Gut Microbiota Dependent and Independent Impacts of Dietary Pulses on Pre- and Postprandial Metabolism and Inflammation in Overweight/Obese Humans

NCT04283448

Study Protocol and Statistical Analysis Plan

Study Document Date : 10/22/2024

Background and Objectives

Pulse crops have unrealized potential to improve health and reduce disease burden for type 2 diabetes (T2D), cardiovascular diseases (CVD), and others. There is a 2-4-fold risk of CVD in individuals with T2D, and the CDC reported that 9% have diabetes (vast majority are T2D) and 34% of US adults have prediabetes. Postprandial TG predict risk of T2D and CVD as well or better than static biomarkers such as fasting glucose or lipids and are causally linked to low-grade inflammation, which plays a direct role in the progression of T2D, CVD, and other diseases. Thus, postprandial TG and inflammation are high impact targets for disease prevention and treatment. Dietary pulses have established potential to lower disease risk through these mechanisms, but it is likely that interindividual differences in the gut microbiome influence this potential.

Diet and gut microbiota interactions are an emerging area of study. Differences in the gut microbiome may limit or enhance health benefits of pulse consumption, e.g. lowering of postprandial TG and inflammation responses. There is large inter-individual variability in which of >1,000 potential gut microbial species are present, relative abundance of each, and overall function in each individual human host. Makeup of the gut microbiome influences short chain fatty acid production from prebiotic fibers and lipid absorption, which in turn exert lipid lowering effects. Our research group has identified several bacterial groups in the gastrointestinal tract that differ in individuals with lower versus high triglyceridemic responses to a high-fat meal challenge. Changes in the gut microbiota (the community of microorganisms in the human gastrointestinal tract) and lipid lowering have been measured in animal and human research studies for lentils and black beans, but the influence of the gut microbiota changes on the lipid changes has not been determined.

Our overall goal is to determine gut microbiome dependent and independent impacts of pulse consumption on metabolic resilience and metabolic risk profiles for type 2 diabetes (T2D) and cardiovascular disease (CVD) risk. Specifically, pulse crop consumption (i.e. Peas, Dry Beans, Lentils & Chickpeas) has unrealized potential to fundamentally alter how the body responds to disease promoting metabolic stresses of postprandial triglyceride and inflammation responses. Our specific objectives are to:

1. Determine the impact of green lentil consumption on postprandial triglyceride (TG) and inflammation responses to a high-fat meal challenge.
2. Determine the extent to which the gut microbiome and changes in the gut microbiome induced pulse consumption influence health impacts
3. Measure metabolites in the blood and generate metabolomic profiles to elucidate underlying mechanisms linking pulse consumption to improved health.

To achieve these objectives, we will determine the effects of green lentils on high impact risk factors of large postprandial triglyceride excursions and inflammation, composition of and changes in the gut microbiomes, and both gut and serum metabolomes in overweight and obese individuals with elevated risk. We will introduce a 12-week intervention which will consist of

consumption of 3 cups (experimental) or 0 cups (control) of pulses per week across 7 pre-made mid-day meals (matched for macronutrient content except fiber) provided to experimental and control groups.

Study Protocol

Visits 1-4 Time Commitment: Visit 1 and 2 are expected to last less than 60 minutes. Visits 3 and 4 are both expected to last 6 hours each. Total time spent in the laboratory for these visits is approximately 14 hours.

Informed Consent: After confirming eligibility with phone interview, a copy of the informed consent document will be mailed or emailed to participants so they can read it prior to their first visit. Before the first visit, an investigator will telephone participants to discuss the procedures and risks of participation in the study and to answer questions relating to the consent document.

Visit 1:

The first 20-30 minutes of the initial laboratory visit is devoted to walking the participant through the procedures and risks of the study, as well as answering any questions they might have prior to signing the informed consent document. Participants will then be asked to complete a series of questionnaires including: a 1-page health screening (AHA/ACSM Health/Fitness Facility Preparticipation Screening Questionnaire), 1-page Physical Activity Questionnaire, and a 1-page Demographic Questionnaire (Race and Ethnicity Questionnaire). The participant will also complete a body composition analysis as described below.

