FRUIT Study Protocol

Effect of Whole Fruit on Glycemic Control in Adults with Type 2 Diabetes

NCT03758742

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1. Study Design and Rationale

1.1 Objectives and Novelty

We will determine the effects of a whole-fruit-rich diet on glycemic control, liver fat, pancreatic fat, and cardiovascular disease risk factors in weight-stable adults with T2D. Our objectives are three-fold:

1. Determine the effects of a diet rich in whole fruit on glycemic control in people with T2D;
2. Determine whether T2D remission—or weaning off all diabetes medications and achieving non-diabetic glycemia—is possible without losing weight;
3. To determine how eating a diet high in fructose and simple sugars from whole-food sources affects liver fat.

Our clinical trial is novel for three reasons. We will conduct the first rigorously designed clinical trial to support (or refute) current American Diabetes Association (ADA) guidelines that patients with T2D should consume whole fruit. Second, to our knowledge, our study is the first interventional trial to test the effects of eating large doses of whole fruit as a food group. Third, our study is the first dietary trial to test whether patients with T2D can wean off all diabetes medications and achieve non-diabetic glucose levels *without losing any weight*. If this is true, it will be an important milestone achievement.

1.2 Hypotheses

We hypothesize that a whole-fruit-rich diet will improve glycemic control, decrease liver and pancreatic fat, lower blood pressure, and improve serum lipids. We also hypothesize that some participants will wean off their diabetes medications and achieve non-diabetic glycemia during

the 12-week intervention. We expect that improvements in glycemic control will be accompanied by restoration of first-phase insulin secretion and reductions in liver and pancreatic fat—similar to studies of VLCDs.

1.3 Primary and Secondary Outcomes

The primary outcome is glycemic control. Glycemic control is difficult to accurately assess whenever antihyperglycemic medications doses change, as changing doses can mask improvements in standard glycemic outcomes. Therefore, we define an individual has having an improvement in glycemic control if s/he needs a lower dose of antihyperglycemic medications (if medication doses change) or has lower mean glucose levels (if medication doses do not change). We will use the following hierarchy of glycemic outcomes:

1. Achieving non-diabetic glycemia without antihyperglycemic medications (assessed as a binary outcome and analogous to T2D remission without the 3-month requirement);
2. Total dosage of antihyperglycemic medications, as measured by the medication effect score (MES);
3. Mean glucose during a three-hour OGTT; and
4. Mean 24-hour glucose as measured by continuous glucose monitoring (CGM), adjusted for any changes in medication doses via the MES (see Section 4.5).

We decided to use mean 24-hour glucose as measured by CGM in lieu of HbA1c because CGM more accurately assesses the present glycemic status over the previous week, whereas HbA1c is a lagging indicator of glycemic control. The hierarchy implies that a positive or negative outcome in an upper level will always supersede any findings from lower levels in the hierarchy. In particular, if glucose-lowering medication dosages change, then assessments 1 and

2 will supersede assessments 3 and 4 since changes in glucose-lowering medications confound the interpretation of glycemic data. If there are no changes in glucose-lowering medications, then we will use assessments 3 and 4 to determine whether there are changes in glycemic control. We will interpret the data from assessments 3 and 4 as follows. We will interpret assessment 3 (OGTT) to indicate whether the intervention improves or exacerbates glucose intolerance and underlying defects in glucose metabolism. If there is no change in assessments 1-3, we will interpret assessment 4 (CGM) to reflect only acute changes in the glycemic load and macronutrient composition of the diet. For instance, the intervention may increase mean 24-hour glucose levels in a participant who eats a low-carbohydrate diet at baseline, but this increase may simply reflect switching to a higher carbohydrate diet, not a worsening of glucose metabolism.[1] As another example, the intervention may decrease mean 24-hour glucose levels in a participant who eats a higher glycemic load diet at baseline, but this decrease may reflect switching to a lower glycemic load diet, not an improvement in glucose metabolism per se.

The secondary outcomes are liver fat, pancreatic fat, cardiovascular risk factors, and ancillary measures of glucose metabolism:

- Liver fat, as measured by magnetic resonance imaging and spectroscopy (MRI/S);
- Pancreatic fat, as measured by MRI/S;
- Insulin secretion, as measured by the oral minimal model;
- Insulin sensitivity, as measured by the oral minimal model;
- Mean insulin and C-peptide levels during the OGTT;
- Time-in-range metrics from CGM;
- Mean amplitude of glycemic excursions from CGM;
- HbA1c;

- Fasting glucose;
- Blood pressure;
- Heart rate; and
- Lipids (total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides).

Additional outcomes include body composition (subcutaneous abdominal fat, visceral fat, and waist circumference), preferences for and sensitivity to sweet tastes, diet satisfaction, food cravings, eating behaviors, mood, quality of life, and depression.

