Date: 09/19/2019

Title: Potato Research for Enhancing Metabolic Outcomes

ClinicalTrials.gov Identifier: NCT04203238

Meat and Potato Diet for Enhancing Cardiometabolic Health in Adults

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Protocol Version Date: September 16, 2019

ABSTRACT

Background

The potato is a nutritious food that comprises approximately 30% of total vegetable intake in the United States (US). Consumption of pulses in the US is low but its contribution to health is frequently promoted. However, in the US diet, potatoes contribute as much dietary fiber, far more potassium, and a host of similar nutrients as pulses. When prepared to enhance its slowly digested starch content, potatoes produce a moderate glycemic response. In encouraging a shift towards plant-based foods and sustainable diets, the potato can partially replace meat in meat dishes to enhance the overall quality of the diet and reduce meat intake to recommended levels.

Design and Methods

Aim. The aim of the proposed trial is to compare the effect of healthy eating patterns containing potato and lean meat (PLM) in the ratio 40:60 + starchy staple (potato), and pulses and lean meat (LMP) in the ratio of 40:60 + starchy staple (bread, rice, pasta) for eight weeks on the glycemic response, lipid profile, and an inflammatory biomarker (high sensitivity C-reactive protein (hsCRP) in 36 overweight or obese, insulin resistant adults.

Hypothesis. We hypothesize that the PLM and LMP diets will improve blood glucose and lipid responses, and hsCRP compared to baseline, and will be non-inferior each other.

Study Design. We propose to conduct a randomized, controlled, parallel trial. Subjects who satisfy inclusion/exclusion criteria will report to the Pennington Biomedical Research Center (PBRC) in the morning after an overnight fast. Weight and vital signs will be measured and an intravenous catheter will be inserted in the arm to obtain baseline blood samples for measurement of glucose, insulin, lipids, LDL particle size, and high sensitivity C-reactive protein (hsCRP). Subjects will be randomly assigned to receive the PLM or LMP meals and complete a meal tolerance test (MTT) measuring glucose and insulin over three hours.

Following baseline testing, subjects will receive foods each week over eight weeks. All diets will be designed to maintain body weight. PLM and LMP diets will include wholegrains, fruits, vegetables, nuts, seeds, and five ounces of meat/day with the respective substitutions and starchy staples as the main entrées at lunch and dinner. Subjects will be required to eat only the study foods and will be counseled by a dietitian on adhering to the study protocol. Subjects will report to the PBRC weekly to collect the study foods and will receive nutrition counseling. Compliance will be evaluated by the dietitian through monitoring of all foods and containers that subjects will be required to return. Weight and vital signs will be measured weekly. At week nine, baseline testing will be repeated and compliance with the diet will be evaluated. Adverse events will be recorded during the study. It is our expectation that the approach used in this study will provide evidence that in the context of an overall healthy eating pattern, potatoes and pulses will reduce the glycemic and insulinemic responses, lipid profile, and hsCRP concentration but will be non-inferior to each other.

These results will be significant, in that they will potentially provide a cost-effective intervention that will substantially improve the health and quality of life for the growing number of overweight or obese individuals who have developed insulin resistance, and prevent or slow the progression to diabetes.

DESCRIPTION AND RATIONALE

Insulin Resistance

The impaired effect of insulin on regulation of blood glucose characterizes the condition known as insulin resistance.[1] Estimates from the Centers for Disease Control and Prevention suggest that more than one in three US adults (33.9%) have prediabetes,[2] an insulin resistant state that

places them at high risk for developing diabetes. At the insulin resistant stage, preventive treatments are likely to be most effective in individuals at high risk. In similar fashion, guidelines for prevention of other chronic diseases such as cardiovascular disease target individuals at risk for vascular events. Lifestyle interventions, recommended as the first line of treatment are clearly beneficial.[3] However, lack of compliance is common in the general population,[4-6] and is a major shortcoming of lifestyle interventions. Helping people make small sustainable changes that confer health benefits constitute the essential components of a long-term strategy to prevent chronic disease.

Animal- and Plant-based Dietary Patterns

Epidemiologic evidence supports an association between meat and animal protein intake and increased risk or incidence of type 2 diabetes.[7-13] In contrast, plant-based dietary patterns are associated with decreased risk or incidence of type 2 diabetes.[14,15] However, large-scale controlled feeding studies provide a deeper insight into the potential differential effects of plant and animal sources of protein than epidemiologic data. The Dietary Approaches to Stop Hypertension (DASH) diet and its variations emphasize fruits, vegetables, whole grains, nuts, legumes, and seeds.[16-18] The Beef in an Optimal Lean Diet (BOLD) was designed to test the effects of DASH diet variations that emphasize animal protein, primarily from lean beef. Although the DASH diet has a higher ratio of plant to animal protein than a typical American diet, it still provides approximately 1.7 times more animal protein than plant protein. The DASH diet and its variations demonstrated beneficial effects of increasing plant-based foods on cardiovascular risk factors, and vegetarian diets have been shown to improve glycemic control.[19-21] However, the BOLD study demonstrated that the DASH diet variation incorporating lean beef elicited similar cardiovascular benefits as the DASH diet. Thus, the evidence supports the inclusion of some animal sources of protein such as lean meats in a plant-based diet to reduce the risk of type 2 diabetes and cardiovascular disease.