Measurement of muscle and fat tissue using bioelectrical impedance analysis: This test simply involves standing on a scale with your feet and hands on sensors for a few seconds. A very low electrical current that you cannot feel and that is not dangerous is transmitted and received across the sensors. The technology is commonly used in a variety of settings such as gyms and health screenings, but the instrument that we will use is more sophisticated in being able to estimate muscle and fat tissue in different regions of your body, including the abdominal cavity that is particularly important for predicting risk of diabetes.

Visit 2:

During Visit 1, our team will work with participants to determine an appropriate meal (breakfast or lunch) to eat that contains approximately 50 g of fat and to schedule a visit (Visit 2) approximately 3-4 hours after consuming this meal. Participants will come to the lab and have blood drawn according to standard procedures, where briefly blood is collected via venous puncture from a forearm vein.

Random Assignment: Participants will be randomized into Pulse or Control groups after Visit 2 if their non-fasting serum TG concentration 3-4 hours after a meal of the participant's choosing is greater than 175 mg/di. Individuals with non-fasting TG levels less than 175 mg/di will not complete the 12 week dietary intervention and will receive the benefits that were a part of their conducted visits in addition to monetary payments, prorated to the their total time spent in the study.

Dietary intervention: Using methods we have established for an ongoing randomized clinical trial with 8 weeks of lentil supplementation, we will prepare 7 mid-day meals per participant per week to deliver a dose of 0 or 3 cups of lentils per week for the experimental and control groups. Meals are matched for macronutrient content (except for fiber), and ground turkey replaces lentils in the control meals. Participants will be instructed to consume food provided for their midday meal, and then to proactively reduce portion sizes and to not eat beyond fullness at the evening meal. This strategy exploits the satiety effect of pulses at the midday meal and the 'second meal effect' in which volitional consumption is reduced at the next meal, the evening meal.

A brief 4 pm online survey addressing satiety and a brief 8 PM online survey addressing gastrointestinal issues will be delivered once a week during the 12-week intervention to participants through personalized e-mails or texts. Each participant will be surveyed once weekly to determine whether they consumed the experimental meal that day, their perceptions of hunger, fullness, satiety, and satisfaction with that day's meal (at 4:00 pm), and their gastrointestinal comfort (level of bloating, flatulence, cramping, and comfort) throughout the day (at 8:00 pm). This methodology has been successfully implemented in our ongoing study to demonstrate that lentil meals are equally pleasing, produce greater satiety, and are well-tolerated.

Vist 3 and 4:

Blood collection and high fat meal. Standard procedures for collection of blood from a forearm vein will be used to collect blood. In brief, participants will report to the laboratory after an overnight fast (10-12 hours), an indwelling venous catheter will be placed in an antecubital vein by an experienced physician, nurse, or phlebotomy-trained graduate student. Anne Tikkanen, RN, Sarah Bronsky, MD, or Audrey Higley, RN, will be present and/or on-call for all catheter placements. Blood samples will be collected before, and 1, 2, 3, 4, and 5 hours following ingestion of the high-fat meal. The high fat meal is 58.3 grams of salted butter (for 50 g of dietary fat) spread over three pieces of toast. High-fat meal challenges with 40-100 g of dietary fat are an established laboratory test to measure both postprandial triglyceridemic and inflammation responses. We have used a 50 g dose of fat delivered in the form of butter on toast on > 50 individuals because this particular dose is effective at discriminating between low versus high TG and inflammation responders. During this time, participants will not be allowed to leave the laboratory area. There will be down time in between blood draws and participants will be encouraged to bring entertainment or work with them to the laboratory, of which we will assist with connecting them to the campus WiFi network. They will be allowed to consume water, but not allowed to consume additional food or drink outside of the high fat meal. From collected blood samples, we will measure inflammatory cytokines, lipids, insulin, and metabolites (small molecules relating to the biochemical process in your body).

Questionnaires. Participants will be asked to complete a 24-hour dietary recall where they outline the types and quantities of food and beverage consumed in the past 24 hours, and a food frequency questionnaire. Completion of the food frequency questionnaire takes approximately 1-2 hours and details the participants' usual diet. Participants can complete these questionnaires

during the course of their visit. An end of study questionnaire will be completed at the end of Visit 4 to elicit feedback from the participant regarding their experience as a participant.

