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Study Title: Genomic Outcomes of Metformin (GO MET)

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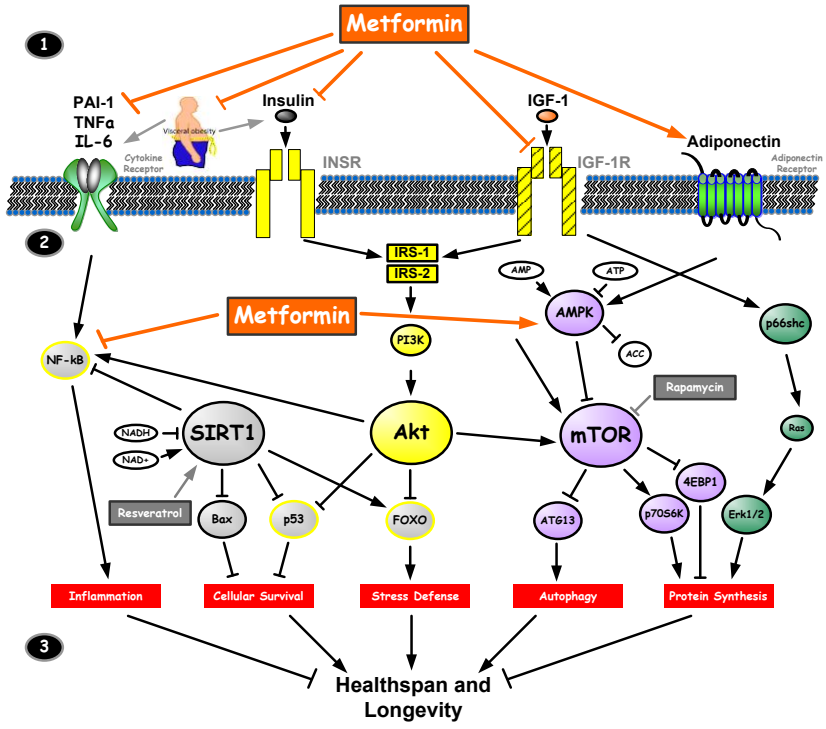
Background, Rationale and Context

The number of older adults is projected to increase dramatically by 2050. Aging-related diseases and conditions still seriously compromise the quality of life among most older adults. Several pharmaceutical agents (1-4), such as metformin, have been tested to extend lifespan and delay aging-related diseases and dysfunctions in mice. Metformin, a biguanide antidiabetic drug, reduces the risk for developing type-2 diabetes in persons at risk by over one-third with few adverse effects (e.g., gastrointestinal irritation) (5). Metformin prevents type-2 diabetes primarily through decreasing hepatic glucose synthesis (6), as well as enhancing insulin sensitivity and increasing peripheral glucose uptake (7-9). The molecular mechanisms remain unclear, although a number of potential mechanism such as activation of AMP-activated protein kinase (AMPK) and inhibition of mitochondrial glycerophosphate dehydrogenase have been proposed, see figure 1 (10;11). The fact that metformin treatment in persons with type-2 diabetes has been associated with reduced risk of other aging-related diseases and conditions, including cardiovascular disease (12), cancer (13) and cognitive decline (14) supports the possibility of the beneficial effects of metformin on healthy aging. It is imperative to capitalize on these leads to extend health span among older adults.

Figure 1. Metformin targets multiple pathways of aging



To translate animal findings to human intervention trials, appropriate aging biomarkers are needed. Methylomic and transcriptomic profiles in relevant cells may reflect molecular features that mediate effects of both genetic and environmental factors on aging-related functional decline and disease. The roles of monocytes have been implicated in development of many aging-related diseases such as cardiovascular disease, cancer and neurodegenerative disease (15). In a cross-sectional association study of 1,200 monocyte samples, we identified 1,794 age-associated methylation sites (16) and 2,704 age-associated transcripts (17), which were over-represented in two networks (autophagy and oxidative phosphorylation) and suggestive of decline in those functions with age. Both autophagy (18) and oxidative phosphorylation (19) are considered as key contributors to the aging process, and their dysfunctions have been linked to aging-related diseases (20;21). Changes in these aging-related omic biomarkers may be early indicators of cellular damage or disruption that eventually leads to age-related dysfunctions. Assessment of these aging biomarkers in response to therapeutic intervention may also

provide molecular insight for personalizing treatment. We propose a pilot study to examine changes in aging-related omic profiles after 3 months of metformin treatment in 35 monocyte samples from older adults using a randomized, double-blind, placebo-controlled crossover study design.

