Randomized, Controlled Dietary Treatment Study of Pediatric NAFLD

Funding Source: Nutrition Science Initiative

Principal Investigators: Miriam Vos, MD, MSPH and Jeff Schwimmer, MD

Co-Investigators: Ariel Feinstein, MD, Jonathan Africa, MD, Kimberly Newton, MD, Claude Sirlin, MD, Michael Middleton, MD, PhD, Jean Welsh PhD, MPH, RN, and Adina Alazraki, MD

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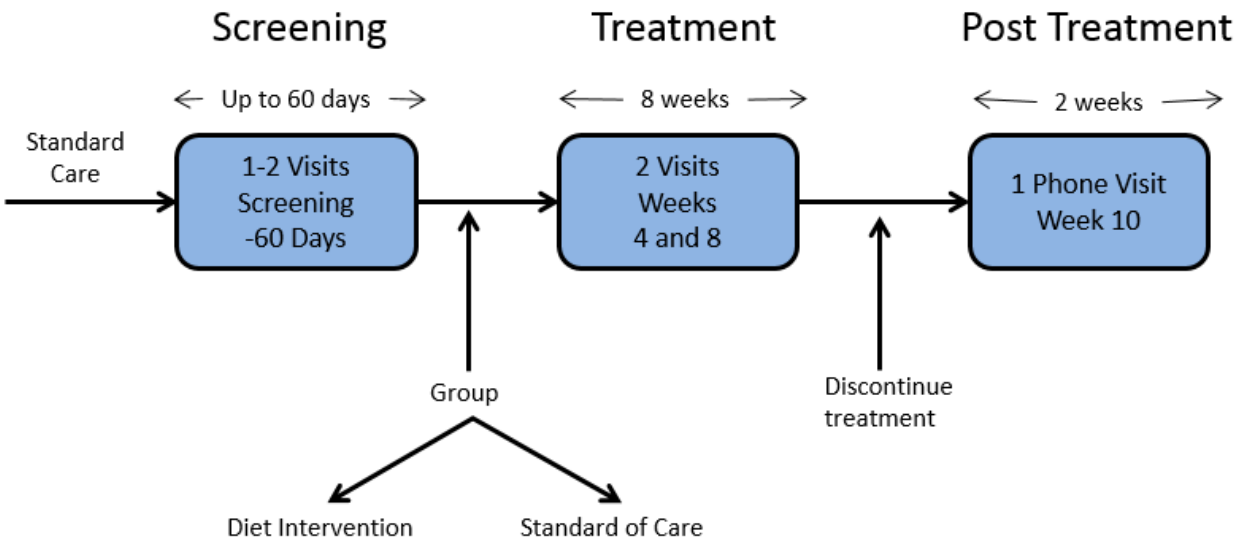
LIST OF ABBREVIATIONS

Adipo-IR	Adipose Tissue Insulin Resistance
AE	Adverse Event
ALT	Alanine Transaminase
AST	Aspartate Aminotransferase
BMI	Body Mass Index
CFR	Code of Federal Regulations
CRF	Case Report Form
GCP	Good Clinical Practice
GGT	Gamma-glutamyl Transpeptidase
HIPAA	Health Insurance Portability and Accountability Act
ICH	International Conference on Harmonization
IRB	Institutional Review Board
MRI	Magnetic Resonance Imaging
NDSR	Nutrition Data System for Research
NAFLD	Nonalcoholic Fatty Liver Disease
NASH	Nonalcoholic Steatohepatitis
NuSI	Nutrition Science Initiative
OGTT	Oral Glucose Tolerance Test
PI	Principal Investigator
RCT	Randomized Control Trial
SOM	School of Medicine
TPN	Total Parenteral Nutrition

PROTOCOL SUMMARY

Title:	**Randomized, Controlled Dietary Treatment Study of Pediatric NAFLD**
Summary:	This is a multisite, randomized, controlled, 8-week outpatient feeding study at Emory University and University of California, San Diego. Participants will be non-diabetic male adolescents with biopsy-proved NAFLD. Two groups of 20 participants will be followed for 8 weeks with hepatic fat content assessed by MRI PDFF at weeks 0, 4, and 8. One group will be a standard of care control to track the naturally-occurring changes in hepatic fat content in children over time. The other group will be provided with a low free sugars (<3% total daily calories) version of their habitual diets. The primary outcome is % change in MRI PDFF over time in the treatment group compared to the control group. Additional parameters of liver function and metabolic status (e.g., serum ALT) will also be assessed.
Objectives:	To evaluate hepatic fat by MRI PDFF before and after 8 weeks of a study-provided low free sugars diet (<3%) compared to a prospective, standard of care, control group receiving only imaging.
Population:	Males age 11 to 16 with a history of liver biopsy confirming NAFLD and hepatic fat by MRI PDFF ≥ 10% during screening.
Type of Trial:	Phase IIb, randomized clinical trial
Study Duration:	2 years
Participant Participation Duration:	Up to 16 weeks
Total Number of Participants:	40 complete (20 per site – 10 interventions and 10 controls) May enroll up to 60 across sites to account for screen fails and drop outs

SCHEMATIC OF STUDY DESIGN



1 KEY ROLES

Principal Investigators:

Jeffrey Schwimmer, MD
University of California, San Diego
UCSD Medical Center
200 West Arbor Drive, MC 8450
San Diego, CA 92103

Miriam Vos, MD, MSPH
Emory University School of Medicine
1760 Haygood Drive, W450
Atlanta, GA 30322

Co-Investigators:

Adina Alazraki, MD
Children's Healthcare of Atlanta

Claude Sirlin, MD
University of California, San Diego

Jonathan Africa, MD
University of California, San Diego

Michael Middleton, MD, PhD
University of California, San Diego

Ariel Feinstein, MD
Children Specialists of San Diego

Joel Lavine, MD, PhD
Columbia University

Jean Welsh, PhD, RN, MPH
Emory University School of Medicine

Institutions:

