STUDY PROTOCOL: The effects of orange juice compared with sugar-sweetened beverage on risk factors and metabolic processes associated with the development of cardiovascular disease and type 2 diabetes

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PROTOCOL TITLE:

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1) Protocol Title

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PROTOCOL MODIFICATIONS TO ACCOMMODATE COVID-19 STUDY SAFETY RESTRICTIONS: *Our original protocol includes inpatient metabolic studies that require multiple close interactions between participants and the research team.* NIH has approved modifications of the inpatient protocol *to an outpatient protocol in order to safely conduct this study while the pandemic continues. The modifications to the original inpatient procedures are described, plus other safety mitigations including the elimination of non-essential interactions between study staff and study participants.*

Protocol Version Date 11-09-2022

2) Objectives

Specific Aims: There is considerable epidemiological evidence that demonstrates associations between added sugar/sugar-sweetened beverage consumption and increased risk for or prevalence of chronic diseases such as cardiovascular disease (CVD), type 2 diabetes (T2D), metabolic syndrome, and gout. Especially concerning is recent evidence from National Health and Nutrition Examination Survey III that demonstrates that there is increased risk of CVD mortality with increased intake of added sugar across quintiles. Even the US mean added sugar intake, 15% of daily calories, was associated with an 18% increase in risk of CVD mortality over 15 years. The results from our recently completed study (1R01 HL09133) corroborate these findings. They demonstrate that supplementing the ad libitum diets of young adults with beverages containing 0, 10, 17.5 or 25% of daily energy requirement (Ereq) as high fructose corn syrup (HFCS) affects lipid/lipoprotein risk factors for CVD in a dose response manner. Specifically, levels of nonHDL-cholesterol(C), LDL-C, apolipoprotein B (apoB), and postprandial triglycerides (TG) increased linearly over a 2-week period with increasing doses of HFCS. Furthermore, even the participants consuming the 10% Ereq dose exhibited increased levels of these risk factors compared to baseline.

These and similar results have helped to lead to reductions in soda consumption in this country, and new dietary guidelines and FDA food labeling requirements to promote reductions in added sugar consumption. However, there are gaps in knowledge about other sugar-containing foods that lead to public confusion concerning healthier options for soda, and impede further progress in implementing public health policies that will promote further reductions in soda consumption. One such food is naturally-sweetened fruit juice. The amount of sugar in fruit juice is comparable to the amount in soda. Because of this, a consumer seeking answers on the internet will find many articles in which experts state or suggest that the effects of consuming fruit juice

are as detrimental as or even worse than those of soda. However, in contrast to soda, fruit juice contains micronutrients and bioactives that may promote health. Therefore the consumer can also find numerous articles on the internet where the health benefits of fruit juice and these bioactives are extolled. There are a limited number of clinical dietary intervention studies that have directly compared the metabolic effects of consuming fruit juice and sugar-sweetened beverage, and their results are not conclusive.

The objectives of this proposal are to address the gaps in knowledge regarding the metabolic effects of consuming orange juice, the most frequently consumed fruit juice in this country, compared to sugar-sweetened beverage. Thus, we will pursue the following Specific Aims:

1. **Specific Aim 1: To compare the weight-independent effects of consuming 25%Ereq as orange juice or sugar-sweetened beverages for 4 weeks on risk factors for CVD and other chronic disease in normal weight and overweight men and women.** We hypothesize that blood concentrations of LDL-C, apoB and uric acid will be lower in subjects consuming orange juice than in subjects consuming sugar-sweetened beverage.

2. **Specific Aim 2: To mechanistically compare the weight-independent effects of consuming 25%Ereq as orange juice or sugar-sweetened beverages on metabolic processes associated with the development of CVD and T2D in normal weight and overweight men and women.** We hypothesize that consumption of orange juice will result in less detrimental changes on hepatic *de novo* lipogenesis (DNL), liver lipid accumulation and insulin sensitivity than consumption of sugar-sweetened beverage.

3. **Specific Aim 3: To relate the changes assessed under Specific Aims 1 and 2 to the changes in the urinary levels of metabolites and catabolites of the main flavanones in orange juice, hesperetin and naringenin.** We hypothesize the increased urinary levels of the hesperetin and naringenin metabolites and catabolites in subjects consuming orange juice will negatively associate their changes in risk factors and metabolic processes associated with chronic disease.

Weight-maintaining, low sugar diets will be provided during the 2-week baseline period, and matched diets that contain 25% energy as the carbohydrate in orange juice or sucrose-sweetened beverage will be provided during the 4-week intervention period. All experimental procedures for assessment of blood risk factors, DNL, insulin sensitivity, liver lipid and abdominal fat distribution are conducted during baseline and during the end of intervention.

The results from this study, whether supportive or not of our hypotheses, will assist the consumer in making informed beverage choices and also aid the efforts to establish evidence-based public health policy aimed at slowing the epidemics of CVD and T2D.

3) Background

As shown in Table 1, orange juice, the most commonly-consumed fruit juice in this country[24], can contribute significant amounts of sugar to the diet. Thus, it is possible that consumption of high amounts of orange juice will result in increases in blood levels of LDL-C, apoB, postprandial TG, and uric acid that are comparable to what we have observed in subjects consuming HFCS-sweetened beverage [7]. However, while the micronutrient content of sugar-

sweetened sodas and beverages is essentially zero, fruit juices contain a wide variety of micronutrients and other bioactive components (e.g. polyphenols, flavonoids, anthocyanins, etc.), which could possibly modify the metabolic processes by which excess sugar consumption leads to increased risk factors for chronic disease. In support of this, there are populations studies that report that incidence/prevalence of metabolic syndrome [25, 26], CVD [27] and T2D [28-30] and their risk factors [31-33] are associated with consumption of SSB, but not with consumption of 100% fruit juice. However, this lack of adverse association may be due to lower levels of fruit juice consumption than SSB consumption in most of these studies. It is also possible that the usual background diets of regular consumers of SSB are less healthy than regular consumers of 100% fruit juice. Furthermore, several studies report both fruit juice and SSB consumption are positively and comparably associated with metabolic syndrome [34], T2D [35, 36] and gout [37].

Table 1: Macronutrient content of 100 kcals orange juice or soda

Beverage	Kcal	Protein (g)	Fat (g)	Available carbohydrate (g)	Total sugar (g)	Glucose (g)	Fructose (g)	Fiber (g)
Orange Juice	100	1.5	0.3	22.7	19.1	9.1	10.0	0.4
Soda (HFCS-sweetened)	100	0.0	0.0	25.0	23.4	10.5	12.9	0.0
Soda (sucrose-sweetened)	100	0.0	0.0	25.0	25.0	12.5	12.5	0.0

The direct experimental data does not resolve the discrepancy in the epidemiological results. A meta-analysis of 19 randomized controlled trials through 2012 investigating the effects of various fruit juices, concentrated fruit juices and fruit juice powders from elderberry, bilberry, grapefruit, blueberry, cranberry, pomegranate and several types of grape, suggested that fruit juice had a borderline significant effect to reduce diastolic blood pressure, and did not affect total or LDL-C [38]. The unaffected lipid levels could be viewed as a positive finding since the majority of the trials utilized control groups that received water intervention or no intervention. However, the dose of provided fruit juice ranged from 40-270 kcal/d, with an approximate mean of 165 kcal/d [38]. Possibly the amount of fruit juice consumed in the majority of the 19 studies [38] was too low to induce unfavorable effects in only 2-12 weeks. The low dose group from our recent study, who exhibited increased levels of LDL-C and apoB in 2 weeks, consumed 234 kcal/d as HFCS. A more recent systematic review of the evidence concerning the metabolic impact of 100% fruit juice consumption concluded evidence-based recommendations were not possible[5].