Objectives

Our overarching goal of the pilot study is to evaluate the utility of using the aging-related omic biomarkers as an indicator of pharmacologic responses in the anti-aging therapeutic intervention trials through the following specific aims. Although this pilot study does not have sufficient power to definitively test all the aims, it will provide essential preliminary data for developing a full scale research program.

- Aim 1A: To test the effects of the metformin treatment on transcriptomic profiles and related functional changes in human monocytes,
- Aim 1B: To explore the effects of the metformin treatment on methylomic profiles in human monocytes,
- Aim 2: To investigate the longitudinal relationship between transcriptional and functional changes in human monocytes during the metformin treatment and
- Aim 3: To test the effects of the metformin treatment on frailty and other aging-related physical and cognitive measures and investigate the longitudinal relationship between these changes and transcriptional changes.

Methods and Measures

Design

A randomized, double-blind, placebo-controlled crossover trial in 35 participants using metformin and matching placebo will be used. In the absence of a treatment by sequence interaction effect, this design can increase study power for evaluating treatment effects by allowing each participant to be his/her own control. The period effect may be minimum because the primary outcomes, methylation and transcriptional measures, are relatively stable overtime.

Setting

All visits will occur in the Geriatric Clinical Research Unit (G-CRU) or the Geriatric Research Center (GRC) in the Sticht Center on Aging.

Subjects selection criteria

All prospective participants in and around Forsyth County, NC will initially be screened via telephone to determine general eligibility. They will be asked about their age, medical history, and current medications. All participants must meet the inclusion/exclusion criteria below.

Inclusion Criteria

Age 65 – 79

Must meet criteria from one or more of the following groups:

Group 1 (Can have 1 or 2 of these, but not all 3)

- History of coronary artery disease (MI/heart attack, stroke, heart failure, or peripheral artery disease)
- Cancer, with no active treatment in the last year
- MCI (MoCA $\geq$18<26 –inclusive of 1 point if <12 years of education

Group 2

- Decline physical function (walking speed < 1 m/s)

Group 3 (Either or both)

- Abdominal obesity (>88cm women, >102cm men) AND hypertension (treated or resting blood pressure >140/90

- Abdominal obesity (>88cm women, >102cm men) AND hyperlipidemia (treated or fasting total cholesterol >240

English literacy
Willing to provide informed consent

Exclusion Criteria

eGFR <45
Type 2 diabetes (HbA1c>6.5) or type 1 diabetes
Any tobacco or nicotine product use in the past year
Low vitamin B12 Levels (< 300 pg/mL)
Self-reported severe difficulty or inability to walk 400m or climb 10 steps (from Q 2 and 19 on PAT-D)
Self-reported difficulty or inability to perform basic ADL functions (from Q 10, 13, 14, 16 on PAT-D)
Excessive alcohol use (>14 drinks/week)
Cancer requiring treatment in past year (except skin)
Dementia – diagnosed and/or MoCA score <18
Parkinson's or other neurological disease
Chronic liver disease or cirrhosis
End stage renal disease or on dialysis
Rheumatic conditions (Rheumatoid arthritis, lupus, and any other autoimmune disease the PI deems them to be ineligible for)
Thyroid problems the PI deems them to be ineligible for
Recent or recurrent exacerbation of gout
Involved in another interventional study
Hemoglobin ≤8 or diagnosed with anemia
Recent unintentional weight change (+/- 10 lbs. in the last 12 months)
BMI <18.5
Likely to not follow the protocol
PI deems unfit to participate
Already taking Metformin or any other drug intended to treat diabetes

Screening, consent and randomization. Individuals who pass the telephone screening will be scheduled for a visit to the Geriatric Research Clinic (GRC) in the Sticht Center on Aging for a screening visit (SV). Before any data collection, all participants will provide written informed consent and complete a HIPAA authorization form in accordance with the Wake Forest School of Medicine Institutional Review Board policies. Individuals who meet all eligibility criteria and provide informed consent will be invited back for the first baseline visit (BV). At the end of BV, participants will be randomized to one of the two study intervention sequences (metformin followed by placebo or placebo followed by metformin) using a web-based randomization scheme (developed by Dr. Miller). The randomization sequences will be unknown to the research staff. Intervention assignment will be generated using a program that the study coordinator will access from his/her PC through the study website.

