RESEARCH PROTOCOL OUTLINE

Title of Project: Does insulin sensitivity impact the potential of metformin to slow aging?

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Abstract

Aging is the number one risk factor for the majority of chronic diseases. There are no pharmaceutical treatments to slow aging and prolong health span. The anti-diabetic drug metformin is considered a likely pharmaceutical candidate to slow aging. In this study, we hypothesize that metformin treatment in subjects free of type 2 diabetes will improve insulin sensitivity and glucoregulation in insulin resistant individuals, but will decrease insulin sensitivity and glucoregulation in insulin sensitive subjects. Further, we hypothesize that long-term metformin treatment will remodel mitochondria in a way that decreases mitochondrial function in subjects that are insulin sensitive, but improves mitochondrial function in subjects that are insulin resistant. We will use a dual-site, 12-week drug intervention trial performed in a double-blind, placebo-controlled manner on 148 subjects recruited from two separate sites (Oklahoma Medical Research Foundation (OMRF) and University of Wisconsin-Madison). After consent and initial subject screening for chronic disease, subjects will be stratified to insulin sensitive (IS) or insulin resistant (IR) groups. Over a 12-week intervention, half of each group will take metformin and half will take a placebo. Pre- and post-intervention, subjects will complete a series of procedures to assess insulin sensitivity, glucose regulation, and biomarkers of aging. The same subjects will provide a skeletal muscle biopsy pre- and post-intervention to assess the change in mitochondrial function and mitochondrial remodeling with and without metformin treatment. By completion of this project, we expect to provide evidence that helps further delineate who may benefit from metformin treatment to slow aging.

Specific Aims

Aim 1: Determine how antecedent metabolic health affects the response to metformin treatment. *Hypothesis 1: Metformin treatment in subjects free of T2D will improve insulin sensitivity and glucoregulation in insulin resistant individuals, but will decrease insulin sensitivity and glucoregulation in insulin sensitive subjects.* We will perform a 12-week randomized, double-blind clinical trial with subjects stratified by insulin sensitivity to directly test if metformin outcomes are dependent on metabolic health. We will perform comprehensive assessments of insulin sensitivity, glucose control, and blood-based biomarkers of aging.

Aim 2: Identify the relationship between skeletal muscle mitochondrial remodeling and mitochondrial function with metformin treatment. *Hypothesis 2: Long-term metformin treatment will remodel mitochondria in a way that decreases mitochondrial function in subjects that are insulin sensitive, but improves mitochondrial function in subjects that are insulin resistant.* We will use novel stable isotope labeling with proteomic analysis to determine mitochondrial, including complex I, remodeling. This approach will be combined with analysis of protein modification and turnover to comprehensively analyze mitochondrial effects of metformin treatment in skeletal muscle.

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Background and Significance

1. Sketch the background leading to this study, evaluate existing knowledge, and identify gaps this study will fill. State the importance of the research by relating the specific aims to the long-term objectives.

The Targeting Aging with Metformin (TAME) trial is a proposed clinical trial that could be the first to examine an intervention to slow aging rather than to treat a specific age-related chronic disease (1). Metformin has 60 years of documented safety and is relatively inexpensive, making it an attractive potential aging treatment. Evidence supporting the use of metformin to increase lifespan and health outcomes originates from lower order model organisms, whereas rodent models have been more mixed. Although the majority of health outcomes improve in rodent models, lifespan either increases or remains unchanged with metformin treatment. In human clinical trials, retrospective data and trials in populations with type II diabetes (T2D) show improved overall survival with metformin, as well as decreased risk of cardiovascular disease, cancer incidence, and cognitive decline. A recent meta-analysis of clinical trials in human subjects demonstrated that diabetics taking metformin have lower all-cause mortality than diabetics and the general population. This study also indicated that diabetics taking metformin had decreased incidence of cancer compared to the general population, and decreased cardiovascular disease compared to diabetics not on metformin. Although these human trials build a strong rationale for proposed study, *the completed studies were in subjects with T2D and/or other co-morbidities, and none were in subjects absent of disease.* Our published data demonstrate that some subjects have *detrimental outcomes* to metformin (4). Our new findings are supported by the landmark Diabetes Prevention Program trial that examined the progression to T2D in 3234 subjects at risk for diabetes (3). Over the three years of study, the metformin treatment group (n=1073) had significantly less progression to T2D compared to controls. However, the effect of metformin was minimal in those with a lower BMI and lower fasting glucose. These data suggest *that the healthier subjects did not reap the same benefits from metformin as their relatively less healthy counterparts*.

The National Institute on Aging (NIA) has specified that there is a significant need to "*develop new therapeutic options to prevent or delay the onset of deleterious changes that contribute to morbidity among older adults"*. There are contradictory findings about chronic metformin treatment, and the effects of metformin treatment in those free of disease are largely unknown. Although there are studies using metformin treatment to delay morbidity in rodent models, there is a noted lack of investigations examining metformin use in a middle-to-older-aged human population absent of metabolic disease. Importantly, within a population of disease-free individuals there is a range of metabolic health and insulin sensitivity, which makes some more at risk for chronic disease than others. In addition, studies are needed to determine the mechanisms through which metformin has potentially varying effects on aging-related outcomes. Two critical questions that remain unanswered in human subjects: 1) does antecedent metabolic health influence responses to metformin, and 2) does long-term treatment with metformin lead to mitochondrial remodeling that changes function. By the conclusion of this study, we will have provided insight into which subjects could benefit from metformin treatment, thereby informing the design of a larger clinical trial.

2. Briefly describe pre-clinical and clinical data, current experience with procedures, drug, or device, and any other relevant information to justify the research.

Recently, we performed a randomized, double-blinded clinical trial on 53 sedentary subjects that had one risk factor for type 2 diabetes (T2D) (4). The subjects completed 12-weeks of aerobic exercise training while taking placebo or metformin. The placebo group had the expected improvements in insulin sensitivity from exercise training and mitochondrial complex I activity. However, metformin abolished the exercise-induced improvement in mitochondrial respiration and insulin sensitivity. Upon further inspection, the group taking metformin, ***but not placebo***, had a striking degree of inter-subject variability with half the subjects in the metformin group having the expected exercise-induced improvement in insulin sensitivity while half had ***no improvement or a decrement*** in insulin sensitivity. We retrospectively identified that the negative responders were subjects that enrolled in the study with lower fasting insulin, greater insulin sensitivity, and greater skeletal muscle mitochondrial complex I activity. These data suggest that in some subjects, metformin has detrimental

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metabolic outcomes that could accelerate aging. In addition, they identify a critical need to directly test if subjects currently free of chronic disease will benefit from metformin treatment in a way that is consistent with slowing the aging process.

