# A Feasibility Study Incorporating DNA Methylation-Predicted Aging with a Dietary Intervention to Improve Cardiometabolic Health

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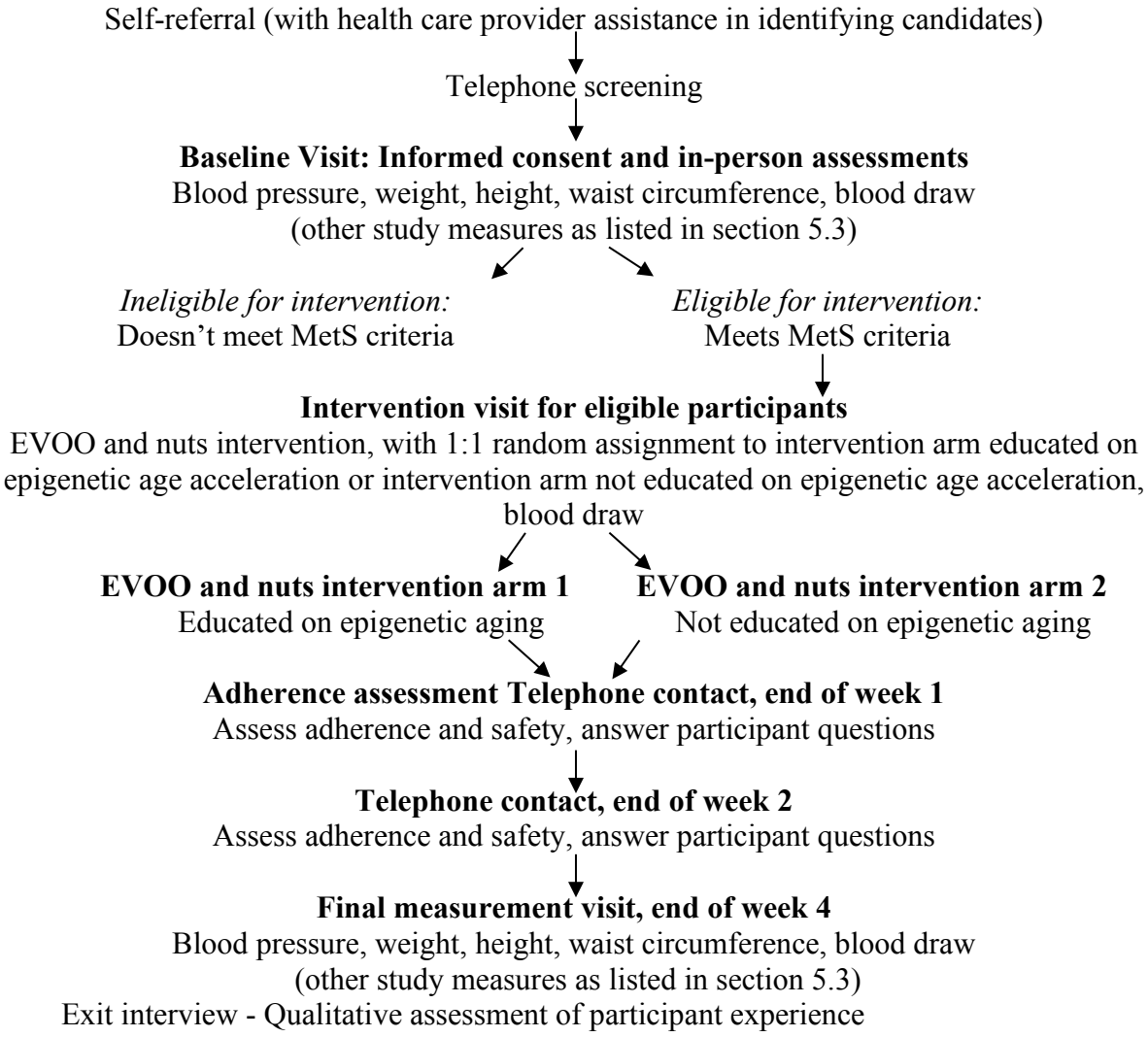
# SCHEMA

We will recruit up to 50 women and men (≥ 35 years) from the surrounding communities with Metabolic Syndrome (Mets). All participants will provide blood samples to determine epigenetic age estimates. Participants who meet eligibility criteria will be asked to participate in a 4-week nuts and extra virgin olive oil (EVOO) intervention. Half of the intervention participants will be randomly selected to be educated about epigenetic age acceleration before the intervention. All participants will have a phone visit after one week of the intervention to assess compliance, and all participants will be contacted by phone after week two of the intervention. Participants will have in person study-related measurements collected at baseline and 4 weeks.



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# 1.0 Introduction and Background

Metabolic syndrome (**MetS**) is a collection of at least three out of five cardiometabolic risk factors including abdominal obesity, hypertension, elevated blood glucose, hypertriglyceridemia, and low high-density lipoprotein cholesterol [1]. The prevalence of MetS is rising worldwide, and as of 2012, more than one third of all US adults met the criteria for MetS [2]. This is important as MetS is a major risk factor for cardiovascular disease and is considered a state of prediabetes [3]. Improving metabolic parameters through dietary, behavioral, and pharmacological interventions can improve or reverse MetS [4, 5]. For instance, a 5-year study in Spain (PREDIMED) evaluated the effects of the Mediterranean diet on primary prevention of cardiovascular disease in subjects at high risk of cardiovascular disease (CVD)[6]. PREDIMED was a multicenter, randomized, controlled clinical trial of 7,447 participants who were randomized to one of three diets: Mediterranean diet supplemented with extra virgin olive oil (EVOO), Mediterranean diet supplemented with mixed nuts, or a low-fat control diet. The PREDIMEDstudy found a reduction in the cardiovascular event rates in both intervention groups compared to the low fat control group. Additionally, post-hoc analysis of individuals meeting the criteria for MetS at baseline revealed EVOO and nut consumption (compared to controls) was more likely to result in **reversion of MetS** (HR = 1.35 95% CI: 1.15 – 1.58, $p<0.001$), and significant reductions in central obesity ($p<0.001$) and high fasting glucose ($p = 0.02$) compared to the low fat control group [7]. However, **lower levels of adherence** to the PREDIMED intervention was found in participants with a higher number of cardiovascular risk factors, larger waist circumference, and lower physical activity levels at baseline [8]. New strategies to convey

one's risk of morbidity and mortality at the onset of a dietary intervention may improve intervention adherence, particularly among individuals meeting the criteria for MetS.