Emory University and Children's Healthcare of Atlanta

University of California, San Diego and UCSD Medical Center

Study staff:

Project Management
Rebecca Cleeton, MPH, CCRP
Research Manager
Emory University School of Medicine

Janis Durelle, BS
Project Manager
University of California, San Diego

Patricia Ugalde Nicalo, MD
Lead Coordinator
University of California, San Diego

Jennifer Sanford, BA
Research Coordiantor
University of California, San Diego

Nutritionists
Juna Konomi, PhD
Emory University School of Medicine

Cynthia Knott, RD
University of California, San Diego

Statistician
Courtney McCracken, PhD
Emory University School of Medicine

Institutional Review Boards:

Emory University IRB
1599 Clifton Road NE, 5th Floor
Atlanta, GA 30322

University of California, San Diego
Human Research Protections Program
9500 Gilman Drive, Mail Code 0052
La Jolla, California 92093

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2 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

The field of pediatric nonalcoholic fatty liver disease (NAFLD) has grown exponentially over the past decade in response to the growth of the disease and increased awareness of this important liver disease. NAFLD is now the most common liver disease for both adults and children in the US and is estimated to affect more than 20 million persons[1, 2]. It is a chronic liver disease closely associated with the metabolic syndrome, although its role as cause, effect or innocent bystander has yet to be defined[3-9]. NAFLD is an umbrella term and it is histologically categorized into nonalcoholic fatty liver (NAFL), defined as hepatic steatosis (fat) without hepatocellular injury and nonalcoholic steatohepatitis (NASH), defined as hepatic fat plus hepatocellular injury with or without fibrosis[10].

In the US, Mexican-Americans are the group most affected by NAFLD and it is estimated that 1 of 4 Mexican American adults has NAFLD [1]. Hispanic-Americans are 2.5 times more likely to have NAFLD compared to African Americans[2, 11, 12]. Liver disease is the 6th leading cause of death for Hispanic adults compared to 12th for the general population[13, 14]. NAFLD increases the risk of liver disease but also increases risk of type II diabetes, cardiovascular disease (CVD) and the metabolic syndrome[15-20]. For example, a person with NAFLD and fibrosis has 2.5 – 3.5 times the risk of cardiovascular disease death and increased risk of type II diabetes compared to a similarly overweight person without NAFLD (reviewed in Armstrong et al[21]). An estimated 1/3 of adults with nonalcoholic steatohepatitis (NASH), a more severe form of NAFLD, will go on to develop cirrhosis and liver cancer[22] and it is the most rapidly increasing reason for liver transplants in adults. The morbidity and mortality of NAFLD, along with its high prevalence in a vulnerable population make NAFLD a public health priority[23].

In a healthy person, almost no fat (<5% of the liver by volume) is stored in the liver, despite the fact that the liver is a major site of metabolism for dietary fat, cholesterol, triglyceride, free fatty acids and more. NAFLD develops when the balance of triglyceride metabolism in the liver becomes dysregulated in the setting of insulin resistance (IR). Triglyceride rich lipoproteins either originate in the intestine or liver and the sources of fatty acids used for lipoprotein assembly are derived either from de novo lipogenesis (DNL), the diet or from the plasma pool of nonesterified FA (NEFA)[24]. Hepatic DNL appears to underlie the mechanism of excess hepatic storage of triglyceride during carbohydrate feeding in individuals with NAFLD[24-26]. Compared to subjects with metabolic syndrome and no increased hepatic fat, subjects with NAFLD have 3 fold higher rates of DNL in addition to higher plasma levels of FFA during the nighttime[25]. The mechanisms of NASH, in which hepatic fat is associated with inflammation and

hepatocellular injury are complex but likely involve lipotoxicity and increased oxidative stress.

Because of the high level of consumption and its metabolic fate, fructose has been studied in relation to many health problems, including NAFLD. Fructose was initially linked to steatosis and NAFLD in animal models but more recently, a number of human studies have demonstrated links. Fructose enters the diet primarily from sugars that are in processed food and beverages, often referred to as *added sugars.* The World Health Organization (WHO) has provided a draft of guidelines on sugar consumption and has clarified the term "free sugars" which includes all monosaccharides (fructose, glucose, galactose) and disaccharides (sucrose, maltose, trehalose) which are added to the foods by the manufacturer, the cook, or the consumer. The "free sugars" category includes naturally occurring sugars in honey, fruit juices, and syrups and excludes sugars from fruits, vegetables or the lactose from dairy. Naturally occurring sources of fructose include fruit and vegetables, but only account for a small percentage of fructose in the diet[27]. In the U.S., added sugar consumption rose over most of the past century [27, 28] until the 2000's when consumption of sugar sweetened beverages began to decline[29]. Recent estimates indicate a slightly declined consumption of total added sugar, fructose, and sugar sweetened beverages[29-32]. Even with this decline, the amount of added sugars, and thus the amount of fructose, in the typical US diet remains elevated at ~16% of total calories, which exceeds the upper recommendation of 5-10%[33].

Fructose is absorbed from the lower part of duodenum and jejunum both passively and actively primarily through GLUT5. After absorption across the brush border of the small intestine into the portal blood supply, fructose is cleared from the blood in the liver on the first pass and almost exclusively metabolized in hepatocytes. Most absorbed fructose is cleaved into glyceraldehyde and dihydroxyacetone phosphate, and these trioses further go to glycerol phosphate and pyruvate metabolic pathways, promoting gluconeogenesis and DNL, respectively. It is this stimulation of DNL that has been studied closely and may be a critical environmental contributor to NAFLD. When compared with a isocaloric diet with the same macronutrient distribution, a fructose containing diet (25% of calories) was associated with both higher DNL and higher liver fat in a short term study of healthy adults[34].

