Effect of Varying Proportions of Low- and High-Energy-Dense Foods Over 5 Days in Preschool Children

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1.0 Objectives

1.1 Study Objectives

Aim: To determine the effect on food and energy intake of varying the proportion of lower-energy-dense vegetables and fruits in meals served over five days to preschool children.

Hypotheses:

1. When low-energy-dense vegetables and fruits are added to children's meals over five days (Addition condition):
    a. Daily energy intake will increase compared to the Baseline condition
    b. Daily vegetable and fruit intake will increase compared to the Baseline condition
    c. Daily food and beverage intake will increase compared to the Baseline condition
    d. Daily food energy density will decrease compared to the Baseline condition

2. When low-energy-dense vegetables and fruits are substituted for foods higher in energy density in children's meals over five days (Substitution condition):
    a. Daily energy intake will
        i. decrease compared to the Baseline condition
        ii. decrease compared to the Addition condition
    b. Daily vegetable and fruit intake will
        i. increase compared to the Baseline condition
        ii. increase compared to the Addition condition
    c. Daily food and beverage intake will
        i. not differ significantly from the Baseline condition
        ii. decrease compared to the Addition condition
    d. Daily food energy density will
        i. decrease compared to the Baseline condition
        ii. decrease compared to the Addition condition

3. Across all three conditions of different proportions of low-energy-dense vegetables and fruits, daily intakes of vegetables, fruit, and energy will begin to converge over the five-day period

1.2 Primary Study Endpoints

1. Differences in food and beverage intake by energy [Time Frame: Day 1-5 in Weeks 1, 2, and 3]

   Energy intake in kcal

2. Differences in food and beverage intake by weight [Time Frame: Day 1-5 in Weeks 1, 2, and 3]

   Weight consumed in grams (g)

1.3 Secondary Study Endpoints

1. Differences in food and beverage intake by energy density [Time Frame: Day 1-5 in Weeks 1, 2, and 3]

   Energy density in kcal/g

2.0 Background

2.1 Scientific Background and Gaps

It has been proposed that preschool children are in a formative stage during which the ability to regulate energy intake is being replaced by eating in response to environmental food cues. In recent studies, we have found that increases in both food portion size (g) and energy density (kcal/g) have been robust in promoting excess energy intake in preschool children [1,2]. These results challenge the proposition that preschool children show self-regulatory behavior by altering their energy intake when it is perturbed by variations in the available foods.

At present no controlled studies have been conducted over a sufficient duration to determine whether preschool children adjust for energy imbalances stemming from changes in the proportions of foods lower and higher in energy density served at meals.

2.2 Previous Data

N/A

2.3 Study Rationale

This study will provide a better understanding of the regulation of energy intake in preschool children over multiple days by determining how intake is affected by changing the proportions of foods lower and higher in energy density that are served at meals.

3.0 Inclusion and Exclusion Criteria

3.1 Inclusion Criteria

1. Children who are enrolled in a participating childcare center
2. Children who are between the ages of 3 and 5 years old at the time of enrollment
3. Parents of children enrolled in the study who are 18 years or older
4. Teacher in a classroom with enrolled children who are 18 years or older

3.2 Exclusion Criteria

1. Allergy to any of the foods served
2. Children whose diets exclude any of the foods served (e.g. vegan, vegetarian, gluten-free)
3. Children who are not planning to be present for all three study weeks (e.g. planned vacation during a study week)

3.3 Early Withdrawal of Subjects

3.3.1 Criteria for removal from study

Subjects will be removed from the study if they miss three or more days in one study week or two meals per day for three or more days in one study week.

Subjects will also be removed from the study if they repeatedly fail to follow study protocol.

All participation is voluntary and a child can refuse a session and parents may withdraw from the study at any time.

3.3.2 Follow-up for withdrawn subjects

N/A

4.0 Recruitment Methods

4.1 Identification of subjects

We will recruit through a parent letter placed in each child's mailbox. Any children enrolled in the childcare center, along with their parents, may participate. A trained staff member will be at the childcare center at the time of child drop off and/or pick up to explain this study.