DNA methylation patterns change with age, and can be used to estimate chronological age [9-12]. A greater DNA methylation-based predicted age relative to chronological age, often referred to as "**epigenetic age acceleration**", has been associated with many lifestyle factors, including physical activity and diet, as well as components of MetS, including obesity, blood pressure, HDL cholesterol and blood glucose levels [13]. A higher number of MetS components are cross-sectionally associated with epigenetic age acceleration [14]. Epigenetic age acceleration is more strongly predictive for the risk of morbidity and mortality than chronological age [9, 11]. We **hypothesize** that the majority of people with MetS have advanced epigenetic aging, and among those that have advanced epigenetic age, learning about epigenetic age and its relationship to morbidity and mortality at the onset of a dietary intervention will improve participant adherence to the dietary intervention. Further, we hypothesize that daily consumption of EVOO and tree nuts in participants with MetS and advanced epigenetic age could help reverse MetS and potentially slow epigenetic aging. Therefore, we plan to conduct this feasibility pilot study to:

### 1.1 Primary Objectives

To determine the proportion of participants with MetS with epigenetic age acceleration

To assess adherence to daily EVOO and tree nut consumption over the 4-week intervention, and compare adherence in participants educated about epigenetic age acceleration at baseline compared to intervention arm not educated about epigenetic age acceleration

Qualitatively explore how participants perceive epigenetic age, and how participants' experiences would impact the feasibility of a larger clinical intervention in terms of challenges and motivators

### 1.2 Secondary Objectives

Compare epigenetic age acceleration before and after the 4-week intervention

### 1.3 Exploratory Objectives

Compare changes in MetS related traits (body mass index, waist circumference, blood pressure, HDL cholesterol, triglycerides, HbA1c, and fasting glucose levels) before vs. after the 4-week intervention

## 2.0 Participant Selection

This study can fulfill its objective only if participants appropriate for this intervention are enrolled. All relevant medical and other considerations will be taken into account when deciding whether this protocol is appropriate for a particular candidate.

### 2.1 Inclusion Criteria

2.1.1 Men and Women ≥ 35 years of age

2.1.2 Metabolic syndrome, defined as ≥ 3 of the following:

2.1.2.1 Waist circumference >102 cm in men and >88cm in women
2.1.2.2 Triglycerides >150 mg/dL AND/OR drug treatment for elevated triglycerides
2.1.2.3 HDL cholesterol <40 mg/dL in men and <50 mg/dL in women AND/OR drug treatment for reduced HDL cholesterol
2.1.2.4 Systolic blood pressure >130 mm Hg or diastolic blood pressure >85 mmHg AND/OR antihypertensive drug treatment
2.1.2.5 Fasting glucose >100 mg/dL or hemoglobin A1c > 5.6% AND/OR oral hypoglycemic medications

2.1.3 Willing to comply with study visits, as outlined in the protocol
2.1.4 Able to read and speak English
2.1.5 No allergies or hypersensitivities to olive oil or nuts
2.1.6 Ability to understand and the willingness to sign a written informed consent document.
2.1.7 Completion of a validated 14-item Mediterranean Diet Assessment Screener to assess baseline dietary consumption of nuts and olive oil

**2.2 Exclusion Criteria**

2.2.1 Plans to move from the study area in the next 12 weeks
2.2.2 Body Mass Index (BMI) $\geq$ 40 kg/m$^2$
2.2.3 Dementia that is medically documented or suspected, or clinical evidence of cognitive impairment sufficient to impair protocol adherence
2.2.4 Candidate with any dietary practice, behavior or attitude that would substantially limit ability to adhere to protocol
2.2.5 Homebound for medical reasons
2.2.6 Living in the same household with another participant
2.2.7 Insulin-dependent Diabetes

**2.3 Inclusion of Women, Minorities and Children**

Males and females of all races and ethnic groups are eligible for this trial. Children will not be included in this study as children with metabolic syndrome may have very different underlying etiologies for metabolic syndrome than adults.

**2.4 Identification of Cohort**

We propose to draw our sample of 50 men and women from the population residing in Winston-Salem/Forsyth County, North Carolina. Over 361,220 persons live in the county. In addition, over 1.3 million people reside in the eight contiguous counties comprising the Piedmont Triad area. We anticipate that recruitment for the study can be accomplished in Forsyth County. However, if needed, we will extend recruitment to the surrounding communities.

## 3.0 Recruitment Procedures

Investigators at Wake Forest School of Medicine have extensive experience in recruitment for both observational and clinical trials. Based on our previous experience in large-scale studies, we have found the use of several approaches for recruitment substantially better than relying on one recruitment strategy. These approaches include: community-wide methods designed to reach large numbers of men and women and contacting participants through MyWakeHealth communications. We will also recruit participants through advertising on the WFBH website

'Be Involved' and using study flyers posted at local medical clinics including Family Medicine and Internal Medicine.

We anticipate it will take approximately 12 weeks to recruit 50 participants for this project. Candidates will complete a telephone screening. The baseline visit will consist of an in-person assessment visit, scheduled for a morning time, and participants will be asked to be fasting and will be instructed to bring their medications to the visit. Candidates will review the informed consent document at the baseline visit for in-person screening with study staff prior to beginning their participation.

