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## **Impact of Portion Size of Fruit Juice on Fruit Juice Consumption and Overall Energy Intake During a Snack in Preschoolers**

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To the Graduate Council:

I am submitting herewith a thesis written by Erin Margaret Norton entitled "Impact of Portion Size of Fruit Juice on Fruit Juice Consumption and Overall Energy Intake During a Snack in Preschoolers." 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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# Impact of Portion Size of Fruit Juice on Fruit Juice Consumption and Overall Energy Intake During a Snack in Preschoolers

A Thesis Presented for the  
Master of Science  
Degree  
The University of Tennessee-Knoxville

Erin Margaret Norton  
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## ABSTRACT

**Background:** The relationship between 100% fruit juice intake and adiposity in children may be a consequence of lack of complete compensation to energy consumed from beverages.

Therefore, this study investigated the impact of beverage type and beverage size on beverage and overall snack intake in preschool-aged children. **Methods:** Using a 2x2x2 design (between-subjects factor of order and within-subjects factors of beverage type [100% fruit juice vs. water] and beverage size [6 oz. vs. 12 oz.]), 26 children ( $3.9 \pm 0.6$  years of age, 50% female, 73% white, and 88.5% non-Hispanic or Latino) completed 20-minute snack sessions on four consecutive Wednesday afternoons. All snacks consisted of 200 g of applesauce, approximately 60 g of graham crackers, and either 6 oz. or 12 oz. of 100% berry fruit juice or water. **Results:** Repeated measures analyses of covariance found a significant effect of beverage size on grams of beverage consumed ( $121.3 \pm 59.9$  g consumed in 6 oz. conditions versus  $173.9 \pm 101.7$  g in 12 oz. conditions,  $p < 0.05$ ). A significant ( $p < 0.05$ ) interaction of beverage type and beverage size was found for calories of beverage consumed, in which more calories were consumed from the beverage in the 12 oz. juice condition than any other condition (12 oz. juice =  $109.5 \pm 56.2$  kcal; 6 oz. juice =  $69.4 \pm 26.4$ ; 6 oz. and 12 oz. water =  $0.0 \pm 0.0$  kcal). No significant difference was observed for total snack energy intake or for energy consumed from applesauce and graham crackers between conditions. However, total caloric intake was approximately 67% higher when juice was served with the snack ( $d = 1.05$ ). **Conclusion:** Serving children larger beverage portions can lead to increased beverage intake during snack time, and leads to increased beverage energy intake if the beverage contains calories. Overall snack energy intake is also elevated when a caloric beverage is served because children do not appear to exhibit

compensation to liquid calories during a snack. Therefore, consuming a caloric beverage alongside a snack may not be the best option if excessive energy intake is of concern.

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## **CHAPTER I: LITERATURE REVIEW**

## **BACKGROUND AND SIGNIFICANCE**

### ***Childhood Obesity and Beverage Consumption***

The prevalence of childhood overweight, defined as a body mass index (BMI) at or above the 85<sup>th</sup> percentile but less than the 95<sup>th</sup> percentile, and obesity, defined as a BMI at or above the 95<sup>th</sup> percentile, in the U.S. is very high. Data from the National Health and Nutrition Examination Survey indicates that as of 2012, 8.4% of toddlers and preschool-aged children (children between the ages of two and five) were obese<sup>1</sup>. Furthermore, nearly 17% of children and adolescents between the ages of two and seventeen in the US were obese<sup>1</sup>. Obesity leads to serious medical implications for children, including type 2 diabetes and increased blood cholesterol and blood pressure, which are contributors to the development of cardiovascular disease later in life<sup>2</sup>. Increased body weight also causes bone and joint problems in children, as well as breathing problems such as sleep apnea<sup>3</sup>. However, it can be argued that from a child's perspective, the most detrimental consequence of childhood overweight and obesity is social discrimination<sup>2</sup>. Children who are overweight or obese are ostracized from their peers and are often teased and bullied<sup>2</sup>.

The rate of overweight and obesity in children has increased dramatically since 1980<sup>3</sup>. Although the rate of increase has slowed in recent years<sup>1</sup>, it remains of serious concern. This significant increase is paradigmatic of the changes that have occurred in children's dietary patterns. Of growing interest is the increase in the proportion of daily calories provided to children's diets by energy-containing beverages, and in particular, 100% fruit juices<sup>4</sup>, which has occurred over the past several decades<sup>4,6</sup>. As of 2004, children and adolescents of all ages were consuming at least 10 to 15% of their daily calories from energy-containing beverages, accounting for about 270 of total daily calories<sup>4</sup>.

The American Academy of Pediatrics (AAP), recommends a 100% fruit juice intake of no more than 4 to 6 ounces per day for children 1 to 6 years old, and an intake of no more than two, 6-ounce servings per day for children 7 to 18 years of age<sup>7,8</sup>. The current guidelines for fruit and fruit juice consumption presented by MyPlate indicate that a cup of 100% fruit juice counts toward the daily fruit recommendation, but that the majority of fruit should be consumed as whole or cut-up fruit due to the fiber content present in these forms and not in juice<sup>9</sup>. Recommended fruit intake according to these guidelines depends on age and gender, but varies upwards of 1 cup per day.

Although 100% fruit juice is more nutritive than other energy-containing beverages (i.e., sugar sweetened beverages), and thus is seen as a more healthful option than sodas or coffee drinks, research has indicated a link between 100% fruit juice consumption and adiposity in children. The majority of studies in this area have been cross-sectional and longitudinal analyses using convenience samples, so some controversy remains in determining a cause and effect relationship between 100% fruit juice intake and childhood obesity<sup>7</sup>.

For example, in a study by Dennison et al., dietary intakes of two- and five-year old children were analyzed for fruit juice consumption and related to anthropometric measures<sup>10</sup>. Results indicated that the prevalence of shorter stature was higher among those children who consumed excess fruit juice. Additionally, among the children reported to have consumed 12 fluid ounces or more of fruit juice per day, there was a higher prevalence of overweight. This indicates a correlation between higher fruit juice intake and increased weight status and decreased stature.

In a follow-up examination by Dennison et al., different fruit juices were analyzed for their correlation with weight and height in children<sup>11</sup>. This study showed a relationship between

weight status and stature for apple and grape juices only. Higher consumption of these two fruit juices was associated with shorter stature, and excess consumption of apple juice was associated with obesity<sup>11</sup>. Lastly, a study by Melgar-Quinonez investigated the effect of juice intake on adiposity in low-income Mexican-American children and provided similar results<sup>12</sup>. In this cross sectional analysis, a significant correlation was observed between higher fruit juice consumption and adiposity.

Longitudinal studies have also found a relationship between fruit juice intake and weight status in children. Libuda et al. found a positive correlation between fruit juice intake and BMI in adolescent girls in a five year German study<sup>13</sup>. The mean age of girls at the beginning of the study was 11.8, and the mean age at the end was 16.8. During this five year study period, increased fruit juice intake over time was associated with increases in BMI. Other studies found this effect for children already at higher weight status. A longitudinal examination by Faith et al. measured changes in adiposity of preschool children between the ages of 18 and 48 months who were from low-income families<sup>14</sup>. This study revealed that in children who were already overweight or at serious risk of overweight, increased fruit juice intake over the study period was correlated with increased body mass<sup>14</sup>. In a study by Welsh et al., data were collected on juice and sweetened drink consumption and body fatness among two- and three-year-old children at two time points<sup>15</sup>. At the beginning of the study, data on children's beverage consumption were gathered from parents, and the children's heights and weights were measured. Height and weight data were then collected one year later, and an association was found between fruit juice consumption patterns at baseline and body fatness in children one year later. This association was observed only for children who entered the study as overweight<sup>15</sup>. For these children, the

results showed a positive association between daily fruit juice intake and an increased risk of remaining overweight one year later.

### ***The Relationship Between Energy Compensation and Beverage Consumption***

Weight gain primarily occurs due to an increase in caloric intake, a decrease in caloric expenditure, or both<sup>16</sup>. An individual is said to be in positive energy balance when caloric intake exceeds caloric expenditure, and the excess energy is stored in the body as fat, causing weight gain that can potentially lead to obesity<sup>17</sup>. Caloric intake consists of the various nutrients taken in from food and beverages, including fat, protein, carbohydrates, and alcohol; when consumption increases, the energy expenditure required to maintain a steady weight also increases.

Caloric beverages are thought to provide energy without offering other properties that solid food provides. Most importantly, liquids lack the ability to satiate the consumer<sup>18</sup>. Compared to solid food calories, compensation does not occur completely for liquid calories<sup>18,19</sup>. In other words, in experimental settings, participants adjust food intake in response to calories consumed from preloads of solid as compared to liquid forms<sup>19</sup>. For example, in a crossover design, DiMeglio and Mattes measured men and women's energy intake throughout the day when accompanied by liquid and solid carbohydrate loads<sup>19</sup>. The liquid load was given as caffeine-free soda, and the solid load was given as jelly beans. Participants consumed each type of preload daily for four weeks, and were allowed to consume the carbohydrate load whenever they chose. For the four weeks, they were also allowed to consume other foods freely throughout the day. Six times during each four-week treatment period, random dietary recalls were used to assess free-feeding energy intake under each condition.

Results of the dietary records in this study showed that free-feeding energy consumption was significantly lower during the solid carbohydrate load phase as compared to the liquid

carbohydrate load phase<sup>19</sup>. Thus, greater compensation occurred in the solid load condition, and less compensation occurred during the liquid load condition. Furthermore, when overall energy intake, intake from the loads and free-feeding situations, was examined, greater intake occurred during the liquid load as compared to the solid load phase. One proposed mechanism for the phenomenon observed in DiMeglio's study is that chewing the solid carbohydrate load offers a satiety signal that the liquid load is not able to provide<sup>19</sup>. The brain does not receive satiety signals as readily after consumption of liquids, so the individual is not as apt to decrease subsequent energy intake in order to compensate for what was consumed as liquid. There are also elements in the stomach that sense the presence of food and provide feedback via endocrine and nervous hormones that influence fullness and hunger. Liquids are able to pass from the stomach more quickly than solids, bypassing these mechanisms and leaving an individual with less of a sense of satiation and satiety<sup>19</sup>.

The lack of compensation to energy-containing beverages may lead to excess caloric consumption. Thus, energy intake from fruit juice may not be compensated in children, leading to the positive relationship between fruit juice intake and adiposity observed in studies on young children<sup>10-14</sup>.

### ***Beverages and Energy Compensation During a Meal***

While evidence suggests that the consumption of energy-containing beverages contributes to the development of childhood obesity<sup>10-14</sup>, little research has examined the impact of these beverages on overall intake when consumed at various times during the day in children. Some studies have shown that consuming these beverages between meals leads to weight gain<sup>20</sup>. Most evidence proposes that this occurs due to the added calories to the diet; however, there has

been less exploration of energy-containing beverages' contribution to dietary intake when consumed alongside other foods, such as at mealtimes, in children.

Ideally, due to the excess calories provided in energy-containing beverages, consumption of caloric liquids at mealtimes would lead to less overall energy intake during the meal. This has been explored in adults, with adults showing poor compensation to energy-containing beverages<sup>18,19,21-24</sup>.

In a study by DellaValle et al., 45 normal weight, overweight, and obese women between the ages of 18 and 60 years participated in a within-subjects experiment testing the effects of five different caloric and non-caloric beverages on food intake during a meal<sup>21</sup>. Beverages of varying energy and nutrient content, including tap water, fruit juice, regular and diet cola, and 1% milk, were tested. Participants were instructed to consume the entire beverage portion intermittently throughout the meal, and to consume pasta, sauce, bread, salad, and cookies ad libitum. Consumed portions of the meal were measured, as were measures of hunger and fullness before and after eating.

Measures of satiety were unaffected by the type of beverage consumed; however, overall energy intake consumed during the meal was greater in the conditions in which energy-containing beverages were consumed. The greater overall energy intake in the meals was due to the excess energy provided by the beverages and the lack of compensatory behavior of participants in adjusting food intake in relation to the amount of energy consumed in liquid form<sup>21</sup>. Neither normal weight nor overweight women compensated for their liquid energy intake by decreasing food intake accordingly.

Most recently, in 2013 Panahi et al. conducted a study to further investigate potential compensation in adults<sup>24</sup>. Twenty-nine male and female participants consumed ad libitum



portions of pizza lunches, alongside ad libitum portions of five test beverages—water, 1% milk, orange juice, regular and diet cola. Results showed that beverage type had no impact on the amount of pizza eaten. Thus, no evidence was found of compensatory behavior when consuming beverages of any type. Due to the greater amount of energy in 1% milk, orange juice, and regular cola as compared to water and diet cola, energy intake was higher in conditions in which energy-containing beverages were consumed.

Studies evaluating the impact of different energy- and non-energy-containing beverages on food intake during meals have mostly been conducted in adults. The role of energy-containing beverages on compensatory behavior in children has received less attention<sup>24</sup>. Understanding children's ability to compensate to energy-containing beverages during a meal or snack would be valuable in gaining a better understanding of how energy-containing beverages influence children's intake and weight status.

One study has examined children's ability to compensate to energy consumed from beverages. In 1989, Birch et al. conducted a study to examine the impact of energy-containing beverages served as preloads on subsequent food intake during a snack in children<sup>25</sup>. The investigation used a within-subjects crossover design. Preschool-aged children's ad libitum consumption of common snack foods was measured at either 0, 30, or 60 minutes after consuming four different beverages. The four preload beverages were water, Kool-Aid sweetened with sugar, an aspartame-sweetened beverage, and an aspartame-sweetened beverage to which a non-carbohydrate calorie load was added. The subsequent ad libitum snack consisted of raisins, cookies, potato chips, crackers, hot dogs, and cheese.

While no significant interaction was found between the length of delay between preload and snack consumption and the amount of food consumed during the snack, this study showed a

significant effect of the type of beverage preload on children's subsequent snack intake<sup>25</sup>.

Children consumed significantly less energy from the snack foods after drinking the sugar sweetened Kool-Aid and the aspartame sweetened/high-caloric drink, compared to consumption after drinking water and aspartame-sweetened Kool-Aid. In these cases, participants exhibited compensation for the calories consumed in the beverages. The results did not show a significant difference between compensation exhibited after drinking the sucrose-sweetened Kool-Aid and the compensation exhibited after drinking the aspartame sweetened/high-caloric drink. However, the compensation observed suggests that young children can compensate to energy-containing beverages consumed prior to a snack.

### ***Portion Sizes***

With the growing rates of obesity in children and adults, there has been increasing concern over the role of portion sizes in this epidemic<sup>26</sup>. Food portions consumed, both within and outside the home, are of interest because they have grown in size over the years, potentially causing excess consumption<sup>26</sup>. In an examination of portion sizes by Young et al. in 2002, it was found that portion sizes available to people have increased steadily and that they are larger than federal standard portion sizes<sup>26</sup>. Larger portions provide more energy, and may encourage an individual to consume more than he or she needs to satisfy energy needs<sup>26</sup>. If an individual habitually consumes excess energy via larger portion sizes, this could influence weight status. No research has been conducted regarding the effect of portion size on beverage consumption in children, but research on adults indicates that portion size does influence beverage intake in adults. Increased intake from energy-containing beverages of larger portion size may produce “worse” compensation in meals, which may contribute to intake being much greater than energy needs.

In a study by Rolls et al. in 1990<sup>22</sup>, forty-two males, between 21 and 39 years of age, underwent a within-subjects experiment testing the impact of three different beverages, each in both the 8 oz. size and 16 oz. size, on food intake during a meal. This experiment examined sucrose-sweetened lemonade, aspartame-sweetened lemonade, and water intake. Participants were allowed to consume the lunch foods freely, but were asked to consume the entire assigned beverage during the experimental session.

Results of this study showed that no differences existed in food intake across caloric and non-caloric beverage conditions<sup>22</sup>. In other words, average food consumption during the lunch did not change when participants consumed a sugar-sweetened beverage compared to an artificially-sweetened, non-caloric liquid. Thus, in the sugar-sweetened beverage condition, total energy consumption during the meal was higher than in the artificially-sweetened beverage condition. Additionally, no compensatory effect occurred due to the size of the beverage consumed. Therefore, total energy consumption during the meals served with a sugar-sweetened beverage was higher in the 16 oz. portion size conditions than in the 8 oz. conditions. This was attributable to a main effect of beverage calories added to the food calories consumed at the meal.

These results are consistent with those observed in a study by Flood et al. in 2006<sup>23</sup>. Thirty-three men and women between the ages of 18 and 45 years participated in a within-subjects crossover design examining the impact of different portion sizes of water, regular cola, and diet cola on caloric intake during an ad libitum lunch of pasta, salad, bread, and cookies. Unlike the previous study, however, participants were allowed to drink as much of their given portion, either 12 oz. or 18 oz., as they wished; they were not required to consume the entire portion.

No significant differences existed in ratings of hunger and fullness after the meal across conditions<sup>23</sup>. Participants consumed significantly more beverage when served the larger portion than when served the smaller portion. On average, women exhibited a 10% increase in beverage intake when served the larger portion compared to when served the smaller portion, while men exhibited a 26% increase with the larger portion. This occurred across all beverage types. Due to this effect, within the caloric beverage conditions, participants consumed an average of 151 kcal from the larger beverage portion compared to 128 kcal consumed from the smaller portion. This caused a significant increase in the overall amount of energy consumed during those meals served with energy-containing beverages.

