

Official title:	Snacks, Smiles and Taste Preferences
NCT number:	NCT03631992
Document:	Protocol
Document date:	4.14.21

1) Abstract of the study

In the United States, preschoolers are consuming, often in the context of snacking, approximately 13 teaspoons of added sugars daily, more than double what the American Heart Association recommends for children. Evidence-based strategies to effectively reduce added sugar intake and promote the liking of beverages and foods containing less added sugar are a clear public health priority. Our proposed study builds upon our prior NIH-sponsored research that demonstrates the plasticity of preferences during early life and the fundamental role that repeated exposure has on shifting preferences. We have established that children have inborn, elevated preferences for sweet taste, that sugary snacks are often offered for nonnutritive reasons by mothers, and that personal variation in the reward sensitivity and appetitive tastes may make some children more susceptible to dietary excess of sugars from snacks. This proposed research will be the first randomized controlled trial to determine whether sweet preferences can be downshifted in preschoolers, whether the impact of the intervention generalizes to other foods as reflected by greater liking and intake of novel low-sugar snacks in the laboratory, and will further examine whether inter-individual variation in taste genotype, appetitive regulation traits, and reward sensitivity determines whether certain children are less successful in shifting preference than others. This research will serve the long-term goals of establishing evidenced-based strategies to reduce children's consumption of added sugar and promoting healthy snacking behaviors.

2) Protocol Title

Downshifting Sweet Preference and Added Sugar Intake during Snacking Among Young Children: A Randomized Controlled Trial

3) Investigators

Principal Investigator

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Co-Principal Investigator

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Associate Member, Monell Chemical Senses Center

4) Objectives

The overarching goal of this study is to experimentally investigate whether preferences for sweet and acceptance of low sweet snacks are modifiable and can be altered in the context of snacking at home. This study has three primary aims and one secondary aim.

Primary aims:

1. To determine whether sweet preference and acceptance of low sweet snacks can be altered by 4 months of daily dietary exposure to low-sugar snacks and whether it persists.
2. To determine whether the reduced sweet preference generalizes to novel low-sugar foods.
3. To identify sources of individual differences in the effectiveness of the intervention

Secondary aim:

1. To explore the impact of the intervention on mothers.

Hypothesis:

- The intervention that repeatedly exposes children to low-sweet snack foods will result in a downward shift in the level of sweetness most preferred and increase their liking and acceptance of other low-sugar snacks.

5) Rationale and Significance

From the age of 2 years, an American is more likely to consume a manufactured sweet than a fruit on any given day. While the use of added sugars and the discovery of chemicals that impart a sweet taste with few or no calories are relatively recent phenomena in human history, our attraction to sweet taste is not. The robust, inborn attraction children have for sweet taste has become entrenched in the current obesogenic environment of pervasive marketing, availability, and high rates of consumption of highly sweetened beverages and foods. Among preschoolers, snacks account for one-third of their caloric intake and almost 40% of added sugar intake, which well exceeds recommended levels. Strong evidence links diets high in added sugar with increased risk of dental caries and heart disease risk factors, including obesity and elevated blood pressure, making recent calls for reductions in added sugar among children a clear public health priority.

One way to reduce added sugar intake would be to downshift children's 'bliss point' for sweet taste and teach them to like less sweet foods. While the use of added sugars and the discovery of chemicals that impart a sweet taste with few or no calories are a relatively recent phenomena in human dietary history, our attraction to sweet taste is not. The heightened preferences children have for sweet tastes and the plasticity of this taste sense have become entrenched in the current obesogenic environment of pervasive marketing, availability, and high rates of consumption of highly sweetened foods rich in added sugars. Because of the lack of evidence-based strategies to effectively reduce added sugar consumption by children, the RCT will determine if repeated exposure to low-sugar afternoon snacks for 4 months changes children's acceptance of low sweet foods, sweet taste preferences, and dietary intake of added sugar.

6) Resources and Setting

a) Setting of the Human Research

This research will be conducted at the Center for Obesity Research and Education (CORE) on Temple University's Health Science Campus (3223 N. Broad Street, Suite 175, Philadelphia, PA 19140) and at Monell Chemical Senses Center (3500 Market Street, Philadelphia, PA 19104).

Protocol: Downshifting Sweet Preference and Added Sugar Intake During Snacking
Among Young Children: A Randomized Controlled Trial [version April 2021]

Both sites are easily accessible via stops on the Septa Broad Street (CORE is at Allegheny) and Market-Frankford (Monell is at 34th Street) lines.

b) Staff

All staff at Temple and Monell will be required to complete necessary human subjects training certifications and background checks prior to commencing work with participants. Staff at Temple University include Lauren Dabritz, MS (LD, Project Coordinator), Elizabeth Carney (pre-doctoral student) and student workers (TBN). LD has been working in clinical research for 4 years. LD will be responsible for developing study protocols, hiring and training student workers, recruitment, data collection, data entry, and analysis. Staff at Monell include Ashley Reiter, MS (AR, Monell Project Coordinator, Mennella Lab Manager), Carly Cherwony (Research Technician), Sara Snell (Research Technician), Alissa Smethers PhD (post-doctoral fellow, Monell), Elizabeth Carney (pre-doctoral Temple student), and part-time workers (TBN). AR will be responsible for overseeing all subject testing and data collection at Monell and works with Dr. Mennella to develop protocols, hire and train student workers, and data management. The Temple-Monell team meets at least weekly to discuss study procedures and to problem-solve any study-related issues that arise.

c) Facilities

Family Eating Laboratory (FEL), Center for Obesity Research Education (CORE), Temple University- Jennifer Fisher, Ph.D.