Each candidate will be greeted by a study staff member who will take the candidate to a private consulting room. A final eligibility checklist and each section of the informed consent will be reviewed with the candidate. Once the candidate fully understands each section of the consent, he/she will sign the document and be given a copy to take home. A copy will be retained by the study staff.

**Recruitment of Participants**

The goal of the recruitment phase is to enroll 50 men and women with MetS into the study.

| | **Female** | **Male** |
|---|---|---|
| American Indian or Alaska Native | 1 | 1 |
| Asian or Pacific Islander | 1 | 1 |
| Black, not of Hispanic Origin | 6 | 6 |
| Hispanic | 2 | 2 |
| White, not of Hispanic Origin | 14 | 14 |
| Other or Unknown | 1 | 0 |
| **TOTAL** | **25** | **25** |

### 3.1 Epigenetic Age Acceleration

For all participants providing informed consent, blood samples will be collected at each in-person visit to calculate epigenetic age acceleration.

## 4.0 Treatment Plan: Tree Nuts and Extra Virgin Olive Oil

At the intervention visit, all participants will receive the full 4-week supply of EVOO and tree nuts, including unsalted English walnuts, almonds or pistachios (approximately 10-day supply of each type). Participants will be asked to supplement their normal diets with these products and will be provided recipes and other information that will allow them to replace other foods with the nuts and oil. We will ask participants to consume one ounce of tree nuts per day and two tablespoons of EVOO per day by incorporating these foods into their diet. Dietary adherence diaries will be given to measure incorporation of the study foods.

Participants will be randomized to learn about epigenetic age acceleration (arm 1) or not to learn about epigenetic age acceleration (arm 2) in a 1:1 allocation at the intervention visit. Those in the intervention arm 1 that are to learn about epigenetic age acceleration will receive some educational materials and a brief description of epigenetic age acceleration.

A telephone visit will be conducted at the end of week 1 for additional diet counseling, to assess safety/potential side effects, to collect adherence diaries. A follow-up phone call for compliance assessment and to answer participant questions will be conducted at the end of week 2. A final measurement visit will be scheduled for the end of week 4 for a morning time and participants will be asked to be fasting.

## 4.1 Toxicities

Side effects will be monitored by staff contact at the end of week 1 visit, and at the end of week 2 (by phone) and at the final measurement visit at the end of week 4. Participants will be encouraged to contact the Study manager during the study period if they feel they are experiencing effects related to the study foods.

EVOO and tree nuts are generally recognized as safe and are consumed by free living adults on a regular basis. The amount to be given in this study are within the range given to the participants in the PREDIMED study [6] and found to have only minor side effects, mainly stomach upset, bloating and nausea, and none of the side effects advanced to serious adverse events.

Administration

Study foods will consist of one ounce of tree nuts per day and two tablespoons of EVOO per day. Participants will be instructed how to consume the proper amount of tree nuts and EVOO and reporting the consumption on the compliance diary (Appendix A). The tree nuts will be provided in daily servings (one ounce per day) and EVOO will be provided in bottles, along with a tablespoon. Participants will be instructed to use the tablespoon to measure EVOO. Participants will also receive recipes and other written information that will assist them in incorporating both the nuts and EVOO into their existing dietary patterns.

Dose Modification/Toxicity management:

Side effects will be monitored via the telephone visit at the end of week 1 and 2 and by participant report. Participants will be encouraged to contact the study team during the study period if they feel they are experiencing side effects related to the study foods. For those participants with existing medical conditions (e.g., gallbladder removal or bariatric surgery) or who are experiencing nausea related to consumption of the olive oil, a titrated dosing plan will be used. These participants will begin their olive oil consumption at 1 tablespoon/day for 5 days and increasing to 2 tablespoons/day on the sixth day if it is well tolerated. If this increase is not tolerated, the participant can be returned to 1 tablespoon per day and attempt an increase after an additional 5 days.

Withdrawal from study intervention

Any side effects experienced by the participant may or may not be due to the study foods. If the side effects become intolerant for the participant, he/she may be withdrawn from the study at the discretion of the study team.

## 4.2 Table of Study-Related Interventions

| Measure | Baseline: In-person screening visit | Intervention visit | End of Week 1 Phone Call | End of week 2 Phone Call | Final in person visit |
|---|---|---|---|---|---|
| **Anthropometrics** | | | | | |
| Body weight | X | | | | X |
| Height | X | | | | X |
| Waist circumference | X | | | | X |
| Blood collection | X | X | | | X |
| Blood pressure | X | | | | X |
| Demographics | X | | | | |
| Mediterranean diet screener | X | | | | X |
| Health History/health habits | X | | | | X |
| Adherence/safety assessment | | | X | X | X |
| Intervention Experience Assessment and Perception of Epigenetic Age Assessment (arm 1 only) | | | | | X |
| Dispense study foods | | X | | | |

## 4.3 Study Visits

### 4.3.1 Baseline Visit: In-person screening

At the in-person screening visit each participant will be greeted by a staff member who will take the candidate to a private consulting room. A final eligibility checklist and each section of the informed consent will be reviewed with the candidate. Once the candidate fully understands each section of the consent, he/she will sign the document and be given a copy to take home. A copy will be retained by the study staff.

After providing informed consent, fasting participants will complete study questionnaires in a quiet private room and show current medications to a staff member that will record the names of medications brought to the clinic. Next, blood pressure will be measured after resting 5 minutes and sitting upright. Blood pressure may be measured twice. Body weight will then be measured using a beam scale with movable weights with participant wearing indoor clothing with no shoes. Height and waist circumference will also be measured. Participant will also have a fasting blood collection. Participants will be asked to complete a validated 14-item Questionnaire of Mediterranean diet adherence [15] to assess their baseline diet including consumption of olive oil and nuts (Appendix B).

