Walnuts to Achieve Lasting NUTrition to prevent Diabetes (WALNUT-Diabetes)

Principal Investigator: **Meghana Gadgil, MD**

Coordinating Center: **University of California, San Francisco**

Protocol Version Date: **September 21, 2017**

TABLE OF CONTENTS

ABBREVIATIONS

AAA	**aromatic amino acids**
AE	**adverse events**
BCAA	**branched chain amino acids**
DM	**diabetes mellitus**
DPP	**diabetes prevention program**
G	**grams**
PI	**principal investigator**
SAE	**serious adverse experience**

1 PROJECT SUMMARY

Prediabetes is a precursor of type 2 diabetes and an independent risk factor for cardiovascular disease, and currently affects one-quarter of the population of the United States. Individuals of overweight or obese BMI are at particular high risk for incident diabetes. A major modifiable risk factor for type 2 diabetes is poor dietary quality, and improvement of dietary quality can effectively delay and even prevent type 2 diabetes. Interventions to improve dietary quality thus far, however, rely on short-term intensive clinically designed meals replacing the entire diet which have poor sustainability. Persistent improvements to daily dietary patterns are often difficult without directed guidance, and overall dietary quality in the United States remains poor. The identification of a practical, daily dietary intervention to improve dietary quality and prevent diabetes in those at high risk remains unknown. We propose to enroll 40 individuals with diagnosed prediabetes into a randomized controlled pilot study and provide a daily walnut supplementation intervention to determine feasibility and acceptability of the supplement. We will then determine preliminary efficacy on metabolic markers and will investigate associations between dietary quality and circulating levels of branched-chain amino acids. Our goal is to implement a whole-food supplement to improve dietary quality in patients with prediabetes as a tool for future type 2 diabetes prevention.

2 SPECIFIC AIMS AND HYPOTHESES

Aim 1: Determine a) feasibility for recruitment and retention into a randomized controlled trial of daily walnut supplementation, and b) acceptability and adherence to the intervention containing 28g of walnuts daily, compared with usual care in individuals with prediabetes.

Aim 2: Determine preliminary efficacy of the walnut intervention vs. usual care on change in a) fasting glucose, HbA1c and lipid levels, b) dietary quality score and c) BCAA and AAA levels. These estimates of efficacy will inform the sample size for a larger future randomized controlled trial.

3 SIGNIFICANCE

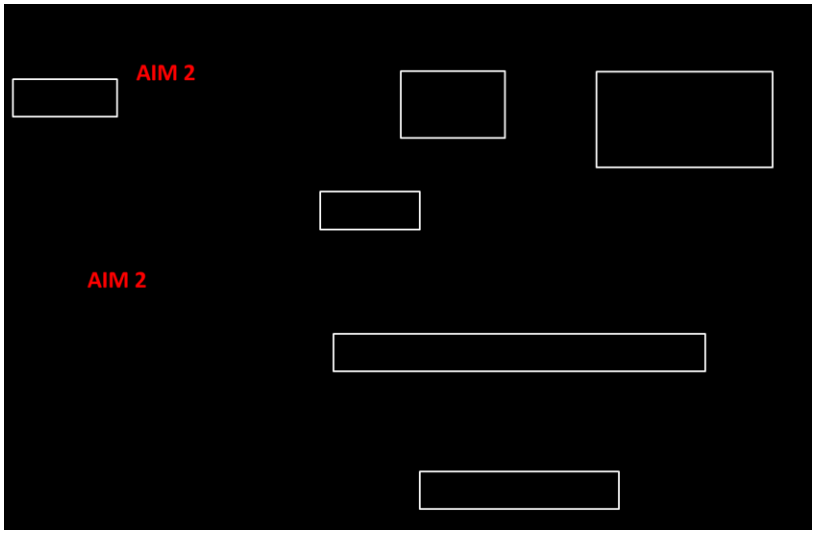
3.1 BACKGROUND

Over one-quarter of the adult population in the United States has prediabetes. Prediabetes is a state of impaired fasting glucose and/or impaired glucose tolerance that is an independent risk factor for type 2 diabetes and cardiovascular disease.[1,2] Up to 30% of people with prediabetes will progress to diabetes in 5 years at current standards.[2] The Diabetes Prevention Program (DPP) is an intensive diet and exercise program that has been shown to decrease incident diabetes in those with impaired glucose tolerance,[3] however this intervention is cost and labor-intensive and is not available to all individuals with prediabetes. In addition, changes to diet and lifestyle must be lasting to significantly delay or prevent diabetes and its complications. The current standard of care to prevent diabetes in those with prediabetes consists only of minimal counseling during primary care visits without significant between-visit follow-up.

Dietary quality is a strong, modifiable risk factor for diabetes and cardiovascular disease. NHANES surveys reveal stably poor dietary quality in the US population over many years, despite improvements in public health messaging.[4] In observational studies, improved dietary quality is associated prospectively with lower incidence of type 2 diabetes and cardiovascular disease.[5-7] Modeling from a longitudinal cohort study suggests that animal protein consumption may promote progression to diabetes at a faster rate than plant-based protein intake.[8,9] There are currently no targeted public health messages to assist those with prediabetes in refining their dietary quality with the goal of preventing diabetes.

