Metformin Effect on Brain Function in Insulin Resistant Elderly People

NCT03733132

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Research Design and Methods

Participants: We will recruit equal numbers of pre-diabetic, elderly men and women using the following criteria: age ≥ 60 yrs. BMI > 25fasting glucose ≥ 100-140 mg/dL. These selection criteria will allow us to recruit participants with reduced insulin sensitivity, but the variations in insulin sensitivity among participants will be evident from hyperinsulinemia glucose clamp that we will perform. People on medications that interfere with primary outcome measurements will be excluded (See under Subject Details). Importantly, people on hypoglycemic drugs including metformin will be excluded. We will exclude T2D/pre-diabetic people on oral hypoglycemic agents due to their variability in insulin secretion and also because most people in Olmsted County with fasting glucose ≥140 mg/dl are on metformin and it would be unethical to withhold treatment from these individuals. People who engage in structured aerobic exercise ≥ 3 d/wk or have active cardiovascular disease, renal failure, or liver function abnormalities will also be excluded (see Subject Details). In the current study, we will screen medical records for any diagnosis of cognitive impairment/dementia. We do not intend to target people with clinical dementia in the current study. We will also administer the Montreal Cognitive Assessment (MoCA) at the screening visit and exclude any individual with a score < 22. English language proficiency will also be included as a criterion for enrollment, as strong English proficiency will be necessary for proper administration of the NIH Toolbox cognitive testing. We will recruit equal numbers of men and women and strive to recruit all ethnic groups represented in the community.

Overall Study Design: We will conduct a placebo-controlled, double-blind randomized trial with longitudinal (pre- & post-intervention) measurements. 40 participants will be randomized to placebo or metformin (2000 mg/d) groups. The initial study visit will include a screen, including testing for the criteria mentioned above. Participants will be asked to report to the CRTU fasting for a blood test, including a CBC, liver function, fasting glucose, lipid panel, and TSH. They will also have height and weight measurements taken and the MoCA test administered. While this is being done, the blood test results will be made available to the study team. If the participant passes the screening criteria, an outpatient visit will include a DEXA scan to assess body composition, a VO_2max test on a cycle ergometer to test aerobic capacity.The first inpatient day will examine baseline brain structure and function using fMRI analysis. For fMRI measurements, a minimum 20 participants are recommended for sufficient reliability for fMRI neuroimaging studies (4). Additionally, cognition will be assessed by the NIH Toolbox-Cognition Battery (NIHTB-CB). Participants will be asked to report to the Charlton CRTU fasting on the day of their fMRI study. They will have a fasting blood draw, followed by the MRI test at Neuroradiology. Imaging will be performed on a 3 Tesla Siemens Skyra equipped with a 32-channel head coil and the multi-nuclear option (MNO) running VE11C software. Imaging sequences include the following: To measure structural information: A sagittal 3D

MPRAGE sequence with 0.7mm isotropic voxels (TR=2400, TE=2.57, TI=1100, FA=8) will be used to acquire high-resolution structural data for volumetric analysis of brain region changes related to metformin. The higher resolution allows for less variable segmentation and parcellation resulting in more accurate volumetric measurements. To measure white matter information: An axial 2D symmetric multi-slice (SMS) diffusion tensor imaging (DTI) sequence with 60 diffusion directions, 5 B0 acquisitions and 2mm isotropic voxels (TR=3000, TE=73, FA=90, ETL 43, both A-P and P-A phase encoding for B0 images) will be used to acquire white matter integrity data related to metformin. To measure global brain blood flow information: An axial 3D pseudo-continuous arterial spin labelling (pCASL) sequence (WIP 818) with 4mm isotropic voxels (TI1=1800, TI2=3600, iPat $\leq$ 2) will be used to acquire quantitative blood flow measurements (flow in ml/100g/min) throughout the brain. A post-label delay of 1800 ms will be used as we are imaging otherwise healthy subjects. To measure global brain functional network information: An axial 2D echo-planar imaging sequence with 3mm isotropic voxels (FA=90, TE=30, TR=2000, # slices=50) will be used to acquire resting state functional MRI data throughout the brain. To measure brain lactate: A single voxel point-resolved MR spectroscopy sequence (PRESS, 2 cm isotropic voxel, TE=30, TR=2000, 5000 Hz, 2048 points, 128 acquisitions with water suppression and 16 acquisitions without water suppression) will be used to acquire metabolite information from the right dorsolateral prefrontal cortex. To measure brain ATP levels: Multivoxel chemical shift imaging (dual-tuned proton (Helmholtz pair)/phosphorus (loop) flex coil applied such that the phosphorus loop overlies the right DLPFC, WIP 1071, axial 1.5 cm slice prescribed to encompass the right dorsolateral prefrontal cortex, 12x12 matrix reconstructed to 16x16 matrix, 1.5 cm nominal isotropic voxels, TR=1500) will be used to acquire phosphorus metabolite information from the right DLPFC.
Following the MRI measurement, the subject will remain at Charlton 6 for a brain PET scan. Brain glucose uptake will be evaluated using 18F-Fludeoxyglucose injection and positron emission tomography (PET) scans at baseline and following 40-weeks of placebo or metformin treatment. The results will provide insight into how brain metabolism changes with metformin treatment.

Imaging: Each patient will be injected with approximately 8 mCi (440 MBq) (range 7-10mCi) of F-18 FDG. The F-18 FDG scan will be performed 30 minutes after injection is performed with an 8-10-minute acquisition. The PET sinograms will be reconstructed using iterative reconstruction into a 256 mm field of view; the pixel size is 1.0 mm and the slice thickness is 3.3 mm. A helical CT image, obtained prior to injection of FDG, will be used for attenuation correction and a model-based scatter correction applied. Random coincidences will be corrected for using single event count rates and corrections for dead-time and decay will be applied. An absolute calibration correction, which converts the image intensity into activity concentration, will be used.

Quantitative PET ROI Analysis: Anatomic standardization and statistical quantification of FDG PET image sets will be performed using a program (Cortex ID, GE Healthcare, WI) that generates standardized three-dimensional stereotactic surface projection datasets for individual subjects. In this algorithm, an individual brain image set will be first realigned to the midsagittal plane. The AC-PC line (a line passing through the anterior and posterior commissures) will be estimated by iterative matching between the individual image set and a standard atlas template. This standard atlas template was produced as an average of FDG images from 66 healthy

volunteers. The individual image sets will be realigned to the standard stereotactic coordinate system based on the estimated AC-PC line. Differences in size between the individual brain and the standard template will be removed by linear scaling. For this process, nine affine transformation parameters will be estimated. To adjust the individual brain shape to the stereotactic atlas coordinate system proposed by Talairach and Tournoux, nonlinear warping along the directions of major neuronal fiber bundles within the brain will be performed. The directions will be predefined in the stereotactic coordinate space on the basis of the fiber origins and their cortical projections. Individual landmarks for cortical projections will be searched iteratively between centers and cortical landmarks predetermined on the template brain using a profile curve analysis. Detected individual landmarks will then be warped to predefined landmarks, resulting in a standardized image set with a uniform voxel size of 2.25 mm, interpolation to 60 slices, and a matrix size of 128 x128. This enables reliable pixel-by-pixel comparisons of these anatomically standardized brain images. To determine projection map values, the program will use a predetermined vector that is 6 pixels (13.5mm) long and oriented perpendicular to the outer and medial surfaces of the right and left-brain hemispheres for each surface pixel. The surface pixel will be assigned the highest pixel value found along this vector. We will normalize surface pixel values to the pons although cerebellar or whole brain normalization measures will be calculated also for comparison. The normalized cortical pixel values will be used to calculate a statistical map, which will show surface pixel-by-pixel z-scores derived from comparing an individual's scan with results in normal controls. These Z-score maps and values will be used for comparison of baseline and post-test scans to detect changes in brain metabolism.

Approximately 1 week after completion of the outpatient study visits, we will assess insulin sensitivity by performing a 6 hour two-stage hyperinsulinemic-euglycemic clamp at Mayo Clinical Research Trial Unit (CRTU) as an inpatient study following an overnight fast. On the evening prior to the clamp, participants will receive a standardized diet from the CRTU research kitchen (10kcal/kg body weight; composition 20% protein, 30% fat and 50% carbohydrate). Following the meal, we will use the computerized NIH toolbox to measure cognitive outcomes. The NIH encourages the use of these tools to enhance collection of data in large studies and for clinical trials (7). The cognitive and behavioral tests were normed across a national sample of persons aged 3-85 years. We will utilize the iPad version of the NIH Toolbox because we have shown that residents within Olmsted County, MN prefer the iPad over a personal computer (8). The NIH Toolbox-Cognition Battery is composed of 7 core tests and 2 supplemental tests (~30-45 minutes) including the Dimensional Change Card Sort (DCCS) Test (executive function), the Flanker Inhibitory Control Test (executive function), the Picture Sequence Memory Test (episodic memory), the Picture Vocabulary Test (vocabulary), the Oral Reading Recognition Test (reading), the List Sorting Working Memory Test (working memory), the Pattern Comparison Processing Speed Test (processing speed), the Rey Auditory Verbal Learning Test Trials 1-3 (episodic memory), and the Oral Symbol Digit Test (processing speed). For each test, the raw scores will be converted to normally distributed standard scores. The NIH Toolbox-Cognition is an in-person test that requires specialized knowledge for administration (use is limited to individuals supervised by a psychologist), as well as back and forth interactions with the patient and a provider throughout its administration. Several measures use computer adaptive testing, which bases subsequent items on a subject's previous response, essentially shortening the time to administer the measure while improving the psychometric properties of the measure (e.g.,

reliability, validity). Because it is administered directly on the iPad, scoring is instantaneous which greatly improves clinical efficiency and reduces costs. Also, iPad administration will help to reduce administration and scoring errors and further increase standardization across sites. Due to this iPad administration and the use of computer adaptive testing, there is no single form for this test than can be reviewed. However, detailed training videos are available online that illustrate test content and administration: http://www.healthmeasures.net/NIH_Toolbox_iPad_e-learning/story_html5.html