Teachers will be recruited verbally in the classrooms of participating children.

4.2 Recruitment process

We will recruit through a parent letter placed in each child's mailbox. A member of our lab approved to do recruitment and consenting of subjects will be available in the center for an evening during child pick up to answer any questions parents may have.

Teachers in classrooms will be asked to participate verbally by study staff.

4.3 Recruitment materials

Parents will receive a letter in their child's mailbox that will describe the study. The consent form will be included with the letter.

Teachers in classrooms will be asked to participate verbally by study staff. A consent form will then be reviewed and signed by each teacher willing to participate.

4.4 Eligibility/screening of subjects

Children with allergies to foods served will not be eligible to participate in this study. Screening questions regarding food allergies and intolerances will be included with the consent form.

5.0 Consent Process and Documentation

5.1 Consent Process

Participation in this study is voluntary.

5.1.1 Obtaining Informed Consent

5.1.1.1 Timing and Location of Consent

Consent will be signed by the parent. Consent will be obtained at the childcare center with a trained member of the lab staff present.

Teachers will consent prior to the first study week in their classroom at the childcare center to fill out questionnaires about children enrolled.

5.1.1.2 Coercion or Undue Influence during Consent

Parents will be given consent form to take home to review on their own and can decide if they want their child to participate.
Teachers will be given the opportunity to review the consent from on their own and can decide if they wish to participate.

5.1.2 Waiver or alteration of the informed consent requirement

N/A

5.2 Consent Documentation

5.2.1 Written Documentation of Consent

Participation in this study is voluntary.

Participants' parents will sign the consent form. Two copies of the consent form will be given, one to be signed and returned to study staff, one to be kept for parents' records.

Teachers will sign a separate consent form for their participation and will be given a copy for their own records.

5.2.2 Waiver of Documentation of Consent

For addendum consent – taking the packed meal(s) implies consent.

5.3 Consent – Other Considerations

5.3.1 Non-English Speaking Subjects

It is not anticipated that there will be any non-English speaking or illiterate subjects.

5.3.2 Cognitively Impaired Adults

5.3.2.1 Capability of Providing Consent

N/A

5.3.2.2 Adults Unable To Consent

N/A

5.3.2.3 Assent

N/A

5.3.3 Subjects who are not yet adults (infants, children, teenagers)

5.3.3.1 Parental Permission

Parents will sign consent for their child to participate. Two copies of the consent form will be given, one to be signed and returned to study staff, one to be kept for parents' records.

5.3.3.2 Assent

A child can refuse to eat any meal if he/she chooses. Before height and weight is measured or the Tasting Game occurs, children are asked if they want to do it and can refuse to participate.

6.0 Study Design and Procedures

6.1 Study Design

This study will have a within-subjects crossover design with repeated measures across five days of meals and three experimental conditions. The investigators will vary the proportions of low-energy-dense vegetables and fruits served at meals by either addition or substitution. In the three experimental conditions, the same foods will be served - only the amounts and proportions of foods will be varied. In the Baseline condition, typical proportions of age-appropriate foods will be served. In the Addition condition, the portion sizes of low-energy-dense vegetables and fruits will be increased, and in the Substitution condition, the portions of low-energy-dense vegetables and fruits will be increased by replacing an equivalent weight of foods higher in energy density. The order of the conditions across the three experimental periods, as well as the order of the five menus within each five-day period, will be counterbalanced by classroom. The three five-day experimental periods will be separated by a two-week washout period. All menus in all conditions will meet the minimum requirements of the United State Department of Agriculture (USDA) Child and Adult Care Food Program (CACFP) [3].