Walnut intake has been shown to improve insulin sensitivity, decrease cardiovascular risk and improve endothelial function. Walnuts contain fiber, polyunsaturated fatty acids and plant-based protein that improve satiety.[10] In conjunction with a Mediterranean diet, supplementation with walnuts has been associated with decreased risk of metabolic syndrome,[11] cardiovascular disease[12] and mortality[12,13] in high-risk populations. Plant-based protein consumption,[8,14] and nut intake in particular, over the long-term have shown inverse associations with incident type 2 diabetes in large cohort studies.[15] Individuals who regularly consume nuts have a lower BMI in epidemiologic studies,[16] and feeding trials have demonstrated an improvement in lipid subfractions with acute and long-term intake of nuts.[17-19] In short-term

Figure 1: Conceptual Model



trials, consumption of nuts improved endothelial function[20] and insulin signaling[17] without caloric restriction. These findings suggest that nut intake and the quality of diet has a major effect on the pathways involved in both the incidence and complications of diabetes. (Figure 1) Increasing nut consumption may also improve satiety, ideally displacing those foods, such as refined carbohydrates, that have been shown to contribute to the incidence of diabetes, cardiovascular disease and obesity.[21]

Practical, daily interventions can result in improved overall dietary quality. An approach to add walnuts to usual dietary intake, exchanging one healthful food for other unhealthful foods, has the potential to improve overall dietary quality, and, in turn, improve surrogate markers of type 2 diabetes and lipids levels. With such cumulative changes, long-term improvement in dietary quality can help to decrease progression to type 2 diabetes in people at high risk. The PREDIMED trial added either nuts or olive oil to a Mediterranean-style diet, compared to a low-fat control diet and found decreased fasting glucose and LDL levels, as well as lower clinical outcomes of incident diabetes and cardiovascular disease.[22,23] Little prior work, however, has assessed the effects on surrogate markers for diabetes, including metabolomics, of the addition of nuts to a usual diet in the United States and in individuals with known prediabetes who are at high risk for both diabetes and cardiovascular disease.

I have examined dietary patterns and quality and their associations with risk factors for diabetes in a population of South Asian Americans, and found that a dietary pattern characterized by refined carbohydrates and high-saturated fat sweets was associated with higher insulin resistance (HOMA-IR) and lower HDL.[24] I have also evaluated dietary quality, as measured by the HEI-2010 score, and its association with glycemic control in women with gestational diabetes mellitus, and found that higher dietary quality is associated with improved post-prandial glycemic control (Gadgil MD, *American Journal of Obstetrics and Gynecology*, submitted). I have an investigation currently underway to examine dietary quality, BCAA intake and incident gestational diabetes mellitus in women who are part of the Coronary Artery and Risk Development in Young Adults (CARDIA) longitudinal cohort study, and our preliminary results show that lower dietary quality and higher BCAA intake is associated with an increase in gestational diabetes incidence. As the preponderance of prior work has shown improved outcomes and decreased incidence of diabetes with improvements in dietary quality, we believe that the next step is to create a practical, daily intervention to aid in improving overall dietary quality.

3.2 STUDY RATIONALE

This study will add to the literature by investigating a practical, daily intervention for individuals with prediabetes to improve dietary quality and, in turn, to prevent diabetes. We will characterize novel metabolomics pathways from dietary patterns to DM incidence that may help explain the mechanism of the effect between diet and glucose tolerance in a high-risk population. In doing so, we will identify previously unknown targets to measure the preclinical effects of dietary influence on risk for diabetes.

2.3 INNOVATION

This proposed investigation will join the burgeoning movement of dietary intervention studies that are performed in a real-world environment, without highly controlled conditions, which is more representative of dietary patterns in people at risk for metabolic disease. The approach we use differs from the current paradigm. We aim to use a whole-food supplement, easily available outside of the research or clinical environment. We also aim to investigate changes in metabolomic measures associated with walnut supplementation as an intermediate outcome. Metabolomics can reflect current conditions for pre-existing prediabetes, and we will examine how short-term interventions can affect levels of these circulating markers. These data will also help us to determine if levels of circulating amino acid levels may be useful as markers of adherence for dietary interventions beyond self-report.

4 EXPERIENCE OF THE INVESTIGATORS

Meghana Gadgil, MD MPH is an Assistant Professor in the Division of General Internal Medicine at the University of California, San Francisco. Over the past 5 years, she has focused her research in the areas of dietary patterns, cardiometabolic disease, and racial/ethnic disparities. Her prior work demonstrates associations of dietary quality and patterns with risk factors for metabolic disease, in both normal weight and overweight/obese individuals, and her objective for this study is to evaluate a walnut supplementation intervention in high-risk individuals with prediabetes. Her long-term goal is to identify and implement practical dietary interventions to improve dietary quality and ultimately prevent diabetes.

5 RESEARCH DESIGN AND METHODS

5.1 Study Participants

Study Population, Study Design and Eligibility criteria: We will enroll 40 individuals with prediabetes identified through an electronic medical record query from patients in the UCSF DGIM primary care clinics and through outside recruitment in the surrounding community. We will exclude patients with tree nut or peanut allergies, who are unwilling to consume a daily nut supplement, are on dietician-managed, restricted diets for any medical condition, have overt diabetes or are on glucose-lowering medications, or who are currently enrolled in a Diabetes Prevention Program activity. See full inclusion and exclusion criteria in next section.