### 4.3.2 Intervention visit

Eligible participants (those that provided informed consent, were fasting at the in-person visit and met the criteria for MetS, and provided blood samples for epigenetic age acceleration

measurements) will be contacted to schedule the intervention visit. The study is set to end on March 21, 2022, which means the study intervention visits need to be completed by March 3, 2022. Participants that have not attended their intervention visit by March 3, 2022 will be discontinued from the study.

At the intervention visit, participants will have a fasting blood collection. Participants will receive counseling and recipes to help them incorporate the nuts and EVOO into daily meals. Dietary adherence diaries will be given to participants to measure incorporation of the study foods and participants will be instructed to complete the adherence diaries on a daily basis. Participants will be given a 4-week supply of EVOO, tree nuts, and a tablespoon for measuring the EVOO.

Participants will be randomized to learn about epigenetic age acceleration (arm 1) or to not learn about epigenetic age acceleration (arm 2) in a 1:1 allocation at the baseline visit. Those in arm 1 will receive some educational materials and a brief explanation of epigenetic age acceleration. At the end of this visit, all participants will be scheduled for the remainder of their telephone and in person study visits.

#### 4.3.3 End of week 1 and week 2 telephone calls

During the end of week 1 telephone call, participants will be contacted via telephone and asked questions about their adherence, and any potential side effects observed. Participants will receive additional counseling for how to incorporate the nuts and oil into a daily meal as needed. A follow up phone call at the end of week 2 will be scheduled to assess compliance and to answer participant questions, and a final measurement visit will be scheduled for the end of week 4 for a morning time and participants will be asked to be fasting.

#### 4.3.4 Final measurement visit, end of week 4

The same measures, as listed in the in-person screening visit will be performed. Study questionnaires, with the exception of demographics and health history, will be distributed and completed, and adherence diaries will be collected.
For participants of the intervention arm 1 (participants educated about epigenetic age acceleration), a self-administered exit questionnaire will be given to qualitatively explore participants' perception of epigenetic age, and challenges and motivators for behavior change during the intervention. The open-ended questions will be designed to ascertain understanding of epigenetic age, and assess the challenges and motivators participants encountered during the intervention.
Participants in both arms will be administered an Intervention Experience Assessment to explore how participants' experiences would impact the feasibility of a larger clinical intervention in terms of challenges and motivators.

## 5.0 Study Measures

### 5.1 Anthropometric Measures

Weight: Weight will be assessed using a digital scale in kilograms with participants wearing indoor clothing with no shoes.

Height: Height will be measured using a stadiometer attached to a beam scale, in centimeters, with no shoes worn.

Blood pressure: First-phase systolic and fifth-phase diastolic Korotkoff blood pressure will be auscultated from the right arm of the subject, after resting 5 minutes and sitting upright. We will use a standard manometer with a cuff sized appropriately. . If the blood pressure reading is elevated, it will trigger an "alert" and subsequent participant/physician notification.

Waist circumference**:** Waist circumference will be measured at the midpoint between the lowest rib and the iliac crest.

### 5.2 Blood-based Biomarkers

HDL cholesterol, triglycerides, HbA1c, and fasting glucose levels will be analyzed using serum samples at a local LabCorp facility.

Epigenetic age acceleration: DNA methylation will be measured from blood samples frozen/ stored at -80 C within 2 hours of collection. After recruitment of 50 participants meeting the criteria for MetS is completed, blood samples will be sent for DNA methylation analysis in the lab of Dr. Howard at WFBMC. DNA will be extracted from whole blood samples, and will be bisulfite-converted using the EZ DNA Methylation Gold kit (Zymo, Irvine, CA). Illumina Infinium MethylationEPIC BeadChip, which targets over 850,000 CpG sites, and the Illumina iScan Reader will be used to determine the proportion of DNA methylation at each site. The ratio of the fluorescence intensity of the methylated versus the combined methylated and unmethylated probes will be determined with Illumina GenomeStudio. As a quality control measure, the cumulative fluorescent signal must be significantly greater than the negative controls included for each sample. We will use a threshold of this "detection p-value" of $\leq 0.05$ in at least 90% of the samples as criteria for adequate assays. Finally, adjustments will be made to normalize the fluorescent signals from the Infinium I and Infinium II fluorescent assays used prior to analysis (in Bioconductor).

DNA methylation profiles (beta-value, after adjustment chip and position effects) at 1,030 unique DNA methylation profiles will be used for epigenetic age acceleration predictions, using the DNA GrimAge predictor [11]. We will calculate predicted lifespan and regress predicted lifespan on chronological age to produce estimates of epigenetic age acceleration.

### 5.3 Questionnaires

Demographic information: Name, marital status, date of birth; racial/ethnic group; occupation; education.

Personal medical history: Select questions about the participant's medical history, past and present.

Dietary intake: Participants will be asked to complete Mediterranean Diet 14 item dietary screener.

Current medications: Prescription medications; participants will be instructed to bring medications to the clinic.

Intervention Assessments (see appendix A)
Compliance-Intake of the study foods, tree nuts and EVOO will be tracked on a daily basis using a compliance diary. The participant will record on a daily diary the following information:
Did you eat the nuts today? - Yes, No
Did you consume the oil today? -Yes, No

Telephone contacts and maintenance of the cohort: Telephone contact will be used to monitor participants for study compliance and side effects by phone at the end of week 1 and 2. During the phone call participants will be invited to ask any questions and to discuss any problems with the study, specifically their adherence to the study foods. Participants' medication history will be reviewed.

Perception of Epigenetic Age Assessment -For participants of the intervention arm 1 (participants educated about epigenetic age acceleration), a self-administered exit questionnaire will be given to qualitatively explore participants' perception of epigenetic age, and challenges and motivators for behavior change during the intervention. The questions will be designed to ascertain understanding of epigenetic age, and assess the challenges and motivators participants encountered during the intervention.

