PROTOCOL TITLE: Examining the Effects of Juice Fasting

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VERSION NUMBER:

1.4

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05-22-18

STUDY SUMMARY:

Investigational Agent(s) (Drugs or Devices)	N/A
IND / IDE / HDE #	N/A
Indicate Special Population(s)	☐ Children ☐ Children who are wards of the state ☐ Adults Unable to Consent ☐ Cognitively Impaired Adults ☐ Neonates of Uncertain Viability ☐ Pregnant Women ☐ Prisoners (or other detained/paroled individuals) ☐ Students/Employees
Sample Size	30
Funding Source	Osher Center for Integrative Medicine at Northwestern University
Indicate the type of consent to be obtained	☒ Written ☐ Verbal/Waiver of Documentation of Informed Consent ☐ Waiver of HIPAA Authorization ☐ Waiver/Alteration of Consent Process
Site	☐ Lead Site (For A Multiple Site Research Study) ☐ Data Coordinating Center (DCC)
Research Related Radiation Exposure	☐ Yes ☒ No
DSMB / DMC / IDMC	☐ Yes ☒ No

OBJECTIVES:

This study aims to examine the impact on epigenetic markers (methylation), gut bacteria, inflammation markers, measures of insulin resistance and patient reported outcomes of well-being in subjects on a 3-day juice-only fast vs calorie restriction vs juice added to regular diet.

BACKGROUND:

Food is increasingly recognized as a core component of preventive and ameliorative health care. An estimated 90% of cardiovascular disease and diabetes, and 70% of all cancers could be prevented with lifestyle measures. Juice fasting has quickly become one of the most popular self-prescribed dietary interventions in the United States. A wide variety of juice fasts are available in the popular market, the most popular variation is the three-day juice and vegetable fast. According to typical juice fast protocol, the participant is instructed to consume only fruit/vegetable juice for the duration of the fast. The purpose of this study is to assess the efficacy of a 3-d fruit/vegetable juice fast as a dietary intervention with the potential to impact epigenetic markers (methylation), increase beneficial gut bacteria, and improve measures of

insulin resistance leading to improved general well-being.

Dietary interventions utilizing calorie restriction without malnutrition have supported for their ability to extend lifespan through reduction of metabolic rate and oxidative damage and improve markers of age-related diseases such as insulin resistance for diabetes since the 1930s.[1] A 2010 analysis of the mechanism of effect linked fasting to changes in IGF-1, IGFBP1, glucose, and insulin.[2] A 30% or more decrease in circulating insulin and glucose and decline in IGF-1 can be observed in mammals after 3 days or more of fasting.[2] Similarly, a 2016 study on 3-day fruit/vegetable juice fasting found significant increases in plasma and urine nitric oxide and a significant decrease in urinary lipid peroxidation.[3]

Fruits and vegetables have also been studied for their antioxidant effects due to the phenolic and prebiotic compounds inherent to the foods. The phenolic and micronutrient compounds contribute to the effectiveness of fruits and vegetables as antioxidants. A 2013 study on the effect of increasing fruit and vegetable consumption found increased levels of -tocopherol, lutein, beta carotene, beta cryptoxanthin, and ascorbic acid through diet intervention.[4] Increasing the amount of antioxidants in the diet can combat the effects of oxidative stress which contribute to the development of chronic disease. The beneficial compounds of fruits and vegetables remain obtainable in pressed fruit/vegetable juice. A 2017 study found pure pressed fruit and vegetable juices contained the necessary polyphenols and vitamins found in whole fruits and vegetables to maintain the positive relationship between fruit/vegetable consumption and lower incidence of chronic noncommunicable disease. Similar to studies with whole fruit, participants consuming pure fruit/vegetable juices had lower levels of lipid peroxidation and higher levels of most plasma/serum antioxidant vitamins.[5] Therefore, pressed fruit/vegetable juices are an appropriate method through which to study the effects of fruit/vegetable consumption on the development of chronic disease.

Despite the popularity of 3-day juice fast, there is little existing literature delineating the effects of the diet intervention over other fasting protocols. 3-day juice fast programs qualify as a fast under the definition of fasting due to the low caloric intake of the participant. The 3-day juice fast program we will be utilizing provides approximately 800 -900 kcal per day, 60% and 70% less than the average recommended daily intake for women and men age 19-30, respectively. The purpose of this study is to delineate the mechanism through which juice fasting interventions affect change in methylation, gut microbiome, and inflammation markers.

STUDY ENDPOINTS:

The primary endpoints of the study are changes in epigenetic markers, gut microbiota, and biomarkers of inflammation and insulin resistance that may potentially occur after juice fasting. Secondary goals of the study include providing a foundation for future studies to examine the mechanisms of the intervention and test the intervention in a larger scale.

STUDY INTERVENTIONS / INVESTIGATIONAL AGENTS:

Group 1: Juice Fasting

This group will be provided with the following juices, 1 of each per day, which together will constitute the 800-900 calorie daily diet.