5.2 Study Overview

This is a single-center, unblinded, randomized-controlled trial. 40 study subjects are planned. Each subject will consume 28g of individually wrapped raw, unsalted walnuts daily for a total of 12 weeks. Participants will be assigned to the treatments in random order. Evaluations will be taken at baseline and at end-of-study.

Screening data will be reviewed to determine subject eligibility. Subjects who meet all inclusion criteria and none of the exclusion criteria will be entered into the study.

5.3 Study Intervention

Participants in the Usual Care group and Walnuts groups will receive usual counseling and care, including general dietary and lifestyle counseling provided to all patients with diagnosed prediabetes, recommending a diet high in vegetables, fruits, whole grains,

lean protein and dairy. Those in the Walnuts intervention group will receive initial dietary counseling to describe the high refined carbohydrate foods to be replaced with the intervention walnut supplement. The intervention group will receive one, individually-wrapped, 28g packet of raw, unsalted walnuts to be consumed daily for a total of 12 weeks to replace specific high refined carbohydrate snacks. Individually packaged walnuts will be donated by the California Walnut Commission, who will not have a role in the design, procedures or interpretation of this study. Participants will be required to log their food exchanges on a daily basis. This study is designed as a non-blinded trial, therefore to protect against greater than usual consumption of walnuts by the Usual Care group during the intervention period, we will offer walnut supplementation at the end of the trial to these participants. Participants in the Usual Care group will be instructed to carry out usual diet and lifestyle changes for the 12-week duration, and will receive walnut supplementation, identical in dose and duration to the intervention group, after the completion of the study.

The following treatment regimens will be used:

- Walnuts “Now”: 28g of individually-wrapped raw, unsalted walnuts consumed daily for 12 weeks during the study period
- Walnuts “Later”: Usual diet during the study period. The participants will be provided with an equivalent amount of individually wrapped walnut packets for their daily use after the study period has concluded.

5.4 Randomization and Blinding

Randomization: Participants will be randomly assigned to two groups of 20 participants each: “Walnuts Now” Or “Walnuts Later”. We will block our randomization by two important potential confounders: sex (female or male) and age categories (<50 or ≥50 years). We will generate stratum-specific sequential ID numbers to randomly allocate individuals to intervention groups in blocks of four. Study staff will be blinded to the linkage between ID numbers and group assignment. Upon enrollment, each participant's stratum will be identified and the next sequential ID number from that stratum was assigned. At the end of the baseline visit, the interviewer will open a sealed, opaque envelope pre-printed with the sequential ID number revealing the intervention group assignment to the participant.

Blinding

Due to the objectives of the study, the identity of test and control treatments will be known to investigators, research staff, or patients.

This study is designed as a non-blinded trial, therefore to protect against greater than usual consumption of walnuts by the Usual Care group during the intervention period, we will offer walnut supplementation at the end of the trial to these participants. Participants in the Usual Care group will be instructed to carry out usual diet and lifestyle changes for the 12-week duration, and will receive walnut supplementation, identical in dose and duration to the intervention group, after the completion of the study.

Because of the behavioral nature of the intervention, study staff who administer the intervention and study participants will not be blinded to their intervention group assignment, however we will blind the outcomes assessments – clinical labs and amino acid analyses will be done by labs without knowledge of intervention group.

5.5 Supply and Dispensing of Intervention Food at the Study Site

A 12-week supply of individually-wrapped walnut packets will be given to intervention-group participants at enrollment in the study. Those randomized to the control group will receive a 12-week supply of walnuts at the final study visit.

The California Walnut Commission will ship the walnuts to the study site in boxes of 200 individually-wrapped samples in 2 installments – in June 2017 and August 2017. Walnuts will be shipped and kept in cold storage according to the attached protocol. If a participant has concerns about the quality of the walnuts, they will contact the study coordinator who will initiate the following protocol from the California Walnut Commission:

1) Have participant return package and remaining product

2) Check walnut batch (lot number, date, size of package) where package obtained, if possible

3) Contact Carol Berg Sloan RD, directly and immediately.

Carol Berg Sloan RDN, FAND

cbsrd@verizon.net

562-221-9869

Dosage/Dosage Regimen

We will test a dose of 28g of walnuts daily for the experimental group participants. The walnuts should ideally be consumed without added salt or roasting. This whole-food supplement will be given in conjunction with dietary advice to replace consumption of added sugars and refined carbohydrates

Dispensing

The study coordinator will provide a 12 week supply of walnuts to the intervention participants at the enrollment visit. The study coordinator will provide an equivalent supply of walnuts to the control participants at the study conclusion visit.

Administration Instructions

The participants will consume one 28-gram packet of walnuts daily. This may be consumed at one sitting or throughout the day. Walnuts should replace added sugars or products with refined carbohydrates.

Supply of Food Supplement at the Site

The individually wrapped walnut packages will be delivered from the California Walnut Commission in 2 installments. The first in early June and the second in early August.

Storage

The individually wrapped walnuts will be stored in cold storage until they are given to the participants.

Measures of Treatment Compliance

Subjects will be asked to keep a patient diary noting the day and date they consume their allocated walnut packet and any adverse events. They will be asked to bring their patient diary to each study visit.