Participants will be offered the opportunity at their screening visit to meet with the dietary staff of the CRTU to discuss dietary preferences and address any concerns or questions. Three hours prior to start of hormone (somatostatin) infusion, priming [6,6'2H2]glucose bolus (approximately 6 mg/kg FFM) will be administered followed by continuous infusion of [6,6'2H2]glucose (approximately 4 mg/kg FFM/h). [6,6'2H2] glucose infusion rate will be titrated downward by 10-30% at 1-2-hour intervals after start of the hormone infusions. For the first 3 hours regular insulin will be infused at a rate of 0.6 mU/kgFFM/minute then 2.4 mU/kgFFM/minute for 3 hours. Euglycemic blood glucose will be maintained with titrated infusion of [6,6'2H2] glucose labeled 40% dextrose solution. Blood will be collected from a catheter inserted in their dorsal hand vein that is kept in a hot box to collect arterialized venous blood. Urine will be collected prior to starting the insulin clamp until the end of the clamp. A stool sample will also be collected during this visit to assess changes to the microbiome based on the study intervention. If participants are unable to provide a stool sample at this time, a kit will be provided with instructions on how to perform the collection at home and how to return the kit to the study team. The stool samples will be sent to the Cell Signaling Clinical Core Lab in the Guggenheim for storage. When the samples are pulled for testing, they will be sent to the University of MN – Genomics Center to have the test performed. After the clamp participants will be able to resume their usual diet and will be discharged from the CRU. We will perform indirect calorimetry of 20 min each at the baseline and during the second phase of hyperinsulinemic-euglycemic clamp.

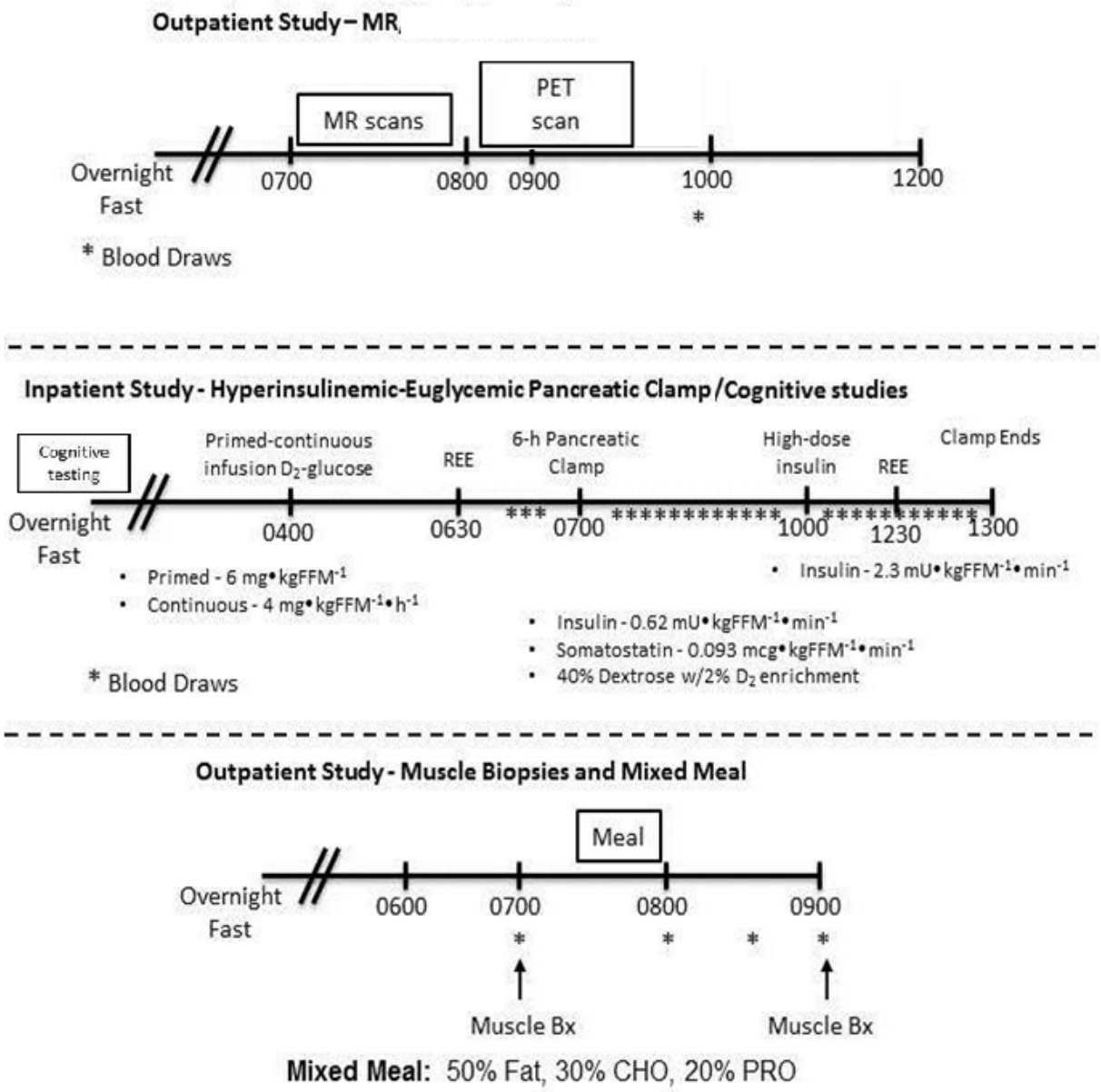
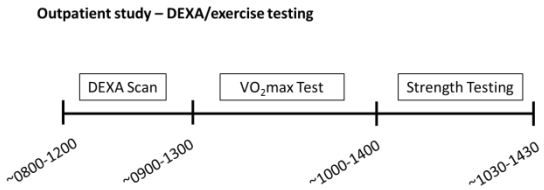


Figure 1. An outline of the study day plan includes an initial study day to examine the primary outcome of brain measures, followed by subsequent hyperinsulinemic euglycemic clamp and muscle biopsy with mixed meal challenge.



Approximately 1 week after the inpatient study visit, we will have a second outpatient visit to assess skeletal muscle changes. Participants will be asked to report to the St. Mary's CRTU fasting at 6am. Percutaneous vastus lateralis muscle biopsies will be performed prior to and 1-h post a 25 kcal/kg FFM mixed meal (50% Fat, 30% Carbohydrate HO, 20% Protein) as we have previously described (9). Briefly, muscle biopsies will be performed with a percutaneous biopsy needle (modified Bergstrom needle). Local subcutaneous injection of 2% lidocaine buffered with 8.4% sodium bicarbonate will be used for analgesia. A small incision will be made through the skin and fascia. After the biopsy, pressure will be held over the incision until hemostasis is achieved. The incision is closed with sterile strips, gauze, and an Ace wrap. Risks of this procedure include hematoma, infection, and pain. Hematoma likelihood is minimized by holding pressure after the biopsy to ensure hemostasis, followed by a pressure dressing. The risk of infection is minimized by using sterile surgical techniques. Pain is managed by local analgesia during the procedure and Tylenol following the procedure. Muscle mitochondrial studies will be performed in muscle samples as we have previously reported (9). We will also be examining muscle samples for changes in protein expression, metabolite concentrations, and gene expression. This visit should last approximately 5 hours.
The plan for various measurements and study days is outlined in Figure 1.

Following the baseline study visits, participants will be randomly assigned to placebo or metformin. A pharmacist will administer identical placebo or metformin tablets and study coordinator will call subjects twice a month for the first 2 months to ensure compliance. Participants will return every 4 weeks to refill their prescription where a tablet tally will be performed to determine the adherence to the drug. We will measure plasma metformin levels at each monthly return visit.

At the conclusion of the 40-week intervention period, subjects will return to repeat the4 study visits outlined above (DEXA scan/VO_2max test, MRI/cognitive study/PET scan, inpatient clamp study, and muscle biopsy study). A study flow diagram is shown in Figure 2 for clarity.

Figure 1. An outline of the study day plan includes an initial study day to examine the primary outcome of brain measures, followed by subsequent hyperinsulinemic euglycemic clamp and muscle biopsy with mixed meal challenge.