No evidence of compensatory behavior was found, as no differences in food intake occurred between the experimental conditions. In other words, participants did not adjust their intake according to the calories consumed in beverages. Instead, individuals consumed the same amount of calories from food when served a caloric beverage as when served a non-caloric beverage at the meal. Thus, the extra calories provided by caloric beverages were simply added to the calories consumed in the meal, causing energy intake during meals served with a caloric beverage to be significantly greater than that during meals served with a non-caloric beverage. However, the difference between overall energy intake during the larger caloric beverage condition, as compared to the smaller caloric beverage condition, was not statistically significant.

While no research has been conducted with children regarding changes in portion size of beverages and beverage intake, research has found that children consume more of solid food when provided the food in larger portions.

A study completed in 2000 by Rolls et al. examined the effect of providing larger portions on intake<sup>27</sup>. Participants of the within-subjects crossover design were 32 children

between the ages of three and five years. Varying portion sizes of a macaroni and cheese entrée were provided to children on different days, alongside fixed portions of carrot sticks, applesauce, and milk.

It was observed that intake increased with portion size among older children, who were on average around the age of five years<sup>27</sup>. Younger children, of mean age 3.6 years, did not exhibit any significant differences in intake between the three portion size conditions. This implies that children respond to larger portion sizes, but potentially only after a certain age. It is possible that younger children are more influenced by internal cues of self-regulation, and that these cues start to diminish as external cues, including increased portion size, take over<sup>28</sup>.

However, in 2003, Fisher et al. replicated this study, using a within-subjects crossover design with children between the ages of three and five years, and did not find younger children to be unresponsive to portion size<sup>29</sup>. On separate occasions, children were provided with a reference-size portion of macaroni and cheese and a larger-size portion of macaroni and cheese, each time served with carrot sticks, applesauce, sugar cookies, and milk. In the larger portion size condition, children of all ages consumed more of the macaroni and cheese meal and more energy overall. The effect of increased consumption when served a larger portion was positively associated with age, meaning children at the upper age range were most responsive to larger portion sizes.

A study by Savage et al. in 2012 further investigated the effect of larger entrée portions on side dish consumption, including consumption of fruits and vegetables<sup>30</sup>. Twenty-one children aged three to five years underwent a series of six experimental lunch sessions, with varied portions of a macaroni and cheese entrée and fixed portions of side dishes. As entrée size

increased, children's intake of the entrée also increased, leading to an increase in total energy consumption during the meal.

A study by Mathias et al. in 2012 also showed that increased portion sizes leads to greater intake<sup>31</sup>. Over five experimental sessions, 38 children between the ages of 4 and 6 years were served fruits and vegetables of varying portion size along with a main entrée of pasta and sauce. Overall energy intake during the meal was measured, as well as the amounts of fruits and vegetables consumed in each condition, to determine whether portion size manipulation resulted in an effect on intake of those foods. The results showed that when served larger portions of fruits and vegetables, children consumed significantly greater amounts of these foods<sup>31</sup>.

### ***Summary***

It is estimated that as of 2012, approximately 8.4% of toddlers and preschool-aged children and 17% of children and adolescents in the United States were obese<sup>1</sup>. Excess body weight can contribute to serious illnesses and consequences can be lifelong and potentially deadly<sup>2,3</sup>. As the prevalence of childhood overweight and obesity has increased since the 1980s, so has the proportion of calories provided daily to children's diets by energy-containing beverages, and in particular 100% fruit juices<sup>4</sup>. Research suggests a link between 100% fruit juice consumption and adiposity in children and adolescents<sup>10-14</sup>. While one study suggests that children may compensate their overall energy intake in response to liquid calories better than adults<sup>25</sup>, other research indicates that the relationship between 100% fruit juice and adiposity in children may be a consequence of lack of complete compensation to energy consumed from beverages. Incomplete compensation could contribute to excessive energy intake, leading to excessive weight gain. Furthermore, over the past few decades, larger portion sizes have become more common and have arisen as a concern<sup>26</sup>, because children appear to be responsive to

portion size and consume greater amounts with larger portion sizes<sup>27,29-31</sup>. Increased portions of juice may further exacerbate the issues related to incomplete compensation.

Neither of these possible contributors to childhood weight status, portion size or beverage type, has been exclusively investigated in children, and doing so would lead to a better understanding of childhood eating tendencies and the factors leading to weight gain and weight management in this population. Thus, the purpose of this study was to investigate whether beverage type (caloric [100% fruit juice] vs. non-caloric) and beverage portion size (6 oz. vs. 12 oz.) impact on energy intake during a snack. For this investigation, four experimental snack sessions occurred, in which beverage portion size and beverage type were manipulated, but provided amount and types of snack foods were kept identical across all four sessions. Participants were 3- to 5-year-old children at the University of Tennessee Early Learning Center for Research and Practice. Four different conditions took place, a 6 oz., caloric beverage (100% fruit juice) condition; 6 oz., non-caloric beverage (water) condition; 12 oz., caloric beverage condition; and 12 oz., non-caloric beverage condition. It was hypothesized that:

1. A main effect of beverage type would occur, such that the caloric beverage conditions would result in a greater energy intake from beverage and greater energy intake from overall snack (which includes the beverage) than the non-caloric beverage conditions.
2. A main effect of beverage portion size would occur, such that the 12 oz. portion size conditions would result in a greater gram intake from the beverage than 6 oz. portion size conditions.
3. An interaction of beverage type and portion size would occur, such that the 12 oz. caloric beverage condition would result in the greatest energy intake from the overall snack (which includes the beverage) as compared to all other conditions.

## **CHAPTER II: MANUSCRIPT**



## INTRODUCTION

As the prevalence of childhood overweight and obesity has increased since the 1980s, so has the proportion of calories provided daily to children's diets by energy-containing beverages, and in particular 100% fruit juices<sup>4</sup>. Research has suggested a link between 100% fruit juice consumption and adiposity in children and adolescents<sup>10-15</sup>. Cross-sectional studies have associated higher levels of fruit juice intake in children with shorter stature and obesity<sup>10,11</sup>, and have reported an association between juice consumption and adiposity in subpopulations such as low-income Mexican-American children<sup>12</sup>. Longitudinal studies have reported correlations between fruit juice intake and body mass index over study periods, mainly in children already overweight or at risk of becoming overweight<sup>14,15</sup>. These studies have sparked an increase in concern over the added energy provided by liquids in children's diets.

Caloric beverages are thought to provide energy without offering other properties that solid food provides. Most importantly, liquids lack the ability to satiate the consumer<sup>18</sup>. Compared to solid food calories, compensation does not occur completely for liquid calories<sup>18,19</sup>. In other words, in experimental settings, participants adjust food intake in response to calories consumed from preloads of solid, but do not compensate as well with liquid preloads<sup>19</sup>. Proposed mechanisms for this phenomenon include the lack of satiety signals that are normally triggered by chewing solid food, the inability of liquids to signal the stomach to fullness and hunger as compared to solids, and the ability of liquid to pass rapidly through the stomach and bypass any mechanisms that control satiety<sup>19</sup>. By way of these mechanisms, the lack of compensation to energy-containing beverages in the diet may lead to excess caloric consumption.

However, studies evaluating the impact of different energy- and non-energy-containing beverages on food intake during meals have mostly been explored in adults, with adults showing

poor compensation to energy-containing beverages<sup>18,19,21-24</sup>. The role of energy-containing beverages on compensatory behavior in children has received less attention<sup>24</sup>.

Only one study has examined children's ability to compensate to energy consumed from beverages. In 1989, Birch et al. conducted a study to examine the impact of energy-containing beverages served as preloads on subsequent food intake during a snack in children<sup>25</sup>. Preschool-aged children's ad libitum consumption of common snack foods was measured at either 0, 30, or 60 minutes after consuming four different beverages. The four preload beverages were water, Kool-Aid sweetened with sugar, an aspartame-sweetened beverage, and an aspartame-sweetened beverage to which a non-carbohydrate calorie load was added. The subsequent ad libitum snack consisted of raisins, cookies, potato chips, crackers, hot dogs and cheese.

While no significant interaction was found between the length of delay between preload and snack consumption and the amount of food consumed during the snack, this study showed a significant effect of the type of beverage preload on children's subsequent snack intake<sup>25</sup>.

Children consumed significantly less energy from the snack foods after drinking the sugar sweetened Kool-Aid and the aspartame sweetened/high-caloric drink, compared to consumption after drinking water and aspartame-sweetened Kool-Aid. In these cases, participants exhibited compensation for the calories consumed in the beverages, suggesting that unlike adults, young children can compensate to energy-containing beverages consumed prior to a snack. This study was based on the supposition that beverages consumed between meals may impact on energy intake at the meal immediately following beverage consumption, due to a time effect. However, little research has examined energy-containing beverages' contribution to dietary intake when consumed alongside other foods, such as at mealtimes, in children. Ideally, due to the excess calories provided in energy-containing beverages, consumption of caloric liquids at mealtimes

would lead to less overall energy intake during the meal. Further investigating children's ability to compensate to energy-containing beverages during a meal or snack would be valuable in gaining a better understanding of how energy-containing beverages influence children's intake and weight status.

Additionally, in recent decades, larger portion sizes have become more common and have arisen as a concern<sup>26</sup>, because children appear to be responsive to portion size and consume greater amounts with larger portion sizes<sup>27,29-31</sup>. Increased portions of juice may further exacerbate the issues related to incomplete compensation. Therefore, beverage type (caloric or non-caloric) and portion size are two environmental factors that may play a role in energy intake, and therefore weight status, in children. Investigating these possible contributors to energy intake in children would lead to a better understanding of the contributing factors that may lead to weight gain in this population. Thus, the purpose of this study was to examine whether beverage type (caloric [100% fruit juice] vs. non-caloric) and beverage portion size (6 oz. vs. 12 oz.) impact on energy intake during a snack in preschool-aged children.

This manipulation took place within snack sessions in which children were provided with a snack (applesauce and graham crackers), and one of four beverage conditions (6 oz., caloric beverage [100% fruit juice] condition; 6 oz., non-caloric beverage [water] condition; 12 oz., caloric beverage condition; and 12 oz., non-caloric beverage condition). It was hypothesized that caloric beverage conditions would have a greater overall snack energy intake (energy from beverage and food combined) than non-caloric beverage conditions and that greater gram amounts of beverage would be consumed in the 12 oz. portion size conditions than the 6 oz. portion size conditions. Lastly, it was hypothesized that the greatest overall snack energy intake would occur in the 12 oz. caloric beverage condition as compared to all other conditions.

## EXPERIMENTAL DESIGN AND METHODOLOGY

### *Study Design*

This study used a crossover design to investigate the impact of different portion sizes of 100% fruit juice on snack food intake in preschool aged children. A 2x2x2 design, with the between-subjects factor of order (order 1 vs. order 2) and the within-subjects factors of beverage type (caloric vs. non-caloric) and portion size (6 oz. vs. 12 oz.) was utilized (see Appendix A Table 1). The dependent variables were the amount in grams of beverage consumed, and energy (in kilocalories) of beverage and food consumed. The Institutional Review Board (IRB) at the University of Tennessee, Knoxville, approved this study.

### *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 2013, standard recruitment procedures took place at the ELC. These procedures involved sending an introductory letter home to the parent/guardian to explain the study, followed by a folder of questionnaires and consent forms for parents/guardians to complete. The questionnaires provided information regarding the children's demographics, eating behaviors, and eligibility, as well as the primary caregiver's feeding style. Parents/guardians willing to allow their child to participate in the study were asked to sign a consent form and return it to the teacher of their child's class.

Participants for this study were preschool-aged children who attend the full week and full day program at the University of Tennessee's ELC at the White Avenue location. To be eligible, all children had to be  $\geq 3$  years of age by September 30, 2013, enrolled at the ELC, be free of allergies to the foods used in the study, like the foods used in the study, be able to use a spoon,

and have parental consent. Children who did not meet these criteria were not included in the study.

There were 36 children enrolled at the ELC at the beginning of the study, with 18 children in each of two classrooms. Parents turned in consent forms for 14 children in classroom 1 and 18 children in classroom 2, for a total of 32 children. Of these children with consent forms, 14 were eligible from classroom 1, and 16 children were eligible from classroom 2. Two children from classroom 2 with consent forms were ineligible because they did not like the foods used in the study. Thus, 30 children participated in the snack sessions. The two children from classroom 2 who were ineligible to participate were served a snack from the ELC during the study period. The researchers provided the four children from classroom 1 who did not have parental consent an identical snack as that served to participants in the study, and these children ate the snack at the same time as participants.

Of the 30 children participating, 26 participants were included in the data analysis. Of the 4 children not included in the analyses, one child consumed all of the provided food and beverage during three out of the four experimental snack sessions, introducing the concern of the ceiling effect<sup>32</sup>. The ceiling effect limits the ability to measure the manipulation's impact on the dependent variables. Three other children were excluded due to being absent from preschool for at least one experimental session. Therefore data were included from 12 children in classroom 1 and from 14 children in classroom 2. See Appendix A Figure 1 for a participant flow chart.

### ***Randomization***

Children were randomized by classroom into one of two orders, with each child within each classroom receiving the same condition at each session. Classroom 1 was assigned to order 1 and classroom 2 was assigned to order 2 (Appendix A Table 1).

## ***Foods***

The snack foods used in this study were graham crackers and applesauce, which are snacks that are used at the ELC and that the children enjoy. The beverages used were water and 100% berry fruit juice. This juice was chosen due to its acceptability among children and in order to avoid too much apple flavor in the snack, as applesauce was also served. A pilot study was done in September 2013, in which approximately 15 preschool-aged children at another ELC facility taste-tested 100% berry and 100% grape juices in order to determine the best juice flavor to use in the study. Based on votes, the majority of participants preferred berry juice, so it was used in the study.

Depending on the condition, children were served either 6 or 12 oz. of juice or water, along with 200 grams (approximately 7 oz.) of applesauce and 3, 2 in. x 4 in. graham cracker rectangles (approximately 60 grams). For the 6 oz. beverage conditions, beverages were served to children in 9 oz. plastic cups and for the 12 oz. beverage conditions, they were served in 16 oz. plastic cups, each with lids and straws to help prevent spills.

Brands of the foods that were served to children were Juicy Juice (100% berry), Mott's applesauce (no sugar added, natural), and HoneyMaid graham crackers (honey grahams). The applesauce contained 45 kilocalories per 100 grams, giving it an energy density of 0.45 kcal/gram, and the graham crackers contained 420 kilocalories per 100 grams, providing an energy density of 4.20 kcal/gram. The berry fruit juice provided 90 kilocalories per 6 oz. serving, and 180 kilocalories per 12 oz. serving. Birch et al. used a beverage containing 90 kilocalories per serving in their examination of children's compensation to energy from a liquid source<sup>25</sup>. A serving twice that size and energy content was used for this investigation's larger portion size condition.

In both conditions in which water was served as the beverage, a total of 279 kilocalories were available in the snack. In the 6 oz. juice condition, 369 total kilocalories were available in the snack (beverage and snack combined), and in the 12 oz. juice condition, 459 total kilocalories were available (beverage and snack combined). During a pilot study at the ELC in September 2013, snack foods were tested, as were 6 oz. and 12 oz. beverages sizes, served in 9 oz. and 16 oz. plastic cups, respectively. Researchers observed the ability of the children to grasp and drink water from the 9 oz. and 16 oz. plastic cups, and also measured the amount consumed of the beverage, in grams. This helped to confirm whether the chosen portions were the appropriate sizes for children of this age.

## ***Procedures***

### ***Snack Session Procedures***

Snack sessions were held at approximately 3:00pm, the ELC's normal snack time, on six consecutive Wednesday afternoons beginning in October 2013. The first two weeks served as practice sessions to help the children become accustomed to the research staff, the process used in the study, and to help them adjust to individualized, pre-portioned snacks. Data was collected from these sessions but was not used in analyses. During practice sessions, each classroom received a practice round with each of the two beverages and portion sizes.

Beginning in Week 3, the order presented in Appendix A Table 1 was followed for measured snack sessions. The lunches served prior to all snack sessions were the same, and consisted of quesadillas, spinach, and orange slices. Children sat at their usual snack tables, in groups of 6 to 10, with research assistants supervising each table. The investigator began each session by informing participants that they would be testing snack foods and beverages that day because the investigators wanted to know how much they liked the foods and beverages. Each

participant then rated their hunger and liking of the foods and beverages used in the investigation on questionnaires. In order to decrease the chance that children would be influenced by their peers in rating their hunger and liking of the foods, children were reminded to answer for themselves, and to not pay attention to the responses given by others. Trained students from the University of Tennessee Healthy Eating and Activity Laboratory (HEAL) and ELC staff assisted children in completing these hunger and liking questionnaires, and then the children received their snack and appropriate beverage.

Students from HEAL distributed each child's food and beverage in containers labeled with the child's identification number. Extreme care was exhibited in order to avoid confusion between participants. Once foods and beverages were distributed, children were told that they should eat and drink as much or as little as they wished, and to avoid sharing any of their food or drink with others. Children were given 20 minutes to consume their snack. Adults observed to ensure the children did not share their foods. Upon completion of snack time, trained HEAL members collected all food and beverage containers and again assisted participants in completing the hunger rating questionnaire.