We will also test measures of walnuts consumption: whole blood RBC fatty acids composition; serum lipids which include LDL HDL, TC ApoA- 1, Apo B, triglycerides.

Allowed Medications and Treatments

Standard therapy for all medical conditions is allowed except for treatments noted in the exclusion criteria described above.

5.6 CLINICAL VISITS AND PROCEDURES

Demographics

Demographic information (date of birth, gender, race/ethnicity) will be recorded at Baseline visit.

Medical History

Relevant medical history, including history of current disease and information regarding underlying diseases will be recorded at Baseline visit.

Vital Signs

Weight, height, blood pressure and waist circumference will be collected and body mass index calculated at initial baseline visit and study completion visit.

Clinical Laboratory Measurements

Blood will be obtained by venipuncture at the UCSF Clinical Research Services site at Parnassus at baseline and study completion visits. We will obtain 2 tubes of blood (serum and plasma and separated into aliquots. One aliquot of serum will be sent to Quest Diagnostics for determination of fasting glucose, lipid Apo A1- and ApoB, s and A1c measurements. An aliquot of plasma will be sent to the West Coast Metabolomics Center at the University of California, Davis, for determination of targeted amino acid metabolomics analysis. The remaining aliquots will be frozen at -80C for future research. See Table 1 for Study procedures at each visit.

Telephone Screening

Individuals who respond to recruitment notices will be provided with a general overview of the program and will complete a brief telephone survey to determine initial eligibility (age, prediabetes diagnosis) before being invited to the screening visit.

Baseline clinic visit

At the baseline visit, the study will be explained in detail, and informed consent will be obtained. Candidates will be told that this is a trial of walnut supplementation to improve dietary quality in overweight or obese individuals with prediabetes.

The research coordinator will perform measurement of height using a stadiometer and weight using a digital scale. Those with a BMI $\geq$ 25 kg/m^2 ($\geq$ 23 kg/m^2 if of Asian or South Asian ethnicity) will be eligible. The research coordinator will them perform a capillary blood glucose measurement using an Accu-check Aviva Connect blood glucose meter and Accu-Check test strips. A capillary blood glucose value of 110mg/dL and higher will be considered eligible for participation in the study.

Eligible participants will be randomized into "Walnuts Now" and "Walnuts Later" groups. They will complete questionnaires about demographic and medical history, health-related behaviors including satiety, and medications. A research coordinator will measure height, weight, waist circumference and blood pressure. The participants will undergo venipuncture and fasting blood draw. One tube of serum will be sent to Quest for measurement of fasting glucose, hemoglobin A1c and serum lipids as well as apolipoprotein A2 and B. One tube will be aliquotted into 1mL cryogenic tubes. One aliquot of plasma will be shipped to the West Coast Metabolomics Center at the University of California, Davis for amino acid and acylcarnitine analyses. The remaining serum and plasma aliquots will be de-identified, frozen at -80C and stored for future analyses.

All participants will receive handouts regarding a healthy diet to prevent diabetes.

The research coordinator will dispense 12-week supply of individually-wrapped, raw walnuts to "Walnuts Now" group.

6-week Telephone Follow-up

The research coordinator will reach out to study participants by phone at 6 weeks to remind intervention participants of daily walnut intervention, give a reminder of healthy eating habits to all participants, and schedule Study Conclusion visit.

Study Completion Visit

At study completion, all participants will be invited back for a study completion visit. At this visit, a research assistant will record height, weight, blood pressure and waist circumference. The participants will undergo venipuncture and fasting blood draw. One tube of serum will be sent to Quest for measurement of fasting glucose, hemoglobin A1c and serum lipids as well as apolipoprotein A2 and B. One tube will be aliquotted into 1mL cryogenic tubes. One aliquot of plasma will be shipped to the West Coast Metabolomics Center at the University of California, Davis for amino acid and acylcarnitine analyses. The remaining serum and plasma aliquots will be de-identified, frozen at -80C and stored for future analyses.

All participants will receive handouts regarding a healthy diet to prevent diabetes.

The research coordinator will dispense 12-week supply of individually-wrapped, raw walnuts to "Walnuts Later" group.

5.7 Measures Collected at Each Visit

Table 1: Measurements at each Study Visit				
Visit	Telephone Screening (Week 0)	Baseline visit (Week 1)	Telephone Mid-Point (6 weeks)	Study completion (12 weeks)
Informed Consent	Verbal informed Consent	Written informed consent		
Questionnaires	Initial eligibility screen	Demographic questionnaire Block food frequency Self-efficacy		Acceptability Palatability Adherence Block food frequency Self-efficacy
Clinical measurements		Height Weight Waist circumference Blood pressure Capillary blood glucose		Height Weight Waist circumference Blood pressure
Laboratory analyses		Fasting glucose HbA1c Lipids BCAA, AAA, acylcarnitines		Fasting glucose HbA1c Lipids BCAA, AAA, acylcarnitines
Counseling		Dietary and exercise counseling for both intervention and control arms	Check-in for diet and exercise changes Adherence to walnut supplement for "Walnuts Now" group.	
Dispensing of Study Intervention		"Walnuts Now" Intervention Group receives a 12-week supply of walnuts		"Walnuts Later" Control Group receives a 12-week supply of walnuts

5.7 Statistical analyses

This trial is designed as a feasibility study and an investigation of the acceptability of the walnut intervention. The second aim is to establish preliminary efficacy for changes in fasting glucose, HbA1c and amino acid levels, for determination of sample size in a future clinical trial. Therefore, we have chosen to enroll a goal of 40 participants, including 20 in the intervention and 20 in the control groups.