Potatoes and Food Applications

The potato is an inexpensive food that comprises approximately 30% of total vegetable intake in the United States (US).[22] Although primarily defined by their starch content, potatoes are a rich source of nutrients and phytochemicals and its starch has unique functionality for food applications such as thickening, bulking, and stabilizing.[23] Moreover, cooling of gelatinized potatoes generates appreciable levels of slowly digested starch and resistant starch (RS) type 3. Therefore optimizing the preparation of starch-rich potatoes such as boiling, cooling, and adding vinegar or small amounts of fat substantially lowers the blood glucose response that potatoes elicit.[24-27] In addition, potatoes contain high molecular weight amylose, which forms insoluble amylose lipid complexes when heated with fatty acids (RS type 5).[23] Meat dishes such as hamburgers made with all lean meat are dry and particularly lacking in texture. The addition of potatoes (prepared to optimize the glycemic response) to a lean meat preparation not only reduces the meat and fat content of the diet but also adds moisture to replace the textural properties of fat in meat dishes.

Potatoes and Sustainability

In the US, per capita meat consumption is approximately three times the global average.[28,29] It is estimated that by 2050, the demand for livestock products will grow by 70%.[30] This scale of animal production is detrimental to the environment and a transition towards low-meat diets is critical if Western consumers are to shift towards a more sustainable diet.[31-33] Based on food disappearance data, per capita meat consumption in the US is approximately 10 ounces/day[34] far in excess of federal dietary guidelines (5-6 ounces meat/day).[35]

Meat is pleasurable, social, and traditional, which hinders a personal willingness to reduce meat in the diet.[36] The most promising pathway appears to be one that does not challenge existing meal formats and hierarchies in which meat plays a central role but makes an incremental

change towards reducing meat in the diet.[36,37] Meat substitutes need to be familiar and appeal to the senses or other attributes such as value.[37] Potato is one of the world's most popular foods providing important nutrients including protein of comparable biological value (90 - 100) to eggs (100) without too many calories.[38] Meat and potatoes are traditionally grouped together. Therefore, using potatoes to expand the concept of meat to encompass non-animal based substitutes without affecting the meal portion size is likely to appeal to consumers, and more research is needed to explore this possibility.

Potatoes v. Pulses

Potatoes have negligible fat and are low in energy density while providing critical nutrients especially dietary fiber and potassium designated by the 2015 Dietary Guidelines as being "shortfall nutrients,"[39] similar to the much acclaimed pulses. The protein content of pulses is much higher than potatoes but pulses are low in methionine, tryptophan, and cysteine, which makes the biological value of its protein (73) lower than that of potatoes (90-100). Similar to potatoes, pulses are inexpensive; however, unlike potatoes the acceptability of pulses is low due to perceptions of flatulence, bland taste, and lack of knowledge of cooking techniques.[40,41] Based on national intake estimates, adults in the US consume a mere 0.5 to 1 cup of pulses per week.[35] Nevertheless, US dietary guidelines and the evidence in the scientific literature unequivocally support increased intake of pulses.[35,42]

Preliminary Data

Acute studies have demonstrated that ingestion of fat with potato remarkably lowers the blood glucose response, without any change or slightly higher insulin concentrations compared to that observed after potato ingestion alone.[25,26] Similar results were observed with meals consisting of lentils and fat.[26] Ingestion of potato, meat, fat and additional fiber from a salad lowers the blood glucose and insulin response compared to mashed potatoes alone.[43] We have previously demonstrated in controlled feeding studies that reducing the glycemic load of the diet improves insulin sensitivity in obese adults measured using the hyperinsulinemic-euglycemic clamp method.[44,45] Additionally, epidemiologic evidence supports the addition of potatoes to meat dishes to reduce the risk of myocardial infarction.[46]

The effect of pulses on the glycemic response has far greater support than potatoes. A systematic review and meta-analysis of 41 randomized controlled clinical trials provided evidence that pulses as part of a low glycemic index (GI) diet improve glycemic control.[47] A study conducted in 121 subjects with type 2 diabetes showed that a low GI diet with legumes reduced glycated hemoglobin more than a diet high in insoluble fiber from whole wheat products.[48] Additionally, pulses contain resistant starch which improves insulin sensitivity and glucose tolerance.[49] Substituting red meat with pulses in a healthy eating plan, improves glycemic control.[50] Given the similarities between potatoes and pulses, we hypothesize that in the context of an overall healthy eating pattern, they are non-inferior to each other.