6.2 Study Procedures

1. Over five consecutive days, enrolled children will be served breakfast, lunch, dinner, and snacks of foods commonly served in the childcare center following their regular meal and snack schedules - menus were developed from the menus at the childcare centers to be as similar as possible to the foods that children are regularly served. Milk or water will be served as beverages. Meals will be served at tables of three to six children and one adult, which is a standard practice at the childcare centers. The adults at the table will be instructed not to discuss the food or to encourage the children to eat. There will be three conditions, with changes only in the proportion of lower-energy-dense vegetables and fruits at the meals. Each participant will be served an individual pre-weighed meal. All food and beverage items will be weighed with a food scale before and after meals and snacks to within 1 gram in order to determine the amount consumed. Participants will be told they can eat as much or as little of the foods as they would like. Trained observers (research personnel) will document food and beverage spillage and any comments made about the food. The children will be provided with an evening snack pre-packaged for consumption at home. Any leftover food from the evening snack will be returned to the childcare center the following day to be weighed.
2. In the event of extenuating circumstances (e.g. unexpected childcare center closure, inclement weather) the researchers may decide to pack out a meal, or meals, which can be consumed outside of the childcare center. Participants will be notified in advance if this should occur. Any leftover food from a packed meal, or meals, will be returned to the childcare center the following day to be weighed. Applicable instructions for serving or reheating will be provided.
3. Parents will be asked not to serve any additional food or beverages to their child apart from those provided by the study and water. If the participant deviates from the procedure and consumes extra food, the estimated food and beverage consumption will be requested.
4. Physical activity levels will be measured over the five consecutive days using triaxial accelerometers. The accelerometers will be worn while the children are present at the childcare center (from before breakfast to after dinner). Research staff will attach a device to the child's waist upon arrival at the center each morning and remove the device upon the child's departure from the center each evening.
5. On the 16th visit to the childcare center, children will have their height and weight measured by a trained staff member of the lab. Body weight will be measured in triplicate using a portable digital scale. Height will be measured in triplicate using a portable stadiometer. The children will also complete the Tasting Game. They will be given small samples of foods served in the study and asked to point to a cartoon face that represents their liking of the food on a scale of yucky to

yummy. Children will also look at three plates (one from each condition) from two meals and be asked which they would prefer.

6. Parents will be asked to complete an Evening Report Form, Background Questionnaire, the Children's Eating Behavior Questionnaire, the Caregiver's Feeding Style Questionnaire, and the Child Feeding Questionnaire. The Evening Report Form will be completed at the end of each study day and returned each morning to research personnel. All questionnaires (the Background Questionnaire, Children's Eating Behavior Questionnaire, Caregiver's Feeding Style Questionnaire and Child Feeding Questionnaire) will be administered during the last five days of the study. All questionnaires will be completed via pencil and paper.
7. Photos of children eating, participating in the tasting game, and having their height and weight measured may be taken.
8. There will be a minimum of a one-week break between study weeks. There will be a four- to six-week wash-out period between running this and any previous experiments at each childcare center.
9. Approved lab staff (on this IRB application) will email parents the Friday before a study week to remind parents of the start of the study.
10. Teachers will be asked to answer two questionnaires (Caregiver's Feeding Style Questionnaire, Children's Eating Behavior Questionnaire) per child in their classroom. Teachers will only complete questionnaires about children who are enrolled in the study. This questionnaire will be administered by paper and pencil. Teachers will be verbally told by study personnel which child to answer each questionnaire about.
11. Data from questionnaires completed by teachers will be linked to child participants by a three-digit number and letter only.

6.3 Duration of Participation

The duration of the study will be five consecutive days, and each participant will be asked to complete three of these five-day periods. Within each day, each meal and snack will take approximately 30 minutes to complete. The estimated time required by the participant each day is 2.5 hrs. The estimated total time required for all of the meals is 37.5 hours. The height and weight measurements and the Tasting Game on the final visit after the 15 days are completed will require an additional hour of time. For the parents, another 30 minutes will be requested to complete the questionnaires. The total time commitment for the study is 39 hours. Teachers will spend approximately 20 minutes per questionnaire.