Schedule and organization of assessment visits

All assessments will be conducted during 4 visits (before and after each intervention) according to the chart below. All examiners are trained in the standardized conduct of all assessments before data collection. Participants will be instructed to wear appropriate and comfortable clothing, and standardized written instructions will be provided prior to each study visit.

Assessment timeline / Activity/assessment	Visit code / Week number	SV -4	BV 0	On trial	Mid 12-14	On trial	FV 24-26
Informed consent, review inclusion/exclusion criteria, demographics, weight, height, BP and pulse, fasting screening blood draw (metabolic blood panel, lipid panel, CBC, Vit B12, Hba1c), eSPPB, cognitive screen (MoCA), waist circumference, medical history, medications PAT-D,		X					
Blood collection for methylomic and transcriptomic, bioenergetic capacity, CBC, and blood for storage, DSST, leg press, grip, , MATsf, and 6MWD			X				
Randomization			X				
Crossover – Dispense New Medication					X		
Vitals, blood collection for methylomic and transcriptomic, bioenergetic capacity, and blood for storage, CBC, CMP, lipids, Hba1c, eSPPB, MoCA, DSST, leg press, grip, PAT-D, MAT-sf, 6MWD					X		X
Health Maintenance							X

The screening visit (SV) will be conducted in the morning following an 8-hour fast for measurement of lipids, blood count, Hba1c, Vitamin B12, and a metabolic panel for screening purposes. Vitals (blood pressure, pulse, height, weight, and waist circumference) are collected and the blood draw completed, participants will be given a snack and continue with the remainder of the visit. First, the expanded short physical performance battery (eSPPB) will be administered and scored and then undergo a cognitive screen, assessed using the Montreal Cognitive Assessment (MoCA), and a medical history, review of medications and dietary supplements We will also ask about the participants demographics and physical abilities using the Pepper Assessment Tool for Disability (PAT-D),

The first baseline visit (BV), will be conducted in the morning following an 8-hour fast for blood collection for methylomic and transcriptomic measures, bioenergetic capacity, CBC, as well as blood for storage. After these measures are complete, participants will be given a snack and will continue with the rest of the visit consisting of lower extremity muscle power (Nottingham Power Rig), grip strength, 6 minute walk distance test (6MWD), and the Mobility Assessment Tool – short form (MAT-sf). Cognitive function will be assessed using the Digit Symbol Test (DSST). Once the visit is complete, the participant will be randomized and placed in one of the following groups discussed below. Participants will be provided with an information sheet that will provide additional details on how and when to take the medication, who to call if they have any problems, possible side effects, and what to do should they encounter any side effects.

Interventions and Interactions

Eligible participants will receive both interventions (3-month metformin treatment and 3-month placebo). Randomization will be used to determine the order in which the participants receive each intervention (i.e., 15 participants with metformin treatment followed by placebo, and 15 with placebo followed by metformin treatment). Neither the participants nor the investigators know the intervention order of a participant. Metformin reaches peak plasma concentration within one to eight hours, has an average elimination half-life of 6.2 hours in plasma, and is cleared but not metabolized from the body by tubular secretion. Treatment with metformin and placebo will be initiated at a dose of 425 mg taken orally once a day at night for 7 days. After the week, the participant will be called to assess tolerance and will be asked to increase dose to one pill at night at a dose of 850 mg for one week. At the end of the second week, participants will be called again and if they tolerated the second dose, they will be asked to take two 850mg pills, one in the morning and one at night, for a total dose of 1700 mg which is within the range of the usual effective dose of 1500 to 2000 mg/day for the remainder of the 3 months. This dosing regimen was created in order to avoid or reduce possible gastrointestinal side effects. This can be modified if the

participant is experiencing any side effects and will be handled on a case by case basis. Each study visit where stored blood is obtained (BV, Mid, FV) may be rescheduled if the subject has a fever, infection, or has taken antibiotics within 24hrs of this scheduled visit.

