describe the linkage between Social Cognitive Theory tenets and the intervention components and outcomes.

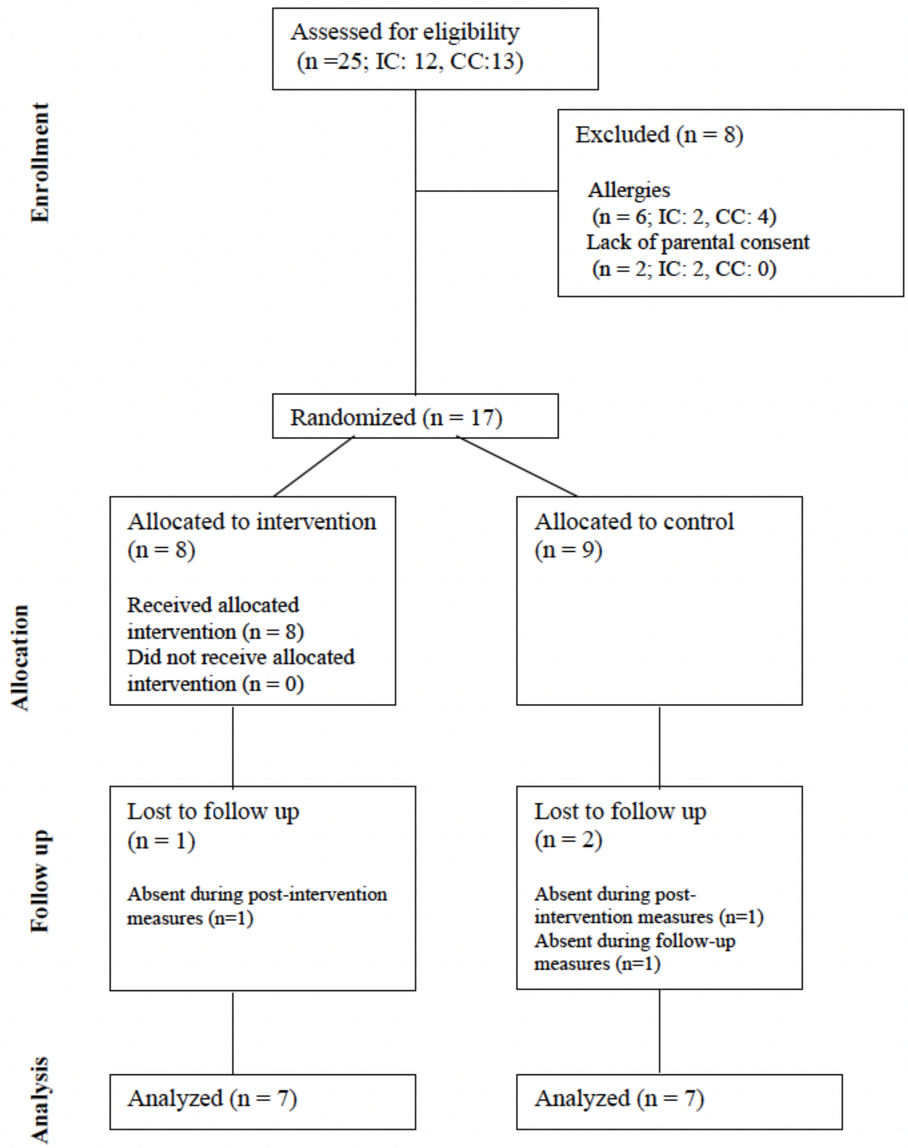


Figure 3. CONSORT diagram for a behavioral intervention to increase fruit and vegetable intake in a sample of preschoolers.