Intervention Experience Assessment- Participants in both study arms will be administered an intervention experience assessment to explore how participants' experiences would impact the feasibility of a larger clinical intervention in terms of challenges and motivators.

### 5.4 Duration of Participation in Intervention

Participation will be for a 4-week period unless the following occur:

- Illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Patient decides to withdraw from the study
- General or specific changes in the participant's condition render the patient unacceptable for further treatment in the judgment of the investigator

### 5.5 Duration of Follow Up

Participants will be involved in the study intervention for a period of 4 weeks. Participants removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

### 5.6 Criteria for Removal from Study

Participants will be removed from study when any of the criteria listed above applies. The reason for study removal and the date the participant was removed will be documented in the Case Report Form.
The study is set to end on March 31, 2022. As such, all study intervention visits need to be completed by March 3, 2022. Any participants remaining on study, that have not attended their intervention visit by that date, will be discontinued from the study.

## 6.0 Adverse Events and Reporting Requirements

The risk of harm or discomfort that may happen as a result of taking part in this research study is not expected to be more than in daily life or from routine physical or psychological examinations or tests.

There is risk, although minor, involved in obtaining blood samples. There may be some discomfort, bruising and/or bleeding where the needle is inserted for blood draws. Some people become dizzy, lightheaded, or feel faint. Infection may occur on rare occasions. Frequent donation of blood can result in low iron in the blood.

There is also a risk of weight gain from the increased caloric intake that may occur as a result of consuming the nuts and oils. Participants will be provided with recipes and other information on incorporating the nuts and oils by replacing other foods to reduce the risk of weight gain and will be encouraged to contact the study team if they believe they are gaining weight.

There is a slight risk that study participants may have an unknown allergy to nuts or olive oil and may therefore have an allergic reaction to one of these substances. Mild allergic symptoms that can occur include: raised red bumps of skin – hives, swelling of the lips, tingling of the throat and mouth, itchy skin and rash, runny nose, tightening of the throat, and digestive symptoms – cramps, stomach pain, nausea, or vomiting. If study participants feel they are experiencing one of these mild allergic reactions, they will be instructed to contact the study team and report the reaction.

Although unexpected in adults without prior knowledge of an allergy to tree nuts, consumption of tree nuts can also result in severe allergic reaction symptoms including: difficult or noisy breathing, swelling of the tongue, swelling or tightness of the throat, difficulty talking or a hoarse voice, wheeze or persistent cough, or persistent dizziness or collapse. If study participants feel they are experiencing one of these severe allergic reaction symptoms, they will be instructed to call 911 and seek immediate medical care. After treatment, they will be instructed to contact the study team and report the reaction.

Study staff will report any adverse events to the PI, Dr. Reynolds, who will review all unanticipated problems involving risk to participants or others, serious adverse events, and provide a written report of the event to the IRB. If necessary, the study team will direct appropriate referrals for health care needed to address study-related adverse events.

Results of routine clinical labs and physical measures obtained as part of study visits that meet the criteria for "Alert Values" will be communicated as described in the table below. The PI, Dr. Lindsay Reynolds, will review medical eligibility criteria as needed, as well as clinical measures and laboratory reports. Dr. Kristina Lewis, the Study Medical Director, will serve as the primary contact for staff and participants regarding medical issues and labs and physical measures meeting alert values. Dr. Lewis will review summaries of alerts on a periodic basis along with the AEs.

<u>Alert Values</u>

| Measure | Alert Value | Notify Participant | Notify PCP/Medical Director |
|---|---|---|---|
| Blood Pressure (Avg.) | Level 1<br>SBP ≥180 mm/Hg OR<br>DBP ≥110 mm/Hg | In clinic. Advise to follow-up with PCP within 1 week. | Within 1 week.<br>IF symptomatic (e.g. chest pain, headache, short of breath), notify safety officer and/or PCP immediately. |
| | Level 2<br>SBP ≥160 mm/Hg OR<br>DBP ≥100 mm/Hg (and not Level 1 BP) | In clinic. Advise to follow-up with PCP within 1 month. | Within 1 week.<br>IF symptomatic (e.g. chest pain, headache, short of breath), notify safety officer and/or PCP immediately. |
| | Level 3<br>SBP ≥140 mm/Hg OR<br>DBP ≥90 mm/Hg (and not Levels 1 or 2) | In clinic. Advise to follow-up with PCP within 2 months. | Per routine reporting.<br>IF symptomatic (e.g. chest pain, headache, short of breath), notify safety officer and/or PCP immediately. |
| | SBP ≤90 mm/Hg OR<br>DBP ≤50 mm/Hg | In clinic. Advise to follow-up with PCP within 1 week. | Within 1 week.<br>IF symptomatic (lightheaded, feels faint), notify safety officer and/or PCP immediately. |
| Blood glucose | < 50 mg/dl OR<br>> 400 mg/dl | Within 1 week or sooner as indicated by exact value and clinician judgment. | Within 1 week or sooner as indicated by exact value and clinician judgment. |
| Triglycerides | TG ≥ 1000 mg/dl | Within 1 week or sooner as indicated by exact value and clinician judgment. | Within 1 week or sooner as indicated by exact value and clinician judgment. |

### 6.1 WFHS IRB AE Reporting Requirements

Any unanticipated problems involving risks to subjects or others and adverse events shall be promptly reported to the IRB, according to institutional policy. Reporting to the IRB is required regardless of the funding source, study sponsor, or whether the event involves an investigational or marketed drug, biologic or device. Reportable events are not limited to physical injury, but include psychological, economic and social harm. Reportable events may arise as a result of drugs, biological agents, devices, procedures or other interventions, or as a result of questionnaires, surveys, observations or other interactions with research subjects.