CORE is located on Temple University's Health Science Campus in North Philadelphia, PA, and consists of 8,669 square feet of dedicated research space. CORE is easily accessible by foot, public transportation (bus and metro), and car. Parking is available on-street and in a parking lot across the street from CORE; validation for parking and/or compensation for transportation costs are provided to participants. CORE is equipped with 3 large group conference rooms for holding clinical and/or research meetings, dedicated rooms for conducting family intervention sessions (equipped with tables and adult/child size chairs), a clinic space with 2 examination rooms and a lab room for drawing blood (equipped with examination beds, blood pressure machines, sinks, calibrated digital scales, wall-mounted stadiometers, and biohazard disposal bins). The Family Eating Laboratory (FEL) is a 1,500-square-foot research space within CORE that is well-designed for observational studies of eating behavior. The FEL consists of two group observation rooms, four private adult observation rooms, two waiting areas, an anthropometric measurement room, a fully equipped research kitchen, a food demonstration kitchen, and an observation monitoring room. Observation monitoring resources include sound recording hardware, PCs with observational monitoring/recording software (XProtect Smart Client, Milestone, Inc.), PC/balance interfacing software (Connect, Labtronics, Inc.) for food intake recording, observational analysis software (Observer XT, Noldus, Inc.), and a network server for data storage. The FEL is outfitted with a wall-mounted stadiometer (Harpenden, Inc.) and a pediatric/adult scale (Detecto, Inc.). The main waiting area contains a flat-screen television, as well as age-appropriate toys, games, and magazines. All observation rooms have state-of-the-art network-based audio and video monitoring/recording capabilities. Observation rooms are equipped with covertly mounted pan-tilt-zoom cameras, microphones, and observational mirrors for remote monitoring. Observation monitoring resources include sound recording hardware, PCs with observational monitoring/recording software (XProtect Smart Client, Milestone, Inc.), PC/balance interfacing software (Connect, Labtronics, Inc.) for food intake recording, observational analysis software (Observer XT, Noldus, Inc.), and a network server for data storage. Dr. Fisher's lab will be responsible for testing participants to determine acceptance of snacks that differ in sweetness and added sugar content, preparing and storing study snacks

inventory; distributing snacks to mothers, preparing and weighing snack stimuli for laboratory-based measures of children's intake of low-added-sugar/sweetness snacks, and digital recording and analyses of children's behaviors during snack test sessions conducted at Temple. They will also be responsible for the diet assessments; maternal questionnaires; anthropometric measures; lessons on nutrition and development to mothers; and collection of hair samples for biomarker analysis of added sugar intake.

Monell Chemical Senses Center- Julie Mennella, Ph.D.

Dr. Mennella's laboratories (Laboratories for Pediatric Studies, Genotyping and Sensory Testing) are housed in the Monell Chemical Senses Center. A 300-square-foot office will function as the epicenter for recruiting, data analysis, and storage, and this office, as well as several other laboratories and clinical facilities described below, form her core facilities. Dr. Mennella and her research team have a combined total of 851 square feet of laboratory space that addresses the special needs of psychophysics and behavioral biology and has all equipment necessary for the proposed research. One laboratory functions as a wet lab for the analysis, processing, and storage of biological samples, and another laboratory functions as a research kitchen for the preparation of test stimuli (e.g., foods and test solutions for psychophysical taste testing) and the cleaning, processing (VacMaster Pro™), and storage of food stimuli. A third room functions as a behavioral coding laboratory that contains all the computers and equipment (Observer™) needed for analysis of behaviors.

Dr. Mennella's laboratories have all the equipment needed for biospecimen (i.e., saliva, hair) sample storage. The genotype laboratory is fully equipped for collecting and analyzing human DNA and includes facilities for genotyping of single-nucleotide polymorphisms, including a freezer farm for sample management, an Agilent Tape Station for DNA quantification, and a 12k Flex Open Array system for targeted genotyping. In addition to equipment in her own laboratory, she has access to Monell's P30 Genotyping and DNA/RNA Analysis Core Facility, of which Dr. Joel Mainland is its Director. He frequently uses core facilities of the University of Pennsylvania (e.g., DNA sequencing, proteomics, and bioinformatics facilities) and will be responsible for the processing, storage, and genotyping of the saliva samples (if collected). The Sensory Testing Laboratory is 1,264-square-foot, seven-room testing suite and waiting room area that is devoted exclusively to testing mothers, infants, and children and is located in the Monell Center Human Sensory Facility. Each room contains a sink, computers for data entry, and all the equipment (e.g., Compusense-equipped computers, sinks, scale, analytic balances) and furniture (e.g., toddler tables and chairs) needed to carry out these studies. In particular, two of the testing rooms (172 square feet each) are carpeted and designed for pediatric studies. Monell has a Research Coordinator for human and clinical research, Mr. Dennis Coleman, who assists with the scheduling and use of communal space and electronic scheduling of research participants. He also ensures that all staff members using the facilities have institutional review board approval and other training in handling human research participants. Monell has a 24-hour telephone hotline for subjects to call should they have any questions, concerns, or complaints. Dr. Mennella's lab will be responsible for the collection of the saliva samples (if collected), storage of the hair samples, and testing of dyads including assessments of taste preferences; waist and hip measurements, completion of diet assessments and questionnaires and assessments on nutrition and child development. Some of the assessments of mothers and children will be audio and video recorded. They will also be responsible for the preparation of taste stimuli, and analysis of subset of the digital recordings (e.g., facial action coding system) obtained during the monthly low-sugar snack acceptance tests at Temple so that we can

determine inter-coder reliability. Finally, Dr. Mennella's and Dr. Mainland's lab will be responsible for the initial processing, storage and genotyping of saliva samples (if collected), storage of de-identified hair samples, and the statistical analysis of the outcome data by genotype.