Self-Collected Stool Sample: A stool sample will be used to analyze the types of microbes present in the gut. The analysis involves measurement of the genetic material of the bacteria. This does not give us any information about the individual's genetic material, only that of the bacteria in their gut. They will be given three self-collection kits that each come with printed step-by-step instructions to collect their stool sample. Two kits will be given at visit 2 for stool sample delivery at Visit 3. An additional kit will be given towards the end of the study in coordination with a meal pick-up for stool sample delivery at Visit 4.

Biospecimen Analysis

All blood and fecal specimens collected during Visits 3 and 4 are de-identified at the point of collection and remain de-identified for the duration of testing and analysis.

Stool samples are sent to the University of Michigan through Dr. Walk's lab for 16S analysis. De-identified, un-processed, microbial DNA results are electronically sent back to MSU Nutrition Lab for data processing and analysis. Serum samples for metabolomic analysis are sent to Dr. Bothner's lab where de-identified results are processed and analyzed. Results are electronically sent back to MSU Nutrition Lab for additional analysis. Remaining biomarker analyses including inflammation, lipid, and blood glucose levels are processed and analyzed in the MSU Nutrition Research Lab as de-identified specimens.

All biospecimen results remain de-identified through study publication.

Statistical Plan

Habitual dietary patterns, represented by DHQ-III HEI scores, were analyzed at base-line through general linear models to detect existing dietary differences between meal groups. Diet components expected to change with a lentil-based intervention including total fiber g, insoluble fiber g, and legumes (cups) as well as total energy intake (kcals) were also assessed for baseline differences. To determine if intervention meal consumption changed participant dietary intake, linear mixed effects models were used to assess dietary components and HEI scores with an interaction predictor between meal and time (pre- or post- intervention) and a random effect for subject. Post-hoc multiple pairwise comparisons were performed to detect significant differences by group using Tukey’s HSD.

Linear mixed effects models were used to assess the impact of a week and meal interaction on satiety measures. GI symptom severity was analyzed through a linear mixed effects model with symptom type and a week and meal interaction as predictors. Subject was the random effect for models assessing satiety and GI symptom severity.

Descriptive summary statistics were performed for participant physical and biological characteristics with general linear models used to determine if participants on average differed between meal groups before the intervention began. General linear models were used to assess

the impact of the dietary intervention on the following categories of variables: anthropometric measures (weight, fat mass percent, visceral adipose, and waist circumference); fasting blood lipid measures (CHOL, HDL, LDL, TG); fasting glycemic measures (GLU, INS, HOMA-IR); and fasting inflammation markers (GM-CSF, IFN-γ, TNF-α, IL-1β, IL-6, IL-10, IL-17, IL-23). Fasting inflammation markers were normalized using r package bestNormalize [38] prior to statistical analysis. Dependent variables were calculated as the change in values from baseline (post-intervention – pre-intervention). Predictor variables were shared within each category of variables and are as follows: anthropometric measures –meal group; blood lipid measures –meal group, visceral adipose tissue; glycemic measures –meal group, visceral adipose tissue, change in BMI, HbA1c; inflammation measures –meal group, visceral adipose tissue. Predictor variables for glycemic measures were chosen based on previous findings by Zeevi and colleagues [39].

Analysis of postprandial metabolic (TG, GLU, INS) and inflammatory (GM-CSF, IFN-γ, TNF-α, IL-1β, IL-6, IL-10, IL-17, and IL-23) measures were conducted by summarizing each makers measurements at timepoints 0 (fasting) and 1, 2, 3, 4, and 5 hours postprandially as net area under the curve (AUC) using the auctime [40] R package. Each dependent variable was then summarized as the change in AUC (post-intervention – pre-intervention). AUC values for inflammation markers were normalized using r package bestNormalize [38] prior to statistical analysis. General linear models were used to assess the impact of intervention meal group on each dependent variable. Predictor variables were identical to variables utilized in corresponding fasting models.