INSTRUCTIONS:

- *Use "TEMPLATE PROTOCOL (HRP-503)" to prepare a document with the information from following sections.*
- *Depending on the nature of what you are doing, some sections may not be applicable to your research. If so mark as "NA" and briefly explain why it doesn't apply. For example, research involving a retrospective chart review may have many sections with NA.*
- *If there is another protocol document (e.g., from a study sponsor), please include that protocol document with your submission in addition to this form. If content requested in this protocol template is already addressed in the other protocol document, please indicate the location by page and paragraph number rather than repeating the information here.*
- *If this research is HHS-supported (e.g., NIH funded) and UNM HSC is the prime awardee or serving as the IRB of record for the prime awardee, include a copy of the grant application and sample consent (if applicable).*
- *All checklists referenced in this protocol can be found in Click under the "IRB" tab, in the "IRB Library".*
- *When you write a protocol, keep an electronic copy. You will need to modify this copy when making changes.*
- *As you are writing the protocol, remove all instructions in italics so that they are not contained in the final version of your protocol.*

PROTOCOL TITLE: A Double-Blind, Placebo-Controlled Trial of Anti-Aging, Pro-Autophagy Effects of Metformin in Adults with Prediabetes

PRINCIPAL INVESTIGATORS: Mark Burge, M.D. Division of Medicine/Endocrinology

Vojo Deretic,Ph.D., Department of Molecular Genetics & Microbiology

VERSION NUMBER:

Version 2

DATE:

May 08,2018

REGULATORY FRAMEWORK:

Please indicate all that apply:

☐	DOD (Department of Defense)
☐	DOE (Department of Energy)
☐	DOJ (Department of Justice)
☐	ED (Department of Education)
☐	EPA (Environmental Protection Agency)
☐	FDA (Food and Drug Administration)
☐	HHS (Department of Health and Human Services)
☐	VA
☒	Other: *CTSC Pilot Study*

Is this a clinical trial under ICH-GCP E6? ☒ Yes ☐ No

If yes, please confirm that the research team is familiar with and agrees to comply with the investigator requirements cited in ICH-GCP E6. ☒ Yes ☐ No

ICH-GCP E6 can be accessed by copying and pasting this URL into your browser: http://www.fda.gov/downloads/Drugs/Guidances/ucm073122.pdf

Table of Contents

1.1. Objectives: Describe the purpose, specific aims, or objectives.

This proposal is for a Phase III study to examine the beneficial, pro-autophagy effects of Metformin in adults with prediabetes.

Our long term goal is to develop a phase III study in response to a specific NIH FOA using high content screening of leucocyte LC3 puncta scores, qRT-PCR transcription factor EB (TFEB) scores, and assays of total DNA methylation and galectin-3, to gauge the magnitude of metformin's effects on autophagy and cell senescence as markers of aging in adults with prediabetes. This study will provide preliminary data for such a proposal and will also fill a knowledge gap regarding the use of validated biomarkers in this field. It will also contribute significantly to the overall anti-aging literature. The primary objective of this proposal is to validate the autophagy experimental design in humans by using leucocyte LC3, TFEB, Galectin-3, and total methylation of DNA scores as markers of autophagy activity and cell senescence.

1.2. State the hypotheses to be tested.

AIM 1: Demonstrate that metformin therapy will increase cellular autophagy as an inverse correlate of aging as measured by increases in LC3 and TFEB scores.
Hypothesis 1: In addition to beneficial effects on glycemia, body weight, and body composition, metformin therapy exerts beneficial effects on surrogate measures of autophagy and aging.
Primary outcome: Increased levels of LC3 in leukocytes**.**

AIM 2: Demonstrate that metformin therapy will mitigate evidence of aging as measured by increased DNA methylation and decreased serum galectin 3 among adults with prediabetes.
Hypothesis 2: Metformin therapy exerts beneficial effects on DNA methylation as measures of aging
Primary outcome: Increased DNA methylation in leukocytes.

