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The influence of dietary variety and course sequence on fruit intake in preschool-aged children

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To the Graduate Council:

I am submitting herewith a thesis written by Chelsi Celcilia Cardoso entitled "The influence of dietary variety and course sequence on fruit intake in preschool-aged children." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Nutrition.

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The influence of dietary variety and course sequence on fruit intake in preschool-aged children

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ABSTRACT

Background: Fruit and vegetable (F&V) intake in preschool-aged children remains below recommendations. As the environment can affect food consumption, this study tested the effects of two environmental factors, dietary variety and course sequence, on fruit and overall intake of a snack in preschool-aged children. **Methods**: Using a 2 x 2 x 2 design (between-subjects factor of order and the within-subjects factors of dietary variety and courses), 16 children (4.1 $\pm$ 0.7 years of age, 56.3% female, 75.0% White, 93.8% non-Hispanic or Latino, and 0.5 $\pm$ 1.3 BMI z-score) from 2 preschool classrooms (classroom 1 [n = 7] and classroom 2 [n = 9]) completed 20 minute snack sessions on Wednesday afternoons over four occasions. All snacks were 200g and included 50g of applesauce, 50g of peaches, and 100g of cheese (variety) or 100g of applesauce, and 100g of cheese (no-variety), served over one 20-minute course (one-course) or one, 10-minute fruit course and one 10-minute cheese course (two-course). **Results**: Repeated measures analyses of covariance found no significant main effects or interactions of fruit or overall snack intake. **Conclusion**: Variety and course sequence may not be an effective strategy to increase low-energy dense foods, like F&Vs, during snacks in preschool-aged children.

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BACKGROUND AND SIGNIFICANCE

Introduction

The importance of fruit and vegetable (F&V) consumption is recognized globally [1]. Recommendations from the United States Department of Agriculture's *Dietary Guidelines for Americans*, which are evidence-based, are used to set guidelines for Federal programs and are supported by the *Academy of Nutrition and Dietetics* (formerly the *American Dietetic Association*) [2, 3] and suggest Americans consume at minimum 2 ½ cups of F&Vs per day. According to nationally representative data from the National Health and Nutrition Examination Survey [4], most Americans are not currently meeting F&V recommendations.

Intake of F&Vs is associated with a variety of health benefits, including reducing the risk of developing several chronic diseases, such as cancer and coronary heart disease [1, 5]. Due to being low in energy density, F&V consumption has also been linked with weight management; foods low in energy density are hypothesized to help with reducing energy intake because they increase satiation [6]. Thus, due to the relationship between F&V intake and chronic disease, increasing F&V consumption has been recommended to improve the health of all populations [5].

Preschool-aged children are in need of a nutrient-rich diet, which can be achieved through increased F&V consumption, to support proper growth and development [7, 8]. Preschool-aged children who are not meeting F&V recommendations may be displacing nutritious, low-energy-dense F&Vs with less nutritious, high-energy-dense options, such as cookies and chips [9]. This tradeoff can increase the risk for excessive weight gain due to increased energy intake [10, 11].

Dietary preferences are established early in life and tend to continue into adulthood [7]. Consuming a diet high in F&V at an early age is important to help establish preferences for these foods for life. Therefore, strategies aimed at young children to increase F&V intake are essential to aid with developing healthy eating patterns that can assist with preventing excessive weight gain, which may lead to overweight and obesity in childhood and adulthood [1, 12].

Childhood Obesity and Risks into Adulthood

The International Obesity Task Force estimates that 1.1 billion adults are overweight and 312 million are obese worldwide [13]. In the United States, the prevalence of overweight and obesity has increased dramatically in the past 40 years [2, 14]. In the 1980s, five percent of children aged 2-to-19 years were obese [15]. These numbers have increased and currently 17% of children and adolescents (ages 2-to-19 years) and 10% of preschool-aged children (ages 2-to-5 years) are obese, according to National Health and Nutrition Examination Survey data [14].

According to the Centers for Disease Control and Prevention (CDC) and the American Academy of Pediatrics, child overweight and obesity is defined as having a body mass index (BMI) at or above the 85th and 95th percentile, respectively, with percentiles based upon a child's age and sex [14, 16]. Overweight and obesity are associated with numerous negative health outcomes in children including asthma, sleep apnea, joint pain, and acanthosis nigricans [17]. More recently, overweight and obese children have even been diagnosed with chronic diseases, such as type 2 diabetes, hypertension, and hepatic steatosis, which were previously seen only in adults [13, 14, 16].

The obesity epidemic is a major public health concern and appears to track individuals throughout their lifetime. Recent evidence shows that overweight and obese adolescents will most likely become overweight and obese adults [2, 18, 19]. In fact, being overweight or obese at

any point in life can increase the risk of obesity related diseases [19]. Targeting children at a young age, such as in preschool, may help reduce weight gain at that time as well as reduce weight related health problems into adulthood.

Fruit and Vegetable Intake, Obesity, and Weight Loss

The most current (2010) Surgeon General's recommendations to combat obesity include increasing F&V intake as a goal [15]. One reason increasing F&Vs is recommended is that F&Vs are low-energy-dense foods. Energy density is defined as the ratio of calories to the weight of the food. Low-energy-dense foods are often high in water and fiber content [6]. These properties not only make low-energy-dense food, such as F&Vs, low in calories, but foods low in energy density also increase satiation and decrease hunger [6, 20]. This report also states that increasing F&V consumption could be beneficial because it may displace the consumption of high-energy-dense foods [20]. However, research conducted on F&V intake and weight loss does not always support a role for F&V intake in weight reduction or obesity prevention.

A prospective cohort study by Field and colleagues [21] aimed to assess if F&V intake was associated with change in BMI among children and adolescents. Participants of the study were children of participants in the Nurses' Health Study. BMI and F&V intake, determined by the Youth/Adolescent Questionnaire, was self-reported annually over a three-year period [21]. F&V intake, as well as calories consumed, was analyzed and no significant association between F&V intake and weight change was found [21]. Researchers concluded that recommending F&V intake as the sole method of body weight regulation of weight may not be warranted.

It is important to note that increasing F&V intake can only improve weight status by reducing total calories consumed. A cross-sectional study compared over 2,000 participants' diets to the 2005 *Dietary Guidelines for Americans* [22]. Data showed that the over-intake of

foods from “discretionary calories,” which are higher in fat and sugar, low in nutrients, and typically high-energy-dense foods, was far greater than the under-intake of F&Vs [22]. The authors concluded that interventions that solely target one behavior, such as increasing F&V intake, may not have sufficient impact on caloric intake due to such over-intake of these “discretionary calories” [22]. Thus, increasing F&V intake alone may not be able to displace enough consumption of high-energy foods to reduce energy intake and improve weight status.

As F&V consumption increases, and as F&Vs contain energy, the only way to decrease total calories consumed would be to decrease or displace consumption energy from other areas of the diet [6, 21, 23]. Only one non-observational study has examined the substitution or displacement relationship of F&Vs with high-energy-dense foods, such as snack foods (i.e. chips, cookies, ice cream), and energy intake in overweight children [24]. Looney and Raynor [24] examined the relationship between changes in consumption of F&Vs, snack foods, and energy through a secondary data analysis in children who were enrolled in a 6-month, family-based, behavioral pediatric weight management trial [25]. Children enrolled in the trial were randomized into one of three conditions: 1) increased growth monitoring with feedback; 2) decreased snack foods and sugar sweetened beverages; or 3) increased F&V and low fat dairy [24]. A subsample (N = 80) of the original trial was used for the secondary analysis [24].

Results of the secondary data analysis showed no relationship between changes in F&V and snack food intake [24]. Increasing F&V intake did not result in decreased consumption of snack foods. In addition, results indicated that decreased snack food consumption was significantly associated with reductions in energy intake, but increased F&V consumption was not associated with decreased energy intake [24]. Thus, it may be more beneficial to focus on increasing F&V intake as well as decreasing intake of calorically dense foods. However, given

the benefits of F&Vs, it is still a goal to increase F&V intake in preschool-aged children. Not only would increasing F&V intake aid in the development of healthy eating behaviors [7, 12], but it may also aid in decreasing the risk of chronic disease, such as cancer or coronary heart disease [1, 5].

Fruit and Vegetable Recommendations for Preschool-aged Children

According to the 2010 *Dietary Guideline for Americans*, [3, 12, 26] children, aged 2-to-3 years are recommended to consume 1 cup of vegetables and 1 cup of fruits daily, and 4-to-8 year olds are recommended to consume 1 ½ cups of vegetables and 1 to 1 ½ cups of fruits daily. Thus, preschool-aged children, aged 2-to-5 years, are recommended to consume between 1-to-1 ½ cups of both F&Vs daily. These are general recommendations and may be different for children who are more physically active and have greater caloric needs [12].

Vegetables can be consumed raw, frozen, canned, juiced or cooked, and fruits can be consumed raw, frozen, canned, juiced, cooked or dried [3, 12, 26]. The amount of F&Vs needed to be consumed to count as a cup depends on the size, shape, and method of preparation. For example, 1 large apple is equivalent to 2 cups of fruit while 1 small apple is equivalent to a ½ cups, and 2 cups of raw dark leafy greens would count as a 1 cup equivalent [3, 12, 26]. One cup of 100% fruit or vegetable juice both count as 1 cup towards the recommended intake of F&Vs [3, 12, 26].

Research on Fruit and Vegetable Intake in Preschool-aged Children

While meeting goals for F&V intake may be helpful for the health of children, unfortunately most children are not meeting F&V recommendations. Guenther and colleagues [4] examined survey data to determine if Americans are consuming recommended amounts of F&Vs. Twenty-four dietary hour recalls from the 1999-2000 NHANES (n = 8,070), and two, 24-

hour recalls from the 1994-1996 Continuing Survey of Food Intakes by Individuals (CSFII, n = 14,963) were utilized in the investigation [4]. F&Vs consumed were converted into serving sizes based on recommendations for age and sex from MyPyramid [4, 5]. Researchers found that approximately 48% of children aged 2-to-3 years consumed the recommended amount of F&Vs [4]. Thus over half of 2-to 3-year-olds were not consuming the recommended amount of F&Vs [4].

Additionally, Lorson and colleagues [27] conducted a study with children aged 2-to-19 years, using data from the 1999-2002 NHANES (n = 6,513). F&V intake was assessed with 24-hour recalls. Researchers found for children the major source of fruit was juice, while french fries accounted for 28% of total vegetable intake [27]. Preschool-aged children (ages 2-to-5 years) consumed significantly more juice (>40%) compared to children aged 5-to-18 years, and children aged 12-to-18 years consumed significantly greater amounts of french fries compared to children aged 2-to-11 years [27]. Overall, Lorson and colleagues [27] found that 50.2% of preschool-aged children were not meeting fruit recommendations, and 78.3% were not meeting vegetable recommendations. These studies show that preschool-aged children are not meeting F&V recommendations. Additionally, it was found that fruit juice and fried potatoes were a large source of F&Vs consumed.

Most recently, Fox and colleagues [9] used data from the Feeding Infants and Toddlers Study to examine consumption patterns of 2-and-3-year-olds (n = 1,461) to determine if children were meeting the *Dietary Guidelines for Americans* recommendations. When analyzing F&V intake, only those F&Vs that were consumed as a distinct portion were counted towards contributing to F&V intake [9]. For example, green peppers from a mixed dish like a pizza would not count towards contributing, but green peppers sliced as a side dish would count

towards contributing to vegetable intake. This rule also applied for counting fruit intake, and servings from 100% fruit juice were counted as contributing to fruit intake [9].

For vegetables, results from this study showed that 70% of the preschool-aged children consumed at least one distinct portion of vegetables daily while the remaining 30% did not consume *any distinct portions of* vegetables [9]. Among the 70% that did consume at least one portion of vegetables per day, the most popular type was white potatoes (consumed by 30.6% of 2- to-3-year-olds), which in many cases was fried (18.5% of cases) [9]. For fruits, approximately 87% of children consumed at least one distinct portion of fruit or 100% fruit juice per day leaving 13% who did not consume *any distinct portions of* fruits [9]. Results showed that children who did not consume distinct portions of F&Vs were more likely to consume high-energy-dense 'junk' foods [9]. Overall, all of these studies show that F&V intake among preschool-aged children are not meeting recommendations set by the *Dietary Guidelines for Americans.*

Interventions to Increase Fruit and Vegetable Intake in Preschool-aged Children

There have only been a few investigations conducted to increase F&V intake in preschool-aged children. *Color Me Healthy* (CMH) [28] which was a nutrition and physical activity intervention with a strong focus on increasing F&Vs, found significant increases in consumption of F&Vs in preschool-aged children participating in CMH compared to preschool-aged children who did not receive the intervention. Two-hundred and sixty-three preschool-aged children 4-to-5 years, from 17 different child care centers, were recruited and randomly assigned to 1 of 2 conditions [28]. One condition (n = 165) received the CMH intervention, which was 6 weeks in length and included a 15-to-20 minute circle-time lesson designed to expose children to F&Vs and provide children with nutrition education on F&Vs. Additionally, CMH had one

imaginary trip per week, in which children imagined being physically active and trying F&Vs [28]. The other condition (n = 98) received no intervention [28].

To assess the impact of CMH, assessments were taken at baseline, 1 week post-intervention and again at 3 months post-intervention. For assessment weeks, F&V snacks were given on Tuesdays and Wednesdays and consisted of 1 cup of mixed fruit, 1 cup of mixed vegetables, 2 ounces of fat-free ranch dip, 2 Ritz crackers, and ½ of a large graham cracker [28]. Consumption was determined by weighing the food before and after and then calculating percentages of intake of the snacks for each child. Children with the CMH intervention consumed 34.2% more fruit and 24.2% more vegetables 1 week post-intervention compared to children with no intervention [28]. Additionally, the CMH intervention showed a 20.8% increased consumption of fruit and a 33.1% increased consumption of vegetables between baseline and the 3 month assessment compared to children receiving no intervention [28]. This intervention suggests that exposing and teaching preschool-aged children about F&Vs can increase F&V consumption during a snack that is maintained over time.

One non-randomized controlled trial, school-based intervention was conducted by Ransley and colleagues [29]. This investigation was aimed at increasing F&V intake in young children aged 4-to-6 years. Ninety-eight schools (53 intervention schools and 45 control schools) participated in the study [29]. Children in intervention schools received a piece of fruit or vegetable on a daily basis. Additionally, educational materials were given to the schools and teachers to encourage the school and children to participate in the program [29].

Dietary intake was collected using an assessment tool for the children's parent to fill out at 3 and 7 months [29]. Results showed that in children aged 4-to-5 years, fruit intake increased by 0.4 servings and 0.2 servings in total at 3 months and 7 months, respectively [29]. Six-year-

old children increased their fruit intake by 0.5 servings in total at 3 months, which was not maintained at 7 months, as children became too old to participate in the program [29]. Overall there were no changes in vegetable intake in the children [29]. This intervention increased fruit intake in the short-term by increasing availability of fruit within the school environment.

Haire-Joshu and colleagues [30] implemented an at home intervention for parents, in which the goal was to increase F&V intake in their children, aged birth-to-5 years of age. The intervention group (*H5-KIDS*) received home visits, newsletters and sing-a-long storybooks, and also taught parents about decreasing coercive feeding practices [30]. The control group received these treatments (but not focused on increasing F&V intake, but only parenting overall), and were part of a parenting program called Parent for Teachers (PAT) [30]. PAT is a program developed to increase parent-child communication and to help parents learn skills to increase their child's social and physical development.

During home visits for *H5-KIDS*, educators delivered sessions that addressed increasing knowledge of benefits in F&Vs, increasing parental modeling of F&Vs, and decreasing coercive feeding behaviors of parents [30]. The major goal was to promote a positive environment for F&V intake and to determine if parenting changes were associated with increased intake [30]. Researchers reported that the *H5-KIDS* group had significant increases in fruit intake, combined F&V intake, but not vegetable intake compared to the control condition [30]. This intervention targeted parenting skills related to eating more F&Vs, and was successful in increasing fruit and F&V intake.

Another intervention designed by Niklas and colleagues [31] utilized television commercials to increase preferences of F&Vs in preschool-aged children. Extensive assessment for content of the commercials was determined through focus groups and interviews with experts

and preschool-aged children [31]. Based on the information gathered, the researchers developed two characters, "Reggie Veggie and Judy Fruity [31]." During the commercials, these characters modeled eating F&Vs, and expressed positive reinforcement, encouragement, and reasons (rationale) towards F&V consumption [31].