1.4 Study Design

The trial is a 12-week, single-arm, eucaloric, controlled-feeding trial. During weeks 1–4 (Ramp-Up Phase), adults with insulin-independent T2D progressively eat more fruit, starting at 10% of daily energy and incrementally increasing to 50% while remaining weight-stable. During weeks 5–12 (Main Phase), participants eat 50% of their energy requirements as whole fruit (~16.4 servings/day) and the remaining 50% as a Mediterranean-style diet. By design, the aggregate diet is a high-carbohydrate diet, rich in simple carbohydrates in whole-food form. Food is prepared for participants in a metabolic kitchen, and participants must eat the provided study food. At week 10, participants who achieve non-diabetic levels of glycemia—defined as mean 24-hour glucose <140 mg/dl (equivalent to HbA1c <6.5% or <)—are taken off their antihyperglycemic medications to see if they can maintain non-diabetic glycemia without the use of medication. All study outcomes are measured at week 0 (baseline), week 4 (end of Ramp-Up Phase), and week 12 (end of Main Phase).

1.5 Participants

We will enroll 16 adults recently diagnosed with insulin-independent T2D. We modeled our eligibility criteria on the landmark DiRECT study, which found that a VLCD intervention induces T2D remission in 46% of patients [3]. Like the DiRECT study, we are recruiting adults with insulin-independent T2D who were diagnosed with T2D within the past six years, as patients with diabetes durations longer than 6–8 years are less likely to achieve remission.[2] Table S1 in the appendix shows the eligibility criteria. To be eligible, applicants must have an HbA1c between 6.0–9.5%, be aged 20–70 years, and have a body mass index (BMI) ≤45.0 kg/m^2. Key exclusion criteria include an estimated glomerular filtration rate (eGFR) <45 ml/min per 1.732 m^2, a change in body weight >5 kg in the past six months, a change in a chronic medication dosage that may affect study endpoints within the past three months, a significant health condition that might compromise the participant's safety or data validity, or inability to follow the study protocol. All participants must provide written informed consent prior to enrolling in the study. Participants are provided with a stipend, food, and a high-speed blender as compensation.

1.6 Setting and Recruitment

The clinical trial is being conducted at the University of Alabama at Birmingham (UAB). The trial was approved (IRB-300001719) by the UAB Institutional Review Board and is registered on ClinicalTrials.gov (NCT03758742). This study is being conducted in accordance with the Declaration of Helsinki.

We are recruiting a convenience sample of participants from the greater Birmingham, Alabama metropolitan area. We recruit participants through flyers placed around the university's campus, ads on the university's clinical trials website, earned media spots on local TV and radio

shows, and by presenting the study to patients enrolled in diabetes education classes at UAB Hospital. We are also engaging Bump Digital Marketing (https://recruitment.bumpdigitalmarketing.com/) to run ads on social media platforms, particularly Facebook and Google.

1.7 Screening

Interested individuals are prescreened via an online questionnaire and/or by phone, and those who pass prescreening are invited to screen in person. At the first screening visit, applicants sign the consent form, are screened against the eligibility criteria, and have their blood drawn to run a metabolic panel and measure HbA1c. After the first screening visit, the study physician reviews each applicant's laboratory results and medical history. Applicants deemed medically eligible then complete a three-day food record before returning for a second screening visit. During the second screening visit, we conduct a 30-45 minute semi-structured interview called a Barriers Interview. During the Barriers Interview, we ensure that each participant understands the demands of the study, and we assess any psychological, behavioral, and logistical barriers that may impede study participation. Only applicants capable of meeting all study demands are enrolled.

1.8 Schedule of Assessments

Table S2 in the appendix shows the schedule of assessments. We measure all outcomes at week 0 (baseline), week 4 (end of the Ramp-Up Phase), and week 12 (end of the Main Phase). Most outcomes are assessed during a 4–5-hour testing visit, with the exception that CGM, stool collection (for exploratory analyses), and the abdominal MRI/S scans are conducted during the

week prior. Before each visit, participants fast for at least 10 hours. During each testing visit, we start by measuring anthropometrics, heart rate, and blood pressure. Thereafter, we collect a fasting blood draw and administer a three-hour OGTT. During the last hour of the OGTT, participants complete a series of questionnaires on food cravings, eating behaviors, mood, quality of life, and depression. Once the OGTT is complete, participants complete a Sweetness Taste Test. At week 12, we also conduct an exit interview.

1.9 Participant Safety

Participants wear a CGM throughout the 12-week controlled-feeding intervention, which allows us to remotely monitor their safety. The study team reviews the CGM data at least twice per week. Values below 70 mg/dl or above 300 mg/dl are grounds for considering medication changes; though, we will attempt not to adjust antihyperglycemic medications until week 10 whenever possible. The study physician (W.T.G.) makes all decisions on whether to adjust medications. In addition, we assess other the safety endpoints of blood pressure, heart rate, and adverse events once a week in our clinic. Every other week, we also draw blood to measure fasting lipids.