Juice Menu (Delivered by Pressed Vibrance)

Name	Calories	Ingredients	Nutrition
Astound, 16oz	140	Spinach, Kale, Romaine, Cucumber, Fennel, Parsley, Celery, Lemon, Apple, Ginger, Turmeric	Sodium: 95mg / 4% Total Carbohydrates: 34g / 11% Sugar: 18g Protein: 4g Vit A: 240% Vit C: 190% Calcium: 20% Iron: 20%
Delightful Greens, 16oz	170	Kale, Spinach, Cucumber, Romaine, Parsley, Celery, Ginger, Lemon	Sodium: 260mg / 11% Total Carbohydrates: 30g / 11% Sugar: 11g Protein: 12g Vit A: 590% Vit C: 420% Calcium: 45% Iron: 50%
Connective, 16oz	120	Aronia Berry, Spinach, Romaine, Cucumber, Parsley, Lemon, Ginger	Sodium: 110mg / 5% Total Carbohydrates: 28g / 9% Sugar: 15g Protein: 5g Vit A: 230% Vit C: 120% Calcium: 15% Iron: 25%
Mindful, 16oz	130	Spinach, Kale, Romaine, Cucumber, Parsley, Celery, Swiss Chard, Lemon, Lime, Ginger, Apple	Sodium: 250mg / 10% Total Carbohydrates: 24g / 8% Sugar: 11g Protein: 8g Vit A: 330% Vit C: 200% Calcium: 20% Iron: 30%
Nourishment, 16oz	120	Aronia Berry, Spinach, Kale, Romaine, Cucumber, Parsley, Celery, Lemon, Turmeric,	Sodium: 150mg / 6% Total Carbohydrates: 23g / 8% Sugar: 8g

		Ginger, Wheatgrass, Broccoli Sprouts, Sunflower Sprouts	Protein: 7g Vit A: 340% Vit C: 230% Calcium: 25% Iron: 30%
Aronia Crunch, 16oz	140	Apple, Celery, Lime, Aronia Berry	Sodium: 40mg / 2% Total Carbohydrates: 36g / 12% Sugar: 22g Protein: 2g Vit A: 10% Vit C: 45% Calcium: 8% Iron: 6%

Group 2: Juice + Ad Hoc Diet

This group will be provided with the same juices as the above, but will also be instructed to supplement their diet as needed with their own food, without any proscribed caloric, liquid vs. solid, or other restrictions.

Group 3: Caloric Restriction

This group will be provided with the meals below to make up their 900 calorie daily diet.

~900 Calorie Plant-Based Daily Meal Plan (Delivered by KitchFix)	**Calories**
Loaded Cauliflower Mash (No Bacon) + Strawberry Cashew Yogurt + Power Green Veggie Quinoa Bowl	881
Herbed Root Vegetable Side + Spice Roasted Sweet Potato Side + Paleo Blueberry Muffin + Broccolini	879
Paleo Blueberry Muffin + Loaded Cauliflower Mash (No Bacon) + Spice Roasted Sweet Potato Side + Power Green Veggie Quinoa Bowl	889
Strawberry Cashew Yogurt + Herbed Root Vegetable Side + Tomato and Roasted Garlic Soup	862

Name	Calories *(Calories from fat)*	Ingredients	Nutrition

Loaded Cauliflower Mash (No Bacon), 141.7 g	75 *(25)*	Cauliflower, scallions, almond, nutritional yeast, parsley, thyme, rosemary, garlic, salt, black pepper	Total Fat (g): 2.5 Sat. Fat (g): 0.2 Trans Fat (g): 0 Cholesterol (mg): 0 Sodium (mg): 130.7 Total Carbs (g): 12.3 Fiber (g): 4.9 Sugars (g): 4.2 Added Sugar (g): 0 Sugar Alcohol (g): 0 Protein (g): 4.7 Vitamin A: 9.80% Vitamin C: 140.70% Calcium: 7.10% Iron: 11.70% Potassium (mg): 601.1 Polyunsat Fat (g): 0 Monounsat Fat (g): 0 Vitamin D: 0.00% Vitamin E: 15.70% Vitamin K: 155.90% Thiamin: 17.30% Riboflavin: 15.30% Niacin: 8.20% Vitamin B6: 19.90% Folate: 33.40% Vitamin B12: 3.80% Pantothenic Acid: 10.80% Phosphorus: 8.70% Magnesium: 8.90% Zinc: 4.10% Selenium: 1.80% Copper: 5.20% Manganese: 17.00%

Strawberry Cashew Yogurt, 198.4 g	453 *(292)*	YOGURT cashew, raw honey, lemon, coconut water, vanilla extract, psyllium, salt STRAWBERRY strawberry, lemon, honey, salt GRANOLA almonds, sunflower seeds, pecans, cashews, almond flour, maple syrup, clover honey, coconut oil, flax seed, vanilla extract, cinnamon, sea salt	Total Fat (g): 32.4 Sat. Fat (g): 6.6 Trans Fat (g): 0 Cholesterol (mg): 0 Sodium (mg): 81.8 Total Carbs (g): 33.1 Fiber (g): 5.1 Sugars (g): 14.8 Added Sugar (g): 7.3 Sugar Alcohol (g): 0 Protein (g): 12.9 Vitamin A: 0.10% Vitamin C: 38.00% Calcium: 5.60% Iron: 23.70% Potassium (mg): 520.4 Polyunsat Fat (g): 0 Monounsat Fat (g): 0 Vitamin D: 0.00% Vitamin E: 15.90% Vitamin K: 21.60% Thiamin: 19.20% Riboflavin: 8.60% Niacin: 5.90% Vitamin B6: 14.30% Folate: 8.30% Vitamin B12: 0.00% Pantothenic Acid: 5.50% Phosphorus: 34.70% Magnesium: 43.90% Zinc: 21.70% Selenium: 17.30% Copper: 60.70% Manganese: 59.90%