All procedures proposed in the current study have been performed previously by the investigators. The majority of these procedures, including metformin administration, stable isotope labeling, muscle biopsies, blood draws, continuous glucose monitoring, subject screening, among others, are in our recent publication (4).

Preliminary Studies/Progress Report

Our previously published study (4) provides the justification for the current study. There are no other unpublished preliminary data that have informed the current study design.

Research Design and Methods (What, When, How, Where)

We will utilize a dual-site, 12-week drug intervention trial performed in a double-blind, placebo-controlled manner. The study design (**Figure 1**) mimics a human metformin intervention previously performed by our team. Dr. Miller will perform all clinical procedures in the Oklahoma Clinical and Translational Science Institute (OCTSI), which is housed in the Harold Hamm Diabetes Center at The University of Oklahoma Health Sciences Center (OUHSC) where he has a joint appointment. In addition, Dr. Miller will perform all analytical procedures in the Aging and Metabolism Research Program at the Oklahoma Medical Research Foundation (OMRF). Dr. Konopka will perform all clinical procedures with human subjects in the Clinical Research Unit located within the Institute for Clinical and Translational Research in the University of Wisconsin Hospital at the University of Wisconsin-Madison (UW-M). Dr. Konopka will perform biospecimen analysis in his laboratory located in the Geriatric Research Education and Clinical Center (GRECC) at the William S. Middleton Madison VA Hospital.

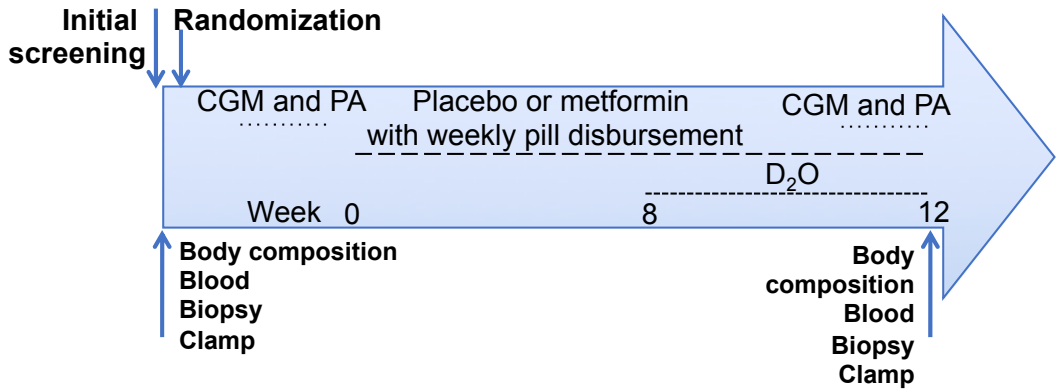


Figure 1. Overall study design at OMRF and UIUC sites.

Study Procedures

Initial phone screening for eligibility

General eligibility (as defined by inclusion/exclusion criteria) will be determined via phone Pre-Screening questionnaire. Prospective participants will be scheduled by phone/email for a screening visit. Final eligibility will be determined (as defined by inclusion/exclusion criteria) on the basis of outcome measures performed after written, informed consent has been obtained, and approved by the medical monitor at each site.

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Visit 1--Medical Screening Visit (2 hours)
Review of informed consent may be an in-person clinic visit or a virtual visit through a secure platform (Microsoft Teams or Zoom). When reviewing the informed consent virtually, the subject will sign the consent in person when arriving for medical screening. After reviewing questions and obtaining a signature, the medical screening visit will be completed at the OSCTI or UW-M. The screening visit will include a medical history, resting ECG, pulmonary function, fasting blood draw, anthropometric measurement (height, and weight). Approximately one tablespoon of blood will be drawn to collect baseline levels for HgA1c, CMP, glucose insulinand B12. From the blood draw we will calculate HOMA-IR for stratification into insulin sensitive (IS) and insulin resistance (IR) groups. Pregnancy test will be completed for women of childbearing potential (WOCB). Medical history will be collected per patient report. The medical history and assessments will be reviewed by the medical monitor per site, along with Inclusion and Exclusion criteria prior to baseline assessments.

Physical Activity (PA) Monitoring. To objectively assess chronic physical activity of the participants, and the potential changes in PA over the course of the study, we will use the 7-Day PA recall questionnaire to monitor the period leading into the study as well as PA during the last week of the study.

Once the screening labs are received and reviewed, and the subject has been confirmed by the medical monitor as eligible to continue on to the 12-week drug intervention, the subjects will be randomized (1:1) between metformin and placebo using the REDCap Randomization database created for this study. The randomization sequence will be stratified by study center (OUHSC or UW-M) and baseline insulin resistance status (HOMA-IR ≤2.2 = insulin sensitive, ≥2.5 = insulin resistant).The randomization code will then be sent to the pharmacy for distribution for the subject.

Visit 2 Baseline DEXA/CGM Placement (1-2 hours) *Up to 90 days after Visit 1*
No more than 90 days following Visit 1, participants will return to the study site for a DEXA scan (body composition) and CGM placement. A pregnancy test is completed for women of childbearing potential before performing the DEXA scan. We will perform real-time continuous glucose monitoring (CGM) with the commercially available Dexcom G6 system as we have previously performed (4), which will collect interstitial glucose values every 5 minutes. The CGM will be optional for participants and will be applied on a first come, first serve basis. We will insert the glucose sensor into the subcutaneous adipose tissue near the umbilical region of the abdomen to monitor interstitial glucose concentrations for 7-10 consecutive days. We will set the CGM device in blinded mode to prevent subjects from manipulating their normal diet or physical activity habits based on their real-time glucose values. This will allow for evaluation of parameters of glucose variability.

Physical Activity (PA) Monitoring. To objectively assess chronic physical activity of the participants, and the potential changes in PA over the course of the study, we will use the 7-Day PA recall questionnaire to monitor the period leading into the study as well as PA during the last week of the study.

At the end of the visit, subjects will be given a standardized meal to consume the night before Visit 3.

Visit 3 (Week 1) *Day 0*—Baseline Muscle Biopsy and Insulin Clamp, Dispense IP (4-5 Hours) *8±2 days after Visit 2*
The evening prior to Visit 3 the subject will consume a standardized evening meal, then complete an overnight fast. They will then come to the clinic (OSCTI or UW-M) the next morning, fasting. Subjects will fast from everything aside from water for a minimum of 8 hours, up to 12 hours. Subjects will also

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fast from alcohol and exercise for 24 hours prior to this visit. The study visit will consist of a fasting blood draw for biomarkers, a muscle biopsy, and a hyperinsulinemic-euglycemic clamp. During this visit, approximately 108 ml of blood will be drawn in total for all procedures.