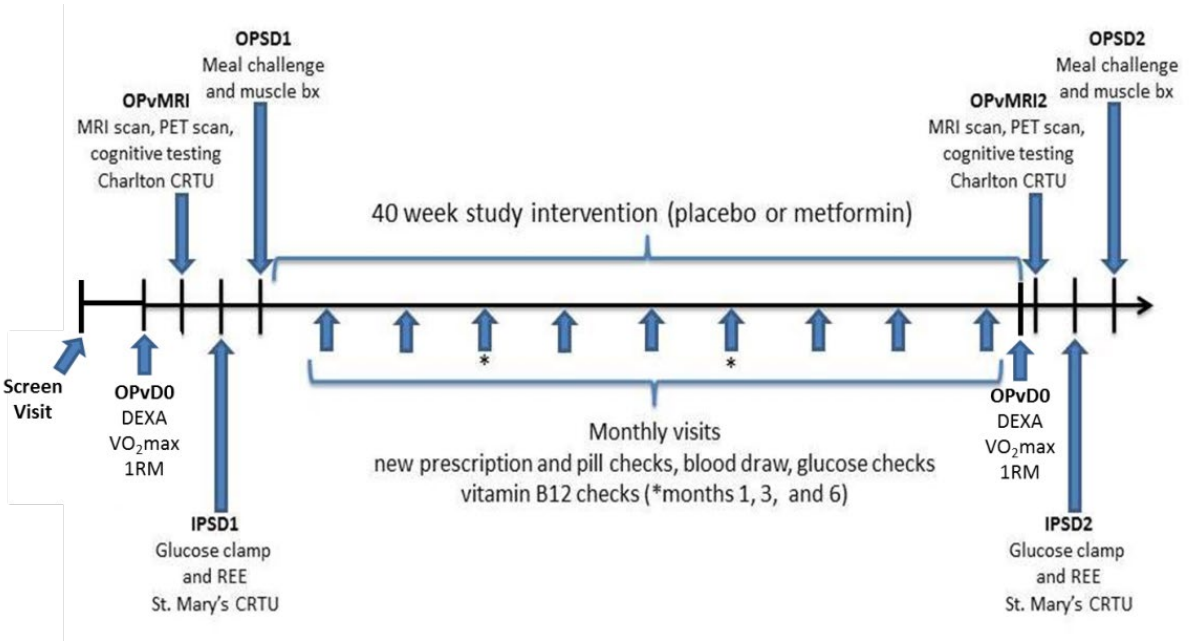


Figure 2. Diagram of the overall study design with visits marked with blue arrows. Study consists of both pre- and post-intervention study visits.

We will escalate the dose of metformin (500 mg metformin and blinded placebo) (week 1, 1 tablet each with breakfast and dinner twice daily; week 2, 2 tablets with breakfast and 1 with dinner, and week 3, 2 tablets with breakfast and 2 tablets with dinner). Administration of tablets with meal reduces the gastro-intestinal (GI) adverse effects which are the most common adverse effects especially when taking metformin on an empty stomach. If the participant develops any adverse GI symptoms on escalating the dose, we will revert back to the previous dose. The maximal dose that is FDA approved is 2500 mg, and the optimum dose clinically used is 2000 mg. We opted to target the maximum dose since some aging literature suggests that for anti-aging effect the higher dose is preferred. The time scale for escalation of metformin dose will be standardized for all participants. Those who develop gastrointestinal symptoms as we escalate the dose to the optimum dose will be analyzed separately from the participants that are able to adhere to the escalation protocol. To account for the anticipated 10% that will experience GI symptoms, we have increased our accrual by 3 participants and will monitor the study to determine if additional participants are needed to complete the per protocol analysis. The PI is a consultant endocrinologist treating exclusively patients with diabetes and lipid disorders at Mayo Clinic and he has extensive experience in using metformin in patients as well as in research (5).

The randomization order will be determined by the statistician and the tablets will be administered by the pharmacy staff in our CRTU pharmacy department. Since vitamin B12 deficiency has been reported (6) (4.3 VS:2.3% Metformin VS Placebo on 5 year) we will check vitamin B12 and more sensitive methyl malonic acid at pre-intervention MRI outpatient visit, 4, 12, 24 weeks, and post intervention MRI outpatient visit. All participants will be given vitamin B12 replacement per DSMB recommendation.

To ensure participant safety we will monitor glucose (fasting) when the participants present for scheduled visits to collect medication / placebo. If fasting glucose is $\geq$ 140 mg/dl we will measure HbA1c. If the HbA1c is $\geq$7% we will refer to our safety officer (Dr. Adrian Vella who is consultant diabetologist) who will determine whether the participants need to be placed on "open label"

metformin. Since the HbA1c target in that age group is 7% we consider up to 7% as a level that does not need intervention. While this management could break study blinding, the cognitive assessments and imaging will be conducted by individuals that remain blind to the treatment.

Participants who do not show a reaction to the high dose insulin during the post-intervention hyperinsulinemic-euglycemic clamp will be asked to repeat this visit to rule out vein infiltration during the previous post-intervention insulin clamp.

Sample size justification. This is a developmental grant, so the sample size is not justified based on power for any of the particular aims. Rather, the estimate is based on precision and reliable estimates of the imaging studies. In particular, for the fMRI study Thirion et al (4) found that 20 to 25 subjects were necessary to achieve reliable results. Given we want to study 20 within-group metformin subjects with fMRI, balancing the randomization 1:1 will yield a total sample size of 40. The effect sizes for between group differences are unknown; however, we will have 80% power to detect an effect size of at least 0.9 SD difference between the groups at alpha=0.05 (two-sided).

Statistical Considerations

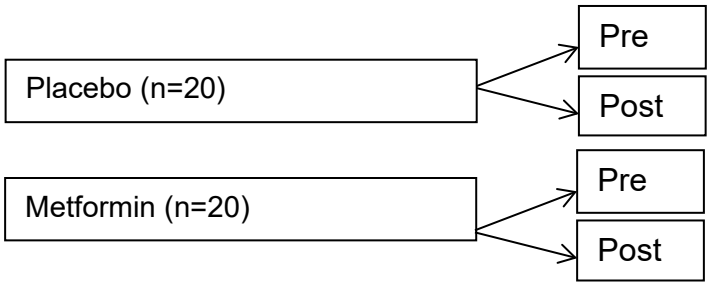
Statistical plan: The analysis of the aims will be focused on estimation – standard deviations of MRS estimates and initial examinations of the changes in measures over time. With 20/group and repeated measures, we will be able to fit repeated measures models (e.g., mixed effects "trajectories"). For MRS with only a pre-post assessment, the mixed model framework is essentially a two-sample t-test on the changes over time, but importantly, the modeling framework will allow inclusion of all available data. This is to provide the most degrees of freedom to estimate the residual error, which will be used to plan a definitive study should these results appear promising (biological engagement as quantified by MRS and fMRI, change in insulin sensitivity, etc.) Supporting these analyses will be standard descriptive and graphical summaries of the data.

Interpretation: In this double-blind placebo-controlled study all data interpretation will be performed at the end of the study and the un-blinding will be done by the statistician. Statistical plan is as outlined above. We will make conclusions on whether our hypotheses are proved or disproved based on statistical significance ($P<0.05$) of the outcomes that are outlined under MR data analysis and cognitive functions. If the observed data reaches statistical significance for one or more outcomes our outlined hypotheses will be proven. However, if the significance level does not reach statistical significance but shows promising results, that will offer opportunities for a larger study with more participants and perhaps longer duration.

SUBJECT DETAILS

1. Overview

We will recruit 40 subjects with equal numbers of men and women age ≥ 60 yrs., BMI > 25, fasting glucose ≥ 100-140 mg/dL (1). Subjects will be randomized to one of 2 intervention groups as described below.



Protocols**:** A common protocol will be followed for baseline studies and post intervention-studies. Our sample size assumes 25% drop-out (see Statistical Analysis section of Research Plan for more details. We will exclude anybody taking anti-hyperglycemic drugs including metformin. We excluded T2D people because of the variability of their insulin secretion

and because in Olmsted County, most people with a fasting glucose >140 mg/dl are on metformin. We will also exclude people who engage in aerobic training ≥ 3 d/wk and anybody with association active cardiovascular disease, renal failure, or liver function abnormalities. Subjects meeting the inclusion criteria will undergo a DEXA scan to determine body composition, which will be repeated following 40 weeks.

Approach to recruitment:
Volunteers will be recruited from advertisements in the Mayo bulletin boards, electronic bulletin boards and local and regional newspapers and magazines. Regional radio advertising will also be used. The Mayo Clinic biobank and Rochester Epidemiology Project will be utilized to identify potential volunteers. Each volunteer will meet one of the investigators who will explain the scientific rationale of the study, the procedures, potential risks involved and rights of the participant in the study. The consent form will be approved by the Mayo Clinic.

2. Risks to subjects

2.1.1. Human Subjects Involvement, Characteristics, Design and Procedures:

We anticipate recruiting a total of 54 adults ages ≥ 60 yrs. All racial/ethnic groups will be eligible for participation. Based on our experience, we expect that approximately 75% in each arm will complete the study (N=20 for each group).

Human Subjects Involvement and Characteristics: All protocols and all techniques to be used will be approved by the Mayo Clinic Institutional Review Board prior to initiation of any studies. Subject characteristics have been briefly mentioned above.

Inclusion criteria: To be eligible for the study, a subject must be willing to be randomized and be retained in the study for its duration and meet the following criteria:

Age ≥ 60 yrs.
BMI ≥ 25 kg/m_2
Fasting glucose ≥ 100-140 mg/dL
Non-smoker
English language proficiency

Exclusion Criteria: The presence of any of the following is an exclusion for the study:

- Active coronary artery disease or heart failure.
- A known medical condition that in the judgment of the investigator might interfere with the completion of the protocol such as the following examples:
  - Inpatient psychiatric treatment in the past 6 months
  - Presence of a known and active adrenal disorder
  - Abnormal liver function test results (Transaminase >2 times the upper limit of normal); testing required for subjects taking medications known to affect liver function or with diseases known to affect liver function
  - Abnormal renal function test results (calculated GFR <60 mL/min/1.73m2); testing required for subjects with diabetes duration of greater than 5 years post onset of puberty
  - Active gastroparesis
  - If on antihypertensive, thyroid, anti-depressant or lipid lowering medication, lack of stability on the medication for the past 2 months prior to enrollment in the study
  - Uncontrolled thyroid disease (TSH undetectable or >10 mlU/L); testing required within three months prior to admission for subjects with a goiter, positive antibodies, or who are on thyroid hormone replacement, and within one year otherwise
  - Abuse of alcohol or recreational drugs
  - Infectious process not anticipated to resolve prior to study procedures (e.g. meningitis, pneumonia, osteomyelitis).