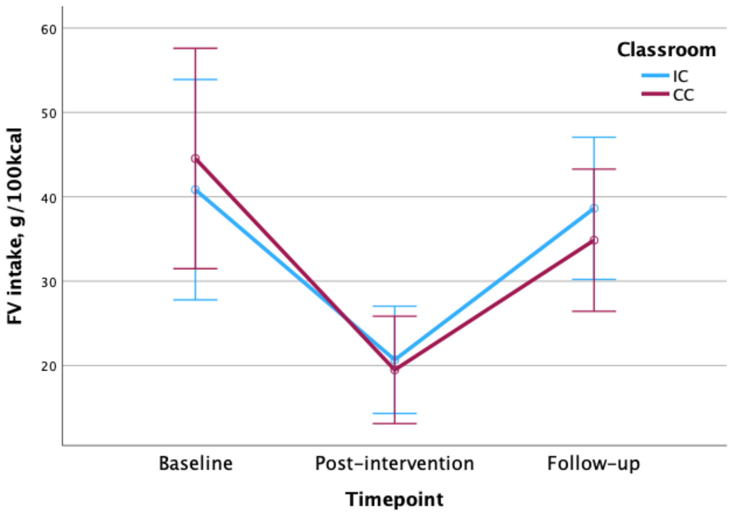


Figure 4. Changes in FV intake from baseline to post-intervention, by classroom.