7) Prior Approvals

This study is being funded by NIH/DHHS/National Institute on Deafness and Other Communication Disorders (NIDOC) grant #1R01DC016616.

Temple University will serve as the IRB of record for the Monell Chemical Senses Center (University of Pennsylvania). All new information, unanticipated problems involving risk to subjects, and protocol modifications will be submitted to Temple's IRB, and the approval letters will be submitted to the other institutions IRBs.

8) Study Design

a) Recruitment Methods

Participants will be a convenience sample of 175 children between the ages of 3 and 6 years and their mothers. We will be placing recruitment brochures and flyers in various child care center and dental clinic waiting areas (**see recruitment brochure and flyer**). Dyads will also be recruited from Temple University Pediatric Clinic, which is located in the same building as CORE (**see letter of support**). We will be placing ads in local newspapers, internet sites, and the Monell Chemical Senses Center website, using the same language used in the submitted brochure and flyer. We will also recruit mothers who have participated in past research at Temple and Monell and have given consent to be contacted about future studies. Temple will be responsible for recruiting, screening, and enrolling eligible participants. Balanced randomization, stratified by child sex, and race/ethnicity, will be achieved by permuted block randomization. A recruitment screener will be used to determine subject's eligibility. We ask potential subjects an extensive list of questions to ensure that the dyad can safely participate in the study as well as give them information about the study and the related timeline. We ask the mother about their child's gender, race/ethnicity, date of birth for both mother and child to determine if they fit the age criteria for participating (child has to be between the ages of 3-6 years of age and mother has to be 18 years of age and older). The other questions on the screener relate to child's eating preferences, mother's feeding responsibility status, current hair length of both mother and child since we collect hair samples during various point of the study, and questions about their child's dental hygiene and behaviors. We will ask potential subjects all of the questions on the screener, despite any eligibility, so that descriptive statistics can be developed for future publications, reporting to the funders, and seeking recruitment trends.

We will be asking participants for their consent to contact them about future research studies.

Compensation. Mother-child dyads can receive up to \$1,500 in the form of Visa gift cards (Temple) or cash (Monell) for participation in this study. Below is a breakdown of compensation.

Payment Schedule		
Visit	Amount	How you will receive payment
Visit 1, 2, 4, 6, 8 (assessments and lessons) Visits at Temple	\$100 x 5= \$500	Visa card given end of each study visit
Visit 10, 12 (assessments and lessons) Visits at Temple	\$150 x 2= \$300	Visa card given end of each study visit
Visit 3, 5, 7, 9 (assessments and lessons) Visits at Monell	\$100 x 4= \$400	Cash given after each study visit
Visit 11, 13 (assessments and lessons) Visits at Monell	\$150 x 2= \$300	Cash given after each study visit
Total amount possible	\$1,500	

Inclusion criteria:

1. English speaking mother ≥ 18 years of age
2. Mother has primary responsibility for purchasing the foods and drinks for the family and for providing the food and drinks to the child
3. Mother is willing to feed the child the daily snack and complete all forms related to snacking.
4. Mother is willing to refrain from eating foods and beverages high in added sugar in the eligible child's presence for the duration of the study.
5. Child's hair is approximately 3cm (1 inch) in length before or at the time of randomization (V1, V2) and mother agrees not to cut the child's hair during the course of the trial.

Exclusion criteria:

1. History of feeding difficulties, severe food allergies, or special diet (e.g. vegan)
2. Medical conditions (e.g. cystic fibrosis) affecting growth and eating
3. Child and/or parent does not comprehend and complete study tasks that occur during baseline study visits (V1, V2) or between (at-home phone interviews) these baseline visits.

b) **Study Timelines**

All dyads will be asked to participate in a study that will last approximately 5 months. We anticipate completing this study within a 5-year timeframe with an anticipated start date of June 2018 and completion of the study in June 2023.

c) **Study Procedures and Data Analysis**

Overview. The overarching goal of the proposed RCT is to experimentally investigate the fundamental question of whether preferences for sweet are modifiable and can be modified in the context of snacking at home. The proposed within- and between-subjects RCT has three Specific Aims and one Secondary Aim, which will be conducted concurrently (see Objectives). The study participants, preschoolers (3-6 y) and their mothers, will be randomized to one of two groups (control, intervention) that differ in the sweetness and sugar content (and consequently energy) of afternoon snacks provided daily to children at home, using established home-based,

repeated-exposure, parent-led interventions. Mothers in the intervention group will also be given dietary instructions, based on recent guidelines, for reducing added sugar in foods offered to children outside of the provided snacks, portion size for sugars and other foods, lessons on dental hygiene and nutrition lessons that support the goals of reducing “sweet” exposure and added sugar intake. Mothers in the control group will be given lessons on development (e.g., sleep habits, portion size, physical activity, screen time); they will receive lessons on dental hygiene and sugar intake at the end of the trial.

Snack Intervention and Instructions and Lessons to Mothers. Mothers in both groups will be blind to the study aims and hypothesis to minimize potential bias. Both groups will be provided good quality snacks for the duration of the study that just vary in sugar content. Immediately following the study, all mothers will be debriefed about the true purpose of the study and mothers in the control group will be provided all of the information given in the intervention group (see debriefing script).