Specific Aim 1: Aim 1: Determine a) feasibility for recruitment and retention into a randomized controlled trial of daily walnut supplementation, and b) acceptability and adherence to the intervention containing 28g of walnuts daily, compared with usual care in individuals with prediabetes.

Feasibility and acceptability. We will assess feasibility based on number screened and enrolled in the proposed randomized clinical trial for our goal of 40 participants. Retention over the 12-week intervention period will be assessed by number of participants who complete the final study visit at the conclusion of the intervention period. Palatability will be assessed by a 100mm visual analog scale. We will also assess acceptability of the walnut intervention, and its dose and frequency through a targeted questionnaire at the end of the intervention period. Adherence will be measured through self-report (food diaries) as well as the food frequency questionnaire administered at study completion. All questionnaires will be administered by our research assistant.

Aim 2: Determine preliminary efficacy measures for change in a) fasting glucose, HbA1c and lipid levels, b) dietary quality score and c) BCAA levels in the intervention group to inform a larger future randomized controlled trial.

Fasting glucose, HbA1c and lipids: We will obtain fasting blood samples at enrollment and at the conclusion of the study. One aliquot of serum tube of blood will be sent for fasting glucose, lipids, ApoA1 and B, and HbA1c measurement, and one aliquot of plasma will be sent for metabolomics analyses. Additional aliquots of plasma and serum will be frozen for future research purposes. We will establish preliminary efficacy for each of these measurements.

Dietary Quality: A Block food frequency questionnaire will be administered to participants at enrollment and completion of the study period. This questionnaire will use patient recall to gather frequency and serving size data for all participants. We will use the responses in this questionnaire to determine consumption of foods grouped based on likeness and culinary usage. These categories will then be transformed into 2 dietary scores: the Healthy Eating Index-2010 score, based on the Dietary Guidelines for Americans, which is a continuous score ranging from 0-100, with a score of 100 representing the optimal diet. We will also calculate the Mediterranean Diet Score, with possible values from 0-55, with 55 representing the diet most congruent with the Mediterranean diet.

BCAA, AAA and acylcarnitines: Targeted amino acid and acylcarnitine levels will be measured at baseline and at 12 weeks, post-intervention using triple quadrupole (MS/MS) and gas chromatography instruments. We will collaborate with the West Coast Metabolomic Center on the metabolomics analyses and interpretation.

Statistical Analysis*:* This is designed as a parallel group controlled trial, randomized on dichotomized age (<50 or ≥ years) and sex (female or male). We will use Fisher's exact test and Student's t-test to compare mean baseline demographic and clinical

characteristics between the intervention and control groups. Using information from the Block food frequency questionnaire, we will construct a continuous dietary quality score using the Healthy Eating Index 2010 and Mediterranean Diet Score criteria. We will use analysis of covariance to compute the adjusted mean for our outcome variables of fasting glucose, HbA1c, HDL, LDL, total cholesterol and amino acid levels. In analysis of covariance, the baseline value of outcome variables will be used as a covariate for the computation of adjusted means between the two groups after intervention.

Expected Results: We believe that walnut supplementation will be acceptable at a dose of 28g daily. We expect to be able to recruit 40 participants within 3 months, and retain >75% for the full 12 weeks of the intervention. We expect to see a trend towards significant change in the BCAA and AAA measurements and some improvements of fasting glucose and HbA1c in the "Walnut" group compared to the "Usual care" group.

5.8 Potential problems and alternative approaches

The dose of raw, unsalted walnuts at 28g, or 1 oz, daily may not be an acceptable dose for study participants with diabetes. Based on our literature review, there have been several studies that have found a dose of 28-56g of walnuts acceptable for a similar duration. The Usual Care study arm may alter their diets to consume more walnuts during the study period given the inability to blind participants for this intervention. We plan to address this by offering walnuts after the study period to the control group, as an incentive to retain the usual care environment during the period in question. We will also be able to determine if the BCAA and AAA levels in the usual care group improve through the 12-week period as a marker of possible co-intervention.

We have considered using an active comparator to walnuts, however with the possibility of confounding biological activity for other whole food supplements, we elected not to include one for this pilot study given our goals of feasibility, acceptability, adherence and preliminary efficacy of a walnut intervention. Instead, we feel that providing walnuts for all of the trial participants, using the approach that the intervention group will receive walnuts during the main study period, and the control group will continue with usual care during the intervention period, but then receive walnuts later as a "walnuts now" vs. "walnuts later" approach will help to control for confounding. If we are successful in our primary outcomes, we will consider an active comparator for our larger randomized controlled trial.

5.9 Study limitations

Generalizability: We aim to recruit subjects who self-identify as overweight or obese, with prediabetes, and will assess their laboratory criteria for prediabetes at the first visit. Our study design is a convenience sample of volunteers who are motivated to participate in a clinical study, all factors which limit generalizability. Our study population should be representative for our target population of overweight or obese adults with prediabetes between the ages of 18 and 65. We will be unable to accommodate participants who do not speak English due to difficulties in finding qualified study personnel who speak other languages.