Power Green Veggie Quinoa Bowl, 326.0 g	353 *(188)*	QUINOA quinoa, arugula, broccoli, asparagus, kale, mustard greens, parsley, salt SAUCE VERTE basil, arugula, EVO, garlic, red onion, lemon, capers, dijon, red fresno pepper, salt	Total Fat (g): 20.9 Sat. Fat (g): 2.9 Trans Fat (g): 0 Cholesterol (mg): 0 Sodium (mg): 545 Total Carbs (g): 35.3 Fiber (g): 7.4 Sugars (g): 4.2 Added Sugar (g): 0 Sugar Alcohol (g): 0 Protein (g): 10.1 Vitamin A: 106.50% Vitamin C: 166.30% Calcium: 19.70% Iron: 24.60% Potassium (mg): 789.4 Polyunsat Fat (g): 0.1 Monounsat Fat (g): 0 Vitamin D: 0.00% Vitamin E: 28.90% Vitamin K: 524.40% Thiamin: 17.80% Riboflavin: 18.40% Niacin: 8.60% Vitamin B6: 23.50% Folate: 41.90% Vitamin B12: 0.00% Pantothenic Acid: 5.80% Phosphorus: 28.30% Magnesium: 32.60% Zinc: 13.90% Selenium: 8.70% Copper: 39.80% Manganese: 67.80%

Herbed Root Vegetable Side, 226.8 g	222 *(34)*	Parsnip, carrot, turnip, redskin potato, high-oleic sunflower oil, parsley, salt, black pepper	Total Fat (g): 3.7 Sat. Fat (g): 0.4 Trans Fat (g): 0 Cholesterol (mg): 0 Sodium (mg): 224.6 Total Carbs (g): 45.6 Fiber (g): 10.3 Sugars (g): 13.3 Added Sugar (g): 0 Sugar Alcohol (g): 0 Protein (g): 4.6 Vitamin A: 306.60% Vitamin C: 83.80% Calcium: 10.30% Iron: 10.50% Potassium (mg): 1231.6 Polyunsat Fat (g): 0.4 Monounsat Fat (g): 2.6 Vitamin D: 0.00% Vitamin E: 16.20% Vitamin K: 85.70% Thiamin: 16.90% Riboflavin: 9.20% Niacin: 14.80% Vitamin B6: 22.30% Folate: 27.80% Vitamin B12: 0.00% Pantothenic Acid: 12.40% Phosphorus: 17.80% Magnesium: 17.20% Zinc: 8.80% Selenium: 4.00% Copper: 17.80% Manganese: 46.40%

Spice Roasted Sweet Potato Side, 226.8 g	229 *(20)*	Sweet potato, chili powder, paprika, coconut oil, salt	Total Fat (g): 2.2 Sat. Fat (g): 1.6 Trans Fat (g): 0 Cholesterol (mg): 0 Sodium (mg): 349.7 Total Carbs (g): 66.1 Fiber (g): 10.4 Sugars (g): 13.7 Added Sugar (g): 0 Sugar Alcohol (g): 0 Protein (g): 5.3 Vitamin A: 929.90% Vitamin C: 13.00% Calcium: 10.30% Iron: 12.70% Potassium (mg): 1126.7 Polyunsat Fat (g): 0 Monounsat Fat (g): 0 Vitamin D: 0.00% Vitamin E: 7.60% Vitamin K: 9.70% Thiamin: 17.10% Riboflavin: 12.60% Niacin: 10.10% Vitamin B6: 35.70% Folate: 9.00% Vitamin B12: 0.00% Pantothenic Acid: 26.10% Phosphorus: 15.80% Magnesium: 20.90% Zinc: 7.00% Selenium: 3.30% Copper: 25.40% Manganese: 43.30%

Paleo Blueberry Muffin, 62.0 g	162 *(106)*	MUFFIN almond flour, coconut flour, banana, almond butter, almonds, baking powder, salt, baking soda, vanilla extract, coconut oil, maple syrup, coconut milk, pastured eggs, cinnamon, blueberries	Total Fat (g): 11.7 Sat. Fat (g): 3.3 Trans Fat (g): 0 Cholesterol (mg): 36.4 Sodium (mg): 87.8 Total Carbs (g): 13.2 Fiber (g): 3.5 Sugars (g): 6.9 Added Sugar (g): 3.4 Sugar Alcohol (g): 0 Protein (g): 4.8 Vitamin A: 1.60% Vitamin C: 3.70% Calcium: 5.40% Iron: 15.90% Potassium (mg): 101.8 Polyunsat Fat (g): 0 Monounsat Fat (g): 0 Vitamin D: 2.00% Vitamin E: 52.20% Vitamin K: 0.10% Thiamin: 0.60% Riboflavin: 1.70% Niacin: 0.80% Vitamin B6: 2.40% Folate: 1.90% Vitamin B12: 1.50% Pantothenic Acid: 0.40% Phosphorus: 3.10% Magnesium: 4.30% Zinc: 1.60% Selenium: 0.20% Copper: 1.40% Manganese: 11.50%