2. Dietary Intervention

2.1 Overview

The 12-week controlled-feeding intervention has two phases: (1) a four-week Ramp-Up Phase, during which participants progressively eat more whole fruit, and (2) an eight-week Main Phase, during which participants eat 50% of their energy requirements as whole fruit and the remaining

50% as a Mediterranean-style diet. During both phases, participants approximately maintain their weight and consume large amounts of whole fruit in three forms:

- frozen fruit smoothies (i.e., frozen whole fruit in blended form);
- fresh fruit in fruit salads; and
- dried fruit.

All study food is prepared in a metabolic kitchen. Participants are instructed to eat all of the provided food while being video-recorded to ensure compliance. Participants are also instructed not to change their current physical activity levels, sleep habits, medication use, and other health behaviors during the study.

2.2 Ramp-Up Phase: Weeks 1–4

We give participants a four-week ramp-up period to acclimate to the high fiber and low energy density of the diet. The ramp-up period allows participants to adapt to eating low-energy-density food and overcome any transient gastrointestinal or bowel symptoms. The ramp-up period also provides preliminary data on the effects of whole fruit alone on cardiometabolic health before the remainder of participants' diet becomes a Mediterranean diet. During the ramp-up period, participants gradually eat more whole fruit as follows:

- Days 1-2: 10% of calories in the form of a frozen fruit smoothie;
- Days 3-7: 20% of calories in the form of a frozen fruit smoothie;
- Week 2: 30% of calories, with 25% as a smoothie and 5% as dried fruit;
- Week 3: 40% of calories, with 25% as a smoothie, 10% as dried fruit, and 5% as a fresh-fruit salad;

- Week 4: 50% of calories, with 25% as a smoothie, 12.5% as dried fruit, and 12.5% as a fresh-fruit salad.

To remain weight stable, participants are instructed to reduce their habitual diet in proportion to the amount of fruit added.

2.3 Main Phase: Weeks 5–12

During weeks 5–12, participants consume a whole-fruit-rich diet that provides 50% of calories as whole fruit. The purpose of the main phase is to determine the effects of a whole fruit-rich diet while matching all aspects of the diet across participants, while also simultaneously maximizing the possibility that participants may be able wean off all their anti-hyperglycemic medications. The fruit composition of the diet is:

- Frozen fruit smoothie: 25% of calories;
- Fresh fruit salad: 12.5% of calories;
- Dried fruit: 12.5% of calories.

We deliberately include dried fruit and other fruits that do *not* have a low glycemic index, instead of only focusing on low-glycemic index fruits, to test fruits that are more representative of what people eat. Although most dried fruit has a moderate glycemic index, it does have a low glycemic load. During the Main Phase, the fruit smoothies also contain a small amount of other foods, such as a cup or two of leafy greens (green smoothies) and/or a couple tablespoons of flax or chia seeds. The remaining 50% of daily calories comes from Mediterranean-style diet. Participants must consume all the provided study food and only consume the provided food during the 12-week controlled-feeding intervention, except for two break meals per week. For the break meals, participants may substitute any meal of their choice for a non-fruit-containing

study meal. Participants are permitted to drink zero-calorie beverages and add zero-calorie spices without salt to meals. Participants can eat study meals in any order as long as they consume either (a) the smoothie or (b) both the fresh salad and dried fruit by noon. We implemented this rule to help participants overcome the challenge of eating a low-energy-density diet by requiring participants to spread out their fruit consumption across the day. Additionally, participants must eat meals in one sitting, except for the fruit smoothies and fresh-fruit salads, which they can eat in two sittings. Finally, participants check off each study food item they eat or do not eat on a provided checklist. We also instruct participants to record any non-study food or beverage they consume on the food checklist.

2.4 Menu Design

For the Main Phase of the trial, we designed a seven-day rotating menu that provides 50% of calories as whole fruit and the remaining 50% of calories as a Mediterranean-style diet (Figure S1 in the appendix). We define a Mediterranean diet as a plant-centered dietary pattern that emphasizes vegetables, whole grains, legumes, nuts/seeds, olive oil, and seafood and minimizes dairy, poultry, eggs, highly refined foods, and red meat. In our menus, we intentionally incorporated small amounts of dairy, bread, and poultry and omitted red meat and processed meat.

The aggregate diet is a high-carbohydrate diet, providing 62% carbohydrate, 26% fat, and 12% protein. The diet is rich in simple carbohydrates and fructose while also being very high in fiber and low in energy density (3.85 kilojoule/g [0.92 kcal/g]). A 10,460 kilojoule (2,500 kcal) diet provides 16.4 servings of fruit, 81 grams of fiber, 243 grams of total sugars, 77 g of fructose, 11 g of saturated fat, and 1,871 mg of sodium. It also provides 6.4 servings of vegetables, 2.6

servings of nuts and seeds, 1.5 servings of legumes, 1.0 servings of whole grains, 0.9 servings of meat, and 0.8 servings of dairy per day. In total, there are 22.8 servings of fruits and vegetables. We designed the menu to meet the estimated average requirements (EARs)—or adequate intakes (AIs) when there is no estimated average requirement—for the mean person aged 31–50 years for all nutrients except Vitamin D. Because it took tweaking to formulate a menu with adequate zinc for males, we do not recommend eating >50% fruit in conjunction with a low-animal product Mediterranean diet without very careful planning or adding more zinc-rich foods.