AIM 3: Monitor the beneficial metabolic effects of metformin in adults with prediabetes. Hypothesis 3: Metformin therapy will elicit the expected beneficial effects on glycemia, body weight, insulin resistance, and body composition demonstrated in previous studies.
Primary outcome: Decreased A1c.

2. Background

2.1. Describe the relevant prior experience and gaps in current knowledge.

Anti-aging medicine is a burgeoning field, and accumulating data implicates the cellular process of autophagy as the primary mechanism of normal aging and the diseases associated with aging. Autophagy is considered to be a process of "cellular recycling" and is known to affect a spectrum of health and disease states associated with aging, including inflammatory disorders, metabolic syndrome and type II diabetes, cardiovascular disease, cancer and neurodegeneration. The dynamics of autophagy are strictly controlled by autophagy-related genes as well as by one of the central regulators of metabolism AMPK, the target of metformin. Autophagy also affects stem cells and cellular senescence. When the process of autophagy fails, the result is a state of chronic inflammation and degenerative diseases in many systems.

2.2. Describe any relevant preliminary data.

Diabetes can be considered a disease of premature aging, and many of the salutary effects of metformin therapy in diabetes can be interpreted as slowing or reversing the aging process. For example, in Western societies, 44% of patients with type 2 diabetes die within 10 years of diagnosis [1], and the incidence of, and mortality from, cardiovascular disease are 2–3 times greater in patients with diabetes than in the general population [2]. In current practice, metformin is the consensus first-line therapy for type 2 diabetes not only for its potent anti-hyperglycemic effects, but also for its well-documented improvements in endothelial dysfunction, hemostasis, oxidative stress, insulin resistance, lipid profiles, and fat redistribution [3]. Metformin may also decrease adverse cardiovascular outcomes independent from its glucose-lowering activity, and it likely has anti-proliferative effects in cancer and may also exert a neuroprotective effect [4]. These apparent pleiotropic effects of metformin make it a prime candidate for investigation as an anti-aging therapy.

What is Autophagy? Autophagy is a key feature of aging cells and a ubiquitous process of cellular senescence in human tissues. It can be described as an irreversible arrest in cellular proliferation. Autophagy is a key cellular homeostatic [5,6] and metabolic process [7-12] that is the center of a rapidly evolving area of biomedical research with broad fundamental and medical significance [13-28]. Autophagy responds to growth factors and is also exquisitely sensitive to cellular energy and nutritional status (e.g.- amino acids, glucose, ATP, NAD^+, Acetyl-CoA, and neutral lipid stores) through very specific regulatory systems [29-38]. Autophagy affects a spectrum of human health and disease states [13, 17, 21-28], including inflammatory and autoimmune disorders [19,21,39,40], metabolic conditions such as obesity and diabetes [11,12,41-43], cardiovascular problems [17,25] cancer [13,15,20,28,44,45] neurodegeneration [18,22,40,46], infectious processes [21], and age-related diseases [47-49].

Autophagy and Metabolism: Autophagy enables regulation of glucose metabolism, and its failure contributes to the Metabolic Syndrome, insulin resistance, and diabetes [12,41,42,60-63]. Drugs such as metformin may affect autophagy [64]. Additionally, autophagy has connections with adipogenesis and lipolysis [11], and the cardinal regulators of metabolism in mammalian cells are also key upstream regulators of autophagy [9-11].

Autophagy and Inflammation: Autophagy directly and indirectly governs (often suppressing) activation of major inflammatory pathways associated with the pathogenesis of many diseases [17,19,39,50-54, 66]. It also modulates other global inflammatory processes, such as interferon responses [39]. These responses are key to maintaining normal immune homeostasis: failure of autophagy can lead to chronic inflammatory conditions (i.e.- Crohn's Disease [57,66-70], cancer [23,28], infections (tuberculosis [21,58, 59, 71,72] or HIV and other viruses [73-76]), and autoimmunity [56,77-82].