NAFLD patients have been found to be high consumers of fructose. In a cross-sectional analysis, Quyang et al. found a 2-3 fold increase of fructose consumption in patients with biopsy-proven NAFLD as compared to their sex, age, and BMI matched controls[35]. A longitudinal study in obese adolescents indicated that energy-adjusted fructose intake was independently associated with NAFLD during a 3-year follow up[36]. And fructose may be associated with the severity of NAFLD. For example, work by Abdelmalek et al. reported a significant association between fructose intake and fibrosis severity in a cohort of 427 adults with histologically-confirmed NAFLD [37].

There are a few small studies demonstrating improvement in features of NAFLD with fructose reduction but this is an area that deserves more research. In a randomized

controlled trial, 4-week fructose restriction in children with NAFLD improved their adipose insulin sensitivity, high sensitivity C-reactive protein (hs-CRP), and LDL oxidation; whereas their liver enzymes and intrahepatic fat remained unchanged [38]. Over a longer intervention as of 6 months, improvement of liver enzyme was seen with lowering dietary component of fructose and glycemic load in NAFLD subjects [39]. Finally, a recently reported 10 day study in children on a fructose-free diet demonstrated a 20 percent drop in liver fat.

Of note, while the studies above isolated fructose as the dietary component being evaluated, in real life, most fructose in the diet is from added sugars. Thus, while fructose is important to evaluate for the mechanism of effect, added sugars are a human health relevant environmental exposure that is being considered in this study.

Another technology utilized in this protocol is magnetic resonance imaging-estimated liver proton density fat fraction (MRI-estimated liver PDFF) for the measurement of hepatic fat. This is a research technology that is not yet clinically available, but has recently been validated for use in children and is highly beneficial because it can be used instead of a liver biopsy to measure hepatic fat[40]. In the validation study, MRI-estimated liver PDFF was used to measure hepatic steatosis and was compared this to histopathologic grading in 174 children with a mean age of 14 years (range 8 to 16 years)[41]. All the children completed the MRI acquisition protocol without difficulty demonstrating the feasibility of MR-based steatosis measurements in young children. The correlation of the MRI-estimated liver PDFF with the pathology assigned steatosis grade was very good (0.725)[41]. Further, it is a precise, low risk technology[42]. No MRI contrast is required and the scan time can be brief (~15 minutes) thus making MRI PDFF ideal for measuring hepatic fat in this research protocol.

In summary, free sugars are associated with severity of NAFLD and may be an important environmental cause of increased DNL leading to hepatic steatosis. In this proposal, we will test the effect of a low “free sugars” (<3% total daily calories) diet in children with biopsy-proven NAFLD using innovative, low risk, non-invasive tools like MRI for hepatic fat measurement.

2.2 Justification

Before designing a large RCT it is prudent to first test effect size of reducing sugar in male children with a history of NAFLD on hepatic fat, ALT, and other markers in a smaller, randomized, controlled study.

Population studies demonstrate that NAFLD is more than twice as likely in boys compared to girls[2, 11]. In the recently completed NIH sponsored NASH randomized, controlled trial, XX% of the subjects enrolled were boys, despite best efforts to increase recruitment of girls. Puberty is a strong modifier of NAFLD severity[43]. Girls undergo puberty earlier than boys. Together, these effects could confound the study results because more girls would be in later stages of puberty compared to the boys.

Increasing the sample size would allow for subgroup analysis and controlling for sex, however budget constraints did not allow for a larger cohort. Thus, the study was designed with boys alone, excluding girls . There are limitations of including only boys including that it makes the study less generalizable. This will hopefully be addressed by proceeding with a larger, longer trial including both sexes justified by the data of this initial study.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

There is minimal risk in this study. Potential risks are related to 1) the MRI PDFF 2) blood draws, 3) NPO status, 4) cumulative blood loss, 5) confidentiality and 6) diet low in free sugars.

1) MRI PDFF – Some children may find the MRI examination to be a fear invoking one. These will be done at a research imaging facility where the staff is experienced in working with children. The MRI scanner also has movies for improving the experience.
2) The placement of an intravenous catheter and drawing of blood specimens has minimal risk of discomfort, bruising, or bleeding. There is minimal risk of infection or extravasation. Experienced staff with pediatric expertise will place all catheters and draw blood.
3) Patients will remain NPO from 8 pm the evening before the study until the fasting portion of the study is concluded. Some children may become agitated with NPO status. Blood sugar will be monitored at the beginning of the study and water will be encouraged to maintain hydration. Each participant will be asked to drink one glass of water at bedtime prior to the study and one glass in the morning before leaving home for the research center. Food and water will be provided immediately following the conclusion of the OGTT.
4) Blood loss: During the study visits, blood will be drawn from an indwelling IV at 19 time-points. To minimize risk to the patient, we will limit amounts in accordance with the guidelines of our IRB and the NIH. The total blood volume drawn over the course of the study will be 108 mL. We expect all of our patients to be >37 kg making these amounts well within the NIH clinical center guidelines recommending less than 3ml/kg at a single research visit and no more than 7ml/kg over any 6 week period.
5) There is a small risk of loss of confidentiality. We will follow all procedures required by respective institutions to protect participants. Efforts will be made to ensure that all personal information remains confidential. All data will be stored in locked offices and password-protected computers. Personal identity will be protected in any publication.
6) Potential risks of a low “free sugars” diet: sugar is not a required nutrient but it is known to have some addictive qualities. Elimination of “free sugars” in the diet may at first result in cravings of sugar and this is expected to fade over 2-3 weeks.

2.3.2 Potential Benefits

The proposed research has substantial potential benefits for children. Sugar is known to increase adiposity in children over time and even a short reduction of 8 weeks is likely to have benefits from a BMI and cardiovascular standpoint, although these may not be sustained after the trial ends. In the standard of care control group, families will receive gift cards to cover groceries and this may allow them to purchase healthier foods such as vegetables.