## ***Measures***

### ***Anthropometrics***

During the week prior to starting the experimental snack sessions, height and weight measurements were taken from participants. Before collecting height and weight measures, children were asked to remove their shoes and excess clothing. Height was measured to the nearest tenth of an inch, using a portable stadiometer (SECA, ITIN Scale Company, Brooklyn, NY). Weight was measured using a calibrated portable digital scale (Healthometer Professional, Sunbeam Product Inc. Raton, FL), to the nearest tenth of a pound. Each child's body mass index



( $\text{BMI} = \text{weight}[\text{kg}] / \text{height}[\text{m}^2]$ ) was calculated and BMI scores were compared to the CDC's BMI percentile charts<sup>33</sup>. Children were classified as overweight if they had a BMI at or above the 85<sup>th</sup> percentile but less than the 95<sup>th</sup> percentile, and were classified as obese if they had a BMI at or above the 95<sup>th</sup> percentile<sup>34</sup>. Participants' z-BMI scores were calculated by standardizing the BMI for children's age and gender in relation to the population mean and standard deviation<sup>33</sup>.

### ***Consumption***

Snack foods and beverages were weighed and measured in the Jessie Harris Building, appropriately covered and stored according to food safety standards, and carried by HEAL students next door to the ELC. Foods and beverages were weighed using a calibrated food scale (Denver Instruments SI-8001, Fischer Scientific) before and after snack sessions. Beverages were first measured in ounces and then transferred to 9 oz. or 16 oz. plastic cups to be weighed in grams. Applesauce was served in paper bowls and graham crackers on paper plates, each of which were measured separately before food was added. The total food and container weight were recorded, and the amount consumed was determined by subtracting the final, post-consumption weight of the food and container from this starting, pre-consumption weight. The investigator then calculated energy intake from the gram amounts consumed and the energy density of each food according to the food label.

### ***Liking of Food***

With assistance, participants completed a questionnaire to determine their liking of the foods and beverages used in the study. This was completed once during each session, prior to children being served the snack. The questionnaire, which has been validated in previous research, consists of a three-point Likert-scale. Participants are to choose one of the three points,

represented by a “yucky” face (dislike), a “so-so” face (neutrality), and a “yummy” face (like)<sup>35,36</sup>.

### ***Hunger***

Participants rated their hunger twice in each session, both before and after consuming the snack. The validated questionnaire that was used to assess hunger in this study consists of a three-point Likert-scale. The points on the scale consist of cartoon pictures with stomachs that are shaded to represent fullness or hunger. The cartoon of the empty, unshaded stomach represents “very hungry,” the somewhat shaded stomach represents “a little hungry,” and the fully shaded stomach represents “not hungry.” Previous studies on preschool aged children have included similar scales for measuring hunger<sup>35-37</sup>.

### ***Parental/Guardian Questionnaires***

Responses to four questionnaires were collected from participants’ parents/guardians. These questionnaires included a demographic questionnaire, the Child Feeding Questionnaire (CFQ), the Three Factor Eating Questionnaire (TFEQ), and the Child Eating and Behavior Questionnaire (CEBQ). The demographic questionnaire collected data on parent/guardian and child characteristics including race and ethnicity, as well as parental/guardian marital status and educational background. The CFQ consists of 31 items and 7 factors (perceived responsibility, perceived parent weight, perceived child weight, concern about child weight, restriction, pressure to eat, and monitoring) designed to gather information regarding parental beliefs and habits surrounding the feeding of their children<sup>38</sup>. The TFEQ measures three different aspects of parents’ eating behavior, dietary restraint, disinhibition, and perceived hunger, with a total of 51 items<sup>39</sup>. The final questionnaire, the CEBQ, is designed to examine a child’s tendencies in regards to 8 factors, food responsiveness, enjoyment of food, emotional overeating, desire to

drink, satiety responsiveness, slowness in eating, emotional undereating, and fussiness.

Parents/guardians provided answers regarding their child's behaviors. The tool contains 35 items and gathers information on an individual's eating habits as they relate to the risk of obesity<sup>40</sup>.

### ***Data Analysis***

All statistical analyses were completed using SPSS 21.0, with an alpha level of 0.05. In order to detect any baseline demographic differences between the two classrooms, independent t-tests were completed on continuous data, and Chi-square tests were performed on categorical data. Baseline differences between classrooms were found for two questionnaire scores, so these were included as covariates in subsequent analyses.

To determine any significant differences between the four experimental sessions in each participants' ratings of initial hunger and liking of the foods, a mixed factorial analysis of covariance (ANCOVA), with the between-subjects factor of order, within-subjects factors of beverage type (caloric and non-caloric) and portion size (6 oz. and 12 oz.), and covariates disinhibition and monitoring score (scores from questionnaires that were different between the classrooms) was conducted. A main effect of order on participants' liking of graham crackers was found, so order was used as a covariate in subsequent analyses. A main effect of beverage size on participants' hunger ratings prior to the snack was also noted; to correct for this, simple linear regressions were conducted, using initial hunger ratings as the independent variable and grams of beverage consumed and total energy consumed from the snack as the dependent variables, to generate standardized residual scores.

Repeated measures ANCOVAs with these residual scores were used to analyze two of the dependent variables, grams consumed of the beverage and overall energy intake within the snack (total consumed calories of foods and beverage). The within-subjects factors for these tests

were beverage type and size, and covariates were order, disinhibition, and monitoring scores. A repeated measures ANCOVA was also used to analyze differences in calories consumed of the beverage. The within-subjects factors for this test were beverage type and size, and the covariates were order, disinhibition and monitoring scores, and initial hunger ratings from all sessions. To compare hunger ratings after the snack between conditions, linear regressions were first completed in order to control for differences in hunger ratings before the snack. The regressions, which used initial hunger ratings as the independent variable and final hunger ratings as the dependent variable, generated standardized residual scores. These standardized scores were used in a repeated measures ANCOVA, with the within-subjects factors of beverage type and beverage size, and disinhibition, monitoring score, and order as covariates. For all analyses with repeated measures, Greenhouse Geisser corrections were included to control for sphericity. Post hoc analyses with Bonferroni corrections were completed for significant outcomes.

## RESULTS

### *Participant Characteristics*

#### *Child*

Baseline characteristics of child participants by classroom are listed in Appendix A Table 2. The 26 participants included in the final analyses were  $3.9 \pm 0.6$  ( $M \pm SD$ ) years of age, and predominantly white (73%) and non-Hispanic or Latino (88.5%). Most children were of a healthy weight status (84.6%), and the average BMI percentile of the participants was  $62.2 \pm 20.5$ ile ( $M \pm SD$ ). Between classrooms, no significant differences were found in these characteristics.

#### *Parent*

Baseline characteristics of parents of participants are listed by classroom in Appendix A Table 3. Parents were  $38.1 \pm 5.1$  ( $M \pm SD$ ) years of age, and were predominantly female (80.8%), white (84.6%), and non-Hispanic or Latino (92.3%). Parents were also educated (65.4% Graduate or Professional Education) and married (92.3%). No significant differences were found between classrooms in these characteristics.

Appendix A Table 4 shows mean scores of parental questionnaire responses. Within the TFEQ, the disinhibition factor was significantly different,  $t(24) = 2.509$ ,  $p = 0.019$ , between classrooms. The mean score of classroom 1 was  $6.2 \pm 3.1$ , and the mean score of classroom 2 was  $3.6 \pm 1.9$ . Additionally, the monitoring factor of the CFQ was significantly different,  $t(24) = 2.200$ ,  $p = 0.038$ , between classrooms, with classroom 1's mean score =  $3.5 \pm 0.9$  and classroom 2's mean score =  $2.7 \pm 0.8$ . These variables were included as covariates in subsequent analyses. No differences were found between classrooms for the CEBQ.

### ***Liking of Food***

A mixed-factorial ANCOVA found a significant main effect of order,  $F(1,22) = 5.946$ ,  $p = 0.023$ , for participants' liking ratings of graham crackers. The mean liking rating of graham crackers among sessions for classroom 1 was ( $M \pm SD$ )  $1.5 \pm 0.8$ , and the mean liking rating for classroom 2 was  $1.1 \pm 0.5$ . Thus, order was included as a covariate in subsequent analyses. No significant main effects or interaction occurred for liking of applesauce or beverage. Overall, the mean liking of applesauce and beverage were  $1.3 \pm 0.7$  and  $1.3 \pm 0.7$ , respectively. Mean ratings of liking of foods among conditions are presented in Appendix A Table 5.

### ***Hunger Before Snack***

A mixed-factorial ANCOVA revealed a main effect of beverage size,  $F(1,22) = 6.898$ ,  $p = 0.015$ , on participants' ratings of hunger before the snack. The mean hunger rating before the snack for 6 oz. and 12 oz. conditions were ( $M \pm SD$ )  $1.2 \pm 0.5$  and  $1.3 \pm 0.5$ , respectively. To control for this difference, simple linear regressions were conducted between this variable and the main dependent variables in the investigation. Standardized residual scores were generated for each of the dependent variables for use in subsequent ANCOVAs. There were no other significant main effects or interaction. See Appendix A Table 5 for mean ratings of hunger before and after snack sessions among conditions.

### ***Grams and Energy of Beverage Consumed***

For grams of beverage consumed, a repeated measures ANCOVA revealed a significant main effect of size,  $F(1,22) = 6.812$ ,  $p = 0.016$ , on grams of beverage consumed. When provided with a larger portion size of beverage, children consumed significantly more grams of the beverage than they did when provided with a smaller portion size of the beverage. The mean gram weights of beverage consumed in the 6 oz. and 12 oz. conditions were ( $M \pm SD$ )  $121.3 \pm$

59.9 g and  $173.9 \pm 101.7$  g, respectively. Appendix A Figure 2 shows the mean gram weights of beverage consumed among conditions.

For calories consumed of beverage, a significant interaction of beverage type and size,  $F(1,22) = 5.993$ ,  $p = 0.025$ , occurred. The mean calories consumed of beverage among the conditions were as follows ( $M \pm SD$ ): 6 oz. water =  $0.0 \pm 0.0$  kcal; 6 oz. juice =  $69.4 \pm 26.4$  kcal; 12 oz. water =  $0.0 \pm 0.0$  kcal; 12 oz. juice =  $109.5 \pm 56.2$  kcal. With the exception of the two water conditions, significant difference ( $p < 0.001$ ) was found between all conditions. A main effect of beverage type occurred,  $F(1,22) = 8.896$ ,  $p = 0.008$ , such that significantly more calories were consumed when juice was the beverage than when water was the beverage. The mean calories consumed of beverage in the water and juice conditions were ( $M \pm SD$ )  $0.0 \pm 0.0$  kcal and  $89.5 \pm 41.3$  kcal, respectively. A significant main effect was also observed for beverage size,  $F(1,22) = 5.993$ ,  $p = 0.025$ , in that significantly more calories were consumed in the 12 oz. beverage conditions than the 6 oz. beverage conditions. The mean calories consumed of beverage in the 6 oz. and 12 oz. conditions were ( $M \pm SD$ )  $34.7 \pm 13.2$  kcal and  $54.8 \pm 28.1$  kcal, respectively.

### ***Overall Calorie Intake from Snack***

For caloric intake for the total snack, a repeated measures ANCOVA revealed no significant main effects or interactions ( $p > 0.05$ ). Overall mean total caloric consumption during snack sessions was as follows ( $M \pm SD$ ): 6 oz. juice =  $153.4 \pm 61.9$  kcal; 12 oz. juice =  $198.0 \pm 60.7$  kcal; 6 oz. water =  $92.5 \pm 75.6$  kcal; 12 oz. water =  $117.7 \pm 69.1$  kcal.

As no significant differences were found in total caloric intake during snack sessions, yet significant difference in caloric intake were found for beverage in the snack sessions, a follow-up analysis was completed on calories consumed of applesauce and graham crackers to have a better

understanding of whether compensation was occurring. To do so, regressions matching initial hunger ratings and calories consumed of applesauce and graham crackers from each session were conducted to correct for differences in hunger ratings before the snack. For these regressions, the independent variable was initial hunger rating and the dependent variable was energy from applesauce and graham crackers. Resulting standardized residual scores were used in a repeated measures ANCOVA to compare the amount of calories consumed from applesauce and graham crackers among sessions. The within-subjects factors were beverage type and beverage size, and disinhibition, monitoring score, and order were covariates. No significant main effects or interactions ( $p > 0.05$ ) were found. Mean caloric consumption of applesauce and graham crackers combined in snack sessions was as follows ( $M \pm SD$ ): 6 oz. juice =  $84.0 \pm 66.9$  kcal; 12 oz. juice =  $88.5 \pm 64.1$  kcal; 6 oz. water =  $92.5 \pm 75.6$  kcal; 12 oz. water =  $117.7 \pm 69.1$  kcal. Appendix A Figure 3 shows the amount of calories consumed from applesauce and graham crackers within each snack condition, along with the calories consumed from the beverage.

### ***Hunger After Snack***

A repeated measures ANCOVA revealed no significant differences between hunger ratings after snack among conditions. See Appendix A Table 5 for mean ratings of hunger before and after snack sessions among conditions.



## DISCUSSION

The purpose of this study was to examine the impact of beverage type (caloric [100% fruit juice] vs. non-caloric) and beverage portion size (6 oz. vs. 12 oz.) on energy intake during a snack in preschool-aged children. This study found that beverage type and beverage portion size interacted to influence the amount of energy consumed from the beverage. Children who were given juice in a 12 oz. portion consumed the most energy from the beverage. Additionally, for the amount of beverage consumed, a significant effect of beverage portion size on the amount of beverage consumed occurred. In other words, children consumed significantly more water or juice in the larger portion size conditions than in the smaller portion size conditions. The results of this study suggest that children's beverage intake increases with larger beverage portion sizes.

The results that portion size influences intake is consistent with previous research on portion sizes in children. While no research has previously been conducted with children regarding changes in portion size of beverages and beverage intake, research has found that children consume more solid food when the food is provided in larger portions<sup>27,29-31</sup>. Rolls et al. observed a 58% increase in intake of a macaroni and cheese entrée when 5-year-old children were served a larger portion within a meal, but this effect was not seen in younger children<sup>27</sup>. Savage et al., showed a 37% increase in three- to five-year-old children's caloric intake from a macaroni and cheese entrée when the portion size was increased from 100 grams to 400 grams<sup>30</sup>, while Fisher et al. showed a 25% increase in three- to five-year-old children's intake of a macaroni and cheese entrée within a meal when the size of the entrée was doubled<sup>29</sup>. Lastly, in a study by Mathias et al., four- to six-year-old children consumed 37% more vegetables, and 70% more fruit in an eating bout when served a larger portion<sup>31</sup>. Therefore, the current investigation,

in which a 43% increase in beverage intake was shown between 6 oz. and 12 oz. beverage portion sizes, further supports the proposition that larger portion sizes lead to greater intake.

The finding that greater energy intake from the beverage occurred when the beverages were served in larger portions and when the beverage was juice as compared to water has important implications. This suggests that providing children with larger amounts of a calorie-containing beverage, such as juice, is likely to cause increased consumption of the juice, and in turn, increased energy intake from the calorie-containing beverage. This poses a potential problem when caloric beverages are provided in unmeasured portions at meals or if they are provided throughout the day in containers holding more than one serving size. These practices regarding caloric beverages may increase energy intake from these beverages. This study indicates that monitoring the portion sizes of caloric beverages provided to children may be important.

This study suggests that overall intake in a snack is elevated when a caloric beverage is served with the snack. Average caloric intake in the snack was approximately 67% higher when juice was consumed with the snack compared to when water was consumed. However, while this finding was not statistically significant, it is important to note that the effect size for the difference in total caloric intake between juice and water conditions was large ( $d = 1.05$ ).

While this investigation's sample size is large enough to detect significance with this effect size, the large number of variables that needed to be controlled in the analyses may have decreased the power available to detect differences. Additionally, when energy intake from applesauce and graham crackers was examined across conditions, mean intake from these components was approximately 18% lower in juice conditions than in water conditions. This effect size ( $d = 0.21$ ) is very small, indicating that very little compensation occurred.

The findings of this investigation are inconsistent with those of previous research showing that children compensate their energy intake to liquid energy consumption. Birch et al., using liquid preloads before snack time, showed a significant effect of a liquid caloric preload on subsequent snack intake<sup>25</sup>. Children consumed significantly less energy from snack foods after drinking sugar sweetened Kool-Aid and an aspartame sweetened/high-caloric drink, compared to consumption after drinking water or an aspartame-sweetened Kool-Aid drink. In Birch's study, participants exhibited compensation for the calories consumed in beverages when the beverages were consumed prior to a snack. The results of the current investigation are inconsistent with this finding, and one potential explanation for this discrepancy is that an individual's ability to compensate for energy intake may be different when the beverage and food are consumed simultaneously rather than independently. Methodological differences, therefore, may have contributed to the lack of statistical difference in caloric intake in the present study.

This study had a number of limitations and strengths. One limitation was that the study sample was homogenous, with the majority of participants being white and from families of higher socio-economic status. Additionally, this investigation's analyses did not examine the effect of participant's weight status on outcomes. The study's population was mixed in terms of weight status. It is possible that different outcomes would result from a study population consisting of more overweight and obese children due to these children potentially exhibiting eating behaviors different from those of healthy weight children. However, among the strengths of this study was that objective measures of food and beverage intake were used. Additionally, the snack sessions were held in the children's natural setting at their normal snack time and children consumed the same lunch prior to all experimental snack sessions. The foods chosen for the investigation were also familiar to the children, thus minimizing a novelty effect.