Following the insulin clamp procedure, the subject will return the CGM.

The muscle biopsy procedure involves obtaining a small (30-150 mg) tissue sample through a 6-8 mm incision that is made under local anesthesia. The sample site will be the vastus lateralis, a large muscle of the quadriceps that weighs several kilograms in adults.

Following all procedures, the patients will be dispensed IP per the randomization assignment at Visit 1. Subjects will be instructed to take 1 pill from the bottle once daily in the morning. Subjects will be encouraged to consume food along with the IP to decrease risk of gastric distress.

Medical oversight will be in the room or building for the first few muscle biopsies and hyperinsulinemic-euglycemic clamps.

Visit 4 (Week 2) *7±2 days after baseline* and 5 (Week 3) *14±2 days after baseline* Metformin Titration (1 Hour)
Subjects will return to the clinic approximately 1 week after they begin IP dosing for Visit 4. IP Compliance will be measured. If the study subjects fall out of 80-120% compliance, they will be retrained in IP dosing.

Study site will query the study subjects for Adverse Events, IP tolerance, and concomitant medications. Once the subject has confirmed IP tolerance, they will be titrated up to 1000 mg total daily dose for the next week. IP will be taken 500 mg in the morning and 500 mg in the evening. Subjects are encouraged consume food with the IP.

Visit 5 (*Week 3, 14±2 days after baseline)* is conducted with the same assessments. If the study subjects are tolerating the IP, their dose will be increased to the maximum study dose of 1500 mg total daily dose. This is taken as two 500 mg tablets in the morning and one 500 mg tablet in the evening. IP dosing with food is encouraged. If the subject is unable to tolerate a minimum of 1000 mg total daily dose, they will be discontinued in the study.

Visit 6 (Week 4 *Day 21±2 days after baseline*), 8 (Week 6 *Day 35±2 days after baseline*), 10 (Week 8 *Day 49±2 days after baseline*) and 12 (Week 10 *Day 63±2 days after baseline*) Telephone Visit (15 minutes)
Telephone calls are conducted at visits 6, 8, 10, and 12 to discuss adverse events, IP tolerance and concomitant medications changes with the study subject. At visit 6 if the subject is not tolerating the maximum dose of 1500 mg total daily dose, they will be reduced back to the 1000 mg total daily dose. If the patient confirms tolerance at 1500 mg total daily dose, they will stay at this dose for the remainder of the study. The study coordinator conducting the telephone call visit will also review study compliance at these visits.

Visits 7 (Week 5 *Day 28±2 days after baseline*) and 9 (Week 7 *Day 42±2 days after baseline*) Clinic Visits (1 hour)
Subjects will return to the clinic for AE and concomitant medication assessment. The subjects will return their pill bottle to the site for compliance. Compliance outside of 80-120% will be documented, and subjects will be retrained in study IP dosing. The subjects will also be dispensed a new study IP bottle.

At Visit 7, safety labs will be obtained to assess liver and kidney function, and the B12 level.

Visits 11 (Week 9 *Day 56±2 days after baseline*) and 13 (Week 11 *Day 70±2 days after baseline*) Clinic Visit DO_2 Disbursement (1 hour)

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Subjects will return to the clinic for AE and concomitant medication assessment. The subjects will return their pill bottle to the site for compliance. Compliance outside of 80-120% will be documented, and subjects will be retrained in study IP dosing. The subjects will also be dispensed a new study IP bottle. The subjects will also receive a stable isotope of water called deuterium oxide (D_2O). This isotope is a naturally occurring, **non-radioactive** isotope that is biologically safe and indistinguishable from normal water. The primary endpoints will be evaluated at 12 weeks with one additional visit.

Participants will be asked to take the water three times daily for the first week, and twice daily for the last three weeks. Subjects will be dispensed with 50 mL bottles, and drink directly from the bottle. For the first week, they will be asked to use a schedule of drinking one 50 mL bottle at each morning, noon, and evening. For the last three weeks, the schedule will be adjusted to one bottle in the morning, and one bottle at night.

Visit 14 (Week 12 *Day 77±2 days after baseline*) End of Treatment (2-3 Hour)
Subject will return to clinic during week 12 of the intervention period. The subjects will repeat the DEXA scan and complete the 7-day PA recall questionnaire. Subjects will be disbursed the standardized meal for consumption the evening prior to the end of study insulin clamp procedure. At this visit the subject will also have a CGM placed for 7-10 day collection.

Investigational product compliance will be measured for the final bottle, which the subject will return to the site at this visit. Visit compliance as well as overall study drug compliance will be calculated. Study subject will be queried for adverse events and changes in concomitant medications?

Visit 15 End of Study *Day 84+3 days after baseline* (7-8 Hours)
The subject will return to repeat the fasting blood draw, muscle biopsy, hyperinsulinemic-euglycemic clamp, and CGM removal.

Visit 16 End of Study *Day 91±2 days after baseline* (15 Minutes)
Site personnel will contact the subject to follow up on any ongoing adverse events, or the presence of any new adverse events.

Metformin protocol

We use a “ramp up” dosing protocol in which the amount of metformin (provided by Belmar Pharmaceuticals, Metformin 500mg, NDC: 65862-0008-05 [Aurobindo Pharma]) will begin at 500 mg/day in week 1, increase to 1000 mg/day in week 2, and then to 1500 mg/day in week 3, as tolerated. At week 3 and for the remaining 9 weeks, the dose will remain at 1500 mg/day, which is a standard clinical dose (1500-2000 mg/day). The dosing schedule will be two 500 mg tablets in the morning, and one 500 mg tablet in the evening. If a subject has gastrointestinal discomfort with 1500 mg/day the dose, we will lower the dose to 1000 mg/day. 1000 mg is the minimum dose to stay enrolled in the study.