All members of the research team are responsible for the appropriate reporting to the IRB and other applicable parties of unanticipated problems involving risk to subjects or others. The

Principal Investigator, however, is ultimately responsible for ensuring the prompt reporting of unanticipated problems involving risk to subjects or others to the IRB. The Principal Investigator is also responsible for ensuring that all reported unanticipated risks to subjects and others which they receive are reviewed to determine whether the report represents a change in the risks and/or benefits to study participants, and whether any changes in the informed consent, protocol or other study-related documents are required.

Any unanticipated problems involving risks to subjects or others occurring at a site where the study has been approved by the WFHS IRB (internal events) must be reported to the WFHS IRB within 7 calendar days of the investigator or other members of the study team becoming aware of the event.

# 7.0 Data Reporting

Data will be tracked and entered by study staff on a secure server. No data reporting is necessary.

# 8.0 Statistical Considerations

Objectives: This feasibility study, incorporating epigenetic age estimates with a nuts and EVOO intervention in individuals with MetS, is an initial step in building our understanding of the relationship between metabolic syndrome and epigenetic age and how epigenetic age estimates may be perceived by participants with MetS. This study will provide preliminary longitudinal data examining the potential to slow epigenetic age acceleration after a 4-week nuts and EVOO intervention. This longitudinal study is designed to provide pilot information that will be used to design a larger study of the effects of the nuts and EVOO on epigenetic aging. This line of work will be an important foundation towards translating epigenetic age prediction from a research concept towards a tool to meaningfully promote healthy lifestyle changes to improve cardiometabolic health among individuals at high risk for cardiovascular disease.

## 8.1 Prevalence of epigenetic age acceleration

Primary Outcome 1: proportion of participants with MetS with advanced epigenetic aging

To characterize the relationship between MetS and epigenetic aging, we will examine epigenetic age acceleration prevalence among the 50 participants with metabolic syndrome. Epigenetic age acceleration will be calculated as described in 5.2. Advanced epigenetic age acceleration will be defined as a positive epigenetic age acceleration (higher than expected based on chronological age).

## 8.2 Assess adherence

Primary Outcome 2: proportion of days for which EVOO was taken
Primary Outcome 3: proportion of days for which tree nuts were taken
Primary Outcome 4: proportion of days for which EVOO and tree nuts were taken

For adherence, we will examine the daily diaries for intake of the two study foods, tree nuts and EVOO. Adherence for each participant will be measured as the proportion of days in 4 weeks for which nuts, and of EVOO were taken, and by the proportion of days for which both were taken. Problems associated with consuming the study foods will be defined as non-adherence (ceasing to consume either or both study foods) and low adherence (not consuming both foods

on a daily basis). The number of days on average each food was consumed and the overall completion rate of adherence diaries will be used to refine plans to both monitor and boost adherence in a future longer, larger trial.

### 8.3 Participants' perception of epigenetic age, challenges and motivators

Primary outcome 5: Perception of Epigenetic Aging Assessment and Intervention Experience Assessment

To gain preliminary data supporting the feasibility of testing epigenetic age acceleration as a motivating factor for behavior change in a larger trial, participants of the intervention arm 1 (participants educated about epigenetic age acceleration), will be given a self-administered exit questionnaire to qualitatively explore participants' perception of epigenetic age. All participants will given a self-administered Intervention Experience Assessment to explore participant experiences in the study, and the challenges and motivators for behavior change during the intervention. The \ questions will be designed to ascertain understanding of epigenetic age, and assess the challenges and motivators participants encountered during the intervention. All participants will be asked if they can be contacted for future studies.

### 8.4 Test for changes in epigenetic age acceleration

Secondary outcome: Changes in epigenetic age acceleration

It is not clear if changes in nut and EVOO consumption can slow or reverse epigenetic age acceleration. As a secondary goal, we will compare change in epigenetic age acceleration from the baseline visit to the intervention visit (control period) to change in epigenetic age acceleration from the intervention visit to the final visit using a paired sample t-test.

### 8.5 Test for changes in MetS-related traits

Exploratory outcomes: Changes in body mass index, waist circumference, blood pressure, HDL cholesterol, triglycerides, HbA1c, and fasting glucose levels

Although longer clinical trials involving daily consumption of EVOO and nuts have observed improvement in MetS-related traits, it is unclear if improvements in MetS-related traits will be observed after 4-weeks. As an exploratory analysis, we will test for changes in MetS-related traits before vs. after the 4-week intervention, including changes in body mass index, waist circumference, HDL cholesterol, triglycerides, HbA1c, and fasting glucose levels using a paired sample t-test.

### 8.6 Sample size justification

The prevalence of epigenetic age acceleration in people with metabolic syndrome is unknown. Also, it is unknown if a short term nuts and EVOO intervention will have measureable effects on estimates of epigenetic age. Thus, this pilot study will provide feasibility data necessary to estimate sample sizes for larger trails. Assuming 50 individuals and a type 1 error rate of $\alpha=0.05$, the study has 0.80 power to detect difference of 0.4 standard deviations in the difference in epigenetic age and chronological age. We acknowledge that power is a limitation of the current study, and designed the study to maximize the sample size based on budget constraints to provide prevalence and potential effect size estimates to guide power calculations when planning a future larger, longer trial.