At baseline, with review at each study visit, mothers will be told to offer the child all foods/beverages provided for an afternoon snack. Snacks will be given to both the intervention and control group for the duration of the study. The snacks offered will be common snacks, with regular versions given to the control and reduced sugar options given to the intervention (**see Table 2 at the end of this section**). The foods provided are commercially available in the grocery store. To minimize risk, we will screen for food allergies during recruitment. Mothers will be encouraged to provide physical assistance when necessary (e.g., handing a spoon to the child, helping the child reach food) but otherwise let the child feed independently. They will be told to encourage but not pressure their child to at least taste each food or beverage and to let the child determine whether and how much they consume, after tasting, without force or pressure to eat; effects of repeated exposure have been demonstrated using small tastes of foods.

The groups will differ in whether and when they receive other types of instructions and lessons on nutrition and child development (**see Table 1 at the end of this section**). Mothers in the intervention group will be given instructions and lessons to limit offering high-added-sugar foods at other snacks and meals and will receive toothbrushes, toothpaste and instructions on caries prevention and proper dental hygiene from the Temple School of Dentistry, based on clinical guidelines. Because added sugar content is currently not required on Nutrition Facts labels, we will provide food-based guidance. Following recent AHA recommendations on added sugar intake in children, mothers in the intervention group will be instructed to not feed their child SSBs for the entire 4 months. Mothers will be given a list of other top food sources of added sugars—SSBs, candy (including fruit gummies), grain-based desserts, dairy-based desserts, and sugary ready-to-eat cereal—with instructions to avoid or limit offering these to her child 1 serving/week, as recommended, during the 4-month intervention phase of the trial. This list will also include NNS-containing foods and beverages. Along with this guidance, mothers will be given a list of low-sugar/sweetness alternatives to facilitate adherence. A trained interventionist will provide nutritional lessons that support the goal of reducing added sugar intake: appropriate child serving sizes, identifying sugar content on food labels, and identifying NNSs on ingredient lists. This guidance will facilitate the dietary instructions and help mothers identify food items low in added sugar that could be offered at other snack or meal times. In light of qualitative findings describing mothers’ own liking of snacks, we will ask mothers to refrain from eating sugary snacks and SSBs in the presence of their children.

Protocol: Downshifting Sweet Preference and Added Sugar Intake During Snacking
Among Young Children: A Randomized Controlled Trial [version April 2021]

For those mothers in the intervention group, we will provide them with \$10 each month during the intervention phase of the trial to buy 2-3 different types of fresh fruit (e.g., 2 bananas, 2 peaches, pint of blueberries) and a packet of baby carrots to feed their children for their afternoon snack. We found this necessary to ensure that children were eating fresh ripe fruit and that the carrots were appealing in texture. The money will be provided at the time of snack pickup. To verify the purchase, mothers will be asked to take a photo of the produce and email to the study team.

Mothers in the control group will also receive lessons at the same time points as the intervention group. However, the lessons will differ in content. The control group will receive lessons on sleep habits, television, portion size, and physical activity for children during the course of the trial. All mothers in the control group will receive the lessons given to the intervention group on caries prevention, proper dental hygiene and added sugar but this will occur after the intervention period has ended (Visit 12).

We will implement several measures to determine adherence to the protocol and compliance. First, project staff, blind to the hypothesis or group assignment of the dyads, will systematically review the mothers' logs, which indicate the time of day the child ate the snack items, whether he or she tried/ate each food item or beverage, and how much the mother perceived her child enjoyed eating each snack. These records will be reviewed monthly by project staff and used to 1) calculate compliance rates, 2) provide feedback to mothers, and 3) document dose of the intervention (e.g., whether or not the snack was given/consumed) and adherence. Second, modifying fidelity assessments, we will include a short (e.g., 5-min) questionnaire and/or assessment during each monthly visit at Monell (Visits 3, 5, 7, 9) to assess mothers comprehension of the information provided in the nutrition and child development lessons. For some of these assessments will digitally record the interaction of mothers and their child (e.g. oral hygiene practices) for future evaluation. Five-minute assessments were chosen because this length of time was effective in determining individual variability in fidelity for other interventions. After completion, feedback will be provided for intervention group mothers only; this will allow us to determine whether they are learning the lessons and to provide feedback on snack choices and issues related to acceptance of the snacks by the children. Third, hair samples will be taken before (Visit 1 or Visit 2) and end of the intervention phase (Visit 10) to estimate intake of two major sources of added sugars (i.e., corn, cane) in the US food supply; values reflect intake over the prior three months and will be used as data on compliance among the intervention group. Mothers will be instructed not to cut their child's hair throughout the trial.

Table 1. Description of Randomized Groups		
Snacks and Instructions	Control Group	Intervention Group
Composition of provided afternoon snacks (Average)	Total sugar: 40-50 g Energy: 300-350 kcal	Total sugar: 10-20 g Energy: 260-300 kcal
Dietary instructions outside of provided snacks	None	• No SSBs for the child for 4 months • Avoid top food sources of added sugar; limit to one serving per week¹ • Avoid NNSs • Offer low-sugar alternatives¹
Lesson topics for mothers ²	• Physical activity • Portion size • Sleep habits • Screen time	• Oral health hygiene • Learn about healthy snack and beverage choices • Locate / identify sugar content on food label • Identify NNSs on labels • Portion size
Abbreviations: SSBs, sugar sweetened beverages; NNSs, nonnutritive sweeteners ¹ Per American Heart Association (AHA) guidelines ² The control group will receive the intervention lessons on dental hygiene and added sugar at Visit 12.		