Participants in both groups will benefit from the knowledge that they are helping to contribute to the knowledge of how diet changes can help treat NAFLD.

In addition, at the conclusion of the study, all participants in the studies will receive copies of their liver transaminases, lipid results and baseline MRI PDFF results and these will be discussed with them by one of the investigators. Education will be provided as appropriate regarding the results and standard of care advice for diet and physical activity levels.

2.3.3 Alternative treatments and procedures

Patients that are approached about the study can choose not to participate. If they decide not to participate it will not impact their standard of care treatment.

2.3.4 Protection against risks

Risk will be minimized to the children by using nurses and staff experienced in pediatrics for the research studies. As discussed above, the risks associated with heavy water will be minimized by giving the first dose at the research center under supervision. Risk to confidentiality will be reduced by using non-identifying participant numbers and removing all identifiers as early as feasible. Only study personnel will have access to identifying data. Laboratory personnel will only have access to numeric identifiers and the key will be kept in a password protected database.

Children will provide written assent (11-16 y). Children are able to decline to continue to participate at any time before and during the study or study visit. During study visits, if an IV fails, we will attempt to replace it. If the child does not wish to have it replaced, we encourage them to tell us this and the visit will be stopped immediately. Study stipends are provided at a standardized pro-rated level appropriate for the time/study procedures completed.

3 OBJECTIVES

What is the role of sugar consumption in the etiology and treatment of pediatric NALFD?

3.1 General hypothesis

Sugar restriction will reduce hepatic fat content and reverse liver histopathology in children with NAFLD (and by extension, such foods/beverages that trigger the condition).

3.2 Specific Hypothesis

Restricting sugar in beverages, in food, or in both will decrease hepatic fat content and reverse liver histopathology in children with NAFLD in relation to the type and/or degree of restriction (and by extension, such foods/beverages that trigger the condition).

3.3 Implications

Improvement in NAFLD/NASH in children can be achieved by limiting all sugars, or by limiting a specific form of sugar (free sugars in beverages or in food) alone. Avoiding such foods will prevent development of NAFLD and NASH.

3.4 Primary Outcome Measure

Change in hepatic steatosis (%) by MRI PDFF in the intervention group compared to change in the control group.

3.5 Secondary Outcome Measures

ALT, AST, GGT, Adipo-IR, fasting glucose, insulin, serum lipids, Insulin sensitivity from OGTT (QUICKI), sweetness perception testing, compliance (all baseline compared to 8 weeks, change in intervention group compared to control).

4 STUDY DESIGN

A study that tests nutritional interventions that clinicians currently prescribe, such as *ad libitum* weight-reducing diets, has the potential to directly and rapidly affect clinical practice, but does not necessarily provide significant insight into the dietary trigger of disease or the dietary mechanism driving any observed effects. Conversely, a highly controlled diet study may more clearly identify the nutritional trigger and mechanism (necessary for the successful prevention of the disease in public health campaigns) but may be dismissed by many clinicians as divorced from actual practice. Here we describe a trial that hopes to balance the need for establishing practical clinical guidelines for dietary treatment and experimental controls sufficient to identify the dietary trigger for NAFLD in a pediatric population.4.1 Overview

This is an 2 site, 8-week randomized, controlled, outpatient feeding study at Emory University and UCSD. Participants will be non-diabetic male adolescents with biopsy-proven NAFLD. Two groups of 20 participants will be followed for 8 weeks with hepatic fat content assessed by MRI PDFF at weeks 0, 4, and 8. The participants will be randomized to either standard of care control arm to track the naturally-occurring changes in hepatic fat content over time, or replacement of habitual diet with a low “free sugars” (<3% of total daily calories) version, intervention arm . The primary outcome is percent change in liver fat content over time in the treatment group compared to the control group. Additional parameters of liver function and metabolic status (e.g., serum ALT) will also be assessed.

4.2 Intervention

The intervention is replacement of habitual diet with a low “free sugars” diet (<3% of total calories). The intervention will be applied by family and will aim to alter the diet by specifically targeting the foods that contain free sugars (WHO definition: glucose, fructose, galactose, sucrose, maltose, trehalose) added to food by consumer, cook, or manufacturer, while preserving the family’s other food group choices. At recruitment, families will be randomized to either the low “free sugars” diet (intervention) or remain on their regular diet (standard of care control). Following screening procedures and prior to study initiation, a home visit will be scheduled at which current food consumption, food preferences, and weekly food volume required for the family will be assessed and recorded. Additionally, common recipes used by the family will be collected. One day prior to study initiation (Day 0), the nutritionist/coordinator/staff will assist the parent or guardian in selecting and removing all sugar and sugar-containing products from the home. The items will be replaced with similar foods that contain no free sugars. In general, artificial sweeteners will be avoided although in some instances it will be necessary to use them.

For each week of the study (8 weeks total), families will be able to choose meals from a list of foods similar to their usual diet including ready-to-eat foods, breakfast foods, lunch

items, dinner entrees, fruits, and snacks. Food will be prepared by dieticians/nutritionists at the research metabolic kitchen and supplies for several days at a time will be delivered to the family by the study staff. Each child will be provided with a lunch bag and instructed to bring lunch to school to maintain the study diet.

Families will have the opportunity to choose from a list of pre-prepared evening meals that are similar to what they consumed before study initiation but not containing any free sugars. Dinners will typically be fresh or frozen and re-heating in the oven or microwave may be required. The families will be instructed to not eat any food outside of assigned diet. For the duration of the study a water dispenser will be provided. Families will be instructed to avoid all fruit juice and sweet drinks. Fruit consumption will be allowed but restricted in amount (to 1-2 portions/ day per family member).