Background—Specific Aim 1: We propose to directly compare circulating levels LDL-C, apoB, uric acid and other risk factors in subjects consuming SSB or orange juice. Two published studies have compared the effects of orange juice with SSB on risk factors for chronic disease. In a 4-week trial, consumption of 500 ml/day (180 kcal) orange juice lowered diastolic blood pressure, fasting plasma uric acid, and increased microvascular reactivity in middle-aged, overweight men [39] compared with the SSB control. Overweight, hypercholesterolemic men who consumed 250 ml (118 kcal) orange juice/day for 12 weeks tended (P=0.06) to have lower levels of TG compared with the men who consumed SSB [40]. We have conducted a pilot study comparing the effects of consuming a much higher dose (25% E_{req}) of orange juice or sucrose-SB. Even at this high dose, orange juice consumption does not appear to have detrimental effects on LDL-C and apoB, and 24-hour uric acid concentrations compared with those following sucrose-SB consumption. Studies that investigated the effects of orange juice consumption

without a SSB control also report positive effects. Women who consumed 500 ml/day (180 kcal) of orange juice for 3 months had improved LDL-C and HDL blood values compared with women who did not consume an intervention beverage [41]. Middle-aged, mildly hyperlipidemic adults who consumed 280 kcal/day of orange juice for 3 months exhibited decrease lipid peroxidation, increased total antioxidant status, and unaffected lipids [42]. Men who consumed 750 ml/day of orange juice for 2 months had decreased LDL-C concentrations and increased HDL function (measured by in vitro assay of transfer of radioactive lipid emulsion into the HDL fraction) [43]. Consumption of 750 ml/day orange juice for 4 weeks increased HDL concentrations without affecting LDL and apoB in subjects with elevated cholesterol levels [44]. Thus the available results specific to orange juice do suggest that it may have neutral or positive effects on risk factors and is a healthier beverage choice than SSB.

Background—Specific Aim 2: We propose to directly compare metabolic processes by which excess sugar consumption leads to increased risk factors for chronic disease in subjects consuming SSB or orange juice. These include de novo lipogenesis, fatty acid oxidation, liver lipid accumulation, hepatic insulin resistance, and visceral fat accumulation. To the best of our knowledge none of these processes have been compared in subjects consuming SSB or orange juice.

Background—Specific Aim 3: Our Specific Aim 3 investigation will be guided by Collaborator/Consultant Dr. Alan Crozier, a Thomson Reuters Highly Cited Researcher with more than 100 publications on the absorption, metabolism, disposition, excretion and health benefits of dietary flavonoids. We propose to directly compare the urinary levels of metabolites and catabolites of hesperetin and naringenin in subjects consuming SSB or orange juice. Hesperetin and naringenin are the main flavanones in orange juice and there is evidence from *in vitro* [66-68], animal [69-76], and human [27, 39, 77-79] studies to suggest that hesperetin and naringenin exert a wide range of beneficial actions that could possibly modify the metabolic processes by which excess sugar consumption leads to increased risk for chronic disease. However, 7 years ago Dr. Crozier pointed out the irrelevance of *in vitro* research that focused on the polyphenolic compounds as they are found in fruits and vegetables, because it is not these compounds that are transported around the human body in the circulatory system, or reach body tissues to elicit bioactive effects [80, 81]. It is their metabolites, formed in the small intestine and hepatic cells, and also the low molecular weight catabolic products of the colonic microflora. It is these compounds that carry interest for drug-discovery and for dietary prevention of disease [80, 81]. More recently, Dr. Crozier has published [1, 82, 83] and reviewed [84] the evidence that refutes the general belief that polyphenols are poorly bioavailable with only relatively small amounts of the ingested dose entering the systemic circulation, in the form of metabolites, prior to undergoing renal excretion. Instead, when metabolites and catabolites are included in the overall estimation of absorption, distribution, metabolism, and excretion, it is evident that many polyphenols, are highly bioavailable[1]. In the case of OJ, after acute intake, 0-24 h urinary excretion of hesperetin and naringenin metabolites; principally in the form of hesperetin-3′-*O*-glucuronide and hesperetin-3′-sulfate; was equivalent to 16% of intake[1]. Excretion of colon-derived phenolic catabolites was 88% of intake[1], with 3-(3′-hydroxy-4’-methoxyphenyl)hydracrylic acid being the main catabolite[1] and a good biomarker of hesperetin intake [84]. This work also demonstrated that while plasma pharmacokinetic measurements provide a ‘snapshot’ of absorbed circulating

metabolites, 0-24 h urinary excretion of both metabolites, absorbed in the small intestine, and catabolites, of distal gastrointestinal origin that are products of bacterial processing, provide a more quantitative reflection of polyphenol absorption. We therefore propose to analyze hesperetin-3′-*O*-glucuronide, hesperetin-3′-sulfate and 3-(3′-hydroxy-4'-methoxyphenyl)hydracrylic acid; and also hesperetin-7-O-glucuronide, naringenin-4'-O-glucuronide and naringen-7-O-glucuronide; in urine collected over a 24-hour period at baseline and 4 week later during intervention when subjects consume orange juice or SSB with each meal.

4) Inclusion and Exclusion Criteria

Screening for eligibility: Subjects are first screened for eligibility via a 30-minute survey conducted over telephone or online (UC Davis Qualtrics) in which information about the study is provided. Qualifying respondents are asked to read the human consent form and then contacted to schedule a consenting appointment using Zoom, an online video communications application. During the consenting appointment, the study coordinator engages potential participants in a discussion about usual diet, social commitments, food-related activities and the difficulties that can be anticipated in adhering to the provided diet for approximately 6 weeks. The study coordinator discusses the consent form and all experimental procedures, the time commitment required by the study, and answers all questions. A list of all food ingredients served during the study is provided to determine acceptability, and potential subjects can request and arrange for pick-up of samples for taste testing if they wish. Interested potential participants will sign the consent form using DocuSign and a fasting blood draw appointment will be scheduled at either Ragle Human Nutrition Research Center on campus or at a UC Davis Health System affiliated clinic off campus if the former is unavailable due to scheduling confilicts. Clinical chemistry, CBC, and lipids are analyzed to determine eligibility. A follow up draw may be requested if necessary to determine eligibility. Female participants will be asked to provide the date of their last menstrual period and form of contraception, if applicable. Qualified respondents who are judged by the study coordinators as likely to be responsible and compliant are scheduled for study.

Inclusion criteria: The first submission of this proposal to NIH limited inclusion to participants ≤ 40 years of age with BMI ≤ 28 kgm^2. The NIH reveiwers criticized this plan because it would preclude the ability to assess the effects of the 2 interventions across the metabolic spectrum of BMIs and detect differences in susceptibility between healthy and less healthy subjects. It was noted that inclusion in the statistical analysis of BMI and metabolic syndrome, liver fat and insulin sensitivity will allow us to gain insights on these variables in the response to both interventions. Therefore in the revised NIH application we expanded our inclusion criteria to include participants ≤ 50 years of age with BMI ≤ 35 kgm^2. In order to ensure that we will be able to enroll obese subjects, who are likely to have plasma concentration of glucose, triglyceride or LDL-C that are out of the normal ranges, we also revised our exclusion criteria for these parameters to the values shown below. We will study men and pre-menopausal women (age 18-50 y; BMI 20-35 kg/m^2 with body weight > than 50 kg) with self-reported stable body weight during the prior six months. We will study 24 subjects per group, requiring a total of 48 subjects

to complete research procedures. We anticipate a completion rate of 70% therefore expect to enroll a total of 72 subjects.

Exclusion criteria:

- Fasting glucose >125 mg/dl
- Evidence of liver disorder (AST or ALT >200% upper limit of normal range)
- Evidence of kidney disorder (>2.0 mg/dl creatinine)
- Evidence of thyroid disorder (out of normal range)
- Systolic blood pressure consistently over 140 mmHg or diastolic blood pressure over 90 mmHg
- Triglycerides > 400 mg/dl
- LDL-C > 160 mg/dl in combination with Chol:HDL > 4
- Hemoglobin < 10 g/dL
- Pregnant or lactating women
- Current, prior (within 12 months), or anticipated use of any hypolipidemic or anti-diabetic agents.
- Use of thyroid, anti-hypertensive, anti-depressant, weight loss medications or any other medication which, in the opinion of the investigator, may confound study results
- Use of tobacco
- Use of marijuana
- Strenuous exerciser (>3.5 hours/week at a level more vigorous than walking)
- Surgery for weight loss
- Diet exclusions: Food allergies, special dietary restrictions, routine consumption of less than 3 meals/day or dietary habits that may undermine compliance to dietary protocol, routine ingestion of more than 2 sugar-sweetened beverages or 1 alcoholic beverage/day, unwillingness to consume any food on study menu
- Veins that are assessed by the R.N.s as being unsuitable for long-term infusions and multiple blood draws from a catheter.
- Pre-existing claustrophobia or metal implants that preclude MRI
- Any other condition that, in the opinion of the investigators, would put the subject at risk
- A recent positive COVID-19 test

We will exclude individuals from each of the following special populations:

- Adults unable to consent
- Individuals who are not yet adults (infants, children, teenagers < 18 years)
- Pregnant women
- Prisoners

We will also exclude non-English speakers because study procedures can only be implemented with proficiency in English. At the current time we do not have staff that are proficient in any other language than English and we do not have the funds to hire extra staff with bi-lingual

skills. This is also true for the staff at Touro University, where the 12-hour blood collection procedures are conducted.

