Cardiometabolic Benefits of Potatoes Mediated Along the Gut-Vessel Axis in Adults with Metabolic Syndrome

I. Objectives

The *objective of this application* is to establish evidence that incorporation of potatoes into a diet pattern based on the *Dietary Guidelines for Americans (DGA)* enhances cardiometabolic health in persons with metabolic syndrome (MetS) by improving microbiota- and host-mediated metabolic responses along the gut-vessel axis. Our *central hypothesis* is that the inclusion of potatoes, as part of a *DGA*-based dietary pattern, will potentiate gut barrier function and attenuate postprandial hyperglycemia (PPH)-mediated impairments in vascular endothelial function (VEF). To test this, we will complete the following objectives: 1) define changes in gut barrier function in association with improved gut microbiota composition, increased fecal short chain fatty acid (SCFA) production, and decreased serum endotoxin, 2) define changes in postprandial glycemic responses and endotoxemia, and 3) define changes in gut hormones that promote glycemic control and changes in markers of oxidative stress in relation to improvements in endothelial vascular function, all following 2-week potato consumption. Upon completing this study, we anticipate novel evidence demonstrating gut-level benefits of a potato-containing diet to lower cardiometabolic risk in MetS adults.

II. Procedures

A. Study Design

We will enroll equal numbers of male and female MetS adults (n = 30; 18-50 y) from the Columbus, OH area as we detailed[1] to complete a 2-arm, randomized controlled, cross-over trial. Participants will be randomized in 3-unit blocks to receive a *DGA*-based diet containing Russet Burbank potatoes (350 g/d; 260 kcal; 12.4 g resistant starch) or an energy-matched bagel that is nearly devoid of resistant starch. Our approach closely follows recommendations of the *DGA*[2] and entails high scientific rigor by providing *all* foods during each 14-d intervention. On day 0, 7, and 14 of each period, coinciding with when participants obtain prescribed foods, we will assess anthropometrics (weight, height, waist circumference) and blood pressure. At baseline (day 0) of each trial, fasting glucose, insulin, and endotoxin will be measured to verify stable health status. Following each 14-d intervention, participants will provide a fecal sample and undergo testing in the fasted state for metabolic endpoints, gut barrier function, and VEF. Then, they will receive a glucose challenge (75 g) to examine VEF in relation to PPH at 30 min intervals for 2 h. Gut permeability probes (sucralose, erythritol, lactulose, mannitol) will also be co-administered on day 14, and urine collected for 24-h. After completing a 2-wk washout period (i.e. to restore microbiota composition[3]), participants will receive the alternate treatment (i.e. *DGA* diet + potato or bagel) and repeat all procedures.

B. Study Subjects

Enrollment Criteria. MetS adults will be studied because of their impaired VEF in association with glucose intolerance.[4] We will enroll those who are unmanaged (i.e. no drug therapy) and they will be required to meet these established MetS criteria[5] to improve homogeneity of study outcomes: i) fasting glucose 100-126 mg/dL, ii) waist circumference >102 cm for men or >88 cm for women, and iii) fasting triglyceride >150 mg/dL. We anticipate that they may also fulfill other MetS criteria, specifically HDL-C (<40 mg/dL for men, <50 mg/dL for women) and elevated blood pressure[6] (>120/80 mmHg); these will be considered for co-variate analysis. Other ***inclusion criteria*** are: i) non-smoker, ii) non-dietary supplement user (>1-mo), iii) free of gastrointestinal disorders, CVD,

cancer, and iv) no recent use of antibiotics, or any medications affecting glycemia, lipidemia, or blood pressure. Participants having any of these ***exclusion criteria*** will not be enrolled: i) use of anti-inflammatory agents or probiotics, ii) vegetarian, gluten intolerant, carbohydrate-restricted diet, iii) alcohol intake >2 drinks/d, or iv) ≥7 h/wk of aerobic activity.