All baseline assessments will be completed again at 12 weeks and again at 24 weeks as listed in the assessment table.

Outcome Measure(s)

The primary outcomes will be the eigengene scores (and related individual transcriptional measures) for the two transcriptional networks, the autophagy and oxidative phosphorylation. The eigengene score is defined as the first principal component of a transcriptional network. The secondary outcomes will include functional, methylation and other aging-related measures.

Individual methylation sites and transcripts, especially the eigengenes of autophage, oxidative phosphorylation and protein synthesis networks Methylomic and transcriptomic profiles in monocytes will be quantified using the Illumina HumanMethylation450 BeadChip and the Illumina HumanHT-12 v4 Expression BeadChips, respectively, at Dr. Liu's laboratory (22). Blood will be collected in Vacutainer CPTTM cell separation tubes containing sodium citrate (Becton Dickinson, Rutherford, NJ) to separate peripheral blood mononuclear cells. Then, monocytes will be isolated with the anti-CD14 coated magnetic beads using AutoMACs automated magnetic separation unit (Miltenyi Biotec, Bergisch Gladbach, Germanry). DNA and RNA will be isolated simultaneously using the AllPrep DNA/RNA Mini Kit (Qiagen, Inc., Hilden, Germany).

Functional measures: An autophagic profile in monocytes including macroautophagy, microautophagy and chaperone-mediated autophagy will be measured using fluorescent reporters at Dr. Ana Maria Cuervo's lab in Albert Einstein College of Medicine.

Mitochondrial Function: Respirometric profiling will be used to assess bioenergetic capacity, respiratory control and electron transport chain function at Dr. Molina's lab (23). This will be performed by examining the oxygen consumption profile of blood monocytes. Specific outcomes of mitochondria function include respiratory control ratio (RCR), state 3 (maximal oxygen consumption rate), and state 4 (oxygen consumption upon inhibition of ATP synthase).

Other Data

Vital Signs Prior to randomization data are collected on sitting blood pressure, heart rate, and weight. Body height is measured once prior to randomization. The blood pressure assessments will allow the determination of the incidence of hypertension and serve as basis for a temporary exclusion. The other measures are collected primarily for descriptive purposes.

Demographics and co-morbidities: Medical information regarding co-morbidities and medications will be ascertained at the screening visit. Demographic data such as socio-economic status, education level, age, and race also will be recorded and used as covariates if necessary. Information about preventative health screenings and other health maintenance information is asked at the follow up visit.

Physical performance will be assessed using the expanded Short Physical Performance Battery (SPPB) (24). The expanded SPPB consists of 5 repeated chair stands, standing balance (semi- and full-tandem stands and a single leg stand for 30 seconds), a 4-m walk to assess usual gait speed, and a narrow 4-m walk test of balance (walking at usual pace within lines of tape spaced 20 cm apart). Scores for the traditional 0-12 point SPPB can also be obtained from these tests. We will also assess physical

performance using the six minute walk distance test (6MWD). The participant is asked to walk at a pace they can maintain for six minutes and the distance covered will be recorded.
Lower extremity muscle power will be measured using the Nottingham Power Rig, a safe, convenient method for assessing power output from the lower limb which has been used reliably in older adults. (25) Participants will sit in a chair and unilaterally depress a foot lever attached to a flywheel as hard and as fast as they can. Power output, derived from the acceleration of the flywheel, will be recorded in Watts. Power will be averaged for each leg following five trials at maximal effort. Participants that have had a unilateral hip or knee replacement should not have that side tested.

Grip strength will be measured twice in each hand to the nearest 2 kg using an isometric Hydraulic Hand Dynamometer (Jamar, Bolingbrook, IL) and the mean value from the stronger hand used. Participants will be excluded from performing the test if they report hand-pain or recent hand or wrist surgery.