Summary

Approximately 11% of individuals with untreated prediabetes progress to diabetes every year. Reversion to normal blood glucose concentrations reduces the incidence of diabetes by 56%.[51] Healthy eating patterns such as the DASH and the Mediterranean Diet have shown that high intakes of fruits, vegetables, whole grains, legumes or pulses, and potatoes are associated with cardiometabolic health. In contrast, dietary patterns rich in meat and sugar-rich foods are associated with increased risk of mortality, type 2 diabetes, and coronary heart disease.[52] These findings suggest that it may be prudent to replace certain foods with fruits and vegetables rather than simply embrace plant-based diets. Small changes that bestow health benefits are likely to be sustainable in the long-term. Further research in this area is essential in order to find the most effective diet regimen for the treatment of insulin resistance in our overweight and obese population.

Hypothesis and Specific Aims

The objective of the present application is to develop a diet intervention to reverse insulin resistance in an overweight or obese population. For US adults, the white potato ranks among the top 15 foods providing key nutrients such as potassium and dietary fiber.[23] Pulses are a highly recommended food that contribute as much dietary fiber to the diet of US adults as potatoes.[53] Our central hypothesis is that in the context of an overall healthy eating pattern, potatoes and pulses will not differ in the glycemic and insulinemic responses, lipid profile, and hsCRP concentration they elicit. The rationale for the proposed study is that by developing a small changes sustainable approach to reducing meat intake and reducing the glycemic load of the diet, we can slow and ultimately prevent the progression to diabetes in individuals with untreated insulin resistance. We plan to test our hypothesis and accomplish the objective of this application by pursuing the following specific aims:

Aim 1. Compare the effect of diets containing white potato/lean meat (PLM) and pulses/lean meat (LMP) main entrées for eight weeks on the glycemic response in 36 overweight or obese, insulin resistant adults. This aim requires a randomized controlled feeding trial of potato and lean meat in the ratio 40:60 + starchy staple (potato), and pulses and lean meat (40:60) + starchy staple (bread, rice, pasta). The primary outcome is the glycemic response to the diets.

Hypothesis. The mean glycemic response will improve compared to baseline in the PLM and LMP groups but the two diets will be non-inferior to each other.

Aim 2. Evaluate changes in insulin, total cholesterol, high density lipoprotein cholesterol (HDL) low density lipoprotein cholesterol (LDL), triglycerides, LDL particle size, and high sensitivity C-reactive protein (hsCRP) in response to eating the PLM or LMP diets for eight weeks.

Hypothesis. Blood lipid, insulin, and hsCRP levels will improve, and the LDL particle size will increase compared to baseline in the PLM and LMP groups, but the two diets will not differ from each other.

RESEARCH DESIGN AND METHODS

The experimental plan is presented in Figure 1.

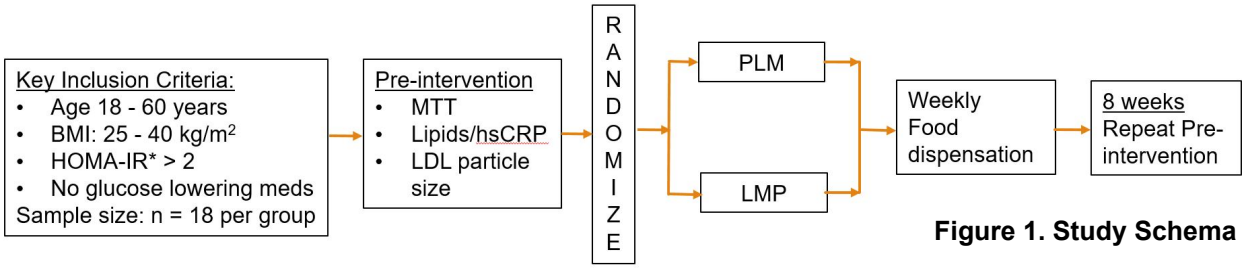


Figure 1. Study Schema

*Homeostatic model assessment of insulin resistance

Study Subjects

This study will enroll 36 overweight or obese male and female subjects. Subjects will be enrolled on the basis of the following eligibility criteria:

Inclusion Criteria

- Adult from 18 - 60 years of age.
- Body mass index (determined at the screening visit) between 25 and 40 kg/m^2.
- Waist circumference > 35 inches (if female) and 40 inches (if male)
- No evidence of diabetes (fasting blood sugar <126 mg/dL).

- HOMA-IR > 2.
- Willing to consume the study foods and refrain from eating other foods for eight weeks.

Subjects will not be eligible for participation if they meet one or more of the following exclusion criteria:

Exclusion Criteria

- Have type 1 or type 2 diabetes currently being treated by medication.
- Are being treated with medications that have a significant effect on insulin resistance, obesity, serum lipids, and metabolic rate, or medications that significantly increase body weight such as tricyclic antidepressants, second-generation antipsychotics, systemic glucocorticoids, and adrenergic blockers or stimulators.
- Current pregnancy or breastfeeding.
- Women of childbearing potential who are not using an effective method of birth control (i.e., barrier method, intrauterine and cervical devices, oral contraceptives, hormonal injections (Depo Provera®), condoms with spermicidal gel or foam, contraceptive patch (Ortho Evra), diaphragm, or abstinence), are not surgically sterilized (including tubal ligation and hysterectomy), or not at least two years postmenopausal. All women of childbearing potential will have a pregnancy test performed prior to starting the study treatment in each cohort. If a subject becomes pregnant during the study, they will be dropped from the study.
- Have clinically significant abnormal laboratory markers (as determined by the medical investigator).
- Have contraindications to participation in a diet intervention.
- Are unable to provide a baseline blood sample.
- Have any condition that impedes testing of the study hypothesis or makes it unsafe to consume the foods being tested in the study (determined by the investigative team).