Biospecimens
Blood markers. To determine whether the proposed biomarkers for the TAME trial (2) predict changes in disease-free individuals, we will assess these outcomes pre- and post-drug intervention. In appropriately de-identified treated blood samples, we will measure IL-6, CRP, Tumor Necrosis Factor-α RII, GDF15, IGF-1, Cystatin- C, B-type natriuretic peptides (NT-proBNP), HbA1C and DNA methylation. These analyses will be performed in the *OMRF Biorepository: Sample Procurement and Management Core* using the Bioplex200 bead-array system for xMAP (Luminex style) in a 4-plex (4 analytes/sample), 96-well format assay, or in the Geroscience Sequencing Core at OMRF. Analysis of the markers will be compared to control (matched age, sex, race) plasma samples. All samples will be randomized across plates in order to control for bias. Protein based measurements will be assessed for quality and reproducibility through our standard QC SOP's for xMAP bead-

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arrays based on CAP and FDA guidelines for similar assays for determining limits of detection and quantitation. Analytes with >50% of the measured values for a particular collection below the LLOQ (lower limit of quantitation) will be scored as present or absent and not used as continuous variables in subsequent statistical analyses. Additionally, biomarkers will be assessed in plasma samples using analyte-specific direct ELISAs that are commercially available. These analyses will be compared to control (matched age, sex, race) plasma samples. All samples will be randomized across plates in order to control for bias. ELISAs will be read on standard plate readers and will be assessed using the manufacture-recommended analytical parameters. A final blood sample from each subject will be analyzed for metformin concentration by the *San Antonio Nathan Shock Center Bioanalytical Pharmacology and Drug Evaluation Core*.

Banking/Repository/Database

Barcoded, de-identified muscle and blood samples that are not exhausted for the proposed measurements will be stored in Dr. Miller's lab. The samples will only be used for additional outcomes that may inform the present study, or for exploratory (non-publishable) data for future studies. The potential samples will be the result of extra tissue from the initial and final blood sample and muscle biopsy. Patients have the option to withdraw from their participation in the biorepository at any time.

Inclusion / Exclusion Criteria

Inclusion criteria

- 40-75 years of age
- Free of chronic disease
- A mental capacity and comprehension where the subject can provide legal consent or understand information regarding the side effects of the study drug.

- Able to use and be contacted by telephone.
- Ability to understand verbal and/or written English
- Independently mobile

Exclusion criteria

- Pregnancy
- Males who expect to conceive in the next 90 days or in the 90 days after trial participation ends.
- Heart disease or an ECG suggestive of a history of coronary ischemia, or other pathology judged by the clinician to increase the risk of the patient taking Metformin
- Cerebrovascular disease (history)
- Cancer or in remission in the last 5 years (history)
- Chronic respiratory disease (history, FEV1/FVC < 70, FEV1 < 80% predicted)
- Chronic liver disease (history, abnormal blood liver panel with more than one value out of range for ALT: >52units per liter (U/L))
- Diabetes (history, HbA1C ≥ 6.5, fasting blood glucose≥126 mg/dl)
- Alzheimer's (history)
- Chronic kidney disease (history, abnormal blood kidney panel including serum creatinine > 1.4)
- Problems with bleeding, on medication that prolongs bleeding time (if subject cannot safely stop prior to biopsy)
- Those on glucose lowering drugs

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- Those planning to have imaging that requires intravenous contrast dye (within 6 weeks) or are on any of the following medications since they are contraindicated with the use of metformin: Dofetilide, Lamotrigine, Pegvisomant, Somatropin, Trimethoprim, Trospium, Gatifloxacin, Cephalexin, Cimetidine, Dalfampridine
- Tobacco use
- Allergies to lidocaine or metformin
- Alcohol use which is equal to or greater than the following guidelines of greater than 3 drinks on any single day or no more than 7 drinks a week for women, and no more than 4 drinks on a single day or greater than 14 drinks a week for men.
- Vitamin B12<193 pg/mL

Gender/Minority/Pediatric Inclusion for Research

The proposed studies will include men and women in equal numbers from 40-75 years of age of all races and ethnic backgrounds. The minority/majority group distribution will be representative of the associated demographics of the Oklahoma City and the Madison Metropolitan area.

Aging affects both sexes and all racial and ethnic groups. In addition, there are no data supporting sex, race, or ethnic differences in metformin efficacy for aging. Therefore, we have targeted our enrollment to be consistent with our subject demographics.

Children under 18 will not be included in this project because the scientific goals of this project focus on aging.

Recruitment and Enrollment

This study will recruit 248 participants who are between 40 and 75 years of age and not being treated with glucose lowering drugs (see inclusion/exclusion criteria). These individuals will be recruited through the Oklahoma Shared Clinical and Translational Resources (OSCTR) at the University of Oklahoma Health Sciences Center (OUHSC) in Oklahoma City, and through the University of Wisconsin-Madison (please see letters of support from the OSCTR and Dr. Konopka). Target recruitment will be 50% of subjects at each site, with half in each insulin sensitivity category (HOMA-IR ≤2.2 versus ≥2.5). Both sites have a history of successful recruitment for studies of this size, and we recently completed a double-blind, study using a combination of 12-weeks of metformin or placebo with exercise. At one site in two years, we completed data collection on 53 older adults (mean age 63 yrs.) who were absent of overt chronic disease but had at least one risk factor for T2D. Recruitment through the OSCTR offers additional unique strategies for recruiting, such as a partnership with the Oklahoma Blood Institute that allows us to selectively reach approximately 290,000 independent blood donors based on their potential eligibility for the study. In addition, the OSCTR can recontact >1,000 previous study participants in the Oklahoma Immune cohort and >1,000 diabetes patients in the Harold Hamm Diabetes Center Registry with flyers that they can share with unaffected relatives and friends. At UW-M, we will use the Institute of Clinical and Translational Research (ICTR) Research Recruitment and Retention (R3) program to ensure timely recruitment and connect with local and university stakeholders. We will also capitalize on the large pool of healthy controls from ongoing studies in the Alzheimer's Disease Research Center, the Osteoporosis and Sarcopenia Research Program, and the Wisconsin Longevity Study. Further, Geriatrics and Gerontology is the largest Division within the Department of Medicine and therefore we will work with primary care clinicians to discuss our study with patients. Last, we will use the Clinical Research Data Service (CRDS) to identify individuals who fit the inclusion/exclusion criteria and contact them through approved advertisements listed below. The study will be advertised through IRB-approved, previously successful activities, such as posting to institutional websites and social media, group e-mails to OUHSC, OMRF, and UW-M personnel, advertisements in local online and print media, and TV coverage of the study. Written, informed consent will be obtained in a private patient room within the OSCTI at OUHSC and UW-M prior to the initiation of the study protocol. A member of the study team will discuss the study protocol and

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answer questions with the prospective participant. Comprehension will be assessed by reviewing all elements of the protocol with the prospective participant, encouraging questions and recanting risks.

Due to the risks associated with the current protocol, individuals with a diminished capacity to consent will be excluded.

Non-English-speaking participants will be excluded due to study materials being available only in English.