Table 2. Examples of type of snacks provided for control and intervention groups			
Snack	Serving size	Energy (kcal)	Sugar (g)
Control group			
Beverages			
Mott's 100% apple juice	6.8 oz	100	23
Horizon low fat chocolate milk	8 oz	150	22
Horizon low fat vanilla milk	8 oz	140	22
Juicy Juice fruit punch	6.8 oz	100	22
Food			
Nabisco Handi snacks, cookies and cream	1.1 oz	140	12
Mott's regular applesauce	4 oz	90	22
Quaker rice cake, chocolate crunch	0.5 oz	60	4
Dannon fruit at the bottom yogurt, strawberry banana	5.3 oz	130	22
Honey nut Cheerios	1 oz	110	9
Ritz peanut butter bites	1.4 oz	200	5
Dippin' Stix, sweet gala apples & caramel	2.8 oz	100	20
Banana, small	3.6 oz	90	12
Flipz chocolate covered pretzels	1 oz	130	11
Goldfish crackers	1 oz	130	1
Kraft string cheese	1 oz	80	0
Mother's frosted animal crackers	1 oz	140	12
Philadelphia cream cheese dippers, brown sugar	2.5 oz	220	9
Boomchikapop, sweet & salty	1 oz	140	8
Lunchables, Turkey, Cheddar, & Capri Sun Cracker Stackers	3.8 oz	330	25
DelMonte mixed fruit cup	4 oz	70	15
Stacy's pita chips, cinnamon sugar	1.5 oz	200	8
Goldfish s'mores crackers	1 oz	160	12
Intervention group			
Beverages			
Horizon low fat milk	8 oz	110	12
Mott's 100% apple juice	6.8 oz	100	23
Mott's for tots apple juice	6.8 oz	50	12
Water	8 oz	0	0
Food			
Nabisco Handi snacks, breadsticks and cheese	1.1 oz	110	4
Justin's peanut butter, squeeze pack	1.2 oz	190	2
Apple, gala, small	5.5 oz	89	16
Quaker rice cake, chocolate crunch	0.5 oz	60	4
Stonyfield yogurt, whole milk, strawberry	5.3 oz	140	10
Cheerios	1 oz	100	1
Ritz cheese bites	1.4 oz	200	5
Dippin' Stix, baby carrots and ranch dip	2.8 oz	90	5
Banana, small	3.6 oz	90	12
Kraft string cheese	1 oz	80	0
Goldfish crackers	1 oz	130	1

Chips Ahoy cookies	1 oz	140	8
Philadelphia cream cheese dippers, garden vegetable	2.5 oz	200	2
Boomchikapop, sea salt	1 oz	80	0
Lunchables, Turkey, Cheddar with crackers	3.2 oz	260	5
Sabra hummus singles	2 oz	150	0
Rold Gold pretzels	1 oz	110	0
Stacy's pita chips, simply naked	1.5 oz	20	1
Abbreviations: oz, ounce; If snacks are not available, they will be substituted with another			

Procedures

The research sites are the Family Eating Lab at the Center for Obesity Research and Education (CORE) at Temple University and the Human Sensory Facility at Monell Chemical Senses Center. Research sites are located ~5 miles apart and can be reached via major N-S and E-W city thoroughfares. Each site is easily accessible by public transit and nearby parking is available.

To control for effects of time of day on feeding, all testing will occur at the same time of day as the home snack exposures. The design of the within- and between-subjects RCT uses a repeated-exposure paradigm previously shown to change acceptance among children. **As shown in Table 3, the RCT will consist of 13 study visits over the course of a period of 5 months for each mother-child dyad.** There will be 2 baseline lab assessments at Temple (Visits 1-2) where we will obtain questionnaires and assessments, and anthropometric measures. In order to continue in the study and to be randomized to study group at Visit 2, we have established a priori that mothers must complete 2 dietary assessments obtained over the telephone between Visit 1 and Visit 2. As shown in Table 3, at the study visits before, during the 4-month intervention phase (Visits 3-11) and after the intervention (1-month delay Visits 12 and 13) we will conduct testing to determine level of sweetness and saltiness most preferred and the acceptance/liking of novel low-sweet snacks; and anthropometric measures will be obtained in duplicate by trained staff. At V3 we may collect saliva from mother and child for genotyping of taste-related genes and before (V1-V2) and at the end of the intervention phase (V10) we will collect hair samples for analysis of added sugar biomarkers from both mothers and children. During the intervention phase of the trial both the intervention and control group will be given a supply of afternoon snacks that mothers will be instructed to offer their child each day and forms to complete at home.

Outcome Measures

Intakes and liking/acceptance of snacks low in sweetness. Children's liking and intake of low- sweetness foods will be assessed at monthly visits to the Family Eating Laboratory at CORE (Visits 1, 2, 4, 6, 8, 10, 12). We will test all children at each monthly visit for their acceptance of a beverage (e.g., water), and food item (e.g., fruit) to determine changes over time and between groups. Some will not have been provided to either group as a snack, to evaluate the extent to which intervention effects generalize. Weighed intake methods will be used to assess food intake, and children will indicate their liking using the categorical hedonic face scale. Children will be tested individually using age-appropriate chairs and tables, with their mother present. Mothers will be instructed to let the child eat independently, offer physical

assistance if needed, and refrain from prompting or encouraging the child to eat; sessions will be monitored by audio and video recording by digital camera and trained researchers will observe the session remote to verify adherence. Each digital recording will undergo frame-by-frame analysis using an event recorder program; staff at Temple and Monell will be blind to hypotheses and group assignment and trained in behavioral coding including Facial Action Coding System (FACS), which dissects orofacial responses into constituent action units and has been shown to discriminate children's liking/disliking of tastes.