Recruitment. Subjects will be recruited through the use of printed material, targeted solicitation through the Pennington Biomedical Research Center (PBRC) email listserv and social media. Participants will complete an online screening and will be contacted by a PBRC recruiter for a brief telephone interview to assess eligibility criteria, prior to being scheduled for a clinic visit. Subject eligibility criteria will be evaluated at a single screening visit. The screening visit will occur in the outpatient clinic, in the morning following confirmation of an overnight (at least 8 hours) fast. Subjects who provide informed consent will proceed with the tests and measurements of the screening visit. Subjects who satisfy the eligibility criteria will be enrolled in the study. The schedule of assessments is provided in Table 1.

Randomization and Blinding. Subjects will be randomly assigned to one of two groups (n = 18 per group). Dr. Robbie Beyl, the biostatistician from the PBRC Biostatistics group will be responsible for the randomization design, assignment of treatment groups, and statistical analyses plans including power and sample size. Except for the Director of the Metabolic Kitchen and the dietitian assigned to the study, all other researchers conducting the study will be blinded to the treatment group assignment of subjects.

CLINIC VISITS

Subjects will complete one screening visit and nine study visits.

Table 1. Schedule of Assessments				
Procedure	**Screening**	**Baseline**	**Visits 2 - 8**	**Visit 9**
Infomed Consent	x			
Height/Waist Circumference	x			
Weight	x	x	x	x
Vital Signs	x	x		x
Chemistry 15 Panel/CBC	x			x
Insulin	x			
Urine Pregnancy Test**	x			
Lipids/LDL Particle Size/hsCRP		x		x
Medical History Questionnaire	x			
Adverse Events		x	x	x
Medications	x			
Meal Tolerance Test		x		x
Visual Analog Scales (Satiety)		x		x
Study Foods Dispensing		x	x	
Compliance			x	x
Nutrition Counseling		x	x	
*Women of child-bearing potential				

Screening Visit (1½ hours)

Subjects will report to Pennington Biomedical in the morning following an overnight fast (except for water) that began no later than 8 hours prior to the study appointment. The screening visit includes explanation of the study purpose, procedures, and signing of the informed consent. If the participant agrees to participate by signing a consent form, the following procedures will be performed:

- Self-report of personal and family medical history.
- Measurement of height, metabolic weight, waist circumference, vital signs (blood pressure, pulse, and temperature).
- Recording of concomitant medication use.
- Urine specimen collection for urine human chorionic gonadotropin (HCG) (for pregnancy in women of child bearing potential).
- Blood collection for insulin, complete blood count (CBC), and Chemistry 15 panel.

Baseline (5 hours; fasting for at least 8 hours)

To start the study, eligible subjects will arrive at the clinic in the morning following an overnight fast (except for water) that began no later than 8 hours prior to the study appointment. The following procedures will be performed:

- Measurement of metabolic weight and vital signs (blood pressure, pulse, and temperature).
- Blood collection for measurement of lipids and hsCRP.
- Three-hour meal tolerance test (MTT) with glucose and insulin.
- Recording of adverse events.
- Recording of visual analog scale (VAS) ratings of satiety measures
- Nutrition counseling session with registered dietitian.
- Dispensation of foods for one week.

Visits 2 to 4 (1 hour to 1½ hours)

Subjects will report to the clinic and the following procedures will be performed.

- Measurement of weight
- Collection of all containers, and any uneaten food items and assessment of compliance.
- Nutrition counseling session with registered dietitian.
- Dispensation of foods for one week.
- Recording of adverse events.

Visit 5 (1 hour to 1½ hours; fasting for at least 8 hours)

Subjects will arrive at the clinic in the morning following an overnight fast (except for water) that began no later than 8 hours prior to the study appointment. The following procedures will be performed:

- Measurement of metabolic weight.
- Collection of all containers and any uneaten food items and assessment of compliance.
- Dispensation of foods for one week.
- Recording of adverse events.

Nutrition counseling session with registered dietitian will be via the telephone.

Visit 6 - 8 (1 hour to 1½ hours)

Subjects will report to the clinic and the following procedures will be performed.

- Measurement of weight
- Collection of all containers and any uneaten food items and assessment of compliance.
- Dispensation of foods for one week.
- Recording of adverse events.

Nutrition counseling session with registered dietitian will be via the telephone.