- Uncontrolled arterial hypertension (Resting diastolic blood pressure >90 mmHg and/or systolic blood pressure >160 mmHg) at the time of screening.
- Oral steroids
- A recent injury to body or limb, muscular disorder, use of any medication, any carcinogenic disease, or other significant medical disorder if that injury, medication, or disease in the judgment of the investigator will affect the completion of the protocol
- Any metal in the body that could interfere with magnetic resonance imaging (MRI) including pacemaker or implanted defibrillator, neurostimulators, ear implants, metal fragments within the body, metal joints, rods, pins, plates, or screws.
- Subjects without English proficiency

Restrictions on Use of Other Drugs or Treatments:

1. Medications that may impact study end points such as mitochondrial biology e.g. beta blockers
2. Anti-hyperglycemic drugs including metformin
3. Any other medication that the investigator believes is a contraindication to the subject's participation.

Procedures: MRI scanning of the brain: We will be performing pre- and post-intervention MRI scanning to measure brain ATP production by NMR spectroscopy. We will measure brain structure (grey and white matter content) and functions by MRI, fMRI. Participants will be explained all testing procedures and how the test can be terminated if the participant wishes to be removed from scanner. Injury within the MRI will be minimized by having each participant complete a magnetic materials safety questionnaire to ensure that it is safe for them to be exposed to strong magnetic fields. Burns due to touching the sides of the magnet will be avoided by instruction participants of proper positioning.

Interventions: Metformin: We will use Metformin in half of the participants. We will enquire about the prior use of this agent during a detailed history and physical examination as part of the screening visit. We will also ensure that the likelihood of subjects developing gastrointestinal side effects is low by taking tablets in the middle of meals. Subjects will start metformin at 500 mg twice daily and titrate upwards by 500 mg every 1-2 weeks to reach a maximum dose of 2000 mg (week 1, 1 tablet each with breakfast and dinner. Week 2, two tablets with breakfast and 1 with dinner and week 3 two tablets with breakfast and two tablets with dinner).

2.1.2. Sources of Materials:

Samples of blood obtained during the course of the experiments will be used exclusively for research purposes. No use will be made of pre-existing specimens.

Screening visit: All subjects will be consented by one of the study personnel after giving them appropriate time to understand the procedures and commitment needed to complete the study. The study physician will perform a detailed history and physical with special emphasis on history of glucose tolerance. Clinical Research Unit (CRU) personnel will obtain routine vital signs and draw blood for screening labs, including hematocrit, comprehensive chemistry panel (including hematocrit, fasting plasma glucose, serum creatinine, liver function tests and TSH). If these tests are available close to recruitment, the same results will be used, and unnecessary testing avoided. This study is not meant to find out if the participant has any other disease or problem. The study leaders will alert the participant if any of the research results are important to his/her health during the study. The participant may have a copy of the screening tests to discuss with his/her personal physician. When the blood tests are completed, any blood left over will be thrown away. It will not be stored for any future testing.

Metabolic Measures: We will record participants' body weight, height, body mass index (BMI). All subjects will be studied after fasting with a hyperinsulinemic, hyperglycemic clamp. After an overnight fast, participants will have catheter inserted in their dorsal hand vein that is kept in a hot

box to collect arterialized venous blood at Mayo Clinical Research Trial Unit (CRTU) where baseline blood samples and then following an insulin infusion at two different doses at 3 hours interval. We will collect blood samples every 10 min to adjust dextrose infusion to maintain blood glucose between 85 to 90 mg/dl. Another day they will be given a mixed meal and pre and post meal (1 hour) needle biopsy of muscle will be performed.
We will perform muscle biopsy of the vastus lateralis muscle under sterile precautions under local anesthesia. We have performed over 4000 muscle biopsies without any major adverse effects. The potential side effects are local hematoma that is mostly prevented by pressure applied for 10-15 min following biopsy. Infection is extremely rarely occurred as we perform the biopsies under strict sterile precautions and follow up carefully.
There is an institutional electronic tracking system to ensure that a patient is not in more than one study at a time. Intravenous risks will be minimized by having experienced CRU staff place the lines and infusing normal saline to decrease the risk of phlebitis or occlusion.

Randomization: A participant who meets all the inclusion and exclusion criteria will be enrolled in the study. After completion of baseline studies, the participant will be randomized to one of the study groups as described above.
Medical/Technical support: Study personnel will be available at all times during the length of the trial to answer any question or troubleshoot any problem.
Access to individually identifiable private information about human subjects: Permission to gather, use and share information about the participant will be obtained in the consent forms. If the participant decides not to give permission, he/she cannot be in the study. This collected information may include personal information such as name, address, date of birth, social security number, medical records and test results from before, during and after the study from any of the participant's doctors or health care providers (including mental health, substance abuse, and HIV/AIDS records) and information needed to bill others for participants care. These records may be accessed by the study team, people or committees that oversee the study, people who pay for the study, including insurance companies, tax reporting offices, people who evaluate study results, and government agencies that provide oversight such as the Office for Research Subject protection. The information collected might be published in a medical journal in a way that protects participant privacy. The participant may change his/her mind at any time; however, permission does not end unless the participant cancels it by sending a letter or email to the researcher. The researchers will still use information about the participant that was collected before his/her participation ended. All researchers will do everything possible to protect participant privacy.

2.1.3. Potential Risks and minimization:

Feasibility, Recruitment, and Informed Consent: These have been described earlier in this section.
Each willing participant will meet one of the investigators who will explain the scientific rationale of the study, the procedures and potential risks involved in the study. The consent forms will be approved by the Mayo Foundation IRB prior to use. Informed written consent from the participant will be obtained by one of the investigators prior to participation. An electronic note will be entered in each participant's medical record regarding the consent and a copy of the consent will be electronically kept with the participant's medical record. A copy of the consent will be given to the subject and the original kept in the study records.

The principal investigators or their co-investigators meet with each participant, review the consent form in detail and confirm the subjects understanding of the study. They answer all questions posed by the participants and when convinced that the subject verbally demonstrates understanding of the protocol obtain a signed consent. Only designated study staff is authorized to obtain informed consent.

All experimental protocols will be conducted at Mayo Clinic Rochester CRU of the Mayo Clinic Center for Clinical and Translational studies (CCaTS).
Protection during in-patient studies: All protocols and all techniques to be used will be approved by the Mayo Clinic Institutional Review Board prior to initiation of any studies. All participants will have the Mayo Clinic paging operator available to them 24 hours a day to contact the investigators for any problems. The following protection will be taken for the risks identified above:

1. Intravenous risks will be minimized by having experienced CRU and study staff place the lines and infusing 0.9% normal saline throughout the study to decrease the risk of phlebitis or occlusion.
2. The amount of blood drawn is within the amounts acceptable by the institution. There is a Center for Clinical and Translational Studies (CCaTS) electronic tracking system that monitors if a subject is in more than one research study at a time.

The following are the potential risks for this study with minimization strategies.

a) Blood will be withdrawn. The total blood withdrawn during each study protocol will not exceed 550 ml. Hemoglobin measured before starting the study will be greater than 12.0 g/dl in males and 11.0 g/dl in females. All subjects will refrain from giving blood or being in any other research study eight weeks prior to the study and until eight weeks after the completion of the study.

b) Metformin will be administered. We will enquire about the prior use of this agent during a detailed history and physical examination as part of the screening visit. Subjects will start metformin at 500 mg twice daily and titrate upwards by 500 mg every 1-2 weeks to reach a maximum dose of 2000mg (week 1, 1 tablet each with breakfast and dinner twice daily; week 2, two tablets each with breakfast and one with dinner and week 3, two tablets with breakfast and two tablets with dinner). Administration of tablets with meal reduces the gastro-intestinal (GI) adverse effects which are the most common adverse effects, particularly when taking metformin on an empty stomach. If the participant develops any adverse GI symptoms on escalating the dose we will revert back to the previous dose. The maximal dose FDA approved is 2500 mg and the optimum dose clinically used is 2000 mg. Since vitamin B12 deficiency has been reported (4) (4.3 VS:2.3% Metformin VS Placebo on 5 year) we will check vitamin B12 and more sensitive methyl malonic acid at pre-intervention outpatient MRI visit, 4, 12, 24 weeks, and post-intervention outpatient MRI visit. All participants will be given Vitamin B12 replacement per DSMB recommendation

c) Vascular catheters will be placed. Catheter insertion and blood withdrawal are associated with a small risk of phlebitis. This will be minimized by careful attention to sterile technique. If phlebitis occurs, it will be treated conservatively with heat and when appropriate, with antibiotics. In all experiments, “arterialized – venous” blood will be obtained by placing a hand in which a catheter has been inserted, in a heated box during the study. The temperature inside the box is maintained at ~55°C. With prolonged exposure to continuous heat, there is a potential risk of local skin irritation or a minor burn. If this occurs, it will be treated appropriately. However, we have used this technique for the past 25 years and have had no instances of hot box related burns or injuries. The potential risks of catheters and hot box use will be discussed with the volunteers prior to obtaining consent for the study.