Synergy Between Autophagy, Inflammation, and Metabolism: Autophagy and metabolic abnormalities such as obesity, diabetes, atherosclerosis, and inflammation are interrelated [83-86]. For example, cholesterol crystals [87], fatty acids [88, 89], obesity-linked lipotoxicity of ceramide [89], and amylin [91] are known inflammasome agonists and inducers of inflammation. Autophagy suppresses inflammasome activation [17,19,21,39, 50-55]. Autophagy also modulates lipid metabolism [9-11].

All of this is relevant for aging. Rapamycin's effects on aging have been observed for years [92-117], and Metformin's impact on aging implicates similar mechanisms, but with improved metabolic sequelae [93,118].

2.3. Provide the scientific or scholarly background for, rationale for, and significance of the research based on the existing literature and how will it add to existing knowledge.

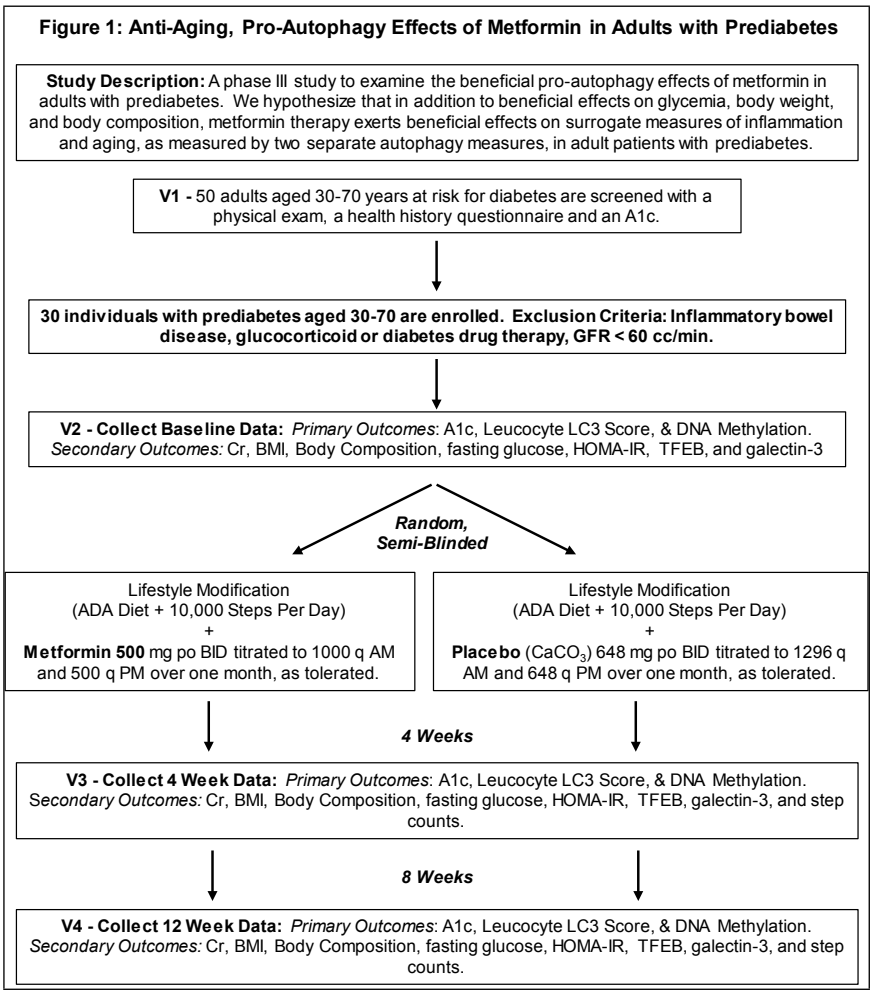
Numerous studies have documented the benefits conferred by the glucose-lowering agent Metformin. In animal models, Metformin is shown to increase both lifespan and healthspan, and a clinical trial (NCT02432287) is currently ongoing to determine whether this effect translates to humans, as well as how the medication alters the adult human transcriptome. *In vitro* studies demonstrate Metformin's ability to mitigate aging- and disease-related inflammation, oxidative damage, and diminished autophagy. Additionally, there are numerous cohort, case-control and meta-analysis studies confirming metformin's reduction in cancer-related death via hypothesized activity in the relevant mTOR, HER2, miRNA and TGF-alpha pathways. As such, NIH has issued FOA PA-17-073 (https://grants.nih.gov/grants/guide/pa-files/PA-17-073.html) to solicit additional clinical studies that will evaluate Metformin's effects on aging and age-related conditions.