Broccolini, 226.8 g	81 *(8)*	Broccolini, garlic, salt, black pepper, lemon zest, chili flake	Total Fat (g): 0.9 Sat. Fat (g): 0.1 Trans Fat (g): 0 Cholesterol (mg): 0 Sodium (mg): 149.9 Total Carbs (g): 16 Fiber (g): 6.1 Sugars (g): 3.9 Added Sugar (g): 0 Sugar Alcohol (g): 0 Protein (g): 6.6 Vitamin A: 29.40% Vitamin C: 338.90% Calcium: 11.20% Iron: 9.60% Potassium (mg): 734.2 Polyunsat Fat (g): 0.1 Monounsat Fat (g): 0 Vitamin D: 0.00% Vitamin E: 9.00% Vitamin K: 289.10% Thiamin: 11.00% Riboflavin: 15.90% Niacin: 7.40% Vitamin B6: 21.10% Folate: 35.80% Vitamin B12: 0.00% Pantothenic Acid: 13.20% Phosphorus: 15.30% Magnesium: 12.20% Zinc: 6.40% Selenium: 8.50% Copper: 6.10% Manganese: 27.90%

Tomato and Roasted Garlic Soup, 453.6 g	187 *(57)*	SOUP tomato, vegetable broth, onion, red bell pepper, garlic, carrot, cashew, tomato paste, olive oil, basil, salt, black pepper, chili flakes GARNISH basil	Total Fat (g): 6.3 Sat. Fat (g): 1.1 Trans Fat (g): 0 Cholesterol (mg): 0 Sodium (mg): 278.3 Total Carbs (g): 27 Fiber (g): 4.4 Sugars (g): 13.8 Added Sugar (g): 0 Sugar Alcohol (g): 0 Protein (g): 5.9 Vitamin A: 114.20% Vitamin C: 135.90% Calcium: 9.50% Iron: 12.00% Potassium (mg): 693.6 Polyunsat Fat (g): 0 Monounsat Fat (g): 0 Vitamin D: 0.00% Vitamin E: 4.00% Vitamin K: 25.50% Thiamin: 5.60% Riboflavin: 2.50% Niacin: 2.30% Vitamin B6: 12.20% Folate: 4.30% Vitamin B12: 0.00% Pantothenic Acid: 2.50% Phosphorus: 8.70% Magnesium: 9.80% Zinc: 5.30% Selenium: 4.90% Copper: 14.10% Manganese: 22.80%

PROCEDURES INVOLVED:

Up to 30 participants (15 males and 15 females) will be enrolled in the study and will be randomized into one of the three arms (we expect each arm will have three male and three female participants after attrition): the “juice fasting” arm, the “juice in addition to normal diet” arm, and the “caloric restriction diet” arm. Participants will come into the lab for an initial visit, during which they will complete questionnaires on their usual diet and demographics and will be informed about their assignment to one of the three arms.

In the “juice fasting” arm, participants will be given vegetable/fruit pressed juices and be instructed to engage in a three-day juice fast diet totaling 800-900 kcal-per-day (nutrient information of juices attached). The specific juices will be assigned for each day in order to maintain the calorie level. In the “juice in addition to normal diet” arm, participants will be given the same juice for three days but continue with their usual diet in addition to the juice. For this

arm, there is no restriction of caloric intake or restriction to liquid only. In the “caloric restriction diet” arm, participants will be on a whole-food plant-based diet totaling 800-900 kcal-per-day (matching the daily calories of juice fasting).

Packaged food and drink will be provided, delivered by the manufacturers (KitchFix and Pressed Vibrance), but participants will need to have access to their own food storage, such as a refrigerator. Deliveries will occur at a place and time of day specified by the participant to ensure delivery to the participant and immediate refrigeration, and both the participant and study coordinator will be notified when deliveries occur. Pressed Vibrance deliveries will be in cooler bags lined with ice packs on all sides, kept roughly 37-39 degrees Fahrenheit. KitchFix deliveries will be in cooler bags with a frozen water bottle, often delivered via refrigerated vehicle.

In all three arms, the three-day diet intervention will be preceded and followed by three days of self-conducted elimination diet. The pre-intervention diet will encourage participants to eat a healthy, light diet for three days (basic instructions: eat only fresh raw or cooked fruits and vegetables (preferably organic), whole grains and eggs; drink 8 glasses of water a day; avoid or eliminate alcohol, caffeine, sugar, processed foods, dairy, red meat, and gluten). The post-intervention diet will instruct participants to gradually return to eating solid foods, and limit strenuous exercise (basic instructions: follow a diet similar to the pre-intervention elimination diet; day 1 after the intervention: eat fruits & green vegetables, and drink 8 glasses of water; day 2 add in: nuts, gluten-free grains like whole oats, quinoa, or brown rice; day 3 add in: organic meats, fish, & gluten-containing grains).

Biological samples and self-reported outcomes will be collected at four time points: baseline (prior to the first elimination diet period), pre-intervention (after the elimination diet and before the three-day intervention), post-intervention (after the intervention), and 14-day post-intervention. At each time point, participants will complete questionnaire on quality of life (PROMIS - Global Health Scale, which is an NIH measure on an individual's’ overall physical and psychological well-being; see appendix) and collect their own saliva and stool samples. Participants will be given self-administered toolkits and be taught how to collect the samples at their first lab visit. At each of the four time points, the research assistant will coordinate the collection of cheek swabs and bloodspot samples. Stool samples will be collected using a pre-moistened wipe, with written instructions provided to participants, and both stool and saliva samples will be collected by the participant at home, then provided to the research assistant at their next visit.

Over the course of the study, participants will be instructed to keep a diet diary days one through nine to record their dietary intake of each day (diet diary forms with instructions attached), as well as fill out a modified version of the PROMIS Global Health Scale. During the intervention period, the research team will also contact the participants to check in with them. Participants will not need to record diet data for the fourteen day post intervention period.