In conclusion, this study found an impact of beverage type and beverage portion size on the amount of beverage consumed. Children consume more of a beverage when it is served in a larger portion, and due to the caloric nature of juice, additional energy is consumed when juice is served in larger portions. Since this study suggests that increased energy from a beverage at a snack is not compensated for, overall energy intake in a snack served with a caloric beverage may then be elevated.

This study found no significant difference in total caloric snack intake between conditions, although total caloric intake was higher when children consumed juice with the snack, by approximately 67%. While calories consumed from applesauce and graham crackers tended to be slightly lower when juice was consumed with the snack, the effect was very small. This indicates that the children did not exhibit compensation to calories consumed from the beverage. Therefore, it can be surmised that consuming any caloric beverage, including juices, flavored milks, and soft drinks, with a meal or snack may increase overall meal or snack intake. Future studies should investigate potential compensation within a larger eating bout, such as during lunch or dinner as opposed to a snack, as well as the mechanism behind compensatory behavior with beverages served before and alongside other food. This will enhance current understanding of energy intake and regulation in children.

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## **APPENDICES**

## **APPENDIX A: TABLES AND FIGURES**

Table 1. Visual of Study Design

	Session						
	Week 0	Week 1 (practice)	Week 2 (practice)	Week 3	Week 4	Week 5	Week 6
<b>Order 1 N=14</b>	Informed consent and questionnaires	6 oz. water + snack	12 oz. juice + snack ----- Anthro- pometrics	6 oz. juice + snack	12 oz. water + snack	6 oz. water + snack	12 oz. juice + snack
<b>Order 2 N=16</b>	Informed consent and questionnaires	12 oz. juice + snack	6 oz. water + snack ----- Anthro- pometrics	12 oz. water + snack	6 oz. juice + snack	12 oz. juice + snack	6 oz. water + snack

Table 2. Characteristics of Child Participants at Baseline

	Classroom 1 n= 12	Classroom 2 n = 14
<b>Age</b> (y), M $\pm$ SD	3.8 $\pm$ 0.6	4.0 $\pm$ 0.5
<b>Height</b> (in), M $\pm$ SD	39.2 $\pm$ 2.0	40.6 $\pm$ 1.7
<b>Weight</b> (lbs), M $\pm$ SD	35.0 $\pm$ 3.5	38.3 $\pm$ 4.5
<b>zBMI</b> , M $\pm$ SD	0.2 $\pm$ 0.6	0.5 $\pm$ 0.7
<b>BMI Percentile</b> , M $\pm$ SD	58.2 $\pm$ 20.3	65.6 $\pm$ 20.7
<b>Overweight or obese</b> (%)	8.3	21.4
<b>Sex</b> (%)		
Male	58.3	42.9
Female	41.7	57.1
<b>Race</b> (%)		
White	50.0	92.9
Asian	25.0	0.0
American Indian/Alaskan Native	8.3	0.0
Other	16.7	7.1
<b>Hispanic or Latino</b> (%)	8.3	14.3

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

Table 3. Characteristics of Parents of Participants at Baseline

	Classroom 1 n = 12	Classroom 2 n = 14
<b>Age (y), M <math>\pm</math> SD</b>	37.0 $\pm$ 4.2	39.1 $\pm$ 5.8
<b>Sex (%)</b>		
Male	16.7	21.4
Female	83.3	78.6
<b>Race (%)</b>		
White	66.7	100.0
Asian	16.7	0.0
American Indian/Alaskan Native	8.3	0.0
Other	8.3	0.0
<b>Hispanic or Latino (%)</b>	8.3	7.1
<b>Relationship to Child (%)</b>		
Father	16.7	21.4
Mother	83.3	78.6
<b>Education (%)</b>		
Some College (Less than 4 years)	16.7	0.0
College/University degree	8.3	42.9
Graduate or Professional Education	75.0	57.1
<b>Marriage Status (%)</b>		
Married	91.7	92.9
Separated	0.0	7.1
Never Married	8.3	0.0

M  $\pm$  SD = Mean  $\pm$  standard deviation; % = percentage.

Table 4. Mean Score Responses from Parental Questionnaires (M  $\pm$  SD)

Questionnaire	Classroom 1 (n = 12)	Classroom 2 (n = 16)
<b>Child Feeding Questionnaire factors</b>		
Perceived responsibility <sup>1</sup>	4.3 $\pm$ 0.7	4.5 $\pm$ 0.5
Perceived parent weight <sup>2</sup>	3.0 $\pm$ 0.5	3.0 $\pm$ 0.2
Perceived child weight <sup>2</sup>	2.7 $\pm$ 0.4	2.8 $\pm$ 0.4
Concern about child weight <sup>3</sup>	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
Restriction <sup>4</sup>	2.5 $\pm$ 0.7	2.7 $\pm$ 0.4
Pressure to eat <sup>4</sup>	2.9 $\pm$ 1.2	2.5 $\pm$ 0.8
<b><i>Monitoring</i><sup>1</sup></b>	<b>3.5 <math>\pm</math> 0.9</b>	<b>2.7 <math>\pm</math> 0.8</b>
<b>Three Factor Eating Questionnaire factors</b>		
Dietary restraint <sup>6</sup>	10.5 $\pm$ 5.7	11.1 $\pm$ 4.9
<b><i>Disinhibition</i><sup>7</sup></b>	<b>6.2 <math>\pm</math> 3.1</b>	<b>3.6 <math>\pm</math> 1.9</b>
Perceived hunger <sup>8</sup>	4.2 $\pm$ 2.0	2.8 $\pm$ 2.3
<b>Child Eating &amp; Behavior Questionnaire factors</b>		
Food responsiveness <sup>8</sup>	9.8 $\pm$ 2.5	11.5 $\pm$ 3.9
Enjoyment of food <sup>8</sup>	13.8 $\pm$ 4.8	14.3 $\pm$ 3.6
Emotional overeating <sup>8</sup>	6.1 $\pm$ 2.1	6.5 $\pm$ 1.7
Desire to drink <sup>8</sup>	7.7 $\pm$ 3.1	7.3 $\pm$ 3.2
Satiety responsiveness <sup>8</sup>	18.3 $\pm$ 3.8	16.3 $\pm$ 2.9
Slowness in eating <sup>8</sup>	13.8 $\pm$ 4.0	11.4 $\pm$ 2.7
Emotional undereating <sup>8</sup>	11.0 $\pm$ 2.6	12.0 $\pm$ 3.9
Fussiness <sup>8</sup>	18.2 $\pm$ 7.0	17.6 $\pm$ 4.8

M  $\pm$  SD = Mean  $\pm$  standard deviation; Bolded and italicized numbers denotes significant difference (p<0.05) between classrooms.

CFQ Factors: <sup>1</sup>Perceived 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. <sup>2</sup>Perceived parent weight and perceived child weight scored on a 5-point scale: 1 = markedly underweight; 2 = underweight; 3 = normal; 4 = overweight; 5 = markedly overweight. <sup>3</sup>Concern about child weight scored on a 5-point scale: 1 = unconcerned; 2 = a little concerned; 3 = concerned; 4 = fairly concerned; 5 = very concerned. <sup>4</sup>Restriction and pressure to eat scored on a 5 point scale: 1 = disagree; 2 = slightly disagree; 3 = neutral; 4 = slightly agree; 5 = agree.

TFEQ Factors: <sup>5</sup>Dietary restraint (factor I) is scored over a range of 0 to 21 with lower values representing lower dietary restraint. <sup>6</sup>Disinhibition (factor II) is scored over a range of 0 to 16 with lower values representing lower disinhibition. <sup>7</sup>Perceived hunger (factor III) is scored over a range of 0 to 14 with lower values representing lower perceived hunger.

CEBQ Factors: <sup>8</sup>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 5. Liking of Food and Hunger Ratings (M  $\pm$  SD)

	<b>6 oz. Juice</b>	<b>12 oz. Juice</b>	<b>6 oz. Water</b>	<b>12 oz. Water</b>
<b>Liking of Applesauce*</b>	<b>1.2 <math>\pm</math> 0.7</b>	<b>1.4 <math>\pm</math> 0.8</b>	<b>1.3 <math>\pm</math> 0.7</b>	<b>1.2 <math>\pm</math> 0.7</b>
Classroom 1 (n = 12)	1.0 $\pm$ 0.0	1.0 $\pm$ 0.0	1.0 $\pm$ 0.0	1.0 $\pm$ 0.0
Classroom 2 (n = 14)	1.4 $\pm$ 0.9	1.7 $\pm$ 1.0	1.6 $\pm$ 0.9	1.4 $\pm$ 0.9
<b>Liking of Graham Crackers*</b>	<b>1.3 <math>\pm</math> 0.7</b>	<b>1.3 <math>\pm</math> 0.7</b>	<b>1.2 <math>\pm</math> 0.6</b>	<b>1.3 <math>\pm</math> 0.7</b>
Classroom 1 (n = 12)	1.4 $\pm$ 0.8 <sup>a</sup>	1.5 $\pm$ 0.9 <sup>a</sup>	1.4 $\pm$ 0.8 <sup>a</sup>	1.5 $\pm$ 0.9 <sup>a</sup>
Classroom 2 (n = 14)	1.1 $\pm$ 0.5 <sup>b</sup>	1.1 $\pm$ 0.5 <sup>b</sup>	1.0 $\pm$ 0.3 <sup>b</sup>	1.1 $\pm$ 0.5 <sup>b</sup>
<b>Liking of Juice*</b>	<b>1.2 <math>\pm</math> 0.6</b>	<b>1.1 <math>\pm</math> 0.4</b>		
Classroom 1 (n = 12)	1.2 $\pm$ 0.6	1.2 $\pm$ 0.6		
Classroom 2 (n = 14)	1.2 $\pm$ 0.6	1.0 $\pm$ 0.0		
<b>Liking of Water*</b>			<b>1.3 <math>\pm</math> 0.7</b>	<b>1.5 <math>\pm</math> 0.8</b>
Classroom 1 (n = 12)			1.2 $\pm$ 0.6	1.5 $\pm$ 0.8
Classroom 2 (n = 14)			1.5 $\pm$ 0.9	1.4 $\pm$ 0.9
<b>Hunger Before Snack**</b>	<b>1.1 <math>\pm</math> 0.3<sup>a</sup></b>	<b>1.2 <math>\pm</math> 0.4<sup>b</sup></b>	<b>1.3 <math>\pm</math> 0.7<sup>a</sup></b>	<b>1.3 <math>\pm</math> 0.5<sup>b</sup></b>
Classroom 1 (n = 12)	1.1 $\pm$ 0.3	1.3 $\pm$ 0.5	1.1 $\pm$ 0.3	1.3 $\pm$ 0.7
Classroom 2 (n = 14)	1.1 $\pm$ 0.4	1.1 $\pm$ 0.4	1.5 $\pm$ 0.9	1.2 $\pm$ 0.4
<b>Hunger After Snack**</b>	<b>2.8 <math>\pm</math> 0.6</b>	<b>2.8 <math>\pm</math> 0.6</b>	<b>2.8 <math>\pm</math> 0.6</b>	<b>2.7 <math>\pm</math> 0.7</b>
Classroom 1 (n = 12)	3.0 $\pm$ 0.0	3.0 $\pm$ 0.0	3.0 $\pm$ 0.0	2.8 $\pm$ 0.6
Classroom 2 (n = 14)	2.6 $\pm$ 0.8	2.6 $\pm$ 0.8	2.6 $\pm$ 0.8	2.6 $\pm$ 0.8

M  $\pm$  SD = Mean  $\pm$  standard deviation. Superscripts denote significant difference (p<0.05).

\*Scoring for Liking questionnaires was as follows: 1 = Yummy; 2 = So-So; 3 = Yucky

\*\*Scoring for Hunger questionnaire was as follows: 1 = Very hungry; 2 = Kind-of hungry/A little hungry; 3 = Not hungry



Figure 1. Participant Flow Chart



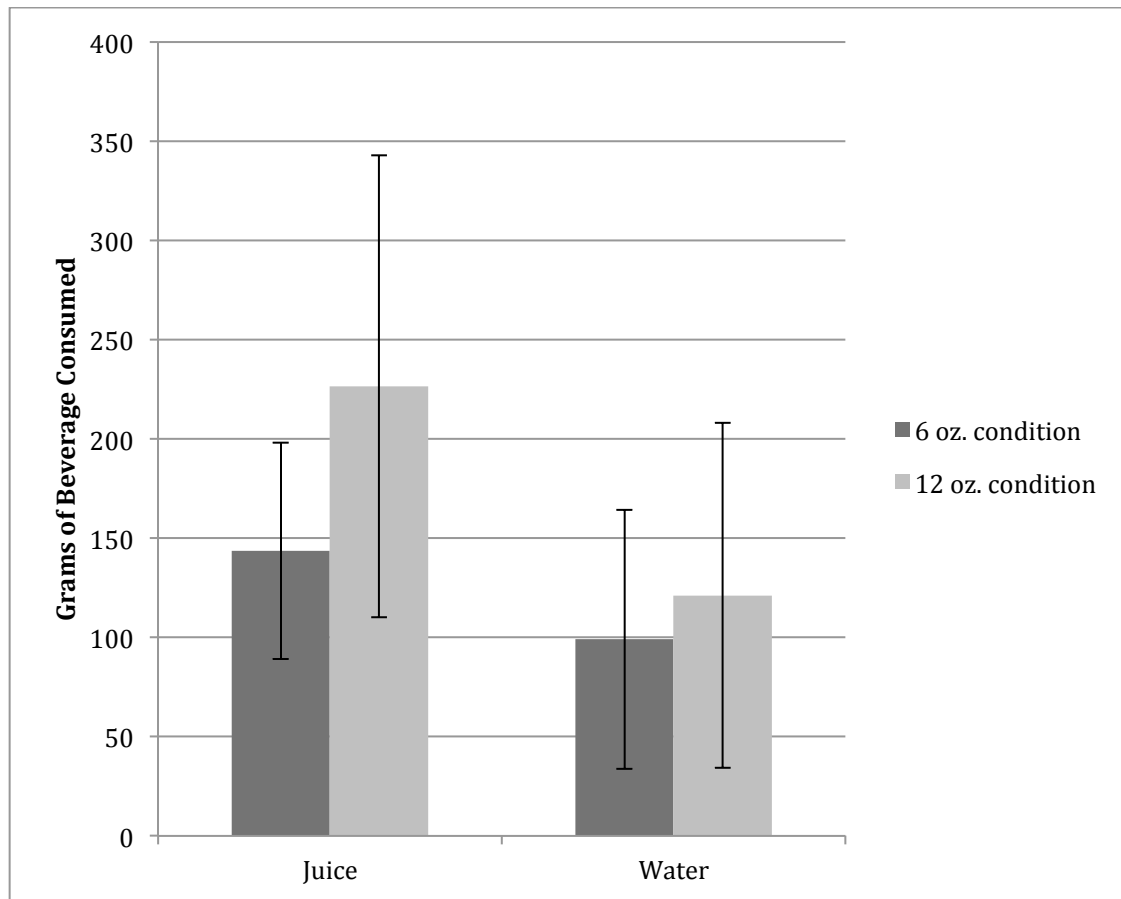


Figure 2. Grams of Beverage Consumed by Beverage and Size Conditions. Data are  $M \pm SD$ . A significant main effect of size was found ( $p = 0.016$ ) with more grams consumed in 12 oz. conditions than in 6 oz. conditions.

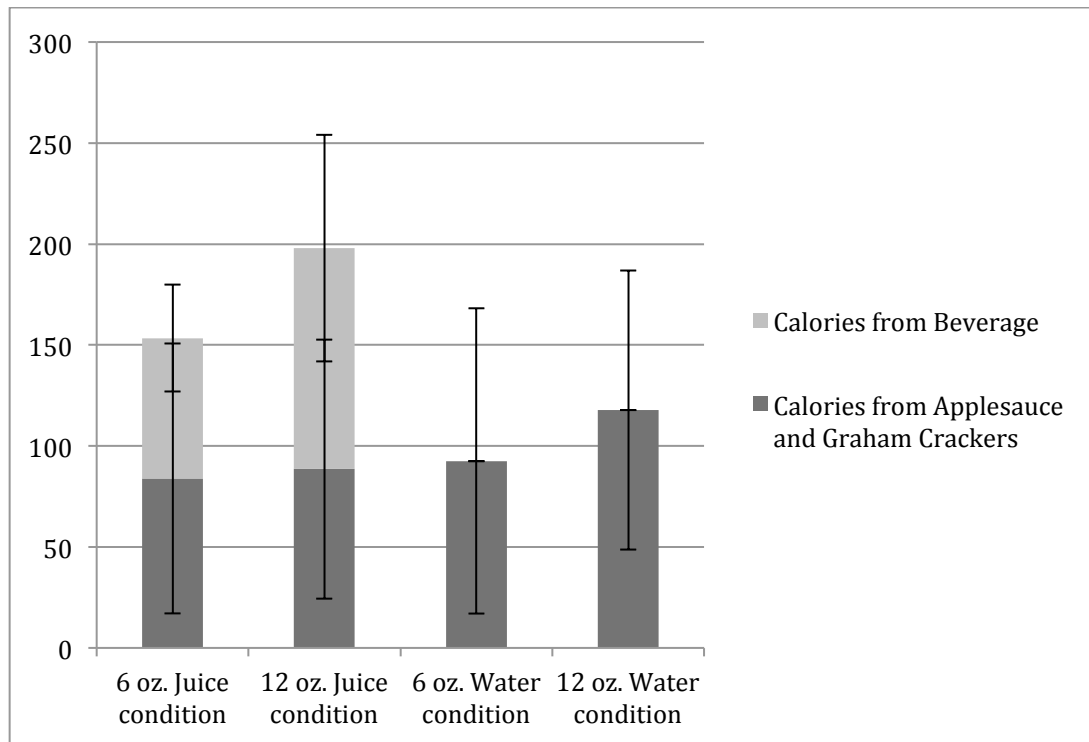


Figure 3. Calories Consumed from Beverage and Snack Foods Among Conditions. Data are  $M \pm SD$ . There were no significant main effects or interactions ( $p > 0.05$ ) found among conditions for calories consumed from applesauce and graham crackers combined.