Effective and clear communication between the study staff and between the subjects is essential in order to properly implement the experimental and dietary protocols required for this study. Before confirming their willingness to participate in this study, the subjects must have a clear understanding of what is required of them in terms of time commitment, diet commitment, and also an understanding of procedures that they will undergo. For example, it is very important that potential subjects clearly understand that we need to know if they have metal implants in their bodies because certain metal implants can cause great harm to them during magnetic resonance imaging. Potential subjects need to clearly understand that this study requires many blood samples and realize that if the insertions required for needle-stick collections or catheters makes them nervous or unhappy, this study is not for them.

Throughout the study more than 30 text messages and/or emails get exchanged between subjects and the study coordinators regarding schedules, body weight, diet issues such as hunger or food that needs to be replaced. Those subjects who are most able to clearly communicate any problems they are having regarding schedules, diet, beverages, are the subjects who are most likely to consider participation in our study to be a rewarding experience.

5) Study Timelines

Timeline: Our goal is to study 24 subjects (12/group) using the modified protocol and 24 subjects (12/group) using the original protocol. Ten subjects have already completed the original protocol. Based on an expected retention rate of 70%, we will enroll 35 subjects during Years 4-5 (spring 2021 to summer 2022) into the modified protocol. When we have achieved our sample size of 24 subjects completing the modified protocol, we will seek approval to resume conduct of the original protocol. If approval is not obtained due to the pandemic, we will enroll up to 24 more subjects into the modified protocol to achieve a final sample size of 48 (10 on original protocol, 38 on modified protocol).

Duration of individual subject's participation: ~6 weeks (40-47 days)
Duration of anticipated enrollment of all subjects: April 1, 2018-August 30, 2023
Estimated date of investigator completion of primary analyses: January 31, 2024

6) Study Endpoints

Primary and secondary study endpoints: The primary endpoints are the changes in risk factors for metabolic disease which include blood lipid and lipoprotein levels. The secondary endpoints are hepatic lipid content, the fractional rate of *de novo* lipogenesis, and whole body insulin sensitivity.

Primary and secondary safety endpoints: The study intervention, which consists of 4-weeks consumption of 25%Ereq as orange juice or sucrose-sweetened beverages, is not associated with any short term safety risks.

7) Procedures Involved

Anyone coming onto campus (students, employees or visitors) is required to follow the university COVID-19 safety guidelines that are posted on the campus website: https://campusready.ucdavis.edu/, which may include filling out the UC Davis Daily Symptom Survey during each visit and completing COVID-19 testing. Additionally, participants will be required to wear a mask during all study interactions, have their temperature monitored and maintain a 6-foot distance when possible (e.g., except Registered Nurses performing blood draws). For transport by study staff in UCD vehicles to study procedure sites, participants will be provided and required to wear a KN95 or N95 mask, face shield and to sit 6 feet away from the driver and any other passenger.

General study design: The study is designed as a parallel, 6-week study with a 2-week baseline period during which all subjects consume the provided low-sugar baseline diet and a 4-week intervention period during which the experimental beverages are consumed along with a low-sugar diet.

Baseline (Week 1-2): All subjects will consume a provided, standardized, low sugar, energy-balanced diet for approximately 9-13 days. All experimental procedures are conducted.

Intervention (Week 3-6): Subjects will be stratified by gender and normal weight/overweight status and randomized to one of the two groups listed below:

1. **Sugar-sweetened beverages (SSB)**: 25% of estimated Ereq/day as sucrose in water (11% weight/w/w), flavored with unsweetened Kool-Aid and divided into 3 servings/day.
2. **Orange Juice (OJ)**: 25% of estimated Ereq/day as the carbohydrate in OJ divided into 3 servings/day.

Subjects will consume three provided beverages/day along with provided, standardized, energy-balanced meals and snacks for approximately 28-33 days. All experimental procedures are repeated.

Diets & weight monitoring: Approximately 2 weeks prior to the start of study, enrolled subjects will be scheduled at their convenience to report to Ragle HNRC. If Ragle HNRC is unavailable due to facility safety restrictions (due to the small dining and procedure rooms, the number of subjects that can be accommodated at this time is very limited) or schedules conflicts, the foyer at the entrance to Ragle HNRC will be used. Their height and body weight will be measured with a portable stadiometer and body weight scale. They will be provided with a body weight scale and asked to provide (via text or email) a daily, minimally clad, fasted body weight for the next 2 weeks and throughout the study. The purpose is to improve the accuracy of the initial estimation of Ereq and to facilitate the adjustment of energy intake required to maintain stable body weight. Also at this visit, female participants will be asked to take a urine pregnancy test.

Study diet is prepared by the study staff in the kitchen of the Ragle HNRC on the UC Davis campus. Subjects are restricted to eating only the study food provided during the entire study.

The initial daily Ereq of each subject will be calculated by the Mifflin equation with an adjustment of 1.5 for activity [108]. During the 2-week baseline period, all subjects consume an energy-balanced, low sugar diet that is 50% E_{req} carbohydrate, 35% fat and 15% protein. During the 4-week beverage intervention, the subjects receive diets that are matched as closely as possible to the baseline diet with the exception of the substitution of 25% E_{req} as the carbohydrate in sucrose-sweetened beverage or orange juice for 25% E_{req} complex carbohydrate. Subjects will return all uneaten food for calculation of daily intake. Diet energy will be adjusted as required to maintain stable body weight. If energy adjustment of the meals consistently fails to prevent weight gain or weight loss the subject will not be studied further.

Food is served/provided as 3 meals and a snack, with 23% of the energy provided as breakfast, 32.5% as lunch, 37% as dinner and 7.5% as snack. Meals and snacks mainly consist of a heterogeneous mix of ingredients (i.e. stir-fry or casserole rather than separate servings of meat, grain and vegetable) formulated to the standardized specifications. This ensures that a subject who does not eat an entire meal is still consuming the required macronutrient distribution. The intervention meals contain 25% more fiber than the baseline meals in order that the fiber content of both the overall baseline and intervention (which includes the beverages) diets is 12.5 g/1000 kcals. This is mainly achieved by a greater proportion of unrefined grains (e.g. whole wheat pasta) in the intervention meals. The overall fat content of the baseline and intervention diets is <10% E_{req} saturated fat, and ~12% and ~13% E_{req} polyunsaturated and mono-unsaturated fat, respectively.

Beverages: We will utilize unfortified 100% orange juice that is pasteurized, ready-to-serve, and is not reconstituted from concentrate. We will utilize a top-selling brand and type that is widely available. The SSB will be made with sucrose from beet sugar at a weight/weight concentration to ensure that subjects with identical energy requirements will receive the identical volume of beverage, whether it be orange juice or SSB. Samples of each beverage batch are retained and frozen for future quality control and nutrient/bioactive analyses. Subjects are provided with 25% of daily E_{req} as the total carbohydrate in their assigned beverage (carbohydrate in orange juice is approximately 90% sugar and 10% oligosaccharides), divided into 3 daily servings to be consumed with meals (25% at breakfast, 35% at lunch, 40% at dinner). Subjects are required to consume all the beverage provided. The beverages will contain a biomarker (riboflavin) that will be assayed fluorometrically in urine collected 2 times/week during meal pick-up appointments (See Compliance monitoring section below).

Beverage and meal pick-up/return: At appointments scheduled at their convenience, subjects will report to Ragle HNRC two times/week to pick up a 3- or 4-day supply of meals/snacks/beverages, and to return uneaten food and packaging. If Ragle HNRC is unavailable due to facility safety restrictions or schedules conflicts, the foyer at the entrance to Ragle HNRC will be used. Temperature will be measured using a contactless thermometer. Subject will be given a urine collection cup and and asked to provide a urine sample using the restroom in the foyer area. All exchanges of meal & beverage carrying cases and urine cup & urine sample will be conducted with both subject and study staff member wearing masks, face shields and maintaining a 6-foot distance at all time. Thus there will be a designated cart or area in the foyer upon which all carrying bag/supplies/samples will be set for dispensation or for turn-in. The cart or area will also have a supply of sterile wipes and disposable gloves so all items can

be decontaminated prior to being picked up. Subjects who fail to pick up a new supply of meals/beverages (or make arrangements for it to be delivered to their homes) in time to allow for uninterrupted consumption as required by the protocol, will be dismissed from the study for non-compliance. Subject will need to fill out study diet report forms, physical activity questionnaires and food surveys. These forms will be made available to complete electronically and are used to assess diet palatability and quantity, activity level changes and to describe diet deviations. These reports will be reviewed by the Study Coordinator and communication through text, email or Zoom call will be used to discuss issues or request clarifications.