During this pilot test, the intervention group (n = 106) was given a pre-test for F&V preferences before children were exposed to the commercials, with a post-test given two weeks later [31]. The control group (n = 77) was given the pre-test and post-test in the same manner, but this group did not receive the commercial intervention [31]. Fifteen different F&Vs were scored for preferences during these tests, and the characters in the commercials highlighted apples, bananas, broccoli, and carrots. Results found that after the intervention, the intervention group displayed a significantly higher preference for broccoli and carrots compared to the control group [31]. There was no difference in fruit preferences.

These studies suggest that teaching and exposing preschool-aged children to F&Vs may increase their consumption, increasing the availability of F&Vs can increase intake in the short-term in preschool-aged children, conducting family interventions may be beneficial in increasing F&V consumption, and the use of television commercials may increase F&V preferences of preschool-aged children. Research focused on increasing F&V intake in preschool-aged children has been limited, therefore novel ways to increase F&V intake should be investigated. While one environmental variable, availability, has been targeted for increasing F&V intake, additional environmental variables should be examined.

Environmental Variables that Influence Consumption

The environment can have considerable impact on intake of food, and it has been proposed that the environment may even be the driving force behind the obesity epidemic [32].

Environmental changes, including greater dietary variety and availability of sedentary activities, have made it more difficult to balance energy intake and expenditure, which is essential to maintain body weight [32]. One theoretical perspective that can help explain how the environment may impact on behaviors is behavioral theory (BT) [33]. BT suggests that behaviors are learned through interactions with the environment [33]. Antecedents, which are stimuli or cues, can trigger or not trigger behaviors [33]. Thus, antecedents in the environment can alter behavior. Food availability is an antecedent for consumption of food and beverages, and a systematic review by Jago and colleagues [34] found that availability of F&Vs was a major factor in consumption of F&Vs, so that increased availability was related to increased consumption.

Another known environmental antecedent that influences F&V intake in both children and adults is portion size [35, 36]. Spill and colleagues [35] conducted a study with 3-to-6-year-old children to determine the effect of portion size on intake of F&Vs. Children in this study were provided meals on four different occasions. On three of the occasions, carrots were served in three difference portion sizes (30, 60, or 90 g) before serving an entree which consisted of macaroni and cheese, steamed broccoli, applesauce and milk [35]. The control meal was the entrée meal without the carrots [35]. Results showed that increasing the portion size of the carrots from 30 g to 60 g significantly increased carrot intake [35]. In comparison to the control meal with no carrots, when 30 g of carrots were served intake of carrots doubled, and when 60 g of carrots were served carrot intake tripled [35].

Similarly, Rolls and colleagues [37] served a meal to adults consisting of a vegetable, grain, and meat with the vegetable differing in portion size (180, 270, or 360 g). Increasing portion size of the vegetable increased vegetable consumption significantly [37]. Increasing the

vegetable portion from 180 to 360 grams increased vegetable consumption by 34 ± 4 g (M ± SE) [37]. Thus changing a dietary environmental antecedent, portion size, produced a desired behavior (increasing vegetable intake) in both preschool-aged children and adults.

Dietary Variety

Another environmental variable that appears to influence F&V intake is dietary variety. Dietary variety has been shown to significantly influence dietary intake in both animals and humans [38, 39], such that greater variety is related to increased intake [40]. In humans, research investigating dietary variety has predominantly examined the effect of variety within a meal [41]. A meal containing variety is composed of foods with sensory distinctiveness [42]. Variety can be manipulated within a meal by introducing variety successively (courses of different foods served during a meal), or simultaneously (different foods served all at once in one course) [40, 42]. Variety is believed to influence intake through sensory-specific satiety. Sensory-specific satiety is the decline in pleasantness of food just consumed, compared to non-consumed foods [38]. For example, if a person recently ate an apple, liking of apples would show a larger decrease as compared to liking ratings of bananas, because bananas had not been consumed. As liking of a food decreases, consumption of the food would also decrease. With greater variety, sensory-specific satiety is considered to be delayed, thus lengthening an eating bout.

Rolls and colleagues [39] conducted a series experiments to test the effects of variety on eating behavior. One of these studies examined the effect of variety in yogurt on intake. In the experiment, participants attended four sessions, in which three sessions were labeled the "plain" conditions and one session was labeled the "variety" condition. In each session participants were served three successive, 10 minute, courses. In the plain conditions participants received the same yogurt during the three-courses, and in the variety condition participants received a

different yogurt for each course [39]. Participants received hazelnut, blackcurrant, or orange flavored yogurt in all three-courses for the plain conditions, and those in the variety condition would receive all three yogurts in succession [39]. These yogurts differed not only in flavor but also in texture, depending on the fruit or nut (nuts, chewy fruit, and either small pieces of fruit or whole berries). When consumption across the three courses was combined, researchers found significantly increased intake in the "variety" condition (735 $\pm$ 55 g) compared to the "plain" condition (619 $\pm$ 62 g) (M $\pm$ SEM) [39].

Brondel and colleagues [38] conducted an experiment examining dietary variety in 21 normal-weight males. The participants completed three sessions, with one session occurring per week. In each session participants were fed a two-course eating bout, with the first course consisting of fries and the second course consisting of brownies. Depending on the groups to which participants were randomized, participants either received their courses with or without condiments [38]. The condiments were added to increase the dietary variety of the meal. Participants were randomized into one of three groups; monotonous, successive, or simultaneous [38]. The monotonous group received fries and brownies with no added condiments during all three sessions. The successive group received fries and brownies each with no condiments at the first session, fries and brownies with ketchup and vanilla cream at the second session, and fries and brownies with mayonnaise and whipped cream at the third session (condiment order was randomized between participants in this group). The simultaneous group received fries and brownies with all condiments openly available at all three sessions. Food consumption was measured every 2 minutes during the sessions [38]. Results showed that total energy intake was 41% greater in the successive group (1682 $\pm$ 777 kcal) and 24% greater in the simultaneous group (1485 $\pm$ 582 kcal) than the monotonous group (1195 $\pm$ 552 kcal) (M $\pm$ SD) [38]. In the

short-term, increasing dietary variety by adding a simple food item such as condiments can significantly increase energy intake.

Bucher and colleagues [43], utilized an increase in variety of vegetables to influence meal composition. The purpose of the study was to determine if increasing vegetable variety during a meal improves meal composition [43]. To investigate this, a fake food buffet was used, and theoretical energy content of the food replicas were determined by preparing real foods and comparing shapes and weight to the replicas [43]. Ninety-eight subjects, 53 men and 45 women, participated in this randomized experiment [43]. Participants were randomly assigned to 1 of 3 conditions, which included two, one-vegetable conditions and one, two-vegetable condition [43]. Participants in condition A served themselves a buffet of cooked carrots, pasta, and chicken, condition B served themselves a buffet of cooked garden beans, pasta, and chicken, and condition AB served themselves a buffet of cooked carrots, cooked garden beans, pasta, and chicken [43].

Results showed significantly higher energy derived from vegetables when two vegetables were offered, with condition AB (carrots and beans) being significantly different from condition A (carrots only) and B (beans only) [43]. Additionally, the percentage energy from vegetables in condition AB was significantly higher than condition A and condition B [43]. This study shows that increased vegetable variety can increase selection f vegetables if available.

Recently, Meengs and colleagues examined the effects of increased vegetable variety on consumption of the vegetables, and any differences of consumption based on potential preference of the vegetables served [44]. Using a crossover design, 66 participants (32 men and 34 women) consumed a lunch once a week for 4 weeks [44]. For lunch, participants received 600 g of pasta with sauce and 600 g of vegetables, each visually presented as taking half of the space

on the plate [44]. Vegetables provided during the course of the study included broccoli florets with butter, snap peas, and carrots. Participants would either receive a vegetable individually (600 g), or a mix of the 3 vegetables (200 g of each respectively) [44]. In addition, participants received a standard breakfast and were instructed to receive lunch at least 3 hours after the breakfast meal [44]. All aspects of the study were conducted in a laboratory setting.

Results showed that serving the combined three vegetables as compared to each individually, produced significantly greater vegetable intake in men and women (48 $\pm$ 6 g) [44]. Additionally, the effect of variety was significant regardless of which vegetable was preferred by participants [44]. Thus, increasing vegetable variety increased vegetable consumption, meaning increased variety could be used as a strategy to promote eating of healthy foods.

Another recent study, by Raynor and Osterholt [45], examined the influence of fruit variety on consumption of fruit as well as on sensory-specific satiety. Twenty adults, aged 18-45 years completed the study sessions [45]. During the sessions, participants received a snack in four courses. The four-course snack was either served as four of the participant's most preferred fruits of six (VARIETY), or as the participants most overall preferred fruit for each of the four courses (NON-VARIETY) [45]. In addition, each participant was asked to eat their most highly rated meal bar 2 hours prior to receiving the four course snack [45]. To determine the participant's most preferred flavor of the meal bar and fruit, pleasantness was rated using a 100 mm Visual Analogue Scale (VAS) before the snack session occurred [45].

Before each four-course session, participants rated hunger and fullness with a 100 mm VAS [45]. Participants then sampled 3 grams of their top 4 rated fruit and rated the pleasantness again with a 100 mm VAS, thus before each session each participant rated the pleasantness of their top 4 rated fruits regardless of what fruit they consumed during the snack [45]. NON-

VARIETY condition received 200 g of their highest rated fruit during each of the 4 courses, and the VARIETY condition received 200 g of the participant's 4 highest rated fruit and received them in random order for the 4 courses [45]. Each course allowed the participants 7 minutes to consume [45]. After each course, participants were asked to rate their hunger, fullness, and pleasantness of the four fruits again [45].

Raynor and Osterholt [45] found that gram intake of fruit during course 4 was significantly greater in the VARIETY condition compared to the NON-VARIETY condition (65.2 $\pm$ 42.2 g vs. 41.0 $\pm$ 46.8 g) [45]. In the NON-VARIETY condition, course 2, 3, and 4 had significantly lower intake compared to course 1 [45]. However, in the VARIETY condition, only course 4 had significantly lower intake compared to course 1 [45]. Additionally, significantly more energy was consumed in course 4 of the VARIETY condition compared to the NON-VARIETY condition (34 $\pm$ 24 kcal vs. 21 $\pm$ 23 kcal) [45]. Finally, a greater reduction of rating, or pleasantness of fruit, was seen in the NON-VARIETY condition compared to the VARIETY condition [45]. Overall, this study shows that fruit consumption and rating of pleasantness differs in courses depending of if the fruit is served with variety or without variety.

These studies suggest that increasing dietary variety can increase intake of foods consumed [38, 39, 45]. As F&V intake needs to be increased, increasing variety of F&Vs within an eating bout may be an effective strategy for increasing F&V intake. However, most research regarding variety and intake has been conducted with adults, thus the effectiveness of utilizing variety to increase intake of F&Vs in children needs to be further investigated.

Course Sequence

Another dietary environmental variable that may impact eating behavior is course sequencing. Course sequencing refers to serving several courses within an eating bout. When an

eating bout contains courses, consumption is usually greatest in the first course and declines with each subsequent course [36, 38]. Thus, serving a food that needs to be increased, such as F&Vs, as a first course may assist with increasing intake of F&Vs. Additionally, as the food served in the first course appears to act as a preload, decreasing consumption in subsequent courses, if the first course is a low-energy-dense food and the second course is a high-energy-dense food, overall energy intake in the meal may decrease due to increased consumption of the low-energy-dense food and decreased consumption of the high-energy-dense food. However, little research has been conducted on the use of course sequencing with F&Vs, F&V intake, and overall energy intake in a meal.

The study by Brondel and colleagues [38], previously described, shows how intake decreases across courses served sequentially. In this study fries were served as the first course and brownies were served as the second course. When all conditions were combined, gram intake decreased from the first course of fries to the second course of brownies [38]. Overall the monotonous (268 $\pm$ 116 g vs. 98 $\pm$ 62 g), successive (319 $\pm$ 139 g vs. 157 $\pm$ 101 g), and simultaneous (296 $\pm$ 146 g vs. 104 $\pm$ 50 g) (M $\pm$ SD) groups consumed a higher gram amount in the first course compared to the second course [38]. The same effect was shown by Rolls and colleagues [39] when serving courses of yogurt (previously described). All conditions, either plain or variety, received three courses of yogurt. After the first course of yogurt, intake significantly declined with the second course of yogurt, regardless of the condition. These are examples of how consuming a first course meal can act as a preload, thus decreasing the intake of a second course.

In a sequential course meal, if the first course is low in energy-density, overall intake in a meal may decrease. Rolls and colleagues [36] examined energy intake in 42 women after

consumption of a first course meal varying in energy density (0.33 kcal, 0.67 kcal, and 1.33 kcal) and portion size (150 g and 300 g). The women met to consume lunch on 7 different occasions. On six of the visits, a first course meal of a salad was served before a main entrée of pasta and at the control visit only pasta was served [36]. Additionally, the researchers examined if serving a low-energy-dense first course (salad) can decrease the intake of the second course (pasta entrée). Results found that total meal energy intake was strongly influenced by the energy-density of the first course salad [36]. When served the small (150 g) or large (300 g) low-energy-dense salad, energy intake decreased by 7% (64 $\pm$ 26 kcal) and 12% (107 $\pm$ 29 kcal) (M $\pm$ SEM) for the entire meal, respectively, compared to the control meal [36]. Additionally, consuming the high-energy-dense salad increased meal intake by 8% (71 $\pm$ 27 kcal) for the small portion and 17% (145 $\pm$ 22 kcal) (M $\pm$ SEM) for the large portion [36]. Researchers concluded that low-energy-dense fruits and/or vegetables served as a first course in a sequential course meal can decrease overall meal energy intake [36].

Spill and colleagues demonstrated that serving a low-energy-dense first course can influence energy and vegetable intake in children [46]. Using a crossover design, the researchers examined the effect of serving tomato soup as a low-energy-dense first course in varying portion sizes (150, 225, or 300 g), on energy and vegetable intake in preschool-aged children [46]. In a daycare setting, children were given breakfast, lunch with tomato soup varying in portion sizes (150, 225, or 300 g) or no soup, and a snack one day a week over four weeks [46]. During lunch, the first course of tomato soup was followed by a main entrée (pasta with cheese sauce, broccoli, applesauce, and milk) [46]. Results showed serving tomato soup as a first course significantly reduced energy intake of the pasta, no matter which portion of soup was given [46]. Total meal energy consumed (soup + entrée) only significantly decreased when children were served the

150 g portion of soup, compared to either serving no soup first or 300 g of soup [46]. In addition, the average mean energy-density of the entire meal significantly decreased when serving any portion of soup first (0.92 ± 0.02 kcal/g) compared to no soup (1.14 ± 0.03 kcal/g) [46]. For vegetable intake (soup + broccoli), gram intake significantly increased as the portion of soup increased (no soup: 22.2 ± 2.5 g; 150g: 108.8 ± 5.7 g; 300: 128.9 ± 9.2 g) [46]. Thus, serving a low-energy-dense food, such as F&Vs, as a first course can be used to decrease energy intake of a main entrée and increase the intake of F&Vs in children.

Summary

Increasing F&V intake is associated with a decreased risk for chronic disease, as well as weight gain and obesity, particularly if F&Vs replace the consumption of less healthy high-energy-dense foods [9, 47]. Even with these well-known benefits, most Americans do not consume the recommended amounts of F&Vs [4]. Therefore, novel ways of increasing F&V intake need to be investigated. As the environment can affect food choices, a food environment that contains cues that prompt F&V intake could be very helpful for increasing F&V intake.

Two environmental cues, dietary variety and course sequence, have both been shown to impact food intake and may thus be environmental factors that could be used to increase F&V intake. Greater variety, defined as greater amounts of food that differ in color, size, or shape, can increase consumption of food. If F&V variety were increased within an eating bout, F&V intake within that eating bout should increase. Additionally, providing an eating bout in courses may alter eating behavior. When courses are used, intake is usually greatest in the first course and consumption drops in successive courses. Thus, to help children with meeting recommendations, those foods that need to be increased in consumption, such as F&Vs, could be served as a first course in an eating bout, and could be provided in greater variety in that first course. Those foods

that should be decreased in intake, such as energy-dense foods, could be served as later courses in an eating bout. If later courses contain the higher-energy-dense foods of the eating bout, the reduction in consumption that occurs in later bouts should help with reducing intake of energy-dense foods.

Thus, the purpose of this study was to examine two dietary environmental variables, variety and course sequencing, on F&V intake in preschool-aged children. These variables were examined within a snack that potentially contained three foods, two low-energy-dense fruits (peaches in light syrup and applesauce), and one high-energy-dense food (cheese cubes). Preschool-aged children were given snacks that varied in existence of fruit variety (no-variety or variety) and number of courses (one or two), with fruit served as the first course in the two course conditions. Amount of food served in the snack remained consistent across conditions. It was hypothesized that:

1. Two-course conditions will have greater intake of fruit and overall lower energy intake than the one-course conditions.
2. Variety conditions will have greater intake of fruit and overall lower energy intake than the no-variety conditions.
3. The two-course, variety condition will have greater intake of fruit and overall lower energy intake as compared to all other conditions.