Taste tests. The test methods chosen have been used in the clinical evaluation of taste among pediatric and adult populations. We will determine the children's and/or mothers' level of sweetness and saltiness most preferred in gelatin and water or broth using the NIH Toolbox forced-choice tracking procedure at the Monell Sensory Testing Facility (Visits 3, 5, 7, 9, 11, 13). We choose to evaluate sweetness in two contexts to determine if change in taste preferences generalizes to different types of foods (beverage, gelatin) and to other tastes (salt preference in broth). To determine the level of taste most preferred, participants will be presented with pairs of either sucrose or salt solutions (5 ml each) or gelatins that differ in sucrose content. They will taste each pair item for 5s and then point to which they like better, without instruction on how the stimuli differ. Each subsequent pair will contain the selected concentration paired with an adjacent stimulus concentration. This pattern continues until the participant either chooses two consecutive times the same concentration paired with both a higher and a lower concentration, or chooses the highest or lowest concentration twice. The entire task will be repeated after a 3-min break, with stimulus pairs presented in reverse order. This enables research to assess whether children understand the task and that their responses are not random (e.g., choose whatever comes first which is evidence of position bias). For each method, practice sessions are also embedded in the task to determine if children understands the task. Because children of this age range have a limited attention span, taste testing will last 30 min or less.

Eating motivation and reward. Appetitive traits that reflect motivation to eat will be assessed by maternal report using the Children's Eating Behavior Questionnaire, which consists of 8 subscales. We will focus on the "food responsiveness" and "enjoyment of food" subscales, constructs thought to reflect individual differences in appetitive drive, with higher levels observed among heavier children and those with poorer appetite regulation. Internal consistency for scales of food responsiveness and enjoyment of food were found to be acceptable ($\alpha > 0.70$) in studies on preschool-age children. Sensitivity to rewards, which has been associated with body mass index in children and snack and SSB intake in teens will be assessed using the adult and child version of the Palatable Eating Motives Scale and the Behavioral Activation Scale; for the latter we will focus on total and "Drive" subscale scores because each demonstrated acceptable internal consistency ($\alpha > 0.70$) among preschoolers.

1. **Hair biomarkers.** To estimate free-living intake of the main forms of added sugar (corn, cane), we will collect at least 50 and no more than 200 strands (at least ~ 3 cm in length, which is equivalent to 1 inch) hair samples before (Visit 1-Visit 2) and at the end of the intervention phase (Visit 10) from mothers and children. The hair samples will be analyzed using stable isotope ratio mass spectrometry to estimate added sugar intake over the prior months. The Principal Investigators (PI) will provide samples of their own hair to be used as internal standards for the stable isotope analysis of hair samples. The internal standards will be used to monitor and ensure the reliability of the assay across repeated measurements during the analysis trial samples. PI consent will not be obtained because the PIs are fully aware of the procedures (i.e., cutting a section of their own hair), purposes, and the risk/benefits involved in obtaining hair samples and performing the assay.

Estimates, based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios, have shown sensitivity to detect changes in added sugar intake by adults following dietary interventions and have been significantly associated with tooth decay in children. We ask that mothers do not cut their child's hair during the course of their enrollment in the study and that they keep their own hair at least 2 inches in length.

Dietary assessment of added sugars and other foods. Dietary intake of children and mothers will be assessed using a variety of methods including the National Cancer Institute's web-based Automated Self-Administered 24 hour recall (ASA24) and Diet History III Questionnaire (food and beverage frequency) as well as the pediatric and adult liking questionnaires (e.g., PALS, ALS). Assessments will be made by project staff at Monell and Temple over the phone or in person at each site. ASA24 incorporates the multiple pass methodology by the USDA and utilizes multiple databases to derive nutrient intakes, particularly Food and Nutrient Database for Dietary Studies. Mothers will report about the child's dietary intake over the past 24-hour period, including periods outside the mother's care with the assistance of other caregivers (e.g. grandparents). Mothers will complete dietary assessments at baseline and throughout the study for both them and their child. On the day before each visit, we ask that mothers write down or take photos of all the foods and beverages and amount that they AND their child eat and drink. We will provide them with diet logs to complete for these days to help in the dietary recall. If the child is out of the mother's care any time during that day, we will ask them to have the caregiver to record on the diet log what and how much the child eats and drinks. Caregivers will complete dietary assessments at baseline and throughout the study for both them and their child. Because ASA24 and Compusense (for collection of PALS and ALS) are web-based applications, no identifiable data will be entered into the recall records. Data will be exported to excel spreadsheets and will be stored on a HIPAA password-protected drive.

Genotyping and banking. If saliva samples collected at V3 from mothers and/or children, we will extract genomic DNA from saliva samples and then analyze the DNA with a panel of validated markers using the Open Array system. We will first focus on identifying known alleles of bitter receptor gene family (T2R genes) that can be related to sweet taste, the sweet receptor gene TAS1R3, and the gustducin gene GNAT3. If others identify other molecular targets during the course of this research, we will assay for those variants and assess impact on outcome measures, such as level of sweetness most preferred.

Anthropometry. Weight and height measurements of children and mothers in light clothing will be taken in duplicate by trained and certified staff following procedures outlined by Lohman. Weight will be measured to the nearest 0.1kg using a research-grade digital platform scale, and standing height will be measured to the nearest 0.1cm using a stadiometer. From these data, body mass index (BMI; kg/m²) for mothers and BMI-for- age z-scores for children will be calculated and used to categorize child weight status. We will also obtain waist and hip measurements from the children only.

Demographic variables. Basic demographic information (e.g., education, income, race/ethnicity, employment) and family demographics will be self-reported by mothers.

Food security. Household food security status will be measured using the US Household Food Security Module; families will be scores as food secure (high or marginal security) or food insecure (low or very low security).