6 PROTECTION OF HUMAN SUBJECTS

6.1 Risks to the Subjects

6.1.a Human Subject Involvement and Characteristics: We will be enrolling approximately 40 volunteers who are overweight or obese and who fulfill criteria for prediabetes.

Eligibility criteria are listed below:

Inclusion Criteria

1. Male or female between 18-65 years of age at baseline living in the San Francisco Bay area.
2. BMI>25 m/kg^2 (or >23 m/kg^2 for individuals of Asian or South Asian ethnicity)
3. Documentation of prediabetes diagnosis as evidenced by the following criteria:
    a. A fasting glucose 100-125 mg/dL, or a HbA1c measurement of 5.7-6.4%, OR a diagnosis of “prediabetes” or “impaired fasting glucose” in the past 6 months, identified through an electronic medical record query from patients at UCSF and through outside recruitment in the surrounding community
    b. We will confirm eligibility of potential participants by repeating fasting capillary blood glucose measurements at the baseline visit to ensure that they have prediabetes
4. Written informed consent and ability for subject to comply with the requirements of the study.

Exclusion Criteria

1. Pregnant or breastfeeding women at enrollment.
2. Presence of a condition or abnormality that in the opinion of the Investigator would compromise the safety of the patient or the quality of the data, such as diverticulosis or diverticulitis.
3. Tree or peanut allergies
4. Unwilling to consume a daily walnut supplement.
5. Diagnosis of diabetes
6. On glucose lowering medications
7. Dietician-managed dietary intake, or personal or medical dietary restrictions that do not allow consumption of walnuts
8. Malabsorptive conditions including intestinal bypass surgery, pancreatitis, inflammatory bowel disease

All subjects should be maintained on the same medications throughout the entire study period, as medically feasible, with no introduction of new glucose-lowering therapies.

6.1.b Sources of Materials: All personal, medical, and biochemical, data collected during the studies are for research purposes only, although test results that come from

certified laboratories can be provided to patients or their physicians upon request with the subject's permission.

6.1.c Potential Risks:

Capillary blood glucose measurement: The baseline visit will require fingerstick collection of one drop of blood for determination of eligibility. This may result in pain or discomfort. We do not anticipate other problems from this procedure in healthy adults.

Phlebotomy and venipuncture: The baseline visit and study conclusion visit 3-month visit will require approximately 35-45 ml of venous blood collection. We do not anticipate any problems with anemia with a total of 45 ml of blood collection in our study of healthy volunteers. Venipuncture may result in a hematoma, which can be painful but carries no significant risks. Infections rarely occur from such brief interventions.

Walnut supplementation: There is minimal risk associated with participation in the walnut intervention. There is the possibility of an allergic reaction to the walnut supplement. We will actively exclude any participants with a known tree nut or peanut allergy from enrollment in the study. There is the small possibility that an as-yet, unknown, allergy to walnuts will occur during the course of the study. In the event of signs or symptoms of allergy, the walnut intervention will be discontinued. Participants will be educated on signs and symptoms of a walnut allergy at the baseline visit. In order to protect the integrity and freshness of the walnuts, they will be kept in cold storage at the study site, and participants will be instructed to keep them in cold storage in their private homes before use.

6.2 Adequacy of Protection Against Risks

6.2.a Recruitment and Informed Consent: Recruitment will be accomplished primarily through a medical records query, and through flyers at Parnassus and Mount Zion UCSF campuses. At the beginning of the baseline visit, a member of the research staff will review the consent form in detail with the patient and answer all questions before inviting the patient to sign the consent form. A photocopy of the signed consent form with the Experimental Subjects' Bill of Rights is given to the patient. The protocols are reviewed by the Institutional Committee on Human Research, University of California, San Francisco and University of California, San Diego. All subjects will be reimbursed for the time and possible inconvenience associated with participation in the studies.

6.2.b Protection Against Risk: The specific measures to minimize each risk are described in the relevant sections above, as well as the Data Safety and Monitoring Plan that is outlined in a subsequent section. In addition, there will be continuous safety surveillance with emphasis on the potential side effects of each procedure, as detailed above. Participation in the study will be discontinued if the subject fails to adhere to the study requirements in a way that may cause harm to him or herself or seriously interfere with the validity of the study results; or the investigator determines that further participation would be detrimental to the subject's health or well being.

Subjects who are injured as a result of being in this study will have treatment available.

The costs of such treatments may be covered by the University of California depending on a number of factors.

Participation in research may involve a loss of privacy. Research records will be kept confidential to the extent permitted by law. Subjects will be identified by a code, and personal information from records will not be released without written permission. Subjects will not be personally identified in any publication about this study.

6.3 Potential Benefits of the Proposed Research to the Subjects and Others

There is no guaranteed benefit to the subjects who participate in this study. To the extent allowable, results of laboratory tests are made available to subjects and/or their primary care providers that might prove useful in their clinical management or prevention of future disease.

6.4 Importance of the Knowledge to be Gained

Benefits to society may include a better understanding of the potential effectiveness of including walnuts in a daily diet to improve dietary quality, and to understand the mechanisms of improvement in blood markers after a walnut intervention.