## References


1. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart JC, James WP, Loria CM, Smith SC, Jr.: **Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity.** *Circulation* 2009, **120:**1640-1645.
2. Moore JX, Chaudhary N, Akinyemiju T: **Metabolic Syndrome Prevalence by Race/Ethnicity and Sex in the United States, National Health and Nutrition Examination Survey, 1988-2012.** *Prev Chronic Dis* 2017, **14:**E24.
3. Grundy SM: **Pre-diabetes, metabolic syndrome, and cardiovascular risk.** *J Am Coll Cardiol* 2012, **59:**635-643.
4. Powell LH, Appelhans BM, Ventrelle J, Karavolos K, March ML, Ong JC, Fitzpatrick SL, Normand P, Dawar R, Kazlauskaite R: **Development of a lifestyle intervention for the metabolic syndrome: Discovery through proof-of-concept.** *Health Psychol* 2018, **37:**929-939.
5. Grundy SM: **Metabolic syndrome update.** *Trends Cardiovasc Med* 2016, **26:**364-373.
6. Estruch R, Ros E, Salas-Salvado J, Covas MI, Corella D, Aros F, Gomez-Gracia E, Ruiz-Gutierrez V, Fiol M, Lapetra J, et al: **Primary Prevention of Cardiovascular Disease with a Mediterranean Diet Supplemented with Extra-Virgin Olive Oil or Nuts.** *N Engl J Med* 2018, **378:**e34.
7. Babio N, Toledo E, Estruch R, Ros E, Martinez-Gonzalez MA, Castaner O, Bullo M, Corella D, Aros F, Gomez-Gracia E, et al: **Mediterranean diets and metabolic syndrome status in the PREDIMED randomized trial.** *Cmaj* 2014, **186:**E649-657.
8. Downer MK, Gea A, Stampfer M, Sanchez-Tainta A, Corella D, Salas-Salvado J, Ros E, Estruch R, Fito M, Gomez-Gracia E, et al: **Predictors of short- and long-term adherence with a Mediterranean-type diet intervention: the PREDIMED randomized trial.** *Int J Behav Nutr Phys Act* 2016, **13:**67.
9. Chen BH, Marioni RE, Colicino E, Peters MJ, Ward-Caviness CK, Tsai PC, Roetker NS, Just AC, Demerath EW, Guan W, et al: **DNA methylation-based measures of biological age: meta-analysis predicting time to death.** *Aging (Albany NY)* 2016, **8:**1844-1865.
10. Horvath S: **DNA methylation age of human tissues and cell types.** *Genome Biol* 2013, **14:**R115.
11. Lu AT, Quach A, Wilson JG, Reiner AP, Aviv A, Raj K, Hou L, Baccarelli AA, Li Y, Stewart JD, et al: **DNA methylation GrimAge strongly predicts lifespan and healthspan.** *Aging (Albany NY)* 2019, **11:**303-327.
12. Hannum G, Guinney J, Zhao L, Zhang L, Hughes G, Sadda S, Klotzle B, Bibikova M, Fan JB, Gao Y, et al: **Genome-wide methylation profiles reveal quantitative views of human aging rates.** *Mol Cell* 2013, **49:**359-367.
13. Quach A, Levine ME, Tanaka T, Lu AT, Chen BH, Ferrucci L, Ritz B, Bandinelli S, Neuhouser ML, Beasley JM, et al: **Epigenetic clock analysis of diet, exercise, education, and lifestyle factors.** *Aging (Albany NY)* 2017, **9:**419-446.

14. Nannini DR, Joyce BT, Zheng Y, Gao T, Liu L, Yoon G, Huan T, Ma J, Jacobs DR, Jr., Wilkins JT, et al: **Epigenetic age acceleration and metabolic syndrome in the coronary artery risk development in young adults study.** *Clin Epigenetics* 2019, **11:**160.
15. Martinez-Gonzalez MA, Garcia-Arellano A, Toledo E, Salas-Salvado J, Buil-Cosiales P, Corella D, Covas MI, Schroder H, Aros F, Gomez-Gracia E, et al: **A 14-item Mediterranean diet assessment tool and obesity indexes among high-risk subjects: the PREDIMED trial.** *PLoS One* 2012, **7:**e43134.

## Appendix A

### NUTS and OIL CONSUMPTION DIARY

1. Make an entry in this diary every day during the study, at roughly the same time each day.
2. For each day, circle either "Yes" or "No" to indicate whether you ate the nuts and oil.
3. Return the completed assessment forms to the study staff at your visit to the clinic.
4. There is space at the bottom of the form for you to add comments (good or bad) regarding your daily consumption of the nuts and oil.
5. THANK YOU FOR BEING A PART OF THIS STUDY!

| | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 |
|---|---|---|---|---|---|---|---|
| Day of Week | MON | TUE | WED | THU | FRI | SAT | SUN |
| Date (MM/DD) | □□/□□ | □□/□□ | □□/□□ | □□/□□ | □□/□□ | □□/□□ | □□/□□ |
| Did you consume a one ounce portion of nuts today? | Yes / No | Yes / No | Yes / No | Yes / No | Yes / No | Yes / No | Yes / No |
| Did you consume 2 tablespoons of olive oil today? | Yes / No | Yes / No | Yes / No | Yes / No | Yes / No | Yes / No | Yes / No |

Adherence Diary

Comments:

**Appendix B.**

**14-item Questionnaire of Mediterranean diet adherence**

**1. Do you use olive oil as your main cooking fat?**

1☐ Yes

2☐ No

**2. How much olive oil do you consume in a given day (including oil used for frying, salads, restaurant meals, etc.)?**

☐☐ tablespoons

**3. How many vegetable servings do you consume per day? (1 serving is ½ a cup of cooked vegetables or 1 cup of raw vegetables)**

☐☐ servings

**4. How many fruit servings, including natural fruit juices, do you consume per day? ( 1 serving is a medium fresh fruit; ½ cup of chopped, cooked, or canned fruit; ½ cup of juice)**

☐☐ servings

**5. How many servings of red meat, hamburger, or meat products (ham, sausage, etc.) do you consume per day? (1 serving is 2-3 ounces)**

☐☐ servings

**6. How many servings of butter, margarine, or cream do you eat per day? (1 serving is 1 Tbsp.)**

☐☐ servings

**7. How many sweet or carbonated beverages do you drink per day?**

☐☐ beverages

**8. How much wine do you drink per week?**

☐☐ glasses

**9. How many servings of legumes do you consume per week? (1 serving is ½ cup of beans or lentils)**

☐☐ servings

**10. How many serving of fish or shellfish do you consume per week? (1 serving is 2-3 ounces)**

☐☐ servings

**11. How many times per week do you consume commercial sweets or pastries (not homemade), such as cakes, cookies, pies, or pudding?**