Other questionnaires and assessments. We will ask each mother to report how much her child ate and how much she thought he or she liked snacks provided. In addition, we will ask her to complete questionnaires to assess the following:

- 1) **Maternal Feeding Styles.** The 19-item 'Child Feeding Style' questionnaire categorizes caregivers' feeding style (e.g., authoritative, authoritarian, indulgent, uninvolved).
- 2) **Children's Eating Behavior.** The 35-item 'Child Eating Behavior' questionnaire assesses caregivers' perception of children's eating behaviors.
- 3) **Snack Parenting Practices.** The 51-item 'Parenting around Child Snacking' questionnaire assesses snack parenting practices of young children.

All questionnaires are published and have been uploaded to eRA.

Timeline of Measures

The table below describes the timing and sites of assessment visits and procedures common to both groups (see Table 3).

Schedule of Snacks & Smiles Activities													
	Timing												
Month of Study	0			1		2		3		4		5	
Phase of Study	Baseline			4-month intervention phase									1 mo delay
Visit Number	1 ¹	2	3	4	5	6	7	8	9	10	11	12	13
CORE, Temple													
Questionnaires and assessments	X	X		X		X		X		X		X	
Height/weight	X					X				X		X	
Snack food tests	X	X		X		X		X		X		X	
Hair collection	X ²									X			
Lessons		X		X		X		X		X			
Snack Distribution		X		X		X		X		X			
Monell													
Questionnaires and assessments			X		X		X		X		X		X
Waist and hip measurements			X				X				X		X
Taste tests			X		X		X		X		X		X
Saliva collection ³													
¹ In between Visits 1 and 2, you must complete both of the 2 requested phone interviews on dietary recall to continue in the study. Randomization to study group will occur at Visit 2, after which your child will begin feeding the provided snacks for 4 months; which is referred to as the intervention phase of the study. ² First hair collection will be delayed until V2 is child's hair is not long enough at start of study. ³ Saliva collected during V3, but dependent on funding.													

d) Withdrawal of Subjects

The Principal Investigators can remove a subject from the research study without the subject's approval. Possible reasons for removal include failure to follow instructions of the research staff, do not comprehend the tasks, do not complete the diet assessments between V1 and V2; do not show up for appointments; do not complete study visits during scheduled week of the

intervention; or if the principal investigators decided the research study is no longer in the subject's best interest.

Participants may also withdraw from the study at any time.

e) Privacy & Confidentiality

Each mother will sign an informed consent form (see **Consent Process** below) detailing confidentiality measures. They will also be asked to sign a video/photo release form.

All the data collected will be stored at CORE for three years after the study ends. All data collected, transmitted and stored is protected by proprietary encryption methods compliant with 21 CFR Part 11. Code numbers will be assigned to each subject to de-identify and maximize anonymity while entering data. The Principal Investigators, Co-Investigators, and study coordinators will have access to the data. All paper copies of data forms will be stored under a double-lock system to ensure confidentiality (locked cabinet in a locked office). Only the Principal Investigators, co-investigators and assigned study personnel will have access to the cabinet. De-identified data will be stored in separate, locked cabinets from identifying information (i.e., consent forms, phone screens). The only places where the subjects' names or other personal identifiers will appear are on the paper screening forms (see recruitment eligibility screener) and on the consent forms. As mentioned earlier, additional questions will be asked once the subject has been determined to be ineligible and those and screeners will also be stored in a secure manner as those subjects that are eligible. Paper forms containing personal identifiers will be contained in a locked office at CORE in a locked filing cabinet. Only the research team, including the PI and her staff, will have access to those paper files. Electronic files containing contact information of participants will be stored on CORE's HIPAA compliant network.

If at any time during the study a participant decides they want to withdraw their permission for the use of their study information, they are informed in the consent form that may cancel their permission to use and disclose the study information in the NIH databases at any time by writing to the study's Primary Investigator, at the name and address listed in the consent form (Dr. Jennifer Fisher, 3223 North Broad Street, Suite 175, Philadelphia, PA 19140). If they do cancel permission to use and disclose information, their part in the study will end and no further information will be collected. They will be informed that cancellation will not affect information already collected in this study.

All data, such as digital recordings, data sheets, saliva (if collected) and hair samples and questionnaire forms, will be coded and de-identified. After data collection and coding, all subsequent analyses will refer to these coded data in such a manner that individual identities cannot be traced. To maintain the safety and confidentiality of the subjects, all records will be stored under double lock system (locked cabinet in a locked office). Only Drs. Fisher and Mennella, and their study coordinators will have access to the cabinet. De-identified data will be stored in separate, locked cabinets from identifying information (i.e., consent forms, phone screens). Training will be provided for all employees at all sites on a yearly basis on the proper handling of blood-borne pathogens and bodily fluids, such as saliva. If collected, the saliva cells will be kept for 20 years but will not contain any identifying information that will be able to link it to the participant. All identifying data will be destroyed upon completion of the protocol. One of Dr. Mennella's laboratories is devoted exclusively to the proper handling and processing of

human bodily fluids (e.g., saliva) and hair. All personnel have been (and future hires will be) trained at Temple University or the University of Pennsylvania, on the rights of human subjects. If collected, saliva samples will be stored in a -80C freezer in a secured location at Monell which is reserved for biological specimens under the supervision of Drs. Mennella and Mainland. Hair samples will be stored at room temperature in sealed containers labeled with ID number in a secured location under the supervision of Drs Fisher and Mennella until processing. Hair samples will be sent to an outside laboratory for analysis following standard protocols for biological specimens.

9) Risks to Subjects

We consider the risks to research subjects in this study to be minimal. Saliva samples from children and mothers and hair samples from children can be obtained with little or no difficulty. Because genetic data are considered unique to each individual, they could theoretically identify the participants. All data and samples, including digital records, hair and saliva samples, will be de-identified and no information that could link the subjects' names or any type of personal identifier will be shared outside the Investigators on this project.