Methods to improve compliance: Twice a week, a coordinator/study staff member will conduct home visits to assess food satisfaction as well as diet compliance in the intervention arm. In the standard of care control arm, study staff will visit the home once a week to check on their compliance and provide them with any study-related items (water, gift cards etc.). To enhance compliance, the family will be allowed 1 meal “off” of the diet 4 times during the study. These meals will be planned in coordination with the staff and may include holidays or special family events or other days that they select.

Compliance will also be increased by informing participants that the investigators can assess compliance with the diet by measuring sugar in the blood work. If there is evidence of the participant not complying, the study coordinator or investigator will meet with the participant and family members and explain the importance of only consuming the study diet. If the study coordinator/staff perceives evidence of persistent non-compliance by the participant, families will be withdrawn from study and the diet will be stopped.

4.3 Dietary Assessment

Baseline diet by 24-hour recalls will include 2 x 24-hour recalls to reflect diet during weekdays and 1 x 24-hour recall to reflect diet on a weekend day. Families will be asked to specify times that work best to conduct the 24-hour recalls and will be called at random, to minimize diet change. The 24-hour recalls will aid in getting a better sense of the food groups consumed by the participant and his family and in the preparation of a personalized, tailored diet. Following study participant randomization, two weeks prior to study initiation, a home visit will be scheduled where current food consumption, food preferences, and weekly food volume required for the family will be recorded. Additionally, common recipes will be collected. During the study, every week a coordinator/study staff member will conduct two home visits to assess food satisfaction and diet compliance. If the study coordinator/staff perceives non-compliance by the family at any point of the study, the intervention will be stopped and families will be withdrawn from the study.

4.4 Sweetness Perception Testing

4.4.1 Participant preparation

Participants will be asked to undergo sweetness taste perception testing at three points in the study: baseline (study initiation, week 0), follow-up visit 1 (week 4), and follow up visit 2 (study end, week 8). Participants will arrive at the clinic after an overnight fast to conduct an oral glucose tolerance test (OGTT), sweetness perception testing, and MRI. Prior to each study visit, participants will be reminded not to eat or drink anything except for water after 8 pm the night before the visit. At each visit height, weight, and vital signs will be collected.

4.4.2 Sensory Ratings

Sweetness perception will be assessed in a test session of about 1 hour. Participants will rate both the intensity and pleasantness of model beverages that vary widely in sucrose concentration. The model beverages will be formulated using unsweetened Kool-Aid ™ drink mix at a fixed concentration. Concentrations of sucrose (ranging from barely sweet to very sweet) will be added to this fixed concentration of drink mix. Model beverages will be presented chilled. Over the course of a 1-hour session, participants will consume about 150 ml of model beverage, or the equivalent of about 42% of a 12 oz can of soda.

The first 10-15 minutes of each session will be devoted to instructions and practice ratings. Participants will rate perceived sweetness intensity using visual analog scales (i.e., horizontal lines with the anchor labels “Not Sweet at All” on the left and “Very Sweet” on the right). Such scales are easy for participants to understand and use. Participants will practice by rating the sweetness of 3-4 sucrose solutions (in plain water, not Kool-Aid) that cover the range of sucrose concentrations to be using in sensory testing. Participants will also rate water (negative control to ensure that participants do not rate non-sapid stimuli as sweet). Participants will rate pleasantness using a standard category scale ranging from “Very Unpleasant” (-11), to “Neutral” (0), to “Very Pleasant” (+11). Again, the use of such scales is fairly intuitive. Participants will practice by rating the pleasantness of a sucrose solution (which should be rated as pleasant), water (which should be rated as neutral), and a non-toxic bitter substance (sucrose octaacetate, an FDA approved food additive, which participants should rate as unpleasant).

After scaling practice, participants will be instructed to rate the sweetness and pleasantness of 30 model drink samples. The 30 samples will be presented in 5 ml aliquots (in 30 ml disposable plastic medicine cups). The 30 samples will be presented in three blocks of 10 trials. Each block will include all 10 sucrose concentrations, presented in random order. Prior to the first trial of each block, participants will be asked to rinse their mouth four times with water (same water used to make test beverage) and spit it

out. Next, participants will sample each of the 10 cups by sipping, rolling the liquid sample around in the mouth for several seconds, and then swallowing the sample. After swallowing, participants will rate both the sweet taste intensity and pleasantness of the sample. After each trial, the participant will rinse the mouth a few times with water (same water used to make test beverage), spit, and wait ~45 seconds before tasting the next sample. Each block (all 10 concentrations, once each, in randomized order) will be followed by a five minute break. The first block will be used as practice and will not be included in analysis. The second and third blocks (replicate ratings for each of the 10 concentrations) will be used for analysis.

4.5 Insulin Sensitivity Measurements

These assessments will only be conducted at the Emory University site.

Assessment of Insulin sensitivity using oral glucose tolerance test (OGTT): Plasma glucose and insulin response during the OGTT reflect the ability of pancreatic β-cells to secrete insulin and the sensitivity of tissues to insulin. Many investigators have validated simple surrogate indices of β-cell function and insulin resistance based on values obtained during the 75-gram OGTT, against indices assessed by the clamp technique[44-50]. We will assess insulin dynamics using a battery of OGTT-indices including: 1. A*ssessment of whole body insulin sensitivity by the* reciprocal of the Homeostasis Model Assessment of insulin resistance (1/HOMA), Quantitative Insulin Sensitivity Check Index (QUICKI) and the Composite Insulin Sensitivity Index (CISI). During the OGTT blood samples will be obtained at 0, 30, 60, 90, and 120 minutes for measurement of plasma glucose and insulin.