Compliance monitoring: The sweetened beverages will contain a biomarker (riboflavin). The biomarker will be assayed in urine collected 2 times/week during meal pick-up appointments. The subjects will be informed that they are being monitored for compliance, but not provided specifics as to the method. We have successfully used this method of enhancing compliance in three previous studies[7, 14, 15].

To identify subjects who consume or consumed non-study foods that contained HFCS or cane sugar or consume alcoholic beverages during the study period, we will measure biomarkers for added sugar and alcohol intake. We will measure the delta ^{13}C value of blood drops collected at baseline and at the end of 4 weeks of intervention. The Delta ^{13}C content in the blood drop will be measured at the UCD Stable Isotope Facility. Delta ^{13}C is a novel validated biomarker of added sugar intake because most plants have a low natural abundance of delta ^{13}C, while that of corn and sugar cane is conspicuously higher [110, 111]. The foods with the highest amounts of delta ^{13}C are pure corn products, and beverages, hard candies and desserts sweetened with HFCS or cane sugar. Sugar from sugar beets has a low natural abundance of delta ^{13}C, therefore beet sugar will be used to sweeten the sucrose-sweetened beverages provided as part of the intervention. Delta ^{13}C in blood is an indicator of added HFCS and sugar cane sucrose consumption over the prior 2 weeks [3]. Because all the meals and snacks provided during the 6-week study have a very low HFCS and sugar cane content, and all subjects are consuming the same foods, all compliant subjects will exhibit low and comparable levels of blood delta ^{13}C at both the end of the 2nd week and 6th week of study. Data from subjects with delta ^{13}C blood values that are more than 2 standard deviations above the mean for all subjects will not be included in the statistical analyses.

To identify subjects who routinely consume alcohol we will assay urinary ethyl glucuronide (immunoassay, Thermo Scientific, Fremont, CA) in the urine samples collected at each meal pick-up visit. Detection of ethyl glucuronide in urine indicates recent alcohol consumption [112] and is considered to be a specific, highly sensitive, and reliable marker of recent alcohol intake within the previous 5 days [4]. Urinary ethyl glucuronide concentrations above the 500 ng/ml will be considered positive for alcohol intake. Urine will be monitored for alcohol intake upon collection, and subjects with positive values will be considered for dismissal. The subjects will be informed that they are being monitored for compliance to the requirement that they not consume non-study food, but will not be provided specifics as to the method.

Experimental procedures: Procedures are performed as shown in the study schedule below (Table 2). This schedule is approximate and the 6-week length is approximate, as changes may

be made to accommodate subject availability and availability of the study facilities, equipment & personnel. Outpatient procedures will occur at the Ellison Building, University of California Davis Medical Center (UCDMC), and at Touro Metabolic Research Center (TMRC) in Vallejo, CA. Transportation from Davis to UCDMC and TMRC and will be offered by study using UCD Fleet Service vehicles. Subjects will be provided and required to wear a KN95 or N95 mask and face shield while in the vehicle and maintain a 6-foot distance from the driver and any other passenger. Participants who wish to provide their own transportation to the facilities will be reimbursed at the UCD mileage reimbursement rate. For subjects who provide their own transportation, a study staff commucation plan that will include a wake-up call if needed will be set up to ensure their arrival at TMRC or the Ellison Building on schedule.

Table 2: Study schedule with procedures, timing and compensation

Week	Procedure	Location	Time	Details	Compensation
Baseline ~ 2 weeks		**Energy-balanced baseline diet: carbohydrate (mainly starch--50%Ereq), fat (35%Ereq), protein (15%Ereq)**			
-2	*Scale Pick-up*	*Ragle HNRC*	*TBD by subject*	*2 weeks prior to study start; at-home body weight scale pick-up visit*	$10
-2 - 6	*Fasting BW*	*Home*	*pre-breakfast*	*Text, email or phone in fasting BW daily*	$65
0	Study Overview	Home	TBD by subject	Zoom meeting for study/schedule review & questions	$10
0	1st Meal Pick-up	Ragle HNRC	TBD by subject	1st meal pick-up visit	$10
1 - 2	Diet	Home/Ragle HNRC	TBD by subject	Daily consumption of provided baseline study meals Pick-up/dropoff for study meals; urine collection: 2 times/week	$40
1	MRI #1	UCDMC Radiology	7:00	Hepatic & abdominal magnetic resonance imaging	$50
1	Stool Collection #1	Home	TBD by subject	Stool collection	$20
2	24-h UC #1	Home	TBD by subject	24 hour urine collection	$30
2	Pre-TMRC Evening	Home	20:00, 23:00	Consumption of acetate tracer dose	$40
2	TMRC Visit #1	TMRC	7:00	Transport to TMRC, Consumption of acetate tracer dose	$150
			8:00	Check-in, vitals, anthropometrics, catheter insertion	
			8:45	Start blood collection every 30 or 60 minutes	
			9:00 - 12:00	Oral glucose tolerance test	
			12:00	Lunch, Consumption of acetate tracer dose	
			13:00 - 15:00	Study questionnaires, DEXA scan	
			16:00	Dinner, Consumption of acetate tracer dose	
			20:00	Final blood draw, remove catheter, check-out	
Intervention ~ 4 weeks		**Energy-balanced intervention diet: Orange juice or SSB (25%Ereq), carbohydrate (mainly starch--25%Ereq), fat (35%Ereq), protein (15%Ereq)**			
3-6	Diet	Home/Ragle HNRC	TBD by subject	Consumption of provided intervention study meals & beverages Pick-up/dropoff for study meals/beverages; urine collection: 2 times/week	$140
3	24-h UC #2	Home	TBD by subject	24 hour urine collection	$40
5	Stool Collection #2	Home	TBD by subject	Stool collection	$20
6	24-h UC #3	Home	TBD by subject	24 hour urine collection	$50
6	MRI #2	UCDMC Radiology	7:00	Hepatic & abdominal magnetic resonance imaging	$50
6	Pre-TMRC Evening	Home	20:00, 23:00	Consumption acetate tracer dose	$45
6	TMRC Visit #2	TMRC	7:00	Transport to TMRC; Consumption of acetate tracer dose	$200
			8:00	Check-in, vitals, anthropometrics, catheter insertion	
			8:45	Start blood collection every 30 or 60 minutes	
			9:00 - 12:00	Oral glucose tolerance test	
			12:00	Lunch, Consumption of acetate tracer dose	
			13:00 - 15:00	Study questionnaires, DEXA scan	
			16:00	Dinner, Consumption of acetate tracer dose	
			20:00	Final blood draw, remove catheter, check-out	
End of Study					
				TOTAL	**$970**

Procedure days are approximate and dependent on clinic and subject availability (baseline visits will occur in the first 2 weeks of the study and intervention visits will occur between the 5th and 6th week of the study)

Ragle HNRC: Ragle Human Nutrition Research Center at UCD campus, Department of Nutrition; UCDMC: University of California Davis Medical Center; TMRC: Touro (University) Metabolic Research Center; Ereq: energy requirement; BW: Body weight

Procedures at UCD or UCDMC:

Urinary flavanone metabolites and catabolites: During baseline and two times during intervention study subjects will collect all urine over a 24-hour period. The urine collection period will begin with the first collection upon waking in the morning (no later than 9:00) and end at approximately 9:00 the next day. Subjects will be provided with pre-labeled bottles (enough to ensure all urine can be collected) containing a preservative solution (30 ml of 10% phosphoric acid) in a designated ice chest with enough cold packs to maintain refrigeration temperatures. Bottles will be transported to our laboratory in the designated ice chest (by either the subject or study staff). All collections are pooled by study staff, weighed and multiple aliquots are saved for metabolic analyses.

The pooled urine samples from baseline and from the two intervention collections will be assayed by the NIH West Coast Metabolomics Center at UC Davis for hesperetin-3'-sulfate, hesperetin-3'-O-glucuronide, 3-(3'-hydroxy-4'-methoxyphenyl)hydracrylic acid, hesperetin-7-O-glucuronide, naringenin-4'-O-glucuronide and naringen-7-O-glucuronide using liquid chromatography-high resolution mass spectrometry [83]. Standards for each compound will be obtained from Toronto Research Chemicals (Toronto, Canada). All outcomes will be adjusted for urinary creatinine concentration, which will be measured on a PolyChem Chemistry Analyzer.