Power Calculation. Plasma glucose and FMD during the 2-h postprandial period ($AUC_{0\text{-}120\ min}$) and fasting endotoxin following 14-d controlled feeding have been selected as the primary outcome variables. These endpoints had no gender differences ($P>0.05$) in our prior studies. PPH was also inversely related ($P<0.001$) to FMD.[4,7] MetS adults had improved FMD following low-fat milk ingestion compared with rice milk ($AUC_{0\text{-}3\ h}$: 1150±145 vs 908±118, CV=56%) and attenuated PPH.[4] For this study, we predict that FMD_{AUC} will be 25% greater in DGA+Potato during the oral glucose challenge on day 14. Thus, 26 MetS subjects would be needed to detect a treatment effect with >80% power ($P<0.05$). We will therefore enroll 30 subjects to account for unexpected attrition and increase power. A similar power calculation was performed using endotoxin measured from MetS vs. healthy adults.[1] To detect a 25% predicted lowering of endotoxin, 22 MetS subjects would be needed. Based on our preliminary data, DGA+Potato could lower endotoxin by increasing vitamin C status in addition to alleviating gut dysbiosis in a resistant starch-dependent manner. This secondary hypothesis will be tested by measuring vitamin C on day 14 using our routine HPLC-ECD method.[1]

Statistical Analysis Plan

Most data in will be analyzed by linear mixed effects models (LMMs) with random effect for subjects (to account for repeated measures) and fixed effects for gender, time period, and their interactions. Multivariate regression analysis will define pairwise correlations between study variables in the presence or absence of potential study covariates. Statistical significance for all analyses will be set at $P<0.05$.

C. Detailed Study Procedures

Overview of Study Procedures. Potential participants who call the study center in an anonymous manner for more information as well as those identified through ResearchMatch will be given a brief description about the study and asked a few questions to determine their eligibility (*see Phone Script* attachment). If they meet the eligible criteria, they will be invited to the study center for a screening meeting. During the meeting, the Informed Consent (see *Informed Consent* attachment) will be explained and provided for them to review. The participant will then be given the opportunity to review the Informed Consent form. If he/she chooses to participate in the study, they will then be asked to provide written consent. Women choosing to participate will also be asked to complete the *Menstrual History Questionnaire*After receiving informed consent, the participants' height, weight, waist circumference, and blood pressure will be measured. Additionally, a small fasting blood sample will be collected for blood chemistry analysis. If they are not fasted at least 10 hours, they will be asked to come back in the fasted state at time of mutual convenience. These blood results in combination with anthropometric parameters will determine the participant's eligibility. Eligible participants who agree to proceed with the study will then complete two 2-week intervention periods in a randomized order, each followed by a 2-h postprandial trial. We estimate that completion of all study procedures will take ~6-12 weeks per participant. Each step of the study procedure will be discussed in detail below:

Screening Meeting. Potential participants who have met the initial criteria of the study (based on the telephone interview) will be invited to the study center at a mutually convenient time. During this time, the participant and a member of the research team will meet in a private, quiet conference room or office. The individual will be provided the informed consent form, and its contents will be

described to the potential participant. The participant will then can review it, and if they choose to participate in the study, they will be asked to provide written consent. Although the participant will be asked to sign the informed consent, the participant will be told that they will not be asked to participate if their body measurements, blood pressure, or plasma chemistries (see *Enrollment Criteria*) do not meet the study criteria. If the participant has provided consent, we will then measure the participant's height, weight, waist circumference, and blood pressure. Next, if the participant is fasted for at least 10 hours, we will ask if a trained individual can draw a small blood sample (10 mL; 1 tube) so that we may measure blood chemistries (glucose, HDL-cholesterol, triglyceride). All samples will be coded to maintain participant anonymity. If the participant's anthropometrics, blood pressure, or plasma chemistries do not meet the study criteria, they will be told that they do not meet the study criteria.

Potential participants who meet the criteria will be contacted within a few days after their screening meeting to provide them with their blood and body measurement results and inform them of their eligibility to participate in the study. Consistent with our CLIA exemption, blood results will be provided in a categorical manner (i.e. "normal", "marginally high", "high") rather than providing actual blood concentrations of lab values (see *Subjects Results Sheet*). Potential participants having any blood values outside of the "normal range" will be directed/encouraged to follow-up with their own physician. Those having body measurements, blood pressure, and blood values within acceptable limits (see Inclusion/Exclusion criteria), will be invited to participate in the study. Subjects will be included or excluded based on a best fit of the inclusion and exclusion criteria (an example of best fit would be if a potential subject says he/she exercises 8 h/wk, which is close to our exclusion criteria of 7 h/wk, they might still be included in the study if they meet all other inclusion and exclusion criteria more closely than other potential subjects). Participants will be read one phone script if they qualify and another phone script if they do not qualify (see *Participant Eligibility Phone Script* attachment). Potential participants who qualify for the study will be communicated a message as follows: "Congratulations! You have been selected for participation in our study based on your blood testing results. A study investigator will be telephoning you to invite you to the testing session. Would you like to know your blood chemistry results?" Subjects not selected for study will be told the following message: "You have not been chosen for our study, but thank you for your interest." This message will be followed by an explanation why they were not chosen, such as lab values outside of the range we are looking for: "Your blood testing data is…..we were looking for participants who had levels less than …..". "We can provide you a copy of your results if you would like…..how would you like us to provide them to you?". "You should also consider sharing these results with your physician."