## **APPENDIX B: RECRUITMENT, FORMS, AND QUESTIONNAIRES**

## FORM B APPLICATION

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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.

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### FORM B

IRB # \_\_\_\_\_

Date Received in OR \_\_\_\_\_

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## THE UNIVERSITY OF TENNESSEE

### Application for Review of Research Involving Human Subjects

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#### I. IDENTIFICATION OF PROJECT

**1. Principal Investigator:**

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**Department:**

Nutrition

**2. Project Classification:**

Thesis

3. **Title of Project:**  
**Impact of Portion Size of Fruit Juice on Fruit Juice Consumption and Overall Energy Intake During a Snack in Preschoolers**
4. **Starting Date:**  
Upon IRB approval or September 2013
5. **Estimated Completion Date:**  
**May 2013**
6. **External Funding (if any):**
  - **Grant/Contract Submission Deadline:**
  - **Funding Agency:**
  - **Sponsor ID Number (if known):**
  - **UT Proposal Number (if known):**

## **II. PROJECT OBJECTIVES**

### **Specific Aims**

It is estimated that as of 2010, approximately 12.1% of toddlers and preschool-aged children and 17% of children and adolescents in the United States were obese<sup>1</sup>. Excess body weight can contribute to serious illnesses and consequences can be lifelong and potentially deadly<sup>2,3</sup>. As the rate of childhood overweight and obesity has climbed, so has the proportion of calories provided daily to children's diets by energy-containing beverages, and in particular 100% fruit juices<sup>4</sup>. Research has suggested a link between 100% fruit juice consumption and adiposity in children and adolescents<sup>5-9</sup>. While one study suggests that children may compensate their overall energy intake in response to liquid calories better than adults<sup>10</sup>, it has been suggested that the relationship between 100% fruit juice and adiposity in children may be a consequence of lack of complete compensation to energy consumed from beverages. Incomplete compensation could contribute to excessive energy intake, leading to excessive weight gain. Furthermore, over the past few decades, larger portion sizes have become more common and have arisen as a concern<sup>11</sup>, because children appear to be responsive to portion size and consume greater amounts with larger portion sizes<sup>12-15</sup>. Increased portions of juice may further exacerbate the issues related to incomplete compensation. Thus, this study will investigate whether beverage type (caloric [100% fruit juice] vs. non-caloric) and beverage portion size (6 oz vs. 12 oz) impact on energy intake during a snack. For this investigation, four experimental snack sessions will occur, in which beverage type and portion size will be manipulated, but provided amount and types of snack foods will be kept identical across all four session. Participants will be approximately 36, 3- to 5-year-old children at the Early Learning Center. These participants comprise two classrooms, each of which will be randomized to a different order of experimental conditions. Four different conditions will take place, 6 oz., caloric beverage (100% fruit juice) condition; 6 oz., non-caloric beverage (water) condition; 12 oz., caloric beverage condition; and 12 oz., non-caloric beverage condition. It is hypothesized that:

1. A main effect of beverage type will occur, such that the caloric beverage conditions will result in a greater energy intake from beverage and greater energy intake from overall snack (which includes the beverage) than the non-caloric beverage conditions.
2. A main effect of beverage portion size will occur, such that the 12 oz. portion size conditions will result in a greater gram intake from the beverage than 6 oz. portion size conditions.
3. An interaction of beverage type and portion size will occur, such that the 12 oz. caloric beverage condition will result in the greatest energy intake from the overall snack (which includes the beverage) as compared to all other conditions.

## Background and Significance

### ***Childhood Obesity and Beverage Consumption***

The prevalence of childhood overweight, defined as a body mass index (BMI) at or above the 85<sup>th</sup> percentile but less than the 95<sup>th</sup> percentile, and obesity, defined as a BMI at or above the 95<sup>th</sup> percentile, in the U.S. has steadily increased over the past several decades<sup>16</sup>. Data from the National Health and Nutrition Examination Survey indicates that as of 2010, 12.1% of toddlers and preschool-aged children (children between the ages of two and five) were obese.<sup>1</sup> Furthermore, nearly 17% of children and adolescents between the ages of two and seventeen in the US were obese<sup>1</sup>. Obesity leads to serious medical implications for children, including type 2 diabetes and increased blood cholesterol and blood pressure, which are contributors to the development of cardiovascular disease later in life<sup>2</sup>. Increased body weight also causes bone and joint problems in children, as well as breathing problems such as sleep apnea<sup>3</sup>. However, it can be argued that from a child's perspective, the most detrimental consequence of childhood overweight and obesity is social discrimination<sup>2</sup>. Children who are overweight or obese are ostracized from their peers and are often teased and bullied<sup>2</sup>.

The rate of overweight and obesity in children has increased by over 200% since 1980<sup>3</sup>. This significant increase is paradigmatic of the changes that have occurred in children's dietary patterns. Of growing interest is the increase in the proportion of daily calories provided to children's diets by energy-containing beverages, and in particular, 100% fruit juices<sup>4</sup>, which has occurred over the past several decades<sup>4,17,18</sup>. As of 2004, children and adolescents of all ages were consuming at least 10 to 15% of their daily calories from energy-containing beverages, accounting for about 270 of total daily calories<sup>4</sup>.

The American Academy of Pediatrics (AAP), recommends a 100% fruit juice intake of no more than 4 to 6 ounces per day for children 1 to 6 years old, and an intake of no more than two, 6-ounce servings per day for children 7 to 18 years of age<sup>19,20</sup>. The current guidelines for fruit and fruit juice consumption presented by MyPlate indicate that a cup of 100% fruit juice counts toward the daily fruit recommendation, but that the majority of fruit should be consumed as whole or cut-up fruit due to the fiber content present in these forms and not in juice<sup>21</sup>. Recommended fruit intake according to these guidelines depends on age and gender, but varies upwards of 1 cup per day.

Although 100% fruit juice is more nutritive than other energy-containing beverages (i.e., sugar sweetened beverages), and thus is seen as a more healthful option than sodas or coffee drinks, research has indicated a link between 100% fruit juice consumption and adiposity in children. The majority of studies in this area have been cross-sectional and longitudinal analyses using convenience samples, so some controversy remains in determining a cause and effect relationship between 100% fruit juice intake and childhood obesity<sup>19</sup>.

For example, in a study by Dennison et al., dietary intakes of two- and five-year old children were analyzed for fruit juice consumption and related to anthropometric measures<sup>5</sup>. Results indicated that the prevalence of shorter stature was higher among those children who consumed excess fruit juice. Additionally, among the children reported to have consumed 12 fluid ounces or more of fruit juice per day, there was a higher prevalence of overweight. This indicates a correlation between higher fruit juice intake and increased weight status and decreased stature.

In a follow-up examination by Dennison et al., different fruit juices were analyzed for their correlation with weight and height in children<sup>6</sup>. This study showed a relationship between weight status and stature for apple and grape juices only. Higher consumption of these two fruit juices was associated with shorter stature, and excess consumption of apple juice was associated with obesity<sup>6</sup>. Lastly, a study by Melgar-Quir6nez investigated the effect of juice intake on adiposity in low-income Mexican-American children and provided similar results<sup>7</sup>. In this cross sectional analysis, a significant correlation was observed between higher fruit juice consumption and adiposity.

Longitudinal studies have also found a relationship between fruit juice intake and weight status in children. Libuda et al. found a positive correlation between fruit juice intake and BMI in adolescent girls in a five year German study<sup>8</sup>. The mean age of girls at the beginning of the study was 11.8, and the mean age at the end was 16.8. During this five year study period, increased fruit juice intake over time was associated with increases in BMI. Other studies found this effect for children already at higher weight status. A longitudinal examination by Faith et al. measured changes in adiposity of preschool children between the ages of 18 and 48 months who were from low-income families<sup>9</sup>. This study revealed that in children who were already overweight or at serious risk of overweight, increased fruit juice intake over the study period was correlated with increased body mass<sup>9</sup>. In a study by Welsh et al., data was collected on

juice and sweetened drink consumption and body fatness among two- and three-year-old children at two time points<sup>22</sup>. At the beginning of the study, data on children's beverage consumption were gathered from parents, and the children's heights and weights were measured. Height and weight data were then collected one year later, and an association was found between fruit juice consumption patterns at baseline and body fatness in children one year later. This association was observed only for children who entered the study as overweight<sup>22</sup>. For these children, the results showed a positive association between daily fruit juice intake and an increased risk of remaining overweight one year later.

### ***The Relationship Between Energy Compensation and Beverage Consumption***

Weight gain primarily occurs due to an increase in caloric intake, a decrease in caloric expenditure, or both<sup>23</sup>. An individual is said to be in positive energy balance when caloric intake exceeds caloric expenditure, and the excess energy is stored in the body as fat, causing weight gain that can potentially lead to obesity<sup>24</sup>. Caloric intake consists of the various nutrients taken in from food and beverages, including fat, protein, carbohydrates, and alcohol; when consumption increases, the energy expenditure required to maintain a steady weight also increases.

Caloric beverages are thought to provide energy without offering other properties that solid food provides. Most importantly, liquids lack the ability to satiate the consumer<sup>25</sup>. Compared to solid food calories, compensation does not occur completely for liquid calories<sup>25,26</sup>. In other words, in experimental settings, participants adjust food intake in response to calories consumed from preloads of solid as compared to liquid forms<sup>26</sup>. For example, in a crossover design, DiMeglio and Mattes measured men and women's energy intake throughout the day when accompanied by liquid and solid carbohydrate loads<sup>26</sup>. The liquid load was given as caffeine-free soda, and the solid load was given as jelly beans. Participants consumed each type of preload daily for four weeks, and were allowed to consume the carbohydrate load whenever they chose. For the four weeks, they were also allowed to consume other foods freely throughout the day. Six times during each four-week treatment period, random dietary recalls were used to assess free-feeding energy intake under each condition.

Results of the dietary records in this study showed that free-feeding energy consumption was significantly lower during the solid carbohydrate load phase as compared to the liquid carbohydrate load phase<sup>26</sup>. Thus greater compensation occurred in the solid load condition, and less compensation occurred during the liquid load condition. Furthermore, when overall energy intake, intake from the loads and free-feeding situations, was examined, greater intake occurred during the liquid load as compared to the solid load phase. One proposed mechanism for the phenomenon observed in DiMeglio's study is that chewing the solid carbohydrate load offers a satiety signal that the liquid load is not able to provide<sup>26</sup>. The brain does not receive satiety signals as readily after consumption of liquids, so the individual is not as apt to decrease subsequent energy intake in order to compensate for what was consumed as liquid. There are also elements in the stomach that sense the presence of food and provide feedback via endocrine and nervous hormones that influence fullness and hunger. Liquids are able to pass from the stomach more quickly than solids, bypassing these mechanisms and leaving an individual with less sense of satiation and satiety<sup>26</sup>.

The lack of compensation to energy-containing beverages may lead to excess caloric consumption. Thus energy intake from fruit juice may not be compensated in children, leading to the positive relationship between fruit juice intake and adiposity observed in studies on young children<sup>5-9</sup>.

### ***Beverages and Energy Compensation During a Meal***

While evidence suggests that the consumption of energy-containing beverages contributes to the development of childhood obesity<sup>5-9</sup>, little research has examined the impact of these beverages on overall intake when consumed at various times during the day in children. Some studies have shown that consuming these beverages between meals leads to weight gain<sup>27</sup>. Most evidence proposes that this occurs due to the added calories to the diet; however, there has been less exploration of energy-containing beverages' contribution to dietary intake when consumed alongside other foods, such as at mealtimes, in children.

Ideally, due to the excess calories provided in energy-containing beverages, consumption of caloric liquids at mealtimes would lead to less overall energy intake during the meal. This has been explored in adults, with adults showing poor compensation to energy-containing beverages<sup>25,26,28-31</sup>.

In a study by DellaValle et al., 45 normal weight, overweight, and obese women between the ages of 18 and 60 years participated in a within-subjects experiment testing the effects of five different

caloric and non-caloric beverages on food intake during a meal<sup>28</sup>. Beverages of varying energy and nutrient content were tested, and beverages included tap water, fruit juice, regular and diet cola, and 1% milk. While instructed to consume the entire beverage portion intermittently throughout the meal, participants consumed pasta, sauce, bread, salad and cookies ad libitum. Consumed portions of the meal were measured, as were measures of hunger and fullness before and after eating.

Measures of satiety were unaffected by the type of beverage consumed; however overall energy intake consumed during the meal was greater in the conditions in which energy-containing beverages were consumed. The greater overall energy intake in the meals was due to the excess energy provided by the beverages and the lack of compensatory behavior of participants in adjusting food intake in relation to the amount of energy consumed in liquid form<sup>28</sup>. Neither normal weight nor overweight women compensated for their liquid energy intake by decreased food intake accordingly.

Most recently, in 2013 Panahi et al. conducted a study to further investigate potential compensation in adults<sup>31</sup>. Twenty-nine male and female participants consumed ad libitum portions of pizza lunches, alongside ad libitum portions of five test beverages—water, 1% milk, orange juice, regular and diet cola. Results showed that beverage type had no impact on the amount of pizza eaten. Thus, no evidence was found of compensatory behavior when consuming beverages of any type. Due to the greater amount of energy in 1% milk, orange juice, and regular cola as compared to water and diet cola, energy intake was higher in conditions in which energy-containing beverages were consumed.

Studies evaluating the impact of different energy- and non-energy-containing beverages on food intake during meals have mostly been conducted in adults. The role of energy-containing beverages on compensatory behavior in children has received less attention<sup>31</sup>. Understanding children's ability to compensate to energy-containing beverages during a meal or snack would be valuable in gaining a better understanding of how energy-containing beverages influence children's intake and weight status.

One study has examined children's ability to compensate to energy consumed from beverages. In 1989, Birch et al. conducted a study to examine the impact of energy-containing beverages served as preloads on subsequent food intake during a snack in children<sup>10</sup>. The investigation used a within-subjects crossover design. Preschool-aged children's ad libitum consumption of common snack foods was measured at either 0, 30, or 60 minutes after consuming four different beverages. The four preload beverages were water, Kool-Aid sweetened with sugar, an aspartame-sweetened beverage, and an aspartame-sweetened beverage to which a non-carbohydrate calorie load was added. The subsequent ad libitum snack consisted of raisins, cookies, potato chips, crackers, hot dogs and cheese.

While no significant interaction was found between the length of delay between preload and snack consumption and the amount of food consumed during the snack, this study showed a significant effect of the type of beverage preload on children's subsequent snack intake<sup>10</sup>. Children consumed significantly less energy from the snack foods after drinking the sugar sweetened Kool-Aid and the aspartame sweetened/high-caloric drink, compared to consumption after drinking water and aspartame-sweetened Kool-Aid. In these cases, participants exhibited compensation for the calories consumed in the beverages. The results did not show a significant difference between compensation exhibited after drinking the sucrose-sweetened Kool-Aid and the compensation exhibited after drinking the aspartame sweetened/high-caloric drink. However, the compensation observed suggests that young children can compensate to energy-containing beverages consumed prior to a snack.

### **Portion Sizes**

With the growing rates of obesity in children and adults, there has been increasing concern over the role of portion sizes in this epidemic<sup>11</sup>. Food consumed both within and outside the home are of interest, because they have grown in size over the years, potentially causing excess consumption<sup>11</sup>. In an examination of portion sizes by Young et al. in 2002, it was found that portion sizes available to people have increased steadily and that they are larger than federal standard portion sizes<sup>11</sup>. Larger portions provide more energy, and may encourage an individual to consume more than he or she needs to satisfy energy needs<sup>11</sup>. If an individual habitually consumes excess energy via larger portion sizes, this could influence weight status. No research has been conducted regarding the effect of portion size on beverage consumption in children, but research on adults does indicate that portion size does influence beverage intake in adults. Increased intake from energy-containing beverages of larger portion size may produce "worse" compensation in meals, which may contribute to intake being much greater than energy needs.