Abdominal and hepatic fat/fasting blood sample: A fasting Magnetic Resonance Imaging (MRI) for hepatic lipid content and quantification of visceral and subcutaneous fat will be performed at the beginning and at the end of the study at the UCDMC Department of Radiology. These appointments are usually scheduled at 7:00 and study staff provide transport in university vehicles to subjects. Due to the pandemic, some subjects may prefer to drive themselves, and these subjects will be reimbursed at the UCD mileage reimbursement rate. Female participants will have a urine pregnancy test prior to the MRI. Dr. John McGahan, Department of Radiology UCD Medical Center, will supervise the pre- and post- quantification of images for intra-/extra-abdominal fat. Image post-processing, tissue segmentation, and analysis of liver fat will be performed by Post-doctoral fellow Dr. Desiree Sigala using a commercial semi-automated software tool (SliceOmatic; Tomovision, Inc.).

Microbiome - Fecal collection: Each subject will receive instructions and a sample collection kit containing ice packs, labeled sample vials, a fecal collection kit, zip-lock bags, and a sealable secondary container (freezer box). Feces will be collected in a disposable fecal collection vessel that attaches to the toilet. Subjects will be instructed to label sample vials with date and times of sample production. Subjects will transfer approximately 10mL of sample into the sample vials using the scoop that is attached to the sample vial lid. The lid is replaced on sample vial and the vial is placed in the ziplock bag and then in the secondary container and stored in the subject's freezer until subject is ready to transport sample back to UC Davis. Upon arrival at UC Davis, labeled sample will be placed in a -80°C freezer until processed for future analyses. Subjects will be asked to collect one sample during baseline and one sample during the intervention period.

Procedures at TMRC: These tests will be conducted during a 13-hour period at TMRC, which will occur during the baseline period and be repeated 4 weeks later during the intervention period.

Isotope consumption for assessment of DNL (Figure 1): We are replacing our original 26-hour isotope infusion protocol with an oral bolus protocol. Each subject will be provided with two doses of an oral stable-isotope tracer (1-^{13}C acetate) in water that will be consumed at 20:00 and 23:00 the night before the outpatient visit to the TMRC. Subject will be picked up at 6:30 - 7:00 by study staff for transport to TMRC. Due to the pandemic, some subjects may prefer to drive themselves to TMRC, and these subjects will be reimbursed at the UCD milage reimbursement rate. A third dose of the acetate tracer will be consumed at 7:00. Additional doses of acetate tracer will be provided and consumed at the TMRC at 9:00, and with meals at 12:00 and 16:00. Each oral dose of the acetate tracer will consist of 1-2 grams 1-^{13}C acetate in approximately 4 ounces of water.

Blood pressure: Blood pressure will be measured at check-in, before dinner, and prior to check-out. Weight, height, waist and hip will be measured.

Blood collection (Figure 1): A catheter will be inserted at 8:15-8:30 and blood sampling will start at 8:45 and continue until 20:00 at the timepoints indicated on Figure 1 and Table 2.

Urine Collection: A urine sample will be collected before 9:00 and after consumption of lunch and dinner.

Oral glucose tolerance test (OGTT) for assessment of insulin sensitivity (Figure 1): We are replacing our original hyperinsulinemic euglycemic clamp protocol with a 3-hour oral glucose tolerance test. Following a second fasting blood draw at 9:00, subjects will consume 75 grams of glucose in 300 ml of water followed with blood draws at 9:30, 10:00, 10:30, 11:00, 11:30, 12:00.

Lunch: Lunch will be provided following the 12:00 blood draw.

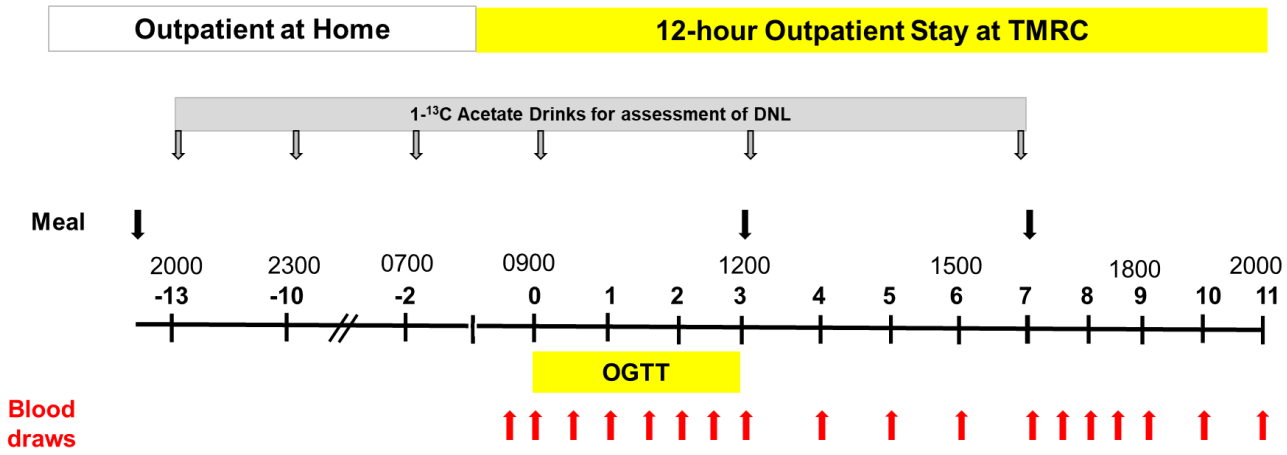
Dual energy X-ray absorptiometry (DEXA): Total body fat will be determined by DEXA. In the event that the DEXA scanner at TMRC cannot be used, the DEXA scanner at CCRC will be used. Each subject's baseline and intervention scans will be conducted using the same scanner.

Questionnaires: Questionnaires about eating behaviors, food preferences and personality traits will be administered.

Dinner: Dinner will be provided following the 16:00 blood draw.

Check-out: Following the final blood draw at 20:00, subjects will be transported home.

Figure 1: Timeline for TMRC Visits



Analyses: %Fractional DNL: %Fractional DNL (fasting, during OGTT, during and following meal feeding) will be assessed in the sample collected from 8:45 – 20:00.

OGTT: Glucose and insulin will be measured in the samples collected at 9:00, 9:30, 10:00, 10:30, 11:00, 11:30 and 12:00. The 3-hour area under the curve (AUC) for glucose and insulin and the Matsuda insulin sensitivity index will be calculated.

Fasting and postprandial blood profiles: Fasting blood samples will be collected at 8:45 and 9:00. Pre-dinner and postprandial blood samples will be collected hourly until 20:00. These samples will be used to analyze hormones and metabolites that include insulin, leptin, lipids, lipoproteins, glucose, lactate, liver enzymes, free fatty acids and uric acid.

The timing and volume of the blood samples to be collected during TMRC visit 1 & 2 are shown in Table 3.

Table 3: Timing and volume of blood samples collected for procedures

Scheduled time of draw	TMRC visit 1 (baseline) blood draws (cc)	TMRC visit 2 (intervention) blood draws (cc)	EXIT study blood draw (cc)
8:00			6
8:45	27	27	
9:00	27	27	
9:30	13	13	
10:00	13	13	
1030	13	13	
11:00	13	13	
11:30	13	13	
12:00	17	17	
13:00	13	13	
14:00	13	13	
15:00	13	13	
16:00	13	13	
16:30	8	8	
17:00	13	13	
17:30	4	4	
18:00	13	13	
19:00	23	23	
20:00	23	23	
Total	*272*	*272*	*6*
Total blood volume (cc) for study			**550**

Questionnaires: Subjects will fill out questionnaire about physical activity, eating behaviors, food preferences and personality traits during the study. Some of these questionnaires will be filled out during the TMRC visits, and some will be provided and returned electronically. These questionnaires are a consolidation of the eleven tests described below:

Reward-Based Eating Drive (RED). The RED taps conditioned hypereating by assessing adaptive (flexible) and maladaptive (rigid) dietary restraint, as well as three aspects of drive to eat (loss of control, lack of satiety and preoccupation with food). Items are answered on a Likert scale from 1 (*not at all like me*) to 5 (*very much like me*). The title of the RED questionnaire provided to the subjects is "Eating thought & experiences".