D) We will be performing pre- and post-intervention MRI scanning to measure brain ATP production by NMR spectroscopy. We will measure brain structure (grey and white matter content) and functions by MRI, fMRI. The main risk of MRI procedures is claustrophobia. Participants will be explained all testing procedures and how the test can be terminated if the participant wishes to be removed from scanner. Injury within the MRI will be minimized by having each participant complete a magnetic materials safety questionnaire to ensure that it is safe for them to be exposed

to strong magnetic fields. Burns due to touching the sides of the magnet will be avoided by instruction participants of proper positioning.

2.2. Potential Benefits of the Proposed Research to the Subjects and Others

The risks to the subjects, including the risk of blood drawing, metformin administration, vascular catheters, muscle biopsies, and MRI scanning are minimal. In our opinion, the risks are justified by the expected knowledge gained from the studies proposed in this application.

2.3. Importance of the Knowledge to be Gained

Our work seeks to build on more than 25 years of clinical investigative experience and more than 20 years of technology development. Both age and insulin resistance are associated with a high prevalence of dementia. Insulin resistance is highly prevalent with advancing age. Metformin is an insulin sensitizer and is currently being extensively investigated for its potential anti-aging effect. However, only very limited information is available on metformin effect on brain, which is a major organ affected by aging. With appropriate experimental design, we are attempting to understand the mechanism of metformin treatment on the physiology of the brain as well as cognitive effects. Our studies may uncover relationships that could be favorably manipulated to decrease health risks associated with insulin sensitivity and the effect on the brain.

VI. Gender/Minority Mix

There are no exclusions based on gender or minority.

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Statistical Analysis Plan

Statistical plan: The analysis of the aims will be focused on estimation – standard deviations of MRS estimates and initial examinations of the changes in measures over time. With 20/group and repeated measures, we will be able to fit repeated measures models (e.g., mixed effects "trajectories"). For MRS with only a pre-post assessment, the mixed model framework is essentially a two-sample t-test on the changes over time, but importantly, the modeling framework will allow inclusion of all available data. This is to provide the most degrees of freedom to estimate the residual error, which will be used to plan a definitive study should these results appear promising (biological engagement as quantified by MRS and fMRI, change in insulin sensitivity, etc.) Supporting these analyses will be standard descriptive and graphical summaries of the data.

Sample size justification. This is a developmental grant, so the sample size is not justified based on power for any of the particular aims. Rather, the estimate is based on precision and reliable estimates of the imaging studies. In particular, for the fMRI study Thirion et al (1) found that 20 to 25 subjects were necessary to achieve reliable results. Given we want to study 20 within-group metformin subjects with fMRI, balancing the randomization 1:1 will yield a total sample size of 40. The effect sizes for between group differences are unknown; however, we will have 80% power to detect an effect size of at least 0.9 SD difference between the groups at alpha=0.05 (two-sided).

MR Data Acquisition: As the effects of metformin on the human brain are not known, a multi-parametric imaging approach will be used to probe different aspects of the CNS. Imaging will be performed on a 3 Tesla Siemens Skyra equipped with a 32-channel head coil and the multi-nuclear option (MNO) running VE11C software. Imaging sequences include the following: To measure structural information: A sagittal 3D MPRAGE sequence with 0.7mm isotropic voxels (TR=2400, TE=2.57, TI=1100, FA=8) will be used to acquire high-resolution structural data for volumetric analysis of brain region changes related to metformin. The higher resolution allows for less variable segmentation and parcellation resulting in more accurate volumetric measurements. To measure white matter information: An axial 2D symmetric multi-slice (SMS) diffusion tensor imaging (DTI) sequence with 60 diffusion directions, 5 B0 acquisitions and 2mm isotropic voxels (TR=3000, TE=73, FA=90, ETL 43, both A-P and P-A phase encoding for B0 images) will be used to acquire white matter integrity data related to metformin. To measure global brain blood flow information: An axial 3D pseudo-continuous arterial spin labelling (pCASL) sequence (WIP 818) with 4mm isotropic voxels (TI1=1800, TI2=3600, iPat ≤ 2) will be used to acquire quantitative blood flow measurements (flow in ml/100g/min) throughout the brain. A post-label delay of 1800 ms will be used (2) as we are imaging otherwise healthy subjects. To measure global brain functional network information: An axial 2D echo-planar imaging sequence with 3mm isotropic voxels (FA=90, TE=30, TR=2000, # slices=50) will be used to acquire resting state functional MRI data throughout the brain. To measure brain lactate: A single voxel point-resolved MR spectroscopy sequence (PRESS, 2 cm isotropic voxel, TE=30, TR=2000, 5000 Hz, 2048 points, 128 acquisitions with water suppression and 16 acquisitions without water suppression) will be used to acquire metabolite information from the right dorsolateral prefrontal cortex (DLPFC, chosen due to the high number of insulin receptors in this region (3). As metformin has been shown to alter mitochondrial function in other organs, we will measure lactate in study participants to determine if they have significant anaerobic metabolism. To measure brain ATP levels: Multivoxel chemical shift imaging (dual-tuned proton (Helmholtz pair)/phosphorus (loop) flex coil applied such that the phosphorus loop overlies the right DLPFC, WIP 1071, axial 1.5 cm slice prescribed to encompass the right dorsolateral prefrontal cortex, 12x12 matrix reconstructed to 16x16 matrix, 1.5 cm nominal isotropic voxels, TR=1500) will be used to acquire phosphorus metabolite information from the right DLPFC. B1 in homogeneities will be corrected using a second high-speed acquisition (4). We may be able to detect changes in ATP levels in metformin subjects due to less efficient ATP production.

MR Data Analysis: Brain volumetrics: MPRAGE data will be analyzed using Freesurfer 6.0 (http://surfer.nmr.mgh.harvard/edu). Briefly, MPRAGE images are converted to a NIFTI volume using mri_convert, then processed using recon_all with manual inspection of data between each recon step. Cortical and subcortical parcellation (5;6) is then performed using the freesurfer DKT40 classifier atlas (reportedly more accurate than the Desikan-Killiany atlas) (7) using mris_ca_label. Volumetric data from each brain region will be compared by group (metformin vs. control) and across time (timepoint A and B). As an exploratory analysis, we may also perform hippocampal subfield parcellation (8), and see how the longitudinal processing stream compares to conventional two-timepoint analysis (Reuter et al 2012). DTI analysis: DTI data will be processed using dt recon with eddy current and motion correction. MPRAGE structural data will then be resampled into DTI space mri_vol2vol, and various DTI metrics (fractional anisotropy (FA), apparent diffusion coefficient (ADC), trace, radial diffusivity (RD) and axial diffusivity (AD) tabulated for each cortical, subcortical and white matter region using mri_segstats. ASL analysis: CBF maps will be calculated per the method of Wang et al (9). CBF data will then be converted into a volume, then co-registered to the MPRAGE data using bbregister. Parcellation masks will then be used to calculate the average CBF in each region. rsFMR analysis: rsFMRI data will be denoised by a well-established preprocessing pipeline (10;11), and registered to the MPRAGE structural data to get functional connectivity matrices across cortical and subcortical parcellations from FreeSurfer. Individual connectivity matrices for whole subjects will be used for the group comparison between placebo and drug-dose groups, and also for canonical correlation analysis between connectivity strength and clinical outcomes (behavioral scores)(12). Proton MRS analysis: single voxel proton MRS data will be processed using LCModel 6.3-1L (13). Phosphorus MRSI analysis: multivoxel phosphorus MRSI data will be reconstructed and quantified using jMRUI 6.0 (http://www.jmrui.eu). For the primary and calibration acquisitions, a simulated 7 metabolite basis set (PME, Pi, PDE, PCr, γ-ATP, α-ATP, β-ATP) will be fit to data following zero- and first-order phasing, and 8 Hz spectral apodization. Data will then be corrected for B1 inhomogeneity per the method of Chmelik (14). Metabolite data from the voxels incorporating the right DLPFC will be averaged and used in the statistical analysis.

Cognitive function: We will use the computerized NIH toolbox to measure cognitive outcomes. The NIH encourages the use of these tools to enhance collection of data in large studies and for clinical trials (15). The cognitive and behavioral tests were normed across a national sample of persons aged 3-85 years. We will utilize the iPad version of the NIH Toolbox because we have shown that residents within Olmsted County, MN prefer the iPad over a personal computer (16). Dr. Mielke will oversee this aspect of the proposal. She is an expert in cognitive function and was a member of the NIH task force on computerized cognitive batteries.
Cognitive Outcomes. The NIH Toolbox-Cognition Battery is composed of 7 tests (~30 minutes) including the Dimensional Change Card Sort (DCCS) Test (executive function), the Flanker Inhibitory Control Test (executive function), the Picture Sequence Memory Test (episodic memory), the Picture Vocabulary Test (vocabulary), the Oral Reading Recognition Test (reading), the List Sorting Working Memory Test (working memory), and the Pattern Comparison Processing Speed Test (processing speed). For each test, the raw scores will be converted to normally distributed standard scores (scaled scores) having a mean of 10 and a standard deviation of 3. To reduce the number of outcomes, *our primary outcomes will focus on the following three composite scores:* Crystalized Cognition Composite (Picture Volabulator Test and Oral Completion Test), Fluid Cognition Composite (DCCS, Flanker test, Picture Sequence Memory Test, List Sorting Working Memory, and Pattern Comparison Processing Speed), and the Total Cognition Composite score. Both the individual tests and composite outcomes have been found to have good convergent and discriminant validity and test-retest reliability (17-19).