2.5 Food Preparation

Food Handler-Certified staff prepare all study food within a metabolic kitchen. Carbohydrate- and protein-rich foods are weighed to the nearest 0.4 grams, while fat-rich foods are weighed to the nearest 0.2 grams. This permits fluctuations of <8 kilojoules (2 kcal) per food item. All food is ready-to-eat, except for the frozen fruit smoothies, which participants prepare at home using a high-speed Vitamix blender (Vita-mix Corporation; Olmstead Township, OH). Participants pick up the study food twice per week.

2.6 Weight Stability

Participants are kept weight stable by being fed enough to maintain their baseline body weight. At baseline, daily energy requirements for each participant are estimated using the National Institute of Diabetes and Digestive and Kidney diseases (NIDDK) Body Weight Planner (BWP)[3], using an assumed activity factor of 1.5., and the results are rounded to the nearest 105 kilojoules (25 kcal). To ensure participants are weight stable, we weigh participants once a week, in tandem with their food pick-ups. Weight stability is defined as being within 2.3 kg (5 lbs) or

2% (if they weigh more than 113.4 kg [250 lbs]) of baseline body weight. Participants whose weight drifts toward the outer limits of the target range will have their energy intake adjusted, typically in 418-837 kJ (100-200 kcal) increments, to maintain weight stability.

2.7 Compliance

To ensure compliance during the controlled-feeding intervention, participants record themselves while preparing smoothies and eating all provided study food. We are using a previously validated method called remote video monitoring.[4] In brief, we provide participants with an encrypted smartphone and tripod and teach them how to record themselves. Participants record themselves while eating all the provided food and preparing the smoothies. Research staff review the recorded videos for compliance and estimate the portion sizes of any uneaten food. We quantify compliance in based on the percent of the provided food calories consumed. Compliance rates below 80% or dishonesty are grounds for withdrawal.

3. Assessments

We will perform all assessments as detailed below.

3.1 Achieving Non-Diabetic Glycemia Without Antihyperglycemic Medications

One of the three objectives of this trial is to determine whether T2D remission may be possible without losing weight. After this trial was launched, an ADA-convened consensus group published new guidelines in August 2021 for defining diabetes remission,[5] which conflicted with the original definition we had planned to use. Diabetes remission is now defined as achieving HbA1c <6.5%, or in some cases, a mean 24-hour mean glucose levels over a two-week period

(also called the Glucose Management Index [GMI]) <140 mg/dl, at least three months after ceasing all glucose-lowering medications. Given the new definition, we will assess whether participants can wean off all antihyperglycemic medications and achieve the same glycemic criteria, as a proxy for whether T2D remission may be attainable. (The only difference is that the new remission guidelines require a 3-month minimum period without antihyperglycemic medications.) In this clinical trial, non-diabetic glycemia will be defined as achieving mean 24-hour glucose values of <140 mg/dl (equivalent to HbA1c<6.5%) over the past week.

3.2 Cessation of Glucose-Lowering Medications

At the end of week 10, we will cease all antihyperglycemic medications in participants who achieve a mean 24-hour glucose of <140 mg/dl for at least one week during weeks 8–10. Participants whose baseline 24-hour glucose values are <140 mg/dl must additionally experience a 10% reduction in mean 24-hour glucose to ensure they have genuinely experienced a clinically meaningful improvement. Prior to week 10, participants' medications will not be adjusted unless there are safety concerns or associated adverse events.

3.3 Medication Effect Score

We will quantify the total dosage of antihyperglycemic medications using the MES, which accounts for the potencies and dosages of diabetes medications. The medication score for each individual medication is calculated as: (dose of drug / maximum drug dose) × a drug-specific adjustment factor,[6] where the adjustment factor is the expected HbA1c reduction at the maximum drug dose. We will use the adjustment factors determined by Alexopoulos et al.[7] The

individual scores will be summed to give the MES, which reflects the overall pharmacologic intensity of diabetes treatment.

3.4 OGTTs

At weeks 0, 4, and 12, participants undergo a three-hour OGTT. First, intravenous lines are inserted into participants' antecubital veins. Thereafter, participants consume a 75 g load of glucose. Blood is collected at -5 (fasting), 10, 20, 30, 60, 90, 120, 150, and 180 minutes after glucose ingestion to measure glucose, insulin, and C-peptide levels. The primary OGTT-derived outcome is three-hour mean glucose, which is equivalent to the glucose AUC divided by the duration of the OGTT. Additional outcomes include three-hour mean insulin levels, three-hour mean C-peptide levels, insulin sensitivity, and insulin secretion. Insulin sensitivity and secretion will be estimated using the oral minimal model, a differential equation model that has been validated against the gold-standard hyperinsulinemic-euglycemic clamp method.[8]