☐☐ times

**12. How many servings of nuts (including peanuts) do you consume per week? (1 serving is ¼ cup of nuts or 2 tablespoons of nut butter)**

☐☐ servings

**13. Do you preferentially consume chicken, turkey, or rabbit meat instead of veal, pork, hamburger, or sausage?**

1☐ Yes

2☐ No

**14. How many times per week do you consume vegetables, pasta, rice, or other dishes seasoned with sauce made with tomato and onion, leek, or garlic and simmered with olive oil?**

☐☐ times

**Appendix C**

## STUDY EVALUATION

1. **How often during the course of your participation in this study did you <u>replace</u> foods in your diet with the tree nuts and olive oil?**

| -2 | -1 | 0 | 1 | 2 |
|---|---|---|---|---|
| Never | Very Rarely | Occasionally | Very Often | Always |

2. **How often during the course of your participation in this study did you eat the tree nuts and olive oil in <u>addition</u> to the other foods in your diet?**

| -2 | -1 | 0 | 1 | 2 |
|---|---|---|---|---|
| Never | Very Rarely | Occasionally | Very Often | Always |

3. **On a scale of 1 to 10, how difficult did you find it to eat the tree nuts and olive oil every day?**

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|
| ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |

NOT AT ALL DIFFICULT — MODERATELY DIFFICULT — VERY DIFFICULT

4. **On a scale of 1 to 10, how difficult did you find it to consume the olive oil every day?**

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|
| ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |

NOT AT ALL DIFFICULT — MODERATELY DIFFICULT — VERY DIFFICULT

**5. On a scale of 1 to 10, how did eating the tree nuts and olive oil daily make you feel?**

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|
| ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |

NOT GOOD AT ALL — NO DIFFERENCE — VERY GOOD

**6. On a scale of 1 to 10, how did consuming the olive oil daily make you feel?**

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|
| ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |

NOT GOOD AT ALL — NO DIFFERENCE — VERY GOOD

**7. Did you use the provided recipes to help incorporate the tree nuts and olive oil into your diet?**

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| Not at all | Not much | Sometimes | Often | Very often |

**8. On a scale of 1 to 5 (with 5 being the very best I have tasted), how much did you like the flavor of the olive oil?**

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| Did not like at all | Disliked somewhat | Neither liked or disliked | Liked somewhat | Liked a great deal |

**9. On a scale of 1 to 5 (with 5 being the very best I have eaten), how much did you like the walnuts?**

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| Did not like at all | Disliked somewhat | Neither liked or disliked | Liked somewhat | Liked a great deal |

**10. On a scale of 1 to 5 (with 5 being the very best I have eaten), how much did you like the almonds?**

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| Did not like at all | Disliked somewhat | Neither liked or disliked | Liked somewhat | Liked a great deal |

**11. On a scale of 1 to 5 (with 5 being the very best I have eaten), how much did you like the pistachios?**

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| Did not like at all | Disliked somewhat | Neither liked or disliked | Liked somewhat | Liked a great deal |

**12. Will you continue to eat tree nuts and olive oil daily after completing the research study?**

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| Very unlikely | Unlikely | Neutral | Somewhat likely | Very likely |

**13. Will you continue to eat olive oil daily after completing the research study?**

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| Very unlikely | Unlikely | Neutral | Somewhat likely | Very likely |

**14. If eating tree nuts and olive oil improved your metabolic health, how likely would you be to continue to eat tree nuts and olive oil daily?**

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| Very unlikely | Unlikely | Neutral | Somewhat likely | Very likely |

**15. If consuming olive oil improved your metabolic health, how likely would you be to continue to consume olive oil daily?**

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| Very unlikely | Unlikely | Neutral | Somewhat likely | Very likely |

**16. Do you think that you would be able to participate in a study like this one that lasted 3-4 years? In other words, would you be able to continue eating the tree nuts and olive oil for 3-4 years?**

1☐ Yes
2☐ No

If no, why not?

**17. Please describe any specific problems or barriers that you encountered that made it difficult for you to eat the tree nuts and olive oil each day.**

**18. Please describe any suggestions you have that would make eating the tree nuts and olive oil each day easier for participants in future studies.**

**19. Can Dr. Reynolds or someone she designates contact you for future studies?**

1 ☐ Yes
2 ☐ No

**Appendix D.**

**We would like to ask you some questions about epigenetic age. Epigenetic age is a DNA-based measure that predicts the biological age of your cells. Having an accelerated epigenetic age means that the biological age of your cells may be more advanced than your chronological age.**

**20. Using the scale below, indicate how much you want to know your epigenetic age:**

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| Not at all | Not much | Neutral | Somewhat | Very much |

**21. For question #1, why did you select that answer?**

**22. Before beginning this study, if you were told your epigenetic age appeared <u>more advanced than your chronological age</u>, do you think you would be more or less motivated to eat the nuts and olive oil everyday?**

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| Not motivated at all | Less motivated | Neutral | More motivated | Very motivated |

**23. Before beginning this study, if you were told your epigenetic age appeared <u>younger than your chronological age</u>, do you think you would be more or less motivated to eat the nuts and olive oil everyday?**

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| Not motivated at all | Less motivated | Neutral | More motivated | Very motivated |

**24. If eating tree nuts and olive oil slowed biological aging, how likely would you be to continue to eat tree nuts and olive oil daily?**

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| Very unlikely | Unlikely | Neutral | Somewhat likely | Very likely |

**25. If consuming olive oil slowed biological aging, how likely would you be to continue to consume olive oil daily?**

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| Very unlikely | Unlikely | Neutral | Somewhat likely | Very likely |

**26. Do you feel we did a good job explaining the concept of epigenetic aging?**

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| Not at all | Not much | Neutral | Somewhat | Very much |

**27. For question #76, can you comment on why you selected that answer?**