General State and Trait Food Craving Questionnaires (reduced). The G-FCQ-T assesses general trait and state food cravings, and was created from the FCQ-T and FCQ-S, which assesses specific food craving. The 15-item G-FCQ-T is a reliable measure of a general 'desire for food' or 'desire to eat' that has evidenced good internal consistency ($\alpha = .94$). The G-FCQ-T comprises four factors: (1) preoccupation with food (i.e., obsessive thought about food and eating), (2) loss of control (i.e., difficulty regulating eating behavior when exposed to food cues), (3) positive outcome expectancy (i.e., believing eating to be positively reinforcing), and (4) emotional

craving (i.e., tending to crave food when experiencing negative emotion). Internal consistencies for the entire G-FCQ-T (α = .94) and its subscales (αs = .72 - .87) are good. The title of the G-FCQ-T questionnaire provided to the subjects is "General food cravings".

Snacking On Sweets. We will assess participants' tendency to snack on sweet foods and drinks using two items created for this purpose: "How many servings a day did you eat of desserts or other sweets (cookies, ice cream, candy, cake, or other sweet foods)?" and "How many servings a day did you drink of regular (not diet) soda, or other sweet drinks (sweet tea, any juices, blended coffees, milkshakes)?" Items are rated on a scale from 0 (*0 servings*) to 5+ (*5 or more servings*). The title of the SOS questionnaire provided to the subjects is "Snacking".

Salty and Sweet. Participants will be asked if they tend to prefer salty foods or sweet foods on 7-point Likert scale, ranging from preferring sweet foods much more / somewhat more / a bit more than salty foods, to equal preferences for sweet and salty foods, to preferring salty foods much more / somewhat more / a bit more than sweet foods. Participants will be asked to endorse this question (1) in general, over the course of their lives (Time 1 only), and (2) over the prior 3 days.

Subjective Opiate Withdrawal Scale. The SOWS is designed to tap the experience of withdrawing from a substance, and items tap common motoric, autonomic, gastrointestinal, musculoskeletal, and psychiatric symptoms of opiate withdrawal. We will add 11 items tapping typical naltrexone-related responses (e.g., nausea, headache) and food (e.g., "I have trouble getting food off of my mind"). We will modify one existing SOWS item (replacing word 'using' with word 'eating'). Items are answered on a scale from 0 (*not at all*) to 4 (*extremely*). The title of the SOWS questionnaire provided to the subjects is "In this moment".

Stress Eating. To tap stress eating, we will ask participants to respond to three general questions related to whether and how often they eat in response to stress, and how they feel when they do. The items are answered on 4-6 point Likert scales that grade the frequency of the behavior or the emotional response to the behavior.

Palatable Eating Motives Scale (PEMS) The PEMS is a 19-item questionnaire that aims to gather information on what motivates the participant to consume food. Subjects will respond to questions ranging from 1 (Almost never/never) to 5 (Almost always/always). The questionnaire will be provided to subjects as the "Palatable Eating Motives Questionnaire".

Control of Eating Questionnaire (CoEQ): The CoEQ asks 21 questions related to appetite, hunger and cravings over the last 7 days. Subject responses regarding frequency or strength of emotional responses are marked on a continuous scale anchored by "not at all" and "extremely". The title of the CoEQ provided to the subjects is "Food feelings during the last 7 days".

Food Security: The Food Security questionnaire will be administered at the beginning of the study and identifies subjects who have experienced food insecurity. All 6 questions include the option to respond "Don't know/Prefer not to answer". The title of the Food Security questionnaire provided to the subjects is "Budget and Food".

8) Data and/or Specimen Management and Confidentiality

Data analysis plan: Janet Peerson, Senior Statistician (USDA, Western Human Nutrition Research Center), will assist with statistical analyses. All fasting and postprandial outcomes will be assessed for conformance to the normal distribution and transformed if appropriate. The dependent variable will be calculated as the change obtained at 4-wk intervention compared with baseline. All outcomes, excepting those related to insulin sensitivity, will be analyzed in a 3-factor (beverage, gender, original or modified protocol) analysis of covariance (SAS 9.4) with adjustment for BMI and outcome at baseline [7]. Other subject characteristics or baseline outcomes will be included as continuous or ranked covariables (e.g. metabolic syndrome risk factors, visceral fat, liver fat, insulin sensitivity) and retained if they improve the sensitivity of the model. Tukey's posttest will be used to identify significant differences between beverage x gender groups. Significant changes compared to baseline will be identified as adjusted means significantly different from zero. Insulin sensitivity outcomes will mainly be analyzed separately by protocol (clamp or OGTT).

Multivariable linear regression analyses will be used to evaluate the association between cardiometabolic risk markers and creatinine-adjusted urinary concentrations of the flavanone metabolites and catabolites, individually, and in hesperetin- and naringenin-specific groups.

Sample Size: The proposed sample size of 24 subjects/group will detect an effect size (difference between group means/pooled standard deviation) of 0.83 at $P<0.05$, 80% power in a 2-group comparison, two-sided test. The effect sizes for the 2-week comparison of 25% Ereq orange juice and sucrose-SB on LDL-C, apoB and the 24h uric acid were 0.89, 0.84 and 1.02 respectively. Thus we expect 24 subjects/group to be sufficient to detect differences in these primary outcomes between the groups consuming orange juice and SSB.

Confidentiality and data management: All research staff requiring access to subject data and specimens will be trained on HIPAA and confidentiality by completing Privacy and Security Training courses offered by the UCDHS Compliance Department. All enrolled subjects will be assigned an identification number upon start of study. Documentation of subject ID number and subject name will be stored in each subject's patient medical chart, which is maintained by the TMRC or CCRC research and medical staff, and in each subject's hard copy file, which is maintained by the Study Coordinator. During the study, each subject's patient medical chart will be stored in a locked file cabinet that is located in a locked room in restricted area at the TMRC or CCRC. The TMRC or CCRC nursing staff has access to these files. When a subject has completed study, copies of the medical chart are placed in the hard copy subject files. When the 5-years study is completed, copies of the CCRC patient medical charts from all subjects will be uploaded into UC Davis Health System Electronic Medical Records (EMR) and hard copy patient medical charts will be destroyed. During study, the hard copy subject files are transported to the various study sites by the Study Coordinator in a locked file case. The Study Coordinator and UCD research team have access to this locked file case. Upon completion of study, each subject's hard copy file will be stored in a locked file cabinet in Dr. Stanhope's locked office in VM3B, 1089 Veterinary Medicine Drive, on the UCD campus. The Study Coordinators, Dr. Stanhope and Dr. Sigala have access to these files. These files will be de-identified within 5 years of publication.

Quality control and specimen management: Samples needing transport will be done so in collection containers and a secondary hard-walled container such as an ice chest. Containers will be labeled as required, i.e. plasma samples will have a dry ice label and UN3373 Category B label on the outside. Study specimens will be stored in -80ºC freezers in the laboratories of Drs. Stanhope and Jean-Marc Schwarz. All specimens and data sent to and analyzed by other collaborators will only be identified by subject ID number. The subject ID number will also be used for all the analyses conducted in the laboratories of Dr. Stanhope and Dr. Schwarz and all collaborators. All electronic databases will be identified only by ID number and will be stored on secured servers. Therefore all laboratory staff will be blinded to subject identity and intervention during all sample processing and analyses. The only exception is when subject identity information is essential to resolve a safety concern (i.e. human pathogens).

All assays are run with in-house quality control samples to allow calculation of the inter- and intra-assay coefficients of variation. All procedures are scheduled such that they are repeated in the time frame relative to the last meal at both baseline and intervention. Identical meals are served before and on procedure days at baseline and intervention and any deviations from the baseline trials are replicated. Samples of each beverage batch are retained and frozen for future quality control and nutrient/bioactive analyses.

9) Data and/or Specimen Banking

Blood, feces and urine will be banked for future use in -80ºC freezers in the laboratory of Dr. Stanhope/Havel. These specimens will be stored for as long as they are viable (for up to 20 years). Dr. Stanhope's study staff will maintain records of the storage location of all specimens and have access to them. Dr. Schwarz's study staff will have access to the blood samples collected for the purpose of measuring glucose production, insulin sensitivity, and DNL and lipid kinetics. Numerous other outcomes (metabolites, enzymes, hormones) will be measured from the blood samples, and the data will be stored on a secured server in excel files that are identified only by subject number.