At the conclusion of the study, participants will bring back the final biological samples (stool wipe and saliva), provide the fourth bloodspot sample, and be debriefed about the purposes of the study.

The biological samples collected will then be processed and assayed for microbiome, methylation, inflammatory markers, and glycemic markers.

All questionnaire data will be stored in a REDCap database, separate from screening surveys, which are not to be used as study data. REDCap is a secure, web-based application designed to support data collection and management activities for research studies, and these databases will be hosted by the Feinberg School of Medicine.

DATA AND SPECIMEN BANKING:

If fewer participants drop out than anticipated, any excess blood/stool/saliva samples will be banked for later assay. All such specimens will be stored at the Center for Population Epigenetics.

Consent forms and demographic information will be added to Study Tracker, per FSM participant tracking policy.

The specimens will be stored in a -80C freezer at the Center for Population Epigenetics, Northwestern University, located at 680 N Lake Shore Drive, Suite 1400, Chicago, IL 60611. The specimen can be accessed through the principal investigators and lab manager.

The procedure to release specimen and data:

1. Requester submits application to principal investigators with specific aims and specimen number and list;
2. The principal investigators review and approve the request of specimen and related data;
3. The request is forwarded to lab manager, who will conduct the sample recording and retrieval from the freezer.
4. The data associated with specimens related to the study including patient ID, date of collection, time of collection, and time point, will also be released.

SHARING OF RESULTS WITH PARTICIPANTS:

We have no plans to share individual participants' results. Aggregate data will be available to participants upon request.

STUDY TIMELINES:

Recruitment and data collection will last for three months. Each participant will stay in the study for 21 days (if they choose to complete the study). Primary analyses are expected to be

completed three months later, for a total of six months.

INCLUSION AND EXCLUSION CRITERIA:

Participants have to meet the all of the following criteria to participate in the study:

1. Subjects considered as healthy by the investigator based on medical history and completion of the screening questionnaire.
2. Subjects who, according to the investigator, can and will comply with the requirements of the protocol and are available for all scheduled visits at the investigational site.
3. Healthy male or female aged between 18 and 35 (included) years
4. 18.5 ≤BMI ≤ 30 kg/m²
5. Ability to give their informed consent in writing

Individuals meeting any of the following criteria may not participate in the study:

1. Documented history of previous cardiovascular disease, including CHD (angina, myocardial infarction, coronary revascularization procedures or existence of abnormal Q waves in the electrocardiogram (EKG)), stroke, syncope, and clinical peripheral artery disease with symptoms of intermittent claudication.
2. Severe medical condition that may impair the ability of the person to participate in a nutrition intervention study (e.g. digestive disease with fat intolerance, advanced malignancy, or major neurological, psychiatric or endocrine disease including diabetes).
3. Daily use of any prescription or non-prescription medication that has a high likelihood of impacting systemic inflammation (e.g. non-steroidal anti inflammatories or steroids), blood sugar control (e.g. medication for diabetes) or the human microbiome (e.g. antibiotics).
4. Any other medical condition thought to limit survival to less than 1 year.
5. Known immunodeficiency disorder
6. Illegal drug use or chronic alcoholism or total daily alcohol intake >80 g/d.
7. Difficulties or major inconvenience to change dietary habits
8. Impossibility to follow an elimination or juice fast diet, for religious reasons or due to the presence of disorders of chewing or swallowing (e.g., difficulties to consume nuts)
9. A low predicted likelihood to change dietary habits according to the Prochaska and DiClemente stages of change model (Nigg, 1999).
10. History of food allergy with hypersensitivity to any of the components of the juice or diet
11. Patients with an acute infection or inflammation (e.g., pneumonia) are allowed to participate in the study 3 months after the resolution of their condition.
12. Dietary restrictions due to medical (including allergies), religious, or other concerns

13. Any diagnosis of allergic rhinitis, eczema, asthma, or inflammatory bowel disease (e.g. ulcerative colitis or Crohn's) from a health professional

VULNERABLE POPULATIONS:

This study will not include vulnerable populations (adults unable to consent, individuals who are not yet adults, pregnant women, and prisoners).

PARTICIPANT POPULATIONS:

Accrual Number:	Category/Group: (Adults/Children Special/Vulnerable Populations)	Consented: Maximum Number to be Consented or Reviewed/Collected/Screened	Enrolled: Number to Complete the Study or Needed to Address the Research Question
Local	Adults	30	18
Study-wide	N/A	N/A	N/A
Total:		30	18

RECRUITMENT METHODS:

Flyers will be posted on the Evanston campus of Northwestern University. Interested individuals will be directed to complete a screening questionnaire online via REDCap which will include questions about general health conditions (from SF-36) and demographics. Based on the inclusion and exclusion criteria, qualified individuals would be contacted for a phone call to gauge their interest in participating in the study. This data will not be used in the study – it will be solely for the purposes of determining eligibility, and will be housed in a separate REDCap database. During the phone conversation, detailed information will be given on what participating in the study entails, and participants will be asked at the end whether they are willing to participate. If the answer is yes, they will be invited for the initial lab visit during which hard-copy consent form will be provided.

COMPENSATION FOR PARTICIPATION IN RESEARCH ACTIVITIES

Participants will be compensated for their participation in the study. Each participant will be given a gift card for $50 when they complete the third collection of biological samples and surveys, and $50 when they complete the final collection of biological samples and surveys.