Interpretation: In this double-blind placebo-controlled study all data interpretation will be performed at the end of the study and the un-blinding will be done by the statistician. Statistical plan is as outlined above. We will make conclusions on whether our hypotheses are proved or disproved based on statistical significance ($P<0.05$) of the outcomes that are outlined under MR

data analysis and cognitive functions. If the observed data reaches statistical significance for one or more outcomes our outlined hypotheses will be proven. However, if the significance level does not reach statistical significance but shows promising results, that will offer opportunities for a larger study with more participants and perhaps longer duration.

Limitations: We appreciate that this exploratory (R21) study plan is without having sufficient preliminary data to determine the optimum study duration and the number of participants required to prove or disprove our hypotheses definitively. The primary objective of this type of grant is to obtain robust preliminary data for larger more definitive clinical trials. However, the robust study design, reasonable studies duration (e.g. a preliminary report of 8 weeks administration of metformin reported cognitive improvements (20) may help to overcome the limitation. The objective of acquiring robust preliminary data to determine whether metformin has any definitive or potential benefit is likely accomplished from the current study proposal. Although it has been shown that brain protein synthesis is low at 0.6%/h and half-life is 10 days (21), and may vary in different regions, a 40 week study duration to determine if metformin has any effect on brain structure has a very reasonable chance of detecting changes with our MR approach, especially as suggested by our preliminary studies in diabetic mice. Assuming that human brain tissue turnover is slower than in rodents we are still likely to observe changes in brain structure and functions in 40 weeks. ^{31}P-MRS based ATP production changes are likely to occur relatively rapidly because the enzymes involved rapidly are responsive to stimuli. Here we target our study in a susceptible population without a clinical diagnosis of dementia. Other interventions such as aerobic exercise in our experience enhances brain metabolism even in healthy people with no known cognitive issues with normal fasting glucose up on enhancing insulin sensitivity. Moreover, our main interest is whether metformin reported to have anti-aging effects has any effect on brain in an elderly population who is relatively sedentary and have insulin resistance. Eventually the goal is to combine metformin with other lifestyle measures such as exercise program.

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MAYO CLINIC

Name and Clinic Number

Approval Date: **August 5, 2022**
Not to be used after: August 26, 2022

RESEARCH PARTICIPANT CONSENT AND PRIVACY AUTHORIZATION FORM

Study Title: Metformin Effect on Brain Function in Insulin Resistant Elderly People

IRB#: 18-004012

Principal Investigator: K. Sreekumaran Nair, M.D., Ph.D., and Colleagues

Please read this information carefully. It tells you important things about this research study. A member of our research team will talk to you about taking part in this research study. If you have questions at any time, please ask us.

Take your time to decide. Feel free to discuss the study with your family, friends, and healthcare provider before you make your decision.

To help you decide if you want to take part in this study, you should know:

- Taking part in this study is completely voluntary.
- You can choose not to participate.
- You are free to change your mind at any time if you choose to participate.
- Your decision will not cause any penalties or loss of benefits to which you are otherwise entitled.
- Your decision will not change the access to medical care you get at Mayo Clinic now or in the future if you choose not to participate or discontinue your participation.

For purposes of this form, Mayo Clinic refers to Mayo Clinic in Arizona, Florida and Rochester, Minnesota; Mayo Clinic Health System; and all owned and affiliated clinics, hospitals, and entities.

If you decide to take part in this research study, you will sign this consent form to show that you want to take part. We will give you a copy of this form to keep. A copy of this form will be put in your medical record.

MAYO CLINIC

Name and Clinic Number

Approval Date: **August 5, 2022**
Not to be used after: August 26, 2022

CONTACT INFORMATION

You can contact …	At …	If you have questions about …
Principal Investigator: K. Sreekumaran Nair, M.D., Ph.D. **Study Team Contact:** Fred de Ruiter	**Phone:** (507) 255-2415 **Phone:** (507) 255-8932 **Institution Name and Address:** Mayo Clinic 200 First Street SW Rochester, MN 55905	▪ Study tests and procedures ▪ Research-related injuries or emergencies ▪ Any research-related concerns or complaints ▪ Withdrawing from the research study ▪ Materials you receive ▪ Research-related appointments
Mayo Clinic Institutional Review Board (IRB)	**Phone:** (507) 266-4000 **Toll-Free:** (866) 273-4681	▪ Rights of a research participant
Research Participant Advocate (The RPA is independent of the Study Team)	**Phone:** (507) 266-9372 **Toll-Free:** (866) 273-4681 **E-mail:** researchparticipantadvocate@mayo.edu	▪ Rights of a research participant ▪ Any research-related concerns or complaints ▪ Use of your Protected Health Information ▪ Stopping your authorization to use your Protected Health Information ▪ Withdrawing from the research study
Patient Account Services	**Toll-Free:** (844) 217-9591	▪ Billing or insurance related to this research study

Approval Date: **August 5, 2022**
Not to be used after: August 26, 2022

Other Information:
A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

1. Why are you being asked to take part in this research study?

You are being asked to take part in this research study because you are insulin resistant, a non-smoker and 60 years or older and otherwise healthy.

2. Why is this research study being done?

This study is being done to understand metformin's mechanisms of action regarding glucose production, insulin sensitivity, and brain function.

The plan is to have 40 people complete this study at Mayo Clinic.

3. Information you should know

Who is Funding the Study?
The National Institutes of Health is funding the study. The National Institutes of Health will pay your study doctor or the institution to cover costs related to running the study.

Information Regarding Conflict of Interest:
Your healthcare provider may be referring you to this research study. If your healthcare provider is also an investigator on this study, there is the chance that his or her responsibilities for the study could influence his or her recommendation for your participation. If you prefer, your healthcare provider will be happy to refer you to another investigator on the research study team for you to decide if you want to participate in the study and to see you for the research study activities while you are in the study.

4. How long will you be in this research study?

You will be in the study for approximately 48 weeks.

5. What will happen to you while you are in this research study?

If you agree to be in the study, you will be asked to participate in the following:

The initial study visit will be a Screening Visit. You will be asked to report to the Clinical Research and Trials Unit (CRTU) fasting (nothing to eat or drink after 7 pm the evening before except water) for a blood test, including a CBC, liver function, fasting glucose, lipid panel, and TSH. You will also have height and weight measurements taken and the MoCA test administered. While this is being done, the blood test results will be made available to the study team, and if you will be told if you pass the screening criteria. You will also be offered the opportunity to meet with the dietary staff of the CRTU to discuss dietary preferences and address any questions or concerns. This visit will take approximately 2 hours.

Within 60 days after the Screening Visit, you will have your first outpatient visit:

- You will have a Dual-energy X-ray absorptiometry (DEXA) scan to assess body composition
- You will perform a VO_2max test on a cycle ergometer to assess aerobic capacity. This test involves running on a treadmill while you are hooked up to a breathing mask. The test aims to measure your rate of oxygen consumption while your heart rate is elevated. It will be a progressively difficult test.
- This visit will take approximately 3 hours

Approximately 7 days after your first outpatient visit you will have your second outpatient visit:

- You will be asked to start fasting, take nothing by mouth except water after 7 pm.
- You will then go to the MRI unit in the Charlton building for your MRI testing in the a.m.
- A small blood sample will be taken.
- You will then undergo a special brain MRI that will not use contrast. We will not need to give you any medications for the MRI. The MRI testing will take approximately 1 hour.

- Lastly, you will have a PET scan done on your brain. Before beginning, we will check your blood glucose levels by doing a finger stick. The PET scan is done with a very small amount of a radioactive form of sugar, called 18F-fluorodeoxyglucose, or FDG. A small catheter will be placed in your arm to inject this compound. Once this is injected you will sit for 30 minutes before the pictures begin.
  You will be placed on a scanner bed for 10 minutes while the scans are completed. Afterwards you will be asked to drink fluids and go to the bathroom. This will take about 1 hour.
- Arrive at the CRTU on Charlton 07.
- A small blood sample will be taken.
- Vitamin B12 levels will be assessed.
- You will then be given a meal and allowed to leave after the visit is completed
- This visit will take approximately 3-4 hours.

Approximately 1 week after completion of the MRI study visit, you will be admitted to the CRTU to stay overnight. The following will happen during your first overnight stay:

- You will be given a standardized meal at 6:00 pm.
- We will then ask you to perform approximately 1 hour of cognitive testing. These tests check your memory, thinking speed and other aspects of your thinking.
- You will be asked to fast and take nothing by mouth except water after 7 pm. On the day after admission, we will ask that you have nothing at all by mouth except water for the 6 1/2 hours of this study day.
- Your resting energy expenditure (REE) will also be measured by performing indirect calorimetry during this visit. We will measure how much energy your body uses at rest by placing a special plastic dome over your head and having you rest quietly for 20 minutes. We will be measuring how much oxygen you breathe in and how much carbon dioxide you breathe out. One indirect calorimetry (REE) will be done at the beginning of the clamp and a second indirect calorimetry (REE) will be done during the second phase of the clamp.
- We will be placing a special kind of "backward" intravenous catheter into your hand and then placing that hand into a "hot box." This special kind of IV is the easiest way for us to get samples of blood during the study. The "hot box" is a transparent plastic box that is heated with a small motor to 50 degrees Celsius (122 degrees Fahrenheit). We will be taking frequent samples of blood throughout the study. The total amount of blood taken during study (including blood taken during the medical evaluation and all stays on the CRTU) will be about one pint (about as much as a standard blood donation).
- At approximately 4 a.m. we will be placing intravenous (IV) needles to give you insulin and somatostatin. When these IV fluids are prepared, a small amount of albumin, a protein common in our bodies, is used. Both insulin and somatostatin are hormones that are normally present in your body. The somatostatin used in this study is considered

investigational. It has not been approved by the Food and Drug Administration (FDA) for routine clinical use. However, the FDA has allowed the use of somatostatin in this research study. Giving you insulin and somatostatin into your veins is called an "insulin clamp."