4.6 Study duration for participants

- Screening MRI must occur within 10 days of baseline (Day 0)
- 56 days of treatment
- 14 days post-treatment assessment (by phone)

4.7 Study Timeline

- Study initiation phase: 3 months (IRB, meal testing, etc.)
- Recruitment phase: 4 – 8 months depending on enrollment pace
- Follow-up phase: 10 months
- Close out phase: 2 months (data analysis etc.)
- Expected recruitment is 8 intervention participants and 8 control participants per clinical center, approximately 1-2 of each type per month per center

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 Participant Inclusion Criteria

- Boys age 11-16
- Liver biopsy for standard of care within 2 years of screening for the study
- Clinical history consistent with NAFLD
- Definite NAFLD based on liver histology
- Hepatic fat by MRI PDFF ≥ 10% on baseline MRI
- Serum ALT ≥ 45 u/L
- Written informed consent from parent or legal guardian
- Written informed assent from the child
- Currently consumes ≥ 3.5 eight ounce sugar drinks (or juice) per week

5.2 Participant Exclusion Criteria

- Participants with a history of health issues that make it unsafe for them to participate in the opinion of the investigators
- History of significant alcohol intake (AUDIT questionnaire) or inability to quantify alcohol consumption
- Chronic use (more than 2 consecutive weeks) of medications known to cause hepatic steatosis or steatohepatitis (systemic glucocorticoids, tetracycline, anabolic steroids, valproic acid, salicylates, tamoxifen) in the past year.
- The use of other known hepatotoxins within 90 days of liver biopsy or within 120 of baseline
- Medications with the intent to treat NAFLD/NASH in the past 60 days
- History of total parenteral nutrition (TPN) use in the year prior to screening
- History of bariatric surgery or planning to undergo bariatric surgery during the study duration
- Significant depression
- Non-compensated liver disease with any one of the following hematologic, biochemical, and serological criteria on entry into protocol:
    a. Hemoglobin < 10 g/dL
    b. White blood cell < 3,500 cells/mm
    c. Neutrophil count < 1,500 cells/mm3 of blood
    d. Platelets < 130,000 cells/mm3 of blood
    e. Direct bilirubin > 1.0 mg/dL
    f. Total bilirubin > 3 mg/dL
    g. Albumin < 3.2 g/dL
    h. International normalized ratio (INR) > 1.4
- Diabetes
- Evidence of other chronic liver disease

- Children who are currently enrolled in a clinical trial or who received an investigational study drug within the past 60 days
- Participants who are not able or willing to comply with the diet protocol or have any other condition that would impede compliance or hinder completion of the study, in the opinion of the investigator
- Unable to have an MRI due to metal device, claustrophobia or other reason
- Failure to give informed consent
- Families with > 5 individuals
- Recipient of a liver transplant

5.3 Strategies for Recruitment

Recruitment will be through discussions of the study in the pediatric clinics, community gastroenterology offices and through recruitment of eligible previous research participants who have previously consented to be contacted regarding future studies.

5.4 Reasons for Withdrawal

Participants are free to withdraw from participation in the study at any time. They can notify the study coordinator that they would like to discontinue from the study for any reason during any part of the study. They may also mail in the letter of revocation given along with the consent form if they desire.

5.4.1 Handling of Withdrawal

Collected data for participants who ask to be withdrawn from the study will be maintained in the database and will be used for future studies unless the participant specifically requests that data be destroyed. The participant's file will be flagged to ensure that no further contact is made.

5.4.2 Termination of Study

This study may be prematurely terminated if, in the opinion of the investigator or the sponsor, there is sufficient reasonable cause. Written notification, documenting the reason for study termination, will be provided to the investigator or sponsor by the terminating party.

Circumstances that may warrant termination include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants.
- Insufficient adherence to protocol requirements.
- Data that is not sufficiently complete and/or evaluable.

If the study is prematurely terminated or suspended, the sponsor will promptly inform the investigator/institution, of the termination or suspension and the reason(s) for the

termination or suspension. The IRB will also be informed promptly and provided the reason(s) for the termination or suspension by the sponsor or by the investigator/institution, as specified by the applicable regulatory requirement(s).

6 STUDY SCHEDULE

The schedule of events (Section 11.1) summarizes the procedures to be done at each visit. The visit windows are the goal for the study and serve as a guideline for the clinical sites. These visit windows are not strictly set; conducting a study visit outside of the visit window will not be considered a protocol deviation.

6.1 Screening

Day –30 Screening visit: Participants will provide verbal consent or in-person written consent indicating that they wish to participate in the study so that a screening visit may be scheduled to assess eligibility. Informed consent will be obtained prior to initiating any research procedures. The screening visit may be conducted at a clinical visit if that is convenient for the participant. The ALT for eligibility must be drawn at or within 7 days before this visit.

Vital signs, height, and weight will be obtained at the screening visit as well as a physical exam. Alcohol use will be assessed using the Alcohol Use Disorders Identification Test (AUDIT) and baseline beverage consumption will be assessed using the Bev-Q beverage questionnaire. A background medical history will be obtained and concomitant medications will be reviewed as well.

Day –25 Dietary Assessment: After the screening visit, the baseline usual diet will be assessed using three 24-hour, interviewer assisted, dietary recalls (1 weekend day and 2 week days). These will be administered by telephone by a trained study nutritionist or coordinator after the screening visit/enrollment and prior to Day 0.

Day –10 MRI, baseline labs: MRI will be used to assess level of liver fat within 10 days of Day 0 (diet initiation). At the Emory site, both the usual MRI based quantification of hepatic fat and a 10-minute HISTO protocol will be collected during a single 30 minute MRI. The HISTO protocol is a highly precise measurement of hepatic fat and will be used to determine eligibility for the study. If the hepatic fat is >10% by MRI PDFF, the participant will be enrolled. The % hepatic fat for the outcome measurements will be determined by the UCSF center's hepatic PDFF MRI protocol.