Risks and Benefits

Potential Risks

We will use a dual-site, 12-week drug intervention trial performed in a double-blind, placebo-controlled manner. We will obtain blood and muscle tissue with methods explained in the proposal for the sole purpose of the proposed project and secondary analyses. We will record information pertaining to age and health during the initial screening. All subject identities and records are confidential. All subject identities and records will be coded and stored on password-protected computers and in locked file cabinets. Only members of the research team have access to subject identities and data. All procedures are low risk in a healthy population and include the following.

- There can be temporary physical discomfort during the blood sampling procedure, the possibility of bruising or fainting, and an extremely slight risk of infection. Discomfort during the blood sampling is minor and brief (<30s) and bruising does not last more than one week.
- With all infusions there is a risk of infection. To minimize the risk, all IV fluids will be prepared by a pharmacy, and we will use sterile techniques to administer the infusion. As a final precaution, infusions will be passed through a sterile filter.
- There is a risk of low blood sugar during the insulin infusion. Low blood sugar can make you dizzy, sweaty, shaky or weak. We will be checking your blood sugar every 10 minutes during the insulin infusion and giving you sugar in your IV to keep your blood sugar at a normal, healthy level. As with all infusions there is a risk of infection. To minimize the risk, all IV fluids will be prepared by a pharmacy, and we will use sterile techniques to administer the infusion. As a final precaution, infusions will be passed through a sterile filter.
- There is a small risk of infection at the site of the incision for the muscle biopsy, and a small risk of the biopsy incision reopening or bleeding after leaving the lab. The risk of allergic reaction to lidocaine (the anesthetic used for the biopsy incision) is extremely low. There is also a risk of fainting or muscle cramping during the procedure, loss of feeling in the leg, damage to the skin (cutaneous) nerve and a risk of bruising around the biopsy site. The procedure leaves a small scar at the incision site. The rate and degree of healing varies considerably, but it is expected that scars are difficult to see 6-12 months after the procedure. Dr. Miller has performed approximately 700 biopsies with many of these performed in older subjects. Dr. Konopka has performed the procedure approximately 250 times.
- The risks associated with the DEXA are very low. The maximum radiation dose we provide is less than 1/1000th of the federal and state occupational whole body dose limit allowed to radiation workers (5,000 mrem). Although minimal, the more radiation received over the course of a lifetime, the more the risk increases of developing a fatal cancer or inducing changes in genes. The radiation from our scans is not expected to significantly increase these risks, but the exact increase in such risks is not known. There are no discomforts associated with this procedure.
- Placement of the continuous glucose monitor (CGM) poses a small risk of infection at the site of insertion. There is also a risk of discomfort (skin irritation, redness, rash, or itchiness) from the adhesive patched to secure the device to the skin.
- In a small number of cases, transient dizziness may result from the initial consumption of heavy water.
- Violation of privacy and loss of confidentiality are both risks to which research participants are exposed. The possibility of these risks increases when protected health information is collected.
- Metformin has the risk of causing several side effects, however, there are nearly 50 million people in the US who use metformin on a daily basis to treat their diabetes and do not experience any side effects

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from metformin use. Metformin may cause lactic acidosis. Lactic acidosis can start slowly and get worse over time and is more prevalent in those with liver or kidney disease or heart failure, conditions that are screened out for this study. Less serious side effects of metformin include headache or muscle pain; weakness; or mild nausea, vomiting, diarrhea, gas, stomach pain. Although very rare metformin may cause low blood sugar, which could be masked if a subject is taking beta-blockers to control blood pressure.

- There is a recent study that indicates that there is a potential increased risk in males taking metformin versus males not taking metformin in the 90 days pre-conception and birth defects (risk 5.2% with metformin versus 3.3% without metformin). There was no increased risk when metformin was taken before or after the 90 days before conception.

The list of possible side effects of metformin collated into most likely, less likely, rarely, and rare but serious.

- Most likely (greater than 20%): These symptoms are generally temporary, occur during the start of treatment, and disappear without stopping the drug.
    - Diarrhea
    - Nausea
    - Vomiting
    - Abdominal bloating
    - Flatulence
    - Anorexia (loss of appetite)

- Less likely [occasional] (5 to 20%):
    - Loss of taste or metallic taste (during start of therapy)
    - Minor weight loss (less than 1 kilogram or 2 pounds)
    - Reduced appetite

- Rarely (1 to 4%): These effects will disappear when metformin is stopped.
    - Rash, redness or itchiness
    - Decrease in the level of vitamin B12 that does not cause symptoms
    - Decrease in the red blood cell count
    - Liver function test abnormalities as seen by blood tests or hepatitis (inflammation of the liver)

- Rare but Serious (less than 1%):
    - Lactic acidosis occurs rarely (3 cases per 100,000 years of use) and can cause death. Individuals at risk of this complication include people with diabetes who have kidney, liver or heart abnormalities. Symptoms of lactic acidosis may include tiredness, muscle aches, difficulty breathing, vomiting or severe abdominal pain.

- There are reproductive risks, as well, so women of childbearing potential should not become pregnant. Men should not father a child during the trial or during the 90 days after trial participation ends.

- There are alcohol consumption risks: Participants should not drink excessive alcohol while taking the drug. There is a limit of less than 3 alcoholic beverages/day.

Protections Against Risk

The general risks associated with the proposed studies will be minimized by: 1) recruiting only men and women who meet the inclusion/exclusion criteria; 2) monitoring health status with the medical

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oversight; 3) using only safe, well-established procedures that will be administered by qualified and experienced personnel; 4) ensuring constant monitoring of each experimental session by the investigators; 5) providing appropriate clinical supervision and emergency equipment; 6) using GCP with established SOPs and documentation: and 7) employing record-keeping procedures that maximize confidentiality. All subject identities and records will remain confidential; individual subject data will not be associated with the individual's name.

Protections against risk for specific procedures include the following: Applying direct pressure to venous sites for five minutes following removal of the sampling needle will minimize risk of bruising due to venipuncture. Conducting all blood-sampling procedures with the subjects in the supine position will minimize the dangers associated with fainting. Regarding the risks associated with biopsy, the consent form will screen for lidocaine allergy and verbal screening for lidocaine allergy will be conducted on all subjects prior to any biopsy being taken. The medical history questionnaire includes screening for current medications and for bleeding disorders. The use of sterile procedures and the provision of detailed written instructions for care of the biopsy site greatly reduces the chance of infection or any reopening of the incision. Constant monitoring of blood glucose concentrations every 10 minutes through use of an YSI, and adjustment of the infusion of dextrose to maintain euglycemia. Monitoring subjects during the first week of ingestion and decreasing the dose in smaller subjects will mitigate initial risk of dizziness associated with D_2O.