WITHDRAWAL OF PARTICIPANTS:

Participants will have the opportunity to voluntarily withdraw throughout the study. Involuntary withdrawals will be initiated when participants report experiencing an adverse medical event (e.g., fainting or other health effects that may be related to fasting and caloric

restriction).
Failure to collect samples for one or more time periods will be reviewed on a case-by-case basis.

RISKS TO PARTICIPANTS:

Risks to participants are expected to be minimal, particularly in light of the valuable information to be gained.

Diet intervention: Fasting and juice cleanse diets over short periods are generally safe, but may cause some minor discomfort during the study period. For those on restricted intake diets, several minor adverse effects may occur and include constipation, headache, muscle cramps, diarrhea, general weakness, and rash. Most of these are short-lived, and are generally alleviated by adequate fluid intake and other minor diet modifications. The restricted diet may lead to decreases in glucose levels; this risk will be mitigated by screening participants for the exclusion criteria of diabetes, hypoglycemia, steroid-dependent diseases and use of medications that impact on blood sugar such as sulfonylureas and insulin.

Blood Spot: There is a small risk that the puncture associated with the blood spot may cause bruising or infection at the site of the needle insertion.

Weight, height and waist circumference measurements: There is no physical risk involved in measuring weight, height, and waist circumference but there is a risk of embarrassment or discomfort at being measured in one's underclothes.

Behavioral and psychological questionnaires: These carry a minimal risk for emotional discomfort and stress.

Participants will be fully informed of all of these risks during the informed consent process and be given the opportunity to withdraw from the study at any time without any further obligation. No data will be collected until informed consent has been obtained. We expect all of these risks to be temporary, and to quickly subside after the study is concluded.

Efforts to minimize risk are as follows:

Dietary intervention: Participants will be instructed to speak to the research associate or principal investigator if they experience discomfort, such as constipation, diarrhea, weakness, muscle cramps, or dizziness. There is instruction provided with the juice cleanse on how to address any potential side effects stemming from the diet. Participants will be told that their participation is voluntary and they can choose to withdraw from the study at any time without any further obligation.

Blood spot: Bloodspot collection will be done as professionally and comfortably as possible. Blood is collected for the in-person assessments in a sterile manner to reduce the threat of infection. Likewise, pressure is applied to the puncture site to prevent hemorrhage and ecchymosis.

Weight, height and waist circumference measurements: These measurements will be done in a private setting by a trained research assistant.

Psychological questionnaires: Participants will be told that they can refuse to answer questions that they feel are too personal or that they feel would be too distressing to answer.

Adverse events: Participants will be asked to report significant adverse events as soon as possible to study staff, and will be provided with a study phone number for this purpose. Study physicians will be available to study staff by pager if needed to assess possible adverse events and provide clinical input, including calling participants if indicated.

POTENTIAL BENEFITS TO PARTICIPANTS:

Participants may or may not experience health benefits from juice cleansing and/or fasting. Participants will receive juice or packaged caloric-restriction meals for the 3-day intervention. They will also be compensated for their participation in the study (up to $100). Other possible immediate and/or direct benefits to participants and society at large include: health and lifestyle changes may occur as a result of participation; knowledge may be gained about their health and health conditions; feeling of contribution to knowledge in the health or social sciences field.

DATA AND SPECIMEN MANAGEMENT AND CONFIDENTIALITY:

Biological sample collection/storage and shipment: All biological samples that will be used for this project will be collected and then de-identified and labeled with the following information: patient ID, date of collection, time of collection, and time point (baseline, pre, post and 3 days post). All samples will be stored in a -80C freezer in the lab located at Northwestern University, Department of Medical Social Sciences, 2205 Tech Drive, Suite 2-120, Evanston, IL 60208 until ready for transfer for laboratory analysis.

Data handling for Methylation/Microbiome Testing

One set of de-identified DNA samples of stool, bloodspot, cheek swab, and saliva will be collected in sealed plastic bags and placed with a freeze pack inside of an insulated shipping container. The container will be shipped to Northwestern University NUseq Core Facility, 300 E Superior St, Tarry 2-770, Chicago, IL 60611 for laboratory assays.

Data handling for Glycemic and Inflammatory Biomarker Testing

One set of de-identified labeled bloodspot samples will be collected in sealed plastic bags and placed with a freeze pack inside of an insulated shipping container. We will seal the box tightly with tape and ship specimens via courier to:

Laboratory for Human Biology Research at Northwestern University Department of Anthropology, 1810 Hinman Avenue, Evanston, IL 60208; Phone: 847-467-6965

Contact: Dr. Thomas McDade; email: t-mcdade@northwestern.edu

No names or individual identities will be used in publications resulting from the study. Physical records will be kept in an area accessible only to research staff. Research data will be stored on a secure, HIPAA-compliant server and drive with monitored and controlled access for study staff and investigators. Survey data will be hosted on secure servers. A key linking study ID to participant names will be kept in a password-protected file on a secure FSM server, separate from study data.

Biomarker Testing

Whole Blood Testing

Inflammatory Stress and Glycemic Biomarkers will include (C-reactive protein, interleukin-6, insulin, leptin). These biomarkers will be assayed using the finger prick/filter paper collection procedure. Blood spot samples will be collected at each scheduled time point in the protocol at the Osher Center for Integrative Medicine clinic or a mutually determined region on the Northwestern campus (in order to accommodate participants' schedules). Throughout all stages of blood collection and processing, Universal Precautions will be followed. Each participant's finger will be cleaned with alcohol and then pricked with a sterile, disposable, widely available micro-lancet commonly used by diabetics. Five drops of whole blood will be collected on standardized filter paper.