In a study by Rolls et al. in 1990<sup>29</sup>, forty-two males, between 21 and 39 years of age, underwent a within-subjects experiment testing the impact of three different beverages, each in both the 8 oz. size and



16 oz. size, on food intake during a meal. This experiment examined sucrose-sweetened lemonade, aspartame-sweetened lemonade, and water intake. Participants were allowed to consume the lunch foods freely, but were asked to consume the entire assigned beverage during the experimental session.

Results of this study showed that no differences existed in food intake across caloric and non-caloric beverage conditions<sup>29</sup>. In other words, average food consumption during the lunch did not change when participants consumed a sugar-sweetened beverage compared to an artificially-sweetened, non-caloric liquid. Thus, in the sugar-sweetened beverage condition, total energy consumption during the meal was higher than in the artificially-sweetened beverage condition. Nor did a compensatory effect occur due to the size of the beverage consumed. Therefore, total energy consumption during the meals served with a sugar-sweetened beverage was higher in the 16 oz. portion size conditions than in the 8 oz. conditions. This was attributable to a main effect of beverage calories added to the food calories consumed at the meal.

These results are consistent with those observed in a study by Flood et al. in 2006<sup>30</sup>. Thirty-three men and women between the ages of 18 and 45 years participated in a within-subjects crossover design examining the impact of different portion sizes of water, regular cola, and diet cola on caloric intake during an ad libitum lunch of pasta, salad, bread, and cookies. Unlike the previous study, however, participants were allowed to drink as much of their given portion, either 12 oz. or 18 oz., as they wished; they were not required to consume the entire portion.

No significant differences existed in ratings of hunger and fullness after the meal across conditions<sup>30</sup>. Participants consumed significantly more beverage when served the larger portion than when served the smaller portion. On average, women exhibited a 10% increase in beverage intake when served the larger portion compared to when served the smaller portion, while men exhibited a 26% increase with the larger portion. This occurred across all beverage types. Due to this effect, within the caloric beverage conditions participants consumed an average of 151 kcal from the larger beverage portion, compared to 128 kcal consumed from the smaller portion. This caused a significant increase in the overall amount of energy consumed during those meals served with energy-containing beverages.

No evidence of compensatory behavior was found; no differences in food intake occurred between experimental conditions. In other words, participants did not adjust their intake according to the calories consumed in beverages. Instead, individuals consumed the same amount of calories from food when served a caloric beverage as when served a non-caloric beverage at the meal. Thus, the extra calories provided by caloric beverages were simply added to the calories consumed in the meal, causing energy intake during meals served with a caloric beverage to be significantly greater than that during meals served with a non-caloric beverage. However, the difference between overall energy intake during the larger caloric beverage condition as compared to the smaller caloric beverage condition was not statistically significant.

While no research has been conducted with children regarding changes in portion size of beverages and beverage intake, research has found that children consume more of solid food when provided the food in larger portions.

A study completed in 2000 by Rolls et al. examined the effect of providing larger portions on intake<sup>12</sup>. Participants of the within-subjects crossover design were 32 children between the ages of three and five years. Varying portion sizes of a macaroni and cheese entrée were provided to children on different days, alongside fixed portions of carrot sticks, applesauce, and milk.

It was observed that intake increased with portion size among older children, who were on average around the age of five years<sup>12</sup>. Younger children, of mean age 3.6 years, did not exhibit any significant differences in intake between the three portion size conditions. This implies that children respond to larger portion sizes, but potentially only after a certain age. It is possible that younger children are more influenced by internal cues of self-regulation, and that these cues start to diminish as external cues, including increased portion size, take over<sup>32</sup>.

However, in 2003, Fisher et al. replicated this study, using a within-subjects crossover design with preschool children between the ages of three and five years, and did not find younger children to be unresponsive to portion size<sup>13</sup>. On separate occasions, children were provided with a reference-size portion of macaroni and cheese and a larger-size portion of macaroni and cheese, each time served with carrot sticks, applesauce, sugar cookies, and milk. In the larger portion size condition, children of all ages consumed more of the macaroni and cheese meal and more energy overall. The effect of increased consumption when served a larger portion was positively associated with age, meaning children at the upper age range were most responsive to larger portion sizes.

A study by Savage et al. in 2012 further investigated the effect of larger entrée portions on side dish consumption, including consumption of fruits and vegetables<sup>14</sup>. Twenty-one children aged three to five years underwent a series of six experimental lunch sessions, with varied portions of a macaroni and cheese entrée and fixed portions of side dishes. As entrée size increased, children's intake of the entrée also increased, leading to an increase in total energy consumption during the meal.

A study by Mathias et al. in 2012 also showed that increased portion sizes leads to greater intake<sup>15</sup>. Over five experimental sessions, 38 children between the ages of 4 and 6 years were served fruits and vegetables of varying portion size along with a main entrée of pasta and sauce. Overall energy intake during the meal was measured, as well as the amounts of fruits and vegetables consumed in each condition, to determine whether portion size manipulation resulted in an effect on intake of those foods. The results showed that when served larger portions of fruits and vegetables, children consumed significantly greater amounts of these foods<sup>15</sup>.

### **Summary**

It is estimated that as of 2010, approximately 12.1% of toddlers and preschool-aged children and 17% of children and adolescents in the United States were obese<sup>1</sup>. Excess body weight can contribute to serious illnesses and consequences can be lifelong and potentially deadly<sup>2,3</sup>. As the rate of childhood overweight and obesity has climbed, so has the proportion of calories provided daily to children's diets by energy-containing beverages, and in particular 100% fruit juices<sup>4</sup>. Research has suggested a link between 100% fruit juice consumption and adiposity in children and adolescents<sup>5-9</sup>. While one study suggests that children may compensate their overall energy intake in response to liquid calories better than adults<sup>10</sup>, it has been suggested that the relationship between 100% fruit juice and adiposity in children may be a consequence of lack of complete compensation to energy consumed from beverages. Incomplete compensation could contribute to excessive energy intake, leading to excessive weight gain. Furthermore, over the past few decades, larger portion sizes have become more common and have arisen as a concern<sup>11</sup>, because children appear to be responsive to portion size and consume greater amounts with larger portion sizes<sup>12-15</sup>. Increased portions of juice may further exacerbate the issues related to incomplete compensation.

Neither of these possible contributors to childhood weight status, portion size or beverage type, has been exclusively investigated in children, and doing so would lead to a better understanding of childhood eating tendencies and the factors leading to weight gain and weight management in this population. Thus, this study will investigate whether beverage type (caloric [100% fruit juice] vs. non-caloric) and beverage portion size (6 oz vs. 12 oz) impact on energy intake during a snack. For this investigation, four experimental snack sessions will occur, in which beverage portion size and beverage type will be manipulated, but provided amount and types of snack foods will be kept identical across all four session. Participants will be approximately 36, 3- to 5-year-old children at the Early Learning Center. These participants comprise two classrooms, each of which will be randomized to a different order of experimental conditions. Four different conditions will take place, 6 oz., caloric beverage (100% fruit juice) condition; 6 oz., non-caloric beverage (water) condition; 12 oz., caloric beverage condition; and 12 oz., non-caloric beverage condition. It is hypothesized that:

4. A main effect of beverage type will occur, such that the caloric beverage conditions will result in a greater energy intake from beverage and greater energy intake from overall snack (which includes the beverage) than the non-caloric beverage conditions.
5. A main effect of beverage portion size will occur, such that the 12 oz. portion size conditions will result in a greater gram intake from the beverage than 6 oz. portion size conditions.
6. An interaction of beverage type and portion size will occur, such that the 12 oz. caloric beverage condition will result in the greatest energy intake from the overall snack (which includes the beverage) as compared to all other conditions.

## **III. DESCRIPTION AND SOURCE OF RESEARCH PARTICIPANTS**

### **3.a. Recruitment**

Participants for this study will be preschool-aged children who attend the full week and full day program at the University of Tennessee's Early Learning Center (ELC) at the White Avenue location. At the time that this study is expected to take place, September 2013, there will be an estimated 36 children enrolled at the ELC. Upon IRB approval, in August 2013, standard recruitment procedures will take place at the ELC. These procedures involve sending an introductory letter (*Appendix 1*) home to the

parent/guardian to explain the study, followed by a folder of questionnaires and consent forms (*Appendices 2-6*) for parents/guardians to complete. The questionnaires will provide information regarding the children's demographics (*Appendix 2*), eating behaviors (*Appendix 5*), and eligibility (*Appendix 6*), as well as the primary caregiver's feeding styles (*Appendices 3-4*). Parents/guardians willing to allow their child to participate in the study will be asked to sign a consent form (*Appendix 7*) and return it to the teacher of their child's class.

### 3.b. Eligibility

An eligibility questionnaire (*Appendix 6*) will be contained in the packet of materials sent home with children, and parents/guardians will fill out the questionnaire to determine eligibility. Eligible children will be  $\geq 3$  years of age by September 31<sup>st</sup>, 2013, enrolled at the ELC, be free of allergies to the foods used in the study, like the foods used in the study, be able to use a spoon, and have parental consent. Children not meeting these criteria will not be included in the study.

### Randomization

Children will be randomized by classroom. Each of the two preschool classrooms will be randomized to either Order 1 or Order 2 [see Table 1]. Only data from eligible children will be collected and used in analyses.

## IV. METHODS AND PROCEDURES

### 4.a. Study Design

This study will be a randomized controlled trial that will investigate the impact of different portion sizes of 100% fruit juice on snack food intake in preschool aged children. This study will have a 2x2x2 design, with the between-subjects factor of order (order 1 vs. order 2) [see Table 1] and the within-subjects factors of beverage type (caloric vs. non-caloric) and portion size (6 oz. vs. 12 oz.). On four consecutive Wednesday afternoons beginning in September 2013, snack sessions will be held with participants at the Early Learning Center (ELC). Two classrooms of children, each containing approximately 18 children between the ages of 3 and 5 years, will be randomized to either order 1 or order 2, with each child within each classroom receiving the same condition at each session. The dependent variables will be the amount in grams of beverage consumed, and energy (in kilocalories) of beverage and food consumed.

**Table 1. Study Design**

	Session						
	Week 0	Week 1 (practice)	Week 2 (practice)	Week 3	Week 4	Week 5	Week 6
<b>Order 1 N=18</b>	Informed consent and questionnaires	6 oz. water + snack	12 oz. juice + snack ----- Anthro-pometrics	6 oz. juice + snack	12 oz. water + snack	6 oz. water + snack	12 oz. juice + snack
<b>Order 2 N=18</b>	Informed consent and questionnaires	12 oz. juice + snack	6 oz. water + snack ----- Anthro-pometrics	12 oz. water + snack	6 oz. juice + snack	12 oz. juice + snack	6 oz. water + snack

### Foods

The snack foods used in this study will be graham crackers and applesauce, which are snacks that are currently used at the ELC, and that the children enjoy. The beverages used will be water and either 100% Berry or 100% Grape fruit juice. These two juices were chosen due to their acceptability among children and in order to avoid too much apple flavor in the snack, as applesauce will also be served. Depending on the condition, children will be served either 6 or 12 oz. of juice or water, along with 200 grams (approximately 7 oz.) of applesauce and 45 grams (about 3, 2 in. x 4 in. rectangles) of graham crackers. A pilot study will be done over summer 2013 at the ELC to determine the acceptability of Berry

and Grape juice among children. Children will taste-test the juices and based on votes, the juice that the majority of the students prefer taste-wise will be used in the study.

Brands of the foods that will be served to children are Juicy Juice (100% Berry or 100% Grape), Mott's applesauce (no sugar added, natural), and HoneyMaid graham crackers (honey grahams). One snack food of high-energy-density and another of low-energy-density were chosen in order to examine any trends in consumption relating to energy density. The applesauce provides 45 kilocalories per 100 grams, thus it has an energy density of 0.45 kcal/gram, and the graham crackers provide 420 kilocalories per 100 grams, thus it has an energy density of 4.20 kcal/gram. 100% Berry and 100% Grape fruit juice both provide 90 kilocalories per 6 oz. serving, and 180 kilocalories per 12 oz. serving. Birch et al. used a caloric beverage containing 90 kilocalories per serving in their examination of children's compensation to liquid energy<sup>10</sup>. A serving twice that size and energy content will be used for this investigation's larger portion size condition.

In both conditions in which water is served as the beverage, a total of 279 kilocalories will be available in the snack. In the 6 oz. juice condition, 369 total kilocalories will be available in the snack (beverage and snack combined), and in the 12 oz. juice condition, 459 total kilocalories will be available (beverage and snack combined). During the pilot study at the ELC over summer 2013, sizes of beverages and snack foods will be tested in addition to juice tastings. This will help determine whether the chosen portions will be the appropriate sizes for children of this age. Choosing the appropriate sizes of foods and beverages is important in ensuring that children will not consume all of the food and beverage.

#### **4.b. Procedures**

Parents/guardians of children attending preschool at the ELC will be asked to allow their children to participate in the study. As described above, parents/guardians will first receive a letter from the investigator (*Appendix 1*) in which the research study will be explained. Each parent/guardian will receive this letter via their individual mailbox at the ELC. Then, parents/guardians will receive a packet of questionnaires and consent forms (*Appendices 2-6*), which will be completed and returned to the child's teacher if they agree to their child's participation in the study. In addition to collecting information about their child's eating habits, two questionnaires collect information on parental eating habits. As parental parent eating behavior can influence child eating behavior<sup>33</sup>, collecting this information from parents will assist with understanding child eating behavior in this investigation. These items will take approximately 20 minutes to complete. Parents/guardians not giving consent for their child to participate will be provided with the option to choose whether or not their child will receive the snack (*Appendix 8*). Children whose parents/guardians have not given permission for them to receive the snack will be provided with a snack from the ELC.

The investigator will visit the ELC every afternoon during the parental consent phase, in order to collect forms which have been returned, as well as to follow up with parents/guardians who have questions regarding the study or who have not yet consented and turned in paperwork. All collected forms will be stored in locked filing cabinets within the Healthy Eating and Activity Laboratory (HEAL), in room 102 of the Jessie Harris Building (JHB) on the University of Tennessee Campus. Data will be stored according to participants' assigned personal identification numbers; therefore, no names will be connected to the data. Completed parental questionnaires will also be stored in this way, in order to protect the confidentiality of the responses.

All snacks prepared, data collected, and contact with ELC children for this study will be done by Ms. Norton, the Co-Principal Investigator, and HEAL staff. These HEAL members have undergone extensive research training under Dr. Hollie Raynor.

#### ***Prior to Start of Snack Sessions***

Each participant will be assigned a personal identification number to protect their identity for the duration of the study. Identification numbers will be used to label questionnaires containing personal information, as well as the dishes used to serve each child's snack foods and beverages during each session. This will ensure that measurements collected from each snack session will correctly be stored together. 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 food containers and keep participation confidential.

### *Snack Session Procedures*

Snack sessions will be held at approximately 3:15pm, the ELC's normal snack time, on six consecutive Wednesday afternoons beginning in September 2013. The lunches served prior to all experimental snack sessions will be the same; this protocol will be approved previously with the ELC's chef.

Sessions will be held for a total of six successive weeks, with the first two weeks serving as practice sessions to help the children get accustomed to the process used in the study. Data will be collected from these sessions but not used in analyses. During practice sessions, each classroom will receive a practice round with each of the two beverages and portion sizes. Practice sessions will familiarize children to the cups and containers holding the foods and drinks, and will also help them to adjust to individualized, pre-portioned snacks, as opposed to serving themselves family-style. Additionally, practice sessions will help children become accustomed to research personnel administering the study. During the second practice snack session, trained students from HEAL will take anthropometric measurements of eligible children who have parental/guardian consent to participate. These measurements will take place in the classroom in a private area.

Beginning in Week 3, the order presented in Table 1 will be followed for measured snack sessions. The investigator will begin each session by informing participants that they will be testing snack foods and beverages that day because the investigators want to know how much they like the foods and beverages, and that they should eat as much or as little as they wish. The investigator or other staff member will then obtain Child Assent (*Appendix 9*). This will ensure that the child agrees to participate, and that the child understands that he or she may choose not to participate at any time. Children will also be instructed not to share any of their food or drink with others. Teachers will also be instructed not to share. Each participant will then rate their hunger (*Appendix 10*) and liking of the foods and beverages (*Appendix 11*) used in the investigation on questionnaires. In order to decrease the chance that children will be influenced by their peers in rating their hunger and liking of the foods, children will be reminded to answer for themselves, and to not pay attention to the responses given by others. Trained HEAL students and ELC staff will be available to assist children in completing questionnaires. Children will sit at tables of 5 or 6, with hunger and liking scales in front of them. The staff member or HEAL student will explain each questionnaire to the group and ask the children to circle the appropriate picture on each questionnaire. Children appearing to have difficulty will be aided individually by a staff member or HEAL student. In order to avoid children not participating from feeling "left out," these children will receive a snack at the same time as those children participating in the investigation. All children will be seated at their usual table during the snack. Children not participating in the investigation will be given a marker and paper to draw on when participating children are given a marker and forms to rate their hunger and liking on.

Once the preliminary questionnaires are complete, the children will be served the snack and appropriate beverage. Students from HEAL will distribute each child's food and beverage in containers labeled with the child's identification number. Extreme care will be exhibited in order to avoid confusion between participants. Once foods and beverages are distributed, children will be given 20 minutes to consume their snack. Adults will observe to ensure children are not sharing their foods. Upon completion of snack time, trained HEAL members will collect all food and beverage containers and again assist participants in completing the hunger rating questionnaire (*Appendix 10*). This will be done in the same way as prior to the snack.