Riboflavin levels and ethyl glucuronide will be measured in the urine samples to monitor dietary compliance. Potential future analyses of urine samples could include indices of inflammation or oxidative stress.

The following resource sharing plan will be utilized.

The final dataset will primarily include data collected specifically for the study, but it will also contain some demographic information and historical data regarding family history of diabetes, obesity, and cardiovascular disease. Upon publication of the primary manuscript, we will consider requests to make the study-related data and relevant demographic information (such as ethnicity) available to other investigators who submit an outline of the rationale for their request. This request will include the following elements:

1. Hypotheses and aims

2. Assurance that the data will be stored only on a secured computer
3. Assurance that access will be limited to those who are named in the proposal
4. Commitment to destroy or return the data after analyses are completed
5. Guarantee that the grant and the primary investigators will be acknowledged in any publications arising from analysis of these data.

The final dataset, which will not include subject names, will be further stripped of any other unique identifiers and prepared in accordance with all HIPAA regulations prior to release for sharing. No unique subject characteristics will be included in any reports generated by use of the data. This plan is specific to data as it is expected that there will not be sufficient blood specimens for sharing.

10) Provisions to Monitor the Data to Ensure the Safety of Subjects

The Study Physician (Dr. Valentina Medici), the TMRC nurses, Dr. Kimber Stanhope, Dr. Jay Shubrook of Touro University, and the Study Coordinators will monitor the performance and safety of each procedure. All adverse events (e.g. bruises, diarrhea) which are reported by subjects or which occur at TMRC, Ragle HNRC or CCRC are noted in the patient medical chart. Upon presentation to Touro Facilities the UC Davis team will provide a paper medical chart with important screening and inclusion and exclusion documentation. This will be used during the TMRC study procedures and will include description of all TMRC procedures and results. A copy of this chart will be housed on the TMRC and a copy will be provided to the UC Davis team. All adverse events reported to the Study Coordinator during meal pick-up appointments or at other times are noted in the subject file. Any unanticipated adverse event is reported to Dr. Stanhope. Dr. Stanhope will discuss this event with Dr. Medici or Dr. Shubrook if she deems it a concern. It will be reported to IRB if Dr. Medici or Dr. Shubrook or Dr. Stanhope deem it a concern. All serious adverse events will be reported immediately to the IRBs and reviewed in the yearly report to the IRBs.

The safety of the intervention (drinking 25%Ereq as sucrose-sweetened beverages for 4 weeks) will be monitored by the Study Coordinators. A fasting blood draw will be scheduled at either Ragle HNRC on campus or at a UC Davis Health System affiliated clinic off campus if the former is unavailable during the final week of study and a clinical chemistry, lipid and blood profile will be performed. These 'end of study results' will be compared with screening results by the Study Coordinator. The Study Coordinator will have the Study Physician review any values that have changed negatively to levels not in the normal range compared to pre-study values, and the Study Physician will determine the appropriate action (e.g. no action, communication with subject, follow-up blood draw, diet counseling, referral to primary care physician).

11) Withdrawal of Subjects

For all subjects, diet energy will be adjusted upward or downward as required to maintain stable body weight. If energy adjustment consistently fails to prevent weight loss or gain, it will be

assumed that the subject is non-compliant with regards to consuming all or only the food provided, and the subject will not be further studied.

Subjects will also be withdrawn if there is failure to pick-up study beverages/meals or make other arrangements that will allow for uninterrupted consumption of study diet, failure to consume study meals or study beverages, or if there is evidence that non-study foods are being consumed.

12) Risks to Subjects

Pre-existing hyperlipidemia: During recruitment/screening, it may be determined that a potential subject qualifies for study, but has TG or LDL-C levels higher than the normal range (TG > 150 and < 400 mg/dl; LDL-C >130 and < 160mg/dl). Subjects identified with existing hypertriglyceridemia will be informed of the potential risk that consuming sugar-sweetened beverages during the study period will further increase their blood levels of TG or LDL-C. They will be informed they have the option to not participate in the study and advised to discuss their clinical report and the decision to participate in this study with their primary care physician.

Hyperlipidemia: Consuming sucrose-sweetened beverages for 4 weeks at 25% of energy requirements is likely to increase risk factors (TG, LDL-cholesterol, apolipoprotein B) for cardiovascular disease in hyperlipidemic and normolipidemic subjects. It is expected that any changes should be fully reversible when the subjects consume a healthy, low fructose diet. Therefore, at conclusion of study, the subjects will receive information about following a low saturated fat, low fructose diet. If analysis of fasting lipids at end of study indicates unfavorable changes to levels out of the normal range, the subject will be asked to return for a follow-up fasting blood draw. If lipids are still elevated, Dr. Medici will recommend further counseling with a Registered Dietitian and referral to primary care physician for treatment.

Diarrhea and gastrointestinal distress: Consuming 3 servings of sugar-sweetened beverage or orange juice/day can cause diarrhea, stomach aches, bloating, heartburn or acid reflux due to fructose malabsorption or the acid content of orange juice. It is also possible that the changes in the subject's normal dietary routine will cause gastrointestinal distress. Imodium is recommended to subjects who experience diarrhea. GasX or Pepto Bismol is recommended to subjects who experience gastrointestinal distress.

Other diet-related effects: Subjects are restricted to only the food provided. They will not be allowed to eat or drink anything outside of the study diet. The subjects are likely to eventually find this diet to be restrictive and boring.

The riboflavin biomarker in the sugar beverages can cause urine to darken or appear to have a greenish tint.

Blood collection: There is potential for reduced hematocrit due to blood collection procedures. Screened participants with low levels of hemoglobin are not enrolled into the study. Screenees with below normal hematocrit/hemoglobin are informed that they may have their levels retested

following iron supplementation or increased meat consumption. The amount of blood collected during the study will be within generally accepted guidelines for drawing blood from research subjects with body weight > 50kg (550 ml/8 weeks: Body weight > 50 kg is an inclusion criterion).
(https://irb.duhs.duke.edu/sites/irb.duhs.duke.edu/files/Blood_Collect_Policy_Statement_12-13-2012.pdf). As shown in Table 3, we will collect 550 ml from each subject throughout the duration of the study.

There is a chance of bruising, or very rarely, an infection at the site where blood will be taken by needle stick or catheter. Proper aseptic blood collection techniques will be used to minimize these risks. There is a small risk that subject may experience a local skin irritation at the site that the catheter was inserted due to sensitivity to the standard dressing that is used to hold the catheter in place. A paper tape dressing will be used instead if a reaction occurs.

There is a chance that insertion of the catheter may not be successful on the first attempt and multiple attempts may be necessary. There is also the chance that a working catheter can stop working later during the 12-hour blood collection period and the nurse will need to insert a new catheter. The subject may request that prior to doing so, that the nurse review other options. If most of the essential blood samples have been collected, collecting the remaining essential samples using a needle and syringe may be an option. The subject's other option will be to end their participation from the study. To prevent clotting of a catheter the nurses will flush the line with saline after each blood collection, and some subjects find this uncomfortable.

There is a moderate risk that subjects will become bored and tired during the 12-hour blood collection period at the TMRC where activity and mobility is limited. There is a moderate risk that subject will become irritable, fatigued, anxious, or nauseated. We recommend subjects bring from home movies and books and any other activities that will help them to pass the time more pleasantly. We inform the subjects that there are some people whose personalities are not suited to the limited mobility and activity required during the long blood collection procedures. We ask the subjects to please carefully consider their ability to undergo these all day and night blood collection procedures.

Stable isotope infusion: There are no risks associated with the consumption of the stable isotope acetate tracer.

Oral glucose tolerance test: Some subjects may experience mild hypoglycemia during the last hour of the OGTT. If a subject complains of dizziness, shakiness, sweating, fast heartbeat or inability to concentrate, a small blood sample will be drawn for testing on the glucometer. If the blood glucose concentration is above 55 mg/dl, a second sample will be tested 10 minutes later. If the blood glucose concentration is still above 55 mg/dl, the OGTT will proceed unless the subject states that they wish to be provided a snack & lunch immediately. If the first blood glucose concentration is below 55 mg/dl, a second blood sample will be drawn and tested immediately. If blood glucose concentration is still below 55 mg/dl, 25 grams of glucose tablets will be provided, followed by snack and lunch. The blood glucose will be rechecked 15 minutes after the glucose tablets/snack/lunch are provided to ensure it has returned to normal range.