Samples will be dried overnight and stored in a -80C freezer at the Northwestern University, Department of Medical Social Sciences, 2205 Tech Drive, Suite 2-120, Evanston, IL 60208 until analysis, at which time specified samples will be transported to the appropriate lab.

Blood spot samples will be analyzed using previously validated ELISA protocols previously developed for use with dried blood spots. Samples will be analyzed for inflammatory and glycemic index markers using a high sensitivity immunoturbidimetric assay on the Hitachi 917 analyzer (Roche Diagnostics—Indianapolis, IN) and the Luminex® 200™ System (Luminex Corporation, Austin, TX), separately. All samples are run in duplicates.

Physiologic Measures

We will measure systolic and diastolic blood pressure, which will be measured manually using a stethoscope and blood pressure cuff. Participants will be assisted in sliding their left arm through the cuff, positioning it 0.5-inches above the elbow, and securing it with Velcro material. They will place their arm on a table so that the cuff is positioned at the same level as their heart. Pulse will

also be checked manually by placing the tips of the index and second fingers of one hand on the inside wrist of the patient and positioning the fingers just below the base of the thumb to take the radial pulse at the patient's wrist.

Weight and waist circumference will be measured at each timepoint. Waist circumference will be measured according to CDC guidelines: the participant will stand, and a tape measure will be placed horizontally around their middle, just above the hip bones. It will be kept snug, but not compressing the skin, and measurement will be taken just after the participant exhales. Height will be measured at the initial intake.

DNA methylation (blood)

We will use QIAamp DNA Blood Midi Kit (Qiagen) to extract cfDNA from blood samples of study participants according to the manufacturer's protocol. We will then undertake unbiased, genome-wide interrogation of DNA methylation using Illumina's newly released Infinium Methylation EPIC BeadChip (EPIC array), an updated version of the widely used Illumina 450K array (terminated in March 2016). The EPIC array covers over 850K methylation sites across the human genome and includes CpG sites outside of CpG islands, non-CpG methylated sites identified in human stem cells, sites differentially methylated in tumor cells, FANTOM5 (Functional Annotation of Mammalian Genome) enhancers, ENCODE (Encyclopedia of DNA Elements) open chromatin and enhancers, DNase hypersensitive sites, microRNA promoter regions, as well as 93% of the content contained in the old Illumina 450K array.

The Illumina Genome Studio-generated detection p-values will be used to distinguish reliable and unreliable probe signals, and to identify failed samples. We will remove samples that contain >5% failed probes at detection p>0.01, as well as CpG sites that failed in >5% of samples (i.e., calling rate <95%). CpG probes mapped to ambiguous locations in the human genome will be detected and removed. To avoid potential SNP-in-probes bias, we will also exclude CpG probes containing common SNPs (MAF [minor allele frequency] >0.01) based on dbSNP. The final analysis set will include only CpG probes located on autosomes. The level of methylation, represented by the ratio of methylated signal to total signal and the M-value (log2 ratio of methylated signal over unmethylated signal) will be determined for each interrogated locus. We will also conduct analyses using the β-value (i.e., the proportion of modified signal in total signals from 0 to 1), which has a more straightforward biological interpretation. The methylation levels will be quantile-normalized across all samples. Potential batch effects and blood cell subpopulation proportion estimated by Houseman's method 41 will be corrected using ComBat.42 To account for multiple comparison issue, we may 1) narrow down the genome- wide scan (~300,000 unambiguous autosomal CpGs) to those well-annotated genic CpG sites (~100,000); and 2) further narrow down to highly variable CpG sites, for example top 10% of highly variable (informative) CpGs (~10,000 CpGs) across the samples.

DNA extraction, 16S rRNA sequencing and microbiome analysis

Saliva samples and buccal cell DNA will be extracted using QIAamp DNA Mini Kit (Qiagen). Stool sample DNA will be extracted using QIAamp DNA Stool Mini Kit (Qiagen). The quality and quantity of the DNA will be examined using Nanodrop spectrophotometer (ThermoFisher Scientific). 16S rRNA amplicon library will be prepared using Nextera XT DNA Library Preparation Kit (Illumina) following the manufactures protocol. DNA (100 ng) will be used as template for PCR

to amplify the 16S rRNA region. The 16S rRNA amplicon libraries will then be sequenced on a MiSeq system (Illumine). For the 16S rRNA amplicon analysis, the individual library read files will be paired and combined in silico (16S–total) and analyzed for taxonomic annotation using the RDP tool.

PROVISIONS TO MONITOR THE DATA TO ENSURE THE SAFETY OF PARTICIPANTS:

The study poses slight risk to participants. The research team will contact each participant each day during the 3-day intervention period to check on their well-being and to monitor for adverse events relating to the diet. Possible mild side-effects include headaches, fatigue, difficulty thinking, moodiness, stomach pain and hunger pangs, and changes in bowel function or frequency of urination. For any participant feeling poorly, the research team member will encourage the participant to stay hydrated, and pass along a report immediately to the PI. The PI will be the primary individual responsible for safety monitoring. If necessary, the participant will be encouraged to cease the diet and resume their usual intake. Any significant adverse side effects including syncope (fainting) or dehydration will lead to immediate cessation of the intervention for that participant.