Visit 9 (4 hours; fasting for at least 8 hours)

Subjects will arrive at the clinic in the morning following an overnight fast (except for water) that began no later than 8 hours prior to the study appointment. The following procedures and measurements will be performed:

- Measurement of metabolic weight and vital signs (blood pressure, pulse, and temperature).
- Blood collection for measurement of CBC, Chemistry 15 panel, LDL particle size, and hsCRP.
- Three-hour MTT with glucose and insulin.
- Recording of VAS ratings of satiety measures
- Recording of adverse events.

STUDY PROCEDURES

Diets. The main entrée in the PLM and LMP arms will consist of a menu item in which 40% of the meat in the original recipe will be replaced with potatoes or pulses, respectively. The diets are designed to provide six ounces or less of meat/day which is consistent with the US dietary guidelines as well as the DASH diet, and constitutes approximately half the average daily meat intake in the US. Potatoes used in the meal preparation will be unpeeled, pre-cooked, refrigerated for 12 - 24 hours, and reheated or served cold.

These partially substituted meat dishes will constitute the main entrée at lunch and dinner and each dish will be accompanied by a starchy staple consisting of potato (PLM diet) and bread,

rice, or pasta (LMP diet). Additionally, the PLM and LMP diets, matched for their carbohydrate and dietary fiber content will consist of whole grains, fruits, vegetables (excluding additional potatoes and pulses), dairy products, seafood, nuts, and seeds as part of a healthy eating pattern. The diets will meet the Institute of Medicine Dietary Reference Intakes recommendations of the acceptable macronutrient distribution range for adults.[54] Caloric needs for weight maintenance will be determined using the Mifflin St. Jeor formula for estimation of basal metabolic rate[55-57] and multiplied by an activity factor based on subjects' activity level. All foods will be provided to the subjects during the eight week study period. Subjects will report to the PBRC each week to collect the foods according to their diet plan. The meals will comprise foods that have been prepared in advance and frozen, dry packaged foods, and frozen foods. Subjects will be instructed to return all containers and any uneaten food items. The Metabolic Kitchen staff will meet with the subject and evaluate compliance.

Nutrition Counseling. Subjects will be counseled on adhering to the diet plan by a PBRC dietitian. The PBRC dietitians specialized in counseling have extensive experience in advocating behavior change messages to subjects instructed to follow structured meal plans. The counseling sessions will be held once per week for 30-60 minutes as needed. For the first four weeks, the subjects will meet with the dietitian followed by four weekly sessions over the telephone. Counseling sessions will focus on identifying barriers to adhering to the diet plan, problem-solving, and how to be compliant on occasions when subjects may have to eat out.

Meal Tolerance Test (MTT). Following a fast which began no later than eight hours before the appointment, an intravenous line will be placed in the arm vein and blood will be drawn for baseline (time 0) glucose and insulin measurement, following standard procedures. Subjects will then receive the PLM or LMP meal according to the randomization assignment. Blood will be collected through the intravenous line at 30, 60, 90, 120, and 180 minutes following the meal for measurement of glucose and insulin.

Biochemical Assessments. Chemistry 15 panel, CBC, fasting insulin, lipid profile, and hsCRP measurements will be performed according to standard PBRC procedures for IV line/blood draws and the relevant measurement. The LDL particle size will be measured using nuclear magnetic resonance (NMR) spectroscopy at LabCorp (Morrisville, NC) in samples collected at and shipped from PBRC.

See Appendix 1 for a detailed listing of all blood draws and their required blood volumes.

Appetite Ratings. A questionnaire (VAS) will be administered at each of the time-points that blood is drawn during the meal tolerance test at baseline and at Visit 9. Subjects will be asked to rate their appetite by making a mark on the line at the point that best represents their feelings at the time that they complete the questionnaire (Appendix 2).

Adverse Events. An Adverse Event (AE) is defined as any untoward medical occurrence in a patient or clinical investigation subject, temporally associated with the subject's participation in research. We define AE as any unfavorable and unintended sign (including a clinically significant abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the subject's participation in research.

DATA SAFETY MONITORING PLAN

For each AE, the seriousness, intensity, and relationship to study product will be assessed, documented, and supported by an entry in the subject's medical records. During the study, each subject will be carefully monitored for any adverse events. After the initial AE report is completed, the investigator is required to follow each subject at subsequent visits/contacts. As currently practiced, it is planned that all AEs will be followed in clinic until: 1) resolution; 2) the condition stabilizes; 3) the event is otherwise explained; or 4) the subject is lost to follow-up.

Additionally, the CBC and Chemistry 15 panel measured at screening and Visit 9 will be reviewed by the medical investigator for clinically relevant changes.

Dr. Robbie Beyl will be responsible for all blinding procedures and for subject randomization. Except for the Director of the Metabolic Kitchen and the kitchen staff assigned to the study, randomization will be blinded to the staff performing the physical measures, metabolic testing, performance of assays, entering or calculating data related to the study and study investigators to assure minimal potential sources of bias. The randomization envelopes will be maintained in a secure location with access limited to authorized personnel. Blinding is critical to the integrity of this clinical trial. However, in the event of a medical emergency in an individual subject, in which knowledge of the random assignment is critical to the subject's management, the blind for that subject may be broken by the medical investigator of the trial. This will be done by a request to the Director of the Metabolic Kitchen to break the blind and report the treatment assignment to the medical investigator.