The following baseline labs will be drawn at the time of the MRI if they were not done at the screening visit:

- CMP
- CBC
- Direct bilirubin
- INR
- HbA1c
- GGT

- Plasma and serum for storage

Day –9 Randomization: Randomization will be assigned after screening and the participant will be informed of their assignment after the 24-hour dietary recalls and MRI but before Day 0.

Day -7 Home Visit Food Assessment: A study coordinator and nutritionist will visit the home of intervention-arm participants and complete the assessment of the family's usual consumption patterns, recipes and the initial menus.

6.2 Day 0: Baseline Visit

Once the screening assessments are complete and the participant is deemed eligible to participate in the study the participant will be scheduled for their Day 0 visit. This visit may be scheduled up to 60 days after the screening visit.
Participants will arrive fasting and will complete an oral glucose tolerance test (OGTT). Participants will have an IV catheter placed to allow for multiple blood draws while minimizing discomfort. Either EMLA cream or cold spray will be used to decrease pain with IV placement. Upon line placement, a 12-hour fasting blood sample will be drawn and an oral glucose tolerance test will be completed. Once both the fasting labs and OGTT are completed, the participant will be provided breakfast according to their randomization. Height, weight, and vital signs will be collected at this visit. Any adverse events and changes to any concomitant medications will be reviewed as well. A sweetness perception test will be administered at this visit.

For the intervention arm, the nutritionist will meet with the family during this visit to review the details of the intervention. The standard of care participants will have diet assessment but no instructions on diet. Instructions will be given for compliance and the nutritionist will arrange a time to meet with the family in the home.

The following labs will also be drawn at this visit:

- CMP
- GGT
- Lipid Profile
- Plasma and serum for storage
- Stool sample collection

After the research visit, the food for the first several days of the study will be delivered to the home of the intervention participants and the sugar-containing non-perishable foods will be boxed up and stored at the home (or another location at the discretion of the family). Perishable sugar containing foods will be disposed of and replaced with no free sugars containing versions.

6.3 Follow-up Visit Day 28

The Day 28 visit will take place 28 days (4 weeks) after baseline. All assessments and testing done at the baseline visit will be repeated at this visits with the exception of the nutrition counseling, stool collection, and oral glucose tolerance test.

6.4 Follow-up Visit Day 56

The Day 56 visit will take place 56 days (8 weeks) after baseline. All assessments and testing done at the baseline visit will be repeated at this visit with the exception of the nutrition counseling.

6.5 End of study follow-up

A phone visit will take place two weeks after the Day 56 visit. The purpose of this call is to discuss feedback about the study with the participant and his or her family. We will ask for details about what they liked or disliked about study participation and ask for any suggestions for future studies.

7 STATISTICAL CONSIDERATIONS

7.1 Sample Size Considerations

Initial analyses will be undertaken to inspect data for errors, inconsistencies, and incomplete information. This will include examining the data with simple frequency tables and dot plots for univariate data and scatter plots and multi-way dot plots with bivariate and multivariate data. Data anomalies and outliers will be examined and corrected if necessary. To summarize bivariate relationships between predictors and hepatic fat percent, Spearman's rank correlation coefficient, r_s, will be used. For reporting inferential statistics, such as differences in means, 95 percent confidence intervals will be used extensively to quantify degree of clinical efficacy. For any models, appropriate assumptions and model conditions will be verified prior to analysis.

Analyses will include descriptive statistics at baseline and for each treatment group for all outcome variables, plots of longitudinal data over time, and examination of distributions within groups at important nodal points (e.g., Baseline, 4 weeks and 8 weeks). All longitudinal models will include baseline measurements as a covariate to adjust for potential differences between treatment groups at baseline. All efficacy analysis will follow the *intention-to-treat* convention (inclusion of all randomized participants in the analysis). Participants that drop out or are lost to follow-up will be compared to those that remain in the study to assess for bias and generalizability of the results. All analyses will be conducted using SAS v9.3 for Windows (Cary, NC, USA).

Power Analysis

The primary outcome is change in MRI PDFF from baseline to 8 weeks in the intervention group compared to the standard of care group. Participants will be screened at baseline and confirmed that their hepatic fat is < 10%. Given 40 completed participants, (20 randomized to the diet low in free sugars and 20 randomized to usual diet), our goal is show a 25% improvement in MRI PDFF with intervention over control. For example:

Intervention: 15% hepatic fat baseline – (30% change = 4.5%) = Hepatic fat of 10.5% at 8 weeks

Control: 15% hepatic fat at baseline – (5% change = 0.75%) = Hepatic fat of 14.25% at 8 weeks

A two sample, two-tailed t test with an overall sample size of 40 participants (20 per group) achieves greater than 90% power to detect a true difference of means of 4% when the sigma is 3%. Power was calculated assuming that 20% of patients in each group will be lost to follow-up with a 0.05 significance level.

Plan for missing data:

We note that attrition, participant compliance and systematic data collection are fundamental requirements for this study. Successful coordination of participants and management of data are important prerequisites for a subsequent trial. Thus, we focus appropriate attention to missing data. Prevention is the first line for controlling bias and loss of power from missing data. Upon entry, alternative contacts will be identified for all participants to minimize loss-to follow-up. Consistent with the intent to treat principle, we will follow-up with all randomized participants regardless of the actual treatment received (we will invite families who miss visits to return for assessments later if possible). Participants who express intent to dropout completely will be asked to attend an early termination visit to collect endpoint measures. Timely data entry combined with monthly missing data reports will prompt tracking down missing outcome assessments. Despite these prevention efforts, missing data will occur. Our primary analysis is valid under the assumption that missing data are missing at random (MAR). To evaluate this assumption, we will examine the extent of missing data and missing data patterns, and determine the reasons and time of dropout.

8 ETHICS/PROTECTION OF HUMAN PARTICIPANTS

8.1 Ethical Standard

The investigator will ensure that this study is conducted in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Participants of Research, as drafted by the US National Commission for the Protection of Human Participants of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and/or the ICH E6; 62 Federal Regulations 25691 (1997).