CHAPTER II: MANUSCRIPT

INTRODUCTION

Intake of fruits and vegetables (F&Vs) is associated with varied health benefits, including reducing the risk of developing chronic diseases, such as cancer and coronary heart disease [1, 5]. Additionally, F&Vs are hypothesized to help with weight management because they are low-energy-dense foods and they increase satiation [6]. As dietary preferences are established early in life and tend to continue into adulthood [7], consuming a diet high in F&Vs at an early age may help to establish preferences for these foods for life.

Survey data has shown that preschool-aged children are not meeting current F&V intake recommendations [4, 9, 27, 47]. Research which targets methods to increase F&V intake in preschool-aged children is limited, thus novel ways to increase F&V intake should be investigated [28-31]. The environment can have considerable impact on intake of food [32]. The most commonly examined environmental variable for F&V consumption is availability, with investigations showing that greater availability enhances intake [34]. However, other environmental variables may influence F&V intake and should be investigated.

One environmental variable that could influence the consumption of F&V intake is dietary variety [43-45] . Dietary variety is defined as food that differs in shape, color, or size [42]. Dietary variety is hypothesized to influence intake through sensory-specific satiety, which is a decline in food pleasantness of recently consumed foods compared to foods which haven't been recently consumed [38]. Research has shown that within meals, greater dietary variety leads to greater consumption as compared to meals with less variety, whether the variety was provided all at once within a meal or within courses during a meal [40, 42].

Research has examined the effect of increasing variety of healthy foods, such as F&Vs, on consumption in adults. One study examined the effect of increased vegetable variety of

choices of what to consume for a meal [43]. Participants selected foods from a 'food replica' buffet which contained different foods for each condition [43]. Participants in the variety condition had two vegetables choices and chose a significantly greater amount of energy to consume from vegetables as compared to conditions with only one vegetable choice [43]. Two other studies examined the effects of variety on actual consumption of F&Vs in adults [44, 45]. Meengs and colleagues sought to determine the effects of increasing vegetable variety during a meal on consumption [44]. Participants ate significantly greater gram intake of vegetables when they were served a variety of vegetables as compared to one vegetable [44]. Similarly, a study by Raynor and Osterholt found that when a greater amount of fruit variety was served within a snack, gram intake of fruit was higher as compared to a condition with no fruit variety [45]. While the effect of variety on F&V intake has been examined in adults, it has not been examined in preschool-aged children.

Another environmental variable that could influence F&V intake is course sequence. Course sequence is described as having more than one course during an eating bout. Serving a first course within an eating bout can act as a preload to decrease intake of the second course, as consumption is usually higher in the first course and declines with subsequent courses [36, 38]. Therefore, if a first course is F&Vs, consumption of F&Vs may increase as compared to if F&Vs were served in a one course eating bout.

An additional benefit of serving a first course of F&Vs is that these foods are low in energy density, and if the second course is higher in energy density, overall energy intake for the eating bout may decrease. For example, one study [36] examined if serving a low-energy-dense first course decreased intake of the second course in adult women. On six occasions, researchers served a first course meal (salad) varying in energy density (0.33 kcal, 0.67 kcal, and

1.33 kcal) and portion size (150g and 300g) followed by a main entrée of pasta [36]. These 6 occasions were compared to consumption of a control meal of the pasta entrée only [36]. Results found that serving the small or large low-energy dense salad decreased overall meal energy intake by 7% and 12%, respectively, compared to the control meal [36]. One study examined the effects of serving a low-energy dense first course on entrée and overall meal intake with children [46]. In the study, children were served a tomato soup varying in portion size (150g, 225g, and 300g) as a first course followed by a second course main entrée (pasta with cheese sauce, broccoli, applesauce, and milk) [46]. These conditions were compared to a control meal of the entrée only [46]. When children were served the 150g portion of soup as a first course, overall energy consumption for the entire meal was significantly decreased, and vegetable intake (tomato soup + broccoli) significantly increased [46]. Therefore, serving a low-energy-dense first course, like F&Vs, in a multiple course eating bout may decrease overall energy intake and increase F&V consumption.

As research suggests that variety and course sequencing can influence consumption, these dietary variables may be helpful in increasing fruit intake and assisting with decreasing overall energy intake within an eating bout in young children. Therefore, the purpose of this study was to determine the extent to which manipulation of dietary variety and course sequence affects fruit intake and overall energy intake during a snack in preschool-aged children. These variables were examined during a snack provided to preschool-aged children that potentially contained three foods, two low-energy-dense fruits (peaches in light syrup and applesauce), and one high-energy-dense food (cheese cubes). Additionally, the children were given snacks that varied in existence of fruit variety (no-variety or variety) and number of courses (one or two), with fruit

served as the first course in the two course conditions. The amount of food served during each snack was consistent across conditions (200g).

It was hypothesized that two-course conditions would have greater intake of fruit and lower energy intake than one-course conditions. Second, it was hypothesized that conditions with greater fruit variety would have greater intake of fruit and lower energy intake than the no-variety conditions. Finally, it was hypothesized that the two-course, variety condition would have greater intake of fruit and lower energy intake as compared to all other conditions.

EXPERIMENTAL DESIGN AND METHODOLOGY

Study Design

The study used a 2 x 2 x 2 cross-over design, with the between-subjects factor of order (order 1 vs. order 2) and the within-subjects factors of dietary variety (no-variety vs. variety) and courses (one-course vs. two-course) [see Appendix A Table 1], to investigate the influence of dietary variety and course sequence on fruit intake in preschool-aged children. The dependent variables in the investigation were the amount, in grams and energy, of fruit and total energy of snack consumed. This study was approved by the Institutional Review Board (IRB) at the University of Tennessee, Knoxville. The trial was registered with ClinicalTrials.gov (Identifier: NCT01414699).

Participants

Participants were recruited from the University of Tennessee's Early Learning Center (ELC) program at the White Avenue location. Upon IRB approval in August 2011, standard procedures of recruitment occurred at the ELC. These procedures included sending a letter home to the parent/guardians that explained the study. Following the introductory letter, all parent/guardians received a folder of questionnaires and consent forms. These questionnaires and

consent forms provided information on demographics, eligibility, eating behavior of the child and parenting styles of the primary caregiver. Parent/guardians who were willing to let their child participate were asked to sign and turn in a consent form to the teacher of their class.

For this study, participants were preschool-aged children who attended the full week and full day program. To be eligible, all children had to be ≥3 years of age, enrolled at the ELC, and have guardian consent to participate. Children had to like the foods being served, be able to use a spoon, and not have any allergies to the foods being served. Additionally, eligible children could not be lactose intolerant. A Cohen's d was used to estimate the sample size needed for this study. Cohen's d is the difference of two means divided by the pooled standard deviation. To determine the sample size, the effect from a feeding study in adults examining dietary variety and course sequence was used. The effect size of the referenced study was 1.9 (large effect) between total grams consumed of variety conditions, yielding a needed sample of 5 children per classroom.

When this study took place, there were 33 students enrolled in the preschool classrooms at the ELC. Of the 33 students enrolled, 16 were in class 1 and 17 were in class 2. Parents turned in consent forms for 10 children from class 1 and 13 consent forms from class 2. Of these children with consent forms, 7 were eligible from class 1 and 11 were eligible from class 2. Thus, 18 children participated in the snack sessions. A final sample of 16 was used for data analysis because 2 children were excluded. Participants were excluded because one child was not present for snack sessions each week, and one child consumed less than 5 grams of the total snack in at least one session [48]. See Appendix A Figure 1 for a participant flow chart.

Randomization

Children were randomized by classroom into one ofr two orders by flipping a coin. There were two preschool classrooms; therefore class 1 was assigned to order 1 and class 2 was assigned to order 2 (see Appendix A Table 2).

Foods

Peaches in light-syrup, applesauce, and cheese cubes were used for the snacks. In all of the conditions, the children received a total of total of 200 grams of food. In the no-variety conditions, children received 100 grams of applesauce, and 100 grams of cheese cubes. In the variety conditions children received 50 grams each of peaches and applesauce, respectively, and 100 grams of cheese cubes.

Mott's applesauce (no sugar added, natural), Del Monte peaches (yellow cling halves in light syrup) and Kraft cheese (natural, mild cheddar) were the specific brands that were served to the children. The applesauce contained 45 kilocalories per 100 grams giving it an energy density of 0.45; the peaches contained 48 kilocalories per 100 grams giving an energy density of 0.48; and the cheese cubes contained 428 kilocalories per 100 grams giving an energy density of 4.28. All of the foods were familiar to the children at the ELC and had been previously served before as snacks at the ELC.

Procedures

Week Prior to Start of Snack Sessions

During the week prior to starting the snack sessions, height and weight measures were taken from the children.

Snack Session Procedures

For each snack sessions, snacks were served after lunch at approximately 3:30 in the afternoon. The same lunch was served on each day in which a measurement was taken. This meal consisted of fish portions, green peas, apple slices, whole wheat bread, and milk. Prior to receiving the snack, each participant at the ELC filled out questionnaires which rated their hunger and liking of the snack foods used in the investigation. Following these questionnaires, the children were given the snack. Research assistants carefully distributed the snack to children with containers that included their identification numbers. Teachers and participants were instructed to avoid sharing their snack with other children and to eat as much or little of the snack as they wished. Water was served to drink during the snack sessions. Each snack session was 20 minutes. For the two-course conditions, each course was provided for consumption for 10 minutes (i.e., 10 minutes for the first course and 10 minutes for the second course), while the one-course condition was served for the whole 20 minutes. Children were told the day of the snack whether they would receive either one-course or two-courses. After snack time was complete, all food containers were collected and participants were asked to fill out the hunger rating questionnaire again.

For seven successive weeks, the procedures described above were used for each snack session. For the first two weeks, two practice rounds, one consisting of a one-course snack with variety and the other of a two-course snack without variety, were conducted to get the children accustomed to the process. These practice sessions were in Weeks 1 and 2, and data were not used from these sessions. Starting at Week 3, the measured snack sessions occurred and followed the order described in Appendix A Table 2.

Measures

Anthropometrics

For height and weight measures, children were asked to remove their shoes and excess clothing beforehand. Height was measured twice and to the nearest tenth of an inch with a portable stadiometer (SECA, ITIN Scale Company, Brooklyn, NY). Weight was measured to a tenth of a pound using a calibrated portable digital scale (Healthometer Professional, Sunbeam Product Inc. Raton, FL). Body mass index [BMI = weight (kg)/height (in^2)] was calculated for each child and BMI scores were compared to the CDC's BMI percentile charts. Children with a BMI of greater than the 85th percentile and less than the 95th percentile for age and gender were classified as overweight, with those greater than the 95th classified as obese [16]. Additionally, children's z-BMI scores were calculated. This was done by standardizing the BMI for children's age and gender in relation to the population mean and standard deviation.

Consumption

Snacks were prepared at the Jessie Harris Building (JHB) at the University of Tennessee's campus, and then carried to the ELC, which is located next to JHB. Once the snacks were weighed, they were appropriately covered and stored following food safety recommendations. Each snack was weighed before and after consumption on a calibrated food scale (Denver Instruments SI-8001, Fischer Scientific) to the nearest tenth of a gram and the container was measured separately then with the snack included. The consumption amount was determined by subtracting the post-snack weight from the pre-snack weight (in grams). This allowed the investigator to calculate energy intake from gram amount consumed.

Liking of Food

The research team and ELC staff assisted the children with filling out a questionnaire which assessed the children's liking of the food presented. The questionnaire has been used in previous studies and consists of a three-point Likert-scale that has faces representing "yucky" (dislike), "so-so" (neutrality), and "yummy" (like) [48-50].

Hunger

This questionnaire is a three-point Likert-scale that represents hunger. This type of tool has been used by several researchers to assess hunger in preschool-aged children [48-50]. The scale consists of cartoons with stomachs that are shaded to show their stomach is empty (hungry), somewhat empty (somewhat hungry) or full (stuffed). The research team and ELC staff assisted the children with filling out this questionnaire.

Parental Questionnaires

Parent/guardians of children with consent were asked to complete four questionnaires: a demographic questionnaire, the Child Feeding Questionnaire (CFQ), the Child Eating and Behavior Questionnaire (CEBQ), and the Three Factor Eating Questionnaire (TFEQ). The demographic questionnaire included questions about parental and child demographics such as race and ethnicity, and parental education level and income level. Second, the CFQ assessed parental beliefs and practices regarding feeding their children [51]. The CFQ contains 31 items and 7 factors: perceived responsibility, perceived parent weight, perceived child weight, concern about child weight, restriction, pressure to eat, and monitoring [51]. Third, the TFEQ, was used to measure the parent's dietary restraint, disinhibition, and perceived hunger [52]. The TFEQ contains 51 items with 3 factors: restraint of eating, disinhibition, and hunger [52]. Fourth, the CEBQ assessed eating behavior and styles related to obesity risk [53]. The CEBQ contains 35

items with 8 factors: food responsiveness, enjoyment of food, emotional overeating, desire to drink, satiety responsiveness, slowness in eating, emotional undereating, and fussiness [53].

Data Analysis

Data analyses were done using SPSS (version 19) with an alpha-level at 0.05. Independent t-tests (continuous data) and Chi-squares (categorical data) were used to analyze differences in demographic information between the two orders. To ensure that liking of foods were not significantly different between the four sessions, a mixed factorial analysis of variance (ANOVA), with the between-subject factor of order and the within-subject factors of course (one and two) and variety (no-variety and variety), was used. Additionally, to ensure that hunger before the snack was not significantly different between the four sessions, a mixed factorial ANOVA, with the between-subject factor of order and the within-subject factors of course and variety, was conducted. To examine change in hunger ratings during the sessions, a mixed factorial ANOVA was used, with the between-subject factor of order and within-subject factors of courses, dietary variety, and time (before and after snack). For the dependent variables (grams and energy consumed) an ANOVA was used with the between-subject factor of order and within-subject factors of courses and dietary variety. Interactions were followed using post-hoc analyses with bonferroni corrections, and Greenhouse-Geiser probability levels were used to control for sphericity in analyses.

The effect size for grams and energy of fruit and total energy from snack consumed between conditions was calculated using G Power [54]. Effect sizes were calculated as Cohen's d using condition means and standard deviations. Effect sizes were classified as small (0.20), medium (0.50), or large (0.80) [55].

RESULTS

Participant Characteristics

Child

Baseline characteristics of child participants by classroom are listed in Appendix A Table 3. The 16 participants included in analyses were 4.1 ± 0.7 (M ± SD) years of age, and predominantly female (56.3%), White (75.0%), and non-Hispanic or Latino (93.8%). Most children were of a healthy weight (75.0%), with the average BMI percentile of 60.7 ± 27.0 (M ± SD). No significant differences in these characteristics were found between classrooms.

Parent

Baseline characteristics of parent participants by classroom are listed in Appendix A Table 4. Parents were 39.0 ± 5.8 (M ± SD) years of age, and they were predominantly female (75.0%), White (87.5%), and non-Hispanic or Latino (87.5%). Parents were also educated (75.0% Graduate or Professional Education) and married (75.0%). No significant differences in characteristics were found between classrooms.

Mean scores of parental questionnaires are in Appendix A Table 5. No differences were found between classrooms for the TFEQ. However, the desire to drink factor from the CEBQ was significantly different [$t(14) = 2.093$, $p = 0.019$] between the classrooms, with classroom 1 higher than classroom 2. Also, the satiety responsiveness factor from the CEBQ was significantly different [$t(14) = 1.699$, $p = 0.037$] between the classrooms, with classroom 2 higher than classroom 1.

To determine if these variables should be included as covariates in subsequent analyses, Pearson correlation analyses were conducted between these variables and the main dependent variables in the investigation (grams and energy consumed of fruit and total energy consumed

from the snack). Significant correlations were found between desire to drink and total fruit grams consumed (two-course, variety [r = -0.613, p = 0.012], one-course, variety [r = -0.690, p = 0.003]); and desire to drink and total fruit calories consumed (two-course, variety [r = -0.609, p = 0.012], one-course, variety [r = -0.683, p = 0.004], one-course, no variety [r = -0.744, p = 0.001], two-course, no variety [r = -0.595, p = 0.015]). No significant correlations were found with satiety responsiveness. Thus, desire to drink was added as a covariate in all analyses (liking of foods, hunger ratings, and consumption).