Before breaking the blind of an individual subject's treatment assignment, the investigator should have determined that the information is necessary, i.e., that it will alter the subjects' immediate management. In many cases, particularly when the emergency is clearly not investigational product-related, the problem may be properly managed without the need for breaking the blind.

In case of an emergency, the investigator may open the emergency randomization envelope to reveal the random assignment for that subject. If such breaking of the blind occurs, the investigator shall notify the IRB immediately. This information, including the reason for the blind being broken must be recorded in the participant chart and regulatory files.

STATISTICAL CONSIDERATIONS

Power and Sample Size. Power for this study is based on a parallel arm design with the potato/lean meat diet (PLM) and pulses/lean meat diet (LMP) groups. Equivalence in blood glucose will be the primary outcome. A similar crossover study by Hatonen *et al.*,[43] using 11 subjects, showed that adding meat, fat, and vegetables to a potato meal reduces the incremental area under the curve for glucose (iAUC) in a MTT. The iAUC was lower after the mixed meal (96 ± 12.5 mmol×min/L) than after potato alone (197 ± 28.2 mmol×min/L). Long interventions using crossover designs with washout periods are associated with high dropout rates.[58] In our experience, recruitment rates are slow and drop-out rates are high, especially, when there is no symptomatic improvement that is obvious to subjects participating in the trial. Therefore, we will use an eight-week parallel arm design.

We assume the standard deviation estimates from the study by Hatonen *et al.* to be similar to this proposed study since the PLM and LMP daily diets will also reduce the meat content in an overall healthy eating pattern which has been shown to lower the glycemic or insulinemic responses.[50,59,60] Further, our trial is a controlled feeding study in which all meals are provided and compliance is tightly monitored by registered dietitians. This study has 80% power to show equivalence between the two groups with at least 15 subjects per group given that the difference between the two groups is no larger than 40 mmol×min/L. Allowing for approximately 20% loss to follow-up, or 3 subjects per group, this study will enroll a total of 36 subjects.

Statistical Analysis. The secondary outcomes are changes in insulin, lipid concentrations, LDL particle size, and hsCRP. Mixed effect linear models will all be used to estimate changes across the eight weeks for primary and secondary outcomes. Normality of model residuals will be checked and transformation will be applied where necessary. An unstructured covariance matrix will model the within-subject effects. Missing data is assessed using a sensitivity analyses which will use several different sets of models under different assumptions. Covariates, like age, race, and gender will be determined using Akaike information criterion (AIC) as the model fit criteria.

Continuous covariate effect will be expressed as partial correlations while categorical effects will be expressed as differences between least square means.

DATA COLLECTION AND QUALITY ASSUARANCE

The only people who will know that these patients are research participants are members of the research team. No information about them, or provided by them during the research, will be disclosed to others without their written permission, except if it is necessary to protect their rights or welfare (for example, in case of injury or emergency care), or if it is required by law. When the results of the research are published or discussed in conferences, no information will be included that would reveal the identity of these patients. All data will be kept in secure files, and subjects will be identified by codes when the data gathered in this procedure is presented or published. Authorized representatives of the sponsor may need to review records of individual participants. As a result, they may see their name; but they are bound by rules of confidentiality not to reveal the patients' identity to others.

Privacy

The subjects will be interviewed in the privacy of an exam room and their records will be protected by a secure medical records area and a password-protected electronic database monitored by the Pennington Research Computing group. Subjects will be asked to sign a written consent after reading it, having it reviewed with them by the study staff, and having all their questions answered. The consent conversation will be conducted in the privacy of an exam room and the subject will be allowed to take the consent home to discuss their decision with their family or counselor, if desired. Study procedures will be conducted by trained staff in accordance with PBRC outpatient and inpatient clinic standards of practice and with subjects' informed consent. Confidential subject information including medical records and test results will be available only to persons authorized by PBRC. Information collected from subjects will be the minimum amount of data necessary to accomplish the research purposes.

Data and Specimen Management

Study participants will be assigned unique subject identification (ID) numbers. Study subject ID numbers will be used on all data collection instruments, to include questionnaires, data collection forms, biological specimen tubes, and computer records. A master list linking the participants' names and ID numbers will be kept in a password-protected computer file with access restricted to the PI, Co-PI and medical investigator. Biological samples that are moved off-site for analysis will not contain any personally identifiable information and will be labeled with only the unique subject ID numbers. Staff at these sites will not have access to the master list at any time.

Data collection forms will be kept secure, or password-protected if computerized, and under the control of the PI, Co-PI, and medical investigator. Only personnel assigned to the research study by the PI will have access to the data. Hard-copy data records will be stored for a minimum of 3 years.

The PBRC has a fully integrated, campus-wide, automated data management system. All data are entered into a central database using existing methodology that has been fully validated. All data are backed up daily, and the Research Computing Core at the PBRC oversees all data management. The research team has extensive experience using the procedures and methods required to conduct this study. Standard operating procedures in place throughout the units at PBRC will be utilized for repeatable, valid data collection and quality.