7.0 Statistical Plan

7.1 Sample size determination

The sample size for the study was based on the statistical power to detect differences in energy intake between the Baseline and Substitution conditions across study days. The sample size was estimated from a linear mixed model and an approximation technique [4] using exemplary data sets based on our previous five-day studies in preschool children [1,2]. We assumed that the minimum clinically significant difference in energy intake across days was 50 kcal, which is about 5% of daily intake for a typical preschool child. To detect this change over time with a Type 1 error rate of 5% and power of 90%, it was determined that 48 participants would be required. To allow for withdrawals and non-compliance to the protocol, 55 to 60 children will be enrolled.

7.2 Statistical methods

The study hypotheses will be assessed using linear mixed models with repeated measures. Differences in mean outcomes will be tested across the three experimental conditions of the proportion of vegetables and fruits served in meals, as well as across the five days of intake.

Hypotheses 1 and 2 test whether variations in the proportion of low- and high-energy-dense foods influence the mean daily outcomes of energy intake, food and beverage intake, and energy density. To test these hypotheses, the fixed factors in the models will be condition, study week, study day, menu, childcare center, classroom, and sex. Interactions between these factors will be tested and removed from the model if not significant and participants will be treated as a random effect. The Tukey-Kramer method will be used to adjust for multiple pairwise comparisons between means. The energy content of parentally reported non-study food consumption will be estimated by research staff. Daily energy intake will be analyzed both with and without the non-study items.

Hypothesis 3, concerning whether daily energy intake in the three conditions converges over each five-day period, tests whether regulation of energy intake occurs over time in response to variations in the proportion of low- and high-energy-dense foods served. To test this hypothesis, a random coefficients model will be used. The trajectories of intake over time will be allowed to vary randomly across subjects; thus, they will be modeled separately for each child. Time (study day) will be treated as a continuous covariate in the model and polynomial factors of time (linear and quadratic coefficients) will be tested as both fixed and random effects to determine the shape of the intake trajectory. The intercept of the trajectory will reflect the magnitude of daily intake at Day 1

and the linear coefficient will represent the rate of change in intake (slope) across time after Day 1. The quadratic coefficient, if significant, will represent the rate of acceleration or deceleration in intake (curvature) across time after Day 1.

It has been predetermined that children who have incomplete intake measures on ≥3 days in all 3 conditions will be excluded from analysis. It has also been predetermined that children who eat >95% of the food weight served on at least 1 day will be classified as “plate cleaners”, and their influence on the results will be tested.

Analysis of covariance with linear mixed models will be used to test the influence on these outcomes of individual subject characteristics such as age, body mass index, and daily level of physical activity. Independent-samples t-tests will be used to assess differences in subject characteristics by sex and weight status (normal weight or overweight/obesity). Results will be considered significant at $p < 0.05$. All data will be analyzed using SAS software (SAS 9.4; SAS Institute, Inc.).

8.0 Confidentiality, Privacy and Data Management

8.1 Confidentiality

We will take photos of the children having a test meal and may use them for poster or oral presentations at scientific meetings. Parents will check on their consent form if they agree to have their child’s photo taken. All images will be stored on a password-protected network drive that only those on this application have access to. Images will be stored with a generic title and will not be labeled with any identifiable information. The images will be deleted from the drive within three years of completion of the study.

8.1.1 Identifiers associated with data and/or specimens

Subjects will only be identified by a three-digit subject number, dot color, and a letter of the alphabet.
Teachers will not have their own identifiers.

8.1.1.1 Use of Codes, Master List

The master list that links subject numbers to personal information will be stored in a locked closet in the Lab Manager's office. Only those listed on this application will have access to it. The master list will be destroyed upon

publication of the study or five years after the close of the study, whichever comes first.

8.1.2 Storage of Data and/or Specimens

Paper data will be stored in a locked cabinet in a locked closet in the Lab Manager's office. Data on network drives will have no identifiable information. All computers and network drives are password protected.

Paper and computer records of data will be stored indefinitely with identifiers removed. Paper records will be stored in a locked filing cabinet in the Lab Manager's office. Computer records will be stored on a password-protected network drive that only those on this application have access to.

This study will be issued a Certificate of Confidentiality. Researchers will not disclose or provide any identifiable information without the subject's prior consent or where permitted according to NIH's Policy on Issuing Certificates of Confidentiality.