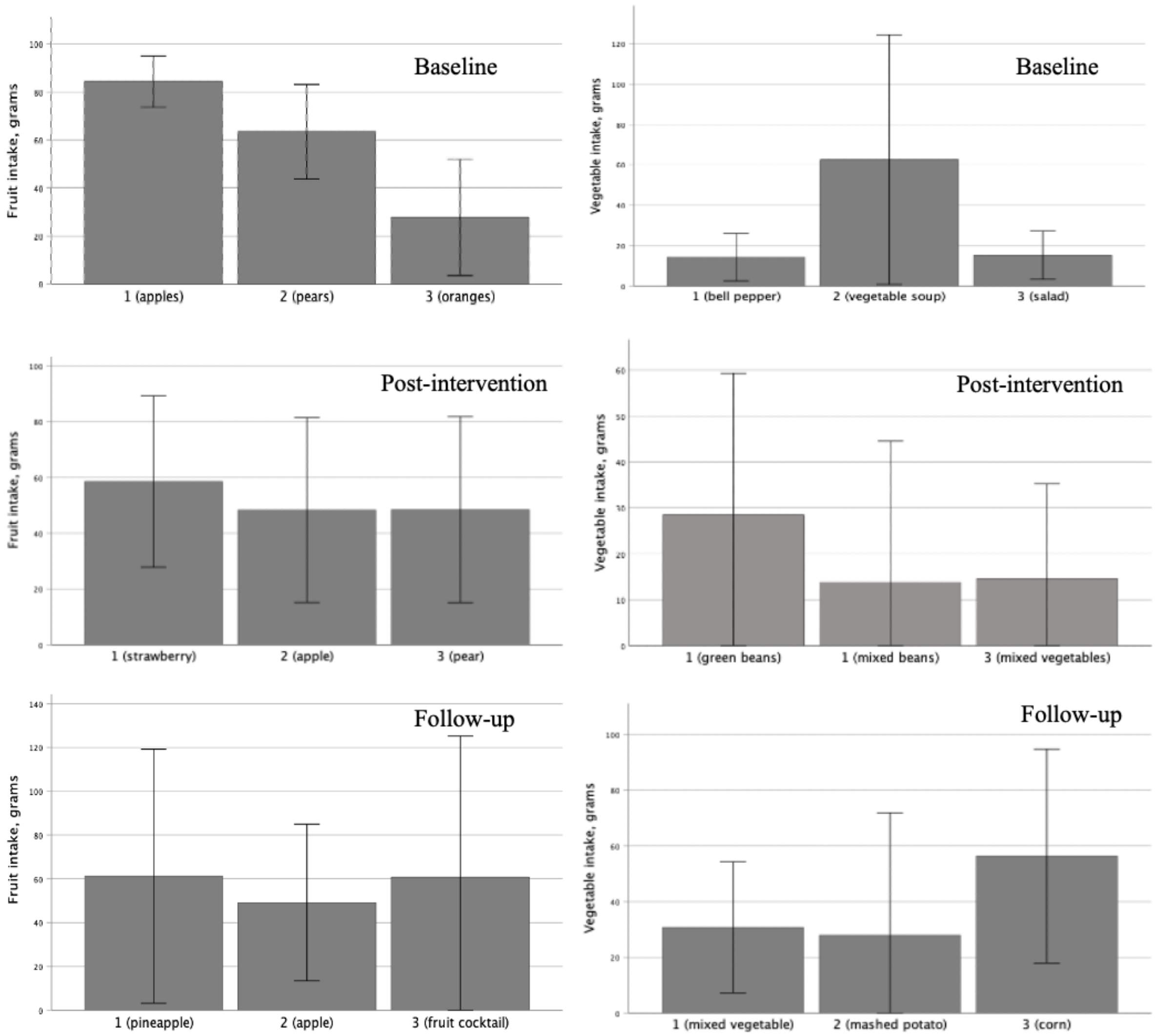


Figure 5. Fruit and vegetable intake (grams) on each day of baseline, post-intervention, and follow-up.

Supplementary Table 1. Timeline of intervention activities

| Week # | Intervention classroom (IC) | Control classroom (CC) |
|---|---|---|
| Curriculum week 1 | **Tuesday**: Digestion/absorption<br>**Wednesday**: Foods are different colors<br>**Thursday**: Purple cabbage guessing game; Cabbage slaw recipe | No activity |
| Curriculum week 2 | **Tuesday**: Nutrients part 1<br>**Wednesday**: Red food focus<br>**Thursday**: Tomato guessing game; salsa recipe | No activity |
| Curriculum week 3 | **Tuesday**: Nutrients part 2<br>**Wednesday**: Orange food focus<br>**Thursday**: Cantaloupe guessing game; melon salad recipe | No activity |
| Curriculum week 4 | **Tuesday**: Food groups<br>**Wednesday**: Yellow food focus<br>**Thursday**: Bell pepper guessing game; bell pepper people recipe | No activity |
| Curriculum week 5 | **Tuesday**: How to build balanced snacks<br>**Wednesday**: Green food focus<br>**Thursday**: Broccoli guessing game; cruciferous forest recipe | No activity |
| Curriculum week 6 | **Tuesday**: Where fruits and vegetables grow<br>**Wednesday**: Blue/purple food focus<br>**Thursday**: Blueberry guessing game; berry parfait recipe | No activity |

Supplementary Table 2. Targeting and measurement of Social Cognitive Theory tenets and constructs in this intervention

| Reciprocal Determinism | Tenet | Constructs, if applicable | How it was targeted | How it was measured |
|---|---|---|---|---|
| Personal/ cognitive factors | Self-efficacy | Mastery experiences, vicarious experiences, and social persuasion | Mastery of experiences was targeted by providing children with taste-test activities where they were given an opportunity to taste new FV recipes. Vicarious experiences were targeted by providing children with taste-test activities where they were given an opportunity to observe their peers receive stamps as a reward for trying their FV recipe. | Self-efficacy was directly measured by asking the children about their willingness to try the various FVs, with questions such as how likely they are to consume the provided FVs if provided with them at school and at home, and whether they are likely to ask for the FVs again at home. |
| | Knowledge | | Knowledge of FVs and the importance of FV consumption was targeted using the nutrition education modules adapted from Color Me Healthy. This included information on the processes of digestion and absorption, the concept of nutrient presence in foods, and knowledge of the MyPlate food groups. | Although the children's knowledge was not directly measured, we asked all children to exhibit their grasp of the concepts by responding to questions throughout the sessions. To quantify their knowledge, we summed the number of days present to yield an exposure score. |
| Behavioral factors | Outcome expectations and expectancies | Short-term outcome expectations | This was targeted by teaching children about the importance of trying new foods to know whether they like them. | Changes in short-term outcome expectancies with regards to consuming FVs were quantified by weighing the amount of FVs children ate during each taste-test session and assessing the change in each child's willingness to taste the recipes. Long-term outcome expectancies were quantified by the number of these unique color-focused sessions (Wednesday sessions) that children attended. |