Liking of Food

A mixed-factorial analysis of covariance (ANCOVA), with the between-subject factor of order, within-subject factors of courses and dietary variety, and desire to drink as a covariate, revealed no significant differences in liking of foods among sessions. Overall, the mean liking rating of the foods served was as follows (M $\pm$ SD): applesauce = 1.2 $\pm$ 0.4; peaches = 1.3 $\pm$ 0.7; and cheese = 1.4 $\pm$ 0.7. See Appendix A Table 6 for mean ratings of liking of foods among conditions.

Hunger

A mixed-factorial ANCOVA, with the between-subject factor of order, within-subject factors of courses and dietary variety, and desire to drink as a covariate, found no significant difference between the sessions for initial hunger ratings. Additionally, a mixed-factorial ANCOVA, with the between-subject factor of order, within-subject factors of course, dietary variety, and time, and desire to drink as a covariate, revealed a significant main effect of time ($F(1, 13) = -10.433$, p = 0.007), with hunger decreasing after the snack. Overall, mean hunger ratings (M $\pm$ SD) before each snack was 1.3 $\pm$ 0.3 and after each snack was 2.7 $\pm$ 0.5. These ratings indicate that children were hungrier before the snacks were presented than after

consuming the snack. See Appendix A Table 6 for mean ratings hunger before and after snack sessions among conditions.

Grams and Energy of Fruit Consumed

For grams of fruit consumed, a mixed-factor ANCOVA, with the between-subject factor of order, within-subject factors of courses and dietary variety, and desire to drink as a covariate, found a significant interaction of course and classroom, ($F(1, 13) = 6.2$, $p = 0.027$), with pairwise comparisons showing that classroom 1 ate significantly less grams of fruit than classroom 2 when snacks were served in one-course conditions (41.9 $\pm$ 30.4 grams versus 81.7 $\pm$ 11.5 grams, respectively [M $\pm$ SD], $p = 0.026$), and that classroom 1 consumed significantly less fruit grams in one-course conditions compared to two-course conditions (41.9 $\pm$ 30.4 grams versus 65.3 $\pm$ 33.3 grams, respectively [M $\pm$ SD], $p = 0.003$). The effect of course was not significant and a small to medium effect size ($d = 0.39$) was found with 64.3 $\pm$ 29.3 grams versus 75.0 $\pm$ 24.9 grams (M $\pm$ SD) consumed, in the one- and two-course conditions, respectively. Additionally, the effect of variety was not significant and a small effect size ($d = 0.10$), was found with 71.0 $\pm$ 30.1 grams versus 68.3 $\pm$ 23.6 grams (M $\pm$ SD) consumed in the variety and non-variety conditions respectively. Effects were in hypothesized directions.

For fruit calories, a mixed-factor ANCOVA, with the between-subject factor of order, within-subject factors of courses and dietary variety, and desire to drink as a covariate, was conducted and no significant main effects or interactions ($p > 0.05$) were found. Overall mean caloric consumption of fruit calories among sessions was as follows (M $\pm$ SD): two-course, variety = 35.0 $\pm$ 13.7 kcals; one-course, variety = 31.4 $\pm$ 15.3 kcals; one-course, no variety = 32.5 $\pm$ 14.5 kcals; and two-course, no variety = 33.9 $\pm$ 11.5 kcals. The effect of course was not significant and a small effect size ($d = 0.19$) was found with 31.9 $\pm$ 14.5 kcal versus 34.4 $\pm$ 11.4

kcal (M ± SD) from fruit consumed, in the one-and two-course conditions, respectively. Effect size was in hypothesized direction. Additionally, the effect of variety was not significant and a negligible effect size (d = 0.01) was found with 33.1 ± 14.1 kcal versus 33.2 ± 11.3 kcal (M ± SD) of fruit consumed in variety and non-variety conditions, respectively.

Grams and Energy of Total Snack Consumed

For both gram and energy intake for the total snack, a mixed-factor ANCOVA, with the between-subject factor of order, within-subject factors of courses and dietary variety, and desire to drink as a covariate, was conducted and there were no significant main effects or interactions ($p > 0.05$) found. Overall mean gram consumption of total snack among sessions was as follows (M ± SD): two-course, variety = 115.4 ± 41.3 grams; one-course, variety = 100.1 ± 43.4 grams; one-course, no variety = 104.0 ± 39.2 grams; and two-course, no variety = 103.8 ± 31.2 grams. The effect of course was not significant and a small effect size (d = 0.21) was found with 102.0 ± 38.9 grams versus 109.6 ± 31.9 grams (M ± SD) of total snack consumed in one- and two-course conditions, respectively. Additionally, the effect of variety was not significant and a small effect size (d = 0.11) was found with 107.8 ± 39.7 grams versus 103.9 ± 32.1 grams (M ± SD) of total snack consumed in variety and no-variety conditions, respectively.

Overall mean caloric consumption of total snack among sessions was as follows (M ± SD): two-course, variety = 185.3 ± 146.7 kcals; one-course, variety = 171.8 ± 127.5 kcals; one-course, no variety = 168.7 ± 107.3 kcals; and two-course, no variety = 156.0 ± 99.9 kcals. The effect of course was not significant and a negligible effect (d = 0.01) was found, with 170.3 ± 108.5 kcal versus 170.7 ± 113.7 kcal (M ± SD) from the total snack consumed in one- and two-course conditions, respectively. Additionally, the effect of variety was not significant and a medium to small effect size (d = 0.14) was found with 178.6 ± 130.4 kcal versus 162.4 ± 101.0

kcal (M ± SD) from the total snack consumed in variety and no-variety conditions, respectively. These effect sizes were not in the hypothesized direction.

DISCUSSION

The purpose of this investigation was to examine the effect of increased fruit variety and course sequence on fruit intake during a snack on fruit intake and overall snack energy intake in preschool-aged children. It was hypothesized that preschool-aged children would have greater intake of fruit in the two-course conditions than one-course conditions. It was also hypothesized that preschool-aged children would have greater intake of fruit in conditions with fruit variety than the no-variety conditions. Finally, it was hypothesized that preschool-aged children would have greater intake of fruit in conditions that combined two courses with variety (two-course, variety condition) as compared to all other conditions. However, contrary to what was hypothesized, this study did not find any significant main effects or interaction of course sequence or variety on fruit intake.

In contrast, to what was found in this investigation, previous research has found that increased variety has consistently produced greater intake of food, whether served simultaneously or over courses, in many types of foods [38, 40, 44, 45]. Additionally, previous research has demonstrated that when serving a meal over courses, consumption is usually greatest in the first course and declines with subsequent courses [36, 38]. While other research has found effects of variety and course sequence on F&V consumption [36, 44, 45], this investigation did not find any main effect or interactions of fruit intake. An additional hypothesis in the investigation was that as the environmental variables increased fruit intake, this increase in fruit intake would lead to a decrease in total energy intake during the snack. The reduction in overall snack energy intake would occur because the increased consumption of fruit, a low-

energy-dense food, would lead to a decrease in consumption of cheese, a high energy-dense food. The reduction in consumption in the high-energy-dense food would then decrease overall snack energy intake. Previous research has shown that increasing intake of low-energy-dense foods, like F&Vs, reduces total energy intake within a meal [36, 46]. Contrary to what was hypothesized, this study did not find a reduction in overall snack energy intake in the variety, two-course, or combined conditions. Potentially the reason why total energy intake did not decrease in conditions with greater variety and two-courses is that fruit intake did not significantly increase in these conditions. Without a significant increase in fruit consumption, consumption of the more energy-dense food in the snack, the cheese, could not occur. Previous research using a low-energy-dense first course found significant decreases in overall energy intake when caloric intake from low-energy-dense foods in the first course exceeded 40 kcals, compared to control conditions with no first course [36, 46]. In this study, the greatest energy intake from fruit was 34.4 $\pm$ 11.4 (M $\pm$ SD) kcals in two-course conditions, which is lower than 40 kcals. In order to see an overall decrease of energy intake during an eating bout utilizing low-energy-dense foods, calories consumed from the low-energy-dense food may need to be larger than what occurred in the current investigation.

One difference in the present study and previous research examining variety and course sequence [43, 44, 46], is that this investigation examined the effect of these variables during a snack rather than a meal. Thus, it is possible that this study did not find main effects of variety and course sequence because the eating bout was smaller and included less food (200g). In an eating bout that is smaller, these variables may produce a smaller effect. Potentially, other reasons for the lack of seeing an effect of variety on intake was that the fruit included in the study had limited sensory distinctiveness (i.e., the two fruits used in the study were of similar

color and texture). While research has shown that greater variety in low-energy-dense foods increases consumption of these foods [43-45] these studies were in adults, thus it is possible the relationship between variety and consumption in low-energy-dense foods is not the same with children.

There were several limitations and strengths to this study. The primary limitation of this study was its sample size. Other than courses and fruit grams consumed (d = 0.39), all other effect sizes among conditions were small to negligible, requiring a sample size ranging from 159 to over 1,000 participants to find significance. In addition, the sample was homogenous, with participants being mostly white and from higher socio-economic status families. Strengths of the study include having participants consume the same lunch prior to each snack sessions; using foods in the study that were familiar to the children; controlling for total amount of food provided in the snack; using objective measures of intake; and serving the snack in a natural setting, which increases generalizability.

In conclusion, this study found no effect of variety and course sequence on fruit intake in preschool-aged children during an afternoon snack. Due to a lack of changes in fruit intake, no overall effects on total snack energy intake were seen. To better understand the effect of variety and course sequence on fruit and overall intake, future studies should examine larger eating bouts in children, such as a meal, and include fruits with greater sensory distinctiveness.

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APPENDICIES

APPENDIX A

TABLES AND FIGURES

Table 1. Visual of Study Design

		Variety	
		No-variety	Variety
Courses	One	1) 100 g Applesauce 100 g Cheese Cubes	1) 50 g Applesauce 50 g Peaches 100 g Cheese Cubes
	Two	1) 100 g Applesauce 2) 100 g Cheese Cubes	1) 50 g Applesauce 50 g Peaches 2) 100 g Cheese Cubes

Table 2. Study Timeline

	Session						
	Week 0	Wk 1 (practice)	Wk 2 (practice)	Wk 3	Wk 4	Wk 5	Wk 6
Order 1 Class 1	Anthropometric measures and questionnaires	One-Course, Variety	Two-Course, No-Variety	One-Course, No-Variety	Two-Course, No-Variety	One-Course, Variety	Two-Course, Variety
Order 2 Class 2	Anthropometric measures and questionnaires	Two-Course, No-Variety	One-Course, Variety	Two-Course, Variety	One-Course, Variety	Two-Course, No-Variety	One-Course, No-Variety

Table 3. Characteristics of Child Participants at Baseline

	Classroom 1 n= 7	Classroom 2 n = 9
Age (y), M ± SD	4.0 ± 0.6	4.1 ± 0.7
Height (in), M ± SD	39.7 ± 3.9	42.0 ± 2.5
Weight (lbs), M ± SD	37.8 ± 4.1	38.8 ± 4.5
zBMI, M ± SD	0.8 ± 1.7	0.3 ± 1.1
BMI Percentile, M ± SD	57.2 ± 25.1	63.5 ± 29.6
Overweight (%)	14.2	33.3
Sex (%)		
Male	57.1	33.3
Female	42.9	66.7
Race (%)		
White	83.3	66.7
Black	16.7	0
Asian	0	11.1
Other	0	22.2
Hispanic or Latino (%)	14.3	0.0

M ± SD = Mean ± standard deviation; zBMI = body mass index z-score; BMI = body mass index; % = percentage.

Table 4. Characteristics of Parent Participants at Baseline

	Classroom 1 **n = 7**	**Classroom 2** **n = 9**
Age (y), M ± SD	37.3 ± 4.1	38.7 ± 4.5
Sex (%)		
Male	14.3	33.3
Female	85.7	66.7
Race (%)		
White	100.0	77.8
Asian	0.0	11.1
Other	0.0	11.1
Hispanic or Latino (%)	20.0	0.0
Relationship to Child (%)		
Father	14.3	22.2
Mother	85.7	77.8
Education (%)		
Some College (Less than 4 years)	0.0	22.2
College/University degree	28.6	0.0
Graduate or Professional Education	71.4	77.8
Marriage Status (%)		
Married	85.7	66.7
Divorced	0.0	22.2
Not Married (Living with Significant other)	14.3	11.1

M ± SD = Mean ± standard deviation; % = percentage.

Table 5. Mean Score Responses from Parental Questionnaires (M ± SD)

Questionnaire	**Classroom 1** (n = 7)	**Classroom 2** (n = 9)
Child Feeding Questionnaire factors		
Perceived responsibility[1]	4.5 ± 0.5	4.7 ± 0.5
Perceived parent weight[2]	3.2 + 0.5	3.0 ± 0.2
Perceived child weight[2]	3.0 ± 0.0	3.0 ± 0.0
Concern about child weight[3]	1.3 ± 0.5	1.3 ± 0.5
Restriction[4]	3.2 ± 0.5	2.8 ± 0.6
Pressure to eat[4]	3.4 ± 0.9	2.4 ± 0.9
Monitoring[1]	3.2± 0.9	2.4 ± 0.5
Three Factor Eating Questionnaire factors		
Dietary restraint[6]	22.9 ± 2.7	23.6 ± 3.4
Disinhibition[7]	21.3 ± 2.4	21.1 ± 1.9
Perceived hunger[8]	18.0 ± 2.8	20.0 ± 1.7
Child Eating & Behavior Questionnaire factors		
Food responsiveness[8]	11.7 ± 3.5	11.2 ± 3.0
Enjoyment of food[8]	12.6 ± 4.2	14.3 ± 1.9
Emotional overeating[8]	7.0 ± 3.9	7.0 ± 3.3
Desire to drink[8]	***9.1 + 3.2 [a]***	***6.8 ± 1.1 [a]***
Satiety responsiveness[8]	***14.1 ± 2.5[a]***	***16.4 ± 2.8[a]***
Slowness in eating[8]	13.6 ± 2.8	11.9 ± 2.1
Emotional undereating[8]	11.0 ± 2.2	9.6 ± 3.0
Fussiness[8]	18.3 ± 5.9	17.6 ± 4.1

M ± SD = Mean ± standard deviation; [a] Denotes significant difference ($p<0.05$) between classrooms.
CFQ Factors: [1]Percieved responsibility and monitoring scored on a 5-point scale: 1= never; 2 = seldom; 3 = half of the time; 4 = most of the time; 5 = always. [2]Percieved parent weight and perceived child weight scored on a 5-point scale: 1 = markedly underweight; 2 = underweight, 3 = normal; 4 = overweight; 5 = markedly overweight. [3]Concern about child weight scored on a 5-point scale: 1 = unconcerned; 2 = a little concerned, 3 = concerned; 4 = fairly concerned; 5 = very concerned. [4]Rescriction and pressure to eat scored on a 5 point scale: 1 = disagree; 2 = slightly disagree; 3 = neutral; 4 = slightly agree; 5 = agree.
TFEQ Factors: [5] Dietary restraint (factor I) is scored over a range of 0 to 21 with lower values representing lower dietary restraint. [6] Disinhibition (factor II) is scored over a range of 0 to 16 with lower values representing lower disinhibition. [7] Perceived hunger (factor III) is scored over a range of 0 to 14 with lower values representing lower perceived hunger.
CEBQ Factors: [8] Food responsiveness, enjoyment of food, emotional overeating, desire to drink, satiety responsiveness, slowness in eating, emotional undereating, and fussiness scored on a 5-point scale: 0 = never; 1 = seldom; 2 = sometimes; 3 = often; 4 = always.