The research team will review cumulative data from patient feedback on a weekly basis to monitor for adverse effects. No outcomes bad enough to warrant termination of the research study are expected. However, it will be on an individual basis that this option will be considered.

PROVISIONS TO PROTECT THE PRIVACY INTERESTS OF PARTICIPANTS:

We will collect no identifying information with the data of interest to this study. All such de-identified data will be stored on a secure, password-protected computer at the Osher Center for Integrative Medicine or the Department of Preventive Medicine. Only the study research assistant and principal investigators will have access to these data. The trained research assistant will explain all study procedures in detail during the informed consent process and answer any of the participants' questions. Participants' names and mailing addresses will be provided to KitchFix or Pressed Vibrance for the purposes of food/drink delivery only. This information will not be stored with any study data.

Encrypted and computerized data of the laboratory measurement will be used. Throughout the conduct of the present study, stringent guidelines will be used to ensure privacy and confidentiality. The methylation data generated from this project will be stored without the original identifying information in the Department of Preventive Medicine at Northwestern University's Feinberg School of Medicine where all data entry and data management will be performed. Double entry with 100% verification will be used to ensure accuracy of the process.

COMPENSATION FOR RESEARCH-RELATED INJURY:

If any participant appears to become ill or get injured as a result of this study, they will be encouraged to seek medical treatment through their doctor or treatment center of choice. They will be told that they should promptly to tell the PI and research team about any illness or injury.

The university will not pay for medical care required because of a bad outcome resulting from

participation in this research study. This does not keep a participant from seeking to be paid back for care required because of a bad outcome.

ECONOMIC BURDEN TO PARTICIPANTS:

This study is not expected to present economic burden to participants. The study will assume the full costs of all special diets used. In addition, the study will take place on Northwestern campus and so it should not incur additional transportation cost for Northwestern students.

CONSENT PROCESS:

Individuals who have completed the screener and who are deemed eligible for the study will be contacted for a phone call. During the phone call, specifics of the study will be described to participants. If they are interested in participating in the study, they will be invited for a lab visit, during which a written informed consent document will be provided. Participants will be given time to read the document and a member of the research team will be available to answer any questions they might have. Participants will then be given a private space to decide if they want to participate in the study, and if so, sign on the informed consent.

Hard-copy informed consent will be provided for the participants to sign during their initial lab visit. The research team will keep the signed documents and offer to make a copy for the participants.

Non-English Speaking Participants

Due to staff limitations, only English-speaking participants will be enrolled in this study.

QUALIFICATIONS TO CONDUCT RESEARCH AND RESOURCES AVAILABLE:

The study procedures will be conducted with a trained research assistant experienced and certified in human subjects research and privacy protections (including HIPAA). It will be supervised by the three PIs, who between them have many years of experience conducting human subjects research. With a small total sample size, recruitment via on-campus advertisements should be achievable in the desired timeframe. Assuming 50% of participants are ineligible or withdraw, we will need access to 36 participants. Participants will be recruited from the multitude of education programs across the Evanston Northwestern campus; Northwestern University is a private institution with a total undergraduate enrollment of 8,353.

The research assistant will be responsible for collecting biospecimen and questionnaire data, and conducting daily follow-up phone calls to monitor participant adherence to the diets of interest. PIs will supervise the study conduct and data collection, monitor potential adverse events, and assist with questions as needed. A trained research assistant will conduct the bloodspot collection and physiologic measurements in a private setting at the Northwestern Medicine Osher Center for Integrative Medicine, located at 150 E. Huron Avenue, Suite 1100, Chicago, IL 60611, or a mutually determined private space on campus.

The research assistant and PIs will review the approved study protocol together, along with all consent forms, scripts, and other participant materials to ensure agreement.

Participants will be asked to report significant adverse events as soon as possible to study staff, and will be provided with a study phone number for this purpose. Study physicians will be available to study staff by pager if needed to assess possible adverse events and provide clinical input, including calling participants if indicated.

All persons assisting with the research will participate in relevant weekly or biweekly team meetings (in person or virtual) to remain adequately informed about the protocol, the research procedures, and their duties and functions during recruitment, implementation and data analysis.

The study will take place at the Evanston and Chicago campuses of Northwestern University.

Bloodspot collection and physiologic measurements will be performed in a private setting at the Northwestern Medicine Osher Center for Integrative Medicine, located at 150 E. Huron Avenue, Suite 1100, Chicago, IL 60611, or a mutually determined private space on campus.

DNA extraction will be performed at 710 N Fairbanks Ct, Olson 8-360, Chicago, where the lab for the Center for Population Epigenetics is located. The lab space and equipment (including centrifuge, thermal cycler, Nanodrop spectrometer, refrigerator, freezer) can be used for DNA extraction, concentration measurement and sample storage.

Biomarker extraction will be performed at Laboratory for Human Biology Research at Northwestern University Department of Anthropology, 1810 Hinman Avenue, Evanston, IL 60208.

STUDY-WIDE RECRUITMENT METHODS:

N/A

REFERENCES:

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5. Zheng, J., Zhou, Y., Li, S., Zhang, S., Zhou, T., Dong-Ping, X., & Hua-Bin, L. (2017). Effects and mechanisms of fruit and vegetable juices on cardiovascular disease. *International Journal of Molecular Sciences, 18*(3), 555; doi:10.3390/ijms18030555.