Potential Benefits of the Proposed Research to Human Subjects

Because the risks of participating in these studies are small, the benefit-to-risk ratio is high. These studies will expand scientific understanding of the potential for metformin to improve physiological outcomes associated with the aging process. Since all subjects will have a health screening, some benefits are immediate. Beyond the immediate benefits, long-term benefits may include improvements of mitochondrial function that may improve health span.

Importance of the Knowledge to be Gained

The study addresses a problem of great significance to the NIA and the health of older Americans - delaying the onset of age-related chronic conditions. The NIA has specifically recognized that there is a need for small-scale clinical studies to assess the potential of metformin as an anti-aging treatment. The overall goal of this work is to determine what subject populations may benefit from metformin treatment to slow aging since the positive benefits may not be universal. In addition, this work examines potential mechanisms through which there are differential responses to metformin treatment. By the conclusion of this study, we will have generated evidence in support or refuting the potential of metformin to slow the aging process to help inform a larger clinical trial.

Multiple Sites

The proposed project is a two-site project at Oklahoma Medical Research Foundation (OMRF) and the University of Wisconsin-Madison . Since Dr. Miller is the PI, OMRF is the coordinating center. The clinical portion of the OMRF site will be carried out at the Harold Hamm Diabetes Center (HHDC) that houses the Oklahoma Shared Clinical and Translational Resources (OSCTR) center on the campus of Oklahoma University Health Sciences Center (OUHSC). There is a reciprocity agreement between OUHSC and OMRF, thus making OUHSC the IRB of record for the Oklahoma site. Due to the two sites, the project falls under the NIH Policy on the use of a single institutional review board (sIRB) for multi-site research. OMRF, OUHSC and UW-M are all members of SMART IRB, so this resource will be used to facilitate all aspects of this project. One blood draw from each clamp (approximately 5 mL) will be sent to the laboratory of Dr. Barbara Nikolajczyk, Ph.D., Associate Director for Translational Research in

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Diabetes, Barnstable Brown Diabetes Center, University of Kentucky. This blood draw will remain deidentified and not traceable to the subject. The sample will be used to assess changes in systemic inflammation.

Provide the name of the IRB that you anticipate will serve as the sIRB of record: Oklahoma University Health Sciences Center IRB (FWA00007961) will serve as the IRB of record.

Indicate that all identified participating sites will agree to rely on the proposed sIRB: Both parties agree that OUHSC will serve as the IRB of record.

Briefly describe how communication between sites and the sIRB will be handled. The Lead PI (OMRF) will designate a central point of contact on the OMRF study team to:

- Coordinate communications with UW-M
- Request and receive information and documentation from UW-M
- Develop template materials for review by the OUHSC IRB and for limited modification by UW-M
- Submit materials from both sites to the OUHSC IRB and coordinate responses to any query
- Provide applicable, IRB-approved documents to UW-M

UW-M, as the Relying institution will designate a central point of contact on the study team to:

- Provide any local context that must be included in the informed consent document
- Provide information on any special circumstances related to obtaining informed consent (e.g. use of a non-English consent form) and/or any HIPPA-specific requirements
- Report to the Lead PI any conflicts of interest; and if so, provide applicable disclosure and management plans
- Communicating and completing any site-specific ancillary reviews prior to beginning local enrollment
- Assure that the study team has appropriate training and qualifications to conduct the study
- Provide pertinent information at time of Continuing Review or Closure information
- Reporting any serious adverse event(s) or non-compliance

Statistical Methods

This is a randomized, controlled clinical trial of healthy older male and female subjects 40-75 years of age. Subjects will be randomized into one of two groups (metformin or placebo) for a 12-week intervention. The study is double-blinded on placebo versus metformin. The randomization sequence will be stratified by study center (OUHSC or UW-M) and baseline insulin status (HOMA-IR ≤2.2 [sensitive] versus ≥2.5 [resistant]) and will be created using randomly chosen block sizes of four or six. At each center, there will be four groups: insulin sensitive (IS) placebo, IS metformin, insulin resistant (IR) placebo, and IR metformin. The study statistician (Dr. Stoner) will implement the randomization sequence through the online REDCap system.

Aim1: Our primary outcome measure is change in insulin sensitivity as determined by a hyperinsulinemic- euglycemic clamp.

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Sample size and power calculations: The proposed sample size affords 80% power to detect an interaction between metformin treatment and baseline insulin sensitivity status on the change in insulin sensitivity. Based on preliminary data from a 12-week treatment period with metformin, the mean (SD) change in insulin sensitivity measures (post- minus pre-treatment) were 0.67 (2.86) among insulin sensitive (HOMA-IR < 2.5) and 1.36 (1.85) among insulin resistant individuals. Assuming no change on average among the placebo-treated participants, a total sample size of 148 (37 per insulin sensitivity group at each center) will result in 80% power to detect an interaction between metformin treatment and baseline insulin sensitivity where the root mean square term (standard deviation) for the interaction is 0.65 or greater (an effect size of 0.25) (PASS13, NCSS, Utah, USA). This calculation is based on a 2x2 factorial ANOVA model and assumes a within-group root mean square error of 2.64, a two-sided 0.05 alpha level, and a withdraw rate of no more than 10% over the 12-week treatment period.

Statistical analysis plans: The mean change in insulin sensitivity (calculated as the 12-week minus baseline measure) will be compared between groups defined by baseline insulin sensitivity (IR or IS) and metformin/placebo assignment using a 2-factor ANOVA model that includes the treatment by insulin sensitivity interaction. A significant interaction will be followed by analyses stratified by baseline insulin sensitivity. Transformations will be used as appropriate to satisfy modeling assumptions. Analyses will be based on the intention-to-treat principle. A separate per-protocol analysis, analyzing data from all participants who took at least 80% of the pills, will also be performed. Sex will be investigated as a biologic variable by including sex, and interactions with sex, in the ANOVA model. Modification by sex will be explored and interpreted cautiously given that the study is not powered to detect modification by sex. A similar approach will be used to explore age, BMI, and physical activity as biologic factors. A mixed effects ANOVA model will be used for the other outcome measures (utilizing day-level average measures; measures including fasting blood glucose, HbA1C, hepatic and peripheral insulin sensitivity, TAME biomarker measures, C-peptide concentrations, body weight and body composition) where the model includes fixed effects for baseline insulin sensitivity category, metformin/placebo assignment, time (baseline or 12-week), and all possible interaction effects. A random participant effect also will be included. The primary term of interest is the time by insulin sensitivity by treatment interaction (indicating that the effect of metformin on the outcome following the 12-week treatment period differs depending on baseline insulin sensitivity). No formal interim efficacy analyses will be performed. Data quality and protocol implementation, as well as adverse events, will be summarized every six months for the data and safety monitoring board. Statistical significance will be declared at $p<0.05$.