5.1.1.1 6.5 Data and Safety Monitoring Plan

6.5.a. Confidentiality

1. Protection of Subject Privacy – During this study, a limited physical examination will be performed and questionnaires will be administered. Fasting blood tests will be done periodically. Data will be kept in strict confidence. No information will be given to anyone without permission from the subject. Confidentiality is assured by use of identification codes. All data, whether generated in the laboratory or at the bedside, will be identified with a randomly generated identification code unique to the subject. Health Information Portability and Accountability Act (HIPAA) guidelines of the two clinical sites will be followed. (See VI. Informed Consent and HIPAA)

2. Database Protection – The database is secured with password protection. The informatics manager receives only coded information, which is entered into the database under those identification codes. Electronic communication with outside collaborators involves only unidentifiable information.

3. Confidentiality during AE Reporting –AE reports and annual summaries will not include subject-identifiable material. Each will include the identification code only.

6.5.b. Adverse Event Information

1. Definition - An adverse event (AE) is any untoward medical occurrence in a subject temporally associated with participation in the clinical study. An adverse finding can include a sign, symptom, abnormal assessment (laboratory test value, vital signs, electrocardiogram finding, etc.) or any combination of these.

2. Classification of AE Severity –AEs will be labeled according to severity which is based on their impact on the patient, per this Event Grading Scale:

Grade 1	**Mild**	Transient of mild discomfort; no limitation in activity; no medical intervention/therapy required.
Grade 2	**Moderate**	Mild to moderate limitation in activity – some assistance may be needed; no medical intervention/therapy required.
Grade 3	**Severe**	Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalizations possible.
Grade 4	**Life-Threatening**	Extreme limitation in activity, significant assistance required significant medical intervention/therapy required, hospitalization or hospice care probable.

3. AE Attribution Scale –AEs will be categorized according to the likelihood that they are related to the study intervention. Specifically, they will be labeled either definitely, probably, possibly or unrelated to the study intervention.

4. Expected Risks – There is minimal risk with participating in the walnut intervention. Expected risks to the subject are mild gastrointestinal symptoms due to inclusion of walnuts in a normal daily diet. These risks are considered to be minimal and are addressed in the protocol and consent form. Participants will have a contact number to report any potential adverse events that occur in between scheduled study visits. They will also have an opportunity to privately discuss any other physical complaints due to inclusion of walnuts.

5. SAE Reporting - SAEs that are unanticipated, serious (grades 3 and 4), and/or possibly related to the study intervention will be reported to the Independent Monitor, IRB, CTSA at UCSF, and NIH in accordance with requirements. Anticipated SAEs or those unrelated to the study intervention will be reported to the same individuals/entities in accordance with requirements.

If abnormal lab values are discovered during the study, the PI will be notified and will address this issue as early as possible. If fasting glucose is found to be abnormally low (greater than or equal to 40 mg/dL and below 70 mg/dL), the participant will receive a call from the study coordinator asking if they are experiencing adverse effects such as nausea or lightheadness This mild hypoglycemia will be treated with sugar intake. A severely low blood sugar (<40 mg/dL) will be flagged as a critically low value by the study coordinator who will notify the principal investigator immediately or within half an hour of receiving the notification. The participant will be called to proceed to the emergency room and will be withdrawn from the study. Abnormal lab values will be reported as adverse events only when the Principal investigator deems them to be clinically significant.

6.5.c. Data Quality and Safety Review Plan and Monitoring

1. Data Quality and Management
   a. Description of Plan for Data Quality and Management – The PI will review all data collection forms on an ongoing basis for data completeness and accuracy as well as protocol compliance. A statement reflecting the results of the review will be sent to the NIH in the annual report.

      Data quality will be assessed using measures such as time from study visit to data entry, time to resolution of data queries, number of missing forms, and proportion of all study variables queried. Guidelines for concern regarding these measures are outlined below:

Measure	Goal Value	Acceptable Value
time from visit to data entry	< 1 week	< 2 weeks
time to resolution of queries	<2 week	< 2 weeks
number of missing forms	0	< 5%
proportion of variables queried	5%	<10%

   b. Frequency of Review –The frequency of data review depends according to the type of data and is summarized in the following table.

Data type	Frequency of review	Reviewer
Subject accrual (adherence to eligibility criteria, randomization)	Monthly	Principal Investigator
Adverse event rates (injuries)	Monthly	Principal Investigator
Out of range laboratory data	Weekly (PI)	Principal Investigator

2. Subject Accrual and Compliance

a. Measurement and reporting of subject accrual, adherence to inclusion/exclusion criteria –Review of the rate of subject accrual, adherence to inclusion/exclusion criteria will occur monthly during the 3 month recruitment phase.

6.5.d Safety Review Plan

Study progress and safety will be reviewed monthly by the PI. A study completion report will be compiled and will include a list and summarization of adverse events. In addition, the annual report will address (1) whether adverse event rates are consistent with pre-study assumptions; (2) reason for dropouts from the study; (3) whether all participants met entry criteria. The report will be signed by the Independent Monitor and will be forwarded to the appropriate IRB and NIH, the UCSF CTSA on an annual basis.