Table 6. Liking of Food and Hunger Ratings (M ± SD)

	One-Course, Variety	Two-Course, Variety	One-Course, No-Variety	Two-Course, No-Variety
Liking of Applesauce	**1.3 ± 0.6**	**1.3 ± 0.6**	**1.1 ± .05**	**1.1 ± 0.5**
Classroom 1 (n = 7)	1.4 ± 0.8	1.1± 0.4	1.3 ± 0.8	1.3 ± 0.8
Classroom 2 (n = 9)	1.1 ± 0.3	1.3 ± 0.7	1.0 ± 0.0	1.0 ± 0.0
Liking of Peaches	**1.2 ± 0.5**	**1.5 ± 0.9**	--	--
Classroom 1 (n = 7)	1.0 ± 0.0	1.6 ± 1.0		
Classroom 2 (n = 9)	1.2 ± 0.5	1.5 ± 0.9		
Liking of Cheese	**1.2 ± 0.5**	**1.5 ± .09**	**1.3 ± 0.7**	**1.4 ± 0.8**
Classroom 1 (n = 7)	1.4 ± 0.8	1.6 ±1.0	1.3 ± 0.8	1.6 ± 1.0
Classroom 2 (n = 9)	1.0 ± 0.0	1.4 ± 0.9	1.3 ± 0.7	1.3 ± 0.7
Hunger Before Snack	**1.2 ± 0.5**	**1.4 ± 0.8**	**1.4 ± 0.7**	**1.1 ± 0.3**
Classroom 1 (n = 7)	1.4 ± 0.8	1.4 ± 0.8	1.3 ± 0.5	1.1 ± 0.4
Classroom 2 (n = 9)	1.0 ± 0.0	1.4 ± 0.9	1.4 ± 0.9	1.0 ± 0.0
Hunger After Snack	**2.7 ± 0.6**	**2.7 ± 0.7**	**2.6 ± 0.7**	**2.8 ± 0.5**
Classroom 1 (n = 7)	3.0 ± 0.0	3.0 ± 0.0	2.9 ± 0.4	3.0 ± 0.0
Classroom 2 (n = 9)	2.4 ± 0.7	2.4 ± 0.9	2.4 ± 0.9	2.7 ± 0.7

M ± SD = Mean ± standard deviation. Scores of liking of foods: 1= yummy, 2= so-so, and 3= yucky. Scores for hunger ratings: 1= hungry, 2= kind of hungry, and 3= not hungry.

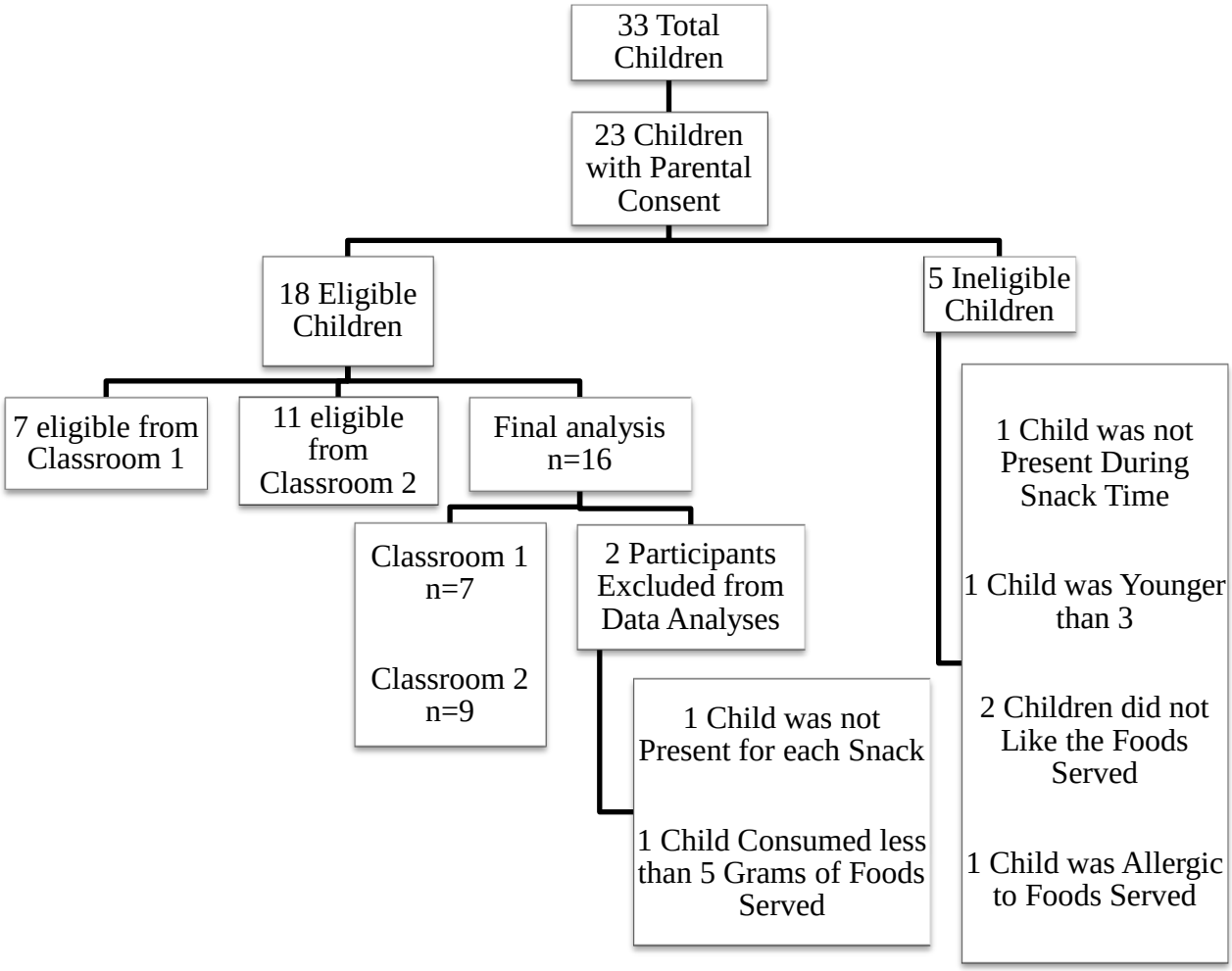

Figure 1**.** Participant Flow Chart.

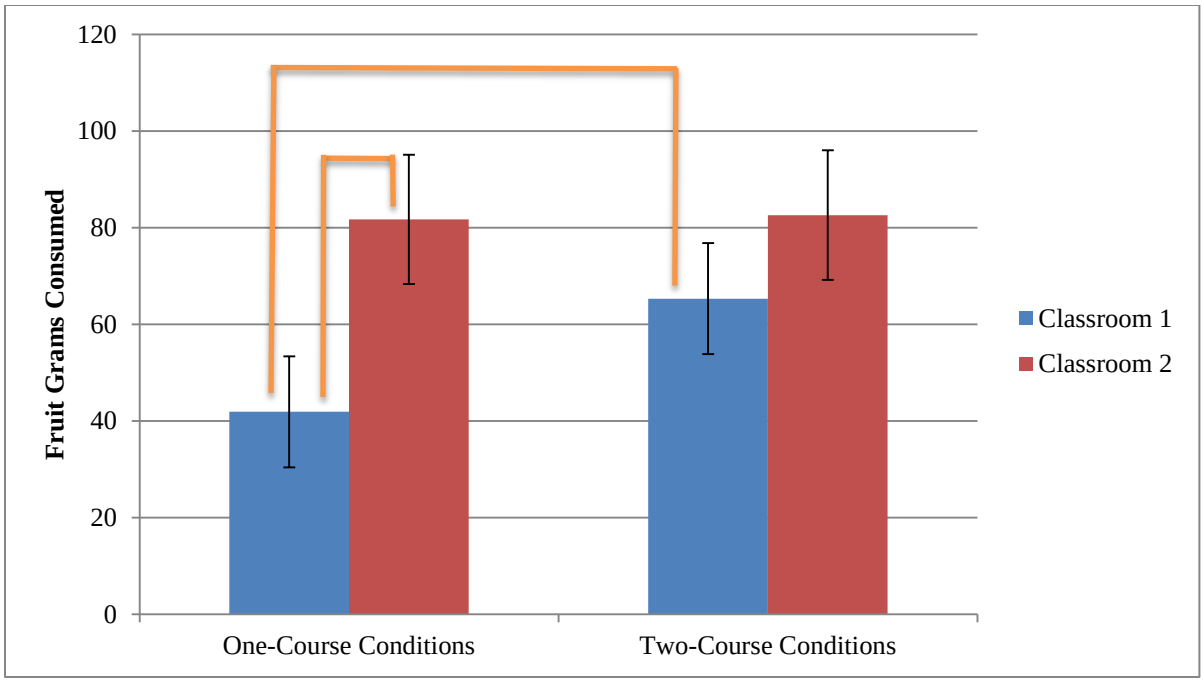

Figure 2. Fruit Grams Consumed by Classroom in One-Course and Two-Course Conditions.

A significant interaction of course and classroom was found ($p = 0.027$), such that classroom 1 consumed less grams of fruit than classroom 2 when snacks were served in one-course conditions, and that classroom 1 consumed significantly less fruit grams in one-course conditions compared to two-course conditions. Data are mean $\pm$ standard deviation. Bars with connecting orange lines are statistically different ($p < 0.05$).

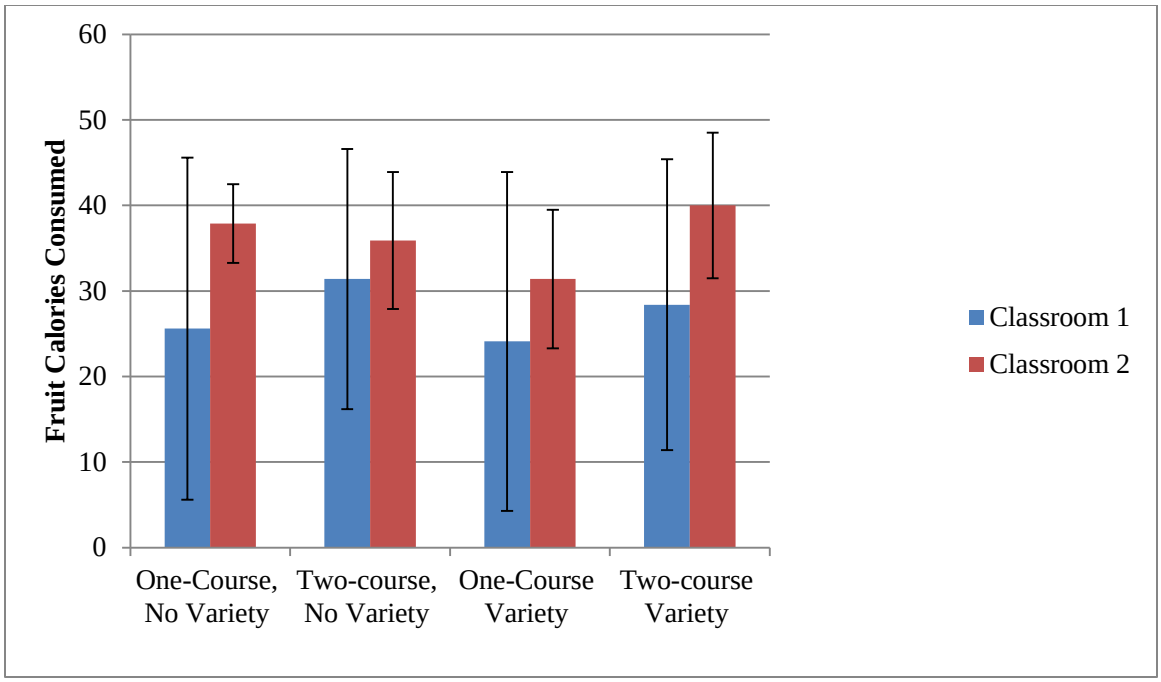

Figure 3. Fruit Calories Consumed by Classroom Across Conditions.

There were no significant main effects or interactions ($p > 0.05$) found among conditions for total fruit calories consumed. Data are mean $\pm$ standard deviation.

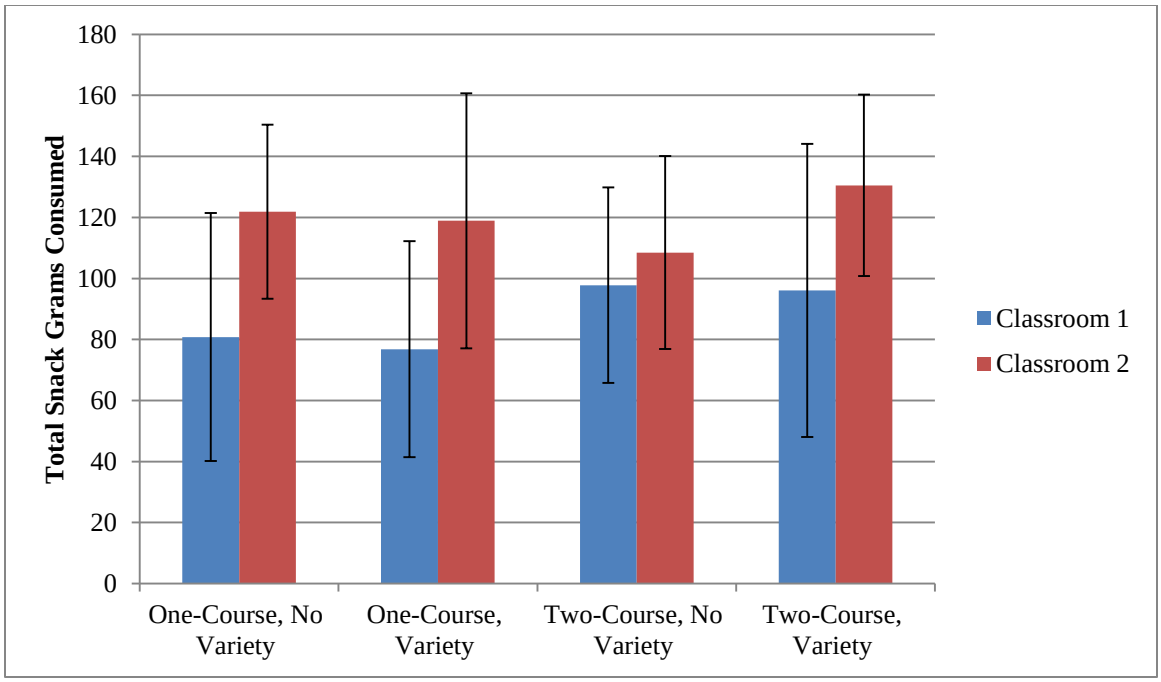

Figure 4. Total Snack Grams Consumed by Classroom Among Conditions.

There were no significant main effects or interactions ($p>0.05$) found among conditions for total snack grams consumed. Data are mean $\pm$ standard deviation.

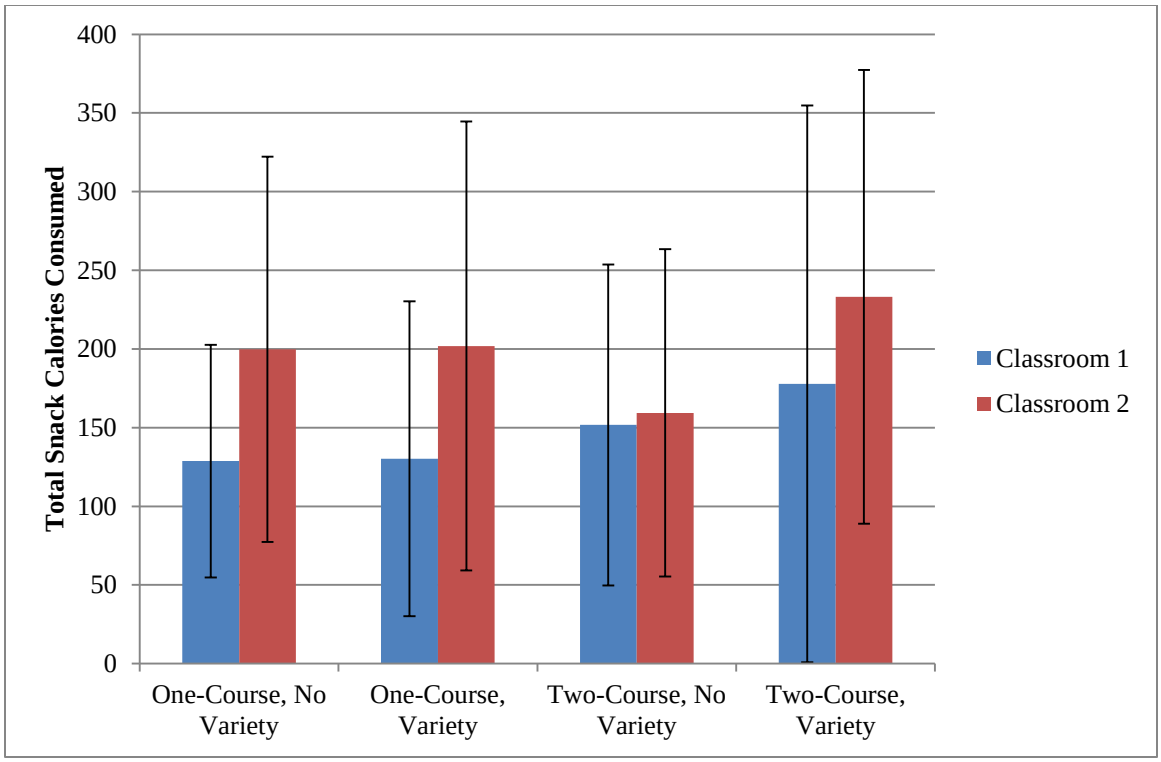

Figure 5. Total Snack Calories Consumed by Classroom Among Conditions.