Aim 2: Our primary outcome measure is mitochondrial function of the electron transport system.

Sample size and power calculations: The proposed sample size affords 80% power to detect an interaction between metformin treatment and baseline insulin sensitivity status on mitochondrial function. Based on preliminary data from a 12-week treatment period with metformin, the mean (SD) change in the complex I activity (post- minus pre-treatment) were 5.86 (16.91) among insulin resistant (HOMA-IR > 2.5) and -0.28 (21.97) among insulin sensitive individuals. Among participants receiving placebo, the estimates were 9.87 (11.93) and 12.01 (15.87), respectively. A total sample size of 148 (37 per insulin sensitivity group at each center) will result in 80% power to detect an interaction between metformin treatment and baseline insulin sensitivity where the root mean square term (standard deviation) for the interaction is 4.0 or greater (an effect size of 0.25) (PASS13, NCSS, Utah, USA). This calculation is based on a 2x2 factorial ANOVA model and assumes a within-group root mean square error of 16.35, a two-sided 0.05 alpha level, and a withdraw rate of no more than 10% over the 12-week treatment period. Similarly, the targeted

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sample size will result in 80% power to detect an interaction between treatment and baseline insulin sensitivity where the root mean square term (standard deviation) for the interaction is 5.25 or greater (an effect size of 0.25) and the within-group root mean square error is 20.73 based on the ADP titration protocol with mean (SD) among metformin-treated participants of 7.65 (22.85) and -10.19(17.40) for IR and IS participants, respectively, and among placebo-treated participants of 19.43 (23.14) and 6.68 (32.69), respectively.

Statistical analysis plans: The mixed effects ANOVA modeling for repeated measures described for Aim 1 will be used to analyze the data from Aim 2. For quantitative proteomics we will use a Benjamini-Hochberg false discovery rate correction (q = 5%). Heatmap and principal component analysis (PCA) plot will be constructed with ClustVis with default settings (Row scaling = unit variance scaling, PCA method = SVD with imputation, clustering distance for rows = correlation, clustering method for rows = average, tree ordering for rows = tightest cluster first).

Data and Safety Monitoring Plan

This multi-site study fits the NIH definition of a clinical trial. Thus, a data safety monitoring plan and data safety monitoring board will be in place. This plan adheres to the guidelines outlined by the National Institute of Aging. We will carry out the study as detailed in Figure 1. Subjects will need to meet strict inclusion/exclusion criteria to participate in the study. These criteria are intended to identify an appropriate sample for the proposed study and to ensure that no individual will experience undue risk by participating in the study.

1.0 PARTICIPANT SAFETY

1.1 Potential Risks and Benefits for Participants

The potential risks and benefits to study participants are detailed in the *Protection of Human Subjects* document. These risks are associated with the blood draw procedure (discomfort, fainting, and bruising), muscle biopsy (discomfort, fainting, bruising, and infection), DEXA scan (low dose radiation), CGM placement (discomfort), deuterium oxide (D_2O) administration (transient dizziness), intravenous infusion of insulin, dextrose, and stable isotope (hypoglycemia, infection) violation of privacy, and metformin administration (side effects). The benefits for subject participation is moderate, but includes the potential benefit from metformin administration for age-related outcomes.

Adverse Events (AE)

Any untoward or unfavorable medical occurrence associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research (modified from the definition of adverse events in the 1996 International Conference on Harmonization E-6 Guidelines for Good Clinical Practice) (from OHRP "Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Subjects or Others and Adverse Events (1/15/07)" at http://www.hhs.gov/ohrp/policy/advevntguid.html#Q2)

For this study, an adverse event will include any untoward or unfavorable medical occurrence associated with:

- Use of the study drug. Attribution to study drugs would not begin until the patient has encountered them (or their corresponding placebo pill). Attention to possible attribution of AE's to metformin will continue up to and including the last study visit.
- Hyperinsulinemic-euglycemic clamp. Attribution to the hyperinsulinemic-euglycemic would occur during the clamp and up to 2 days later.
- Use of deuterium oxide. Attribution to deuterium oxide would not begin until the patient has taken his/her first dose (week 9). Attention to possible attribution of deuterium oxide will continue up to and including the last study visit.

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- Muscle biopsy. Attribution to the muscle biopsy would occur at the time of the biopsy and up to 7 days post-biopsy.
- Blood draw. Attribution to the blood draw would occur at the time of the blood draw and up to 5 days later.
- Study mandated procedures. Although unlikely, AEs attributed to study mandated procedures could occur and be reported as soon as the patient signs informed consent.

1.2 Adverse Event Reporting

Subjects will be instructed to report serious or worrisome side effects to the investigators. The reports will be categorized according to the following criteria:

- Adverse Event – any untoward medical occurrence experienced by a subject that is temporally associated with participation in the clinical study. An adverse finding can include a sign, symptom, abnormal assessment (e.g., laboratory test value or ECG finding) or any combination of these.
- Unanticipated Adverse Event – any adverse event that results in risk or harm to the subject or others that differs from the known, predicted possible effects of the research protocol.
- Serious Adverse Event – any event that is life threatening or results in death, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability or incapacity. Important medical events that may not result in death, be life-threatening, or require hospitalization may be included in this category when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of these outcomes.

Grading: Each adverse event will be classified as mild, moderate, or severe based on the degree to which it affects the individual's function or activities of daily living. Adverse events will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual: Grade 1 = mild adverse event, Grade 2 = moderate adverse event, Grade 3 = severe and undesirable adverse event, Grade 4 = life-threatening or disabling adverse event, Grade 5 = death. Furthermore, each event will be rated as definitely, possibly, or unrelated to the study intervention (**Table 1**). We will report all unanticipated adverse events to the study medical staff and the IRB as they occur. Events grade 3 or higher will be recorded on the appropriate AE case report form for this study which will be a printable pdf form that can be filled out electronically or printed on paper, such that the form can be scanned or uploaded into electronic data system(s). Anticipated adverse events that are graded as moderate or serious will also be reported as they occur. The relationship, or attribution, of an adverse event to the study therapy regimen or study procedure(s) will initially be determined by the site investigator and recorded on the appropriate AE pdf /printed case report form. The PI will be responsible for reporting all adverse events and serious adverse events within 5 days of the occurrence to the IRB. A death will be reported within 24 hours.

Table 1. Attribution of Adverse Events

Code	Descriptor	Relationship (to primary investigational product and/or other concurrent mandated study therapy or study procedure)
UNRELATED CATEGORY		
1	Unrelated	The adverse event is clearly not related: there is insufficient evidence to suggest a causal relationship.