Cognitive function will be assessed during screening using the Montreal Cognitive Assessment (MoCA) (26), participants must score ≥18 to be eligible. We will also assess psychomotor speed, attention, and working memory using the Digit Symbol Substitution Test (DSST) (27; 28). Participants are given a series of numbered symbols and then asked to draw the appropriate symbols below a list of random numbers. The score is the number of correctly made matches in 2 minutes (120 seconds).

Body composition will be measured by circumference measurements of the waist which is made with a tape measure.

Blood lipids (total cholesterol, HDL-Cholesterol, Triglycerides and calculated LDL levels**)** will be measured at a clinical lab and all results will be entered into the data system.

Activities of daily living will be assessed using the Mobility Assessment Tool – Short Form (MAT-sf). This is a novel, computerized tool for self-assessment of functional performance designed to reduce bias from factors such as age, gender and body image. The Pepper Assessment Tool for Disability (PAT-D) will also be used to assess the participant's ability to complete an array of important day-to-day activities without difficulty and, for a subset of activities, without personal assistance.

Medication adherence: Although we will attempt to enhance adherence by monthly calls to participants, there may be inter-individual variability in adherence to the medication. At the end of each phase, participants will be asked to return any unused pills for a pill count to determine participant adherence. Our primary analyses will be an intent-to-treat analysis of the effects of the metformin intervention, but secondary analyses will include adherence as a covariate.

Analytical Plan
We will use an analysis of variance model for crossover design to assess the effect of metformin on transcriptional profiles and other aging-related outcomes. The factors in the analysis will include the baseline measure, sequence, participant within sequence, period, and metformin treatment. We will explore for the metformin by sequence interaction and will base estimates of the metformin effect on whether this interaction effect is significant. We will also perform exploratory, linear regression analysis with transcriptional changes as the explanatory variables and changes in other aging-related measures as the outcome variables. The analysis will be focused on the eigengene scores of the autophagy and oxidative phosphorylation transcriptional networks (and related individual transcriptional measures). As this is a pilot study, focus of analyses will be on estimation of effects and constructing appropriate 95% confidence intervals for these effects.

Human Subjects Protection

Risk to participants

The Diabetes Prevention Program (DPP) study, a placebo-controlled study of metformin vs. lifestyle intervention in persons with pre-diabetes (included subjects with fasting blood glucose 90-125 mg/dL, n = 3,234) provides insight into side effects of long-term metformin administration in the elderly and non-diabetics.[29] Of note, the most common side effect was not hypoglycemia, but gastrointestinal symptoms (see table below).

TABLE 3. ADVERSE EVENTS.

EVENT	PLACEBO	METFORMIN	LIFESTYLE
Gastrointestinal symptoms (no. of events/ 100 person-yr)*	30.7	77.8†	12.9†
Musculoskeletal symptoms (no. of events/ 100 person-yr)‡	21.1	20.0	24.1†
Hospitalization			
One or more admissions (% of participants)	16.1	15.9	15.6
Rate (no. of admissions/100 person-yr)	7.9	8.4	8.0
Median stay (days)	3	3	3
Deaths (no./100 person-yr)	0.16	0.20	0.10

*Gastrointestinal symptoms included diarrhea, flatulence, nausea, and vomiting.

†$P<0.0167$ for the comparison with placebo.

‡Most participants with musculoskeletal symptoms had myalgia, arthritis, or arthralgia.

As far as common side effects, GI side effect and diarrhea are transient and usually pass after a week of therapy. In the DPP study, 3-5% of subjects had continuous diarrhea; this portion of subjects would be excluded in the present trial during the run-in, dose-titration period. No one had metformin associated lactic acidosis. Hypoglycemic events were either not in evidence or reported.

Weight loss is a common occurrence with metformin and will be monitored. In such a case some patients may complain of hypoglycemic symptoms (rapid heartbeat, feeling light-headed or fainting, and nausea), and in this case subjects will be asked to refrain from taking their next dose. Subjects may not need medical interventions, but will be given instruction for fruit juice or other carbohydrate and to call the coordinator immediately to come in for a blood glucose check. Levels will be checked by finger (Accucheck) and labs to LabCorp to confirm levels. If blood glucose levels are < 60 mg/dL they will be removed from the study and replaced by an additional participant. No more side effects were noted in the elderly; in fact, they had a better adherence to therapy. Young and elderly had the same rates of GI side effects and diarrhea.