8.1.3 Access to Data and/or Specimens

Only those listed on this application will have access to the data.

8.1.4 Transferring Data and/or Specimens

The researchers do not plan to release identifiable information collected in the study. However, if researchers consider releasing identifiable information in the future – the individual or institution receiving the identifiable information will be made aware they are also subject to the requirements of subsection 301(d) of the Public Health Service Act.

8.2 Privacy

No personal information other than first name of the child, and telephone number and email address of the parent, is accessible to research staff. Subjects remain anonymous except for study number and letter. Only senior staff members listed on this IRB application will have access to personal information such as address, age, weight.

Data on food consumption is collected in a separate room where only study staff is present. Heights and weights for children are collected in a private area of the child's classroom.

9.0 Data and Safety Monitoring Plan

The proposed study poses minimal risk to participants, since the procedures involve little change from the usual meals and activities at the childcare centers.

10.0 Risks

There are minimal risks associated with participation in this project. The risks are no greater than those associated with the children's usual attendance and consumption of meals at the childcare center and at home, which include the risk of food allergy, food-borne illness, and choking on food.

The amounts of food and beverages served in all conditions meet or exceed minimum requirements for calorie and nutrient content for this age group as specified by the USDA and CACFP.

There are no potential risks for teacher participants.

11.0 Potential Benefits to Subjects and Others

11.1 Potential Benefits to Subjects

There are no direct initial benefits to subjects participating in the proposed study.

11.2 Potential Benefits to Others

The research will benefit society by advancing understanding of children's eating behavior and identifying meal-related strategies for the prevention of obesity in children.

12.0 Sharing Results with Subjects

No results will be shared with the subjects.

13.0 Economic Burden to Subjects

13.1 Costs

N/A

13.2 Compensation for research-related injury

It is the policy of the institution to provide neither financial compensation nor free medical treatment for research-related injury. In the event of injury resulting from this research, medical treatment is available but will be provided at the usual charge. Costs for the treatment of research-related injuries will be charged to subjects or their insurance carriers.

14.0 Other Approvals

Local childcare centers have given permission for the conduct of this research in their facilities. Letters of permissions are on file with the Institutional Review Board.

15.0 Adverse Event Reporting

15.1 Reporting Adverse Reactions and Unanticipated Problems to the Responsible IRB

In accordance with applicable policies of The Pennsylvania State University Institutional Review Board (IRB), the investigator will report, to the IRB, any observed or reported harm (adverse event) experienced by a subject or other individual, which in the opinion of the investigator is determined to be (1) unexpected; and (2) probably related to the research procedures. Harms (adverse events) will be submitted to the IRB in accordance with the IRB policies and procedures.

15.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the Penn State quality assurance program office(s), IRB, the sponsor, and government regulatory bodies, of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory).

16.0 Study Monitoring, Auditing and Inspecting

16.1 Auditing and Inspecting

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17.0 References

[1] Smethers, A. D., Roe, L. S., Sanchez, C. E., Zuraikat, F. M., Keller, K. L., Kling, S. M., & Rolls, B. J. (2019). Portion size has sustained effects over 5 days in preschool children: A randomized trial. *The American Journal of Clinical Nutrition*, 109(5), 1361-1372.

[2] Smethers, A. D., Roe, L. S., Sanchez, C. E., Zuraikat, F. M., Keller, K. L., & Rolls, B. J. (2019). Both increases and decreases in energy density lead to sustained changes in preschool children's energy intake over 5 days. *Physiology & Behavior*, 204, 210-218.

[3] USDA Food and Nutrition Service. Child and Adult Care Food Program (CACFP). Nutrition Standards for CACFP Meals and Snacks. Version current: 26 May 2020 [Internet]. Available from: https://www.fns.usda.gov/cacfp/meals-and-snacks.

[4] Littell R.C., Milliken G.A., Stroup W.W., Wolfinger R.D., Schabenberger O. SAS for Mixed Models, 2nd ed. SAS Institute Inc., Cary, NC, 2006.