| | | | | |
|---|---|---|---|---|
| | | Long-term outcome expectations | The long-term outcome expectations were targeted with the educational sessions that taught children the various roles that FVs play in their health (ie. orange foods are healthy for your eyes). | Although we did not directly assess this, this measure was quantified by the number of these unique color-focused sessions (Wednesday sessions) that children attended. During these sessions, each child was asked to participate by correctly identifying the role of one of the colors that had been discussed. |
| | Self-regulatory capabilities | Performance judgements | Performance judgements were targeted by asking children to evaluate whether they were willing to try the various FV recipes, even if they did not think they were going to like them. This construct was also targeted by asking the children about their liking of the FVs, including aspects like the taste, texture, appearance, smell, to allow children to reflect on the various aspects of the FVs they tasted. | This tenet was assessed by measuring the change in children's self-report of their willingness to try new FVs and whether they responded to the questions regarding liking of the various aspects of each food component. |
| Environmental factors | Social support and social norms | | Social support was targeted by designing the taste-test activities to occur in full group sessions and offering children stamps or stickers if they participated in the activity and tasted each food component. | This measure was quantified as the sum of the number of taste-test sessions that each child attended as a proxy for their exposure to these social norms of trying new recipes. |

Supplementary Table 3. Behavior change techniques (BCTs) included in this intervention.

| BCT domain | BCT | How it was targeted |
|---|---|---|
| Shaping knowledge | Instruction on how to perform a behavior | Children received information about the importance of FV consumption and strategies to practice tasting them (ie. Two Bite Club). |
| Natural consequences | Information about health consequences | Educational sessions, where children were given information about the importance of nutrients found in FVs, were presented. This activity provided and emphasized the health consequences associated with not eating more FVs. |
| | Salience of consequences | Educational sessions, where children were given information about the importance of nutrients found in FVs, were presented. This activity provided and emphasized the salience of consequences associated with not eating more FVs. |
| Comparison of behavior | Demonstration of the behavior | The inclusion of weekly taste test activities as a component of this intervention, demonstrated higher FV consumption. |
| Associations | Exposure | Children were exposed to FVs they might not have seen before. |
| | Prompts/cues | Children were prompted to eat new foods that had been introduced to them. |
| | Associative learning | Children were asked to associate the various FVs to body functions through both taste tests and educational activities. |
| Repetition and substitution | Behavioral practice/rehearsal | Children had the opportunity to engage in and practice higher FV consumption. |
| | Habit formation | Children were able to facilitate habit formation through weekly exposure to different FV. |
| | Generalization of target behavior | Children were given the opportunity to engage in FV consumption during their usual breakfast, snack, and lunch meals and were encouraged to apply their FV knowledge to each of their meals. |
| Comparison of outcomes | comparatives imagining of future outcomes | Hypothetical and imaginary situations related to the benefits of increased FV intake were discussed using a comparative imagining of future outcomes. |

| | | |
|---|---|---|
| Reward and threat | Material incentive (behavior) | Children were incentivized to try FV recipes with verbal motivation and the knowledge of potential material rewards (stamps) to ultimately help the children believe in their ability to eat more FVs. |
| | Material reward (behavior) | Positive reinforcement, in the form of material rewards (stamps), was given for trying FV recipes to, ultimately, help the children believe in their ability to eat more FVs. |
| | Social reward | Positive reinforcement, in the form of verbal praise and social reward, was given for trying FV recipes to, ultimately, help the children believe in their ability to eat more FVs. |
| Antecedents | Restructuring the physical environment | FV recipes that are not typically found in childcare settings were introduced to both restructure and add objects to the children's environment. |
| | Adding objects to the environment | |
| Identity | Framing/reframing | Children were taught that eating more FVs helps with their bodily functions, contrasting with the common instruction for children to eat more FVs simply because they are healthy. This is an example of framing/reframing their perception of FVs. |
| Self-belief | Verbal persuasion about capability | Children were motivated to believe in their ability to eat the fruits and vegetables by being encouraged and persuaded to participate in the taste-test activities. |
| Covert learning | Imaginary reward | Imaginary rewards were provided to the children in terms of the long-term benefits of increased FV intake. |
| | Vicarious consequence | Children observed their peers receiving or not receiving stamps in response to whether or not they tried their FV recipe. |