In accordance the standards of practice followed by the Clinical Chemistry core, blood samples will be stored frozen at PBRC until analysis can be completed. Specific blood samples will be

shipped to LabCorp (Morrisville, NC) for analysis of LDL particle size. Packaging and shipping of biological samples will be overseen by the Co-PI, shipped by the laboratory and will be completed in accordance with International Air Transport Association regulations to ensure that viable biological samples reach their intended destination.

WITHDRAWAL OF SUBJECTS

We will attempt to retain program participants once randomized for study completion through the end of study visit. It is our desire to analyze results on all participants who were enrolled in the study. In accordance with the declaration of Helsinki/Tokyo/Venice/Hong Kong, participants have the right to withdraw from the program at any time for any reason. The investigator also has the right to withdraw participants from the program treatments in the event of inter-current illness, adverse experience, treatment failure, protocol violation, or other reasons. Should a participant decide to withdraw from treatment, all efforts will be made to complete and report follow-up observations as thoroughly as possible.

RISKS/BENEFITS

The study involves the following risks:

- **IV Procedures/Blood Draws:** There is the possibility of discomfort, pain, and bruising at the vein on the arm where the needle is inserted. There may also be a small risk of bleeding and a very small risk of infection at the site of the blood draw. Sterile technique and trained personnel minimize these risks.

- **Study Foods**. The meals provided during the study are foods that are ordinarily consumed by people and are cooked and stored using methods common in food preparation. The research dietitians and staff of the research kitchen are experienced in developing, preparing, storing, and dispensing study-specific food products developed to meet the needs of the study. Additionally, subjects will be asked to inform staff about any food allergies or intolerances. A dietary questionnaire is routinely administered at the screening visit when the study involves a meal service. Research dietitians are responsible for managing the dietary component of specific study protocols. A continuous quality assurance program is followed to check food item weights, recipe procedures, packaging of meals, and food temperatures. Documentation is maintained for each study. All Metabolic Kitchen staff members receive training in food sanitation and in research diet preparation. Therefore, there is no risk associated with the meals provided by the Pennington Metabolic kitchen.

- **Nutrition Counseling.** There is minimal risk associated with behavioral counseling. Sessions are confidential and the counselor is trained to minimize any discomfort that is associated with the discussions during the sessions.

- **Medical History Questionnaire:** Subjects may refrain from answering questions if they do not wish to do so. There are no risks from completing the questionnaire.

- **Anthropometric Measurements and Vital Signs.** The PBRC outpatient and inpatient clinic staff are trained to perform these procedures in accordance with PBRC standards of practice. There are no risks involved.

- **Urine Test.** The staff of the phlebotomy unit collect the urine in accordance with standard operating practices. There are no risks involved.

In addition to the risks listed above, participants may experience a previously unknown risk or side effect.

Minimizing risks

Continuous monitoring by the Co-PI and/or the medical investigator of the study will minimize all potential risks and discomforts. Research participants will be immediately withdrawn from the study if participation in the study becomes an unreasonable risk to the subject.

Potential Benefits to Subjects

Based on the preliminary data, there is high likelihood that subjects may have improvements in the biochemical measures evaluated in the study. Subjects will gain knowledge of effective diet prescriptions for improvements in metabolic health. Since the changes are small, it is expected that subjects will be able to sustain these lifestyle changes over the long term.

PAYMENT FOR PARTICIPATION

At the completion of all study visits and procedures, participants will be paid $200 for the time spent in the clinic. Subjects will receive $75 for the Baseline visit and $125 for Visit 9.This compensation is in line with all the other studies conducted at the Pennington Biomedical Research Center.

EMERGENCY CARE AND COMPENSATION FOR RESEARCH-RELATED INJURY

No form of compensation for medical treatment is available from the Pennington Biomedical Research Center. In the event of injury or medical illness resulting from the research procedures the research volunteer (from any group) will be referred to their physician/surgeon or a treatment facility. The Pennington Biomedical Research Center is a research facility and provides medical treatment only as part of research protocols. Should a volunteer require medical treatments, community physicians and hospitals must provide them to him/her.

SHARING OF RESULTS WITH SUBJECTS

The screening lab results will be shared with the subjects on their first testing visit. At the end of the study, a manuscript will be prepared for submission to a peer-reviewed journal. A summary of the results will be posted on ClinicalTrials.gov which subjects may access.

RESOURCES AVAILABLE

The Outpatient and Inpatient research units and the Metabolic Kitchen are well equipped and staffed to carry out the requirements of this study and appropriate standards of practice are in place to ensure appropriate research procedures.

ECONOMIC BURDEN TO SUBJECTS

There are no costs for which the subjects will be responsible.

CONSENT PROCESS

Written informed consent will be obtained in the outpatient research clinic by the coordinators and one of the physicians will be available to answer questions if needed. A waiting period will be allowed, if desired by the participant. The coordinators and investigators will be available for questions throughout the study.