Subject Recruitment Methods

We will recruit these individuals using community-based recruitment strategies including newspaper ads and mass mailings. We will also advertise in the VITAL newsletter (BG99-559) and participate in community outreach events.

Informed Consent

Written informed consent will be obtained from each subject. The informed consent process will follow

the procedures of the WFSM Institutional Review Board. The study interviewers will explain the purpose, methods and extent of the study to prospective participants. The potential participant is asked to read the informed consent form and ask questions. The form is written in simple easy to understand language. We require study staff to review all of the key aspects of the study verbally with the potential participants. Staff is provided with a structured checklist for this purpose. Staff is then required to question potential participants to ascertain whether s/he has understood the information. Potential participants who are illiterate or have impaired vision must have the consent read to them, followed by review of the checklist, opportunity for questions, and discussion. This process will take place in a quiet, private room. A copy of the signed and dated consent form will be given to participants, and the original document will be placed in subjects' individual study files, which will be stored in a secure location. In compliance with the Health Insurance Portability and Accountability Act (HIPAA) and the Standards for Privacy of Individually Identifiable Health Information of the Department of Health and Human Services, we will access personal health information only after obtaining informed consent.

Confidentiality and Privacy

All data are obtained for research purposes only. Confidentiality of data is maintained by using research identification numbers which uniquely identify each individual. Data will be used only in aggregate and no identifying characteristics of individuals will be published or presented. The information collected has a low potential for abuse because the data do not address sensitive issues. Nevertheless, appropriate measures are taken to prevent unauthorized use of study information. Research records are kept in a locked room. Data access will be limited to study staff. Information linking IDs to individuals is kept on a secure, password-protected server to which only authorized study personnel will have access. Data and records will be kept locked and secured, with any computer data password protected. A web-based data entry system will be created for this study which will allow access only to study staff (user id and password required to access). Blinded staff will not have access to blinded study information on the web-based database. Computer files are stored on file servers, which are backed up each night and stored for easy retrieval in case of emergency. Files may not be obtained from the research unit by persons other than the research personnel, who are asked to sign a document agreeing to maintain the confidentiality of the information. No data files distributed for analysis will include personal information. No reference to any individual participant will appear in reports, presentations, or publications that may arise from the study. After the study is completed, the data will be stored with other completed research studies in a locked secure area.

Data and Safety Monitoring

The principal investigator along with the study physician (William Applegate) will be responsible for the overall monitoring of the data and safety of study participants. The study physician will be unmasked to treatment assignment as needed and appropriate. The principal investigator will also be assisted by other members of the study staff. All participants will have a protocol-driven medical monitoring plan to consistently assess for adverse events or medical safety concerns. In addition, participant safety in all Wake Forest Pepper Center OAIC-supported studies is monitored by a Data and Safety Monitoring Committee (DSMC). It reviews study recruitment, drop-outs, protocol changes, losses to follow-up, and adverse events every 6 months. The DSMC has authority to recommend changing or stopping the protocol. In the latter case, the PI will immediately act upon the recommendation according to institutional policy and in consultation with the Wake Forest Pepper Center OAIC Executive Committee.

Reporting of Unanticipated Problems, Adverse Events or Deviations

Any unanticipated problems, serious and unexpected adverse events, deviations or protocol changes will be promptly reported by the principal investigator or designated member of the research team to the IRB and sponsor.

Use of biological samples by other investigators

Biological samples may be used by investigators other than the investigators of the current study. The use will be limited to non-commercial purposes. The names and other personal identifiers of the study participants will not be sent to any recipients of the blood samples.

Storage and disposal of biological material

Blood samples will be stored at Wake Forest University Medical Center for up to twenty years after the end of the trial at which time the samples will be destroyed. Biological specimens will be stored in locked -70oC alarmed freezers located in a locked room. The lab coordinator and the PIs have access to the keys of the freezers. All the specimens will have numerical study IDs with no personal identifiers of the participants. These are stored under the Pepper Center Tissue Repository (IRB#1219).

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