3.5 CGM

Participants wear a Dexcom G6® CGM (DexCom Inc.; San Diego, CA) at baseline and throughout the 12-week controlled-feeding intervention. Following the manufacturer's instructions, the sensor is inserted into the subcutaneous abdominal fat and replaced every 7-10 days or as necessary. We will use CGM data to measure mean 24-hour glucose levels, glycemic excursions, and time-in-range metrics. To clean the CGM data, all data collected on the calendar day that a new sensor was inserted will be excluded due to a high mean absolute relative difference (MARD) during the first several hours of sensor usage. Days missing >20% of data will also be excluded from the analysis. Data will be analyzed in one-week segments. Mean 24-

hour glucose levels will be adjusted by the MES using the conversion formula between estimated average glucose (eAG) and HbA1c: 24-hour mean glucose levels + 28.7 × MES. The resultant value, which we call the adjusted mean 24-hour glucose level, represents a person's mean 24-hour glucose levels in the absence of any antihyperglycemic medications. It enables us to fairly compare changes in mean 24-hour glucose when medication dosages change. Finally, glycemic excursions will be quantified using the mean amplitude of glycemic excursions (MAGE),[9] while time-in-range (TIR) will be quantified using the standard thresholds of 70 (low) and 180 (high) mg/dl, respectively.

3.6 Blood Analyte Assays

Blood analytes include HbA1c, glucose, insulin, C-peptide, and fasting levels of lipids (total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides). Glucose, total cholesterol, HDL cholesterol, and triglycerides will be measured in serum using a Stanbio Sirrus Clinical Chemistry analyzer (Stanbio Laboratory, L.P.; Boerne, TX). LDL cholesterol will be calculated using the Friedewald equation.[10] Insulin and C-peptide will be measured in serum via immunofluorescence using a Tosoh AIA-II Analyzer (Tosoh Corporation; South San Francisco, CA). HbA1c will be measured in whole blood on a Siemens DCA Vantage Analyzer (Siemens Healthcare Diagnostics; Tarrytown, NY).

3.7 Blood Pressure and Heart Rate

Systolic and diastolic blood pressure are being measured using an automated blood pressure monitor and following the 2019 American Heart Association and American College of Cardiology guidelines.[11] After a five-minute rest, blood pressure and heart rate are measured in

triplicate, with 1-2 minutes separating each measurement. We will use the average of the second and third measurements.

3.8 Body Weight, Height, and Waist Circumference

Metabolic weight is measured in duplicate to the nearest 0.1 kg on a Scale-Tronix 6702 scale (Hillrom Holdings, Inc.; Chicago, IL) and averaged. Height is measured once to the nearest 0.1 cm at each testing visit using a Heightronic Model 235D stadiometer (QuickMedical; Warwick, RI) and averaged across all measurements. Waist circumference is measured in duplicate to the nearest 0.1 cm at the midpoint between the superior aspect of the iliac crest and the inferior border of the rib cage.

3.9 Magnetic Resonance Imaging and Spectroscopy

At weeks 0, 4, and 12, we measure intrahepatic lipid (i.e., liver fat), pancreatic fat, subcutaneous abdominal adipose tissue (SAAT), and visceral adipose tissue (VAT) using magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS). Participants fast for at least four hours before each scan. All MRI scans are performed at 3 Tesla on a Siemens Prisma whole-body scanner (Siemens Healthcare; Erlangen, Germany). MRS and 3-point M Dixon images are acquired using Siemens LiverLab software. This state-of-the-art technology integrates the multi-point 3D Dixon imaging technique and ^{1}H MRS for an immediate, comprehensive evaluation of intrahepatic lipid content (%). We will also use the images collected via the 3-point M Dixon method to assess pancreatic lipids. We will assess pancreatic lipid content (%) by identifying three regions of interest free of artifacts and vessels and computing the 3-point M Dixon pancreatic fat fraction (FF) from the separated water and fat signals on a voxel-by-voxel basis.

The accuracy of the fat fraction metric from this multi-echo fat quantification method has been validated in several fat quantification studies using the following equation:

$$Fat\ Fraction_{3-point\ M\ Dixon} = Signal\ Intensity\ (SI)_{FAT}/(SI_{WATER} + SI_{FAT})\ \text{x}\ 100\% \quad (1)$$

We will use trans-axial abdominal images acquired using the LiverLab 3-point Dixon method to assess visceral adipose tissues and subcutaneous abdominal adipose tissue volume within a 10 cm region of the abdomen between the L1-L5 vertebrae. Images will be analyzed using Slice-O-Matic software. All analyses will be conducted blinded by A.M.G.