SIGNIFICANCE OF THE STUDY

Insulin resistance is a hallmark of obesity and lies along the continuum of type 2 diabetes which remarkably affects 9.4% of the US population.[2] The debilitating complications of diabetes include heart disease, stroke, amputations, end-stage renal disease, and blindness. Thus, research that focuses on preventing diabetes, and finding a treatment that is cost efficient is important. Potatoes are recognized as a starch-rich vegetable and denigrated for their role in contributing to obesity and diabetes, regardless of how they are prepared. However, potatoes

are a low-cost source of critical nutrients, high-quality protein, and phytochemicals which are largely ignored. Given their frequency of consumption, potatoes are clearly available, familiar, and taste good, which gives them an unassailable edge over other plant foods as a means of cutting the meat in a dish. Our randomized controlled feeding study will be the first trial to test this deceptively simple strategy to enhance the overall quality of the diet and provide health benefits. More importantly, these small changes are likely to be sustained over time Further, we expect that the potato will hold its own in a fair comparison with pulses, a food that is frequently promoted for its health benefits.

APPENDIX 1

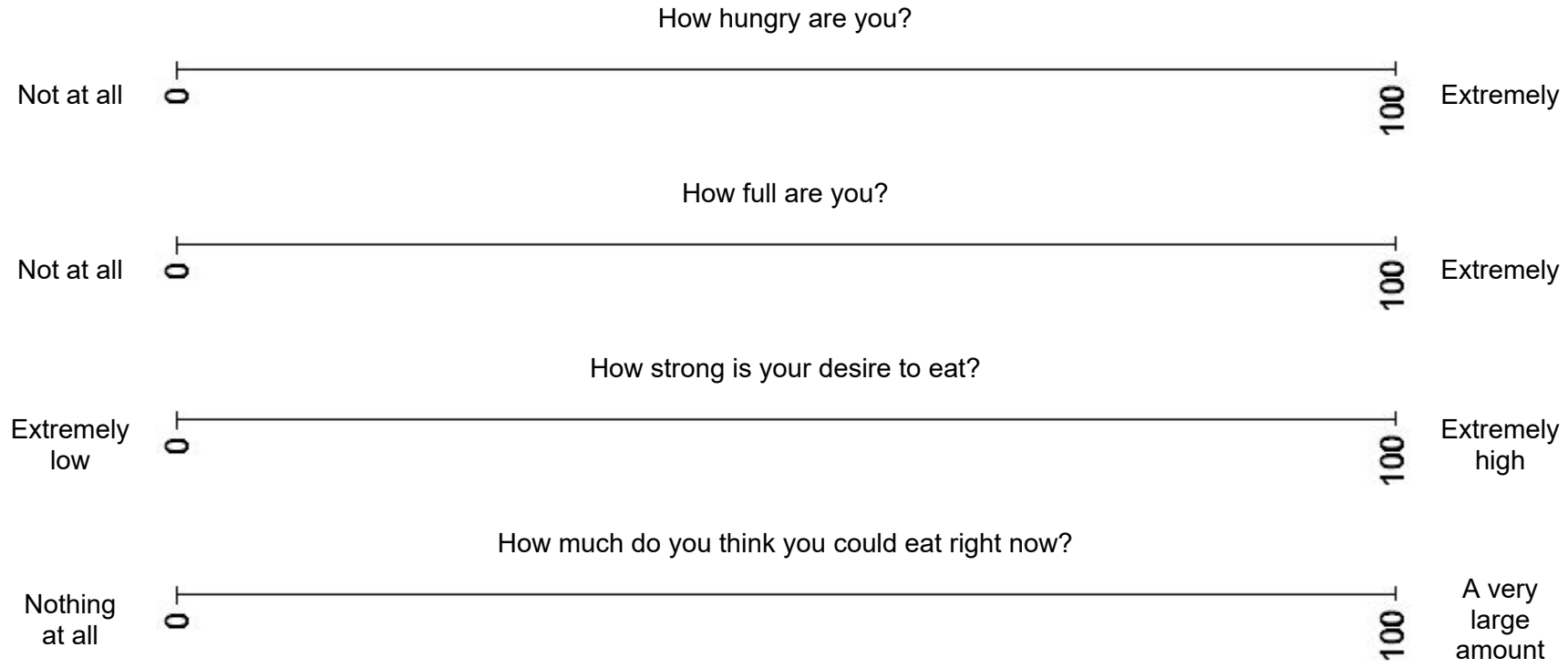
Table 1. Volume of blood drawn at Screening, Baseline, and Visit 9

Test	Screening	Baseline	Visit 9
	Blood (ml)	Blood (ml)	Blood (ml)
CBC	4		4
Chemistry 15/Insulin	4		4
Lipids		4.5	
LDL particle size		1.5	1.5
Glucose and Insulin (MTT)		15	12.5
Total	8	21	22

Notes: (1) Chemistry 15 includes fasting glucose and lipids **(2)** IV blood draw volumes include 0.5 ml returned during blood draw.

Appendix 2.

Appetite Ratings



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