Radiological (DEXA): Subjects will be exposed to a very small amount of x-ray radiation (0.04 mrad) during DEXA procedure. There are no known risks associated with radiation exposure levels this low. There may be some side effects with repeated exposure to radiation, including an increased risk of cancer. However, exposure to doses several thousand times greater than that obtained from this study would be required before an increased risk of cancer could be detected. Thus, the radiation exposure derived from this procedure represents a negligible health risk.

Magnetic resonance imaging (MRI): There is a small risk of that subject will experience anxiety or feelings of claustrophobia during the MRI scan. The radiology technician is in constant communication with the subject and will end the test if subject experiences undue anxiety or claustrophobia. Subjects with pre-existing claustrophobia or metal implants that preclude MRI of the abdominal region will not be enrolled. The MRI scanner makes a loud banging noise that some subjects may find disturbing. Subjects are provided with ear plugs to protect again the loud banging noise. Because the risks to a fetus from MRI are unknown, pregnant participants will not be allowed to participate. Urine pregnancy tests will be conducted before each MRI.

13) Potential Benefits to Subjects

There are no direct benefits to the subjects for participating in the research.

14) Multi-Site Research: N/A

15) Community-Based Participatory Research: N/A

16) Sharing of Results with Subjects

The results that the will be shared with the subjects include the anthropometric results (body weight, waist and hip measurement) and the body composition results generated by DEXA. Upon completion of their participation in the study, subjects will be provided with copies of their clinical chemistry, lipid and blood reports, which were generated on the blood samples collected during screening and during the final week of study. Other study results will not be shared with subjects. In general they will not be available until the end of the 5-year study.

17) Prior Approvals

NIH, the funding agency, will be sent a copy of the IRB-approved Protocol and Consent form for this study, when it is available.

Dr. Schwarz will obtain approval to conduct this research from the Touro University, California Committee on Human Research.

The Human Radiation Use Research Application (Form 35) has approved conduct of the DEXA procedures.

18) Provisions to Protect the Privacy Interests of Subjects

Following the 30-minute screening survey, most of the interactions with the potential and enrolled subjects that involve providing personal information are handled by the Study Coordinators, research and medical team. We anticipate that our experienced study coordinators and medical team will be able to make the subjects feel at ease with the research situation with honest, open answers about any concerns that the subjects may have. The only information we are unable to share with the subjects will be concerning the metabolic effects we anticipate that we will observe in subjects consuming sucrose. They will be able to share that the amount of sugar in the sugar-sweetened beverages represents an amount that is consumed daily by at least 13% of the US.

As required by NIH, this study will be issued the Certificate of Confidentiality. We will ensure that all research personnel and collaborators listed understand the responsibilities for protecting the confidentiality of the participants' information and will comply with the prohibitions on disclosure. No other collaborator receiving specimens or data will receive identifiable information linking to the participants. Sources of information accessed by the research team include the data collected by the team, including test and scan results, and data generated from subject participation. Subject EMR records will be accessed only for the purpose to assigning them to the study for procedure scheduling and billing purposes. All files pertaining to personal and medical information will be safeguarded using locked filing cases and cabinets, locked offices, password-protected computers and encrypted software.

19) Compensation for Research-Related Injury

We will provide this information to the subjects concerning research-related injury:

Subjects will be informed that it is important that they promptly tell the Researchers if they believe that they have been injured because of taking part in this study. If subjects are injured as a result of being in this study, the University of California will provide necessary medical treatment. The costs of the treatment may be covered by University or by the Principal Investigator's Research Grant. The University does not normally provide any other form of compensation for injury. The subject does not lose any legal rights by signing the consent form.

20) Economic Burden to Subjects

There is no cost to the subject for participating in the study, or for the provision of food and beverage. There will likely be costs for the subjects for transportation to Ragle HNRC twice weekly for meal/beverage pickup/drop-off (approximately 12 times total). Subjects will be offered transportation from Davis to UCDMC and Touro University by study staff in university vehicles. In the case of staff shortages, subjects will be offered transportation from Davis to

UCDMC and Touro University via rideshare service, such as Uber or Lyft, that the study will pay for or reimburse. Subjects may prefer to drive themselves due to COVID risk. These subjects will be compensated at the UC mileage reimbursement rate for 44 miles to and from UCDMC and for 100 miles to and from TMRC. Additionally, subjects will be compensated $970 upon completion of the study. This amount may be greater if scheduling adjustments result in a subject remaining on the standardized diet for longer than 47 days. Beyond 47 days, subjects will receive $10/day for each additional day that they consume the study diet and an additional $15 for any extra meal pick-up visits. Subjects who do not complete the study will receive prorated compensation as shown in Table 2. Payment forms for each subject will be processed when participation in the study has ended.

21) Drugs or Devices: N/A

If the research involves drugs or device, describe your plans to store, handle, and administer those drugs or devices so that they will be used only on subjects and be used only by authorized investigators.

☐ I confirm that all investigational drugs will be received by the Investigational Drug Service (IDS). The IDS will store, handle, and administer those drugs so that they will be used only on subjects and be used only by authorized investigators.

☐ I confirm that all investigational devices will be labelled in accordance with FDA regulations and stored and dispensed in such a manner that they will be used only on subjects and be used only by authorized investigators.

22) ClinicalTrials.gov Registration

FDAAA 801 establishes penalties for Responsible Parties who fail to comply withClinicalTrials.gov registration or results submission requirements. **Penalties include civil monetary penalties and, for federally funded studies, the withholding of grant funds.**

For additional information on registration and results submissions requirements click here.
If registration is necessary the following are required:

- *Registration and, in some instances, submission of results at Clinicaltrials.gov*
- *Specific clinicaltrials.gov language in the consent form for this research. The language can be found in HRP 502 Template Consent under the heading "What happens to the information collected for the research?"*

PROTOCOL TITLE:

The effects of orange juice compared with sugar-sweetened beverage on risk factors and metabolic processes associated with the development of cardiovascular disease and type 2 diabetes

Complete each section below. If you finish a section indicating the research must be registered on Clinicaltrials.gov is required you do not have to complete the remaining sections.

Section 1: NIH Funded Studies

If yes to BOTH, the study must be registered on Clinicaltrials.gov.

Yes	
☒	This study is funded by the NIH. (If this study is not funded by NIH, go to Section 2.)
☒	One or more human subjects will be prospectively assigned to one or more interventions (which may include placebo or other control) to evaluate the effects of those interventions on health-related biomedical or behavioral outcomes.

Section 2: Studies subject to FDA jurisdiction

If yes to ANY the study must be registered on Clinicaltrials.gov.

Yes	
☐	This is a prospective clinical study of health outcomes in human subjects that compares an intervention with an FDA-regulated device against a control. This is not a small clinical trial to determine the feasibility of a device, or a clinical trial to test prototype devices where the primary outcome measure relates to feasibility and not to health outcomes.
☐	This is a pediatric postmarket surveillance of a device as required under section 522 of the Federal Food, Drug, and Cosmetic Act.
☐	This is a controlled clinical investigation, other than a phase I clinical investigation, of a drug subject to section 505 of the Federal Food, Drug, and Cosmetic Act or to section 351 of the Public Health Service Act.

To view a flowchart describing applicable clinical trials subject to FDA jurisdiction click here.

Section 3: Publishing the results

If yes to BOTH the study must be registered on Clinicaltrials.gov.

Yes	
☒	This study prospectively assigns people or a group of people to an intervention, with or without concurrent comparison or control groups, to study the cause-and-effect relationship between a health-related intervention *and* a health outcome.
☒	The PI has access to and control over all the data from the clinical trial and has the right to publish the results of the trial and plans to publish the results in a journal that follows the ICMJE recommendations.

This requirement includes studies of behavioral interventions.

Section 4: Registration on Clinicaltrials.gov is not required

Yes	

☐	I have read sections 1-3 above and registration on clinicaltrials.gov is not required for this research.

23) Criteria for 10 Year Approval

If yes to all items below this research may qualify for a 10-year approval period.

Yes	
☐	This research involves no more than minimal risk.
☐	This research does not receive any federal or state government funding or funding from a private funder who requires annual review per contract.
☐	This research is not subject to FDA jurisdiction.
☒	This research does not include prisoners as participants.
☒	This research is not subject to SCRO oversight.
☐	This research is not subject to oversight by the Research Advisory Panel of California (RAP of C).
☐	This research does not involve identifiable information held by the State of California Department or Agency
☐	No personnel involved in the design, conduct, or reporting of this research have a new unreported related financial interest (RFI) in this study.