There were no significant main effects or interactions ($p>0.05$) found among conditions for total snack calories consumed. Data are mean $\pm$ standard deviation.

APPENDIX B

RECRUITMENT, FORMS, AND QUESTIONNAIRES

FORM B APPLICATION

All applicants are encouraged to read the Form B guidelines. If you have any questions as you develop your Form B, contact your Departmental Review Committee (DRC) or Research Compliance Services at the Office of Research.

FORM B

IRB # _____

Date Received in OR _____

THE UNIVERSITY OF TENNESSEE
Application for Review of Research Involving Human Subjects

I. IDENTIFICATION OF PROJECT

1. **Principal Investigator:**
   Hollie Raynor, PhD, RD, LDN (Principal Investigator)
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   Department:

Nutrition

2. **Project Classification:**
   Thesis
3. **Title of Project:**
   The Influence of Dietary Variety and Course Sequence on Fruit Intake in Preschool-aged Children

4. **Starting Date:**
   Upon IRB approval or August 2011

5. **Estimated Completion Date:**
   July 2012

6. **External Funding** *(if any)**:***

   - **Grant/Contract Submission Deadline:**
   - **Funding Agency:**
   - **Sponsor ID Number** ***(if known)*****:**
   - **UT Proposal Number** ***(if known):***

II. PROJECT OBJECTIVES

Background and Significance

In the past two decades overweight and obesity rates in children (ages 2-19) have risen from 5% to 17%, with toddlers (ages 2-5) at 10% [56]. Among children and adolescents the consumption of low-energy-dense foods, such as fruit and vegetables (F&Vs), remain below current recommendations [57]. On the other hand, the consumption of high-energy-dense foods and intake of added sugars has risen [57]. This suggests that there may be a relationship between F&Vs and high-energy-dense foods that is complementary to each other. Dietary preferences are established early in life and can continue into adulthood [7]. Therefore, strategies to increase low-energy-dense F&V intake and decrease high-energy-dense food intake aimed at young children are essential. Thus, not only can these strategies aid with developing healthy eating patterns, but can help with preventing the development of obesity by preventing excess calorie consumption.

Behavior theory can provide a framework to understand eating behavior related to the environment [33]. Antecedents, or cues, can trigger eating. Therefore, manipulating food presentation can be utilized to produce certain behaviors. Dietary variety and course sequence are two examples of this relationship. It has been well established that high dietary variety leads to greater consumption patterns compared to low DV diets in adults [38, 40, 42, 58]. High DV has only been tested with problematic foods, and not with the goal of increasing F&V intake. Additionally, serving a first course meal can act as a preload to decrease intake of the second course entrée [59]. Dietary variety and course sequence manipulations have been experimentally

tested with caloric intake goals but never with the goal of increasing F&V intake**.** Therefore, the purpose of this study is to determine the extent to which manipulation of dietary variety and course sequence affects fruit intake and overall energy intake in preschool-aged children.

These variables will be examined within a snack that potentially contains three foods, two low-energy-dense fruits (peaches in light syrup and applesauce), and one high-energy-dense food (cheese cubes). Preschool-aged children will be given snacks that vary in existence of fruit variety (no-variety or variety) and number of courses (one or two), with fruit served as the first course in the two course conditions. Amount of food served in the snack will remain consistent across conditions. See Table 1 for a description of each condition. It is hypothesized that:

4. Two-course conditions will have greater intake of fruit and overall lower energy intake than the one-course conditions.
5. Variety conditions will have greater intake of fruit and overall lower energy intake than the no-variety conditions.
6. The two-course, variety condition will have greater intake of fruit and overall lower energy intake as compared to all other conditions.

Table 1. Description of Conditions

		Variety	
		No-variety	**Variety**
Courses	**One**	2) 100 g Applesauce 100 g Cheese Cubes	2) 50 g Applesauce 50 g Peaches 100 g Cheese Cubes
	Two	3) 100 g Applesauce 4) 100 g Cheese Cubes	3) 50 g Applesauce 50 g Peaches 4) 100 g Cheese Cubes

III. DESCRIPTION AND SOURCE OF RESEARCH PARTICIPANTS

Recruitment

Participants will be recruited from the University of Tennessee's Early Learning Center (ELC) program at the White Avenue location. For this study, participants will be preschool-aged children who attend the full week and full day program. Upon IRB approval, in August 2011, standard procedures of recruitment will occur at the ELC. These procedures include sending a letter home to the parent/guardian that explains the study. Following the introductory letter, all parent/guardians will receive a folder of questionnaires and consent forms. All questionnaires and documents will be placed into each child's cubby by Chelsi Cardoso, the Co-Principal Investigator. These questionnaires and consent forms will provide information on demographics, eligibility, eating behavior of the child and parenting styles of the primary caregiver. Parent/guardians who are willing to let their child participate will be asked to sign and turn in a consent form to the teacher of their class. When this study takes place, there will be an estimated 34 students that will be enrolled in August 2011.

Eligibility

To be eligible, all children must be ≥3 years of age, enrolled at the ELC, and have guardian consent to participate. Children must like the foods being served, be able to use a spoon and not have any allergies to the foods being served. Additionally, eligible children cannot be lactose intolerant. Children who do not meet these eligibility requirements will not be included.

Parent/guardians will be required to fill out an eligibility questionnaire which will assess these criteria.

Randomization

Children will be randomized by classroom. There are two preschool classrooms; therefore one class will be assigned to order 1 and the other to order 2 [see Table 2]. Data only from eligible children will be collected and used.

IV. METHODS AND PROCEDURES

Study Design

This study will be a randomized trial that will investigate the influence of dietary variety and course sequence on fruit intake in preschool aged children. It will be a 2 x 2 x 2 design with the between-subjects factor of order (order 1 vs. order 2) [see Table 2] and the within-subjects factors being dietary variety (no-variety vs. variety) and courses (one-course vs. two-course). Starting in August 2011, the snack sessions for participants at the ELC will be served on Wednesday afternoons every week. Two preschool classrooms will be randomized to order 1 or order 2 (each child in each classroom will receive the same food). Each classroom contains around 17 preschoolers, aged 3-to-5 years. The dependent variables will be the amount, in grams and energy, of peaches, applesauce, and cheese cubes consumed.

Table 2. Study Design Visual

	Session						
	Week 0	Wk 1 (practice)	Wk 2 (practice)	Wk 3	Wk 4	Wk 5	Wk 6
Order 1 N≈17	Anthropometric measures and questionnaires	**One-Course, Variety**	**Two-Course, No-Variety**	**One-Course, No-Variety**	**Two-Course, No-Variety**	**One-Course, Variety**	**Two-Course, Variety**
Order 2 N≈17	Anthropometric measures and questionnaires	**Two-Course, No-Variety**	**One-Course, Variety**	**Two-Course, Variety**	**One-Course, Variety**	**Two-Course, No-Variety**	**One-Course, No-Variety**

Foods

Peaches in light-syrup, applesauce, and cheese cubes will be used for these snacks. In the conditions, the children will receive a total of total of 200 grams of food. In the no-variety conditions, children will receive 100 grams of applesauce, and 100 grams of cheese cubes. In the variety conditions children will receive 50 grams being of peaches and applesauce, respectively, and 100 grams of cheese cubes.

Mott's applesauce (no sugar added, natural), Dole peaches (yellow cling sliced in light syrup) and Kraft cheese cubes (natural, 2% milk reduced-fat, mild cheddar) are the specific brands that will be served to the children. The applesauce contains 42 kilocalories per 100 grams giving it an energy density of 0.42; the peaches contain 54 kilocalories per 100 grams giving an energy density of 0.54; and the cheese cubes are 297 kilocalories per 100 grams giving an energy density of 2.97. These foods are easy snacks to consume and are typically foods preschoolers will consume. Additionally, all of the foods are familiar to the children at the ELC and have been served before as snacks at the ELC.

Procedures

Parent/guardians with children in preschool at the ELC on White Avenue will be asked to participate. As previously mentioned, the parent/guardians will receive a letter from the investigator explaining the research study and this will be followed by a packet with questionnaires. Parent/guardians who are willing to allow their children to participate will fill out the questionnaires and turn them into their child's teacher. All forms turned in will be collected by the investigator and locked in filing cabinets located at the Healthy Eating and Activity Laboratory (HEAL), in room 102 at the Jessie Harris Building (JHB). For parent/guardians who do not want their child to participate they will have an option to choose whether their child will be provided with the snack or not.

All snacks prepared, data collected, and contact with ELC children for this study will done by Ms. Cardoso, the Co-Principal Investigator, and HEAL staff. These HEAL members have undergone extensive research training under Dr. Hollie Raynor. Additionally, HEAL staff will only be responsible for helping with weighing food before and after the snack, as well as distributing and collecting the food from the children.

Week prior to start of snack sessions.

Children that have parent/guardian consent and meet eligibility criteria will have anthropometric measures taken. Trained students from HEAL will measure height and weight of participants. Additionally, eligible children will be given personal identification numbers to protect their identity throughout the study. These identification numbers will be used to mark each child's bowl to ensure the correct data are with the correct child. At each snack session, only researchers will have a document containing each child's name with their corresponding personal identification number. This will allow the researcher to hand out the correct bowls and keep participation confidential.

Snack Session Procedures.

For each snack session, snacks will be served after lunch at approximately 3:30 in the afternoon. The lunches served on each day in which a measurement is taken regarding snack intake will be the same for each week. Prior to receiving the snack, each participant at the ELC will fill out questionnaires to rate their hunger and liking of the snack foods used in the investigation. Following these questionnaires, the children will be given the snack. HEAL workers will carefully distribute the snack to children with containers that include their identification numbers. Teachers and participants will be instructed to avoid sharing snack with other children and to eat as much or little of the snack as they wish. Water will be served to drink during the snack session. Each snack session will be 20 minutes. For the two-course condition, each course will be provided for consumption for 10 minutes (i.e., 10 minutes for the first course and 10 minutes for the second course), while the one-course condition will be served for the whole 20 minutes. Children will be told the day of the snack whether they will receive either one-course or two-courses. After snack time is complete, all food containers will be collected and participants will be asked to fill out the hunger rating questionnaire again.

For seven successive weeks, the procedures described above will be used for each snack session. For the first two weeks, two practice rounds, one consisting of a one-course snack with variety and the other of a two-course snack without variety, will be conducted to get the children accustomed to this process. These practice sessions will be in Weeks 1 and 2, and data will not be used from these sessions. Starting at Week 3, the measured snack sessions will occur and follow the order described in Table 2.

Measures

Anthropometrics.

For height and weight measures, children will be asked to remove their shoes and excess clothing beforehand. Height will be measured twice and to the nearest tenth of an inch with a portable stadiometer (SECA, ITIN Scale Company, Brooklyn, NY). Weight will be measured to a tenth of a pound using a calibrated portable digital scale (Healthometer Professional, Sunbeam Product Inc. Raton, FL). Body mass index [BMI=weight(kg)/height(in^2)] will be calculated for each child and BMI scores will be compared to the CDC's BMI percentile charts [60, 61]. Children with a BMI of greater than the 85th percentile and less than the 95th percentile for age and gender will be classified as overweight, with those greater than the 95th classified as obese [62]. Additionally, children's z-BMI scores will be calculated. This will be done by standardizing the BMI for children's age and gender in relation to the population mean and standard deviation [60, 61].

Consumption.

Snacks will be prepared at JHB then carried to the ELC, which is located next to JHB. Once the snacks are weighed, they will be appropriately covered and stored following food safety recommendations. Each snack will be weighed before and after consumption by a calibrated food scale (Denver Instruments SI-8001, Fischer Scientific) to the nearest tenth of a gram and the container will be measured separately then with the snack included. The consumption amount will be determined by subtracting the post-snack weight from the pre-snack weight (in grams). This will allow the investigator to calculate energy intake from gram amount consumed.

Liking of Food.

The research team and ELC staff will assist the children with filling out a questionnaire to assess the children's liking of the food presented. The questionnaire has been used in previous studies and consists of a three-point Likert-scale that has faces representing "yucky" (dislike), "so-so" (neutrality), and "yummy" (like) [48-50].

Hunger.

This questionnaire, is similar to the above, in that it is a three-point Likert-scale that represents hunger. This type of tool has been used by several researchers to assess hunger in preschoolers [48-50, 63]. The scale consists of cartoons with stomachs that are shaded to show their stomach is empty (hungry), somewhat empty (somewhat hungry) or full (stuffed). The research team and ELC staff will assist the children will filling out this questionnaire.

Parent/Guardian Questionnaires.

Parent/guardians of children with consent will be asked to complete four questionnaires: a demographic questionnaire, the Three Factor Eating Questionnaire (TFEQ), the Child Feeding Questionnaire (CFQ), and the Child Eating and Behavior Questionnaire (CEBQ). First, the demographic questionnaire includes questions about parental and child demographics such as race and ethnicity, and parental education level and income level. Second, the CFQ assesses parental beliefs and practices regarding feeding their children [51]. The CFQ contains 31 items and 7 factors: perceived responsibility, perceived parent weight, perceived child weight, concern about child weight, restriction, pressure to eat, and monitoring [51]. Third, the TFEQ, or called the Eating Inventory Questionnaire, is used to measure the parent's dietary restraint, disinhibition, and perceived hunger [52]. The TFEQ contains 51 items with 3 factors: restraint of eating, disinhibition, and hunger [52]. Fourth, the CEBQ was developed by Wardle and colleagues in 2001 [53]. This questionnaire was designed to asses eating behavior and styles related to obesity risk [53]. The CEBQ contains 35 items with 8 factors: food responsiveness,

enjoyment of food, emotional overeating, desire to drink, satiety responsiveness, slowness in eating, emotional undereating, and fussiness [53].

Quality Control

Participants absent during any of the 4 days of the snack measures or at any session consume all of the food presented will not be included in the final analyses. However, regardless of inclusion in analyses, all children will be provided with the snack (unless a legal guardian indicates that their child cannot receive the snack by the Parent/Guardian Request for No Snack form). If the parent/guardian does not want the child to receive the study snack, another snack will be provided from the ELC to the child.

Data Analysis

Data analyses will be done using SPSS with an alpha-level at 0.05. Independent t-tests (continuous data) and Chi-squares (categorical data) will be used to analyze differences in demographic information between the two orders. To ensure that liking of foods and hunger rating are not significantly different between the five sessions, a mixed factorial analysis of variance (ANOVA), with the between-subject variable being order and the within-subject factors of course (one and two) and variety (no-variety and variety), will be used. For the dependent variables (grams and energy consumed) an ANOVA will be used with the between-subject factor being order and within-subject factors being courses and dietary variety. Interactions will be followed using post-hoc analyses with bonferroni corrections.

V. SPECIFIC RISKS AND PROTECTION MEASURES

Risks are minimal to participants. Children may be allergic or intolerant to the foods used in this study, but all children will be screened for food allergies. Information collected during this study will be kept confidential. Each participating child will be given a personal identification number with no reference to names, addresses, or phone numbers to ensure confidentiality. No reference will be made in any written or oral material that will link the participant to the study. All collected information will be stored in locked filing cabinets in locked rooms in HEAL, room 102 in JHB. Procedures to protect confidentiality will be approved by the University of Tennessee's Institutional Review Board to ensure they meet standards for the protection of human rights.

VI. BENEFITS

There are no anticipated benefits to the participant. Anticipated benefits for the researcher include a controlled environment to collect valuable information for the advancement of nutrition.

VII. METHODS FOR OBTAINING "INFORMED CONSENT" FROM PARTICIPANTS

Informed consent will be received upon IRB approval from parent/guardians of preschool-aged children from the ELC located on White Avenue. A letter from the investigators will go home to legal guardians with a child to explain the study. During the following week, in the same manner, parent/guardians will be given questionnaires, two consent forms and an envelope. All information and forms for the parents will be placed in the child's corresponding cubby, which is standard procedure for communication at the ELC that has been approved and

used in several studies. Additionally, since a major form of communication with the parent/guardians and the ELC is through their child's cubby, these questionnaire packets will not appear unusual. If the parent/guardian chooses to provide consent for participation they will return the consent form to their child's teacher by sealing the forms into the provided envelope. It will be the responsible of the investigators to collect consent forms daily from the ELC to store in locked filing cabinets in locked rooms in HEAL, room 102 in JHB.

After Informed Consent is collected from the parents, each child will be assented. A formal "Assent Form" will be used to collect this information. Children who do not want the snack will not be forced to consume it and will be offered an alternative snack that the ELC would provide.