## CHAPTER 4: MANUSCRIPT III

# The Associations between Physical Activity and Skin Carotenoid Levels in a of Preschoolers

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## Introduction

The measurement of skin carotenoids is often used as a biomarker to objectively measure fruit and vegetable (FV) intake in preschoolers (48-50) as a proxy for other subjective measures of intake, such as 24-hour dietary recalls, diet records, and food frequency questionnaires (49, 51-54, 192-194). When FVs are consumed, carotenoids are absorbed in the intestines and deposited in various adipose tissues, including the skin, approximately two to eight weeks after consumption (47, 49, 195-197). However, the correlation between FV intake and skin carotenoid scores (SCS) is modest in children, and recent literature has questioned its validity for this use (198).

Skin carotenoid levels and their relationship and response to dietary intake in adults are highly individualized and variable (196). This was formerly believed to be primarily due to carotenoids having unique plasma and skin kinetics within the body (48, 50, 197, 199), although recent studies suggest that oxidative balance may also be responsible (192, 194). Oxidative stress, or the mismatch of oxidant- and antioxidant- activity, is caused by an excess of internal and external oxidants (200). Carotenoids help to counteract oxidative stress within the body by neutralizing these reactive oxygen species (ROS) (201). Hence, higher levels of oxidative stress require more carotenoids for neutralization and, subsequently, this leads to less storage within the skin. Additional factors that influence carotenoid storage within the skin include biological sex, weight status, body fat, genetic and ethnic differences in carotenoid intake, absorption, bioavailability and/or storage, tobacco smoking, environmental pollutants, and more recently discovered, physical activity (PA) (202).

Studies have suggested a potential relationship between PA and SCS: individuals that regularly exercise or have higher aerobic fitness have a higher concentration of stored carotenoids than sedentary individuals or those with lower aerobic fitness (192, 194). PA indirectly lowers oxidative stress by increasing mitochondrial biogenesis and upregulating antioxidant-related gene transcription (203). In response, oxidative stress in the body is lowered, and carotenoids are not relied on as heavily for ROS neutralization, resulting in greater storage of carotenoids within adipose tissue and skin (192, 193, 200, 204). An emerging factor that may also  mediate the association of body fat amount with both PA and skin carotenoids because PA is associated with lower body fat percentage in adults (205), which concentrates the carotenoids within the body, as carotenoids are fat-soluble and stored within fatty tissue (206). These relationships are illustrated in Figure 1.

Despite the established evidence of an existing relationship between PA and SCS, no data to our knowledge exist with regards to preschool-aged children. Hence, the aim of this study was to examine the relationship between measured PA and SCS in a sample of preschoolers.

**Methods**

Study population

This study report focuses on measures completed at baseline of a randomized controlled feeding study in a small sample of preschoolers attending a local childcare center. Parents of eligible children (no developmental delays, food allergies, or medications) were invited to enroll their child. Data were collected in February 2023.

The classrooms chosen to participate in the study included 12 and 13 children. Nineteen children met the inclusion criteria and were invited to participate. The parents of 17 children provided consent to enroll their child in the study, and fourteen children provided baseline data. All parents provided written informed consent and participating children were asked for verbal assent before each study procedure. This study protocol was approved by the University of Virginia Social and Behavioral Sciences Institutional Review Board (UVA IRB-SBS #5304) and was registered with ClinicalTrials.gov (NCT05730530).

## Measures

### Physical Activity

PA was measured using triaxial *ActiGraph GT3X+* (ActiGraph, Pensacola, Florida) accelerometers secured to each child's hip using a colorful belt from Tuesday 8:30am until Thursday 4:30pm. Actigraphy data for each day was converted to time spent in light, moderate, and vigorous PA using the Butte Preschoolers VM cut points (181, 182) on the ActiLife (ActiGraph, Pensacola, Florida ) (181) software program (82, 83). Time spent engaging in each level of PA (light, moderate, vigorous) was summed to yield time spent in total PA for each day and averaged for each child to yield mean total PA (207).