We will be providing foods to children. Risks associated with food consumption are unlikely but include allergies and choking. Risk of food allergy will be minimized through screening, and choking risks will be minimized by avoiding foods associated with choking risk for young children (e.g., uncut grapes, hotdogs, hard candy). All research staff who interact with children will have FBI child abuse clearances and have received training in good clinical practice and all research staff who conduct the food testing (Temple) will receive training in CPR and first-aid.

10) Potential Benefits to Subjects

Benefits to individual subjects are modest. Mothers in the intervention group may benefit from nutritional education. All participating mothers may benefit from education provided by Temple. The provision of free snack foods for a 4-month period may offset family food expenditures. Though modest, these benefits outweigh the risks which are minimal.

11) Costs to Subjects

Mothers may have to pay for babysitting for non-participating children during study visits.

12) Informed Consent

Temple University HRP-803 SOP: Written Documentation of Informed Consent will be followed to document informed consent in writing. Upon subjects' arrival to Temple University for the first visit, the study coordinator will read the written consent form, emphasizing that the subject may, at any time and for any reason, withdraw from the study without and penalty or prejudice. Each mother will be encouraged to ask any questions she might have regarding the study. If the mother understands the study, she will document her informed consent by signing the Consent Form. Also included in the consent form will be a photography release form. This form will be explaining to the mother specific details on what their audio/video recordings may be used for that are research related. This includes having the photographs/recordings included in publications, presentations, and grant applications related to this research study that may be seen by other researchers and by the general public. The videotapes/digital media will be shown to scientists and the media. Neither the mother nor your child will be identified by name. Mothers may choose not to give their permission for audio/video recordings to be shared in that manner, and still participate in the study. Permission of one parent is sufficient even if the other

parent is alive, known, competent, reasonably available, and shares legal responsibility for the care and custody of the child. Prior to initiation of any protocol-specified procedures, mothers must sign the Consent Form. They will be given a copy of the signed consent form.

13) Vulnerable Populations

Participants will be preschool-aged (3-6 years) children and their mothers. The inclusion of children in this research is supported by epidemiological evidence that children's intake of added sugars exceeds recommendation and is influenced by frequent snacking as well as by basic evidence of strong inborn preferences for sweet and the capacity to shift preferences through experiential learning. Dr. Fisher has conducted studies of eating behaviors and dietary intake of young children for more than two decades. These studies have involved both laboratory and free-living assessments of children as well as their caregivers. Dr. Mennella has been studying child development for the past 30 years, and the Monell Center has a long history of doing developmental research and accommodating mothers and their children so that they are comfortable with research participation. Drs. Fisher and Mennella have a history of conducting pediatric research in research facilities that are specifically designed for pediatric studies and that provide privacy, developmentally appropriate furnishings, and study foods appropriate for children. With the experience of the team and the unique clinical and research facilities, we are well suited to conduct the study with the least possible participant burden, optimize their experience, and provide top-quality data in a timely fashion.

We will recruit women of all ethnicities; there is no reason to exclude women of a particular racial group. We plan to recruit from a population that is diverse in race/ethnicity, and we expect the ethnic and other demographic characteristics of the study population to reflect the diversity of the inhabitants of the city of Philadelphia: race 50% Black, 40% White, 7% Asian, 3% more than one race of whom 12% will identify as Hispanic; family income 73% <\$35,000, 11% \$35-\$75,000; and mothers' highest education levels comprising 82% high school graduate or higher and 25% college degree or higher.

We focus on both girls and boys and adult women of all ethnic/minority groups for recruitment based on the higher than recommended intakes of added sugar among all socioeconomic and race/ethnic groups in the United States. We will target the recruitment of mothers of all ethnic/minority groups because we believe the recruitment of a diverse sample will yield greater scientific integrity and generalizability to the findings. While some young children are in care arrangements and/or are cared for by more than one adult, we will focus on mothers who have primary responsibility for feeding their children.

14) Sharing of Results or Incidental Findings with Subjects

We will inform mothers both verbally in the consent process and in the consent form that the National Institutes of Health (NIH), who funds this research project, have set up a health research database of genetic (genotype) and observable (phenotype; such as taste measures) information called dbGaP; database for Genotype and phenotype. The purpose of this database is to allow multiple researchers access to the data to explore new ideas in discovering how genes interact with human diseases. This national database is designed to increase efficiency in both time and money to study disease related by genetics, and the data will be kept indefinitely. Qualified researchers who receive permission to access and share these data may be from other universities or commercial companies. The name or contact information of the participant, or any identifiers will never be released to the databank. However, because genetic data is considered unique to each individual, it could theoretically identify the individual. We will obtain

permission to allow their data to be included in a national genetic database (such as dbGaP; database for Genotype and Phenotype) and inform them that they can withdraw your consent anytime you don't want the data in the national databank. Additionally, if mother agrees to and signs photo release waiver in the consent form, their photos/videos may be included in presentations, grant applications, and publications related to the research.

They also will be informed that a description of this clinical trial will be available on <http://www.ClinicalTrials.gov>, as required by U.S. Law. We will inform them that [ClinicalTrials.gov](http://www.ClinicalTrials.gov) is a website that provides information about federally and privately supported clinical trials. This website will not include information that can identify the participant. At most, the website will include a summary of the results. The participant can search this website at any time.

At Study Visit 12, mothers in the control group will receive the information provided to the intervention group related to dental hygiene and added sugar content. At the final visits (Visit 12 and 13), the mothers will be debriefed about purpose of the research.