- Urine will be collected prior to starting the insulin clamp until the end of the clamp. A stool sample will be collected at this visit. If you are unable to provide a stool sample at this time, a kit will be provided with instructions on how to perform the collection at home, and how to return the kit to the study team.
- You will also receive IV glucose. Glucose is produced in your body mainly by the liver. By giving you glucose that is slightly different than the glucose produced by your body, we will be able to measure how much glucose you produce.
- When the intravenous lines are started, you will also receive a small amount of IV fluid that contains sodium chloride.
- All infusions will be stopped by 1:30pm and a meal will be provided for you by the metabolic kitchen to consume.
- Once deemed stable by the investigative team you will be allowed to leave.

Approximately 1 week after the inpatient study visit, you will have a third outpatient visit to assess skeletal muscle changes.

- You will be asked to fast and take nothing by mouth except water after 7 pm. The next day you will be asked to arrive at the CRTU at 6am for admission. On the day of admission, we will ask that you have nothing at all by mouth except water for the 6 1/2 hours of this study day.
- We will be placing a special kind of "backward" intravenous catheter into your hand and then placing that hand into a "hot box." This special kind of IV is the easiest way for us to get samples of blood during the study. The "hot box" is a transparent plastic box that is heated with a small motor to 55 degrees Celsius (131 degrees Fahrenheit). We will be taking frequent samples of blood throughout the study. The total amount of blood taken during study (including blood taken during the medical evaluation and all stays on the CRTU) will be about one pint (about as much as a standard blood donation).
- When the intravenous lines are started, you will also receive a small amount of IV fluid that contains sodium chloride (i.e., saline).
- We will perform two muscle biopsies to obtain a small piece of your muscle from the upper part of your leg. Muscle biopsies are obtained to measure markers of protein metabolism and function, including your DNA. The area of your leg will be numbed with Lidocaine that is mixed with a small amount of sodium bicarbonate. The Lidocaine can sting, and the sodium bicarbonate decreases the amount of stinging. A needle will then be used to remove the muscle. We will take a small sample at 7:00am and 9:00am.

- After the first muscle biopsy, we will give you a milk shake to drink, and we will take small blood samples for the next 2 hours to see how your body processes the protein, sugar, and fat. We will ask you to complete the entire milk shake within 15 minutes.
- After the second biopsy, you will be given a standardized meal and allowed to leave by 11:00am. This visit will take approximately 5 hours.
- After completion of this visit you will pick up your first month's study medication from the Research Pharmacy and begin taking the capsules as instructed.

We will assign you by chance (like a coin toss) to the metformin group or the placebo group. You and the Principal Investigator cannot choose your study group. You will have an equal chance of being assigned to the metformin group.

Placebo and Metformin
This study uses a placebo. A placebo looks exactly like the study drug, but it contains no active ingredient. We use placebos in research studies to learn if the effects seen in research participants are truly from the study drug. You will be asked to swallow 2 capsules per day for the 1st week. One capsule will be taken with the morning meal and 1 capsule will be taken with the evening meal. For the 2nd week, you will be asked to swallow 3capsules per day. Two capsules will be taken with the morning meal and 1 capsule will be taken with the evening meal. From the 3rd week onward, you will be asked to increase to 4 capsules a day. Two capsules will be taken with the morning meal, and 2 capsules will be taken with the evening meal.

The placebo capsule will contain lactulose and the Metformin capsule will contain 500 milligrams of metformin. You will be given your first month's supply of capsules upon discharge from the CRTU (Clinical Research and Trials Unit) at the end of your baseline studies. Once a month thereafter, you will be asked to report to the CRTU to pick up your new supply of capsules. At this time, you will be asked to return any capsules that you did not consume during the previous month. You will also have a brief appointment where a small amount of blood will be taken to measure markers of renal and liver function. These measurements are done to make sure it is safe for you to continue with the study.

You will then report to the CRTU monthly for the 40 weeks that you will be taking metformin or the placebo. You must report to the CRTU fasting (nothing to eat or drink after 7 pm) for a weight check, blood test, and to pick up new capsules. You will be asked to return any unused capsules at this time as well. Your vitamin B12 levels will be checked pre-intervention, during the, 1, 3, 6 monthly visits, and post intervention. You will be given 1000mg vitamin B12 replacement to be taken weekly. The study coordinator will also call you twice a month for the first 2 months to ensure compliance. After 40 weeks of taking metformin or placebo you will return to the CRTU to repeat the 1 inpatient and 3 outpatient visits as listed above. All procedures will be the same.

You will be asked to refrain from changing any lifestyle habits such as physical activity or diet for the entire duration of the study. You will also need to refrain from giving blood or being in any other research study eight weeks prior to the study and until eight weeks after the completion of the study.

6. What are the possible risks or discomforts from being in this research study?

Metformin may cause nausea or diarrhea. Metformin has been shown to cause lactic acidosis in patients with impaired liver, renal or heart function. Although these side-effects are rare, we will perform routine blood tests to make sure it is safe for you to be in the study. As with any medication, allergic reactions are a possibility.

If you have recently had (<6 weeks) or are planning to have medical imaging performed on your body that requires contrast agents (i.e., dye) to be consumed please inform the investigators.

You will receive the following drug and hormones during the study: Lidocaine, insulin, somatostatin, and labeled glucose. Infusion of insulin may decrease blood glucose (sugar) levels. Low blood sugar levels can cause you to feel sweaty, shaky, dizzy, or weak. Changes in blood glucose will be managed by checking blood glucose every 10 minutes and giving you a glucose solution. Somatostatin is an investigational drug.

Giving somatostatin in the vein can cause nausea and rarely vomiting. If this occurs, we will promptly stop the study and respond appropriately. The special glucose we will be using has been changed very slightly from the glucose we eat every day. This change allows us to tell the difference between the glucose that is already in your system from the glucose we are giving you during the test. No side effects have been reported due to this change in the glucose molecule. A small amount of albumin, a protein found normally in our bodies, is used when the hormone solutions are prepared. No side effects have been reported from intravenous albumin. Lidocaine usually causes a mild burning sensation when it is first given into the skin. This stinging is lessened by our adding some sodium bicarbonate. As with any medication, allergic reactions are a possibility. In addition to the IV fluids containing medications, you will receive fluids that contain sodium chloride (salt). This fluid helps keep the veins open if the other fluids are not running and helps make the sugar water less irritating to your vein.

You will have blood tests, body measurements, and blood pressure measurements that could uncover a condition you did not know you had. This can be stressful. We will fully explain any results that are not normal.

To measure your blood pressure, a cuff will be placed around your arm and this will be pumped up and become tight. This can cause temporary discomfort. You will talk with a dietician so that you will not receive food to which you may be allergic or dislike.

The risks of drawing blood include pain, bruising, and rarely, infection at the site of the needle stick.

The risks of placing the IV catheters include pain, bruising, or, rarely, infection at the site of catheter placement. There is also the possibility that you may have a reaction to the tape or other materials to hold the IV in place.

The "hot box" may cause mild discomfort and reddening of your skin. This will go away when your hand is taken out of the "hot box."

We will be collecting urine and stool samples during your stay in the Clinical Research Unit. We will need to ask you to use a urinal or bedside commode. This may be embarrassing. We will provide privacy to minimize any embarrassment.

A DEXA scan uses X-rays to measure body composition or bone density. You will be exposed to radiation during the DEXA scan. The amount of radiation you will receive has a low risk of harmful effects.

The treadmill exercise test may be uncomfortable because of the mouthpiece and nose clip. Breathing through the mouthpiece during the exercise can cause your throat to feel dry. We will be asking you to exercise as long as possible. Your heart rate and blood pressure will be watched closely, and the test can be stopped at any time.

Participating in unaccustomed resistance exercise may include risk of injury. To reduce this risk, the exercise will be performed under the supervision of qualified study team member, which will also decrease the likelihood of injury.

There is a risk of claustrophobia (fear of closed space) while you are in the transparent plastic hood during the indirect calorimetry (REE). Fresh air does come through the hood. This risk will be decreased by allowing you to watch television or a movie while the hood is on. If you are unable to tolerate the hood, it will be removed.

Side affects you may experience from the muscle biopsy include bleeding, collection of blood under the skin, bruising or infection. By applying proper pressure over the site, bleeding and bruising are usually avoided. Strict sterile precautions will be taken to avoid infection. Pain is unlikely since a local anesthetic is used. However, you may experience a deep pressure feeling at

the site of the biopsy. You will have temporary discomfort for 2 to 7 days following the procedure at the site where the incision was made. In rare instances, people have reported numbness around the biopsy site for up to one year or longer. You may have a scar from the incision.

During tests of memory and thinking you may experience anxiety or fatigue like taking a test in school.

There is no radiation associated with MRI, but people who have metal devices like pacemakers cannot have an MRI and will not be able to participate in the study. People with claustrophobia may feel too closed in and may not tolerate MRI scanning. If you feel too confined in the MRI scanner you can inform the technologist and the MRI scan will be stopped. The MRI machine makes loud knocking sounds when it is scanning. Because of this you will be asked to wear earplugs while getting your MRI scan. The earplugs minimize discomfort from noise and keep the MRI noise within the safety range.

There is a small amount of radiation exposure from the images used in the PET scan. There is also a minor risk of pain and bruising at the injection site.