REFERENCES CITED

1. Merlotti C, Morabito A, Pontiroli AE. Prevention of type 2 diabetes; a systematic review and meta-analysis of different intervention strategies. *Diabetes Obes Metab*. 2014.

2. Tabak AG, Herder C, Rathmann W, Brunner EJ, Kivimaki M. Prediabetes: A high-risk state for diabetes development. *Lancet*. 2012;379(9833):2279-2290.

3. Knowler WC, Barrett-Connor E, Fowler SE, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med*. 2002;346(6):393-403.

4. Rehm CD, Penalvo JL, Afshin A, Mozaffarian D. Dietary intake among US adults, 1999-2012. *JAMA*. 2016;315(23):2542-2553.

5. Bauer F, Beulens JW, van der, A D L, et al. Dietary patterns and the risk of type 2 diabetes in overweight and obese individuals. *Eur J Nutr*. 2013;52(3):1127-1134.

6. Esposito K, Giugliano D. Mediterranean diet and type 2 diabetes. *Diabetes Metab Res Rev*. 2013.

7. Mayer-Davis EJ, Sparks KC, Hirst K, et al. Dietary intake in the diabetes prevention program cohort: Baseline and 1-year post randomization. *Ann Epidemiol*. 2004;14(10):763-772.

8. Satija A, Bhupathiraju SN, Rimm EB, et al. Plant-based dietary patterns and incidence of type 2 diabetes in US men and women: Results from three prospective cohort studies. *PLoS Med*. 2016;13(6):e1002039.

9. van Woudenbergh GJ, Kuijsten A, Tigcheler B, et al. Meat consumption and its association with C-reactive protein and incident type 2 diabetes: The rotterdam study. *Diabetes Care*. 2012;35(7):1499-1505.

10. Brennan AM, Sweeney LL, Liu X, Mantzoros CS. Walnut consumption increases satiation but has no effect on insulin resistance or the metabolic profile over a 4-day period. *Obesity (Silver Spring)*. 2010;18(6):1176-1182.

11. Salas-Salvado J, Guasch-Ferre M, Bullo M, Sabate J. Nuts in the prevention and treatment of metabolic syndrome. *Am J Clin Nutr*. 2014;100(Supplement 1):407S.

12. Bao Y, Han J, Hu FB, et al. Association of nut consumption with total and cause-specific mortality. *N Engl J Med*. 2013;369(21):2001-2011.

13. Fernandez-Montero A, Bes-Rastrollo M, Barrio-Lopez MT, et al. Nut consumption and 5-y all-cause mortality in a mediterranean cohort: The SUN project. *Nutrition*. 2014.

14. Ko BJ, Park KH, Shin S, et al. Diet quality and diet patterns in relation to circulating cardiometabolic biomarkers. *Clin Nutr*. 2015.

15. InterAct Consortium, Romaguera D, Guevara M, et al. Mediterranean diet and type 2 diabetes risk in the european prospective investigation into cancer and nutrition (EPIC) study: The InterAct project. *Diabetes Care*. 2011;34(9):1913-1918.

16. Newby PK, Muller D, Hallfrisch J, Qiao N, Andres R, Tucker KL. Dietary patterns and changes in body mass index and waist circumference in adults. *Am J Clin Nutr*. 2003;77(6):1417-1425.

17. Casas-Agustench P, Lopez-Uriarte P, Bullo M, Ros E, Cabre-Vila JJ, Salas-Salvado J. Effects of one serving of mixed nuts on serum lipids, insulin resistance and inflammatory markers in patients with the metabolic syndrome. *Nutr Metab Cardiovasc Dis*. 2011;21(2):126-135.

18. Wu L, Piotrowski K, Rau T, et al. Walnut-enriched diet reduces fasting non-HDL-cholesterol and apolipoprotein B in healthy caucasian subjects: A randomized controlled cross-over clinical trial. *Metabolism*. 2014;63(3):382-391.

19. Damasceno NR, Sala-Vila A, Cofan M, et al. Mediterranean diet supplemented with nuts reduces waist circumference and shifts lipoprotein subfractions to a less atherogenic pattern in subjects at high cardiovascular risk. *Atherosclerosis*. 2013;230(2):347-353.

20. Katz DL, Davidhi A, Ma Y, Kavak Y, Bifulco L, Njike VY. Effects of walnuts on endothelial function in overweight adults with visceral obesity: A randomized, controlled, crossover trial. *J Am Coll Nutr*. 2012;31(6):415-423.

21. Njike VY, Ayettey R, Petraro P, Treu JA, Katz DL. Walnut ingestion in adults at risk for diabetes: Effects on body composition, diet quality, and cardiac risk measures. *BMJ Open Diabetes Res Care*. 2015;3(1):e000115.

22. Zampelas A. Nuts and not olive oil decrease small and dense LDL: Results from the PREDIMED study. *Atherosclerosis*. 2013;231(1):59-60.

23. Salas-Salvado J, Bullo M, Babio N, et al. Reduction in the incidence of type 2 diabetes with the mediterranean diet: Results of the PREDIMED-reus nutrition intervention randomized trial. *Diabetes Care*. 2011;34(1):14-19.

24. Gadgil MD, Anderson CA, Kandula NR, Kanaya AM. Dietary patterns are associated with metabolic risk factors in south asians living in the united states. *J Nutr*. 2015;145(6):1211-1217.