If a participant is telephoned and is unavailable, a message will be left requesting a callback at a convenient time or that at a member of the study team will try calling again at a later time. No confidential or sensitive information will be shared with third parties or left on answering machines.

Intervention timeline. Each participant will complete a 2-arm (*DGA* + Potato; *DGA* + Bagel), randomized, cross-over study. For this study, each participant will visit the study center a total of 12 times (6 times per study arm, including 1) baseline measurements of anthropometrics, serum glucose, insulin, endotoxin, and pick-up of meals for days 1-3, 2) pick-up of meals for days 4-7 2) pick-up of meals for days 8-11 and measurement of anthropometrics, 3) pick-up of meals for days 11-14 and stool collection kit, 4) 2 h postprandial testing, and 5) return samples from 24-h urine collection. Interventions will be separated by two weeks (for women, interventions will be separated by 2, 6, 10 etc. weeks to ensure vascular testing will be performed at the same time of the menstrual cycle).

Baseline visit. On day 0 of each trial arm, participants will report in the fasted state (10-12 hours) to the study center for assessment of height, weight, waist circumference, blood pressure, serum glucose, serum insulin, and serum endotoxin. They will receive 3-4 days of *DGA* meals plus potatoes (350g/day) or bagels (1/day = 95g).

Dietary intervention. Participants will be provided all foods for the 14-d intervention period. After the baseline visit, participants will report back to the study center on days ~3, 7, and 10 to pick up their meals for the proceeding 3-4 days. On day 7, anthropometrics (height, weight, and waist circumference) will also be measured to ensure weight maintenance. To standardize resistant starch (~12 g/d/350 g potato) and promote dietary compliance, potatoes will be prepared in several ways that minimize resistant starch degradation[8] (e.g. Day 1 = whole baked, Day 2 = mashed, Day 3 = seasoned wedges, Day 4 = sliced, with soy milk and vegan cheese) without creating a food product that is contraindicative to a *DGA* diet (e.g. potato chips, deep-fat fried). The prepared potatoes will be refrigerated by the participants until ready for consumption, at which point they may be microwaved if desired. Participants will consume the potato daily, with a meal of their choice. Participants will be provided non-sodium seasoning (e.g. pepper blend) or may consume the potato with other foodstuffs (e.g. eggs at breakfast). Similarly, participants may choose to co-ingest the bagel with other provided foods.

Test trials. At the end of the intervention period (day 14), participants will report back to the laboratory in the fasted state (10-12 hours) for assessment of height, weight, waist circumference, blood pressure, serum glucose, serum insulin, and serum endotoxin. Participants will return a stool sample collected on day 13 of the intervention period at home. Participants will ingest a sugar solution containing 75 g glucose, which we and others have routinely shown to induce PPH, oxidative stress, and VED. This will be co-ingested with 1 g sucralose,1 g erythritol, 1 g mannitol, 5 g lactulose for assessment of gut permeability. Then, at 30 min intervals during the 2 h postprandial period, we will assess blood markers and brachial artery flow-mediated dilation. During each study visit, participants will have access to drinking water and use of the restroom as needed. Urine will be collected during the 2 h postprandial period in the laboratory, and participants will be instructed on urine collection for when they return home following their laboratory visit. Participants will return to the study center the following day to drop off their 24-h urine collection containers. To ensure that the order by which each participant completes each trial is randomized, a random sequence generator (http://www.random.org/sequences/) will be used to determine the order by which each participant will undergo the two trials.

Sugar Probe Test. On the morning of testing at day 14, participants will arrive at the study center after abstaining from food and only consuming water for 10-12 hours. A sugar probe test will be conducted as described.[9] Upon arrival, participants will be asked to empty their urinary bladder. Subsequently, they will then be asked to ingest a drink containing 1 g sucralose, 1 g erythritol, 1 g mannitol, and 5 g lactulose dissolved in a 75 g glucose solution (240 mL). Within 2-hours after ingesting the sugar test beverage, participants will be asked to consume an additional 500 mL of water. Additionally, participants will be provided with standardized sucrose-, erythritol-, lactulose-, and mannitol-free meals (e.g. English muffin, butter, scrambled eggs) during the 24-h period. All foods consumed during the 24-hour period will be free of artificial sweeteners. Urine will be collected from 0-5 and 6-24 hours in sterile containers. For each urinary collection period, participants will be provided 2-L urine collection containers and an insulated bag with ice packs to store collected urine until they return their samples the next day.