### Skin carotenoids

SCS was measured using pressure-mediated reflection spectroscopy (VEGGIE METER ®; Longevity Link Corporation, Salt Lake City, Utah, USA), which is based on the quantification of the color and intensity of reflected light in the skin after illumination with white light (45). Skin carotenoid concentration is reported as score from 0 to 800. Based on device instructions and as reported in published studies using the device, skin carotenoids were measured in triplicate on each data collection day and subsequently averaged to yield a mean SCS for each child.

### Statistical analysis

Descriptive statistics were used to describe the characteristics of participating children. The test-retest reliability of the skin carotenoid measures across the three days of measurement was assessed using intraclass correlation coefficient (ICC) and two-way mixed effects model. Multiple linear regression modeling was used to assess the relationship between SCS and time spent in PA, controlling for classroom. Statistical analyses were performed using SPSS Statistics (IBM Corp. Released 2020. IBM SPSS for Macintosh, Version 29.0.0.0, Armonk, NY: IBM Corp). The significance level for all statistical tests were set at $p<0.05$.

**Results**

Children were 44±7 months old, 71% male, and predominately non-Hispanic White (86%). On average, children engaged in 137±18 minutes of PA; 79% (n=11) of preschoolers met the guidelines for daily PA (≥ 120 minutes total PA). Mean SCS was 304±94. The ICC indicated moderate test-retest reliability of SCS (ICC= 0.837, 95% CI: 0.513, 0.945). There was a significant association between time spent in PA and SCS ($\beta = 3.448$, $p=0.032$).

**Discussion**

As has been observed in recent research in adults, time spent in PA and concentration of carotenoid storage in the skin were significantly related, thus supporting the notion that numerous factors contribute to skin carotenoid levels.

Previous studies examining this relationship have also reported a significant association between PA or physical fitness with increased skin carotenoids in both school-age children (194) and adults (192), even when there was no observed relationship between FV intake and PA (192). In fact, a study conducted in 134 adults found that aerobic fitness was a predictor of skin carotenoid levels, independent of FV intake, and an increase in fitness over 8 weeks was directly associated with increases in skin carotenoid pigmentation (192). This relationship was attributed to two mechanisms involved: primarily, the common antioxidant effect of both carotenoids and exercise and, secondarily, that PA decreases levels of body fat, therefore concentrating carotenoids within the skin. This relationship between skin carotenoids and PA may partially contribute to the inconsistencies observed in the literature regarding the use of skin carotenoid measurements as a biomarker for FV intake (50, 54, 208, 209). Consequently, researchers have suggested that use of skin carotenoids as biomarker of health status, as opposed to solely FV intake, may be more appropriate (50, 193, 202, 210). These findings support this notion in preschool-aged children. Limitations of this study include the small, homogenous sample of preschoolers from the same childcare center. Additionally, we did not assess additional factors known to influence skin carotenoids, such body fat levels, dietary carotenoids, and other sources of antioxidants and oxidants. Assessments of these factors in larger and diverse samples may further elucidate the use of SCS to evaluate health-related behaviors in preschool-aged children.

In conclusion, we found that PA levels in preschool-age children were associated with SCS. These findings here suggest a potential avenue for future research to further investigate health status in children, thus contributing to the literature on child health research.