### *Training of ELC and HEAL Staff*

All ELC and HEAL Staff members will follow the same Script of Procedures (*Appendix 12*) when interacting with participants. All members of the HEAL lab who will aid in this study will be trained on procedures, including taking anthropometric measurements (*Appendix 13*) and aiding children in completing liking and hunger scales (*Appendices 10-11*). This will be done so that all procedures will be completed in a uniform manner. Likewise, prior to beginning the sessions, the investigator will meet with ELC teachers and teachers' aides who will be present in the classroom during sessions. This will be done in order to inform them of procedures, and train them on helping children complete questionnaires. This training will help maintain uniformity.

Additionally, all staff and HEAL members will complete a Pledge of Confidentiality (*Appendix 14*) to ensure that participants' identities will be protected for the duration of the study.

#### **4.c. Measures**

##### **4.c.i. Anthropometrics**

Anthropometric measurements (*Appendix 13*) will be completed on all participants at the second practice session, the week prior to beginning the four consecutive practice sessions. Before collecting height and weight measures, children will be asked to remove their shoes and excess clothing. Height will be measured twice, to the nearest tenth of an inch, using a portable stadiometer (SECA, ITIN Scale Company, Brooklyn, NY). Weight will be measured using a calibrated portable digital scale (Healthometer Professional, Sunbeam Product Inc. Raton, FL), to the nearest tenth of a pound. Each child's body mass index [ $BMI = \text{weight(kg)} / \text{height(in}^2\text{)}$ ] will be calculated and BMI scores (*Appendix 13*) will be compared to the CDC's BMI percentile charts<sup>34</sup>. Children will be classified as overweight if they have a BMI at or above the 85<sup>th</sup> percentile but less than the 95<sup>th</sup> percentile, and will be classified as obese if they have a BMI at or above the 95<sup>th</sup> percentile<sup>35</sup>. Participants' z-BMI scores will also be calculated by standardizing the BMI for children's age and gender in relation to the population mean and standard deviation<sup>34</sup>.

##### **4.c.ii. Consumption**

Snack foods and beverages will be weighed and measured in JHB, appropriately covered and stored according to food safety standards, and carried by HEAL students next door to the ELC. Foods and beverages will be weighed using a calibrated food scale (Denver Instruments SI-8001, Fischer Scientific) before and after snack sessions. Beverages will first be measured in ounces and then transferred to 16 oz. plastic cups to be weighed in grams. Food will be served in paper bowls, which will be measured separately before food is added. The total food and container weight will be recorded, and the amount consumed will be determined by subtracting the final, post-consumption weight of the food and container from this starting, pre-consumption weight. The investigator will be able to calculate energy intake from the gram amounts consumed.

##### **4.c.iii. Liking of Food**

With assistance, participants will complete a questionnaire to determine their liking of the foods and beverages used in the study (*Appendix 11*). This will be completed once during each session, prior to being served the snack. The questionnaire, which has been validated in previous research, consists of a three-point Likert-scale. Participants are to choose one of the three points, represented by a "yucky" face (dislike), a "so-so" face (neutrality), and a "yummy" face (like)<sup>36,37</sup>.

##### **4.c.iv. Hunger**

Participants will rate their hunger (*Appendix 10*) twice in each session, both before and after consuming the snack. The validated questionnaire that will be used to assess hunger in this study consists of a three-point Likert-scale. The points on the scale consist of cartoon pictures with stomachs that are shaded to represent fullness or hunger. The cartoon of the empty, unshaded stomach represents "very hungry," the somewhat shaded stomach represents "a little hungry," and the fully shaded stomach represents "not hungry." Previous studies on preschool aged children have included similar scales for measuring hunger<sup>36-38</sup>.

##### **4.c.v. Parental/Guardian Questionnaires**

Responses to four questionnaires will be collected from participants' parents/guardians. These questionnaires include a demographic questionnaire (*Appendix 2*), the Child Feeding Questionnaire (CFQ) (*Appendix 3*), the Three Factor Eating Questionnaire (TFEQ) (*Appendix 4*), and the Child Eating and Behavior Questionnaire (CEBQ) (*Appendix 5*). The demographic questionnaire collects data on parent/guardian and child characteristics including race and ethnicity, as well as parental/guardian marital status, income, and educational background. The CFQ consists of 31 items and 7 factors (perceived responsibility, perceived parent weight, perceived child weight, concern about child weight, restriction, pressure to eat, and monitoring) designed to gather information regarding parental beliefs and habits surrounding the feeding of their children<sup>33</sup>. The TFEQ measures three different aspects of parents' eating behavior, dietary restraint, disinhibition, and perceived hunger, with a total of 51 items<sup>39</sup>. The final questionnaire, the CEBQ, was designed to examine a child's tendencies in regards to 8 factors, food responsiveness, enjoyment of food, emotional overeating, desire to drink, satiety responsiveness, slowness in eating, emotional undereating, and fussiness. Parents/guardians provide answers regarding

their child's behaviors. The tool contains 35 items and gathers information on an individual's eating habits as they relate to the risk of obesity<sup>40</sup>.

#### **4.d. Quality Control**

Participants absent during any of the 4 days of the snack measures or who at any session consume all of the food presented will not be included in the final analyses. However, regardless of inclusion in the analyses, all children will be provided with the snack (unless a legal guardian indicates that their child cannot receive the snack by the Parent Request for No Snack form [Appendix 8]).

#### **4.e. Data Analysis**

All statistical analyses will be completed using SPSS 21.0, with an alpha level of 0.05. In order to detect any baseline demographic differences between the two classrooms, independent t-tests will be completed on continuous data, and Chi-square tests will be performed on categorical data. To determine any significant differences between the four experimental sessions in each participants' ratings of hunger and liking of the foods, a mixed factorial analysis of variance (ANOVA), with the between-subjects factor of order and the within-subjects factors of beverage type (caloric and non-caloric) and portion size (6 oz. and 12 oz.), will be conducted. Another mixed factorial ANOVA will examine differences in participants' ratings of hunger before and after the snack. For these tests, the between-subjects factor will be order, and the within-subjects factors will be beverage type (caloric and non-caloric), beverage portion size (6 oz. and 12 oz.), and time (pre- and post-snack). Mixed factorial ANOVAs will also be used to analyze the dependent variables, grams consumed of the beverage, calories consumed of the beverage, and overall energy intake within the snack (total consumed calories of foods and beverage). The between-subjects factor for this test will be order, and the within-subjects factors will be beverage type and size. Greenhouse Geisser corrections will be included in these tests to control for sphericity. Post hoc analyses with Bonferroni corrections will be completed for significant outcomes.

### **V. SPECIFIC RISKS AND PROTECTION MEASURES**

Risks are minimal to participants. Children may be allergic to the foods used in this study, but all children will be screened for food allergies (Appendix 6). 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. Data from this study will be retained for three years, after which it will be destroyed.

### **VI. BENEFITS**

Anticipated benefits for this study are a greater understanding of the influence of energy-containing beverages and portion size on energy intake in children. This information may assist with the development of guidelines related to juice intake in children to prevent over consumption, which could assist with the prevention of obesity in children.

### **VII. METHODS FOR OBTAINING "INFORMED CONSENT" FROM PARTICIPANTS**

Informed consent (Appendix 7) will be received upon IRB approval from parents/guardians of preschool-aged children from the ELC located on White Avenue. A letter from the investigators (Appendix 1) will go home to legal guardians with a child to explain the study. During the following week, in the same manner, all parents/guardians will be given two consent forms. All information and forms for the parents/guardians 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 between parents/guardians and the ELC is through the children's cubbies, these questionnaire packets will not appear unusual. The investigator will also be present at the ELC daily to hand out additional forms that parents have misplaced, and to answer any questions caretakers may have regarding the study. 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. Teachers

from the ELC consistently collect forms from parent/guardians in this manner; therefore, this process will not be abnormal. It will be the responsibility of the investigator to collect consent forms daily from the ELC to store in locked filing cabinets in locked rooms in HEAL, room 102 in JHB.

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

Ms. Norton has extensive training as a graduate research assistant in the Healthy Eating and Activity Laboratory under the direction of Dr. Hollie Raynor. 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. Norton 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)**

#### **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, PhD, RD, LDN

Signature: \_\_\_\_\_ Date: \_\_\_\_\_

Co-Principal Investigator: Erin Norton, BS CMB

Signature: \_\_\_\_\_ Date: \_\_\_\_\_

Student Advisor (if any):

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

☒ Full IRB Review

Chair, DRC: \_\_\_\_\_

Signature: \_\_\_\_\_ Date: \_\_\_\_\_

Department Head: Jay Whelan, PhD

Signature: \_\_\_\_\_ Date: \_\_\_\_\_

Protocol sent to Research Compliance Services for final approval on (Date) :

\_\_\_\_\_

Approved:  
Research Compliance Services  
Office of Research  
1534 White Avenue

Signature: \_\_\_\_\_ Date: \_\_\_\_\_

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

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## Appendix 1: Introductory Letter to Parents

September 25<sup>th</sup>, 2013

Dear Parent or Guardian,

My name is Erin Norton, a graduate student in the Nutrition Department here at the University of Tennessee, Knoxville. I am working on my Master's 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, beverage type and beverage portion size, on beverage intake and overall intake during a snack. The snack will consist of applesauce and graham crackers, with either water or 100% fruit juice provided as a beverage for each snack. Thus all children receiving the snack will receive water at some snacks and 100% fruit juice at other snacks. This snack intervention will take place during afternoon snack on the following **Wednesdays** between September and November:

<b>October</b>	<b>November</b>
October 9, 2013	November 6, 2013
October 23, 2013	November 13, 2013
October 30, 2013	November 20, 2013

During these days, the children will be given applesauce and graham crackers, with either a 6 oz. or 12 oz. portion of water or 100% fruit juice. Additionally, weight and height of children with legal guardian consent will be collected during the week of October 21<sup>st</sup> in a private area in the classroom.

As a part of this study, you will be asked to complete questionnaires on demographics, child feeding style, and your eating behaviors. It will take you about 20 minutes to complete the questionnaires. Today, you have received a packet that includes further information about the study, questionnaires, consent forms, and an envelope. 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 **October 4<sup>th</sup>** to your child's teacher, sealed in the envelope that will be given to you.

If you do not want your child to participate in the study, and do not want your child given the applesauce, graham crackers, and juice 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 **October 4<sup>th</sup>**, sealed in the envelope that will be given to you. If you do not want your child to receive the provided snack, he or she will be provided with a different snack from the ELC on that day.

I will provide a checklist in the envelope to ensure that all forms and questionnaires are included before returning them to your child's teacher by **October 4<sup>th</sup>**. If you have any questions, feel free to contact me by phone at (865)-974-0754 or e-mail at enorton4@utk.edu.

Sincerely,

Erin Norton

## Appendix 2: Demographics

Reference #

DATE //  
M M D D Y Y

### Caretaker 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 ☐ FEMALE  
(1) (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

## Child Demographic Information

Reference #

1. CHILD'S BIRTHDATE //  
M M D D Y Y Y Y
2. SEX: ☐ MALE ☐ FEMALE  
(1) (2)
3. Which of the following best describes this child's 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 \_\_\_\_\_
6. Which of the following best describes your child's ethnic heritage?
- ☐ (1) Hispanic or Latino
  - ☐ (2) Not Hispanic or Latino

## Appendix 3: Child Feeding Questionnaire

DATE //  
M M      D D      Y Y

<b>Office Use Only</b>
Reference #:

## Child Feeding Questionnaire

Please indicate your response in the column based on the question or statement.

	never (1)	seldom (2)	sometimes (3)	often (4)	always (5)
When your child is at home, how often are you responsible for feeding him/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					

<b>Your child as a preschooler</b>					
	<b>markedly underweight (1)</b>	<b>underweight (2)</b>	<b>normal (3)</b>	<b>overweight (4)</b>	<b>markedly overweight (5)</b>
<b>Your child kindergarten through 2<sup>nd</sup> grade</b>					
<b>Your child 3<sup>rd</sup> through 5<sup>th</sup> grade</b>					

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

	<b>disagree (1)</b>	<b>slightly disagree (2)</b>	<b>neutral (3)</b>	<b>slightly agree (4)</b>	<b>agree (5)</b>
<b>I have to be sure that my child does not eat too many sweets (candy, ice cream, cake or pastries).</b>					
<b>I have to be sure that my child does not eat too many high-fat foods.</b>					
<b>I have to be sure that my child does not eat too much of his/her</b>					

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 his/her favorite foods in exchange for good behavior.					
	<b>disagree</b> (1)	<b>slightly disagree</b> (2)	<b>neutral</b> (3)	<b>slightly agree</b> (4)	<b>agree</b> (5)
If I did not guide or regulate my child's eating, he/she would eat too many junk foods.					
If I did not guide or regulate my child's eating, he/she would eat too much of his/her favorite foods.					

	<b>disagree</b> (1)	<b>slightly disagree</b> (2)	<b>neutral</b> (3)	<b>slightly agree</b> (4)	<b>agree</b> (5)
My child should always eat all of the food on his/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 him/her to eat anyway.					
If I did not guide or regulate my child's eating, he/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?					

## Appendix 4: Three Factor Eating Questionnaire



DATE //  
M M    D D    Y Y

### ***Eating Habits***

<b>Office Use Only</b>
Reference #
Assessment #

**Please answer true or false to the following statements.**

	<b>True (1)</b>	<b>False (2)</b>
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.	<input type="checkbox"/>	<input type="checkbox"/>
2. I usually eat too much at social occasions, like parties and picnics.	<input type="checkbox"/>	<input type="checkbox"/>
3. I am usually so hungry that I eat more than three times a day.	<input type="checkbox"/>	<input type="checkbox"/>
4. When I have eaten my quota of calories, I am usually good about not eating any more.	<input type="checkbox"/>	<input type="checkbox"/>
5. Dieting is so hard for me because I just get too hungry.	<input type="checkbox"/>	<input type="checkbox"/>
6. I deliberately take small helpings as a means of controlling my weight.	<input type="checkbox"/>	<input type="checkbox"/>
7. Sometimes things just taste so good that I keep on eating even when I am no longer hungry.	<input type="checkbox"/>	<input type="checkbox"/>
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.	<input type="checkbox"/>	<input type="checkbox"/>
9. When I feel anxious, I find myself eating.	<input type="checkbox"/>	<input type="checkbox"/>
10. Life is too short to worry about dieting.	<input type="checkbox"/>	<input type="checkbox"/>
11. Since my weight goes up and down, I have gone on reducing diets more than once.	<input type="checkbox"/>	<input type="checkbox"/>
12. I often feel so hungry that I just have to eat something.	<input type="checkbox"/>	<input type="checkbox"/>

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

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

**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

## Appendix 5: Child Eating & Behavior Questionnaire

**Office Use Only**

Reference #:

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	①	②	③	④	⑤
2. Given the choice, my child would eat most of the time	①	②	③	④	⑤
3. If allowed to, my child would eat too much	①	②	③	④	⑤
4. If given the chance, my child would always be having a drink	①	②	③	④	⑤
5. If given the chance, my child would always have food in his/her mouth	①	②	③	④	⑤
6. If given the chance, my child would drink continuously throughout the day	①	②	③	④	⑤
7. My child cannot eat a meal if s/he has had a snack just before	①	②	③	④	⑤
8. My child decides that s/he doesn't like food, even without tasting it	①	②	③	④	⑤
9. My child eats less when s/he is angry	①	②	③	④	⑤
10. My child eats less when s/he is tired	①	②	③	④	⑤
11. My child eats less when s/he is upset	①	②	③	④	⑤
12. My child eats more and more slowly during the course of a meal	①	②	③	④	⑤



13. My child eats more when annoyed	①	①	②	③	④
14. My child eats more when anxious	①	①	②	③	④
15. My child eats more when s/he has nothing else to do.	①	①	②	③	④
16. My child eats more when s/he is happy	①	①	②	③	④
	NEVER (0)	SELDOM (1)	SOMETIMES (2)	OFTEN (3)	ALWAYS (4)
17. My child eats more when worried	①	①	②	③	④
18. My child eats slowly	①	①	②	③	④
19. My child enjoys a wide variety of foods	①	①	②	③	④
20. My child enjoys eating	①	①	②	③	④
21. My child enjoys tasting new foods	①	①	②	③	④
22. My child finishes his/her meal very quickly	①	①	②	③	④
23. My child gets full before his/her meal is finished	①	①	②	③	④
24. My child gets full up easily	①	①	②	③	④
25. My child has a big appetite	①	①	②	③	④
26. My child is always asking for a drink	①	①	②	③	④
27. My child is difficult to please with meals	①	①	②	③	④
28. My child is interested in food	①	①	②	③	④
29. My child is interested in tasting food s/he hasn't tasted before	①	①	②	③	④
30. My child leaves food on his/her plate at the end of a meal	①	①	②	③	④

31. My child looks forward to mealtimes	①	②	③	④
32. My child loves food	①	②	③	④
33. My child refuses new foods at first	①	②	③	④
34. My child's always asking for food	①	②	③	④
35. My child takes more than 30 minutes to finish a meal.	①	②	③	④

## Appendix 6: Eligibility Questionnaire

Reference #

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. My child will be 3 years old by September 31<sup>st</sup>, 2013. ☐ Yes ☐ No

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

---

---

---

---

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3. My child can use a spoon. ☐ Yes ☐ No

4. My child likes graham crackers. ☐ Yes ☐ No

5. My child likes applesauce. ☐ Yes ☐ No

6. My child likes (grape or berry) juice. ☐ Yes ☐ No

\_\_\_\_\_  
Parent or Legal Guardian Signature

\_\_\_\_\_  
Date

\_\_\_\_\_  
Researcher Signature

\_\_\_\_\_  
Date

## Appendix 7: Consent Form

## INFORMED CONSENT STATEMENT

### Impact of Portion Size of Fruit Juice on Fruit Juice Consumption and Overall Energy Intake During a Snack in Preschoolers

#### INTRODUCTION

Your son/daughter has been invited to participate in a research study conducted at the University of Tennessee. The purpose of this study is to investigate the impact of beverage type and beverage portion size on food and beverage consumption during a snack.