Sampling Handling. At each time point of the postprandial trial and once at baseline, a blood sample (20 mL; 1.4 tablespoons x 5 time points = 100 mL or 0.42 cups) will be collected into evacuated blood collection tubes. Collecting a total of 100 mL or 0.42 cups during the postprandial trial and once at baseline is necessary to ensure adequate amount of plasma for each time point to accurately analyze levels of glucose, insulin, endotoxin and gut hormones. During blood collection, participants may feel an initial pain when inserting the needle, bruising around the insertion area, lightheadedness, or fainting, which are common when donating blood. However, we do not foresee any additional significant risks for collecting this amount of blood over 2 hours, other than the risks stated previously.

Throughout the span of the study (~8-12 weeks in duration depending on participant/investigator availability) which consists of four weeks total of controlled feeding, a blood collection at wk 0 and 2 and then repeated following a ≥2 week washout period, and 1 screening day, we will be collecting a total of ~210 mL or ~0.88 cups) of blood. Urine will be collected in provided containers (VWR) containing 10% thymol to inhibit bacterial growth. We will then have the participants return their urine samples to the study center or coordinate with study personnel to meet at a public and mutual location to return their samples within 24-h after collection. Feces will be collected using a commercial commode specimen collection system (Fisher Scientific). Briefly, the collection kit consists of the necessary materials (e.g. gloves, waste bag) for participants to easily and hygienically collect their stool without contaminating the sample or themselves. Volumes of urine will be recorded and fecal mass and observations will be recorded based on the Bristol Stool Chart.[10]

During each blood collection, plasma will be obtained by centrifugation and then transferred to cryogenic storage tubes. Serum samples will be obtained by allowing the blood to clot, followed by centrifugation and transfer to cryogenic storage tubes. Tubes will be stored at -80° C until analysis can be completed. Analyses will include plasma glucose, insulin, triglyceride, total cholesterol, nitrite, nitrate, arginine, ADMA, SDMA, MDA, CCK, serum endotoxin. Urine will be stored at -80ºC until analysis can be completed. Analysis from urine will include sucralose and erythritol. Fecal samples will be stored at -80ºC until analysis can be completed. Feces analysis will include microbiota composition and fecal short chain fatty acids (butyrate, acetate, propionate). Remaining plasma, serum, urine, and fecal samples not used for these analyses will be archived for 5 years at -80 C in the event we decide to measure additional inflammatory, antioxidant, or microbiota related markers. Appropriate notation has been made in the informed consent to alert participants that we will be archiving specimens and that they have the right to refuse our use of these specimens for future analyses. Lastly, approval from OSU IRB will be sought via a protocol amendment prior to the analysis of any additional biomarkers not specified herein.

Privacy/Confidentiality. For all data and records that are a part of this study, a number (i.e. code) will be assigned to each participant and will only be available to research personnel. Any records containing the names of participants will be stored in a locked filing cabinet or on a password protected computer in the PI's laboratory or office. Research personnel under the supervision of the PI and the PI himself will be the only individuals that have access to this information. The names of participants will not be used for publication in any form. The records will be maintained until the data are published, up to a maximum of five years. All archived samples will be coded, but the key linking the code to each participant's identifiable information will have been destroyed. In addition, participants will be instructed that their participation in this study is voluntary and that they may withdraw at any time without prejudice. Data (biochemical values) obtained from this study will stored on a computer in the PI's laboratory. In addition, a backup of digital data will be stored on the

PI's computer in his office. Both computers are password protected and both doors are locked when work areas are not in use.

D. Internal Validity

For all data and records that are a part of this study, a number (i.e. code) will be assigned to each participant. This will minimize measurement bias when performing analysis on dietary records, and biochemical markers because all samples/records will be coded. The codes will only be broken once data analysis has been completed and verified by the PI.