VIII. QUALIFICATIONS OF THE INVESTIGATOR(S) TO CONDUCT RESEARCH

Ms. Cardoso has extensive training as a graduate research assistant in the Healthy Eating and Activity Laboratory under the direction of Dr. Hollie Rayor. She is currently a graduate student in public health nutrition and has had a strong educational background in nutrition. Dr. Raynor has conducted several basic eating studies and will provide mentorship to Ms. Cardoso over the course of the investigation.

IX. FACILITIES AND EQUIPMENT TO BE USED IN THE RESEARCH

The primary facility to be used is the kitchen located in HEAL. Food will be stored at proper temperatures in cabinets and the refrigerator depending upon the type of food. The kitchen remains locked when no HEAL staff members are present to prevent tampering with the food.

The two preschool classrooms at the ELC on White Avenue, Knoxville, TN will be the primary location where all interactions with the children will take place.

All equipment is courtesy of HEAL. The following equipment will be used in the research study:

1. Food Scale (Denver Instruments SI-8001, Fischer Scientific)
2. Portable digital scale (Healthometer Professional, Sunbeam Product Inc. Raton, FL)
3. Portable stadiometer (SECA, ITIN Scale Company, Brooklyn, NY)
4. SPSS (version 19.0)

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X. RESPONSIBILITY OF THE PRINCIPAL/CO-PRINCIPAL INVESTIGATOR(S)

By compliance with the policies established by the Institutional Review Board of The University of Tennessee the principal investigator(s) subscribe to the principles stated in "The Belmont Report" and standards of professional ethics in all research, development, and related activities involving human subjects under the auspices of The University of Tennessee. The principal investigator(s) further agree that:

1. Approval will be obtained from the Institutional Review Board prior to instituting any change in this research project.

2. Development of any unexpected risks will be immediately reported to Research Compliance Services.

3. An annual review and progress report (Form R) will be completed and submitted when requested by the Institutional Review Board.

4. Signed informed consent documents will be kept for the duration of the project and for at least three years thereafter at a location approved by the Institutional Review Board.

XI. SIGNATURES

ALL SIGNATURES MUST BE ORIGINAL. The Principal Investigator should keep the original copy of the Form B and submit a copy with original signatures for review. Type the name of each individual above the appropriate signature line. Add signature lines for all Co-Principal Investigators, collaborating and student investigators, faculty advisor(s), department head of the Principal Investigator, and the Chair of the Departmental Review Committee. The following information should be typed verbatim, with added categories where needed:

Principal Investigator: Hollie Raynor

Signature: _____ Date: _____

Co-Principal Investigator: Chelsi Cardoso

Signature: _____ Date: _____

Student Advisor (if any): Hollie Raynor

Signature: _____ Date: _____

XII. DEPARTMENT REVIEW AND APPROVAL

The application described above has been reviewed by the IRB departmental review committee and has been approved. The DRC further recommends that this application be reviewed as:

[ ] Expedited Review -- Category(s): _____

OR

[X] Full IRB Review

Chair, DRC: Michael Zemel

Signature: _____ Date: _____

Department Head: Jay Whelan

Signature: _____ Date: _____

Protocol sent to Research Compliance Services for final approval on (Date) : _____
1
Approved:
Research Compliance Services
Office of Research
1534 White Avenue

Signature: _____ Date: _____

For additional information on Form B, contact the Office of Research Compliance Officer or by phone at (865) 974-3466.

INFORMED CONSENT STATEMENT

The influence of dietary variety and course sequence on fruit intake in preschool-aged children

INTRODUCTION

You and your child have been invited to participate in a research study conducted at the University of Tennessee. For my study, I want to explore how the surroundings of preschoolers may influence how they eat fruit during an afternoon snack. I want to learn about this because it is important to see how a child's environment or surroundings may impact their eating behavior.

This form explains the study. Please read this form and contact the researcher (Chelsi Cardoso (865)-974-0754) with any questions. If you decide to allow your child to participate in the study, please initial each page of the form with your full signature on the last page.

Chelsi Cardoso, a graduate nutrition student at the University of Tennessee, advised by Dr. Hollie Raynor, an Associate Professor in the Department of Nutrition at the University of Tennessee, is doing a study to learn impact of two environmental factors, dietary variety and course sequence, on fruit intake. Dietary variety means having foods that are different in color, shape or size in a meal. Course sequence means having a meal with several courses, like a first course and second course. It is estimated 34 preschool students from the Early Learning Center on White Avenue will participate in the study.

Your child has been asked to participate in the study because they attend preschool at the Early Learning Center on White Avenue.

INFORMATION ABOUT PARTICIPANTS' INVOLVEMENT IN THE STUDY

If you provide consent for you and your child to participate in the study, you will be asked to complete questionnaires on demographics, child feeding style, and your eating behaviors. It will take you about 30 minutes to complete the questionnaires. After you complete these forms, please return a signed and initialed consent form and questionnaires sealed in the envelope that has been provided to you in your child's cubby, to your child's teacher at the Early Learning Center.

After the questionnaires have been completed and returned to the Early Learning Center your child's height and weight will be measured by a trained research team from the Healthy Eating and Activity Laboratory (HEAL), which is directed by Dr. Raynor, at the University of Tennessee. These measurements will take place in a private area (an empty classroom) at the Early Learning Center. Each child will be measured individually by research staff members. Your child will be asked to remove his/her shoes and to stand straight to measure how tall your child is by using a stadiometer, which is a tool that measures height. Your child will also be asked to step onto a portable electronic scale to measure weight. It will take about 5 minutes per child to take measurements.

Your child will then participate in 6 sessions in which he/she will receive an afternoon snack of either approximately 100 grams of applesauce and 100 grams of cheese cubes, or 50 grams of applesauce, 50 grams of peaches with light syrup drained, and 100 grams of cheese cubes. The snack will either be provided to your child all at one time, in one course, or as a two-course snack with the fruit given in the first course and the cheese cubes given in the second course. These sessions will take place during your child's usual afternoon snack time. Sessions will be on Wednesday afternoons and be spaced approximately 1 week apart. These sessions will be take place during the months of August to October of 2011.

In these sessions, your child will be asked to rate how hungry he/she is and how much he/she likes the food. Trained research staff from HEAL will help your child with these ratings. Your child will then be given the applesauce and cheese cubes in a one-course snack or applesauce, peaches and cheese cubes to eat in two-courses with the fruit first followed by cheese cubes during the snack time. When the snack time is done, your child will rate his/her hunger again.

If your child does not want the food for this study, an alternative snack provided by the ELC will be offered to him/her without any penalty. Your child will not be forced into eating the study food and will be allowed to leave the table at any time. All children in the same classroom will receive the same food, and the classrooms will be randomly assigned to the order by which the children receive the foods.

Please contact Chelsi Cardoso at (865)-974-0754 if you have any questions about the procedures.

RISKS

There are no foreseen risks to participants. Any child with allergies to foods in the study will not be able to participate in the study.

BENEFITS

There are no anticipated benefits to the participant. Anticipated benefits for the researcher include a controlled environment to collect valuable information for the advancement of nutrition. Specifically, the information gained from this study could inform teachers and day care centers about how the environment can impact eating behaviors in preschoolers.

CONFIDENTIALITY

All information collected in the study will be kept confidential. A unique code will be used to identify each participant with no reference to individual names, addresses, or phone numbers. No reference will be made in oral or written reports which could link participants to the study. All information will be stored in locked filing cabinets in locked rooms in the Healthy Eating and Activity Laboratory, Room 102 in the Jessie Harris Building. Only the project researchers will have access to participant information. Procedures to protect confidentiality will be approved by the University of Tennessee's Institutional Review Board to ensure they meet standards for the protection of human subjects.

INCLUSION CRITERIA

All children that are at least 3 years of age, enrolled at the Early Learning Center, with parental/guardian consent can participate. Children must not have any allergies to the foods being served, be lactose-intolerant, or dislike the food. Additionally, eligible children must also agree to eat the snack served.

COMPENSATION
There is no compensation for participation in this study.

EMERGENCY MEDICAL TREATMENT

The University of Tennessee does not "automatically" reimburse subjects for medical claims or other compensation. If physical injury is suffered in the course of research, or for more information, please notify the investigator in charge, Chelsi Cardoso at (865)-974-0754.

CONTACT INFORMATION
If you have questions at any time about the study or the procedures, (or you experience adverse effects as a result of participating in this study,) you may contact the researcher, Chelsi Cardoso, at Healthy Eating and Activity Laboratory, 102 Jessie Harris Building, 1215 W Cumberland Avenue and (865) 974-0754. If you have questions about your rights as a participant, contact the Office of Research Compliance Officer, Brenda Lawson, at (865) 974-3466.

PARTICIPATION

Your participation in this study is voluntary; you may decline to participate without penalty. If you decide to participate, you may withdraw from the study at anytime without penalty and without loss of benefits to which you are otherwise entitled. If you withdraw from the study before data collection is completed you data will be returned to you or destroyed. To communicate that you would like to withdraw from participation, please contact the researcher, Chelsi Cardoso, using the contact information listed above.

CONSENT
I have read the above information. I have received a copy of this form. I agree to allow my child to participate in this study.

Legal guardian's printed name_____ Date _____

Legal guardian signature _____ Date _____

Investigator's printed name_____ Date _____

Investigator's signature _____ Date _____

Recruitment Letter

August 10, 2011

Dear Parent or Guardian,

My name is Chelsi Cardoso, a graduate student in the Nutrition Department here at the University of Tennessee, Knoxville. I am working on my thesis, with Dr. Hollie Raynor, an Associate Professor in the Department of Nutrition, and have been granted permission to work with the preschool children at the Early Learning Center. I am very excited to have this opportunity to work with your child.

Your child is being invited to participate in a research study. The study is looking at the influence of two environmental cues, dietary variety and course sequence, on fruit intake during a snack. The snack will consist of applesauce and cheese cubes or applesauce, peaches in light syrup, and cheese cubes, either served in a one-course or two-course snack. Weight and height of children with legal guardian consent will be collected during the week of September 5, 2011 in a private area in the classroom. This snack intervention will take place during afternoon snack on the following **Wednesdays**:

September	**October**
September 14, 2011	October 5, 2011
September 21, 2011	October 12, 2011
September 28, 2011	October 19, 2011

During these days, the children will be given either applesauce and cheese cubes in one a one or two-course snack or applesauce, peaches in light syrup, and cheese cubes in a one or two-course snack during afternoon snack.

On August 17, 2011, you will receive a packet that includes further information about the study, questionnaires, and consent forms. This study is voluntary. If you would like your child to participate, please have the primary caretaker who is responsible for feeding your child fill out the questionnaires, by carefully answering each question. Additionally, please read and sign one consent form, keeping the other for your records. Return all consent forms and questionnaires by **Friday, August 26, 2011** to your child's teacher.

If you do not want your child to participate in the study, and do not want your child given the applesauce, peaches and cheese cubes for afternoon snack on the Wednesdays mentioned above, please fill out the *Parent Request for No Snack* form and return that to your child's teacher by **Friday, August 26, 2011.** I will provide a checklist to ensure that all forms and questionnaires are included before returning them to your child's teacher by **Friday, August 26, 2011.** If you have any questions, feel free to contact me by phone at (865)-974-0754 or e-mail ccardoso@utk.edu.

Sincerely,

Chelsi C. Cardoso

Snack Study

Assent Form

I. Assent is required for children (17 years of age and under) participating in a research study. Assent must be obtained in addition to parental consent.

Examiner: Hello, my name is Chelsi (or research assistant's name). Your mom and your teacher say that you are willing to help me. The first thing you have to do is let me take your weight and height. Then, I will ask you to have a snack on some days that has applesauce, peaches, and cheese. Are you willing to help with this? (Child's response). Great! I think you will find that it will be a fun snack! If you decide that you don't want to do this anymore, all you have to do is tell me. You can just say, "I don't want to do this anymore." Okay? (Child's response).

I really appreciate your help! All of these things will happen here at school. Are you ready? Let's begin.

II. The investigator will use the following procedures during the course of test administration:

- Maintain a pleasant facial expression.
- Give general reinforcement by means of these example comments:

"You're really working hard on that form."

"Good work!"

"I can see that you are standing really tall for me to measure!"

"You are really listening well!" (Child's first name)

"You did turn your eyes and ears on, didn't you?"

"Thank you for listening!"

III. The investigator will use the following procedures at the end of test administration:

- If the child wishes to stop during the weight/height measures, the investigator will maintain a neutral expression, stop measuring, and say, "All right, thank you for helping me again. Let's go back to your teacher."
- When the height/weight measures are complete, the investigator will say, "Thank you for helping me again. You have really worked hard today. Let's go back to the classroom."

- If the child does not want applesauce, peaches, or cheese cubes, the examiner will maintain a neutral expression, and say “would you like something different?” If the child does want a different snack, the investigator will give a snack alternative. If the child does not want any snack, the investigator will say “that is just fine, you may leave the table.”

- When the snack is complete, the investigator will say, “Thank you for helping me again!”

Research Team Member's/Teacher's Pledge of Confidentiality

As a member of or teacher of children participating in this research project, I understand that I will be viewing observations and forms containing confidential information. The information from observations and on the forms has been revealed by research participants who participated in this project on good faith that their observations and personal information would remain strictly confidential. I understand that I have a responsibility to honor this confidentiality agreement. I hereby agree not to share any information on these forms with anyone except other members of this research team and teachers who have also signed a pledge of confidentiality. Any violation of this agreement would constitute a serious breach of ethical standards, and I pledge not to do so.

_____ _____

Research Team Member Date

Office Use Only
Reference #:
Assessment #:

DATE ☐☐/☐☐/☐☐

M M D D Y Y

Eligibility Questionnaire

If you have provided consent for your daughter/son to participate in the research study please answer the following questions to ensure you child is eligible for the study.

1. Please list all allergies your child currently has, if any.

_____

_____

_____

_____

_____

2. My child is lactose-intolerant. ☐ Yes ☐ No

3. My child likes cheese cubes. ☐ Yes ☐ No

4. My child likes applesauce. ☐ Yes ☐ No

5. My child likes peaches. ☐ Yes ☐ No

_____ _____

Parent or Legal Guardian Signature Date

_____ _____

Researcher Signature Date

Office Use Only
Reference #:
Assessment #:

DATE ☐☐/☐☐/☐☐

M M D D Y Y

Parent/Guardian Request for No Snack

If you have **not** provided consent for your child to participate in the study please indicate if you would like your child to receive the foods (cheese cubes, applesauce, and peaches) on the days the study will take place. No data will be collected from your child, but they are welcome to receive the snack. If you do not want your child to receive either snack food used in this study they will receive the snack provided by the Early Learning Center for that day.

☐ Yes, my child can receive the food used in the research study.

☐ No, I do not want my child to receive the food used in the research study.

_____ _____

Parent or Legal Guardian Signature Date

_____ _____

Researcher Signature Date

DATE ☐☐/☐☐/☐☐
M M D D Y Y

Contact Information

Child's Name	
Parent/Guardian's Name	
Phone	**Home**
	Cell
	Work
Address	

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Assessment #:

DATE ☐☐/☐☐/☐☐

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Parent/Guardian's Demographic Information

Please fill out this questionnaire if you are the caretaker primarily in charge of feeding the child that will be involved in this study.

1. AGE ☐☐

2. SEX: ☐ MALE (1) ☐ FEMALE (2)

3. RELATIONSHIP TO CHILD

☐ (1) Mother (biological, adopted, step-parent)
☐ (2) Father (biological, adopted, step-parent)
☐ (3) Grandmother
☐ (4) Grandfather
☐ (5) Aunt
☐ (6) Uncle
☐ (7) Sister
☐ (8) Brother
☐ (9) Cousin
☐ (10) Legal guardian
☐ (11) Other (specify):_____

4. EDUCATION: Check years of school completed. (CHECK ONLY ONE ANSWER)

☐ (1) Grade School (6 yrs or less)
☐ (2) Junior High School (7-9 yrs)
☐ (3) High School (10-12 yrs)
☐ (4) Vocational Training (beyond High School)
☐ (5) Some College (less than 4 yrs)
☐ (6) College/University degree
☐ (7) Graduate or Professional Education

5. MARITAL STATUS:

☐ (1) Married
☐ (2) Separated
☐ (3) Divorced
☐ (4) Widowed
☐ (5) Never Married
☐ (6) Not Married (living with significant other)
☐ (7) Other (specify): _____

6. Which of the following best describes your racial heritage? (you may choose more than one)

- ☐ (1) American Indian or Alaskan Native
- ☐ (2) Asian
- ☐ (3) Black or African American
- ☐ (4) Native Hawaiian or other Pacific islander
- ☐ (5) White
- ☐ (6) Other _____

7. Which of the following best describes your ethnic heritage?

- ☐ (1) Hispanic or Latino
- ☐ (2) Not Hispanic or Latino

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DATE ☐☐/☐☐/☐☐

M M D D Y Y

Child Feeding Questionnaire

	never (1)	seldom (2)	sometimes (3)	often (4)	always (5)
When your child is at home, how often are you responsible for feeding her?					
How often are you responsible for deciding what your child's portion sizes are?					
How often are you responsible for deciding if your child has eaten the right kind of foods?					