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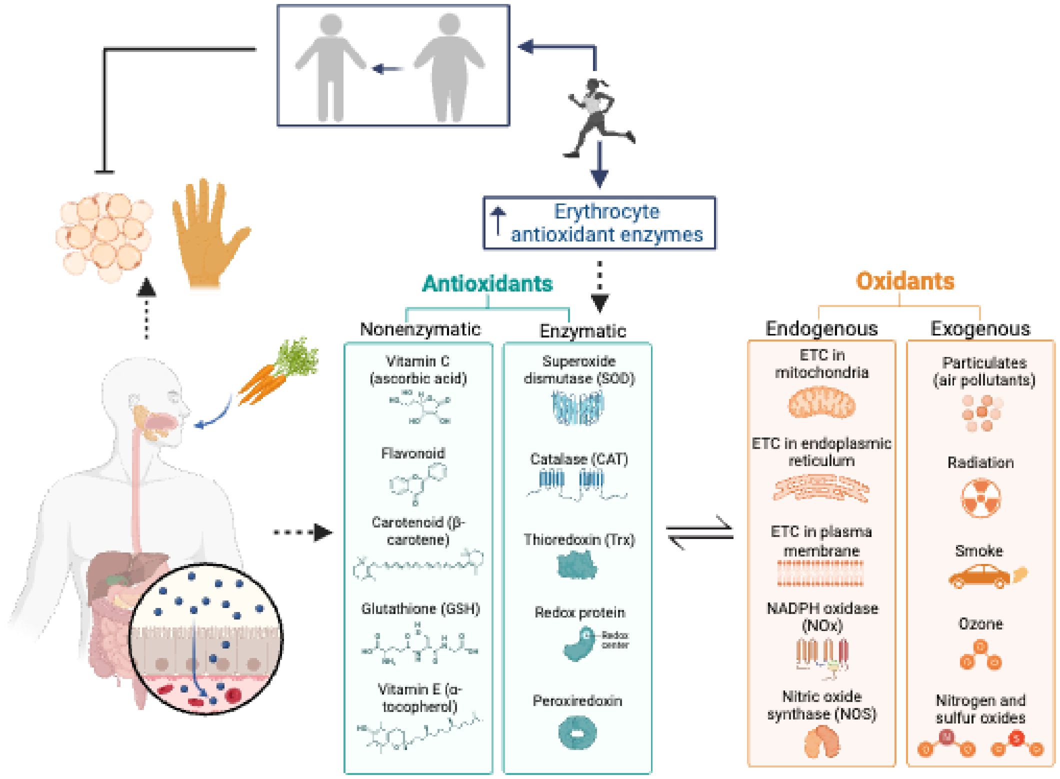


Figure 1. Depiction of the proposed mechanisms regarding the relationship between skin carotenoid levels and physical activity levels. Adapted from (192, 193, 200, 204-206, 211, 212).

# Chapter 5: Summary and Final Comments

Low fruit and vegetable intake in the United States is a significant public health issue. Despite over four decades of recommendations set forth by various governing agencies and well-established research regarding their importance, Americans continue to under-consume both fruits and vegetables (1). To contribute to the existing literature aiming to address low FV intake, we designed and tested the feasibility of a study to assess the effects of a theory-informed behavioral intervention on lunch fruit and vegetable intake in preschoolers attending a local childcare center. Our approach was novel in that our study objectively assessed changes in fruit and vegetable intake, and our intervention was both informed by a behavioral change theory and was adapted from previous studies that successfully increased intake. Notwithstanding, while we successfully established study feasibility, we observed no increase in FV intake.

In our third manuscript, we examined the association between physical activity and skin carotenoids. While the existing literature suggests a relationship between these two health-related variables, this had yet to be examined using objective measures in preschoolers. We observed that time spent in PA was associated with higher skin carotenoid storage in this sample. Previous research has proposed the use of skin carotenoids as a proxy for FV intake. However, our findings, along with those of similar studies, implicate that skin carotenoids may in fact reflect the balance between carotenoid intake and carotenoid-usage to neutralize oxidative stress.

Dietary habits are influenced by a plethora of internal and external factors that uniquely interact to influence behavior and, as such, interventions may need to be tailored to these factors in order to be successful. As such, this field of research may benefit from a shift towards a personalized, precision behavioral nutrition approach, similar to the principles underlying precision medicine. Herein we establish the feasibility of a behavioral intervention approach and present data that highlight the challenges involved with increasing FV intake in preschoolers. We also provide findings that support a more comprehensive interpretation of skin carotenoid measurements. Given the gaps present within this field of research, these findings are of great importance for informing future interventions.