8.2 Institutional Review Board

Permission to perform this study will be sought from the Emory University IRB and the USCD IRB. All future modifications of the study or changes in the protocol will receive IRB approval.

8.3 Informed Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Informed consents and HIPAA waivers will be obtained prior to initiating any study procedure. Participants and their parents or legal guardian will be approached to participate in the study. The research coordinators and PI/Co-I will discuss the study with them and give them all the information listed above in language understandable at the level of the parent/guardian and all information needed to make an informed choice about participation, including information about NAFLD, the study intervention, possible risks to participation, study procedures, study visits/contacts and potential benefits to the participant. Consent will be documented by signature of the parent/guardian.

8.4 Informed Assent Process

All children must provide written assent (11-16 y) in a language appropriate for the age of the child. Written assent will be documented by signature in age 11-16 yrs. Assent will be documented by signature of the child participant.

8.5 Participant Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any third party without prior approval of the participant.

Authorized representatives of the sponsor may inspect all documents and records required to be maintained by the investigator.

8.6 Study Discontinuation

In the event that the study is discontinued, participants will be notified of the date of discontinuation.

8.7 Data Safety Monitoring Plan

Data Management: Data for each subject will be collected in individual folders kept in a locked filing cabinet in a secure office. Data will be entered into a secure RedCap database within the same division. The study statistician will run routine reports for completeness and send to the site PIs.

Adverse Events: The PI at each site will monitor adverse events throughout the course of the study. Any adverse events occurring during the study will be documented and reported according to applicable IRB policies and procedures. AE's will be entered into RedCap in a timely fashion. The study statistician will run quarterly reports and will send out pooled analyses to the PI's at both sites. A status report will be provided to the IRB at the time of continuing review.

Serious Adverse Events: SAE's are not anticipated in this study, however, any SAE that occurs will be reported to the other site, respective IRBs, and sponsor within 10 business days of first knowledge of the event.

Monitoring: A representative from the sponsor will conduct a site monitoring visit after the 1st patient is randomized and then again approximately every 6 months. This site visit will review the informed consent process, eligibility, CRFs, and AE reporting. A monitoring report will be provided to the PIs within 10 business days of review. The PI will review the monitoring report and follow up on any corrective actions on the site monitoring log and will notify the respective IRB according to applicable policies and procedures.

9 DATA HANDLING AND RECORD KEEPING

All data will be entered, stored and processed within REDCap, a secure, web-based application for managing databases. All access to office space containing paper source documents requires badged entry. All computer files are stored on secure servers.

Data management staff members are required to complete and pass an on-line HIPAA course and other confidentiality certification procedures upon employment. All computer systems and programs are password protected, and all web-based electronic communications of study information is encrypted. Good computer security practices (restricting physical access to computers, prohibition of password sharing, timing out of system access interfaces, etc.) is required. Virus protection software is installed on all study machines. The virus protection tools are used, maintained, and updated as necessary on all computers and pathways into the system.

Only study personnel (PI, co-Is, study coordinators, research assistants, and nutritionists) will have access to identifiable data. Data will be de-identified before analysis and will be stored in locked research material cabinets.

9.1 Data Management Responsibilities

Emory University will serve as the data coordinating center for this study and will be responsible for data management, quality review, analysis, and reporting of the study data. The statistician will review data completion monthly during the active portion of the study.

9.2 MRI Data Coordination

UCSD will serve as the radiology coordinating center for this study. CD's will be shipped to UCSD and all MRI's will be read and interpreted at this site.

9.3 Types of Data

Data for this study will include self-report patient data, nutritional data from NDSR, laboratory values, medical records, and imaging data.

9.5 Study Records Retention

Study documents will be retained for the minimum number of years per Emory University and UCSD requirements. No records will be destroyed without the written consent of the sponsor. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

Electronic data, stripped of identifiers, will be stored on a secure server and will be kept indefinitely.

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11 Attachments

11.1 SCHEDULE OF EVENTS

Study Procedure	Screening (up to -60 Days)	-25 Day (± 7 days)	-10 Days (±2 days)	Day -9 (±1 Day)	Day – 7 (±3 Days)	Baseline (Day 0)	Day 28 (±5 Days)	Day 56 (±3 Days)
Informed Consent and HIPAA Authorization	X							
Review Inclusion/Exclusion Criteria	X			X				
Randomization				X				
Demographics & Medical History	X							
Vital Signs	X					X	X	X
Height and Weight	X					X	X	X
Physical Exam	X							
MRI			X				X	X
AUDIT Questionnaire	X							
3 x 24-hour food recalls (NDS-R)		X						
Beverage Questionnaire (Bev-Q)	X							
Instructions for Compliance	X					X		
Home Visit Food Assessment					X			
Sweet Taste Test						X	X	X
Adverse Events Review						X	X	X
Concomitant Medication Review	X					X	X	X
Stool Collection						X		X
Oral Glucose Tolerance Test (Emory)						X		X
Blood Draw	X					X	X	X

11.2 BLOOD DRAW SCHEDULE

Blood Test	***Screening (-60 days)***	***Baseline (Day 0)***	***Follow-up 1 Day 28***	***Follow-up 2 Day 56***	***Total Volume***
Complete Blood Count	1	-	-	-	**1**
Comprehensive Metabolic Panel	1	1	1	1	**4**
HbA1c	3	-	-	-	**3**
Lipid Profile	-	3	3	3	**9**
Direct bilirubin	1	-	-	-	**1**
GGT	-	1	1	1	**3**
Serum	6	6	6	6	**24**
Plasma	12	12	12	12	**48**
Measurements for OGTT (Emory)	-	6	-	6	**12**
PT/INR	3	-	-	-	**3**
Total	**27 mL**	**29 mL**	**23 mL**	**29 mL**	**108 mL**