This form explains the study. Please read this form and contact the researcher 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.

Erin Norton, 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 investigate the impact of two environmental factors, beverage type and beverage portion size, on beverage intake and overall intake during a snack. It is estimated that 36 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 your child attends preschool at the Early Learning Center on White Avenue. There is no cost for participating in this study.

#### INFORMATION ABOUT PARTICIPANTS' INVOLVEMENT IN THE STUDY

If you decide to have you and your child participate in the study, please return a signed and initialed consent form to your child's teacher at the Early Learning Center.

Upon guardian consent, your child will participate in the study over a period of six sessions. 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 20 minutes to complete the questionnaires.

After the questionnaires have been completed and returned to the Early Learning Center your child will then participate in 6 sessions in which he/she will receive an afternoon snack of approximately 45 grams of graham crackers and 200 grams of applesauce, with either a 6 oz. or 12 oz. portion of water or 100% fruit juice. 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 take place during the months of September to November of 2013. In these sessions, your child will be asked to rate how hungry he/she is and

\_\_\_\_\_  
Initials of Participant's  
Legal Guardian

UTK IRB Approval:  
SEP - 3 2013 - SEP - 3 2014

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 graham crackers and applesauce and either 6 oz. or 12 oz. of water or 100% fruit juice. When the snack time is done, your child will rate his/her hunger again.

During the second snack session, 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 the classroom in a private area. Each child will be measured individually by research staff members. Your child will be asked to remove his/her shoes and to stand tall to measure how tall your child is 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.

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 Hollie Raynor at (865)-974-6259 if you have any questions about the procedures.

#### **RISKS**

Participants are at minimal risk as 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 research.

#### **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.

\_\_\_\_\_  
Initials of Participant's  
Legal Guardian

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### 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, Dr. Hollie Raynor at (865)-974-6259.

### 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, Hollie Raynor, PhD, at the Healthy Eating and Activity Laboratory, 102 Jessie Harris Building, 1215 W Cumberland Avenue and (865) 974-6259. 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, Hollie Raynor, using the contact information listed above.

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### 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.

Parent or Legal Guardian Signature \_\_\_\_\_ Date \_\_\_\_\_

Investigator's signature \_\_\_\_\_ Date \_\_\_\_\_

\_\_\_\_\_  
Initials of Participant's  
Legal Guardian

UTK IRB Approval:  
SEP - 3 2013 - SEP - 3 2014



## Appendix 8: Parent Request for No Snack

DATE //  
M M D D Y Y

Reference #:

### Parent 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 (graham crackers, applesauce, and juice) as a snack on the days the study will take place. No data will be collected from your child, but your child is welcome to receive the snack. If you do not want your child to receive the foods provided in this study, your child 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 for a snack.
- ☐ No, I do not want my child to receive the food used in the research study for a snack.

\_\_\_\_\_  
Parent or Legal Guardian Signature

\_\_\_\_\_  
Date

\_\_\_\_\_  
Researcher Signature

\_\_\_\_\_  
Date

## Appendix 9: Child Assent

## 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 Erin (or research assistant's name). Your mom and your teacher said it's okay for me to ask you if you want to help me. First, I would like to see how tall you are and how much you weigh. Then, I would like to ask you to have a snack on some days. The snack has applesauce, graham crackers, and either juice or water. Would you like to help me with this project? (Child's response).

☐ Yes

☐ No

Great! I think you will find that these things are easy and fun to do. 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 play this anymore." Okay? (Child's response).

☐ Yes

☐ No

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

**II. The examiner 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."

"Good work!"

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

"You are really listening well!" (Child's first name), I'm proud of the hard work you are doing."

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

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

- If the child wishes to stop during the weight/height measures, the examiner will maintain a neutral expression, stop measuring, and say, "All right, thank you for helping me again. Let's go back to your teacher."

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**IV. These behavioral management guidelines will be followed during test administration:**

- Prompts will include phrases such as:  
"Remember to stay in your seat so you can do a good job."  
"Keep listening carefully."  
"Please wait until I am finished with the question before you give your answer."  
"Please keep your eyes and your ears turned on."  
"Please don't touch the (tape recorder)."
- If the child is unable to be conditioned to take the test, administration will be discontinued.

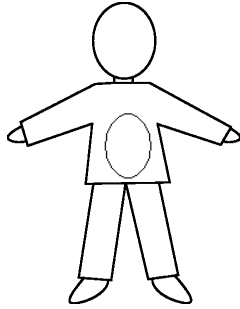
UTK IRB Approval:

SEP - 3 2013 - SEP - 3 2014

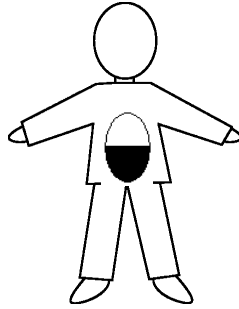
## Appendix 10: Hunger Scale

Letter \_\_\_\_\_  
Before Snack

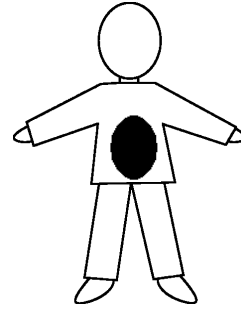
# How hungry are you right now?



Very Hungry



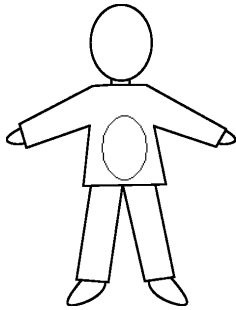
Kind-of Hungry/  
A Little Hungry



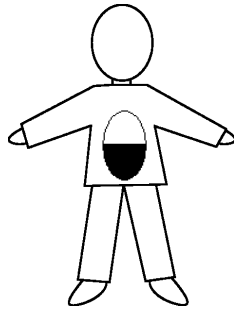
Not Hungry

Letter \_\_\_\_\_  
After Snack

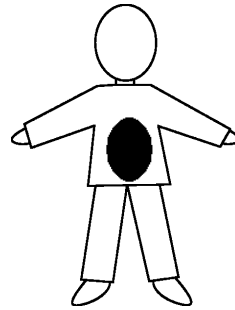
## How hungry are you right now?



Very Hungry



Kind-of Hungry/  
A Little Hungry

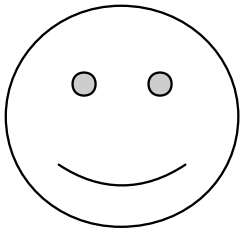


Not Hungry

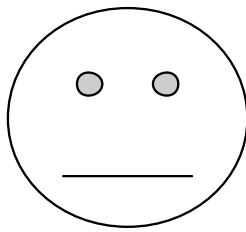


## Appendix 11: Liking Scale

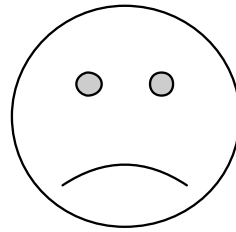
**Do you like applesauce?**



**YUMMY**

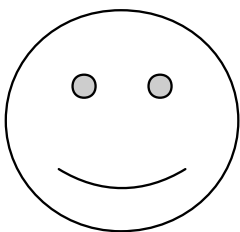


**SO-SO**

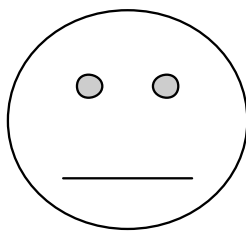


**YUCKY**

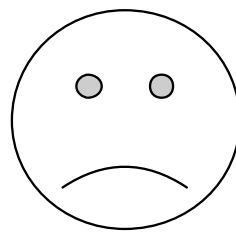
**Do you like graham crackers?**



**YUMMY**

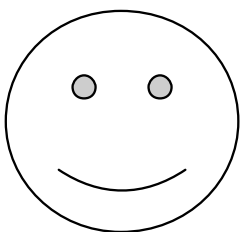


**SO-SO**

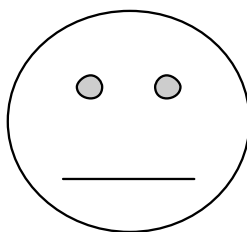


**YUCKY**

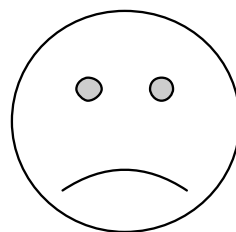
**Do you like berry juice?**



**YUMMY**

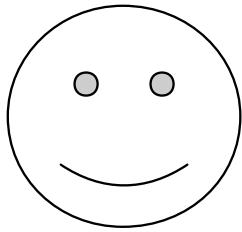


**SO-SO**

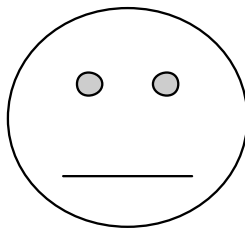


**YUCKY**

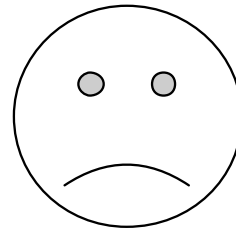
**Do you like applesauce?**



**YUMMY**

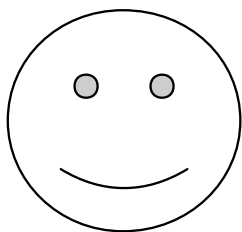


**SO-SO**

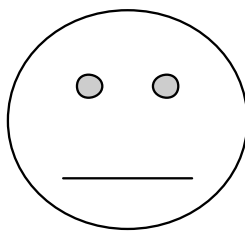


**YUCKY**

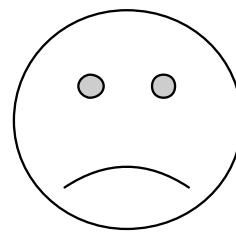
**Do you like graham crackers?**



**YUMMY**

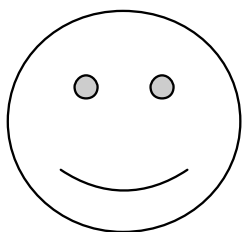


**SO-SO**

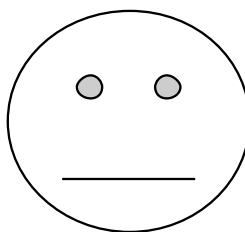


**YUCKY**

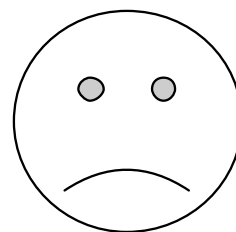
**Do you like water?**



**YUMMY**



**SO-SO**



**YUCKY**

## Appendix 12: Script of Procedures

### Procedures and Scripts for Snack Sessions

Procedures will be the same for all six sessions, and will only vary in the beverage served.

Prior to session prepare:

- \_\_\_ 200 g of applesauce in bowl with spoon, 45 g of graham crackers on plate, and 6 oz. or 12 oz. of water or juice
- \_\_\_ Liking scales
- \_\_\_ Hunger scales
- \_\_\_ Assent forms

1. Arrive at classroom at 3:00 with snacks on tray (snack time is very fluid as to when it starts so it is important to be flexible and work with the staff members).
2. Sit at table with 5-6 children. Identify each child with their PID number and pass out an assent form, hunger scale, and liking scales for applesauce, graham crackers, and water, as well as a marker to each child. Provide markers and paper to children not participating in the investigation as well.

*"I am so excited to see everyone. Today you are going to test some snack foods for me because I'd like to know how you like them."*

Read and complete Assent Form for each participant.

*"Great! Now I am going to ask you how you like the foods in today's snack. If you really like **(applesauce, graham crackers, water)** I want you to circle the smile face **(point to yummy)**, if you like **(applesauce, graham crackers, water)**, but you don't dislike it I want you to circle this face **(point to so-so)**. If you don't like **(applesauce, graham crackers, water)** I need you to circle this face **(point to yucky)**. Please answer for yourself and don't pay attention to what others are circling. Great you have all done a good job circling!"*

Each child may need to be assisted individually after explaining to the small group.

*"Now this is your last task before you get to have snack. I want to know how hungry you are using this scale. If you are very hungry I need you to circle to person with an empty tummy **(point to person with empty stomach)**, if you are a little hungry and you would say your tummy is about half full I need you to circle the middle person **(point to middle person)** and if you are full and not hungry at all I need you to circle this person with a full tummy **(point to person on far right)**. Please answer for yourself and don't pay attention to what others are circling. Great you have all done such a wonderful job and we are now going to hand out your snack. Please do not share any of your food or drink with anyone else, and you may eat and drink as much or as little as you'd like."*

3. Collect liking and hunger scales. Hand out each snack according to the PID on the bottom of the bowl. Allow 20 minutes for snack time.

*“Okay everyone, snack is over now and I am going to ask you to leave your plate, cup, and bowl with your spoon in the bowl on the table and I will pick them up for you.”*

4. Collect any spilled food into the specific child’s container. Then, collect all the plates, cups, and bowls with spoons in the bowls and place on tray to bring back to HEAL. Hand out hunger scales according to PID numbers.

*”Okay everyone, now I just have one more thing for you to do. I want to know how hungry you are using this scale. If you are very hungry I need you to circle to person with an empty tummy (**point to person with empty stomach**), if you are a little hungry and you would say your tummy is about half full I need you to circle the middle person (**point to middle person**) and if you are full and not hungry at all I need you to circle this person with a full tummy (**point to person on far right**). Please answer for yourself and don’t pay attention to what others are circling. Great you have all done such a wonderful job!”*

5. Upon arrival back to the HEAL lab weigh each bowl with the spoon on the scale and record weight for appropriate PID number.
6. Throw away all bowls, spoons etc.

## **Procedure and Script for Weight/Height Measurements Occurring in Second Session after the Snack**

Materials needed to bring to the ELC:

Portable Scale  
2 50 lb. weights for calibration  
Microfiber Glove  
Portable Stadiometer  
Height/Weight Record Document  
Pen

7. Set-up stadiometer and scale.
8. Calibrate scale.
9. Call each child to area one at a time.
10. Have the child remove their shoes and stand on the scale weighing them to the nearest tenth of a pound.
11. Measure the child's height to the nearest  $1/8^{\text{th}}$  of an inch.
12. Have the child put their shoes on and return to whatever activity they were participating in, in the classroom.

*"Hi (student's name) I am going to ask you to take off your shoes and step up here onto the scale keeping your hands right at your side. Great! You did a wonderful job. Now I need to stand on this gray piece of plastic facing me (help child be properly arranged in the stadiometer). I need you to stand up tall with your hands at your side. I am going to just put my hands on your neck to make sure your neck is stretched nice and tall. Great now you are going to feel a piece of plastic slightly touch your head while you are doing a great job standing [record height]. Wonderful! Thank you so much (student's name) you are all set to put your shoes on and go back to your activity."*

## Appendix 13: Anthropometrics



Letter: \_\_\_\_\_

DATE //  
M M D D Y Y

<b>Office Use Only</b>	
Reference #	
Assessment #	

### Anthropometrics

**Assessment:** Height \_\_\_\_\_ inches  
Weight \_\_\_\_\_ pounds  
BMI percentile: \_\_\_\_\_

## Appendix 14: Researcher Pledge of Confidentiality

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

As a member of the research team or teacher of children participating in this research project, I understand that I will be observing children participating in the study and see forms containing data. Data from observations and on the forms has been revealed by research participants who will be participating in this project on good faith that their observations and personal information will 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.

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Research Team Member

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Date

## **VITA**

Erin Norton is originally from Greene, New York. In May 2012, she received her Bachelor of Science degree in Cell and Molecular Biology from the State University of New York at Binghamton. As an undergrad, she gained experiences in nutrition, exercise and wellness, by working as a Nursing Assistant at a hospital, as a Certified Personal Trainer at a health club, and as a representative for a wellness company. Upon completion of her undergraduate degree, she pursued a Master's of Science degree in Public Health Nutrition at the University of Tennessee, Knoxville.

As a graduate student at UTK, Erin served as a Student Research Assistant in the Healthy Eating and Activity Laboratory (HEAL). Here she gained valuable skills and knowledge of research techniques in the area of behavioral weight loss interventions. Furthermore, she was a Graduate Research Assistant for the Metabolic Translational Research Initiative (MTRI) in the department of Nutrition, which provided her with the opportunity to gain experience in developing and implementing a basic research study. During her second year at UTK, Erin also served as a Graduate Teaching Assistant for an introductory Nutrition class offered by the university, which allowed her to advance her teaching skills. Erin will complete her Master's degree in Summer 2014.