3.10 Sweetness Taste Test

At weeks 0, 4, and 12, we assess preferences for and sensitivity to sweet tastes using a modified version of the sweetness taste test described by Asao et al.[12] Participants undergo the test after completing each OGTT but before consuming any subsequent food or beverage. Eight logarithmically spaced quantities of sucrose ranging from 0.053 g to 6.846 g are dissolved in 10 ml distilled water to produce sucrose concentrations ranging from 0.015 M to 2.000 M. Approximately 5 ml of each solution is randomly assigned to a cup numbered 1 through 8, and this is repeated a second time in an identically sequenced set of eight cups. This randomized sequence for this study was determined before the study started and is identical for all participants and across all time points. Participants take a sip of the sucrose solution in each cup, swish it around in their mouths for five seconds, and expectorate the solution. Participants indicate (a) how much they liked the sucrose solution (the pleasantness) and (b) how sweet they thought it was (the intensity) on a 100-point visual analog scale. Participants then rinse their mouths with plain water before continuing this process for the remaining sucrose solutions, with

the interval between tastings being 30-60 seconds. After a three-minute break, the participants repeat the process with the second set of cups.

3.11 Questionnaires

We are assessing diet satisfaction, appetite, food cravings, eating behaviors, mood, quality of life, and depression using questionnaires. At weeks 0, 4, and 12, participants complete the following questionnaires:

- Profile and Mood States-Short Form (POMS-SF): assesses current mood states and total mood disturbances;[13]
- Patient Health Questionnaire-9 (PHQ-9): measures the severity of depression;[14]
- CDC's Health-Related Qualify of Life (HRQoL): measures quality of life;[15]
- Food Craving Inventory II (FCI-II): measures food cravings for various categories of food;[16]
- The fruit subsection and fruit juice questions of the Diet History Questionnaire (DHQ): assesses fruit consumption via a semi-quantitative food frequency questionnaire.[17] Using the same question format as in the DHQ, we also ask participants how often and how much they eat different subcategories of fruit (i.e., raw, frozen, dried, cooked, and juice);
- The fruit and vegetable questions of the 2007 Food Attitudes and Behaviors (FAB) survey: measures attitudes and behaviors related to fruit and vegetable consumption.[18] We are using most of question 2 (except for Q2A, Q2C, Q2H, Q2I, Q2K, Q2L, Q2P, Q2Q, Q2S, Q2T, Q2V, Q2AA, Q2CC, Q2EE) and questions 3, 4, 5, 6, 7, and 9 from

section 1; questions 24, 29, 30, 31, and 32 from section 4; all questions from section 6 except for Q43; and all questions in section 7;

- Fruit Liking Visual Analog Scales (VAS): a 100-mm VAS custom-designed for this study to assess how much participants like subcategories of fruit (i.e., fresh, frozen, fruit smoothies, dried, cooked, canned, and fruit juice);
- Diet Satisfaction Questionnaire: a series of VAS questions to assess both appetite and diet satisfaction. Specifically, we ask about overall diet satisfaction, food tastiness, energy levels, hunger, fullness, stomach fullness, desire to eat, capacity to eat, cravings, and feelings of deprivation. Appetite-related questions are retrospectively assessed over the past week.

3.12 Food Records

Participants complete three-day food records at baseline. Participants complete the records on two weekdays and one weekend day. The records will be analyzed using the Nutrient Data System for Research (NDS-R; University of Minnesota, Minneapolis, MN; version 2014). Dietary endpoints will include energy intake, macronutrient composition, % of calories (or servings/day) of food groups, and the Healthy Eating Index (HEI).[19] We will also test whether baseline HEI predicts changes in glycemic control.

We initially instructed participants to complete baseline food records during baseline CGM data collection and weeks 4 and 12. However, after examining CGM data from the first three completers, we discovered that participants drastically altered their eating patterns and ate substantially less and/or healthier on days that they filled out the food records. To eliminate these data validity issues moving forward, we subsequently amended the protocol to have participants

complete the baseline food records before the second screening visit (i.e., before baseline CGM data is collected) and to eliminate the records at weeks 4 and 12. For the first three completers, since some of their baseline CGM data is invalid, we will use the mean response from the remaining completers to extrapolate which days of CGM data are valid.

3.13 Exit Interview

At the end of the week 12 testing visit, we conduct an exit interview to assess participants' satisfaction with the intervention and their perceptions of fruit. Specifically, we ask participants about any challenges they faced in adjusting to eating a high-fruit diet, their beliefs about the healthfulness of fruit, and for their feedback on the menu, the amount of fruit provided, and the study overall.

3.14 Potential Exploratory Analyses

We are collecting whole blood at weeks 0, 4, and 12 for potential future analyses of whole-transcriptome gene expression and/or epigenetic changes in peripheral blood mononuclear cells (PBMCs), pending available funding. In brief, whole blood is collected and transferred into Leucosep tubes containing Lymphoprep reagent (StemCell Technologies Inc.; Cambridge, MA), following the manufacturer's instructions. The resultant solution is centrifuged at 1000-1200 g for 12 minutes with the brake on. The mononucleocyte layer is then harvested into a new Leucosep tube and centrifuged at 400 g for 6 minutes. Most of the supernatant is discarded, and the white pellet is resuspended in the remaining 1-2 mL of supernatant. After determining cell density, the solution is centrifuged again at 400 for six minutes, and the remaining supernatant is

discarded. The pellet is then resuspended using Invitrogen™ RNAlater™ (Life Technologies Corporation; Carlsbad, CA) and frozen at -80ºC for potential future analysis.