Risk summary
Most side effects go away shortly after the intravenous infusions and muscle biopsies are stopped, but in rare cases side effects can be serious, long lasting, or may never go away. There is the potential for side effects may not be known. Side effects may range from mild to life-threatening. Other drugs may be given to make side effects less serious and less uncomfortable. Talk to the researcher and/or your healthcare provider about side effects and ask any other questions.

7. Are there reasons you might leave this research study early?

You may decide to stop at any time. You should tell the Principal Investigator if you decide to stop and you will be advised whether any other tests may need to be done for your safety in addition, the Principal Investigator or Mayo Clinic may stop you from taking part in this study at any time:

- if it is in your best interest,
- if you do not follow the study procedures,
- if the study is stopped.

If you leave this research study early, or are withdrawn from the study, no more information about you will be collected; however, information already collected about you in the study may continue to be used.

We will tell you about any new information that may affect your willingness to stay in the research study.

8. What if you are injured from your participation in this research study?

Where to get help:
If you think you have suffered a research-related injury, you should promptly notify the Principal Investigator listed in the Contact Information at the beginning of this form. Mayo Clinic will offer care for research-related injuries, including first aid, emergency treatment and follow-up care as needed.

Who will pay for the treatment of research related injuries:
Care for such research-related injuries will be billed in the ordinary manner, to you or your insurance. Treatment costs for research-related injuries not covered by your insurance will be paid by Mayo Clinic.

9. What are the possible benefits from being in this research study?

You will not benefit from taking part in this research study. It is for the benefit of research.

10. What alternative do you have if you choose not to participate in this research study?

This study is only being done to gather information. You may choose not to take part in this study. Your care at the Mayo Clinic or Olmsted Medical Center will not be jeopardized if you choose not to participate.

11. What tests or procedures will you need to pay for if you take part in this research study?

You will not need to pay for tests and procedures which are done for this research study. These tests and procedures are:

- Blood tests
- Urine collections
- Stool collections
- Muscle biopsies
- Brain MRIs
- Cognitive function testing
- REE's (resting energy expenditure)
- Study meals
- Medication (i.e., metformin or placebo)
- DEXA scans
- PET Scans
- Vitamin B12 replacements (if applicable)
- VO_2max tests

However, you and/or your insurance will need to pay for all other tests and procedures that you would have as part of your clinical care, including co-payments and deductibles.

If you have billing or insurance questions call Patient Account Services at the telephone number provided in the Contact Information section of this form.

12. Will you be paid for taking part in this research study?

You will receive $100.00 for each visit (excluding the monthly medication dispensing visits) you complete. If you complete the entire study, you will receive up to $900.00. You will receive one payment at the end of the study.

You will receive reimbursement for driving mileage during the study. Reimbursement will be equivalent to the number of miles traveled multiplied by the IRS standard mileage reimbursement rate. As of January 1, 2022, this reimbursement rate was $0.585 per mile. This

will be calculated from the distance between the address you have in your Electronic Health Record and the St. Marys Campus.

Payment for participation in research is considered taxable income and reportable to the Internal Revenue Service (IRS). Accounts Payable at Mayo Clinic will be given your name, address, and Social Security number to issue a check for your study participation. If you receive research payments totaling $600 or more in a calendar year, a tax Form 1099 will be sent to you. For Mayo Clinic employees, research payments are included in your paycheck with applicable taxes withheld and reported on your Form W2 after calendar year-end.

You will receive a 3D printed model of your brain. The model will be scaled to approximately 1/3rd of the size of your brain.

13. What will happen to your samples?

Your stool samples will be stored at Mayo Clinic and then sent to University of MN for research purposes only as described in the research study and this consent form. Your sample will be sent in a coded format, which protects your identity.

We would like to keep your sample for future research. You can still take part in this current study even if you do not want your sample used for future research. If you agree to give your sample, it will be the property of Mayo Clinic.

Other researchers at Mayo Clinic who are not involved with this study may ask to use your sample for future research. Researchers at other institutions may also ask for a part of your sample for future studies. Your sample will be sent to researchers in a coded format, which protects your identity.

Some future studies may examine your DNA, which is the genetic information you inherited from your parents (genetic testing). The Principal Investigator may contact you if there are findings which may be useful for your health care. You would be given general information on the potential risks, benefits, and costs of choosing to learn about the findings.

There is an exceedingly small chance that commercial value may result from the use of your donated sample. If that happens, you will not be offered a share in any profits.

Approval Date: August 5, 2022
Not to be used after: August 26, 2022

Please read the following statements and mark your choices:

1. I permit my sample to be stored and used in future research of diabetes mellitus at Mayo Clinic:

☐ Yes ☐ No Please initial here: _____Date: _____

2. I permit my sample to be stored and used in future research at Mayo Clinic to learn about, prevent, or treat any other health problems:

☐ Yes ☐ No Please initial here: _____Date: _____

3. I permit Mayo Clinic to give my sample to researchers at other institutions:

☐ Yes ☐ No Please initial here: _____Date: _____

You may request to have your sample destroyed by writing to the Principal Investigator. The address is found in the “Contact Information" section of this consent form.

Because we cannot predict how your sample will be used in the future, we cannot promise that samples can be retrieved and destroyed.

14. How will your privacy and the confidentiality of your records be protected?

Mayo Clinic is committed to protecting the confidentiality of information obtained about you in connection with this research study. The safeguards include coding data, removing identifiers before data analyses, storing electronic data files behind the Mayo security system accessible only to study team members by password protection and by keeping hard copy of subject details in institutionally secure offices. If the results of the research are made public, information that identifies you will not be used.

All materials collected will be used for research purposes only and confidentiality will be assured by use of identification codes. Electronic data will be kept in a secure database, which is only accessible to the study investigators.

During this research, information about your health will be collected. Under Federal law called the Privacy Rule, health information is private. However, there are exceptions to this rule, and you should know who may be able to see, use and share your health information for research and why they may need to do so. Information about you and your health cannot be used in this

research study without your written permission. If you sign this form, it will provide that permission (or "authorization") to Mayo Clinic.

Your health information may be collected from:

- Past, present, and future medical records.
- Research procedures, including research office visits, tests, interviews, and questionnaires.

Your health information will be used and/or given to others to:

- Do the research.
- Report the results.
- See if the research was conducted following the approved study plan, and applicable rules and regulations.

Who may use or share your health information?

- Mayo Clinic research staff involved in this study.

Your health information may be used and shared with:

- Mayo Clinic research staff involved in this study.
- Other Mayo Clinic staff involved in your clinical care.
- Researchers involved in this study at other institutions.
- University of MN
- The sponsor(s) of this study and the people or groups hired by the sponsor(s) to help perform this research.
- The Mayo Clinic Institutional Review Board that oversees the research.
- Federal and State agencies (such as the Food and Drug Administration, the Department of Health and Human Services, the National Institutes of Health, and other United States agencies) or government agencies in other countries that oversee or review research.
- A group that oversees the data (study information) and safety of this research.

How your information may be shared with others:

While taking part in this study, you will be assigned a code that is unique to you, unless otherwise specified in this consent form, but does not include information that directly identifies you unless otherwise specified in this consent form. This code will be used if your study information is sent outside of Mayo Clinic. The groups or individuals who receive your coded information will use it only for the purposes described in this consent form.
If the results of this study are made public (for example, through scientific meetings, reports, or media) information that identifies you will not be used.

In addition, individuals involved in study oversight and not employed by Mayo Clinic may be allowed to review your health information included in past, present, and future medical and/or research records. This review may be done on-site at Mayo Clinic or remotely (from an off-site location). These records contain information that directly identifies you. However, the individuals will not be allowed to record, print, copy (using paper, digital, photographic, or other methods), or remove your identifying information from Mayo Clinic.

Is your health information protected after it has been shared with others?

Mayo Clinic asks anyone who receives your health information from us to protect your privacy; however, once your information is shared outside Mayo Clinic, we cannot promise that it will remain private, and it may no longer be protected by the Privacy Rule.

Your Privacy Rights

You do not have to sign this form, but if you do not, you cannot take part in this research study.

If you cancel your permission to use or share your health information, your participation in this study will end and no more information about you will be collected; however, information already collected about you in the study may continue to be used.

If you choose not to take part or if you withdraw from this study, it will not harm your relationship with your own doctors or with Mayo Clinic.

You can cancel your permission to use or share your health information at any time by sending a letter to the address below:

Mayo Clinic
Office for Human Research Protection
ATTN: Notice of Revocation of Authorization
201 Building 4-60
200 1st Street SW
Rochester, MN 55905

Alternatively, you may cancel your permission by emailing the Mayo Clinic Research Subject Advocate at: researchsubjectadvocate@mayo.edu.

Please be sure to include in your letter or email:

- The name of the Principal Investigator,
- The study IRB number and /or study name, and
- Your contact information.

MAYO CLINIC

Name and Clinic Number

Approval Date: August 5, 2022
Not to be used after: August 26, 2022

Your permission for Mayo Clinic to use and share your health information lasts until the end of this study unless you cancel it. The study does not end until all data has been collected, checked (or audited), analyzed, and reported. Because research is an ongoing process, we cannot give you an exact date when the study will end. Sometimes this can be years after your study visits and/or activities have ended.

There is no expiration or end date related to the Sponsor's use of your health information received from Mayo Clinic as part of this study.

ENROLLMENT AND PERMISSION SIGNATURES

Your signature documents your permission to take part in this research.

Printed Name Date (mm/dd/yyyy) Time (hh:mm am/pm)

Signature

Person Obtaining Consent

- I have explained the research study to the participant.
- I have answered all questions about this research study to the best of my ability.

Printed Name Date (mm/dd/yyyy) Time (hh:mm am/pm)

Signature