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RELATED CATEGORIES		
2	Possible	The adverse event has a reasonable possibility to be related; there is evidence to suggest a causal relationship.
3	Definite	The adverse event is clearly related.

1.3 Protection Against Study Risks

The complete procedures for protecting against study risks are detailed in the *Protection of Human Subjects* document. In general, the risks will be minimized by: 1) recruiting only men and women who meet the inclusion/exclusion criteria; 2) monitoring health status with the medical oversight; 3) using only safe, well-established procedures that will be administered by qualified and experienced personnel; 4) ensuring constant monitoring of each experimental session by the investigators; 5) providing appropriate clinical supervision and emergency equipment; 6) using GCP with established SOPs and documentation; 7) compounding by licensed pharmacies, and 8) employing record-keeping procedures that maximize confidentiality.

2.0 INTERIM ANALYSIS

To ensure consistency between the two research sites, the investigative team will meet once every year to review all active protocols and follow the guidelines established by the NIH National Center for Research Resources: (i) monitoring the progress of the protocol (e.g., reviewing subject recruitment, attrition, and minority involvement) and the safety of the research participants; (ii) assuring compliance with requirements for reporting adverse events; (iii) making sure that any action responsible for the temporary or permanent suspension of the protocol is reported to the relevant authorities (e.g., IRB, DSMB); and (iv) examining data accuracy and protocol compliance. The Data and Safety Monitoring Board (DSMB) shall review safety data at least yearly during planned DSMB Data Review Meetings. Data for the planned safety reviews will include, at a minimum, a listing of all reported AEs.

3.0 DATA AND SAFETY MONITORING

Drs. Miller and Konopka will assume primary responsibility for subject surveillance as well as oversight of scientific rigor and adherence to study protocols. Jonea Lim, MD is a board-certified clinical endocrinologist that will provide medical oversight at the OMRF/OUHSC study site. Samantha Pabich, MD in Endocrinology, Diabetes and Metabolism at the University of Wisconsin School of Medicine and Public Health will provide medical oversight at the UW-M site. Appropriate medical oversight will be present in the building or room for the first few skeletal muscle biopsies or hyperinsulinemic-euglycemic clamps. The study will be performed under the oversight of a Data and Safety Monitoring Board (DSMB -see below). The primary exit criteria will be completion of the protocol. Exclusion criteria include intolerance to medication (as determined by potential metformin side-effects), and referral for a previously undiagnosed chronic disease. If signs and symptoms develop that are consistent with treatment side effects (e.g. nausea) we will decrease medication dose to (1000 mg) to determine if symptoms diminish. If the symptoms do not reduce, the subject will be removed from study. Incidental findings will be reported to the study physician for appropriate referral.

3.1 Frequency of Data and Safety Monitoring

The Data Safety Monitoring Board (DSMB) will review the study every 6 months, with the first meeting scheduled approximately 6 months following the activation of protocol subject entry.

3.2 Content of Data and Safety Monitoring Report

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The DMSB will operate under the rules of an approved charter that will be written and reviewed at the organizational meeting of the DSMB. At this time, each data element that the DSMB needs to assess will be clearly defined. The study statistician will produce an interim report, outlining important aspects of the trial, including accrual, retention, data submission timeliness, data completeness, protocol adherence, and interim safety data. The DSMB will meet (either in person or by telephone conference) to discuss the interim results and make recommendations regarding study conduct. The DSMB will be authorized to make recommendations to the study investigators and NIH regarding the continuation, modification, or termination of the trial.

3.3 DSMB Membership and Affiliation

The DSMB will include 3 members who are independent of the institutions involved in the clinical trial, free of conflict with the trial and investigators, and with expertise in metabolism, aging, muscle and statistics. Katrin Kuhn, PhD will also participate as the study statistician, but will be a non-voting member.

3.4 Protection of Confidentiality

All electronic data will be stored on a Protected Health Information-safe server that is accessible from allowed desktop and laptop computers with appropriate login credentials. The data server is managed by the Data Hosting Center at the University of Oklahoma Health Sciences Center Information Technology Department. Dr. Stoner will train and oversee all data collection and ensure all research personnel are actively engaging in protection of data. She will ensure that data safety measures of the raw data and that hard-copies of data are handled with appropriate protections.

Data Sharing

Data capture, quality assurance, management, and processing are consolidated through the use of the well-established Research Electronic Data Capture (REDCap) system, which is hosted at OUHSC by the Oklahoma Shared Clinical and Translational Resources. REDCap is a secure, web-based application designed to support data capture for research studies, providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources. Online data capture forms can be created using REDCap, exported as pdf files, and printed for off- line use so that a hard copy will be available for data checks. REDCap will allow for multi-personnel access of project files and will be used in the proposed project to capture data. The REDCap data server is hosted in the OUHSC Data Hosting Center and meets security requirements for storage of protected health information (PHI) and back-ups and recovery.

Should any of the research personnel leave the university, their credentials to access hard copy and/or electronic data are immediately restricted. University Tier I personnel have the ability to access all files in the Protected Health Information-safe server. They will be notified if personnel responsible for implementation of the Data Management Plan leave the university. Unit supervisors will also be immediately notified, and replacement personnel will be identified to fulfill essential study roles and responsibilities, specifically regarding data safety and management.

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A. Confidentiality

All the original paper copies of participants' observations will be kept in locked storage cabinets at the OSCTI. Data entered from paper into electronic records in Redcap will be kept on a secure server maintained by OUHSC Information Technology's Secure Data Services (SDS). SDS uses the OUHSC enterprise data center to house critical information assets, including PHI. Centralizing electronic information in a secured data center addresses physical security, applies uniform administrative procedures, provides uniform data custodial activities including backups, off-site storage, and disk drive disposal, and enforces uniform network protection policies.

To ensure data security and to prevent loss of data, electronic data will be accessible on shared drives or folders to which the OSCTI restricts access. Only the PI and certain members of the research team (co-investigators and sub-investigators, statisticians and research assistants) will have access. Data containing patient identifiers will be maintained to track and integrate multiple records from individual participants throughout and at the end of the study. However, lists that contain identifiers, and information that permits linking to participants' identities, will be kept only on the secure server and will be accessible only to the PI. Other research staff will access a database that contains only de-identified data that are not considered PHI. Staff other than the PI will have no access to identifying information but only to non-informative patient codes.

Publications and presentations that result from this research will report only aggregate data and not individual responses. While electronic records of the data will be maintained on the secure server, paper records will be shredded two years after the conclusion of data collection.

B. Literature Cited

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