At weeks 0, 4, and 12, we are also collecting stool samples for potential future analyses following the protocol described by Kumar et al.[20] Briefly, participants wipe their anus with a pre-moistened wipe immediately following a bowel movement on two separate occasions no more than 1-5 days apart. Participants temporarily store the wipes in a zip-lock bag in their freezer. On the day of the testing visit, participants bring in both samples, which are stored at -80 °C until future analysis.

4. Data Management and Statistical Methods

4.1 Data Management

Study data and questionnaires are collected and managed using REDCap electronic data capture tools hosted at UAB.[21,22] REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies, providing (1) an intuitive interface for validated data capture, (2) audit trails for tracking data manipulation and export procedures, (3) automated export procedures for seamless data downloads to common statistical packages, and (4) procedures for data integration and interoperability with external sources. CGM data are uploaded and stored securely in the Dexcom Clarity® app (DexCom, Inc.; San Diego, CA).

4.2 Blinding

By necessity, participants and intervention staff are unblinded. Nursing staff unaffiliated with the study assess blood pressure and heart rate and perform the OGTTs blinded. Laboratory

technicians unaffiliated with the trial conduct the biochemical assays. MRI image analysis will be performed blinded. Data will be cleaned blinded by aggregating all time points together and/or by scrambling the order of timepoints.

4.3 Power Calculation

As this is a pilot and feasibility trial, the sample size was not based on a power calculation. We selected a sample size of 16 completers to provide sufficient data to inform the design of a future trial. Nonetheless, our study has 81% power to detect an 18.0% rate of attaining non-diabetic glycemia vs. a spontaneous remission rate of 0.7%.[23] Further, 16 completers provides 80% power to detect effect sizes of Cohen's $d \geq 0.78$, using a two-tailed paired t-test with $\alpha=0.05$. This corresponds to a large effect size, which we expect, as participants eat a very large amount of fruit. We acknowledge that we are not powered to detect medium or small effect sizes, which may also be clinically relevant.

4.4 Statistical Analyses

All statistical tests will be two-sided with a Type I error rate of $\alpha=0.05$. Because this is a controlled-feeding trial—designed to determine the cardiometabolic effects under conditions of nearly perfect adherence—we will analyze the data per protocol in completers only. The primary comparison will be between weeks 0 and 12, with secondary comparisons between weeks 0 and 4 and between weeks 4 and 12. Data may be Winsorized if there are outliers with an overwhelming degree of influence or values that are non-physiologic . We will test whether a fruit-rich diet can induce non-diabetic glycemia without hyperglycemia medications by testing whether our observed "remission" rate differs from the spontaneous T2D remission rate of

0.07% using an exact binomial test.[23] If there is one or more cases of non-diabetic glycemia, then the true rate is non-zero, and we will report a 95% confidence interval derived from bootstrapping. All other data will be analyzed as change scores. For all continuous data and Likert score data with at least four levels and sufficient variability, we will use linear mixed models with time as either a linear or categorical effect and with a random intercept for participants. For other data types, we will use distribution-appropriate generalized linear mixed models. Alternatively, if spline models are more parsimonious than treating time as a linear or categorical effect, we will use generalized-additive models with penalized adaptive regression splines instead. To analyze the data from CGM, we will perform a trajectory analysis to determine whether there are different "response phenotypes." Since it is conceivable that participants starting from very different baseline diets may have distinct trajectories early on, we may also consider a trajectory analysis of the data with special attention to the Ramp-Up phase and restrict the models to the Main Phase period after the trajectories converge. We will consider adjusting for covariates such as baseline values or mean 24-hour glucose levels only if statistically and clinically merited. For the inferential statistics on continuous variables, we will test for differences using least squares means evaluated at study timepoints, or alternatively, if time is most appropriately treated as a categorical variable, by the significance of the coefficient for each timepoint. We will analyze change in categorical variables using McNemar's test for dichotomous variables and Bowker's test of symmetry for variables with more categories. Finally, to assess inter-individual responses in the vein of precision nutrition, we will also report the percentages of participants who experience an improvement, no change, and a worsening in glycemic control.

5. Protocol Amendments

We made the following protocol amendments since recruitment began. First, the original menu designed for this trial was too challenging to consume due to its low energy density (3.14 kJ/g [0.75 kcal/g]). We revised the menu to be more energy dense (3.85 kJ/g [0.92 kcal/g]), and the new menu is described in this manuscript. Second, after the ADA released new guidelines defining T2D remission in August 2021, we immediately revised our definition of T2D remission to be consistent with the new guidelines. Third, to increase enrollment, we extended the age range from 20-65 years to 20-70 years and removed the lower limit on body mass index (BMI). We also added a criterion to exclude those unwilling to follow university mandates during the COVID-19 pandemic. Fourth, after discovering that participants ate healthier and/or substantially less food when they were filling out food records, we eliminated the food records during weeks 4 and 12 and move the baseline food record to be before CGM data collection started. Finally, as we were preparing this manuscript, we added a new statistician to our team (J.S.R.) and revised the statistical analysis plan to fill in any gaps and be more detailed. At the time we made the last two decisions, C.J.H., W.T.G., and C.M.P. had viewed safety data, medication data, and HbA1c data from the first three completers, and C.J.H. had additionally viewed blood pressure, heart rate, and lipid data.