F. Medical Safety Plan

All aspects of the clinical study will be conducted in Dr. Bruno's clinical lab located in in 262D Campbell Hall. Participants will be fasted for 10-12 hours prior to each study visit. We recognize that certain risks associated with fasting include: hypoglycemia, weakness, and fainting. This duration of fasting is consistent with guidelines set forth by the American Diabetes Association to minimize risk to the individual[62] when determining fasting blood glucose concentrations.

Risks related to hypoglycemia are anticipated to be low due to participants' already elevated fasting blood glucose (study entry criteria = 100-126 mg/dL). They will also be ingesting a glucose beverage containing 75 g of glucose immediately following the fasting period. Throughout each visit to the study center, participants will be closely monitored by Emily Shaw and Geoffrey Sasaki (Doctoral Students), both of whom are members of the research team. They will monitor the safety and well-being of participants for any signs and symptoms of hypoglycemia including: confusion, dizziness, irritability, weakness, headaches, and fainting. Emily Shaw is certified by the Red Cross in CPR and basic first aid. Additionally, Dr. Bruno (PI; 325 Campbell Hall) has an academic office in close proximity to the study center and has significant experience coordinating clinical research studies involving overnight fasting, and other dietary- and carbohydrate-challenges, thereby supporting the competency of our research team in managing potential adverse events relating to fasting glucose and glycemic responses.

Consistent with our prior studies of similar design, and to ensure participant safety, all procedures throughout each study visit will occur while positioned on a hospital bed in the prone position. In the event that a participant was to become weak, dizzy, or faint, they would already be ideally positioned to minimize risks associated with these symptoms. In the event that hypoglycemia-related symptoms occur, the study would be terminated to allow the participant to recover. We are prepared to provide pre-packaged beverages and snacks containing simple carbohydrates (e.g. Gatorade, fruit juice, apple sauce, crackers) that will allow for rapid restoration of blood glucose. These food items will be stored in a refrigerator, located in close proximity (262 Campbell Hall), that is dedicated for foods used in research studies. Participant status (e.g. attentiveness, skin color) will be monitored in our clinical laboratory to ensure recovery. The clinical area where participants undergo vascular assessment is also equipped with first-aid measures (e.g. smelling salt) and all study team members are trained to assist with basic first-aid application if needed.

Any adverse hypoglycemic response that might occur during this study will receive care commensurate to the symptoms. For example, if a participant were to faint, then smelling salts would be administered along with a carbohydrate-containing food upon regaining consciousness. The study PI would also be contacted.

Alternatively, should a more severe hypoglycemic response occur (e.g. contusion or laceration relating to fainting), the research team would immediately contact medical services (i.e. 911). For non-life threatening emergencies, any OSU students participating in these studies would be directed to the Wilce Student Health Center. For other emergencies or those participants who are not OSU students, individuals would be directed to the Wexner Medical Center either by transporting them directly or requesting ambulance service. Regardless of the complexity of the adverse events, the research team would monitor the participant in the interim, provide palliative care as appropriate, and follow-up after any medical care has been provided.

Meal Pattern A:	**Meal Pattern B:**	**Meal Pattern C:**	**Meal Pattern D:**
Breakfast			
Boiled eggs	Grapenuts cereal	Whole wheat waffles	Scrambled eggs
Blueberries	Soy milk	Peanut butter	Diced green bell pepper
Oat milk	Mandarin oranges	Tropical fruit cup	Diced onion
English muffin	English muffin	Fruit & nut bar	Whole wheat tortilla
Butter spread	Honey		Fruit & nut bar
Raisins	Butter spread		
Lunch:			
Turkey breast	Spinach	Spaghetti	Rotini pasta
Wheat bread	Sunflower oil	Chicken meatballs	Ground beef
Baby carrots	Whole wheat bread	Marinara sauce	Marinara sauce
Fruit & nut bar	Tuna		
Cheese slice	Mayonnaise		
	Diced pears		
Dinner:			
Grilled chicken	Taco shells	Turkey burger	Tilapia filets
Wheat bun	Shredded chicken	Wheat bun	Brown rice
Tomato sauce	Grapes	BBQ crisps	Spinach
Riced cauliflower		**Au gratin potatoes or bagel***	Sunflower oil

Broccoli	Grape tomatoes		Diced pineapple
Red apple	**Potato wedges or bagel***		**Mashed potatoes or bagel***
Sun Chips			
Baked potato or bagel*			
Snacks			
Rice cakes	Celery	Craisins	Granola
Peanut butter	Peanut butter	Cashews	Clementines
	Granola		Soy milk

III. Literature Cited

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