	Markedly underweight (1)	underweight (2)	normal (3)	overweight (4)	markedly overweight (5)
Your childhood (5 to 10-years-old)					
Your adolescence					
Your 20s					
At present					
Your child during the first year of life					
Your child as a toddler					
Your child as a preschooler					

	unconcerned (1)	a little concerned (2)	concerned (3)	fairly concerned (4)	very concerned (5)
How concerned are you about your child eating too much when you're not around her?					
How concerned are you about your child having to diet to maintain a desirable weight?					
How concerned are you about your child becoming overweight?					

	disagree (1)	slightly disagree (2)	neutral (3)	slightly agree (4)	agree (5)
I have to be sure that my child does not eat too many sweets (candy, ice cream, cake or pastries).					
I have to be sure that my child does not eat too many high-fat foods.					
I have to be sure that my child does not eat too much of her favorite foods.					
I intentionally keep some foods out of my child's reach.					
I offer sweets (candy, ice cream, cake, pastries) to my child as a reward for good behavior.					
I offer my child her favorite foods in exchange for good behavior.					
If I did not guide or regulate my child's eating, she would eat too many junk foods.					
If I did not guide or regulate my child's eating, she would eat too much of her favorite foods.					

	disagree (1)	slightly disagree (2)	neutral (3)	slightly agree (4)	agree (5)
My child should always eat all of the food on her plate.					
I have to be especially careful to make sure my child eats enough.					
If my child says "I'm not hungry", I try to get her to eat anyway.					
If I did not guide or regulate my child's eating, she would eat much less than she should.					

	never (1)	rarely (2)	sometimes (3)	mostly (4)	always (5)
How much do you keep track of the sweets (candy, ice cream, cake, pastries) that your child eats?					
How much do you keep track of the snack food (potato chips, Doritos, cheese puffs) that your child eats?					
How much do you keep track of the high-fat foods that your child eats?					

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Assessment #:

Child Eating Behavior Questionnaire (CEBQ)

For each statement mark an "x" in the response column (never, seldom, sometimes, often, or always) that best represents how often your child displays that particular behavior.

	NEVER (0)	SELDOM (1)	SOMETIMES (2)	OFTEN (3)	ALWAYS (4)
1. Even if my child is full up, s/he finds room to eat his/her favorite food	⓪	①	②	③	④
2. Given the choice, my child would eat most of the time	⓪	①	②	③	④
3. If allowed to, my child would eat too much	⓪	①	②	③	④
4. If given the chance, my child would always be having a drink	⓪	①	②	③	④
5. If given the chance, my child would always have food in his/her mouth	⓪	①	②	③	④
6. If given the chance, my child would drink continuously throughout the day	⓪	①	②	③	④
7. My child cannot eat a meal if s/he has had a snack just before	⓪	①	②	③	④
8. My child decides that s/he doesn't like food, even without tasting it	⓪	①	②	③	④
9. My child eats less when s/he is angry	⓪	①	②	③	④
10. My child eats less when s/he is tired	⓪	①	②	③	④
11. My child eats less when s/he is upset	⓪	①	②	③	④
12. My child eats more and more slowly during the course of a meal	⓪	①	②	③	④
13. My child eats more when annoyed	⓪	①	②	③	④
14. My child eats more when anxious	⓪	①	②	③	④
15. My child eats more when s/he has nothing else to do.	⓪	①	②	③	④
16. My child eats more when s/he is happy	⓪	①	②	③	④

	NEVER (0)	SELDOM (1)	SOMETIMES (2)	OFTEN (3)	ALWAYS (4)
17. My child eats more when worried	⓪	①	②	③	④
18. My child eats slowly	⓪	①	②	③	④
19. My child enjoys a wide variety of foods	⓪	①	②	③	④
20. My child enjoys eating	⓪	①	②	③	④
21. My child enjoys tasting new foods	⓪	①	②	③	④
22. My child finishes his/her meal very quickly	⓪	①	②	③	④
23. My child gets full before his/her meal is finished	⓪	①	②	③	④
24. My child gets full up easily	⓪	①	②	③	④
25. My child has a big appetite	⓪	①	②	③	④
26. My child is always asking for a drink	⓪	①	②	③	④
27. My child is difficult to please with meals	⓪	①	②	③	④
28. My child is interested in food	⓪	①	②	③	④
29. My child is interested in tasting food s/he hasn't tasted before	⓪	①	②	③	④
30. My child leaves food on his/her plate at the end of a meal	⓪	①	②	③	④
31. My child looks forward to mealtimes	⓪	①	②	③	④
32. My child loves food	⓪	①	②	③	④
33. My child refuses new foods at first	⓪	①	②	③	④
34. My child's always asking for food	⓪	①	②	③	④
35. My child takes more than 30 minutes to finish a meal.	⓪	①	②	③	④

Reference #:
Assessment #:

DATE ☐☐/☐☐/☐☐
M M D D Y Y

Eating Habits

Please answer true or false to the following statements.

	True (1)	False (2)
1. When I smell a sizzling steak or see a juicy piece of meat, I find it very difficult to keep from eating, even if I have just finished a meal.	☐	☐
2. I usually eat too much at social occasions, like parties and picnics.	☐	☐
3. I am usually so hungry that I eat more than three times a day.	☐	☐
4. When I have eaten my quota of calories, I am usually good about not eating any more.	☐	☐
5. Dieting is so hard for me because I just get too hungry.	☐	☐
6. I deliberately take small helpings as a means of controlling my weight.	☐	☐
7. Sometimes things just taste so good that I keep on eating even when I am no longer hungry.	☐	☐
8. Since I am often hungry, I sometimes wish that while I am eating, an expert would tell me that I have had enough or that I can have something more to eat.	☐	☐
9. When I feel anxious, I find myself eating.	☐	☐
10. Life is too short to worry about dieting.	☐	☐
11. Since my weight goes up and down, I have gone on reducing diets more than once.	☐	☐
12. I often feel so hungry that I just have to eat something.	☐	☐

	True (1)	False (2)
13. When I am with someone who is overeating, I usually overeat too.	☐	☐
14. I have a pretty good idea of the number of calories in common food.	☐	☐
15. Sometimes when I start eating, I just can't seem to stop.	☐	☐
16. It is difficult for me to leave something on my plate.	☐	☐
17. At certain times of the day, I get hungry because I have gotten use to eating then.	☐	☐
18. While on a diet, if I eat food that is not allowed, I consciously eat less for a period of time to make up for it.	☐	☐
19. Being with someone who is eating often makes me hungry enough to eat also.	☐	☐
20. When I feel blue I often overeat.	☐	☐
21. I enjoy eating too much to spoil it by counting calories or watching my weight.	☐	☐
22. When I see a real delicacy, I often get so hungry that I have to eat it right away.	☐	☐
23. I often stop eating when I am not really full as a conscious means of limiting the amount that I eat.	☐	☐
24. I get so hungry that my stomach seems like a bottomless pit.	☐	☐
25. My weight has hardly changed at all in the last ten years.	☐	☐
26. I am always hungry so it is hard for me to stop eating before I finish the food on my plate.	☐	☐

	True (1)	False (2)
27. When I feel lonely, I console myself by eating.	☐	☐
28. I consciously hold back at meals in order not to gain weight.	☐	☐
29. I sometimes get very hungry late in the evening or at night.	☐	☐
30. I eat anything I want, any time I want.	☐	☐
31. Without even thinking about it, I take a long time to eat.	☐	☐
32. I count calories as a conscious means of controlling my weight.	☐	☐
33. I do not eat some foods because they make me fat.	☐	☐
34. I am always hungry enough to eat at any time.	☐	☐
35. I pay a great deal of attention to changes in my figure.	☐	☐
36. While on a diet, if I eat a food that is not allowed, I often then splurge and eat other high calorie foods.	☐	☐

Please answer the following questions with one of the response that is appropriate for you.

37) How often are you dieting in a conscious effort to control your weight?

☐ (1) Rarely
☐ (2) Sometimes
☐ (3) Usually
☐ (4) Always

38) Would a weight fluctuation of 5 lbs affect the way you live your life?

☐ (1) Not at all
☐ (2) Slightly
☐ (3) Moderately
☐ (4) Very Much

39) How often do you feel hungry?

☐ (1) Only at meal times
☐ (2) Sometimes between meals
☐ (3) Often between meals
☐ (4) Almost always

40) Do your feelings of guilt about overeating help you to control your food intake?

☐ (1) Never
☐ (2) Rarely
☐ (3) Often
☐ (4) Always

41) How difficult would it be for you to stop eating halfway through dinner and not eat for the next four hours?

☐ (1) Easy
☐ (2) Slightly difficult
☐ (3) Moderately difficult
☐ (4) Very difficult

42) How conscious are you of what you are eating?

☐ (1) Not at all
☐ (2) Slightly
☐ (3) Moderately
☐ (4) Extremely

43) How frequently do you avoid "stocking up" on tempting foods?

☐ (1) Almost never
☐ (2) Seldom
☐ (3) Usually
☐ (4) Almost always

44) How likely are you to shop for low calorie foods?

☐ (1) Unlikely
☐ (2) Slightly unlikely
☐ (3) Moderately likely
☐ (4) Very likely

45) Do you eat sensibly in front of others and splurge alone?

☐ (1) Never
☐ (2) Rarely
☐ (3) Often
☐ (4) Always

46) How likely are you to consciously eat slowly in order to cut down on how much you eat?

☐ (1) Unlikely
☐ (2) Slightly unlikely
☐ (3) Moderately likely
☐ (4) Very likely

47) How frequently do you skip dessert because you are no longer hungry?

☐ (1) Almost never
☐ (2) Seldom
☐ (3) At least once a week
☐ (4) Almost every day

48) How likely are you to consciously eat less than you want?

☐ (1) Unlikely
☐ (2) Slightly unlikely
☐ (3) Moderately likely
☐ (4) Very likely

49) Do you go on eating binges though you are not hungry?

☐ (1) Never
☐ (2) Rarely
☐ (3) Sometimes
☐ (4) At least once a week

50) On a scale from 0-5, where 0 means no restraint in eating (eating whatever you want, whenever you want) and 5 means total restraint (constantly limiting food intake and never "giving in"), what number would you give yourself?

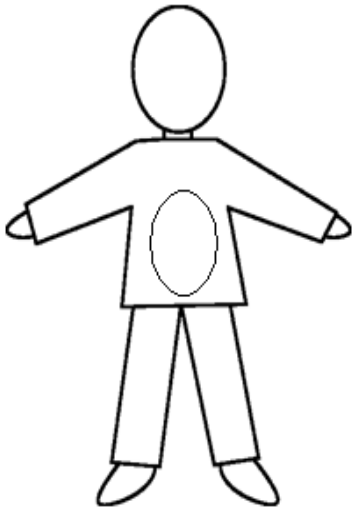
☐ (0) – eat whatever you want, whenever you want
☐ (1) – usually eat whatever you want, whenever you want
☐ (2) – often eat whatever you want, whenever you want
☐ (3) – often limit food intake, but often "give in"
☐ (4) – usually limit food intake, rarely "give in"
☐ (5) – constantly limiting foods intake, never "giving in"

51) To what extent does the statement describe your eating behavior? "I start dieting in the morning, but because of any number of things that happen during the day, by evening I have given up and eat what I want, promising myself to start dieting again tomorrow."

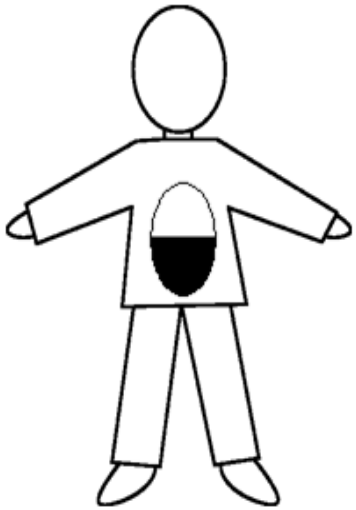
☐ (1) Not like me
☐ (2) Little like me
☐ (3) Pretty good description of me
☐ (4) Describes me perfectly

Official Use Only
Reference #:
Assessment #:

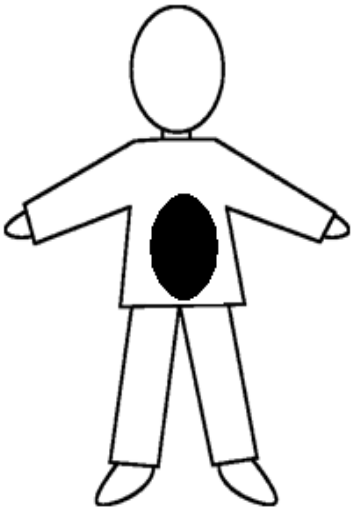
How hungry are you right now?



Very Hungry



Kind-of Hungry/
A Little Hungry

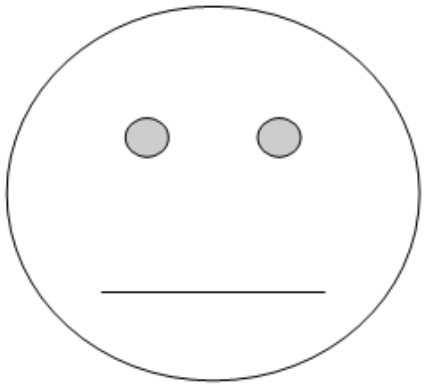


Not Hungry

Official Use Only
Reference #:
Assessment #:

Do you like applesauce?

YUMMY



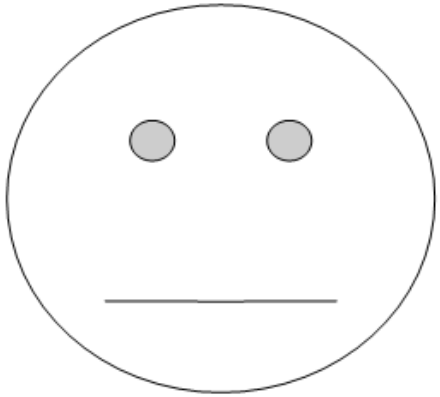
SO-SO

YUCKY

Official Use Only
Reference #:
Assessment #:

Do you like cheese cubes?

YUMMY



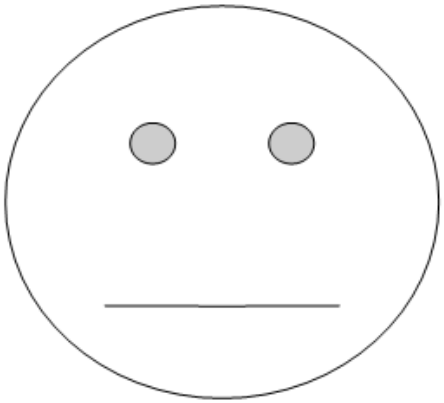
SO-SO

YUCKY

Official Use Only
Reference #:
Assessment #:

Do you like peaches?

YUMMY



SO-SO

YUCKY

VITA

Chelsi Cardoso is originally from Virginia Beach, Virginia. She earned her Bachelor of Science degree in Nutrition with a minor in Psychology at the University of Tennessee, Knoxville (UTK) in May 2010. During undergraduate school, she had a variety of exposure to various aspects of nutrition. These experiences included working in a clinical, community, food safety, and research setting. Following undergraduate school, she pursued her Masters of Science in Public Health Nutrition and Dietetic Internship at UTK.

While pursuing her Masters, Chelsi served as a Maternal and Child Health Graduate Assistant, which provided her with the ability to sharpen her leadership skills working in public health nutrition, specifically focused on maternal and child health. She also served as a Student Research assistant in the Healthy Eating and Activity Laboratory (HEAL). While working in HEAL, Chelsi gained a large amount of experience in behavioral weight management in adults and children, data and project management, and team collaboration. In addition, she was able to provide nutrition counseling to a low-income, racially/ethnically diverse population through Cherokee Health Systems, and maintained a position working in a pediatric clinical setting. All of Chelsi's experiences lead her to believe that nutrition intervention at a young age may provide the most potential for healthful, lifelong changes. Thus, Chelsi seeks to work with children following graduation. Chelsi completed her Dietetic Internship in August 2012 and will complete her Masters during the Fall of 2012.