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Appendix

Table S1. Eligibility Criteria.

Inclusion Criteria

- Aged 20–70 years
- BMI ≤45.0 kg/m^2
- First diagnosed with T2D within the past six years
- HbA1c between 6.0–9.5%

Exclusion Criteria

- On insulin
- Diagnosis of diabetes before age 18
- eGFR <45 ml/min per 1.732 m^2
- Heart attack in the past six months or severe or unstable heart failure
- Significant gastrointestinal disease, major gastrointestinal surgery, or gallstones
- Significant cardiovascular, renal, cardiac, liver, lung, adrenal, or nervous system disease that might compromise the participant's safety or data validity
- Evidence of cancer (other than non-melanoma skin cancer) within the last five years
- Clinically significant laboratory abnormality
- Change in the dosage of a chronic medication that may affect study endpoints within the past three months
- On weight loss medication
- Lost or gained >5 kg of weight in the past six months
- Pregnant, planning to become pregnant in the next 12 months, or breastfeeding
- Major psychiatric condition that would affect the ability to participate in the study
- Not able to eat the provided study meals (e.g., food allergies)
- Behavioral factors or circumstances that may impede adhering to the dietary intervention
- Not able to do the abdominal MRI scan (e.g., claustrophobia, implanted metal objects, body diameter >60 cm)
- Not willing to wear a mask and/or comply with other COVID-19 precautions

Figure S1. Study Menu. Shown below is the 7-day menu during the main phase of the study (weeks 5-12).

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Breakfast						
Mixed Berry Smoothie	Raspberry Smoothie	Peach Smoothie	Strawberry Smoothie	Cherry Smoothie	Mango Smoothie	Blueberry Smoothie
Lunch						
Tomato-Garlic Lentil Bowl Walnuts Dried Mango	Corn, Edamame, and Sweet Pepper Salad Brazil Nuts Dried Peaches	Farro and Roasted Vegetables with Mozzarella Almonds Dried Apricots	Chickpeas and Broccoli with Pesto Parmesan Dried Figs	Sweet Potato and Kidney Bean Bowl Pumpkin Seeds Dried Pineapple	Oatmeal Soy Milk Pecans Dates	Vegetable and Hummus Sandwich on Ezekiel Bread Almonds Dried Figs
Snack (Fruit Salad)						
Clementines, Honeydew, Red Grapes	Strawberries, Blueberries, Green Grapes	Blueberries, Peaches/Mango, Pineapple	Cantaloupe, Strawberries, Raspberries	Clementines, Honeydew, Red Grapes	Strawberries, Blueberries, Green Grapes	Cantaloupe, Strawberries, Raspberries
Dinner						
Lemon Pepper Chicken Seasoned Carrots	Black Bean and Quinoa Stuffed Bell Pepper with Parmesan Soy Milk	Mediterranean Chicken Lettuce Wraps Avocado Tzatziki Sauce	Catfish Almondine Mixed Vegetables Brown Rice	Balsamic Chicken Breast Green Peas Roasted Potatoes Soy Milk	Chickpea Pasta with Marinara and Vegetables	Garlic Herb Salmon Broccoli Barley

Table S2. Study Assessments.

Phase	BL	Ramp-Up				Main Phase							
Month #	0	1				2				3			
Assessments Week	0	1	2	3	4	5	6	7	8	9	10	11	12
Medication	X	X	X	X	X	X	X	X	X	X	X	X	X
OGTT	X				X								X
CGM	X	X	X	X	X	X	X	X	X	X	X	X	X
Fasting Blood Draw	X		X		X		X		X		X		X
HbA1c	X				X								X
Glucose	X				X								X
Insulin, C-Peptide	X				X								X
Lipids	X		X		X		X		X		X		X
BP, Heart Rate	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight	X	X	X	X	X	X	X	X	X	X	X	X	X
Abdominal MRI/S	X				X								X
Waist Circumference	X				X								X
Stool Collection	X				X								X
Sweetness Taste Test	X				X								X
Questionnaires	X				X								X
Food Record	X												
Medication Cessation											X		
Exit Interview													X

BL, baseline; OGTT, oral glucose tolerance test; CGM, continuous glucose monitoring; MRI/S, magnetic resonance imaging/spectroscopy; BP, blood pressure; Medication Cessation, cessation of glucose-lowering pharmacotherapy in participants who attain non-diabetic glycemia by the end of week 10.