3. Study Design

3.1. Describe the study design (e.g., observational; randomized placebo-controlled clinical trial, etc.)

We will perform a randomized, quasi-double blind, placebo-controlled trial of metformin in adult patients with prediabetes. Inclusion criteria will comprise individuals aged 30-70 years (inclusive) with prediabetes (defined as an A1c of 5.7-6.4%) and a BMI between 27 and 40 kg/m^2 (inclusive). Exclusion criteria will include prior treatment with metformin or other diabetes medications, pregnancy, the presence of significant renal dysfunction (Serum Creatinine > 1.3 mg/dl for women, > 1.4 mg/dl for men), severe hepatic dysfunction (AST or ALT > 3 times the upper limit of normal), ongoing alcohol or substance abuse, inflammatory bowel disease, ongoing glucocorticoid therapy, or inability to render informed consent.

Figure 1: Anti-Aging, Pro-Autophagy Effects of Metformin in Adults with Prediabetes



As summarized in the accompanying **Figure 1**, all subjects will complete four study–related visits. All visits will occur within a 7-day window of the target date. At Visit 1, informed consent will be obtained, a brief medical history and physical examination will be performed, and a health-history questionnaire will be completed by the participant. Blood samples will also be obtained for eligibility criteria at this visit. Women of child-bearing age will receive a urine pregnancy test at Visit 1.

At Visit 2, randomization to placebo or active Metformin will occur. All subjects will receive standardized instruction about diet and exercise for the prevention of diabetes and will be provided with a pedometer. Participants will subsequently be instructed to attempt to walk at least 10,000 steps per day five days during each week for the 12-week duration of the study. A step diary will be provided for subjects to complete daily to collect their step data.

As shown in **Table 1**, the following data will be collected at visit two (Week 0), visit three (4 weeks), and visit four (12 weeks).

Table 1: Study Visit Summary

Visit Number	Data Obtained	Blood Volume
1 (Screening)	*Blood:* A1c, Lytes, BUN, Cr, Fasting Glucose, AST, ALT. Urine Pregnancy. *Anthropomorphics:* Height, Weight *Other:* Health History, Physical Exam, AUDIT Questionnaire	22 ml
2 (Week 0, Baseline)	*Blood:* A1c, Fasting BMP, Insulin, LC3, TFEB, Galectin-3, DNA Methylation. *Anthropomorphics:* Height, Weight, Body Composition by BIA. *Other:* Health History, Physical Exam, Distribute Step Diary.	60 ml
3 (Week 4)	*Blood:* A1c, Fasting BMP, Insulin, LC3, TFEB, Galectin-3, DNA Methylation. *Anthropomorphics:* Height, Weight. *Other:* Health History, Physical Exam, Step Diary, Pill Counts.	60 ml
4 (Week 12)	*Blood:* A1c, Fasting BMP, Insulin, LC3, TFEB, Galectin-3, DNA Methylation. *Anthropomorphics:* Height, Weight, Body Composition by BIA. *Other:* Health History, Physical Exam, Step Diary, Pill Counts.	60 ml

After randomization, all subjects will receive 90 days worth of study medication in the form of either Metformin or Placebo (i.e.- nearly identical Calcium carbonate [$CaCO_3$]) as prepared and labelled by the UNM Hospital research pharmacist, Susan Kunkel, PharmD. Subjects will start on doses of Metformin 500 mg po BID, and then the dose will be titrated up to 1000 mg po q AM and 500 mg po q PM over the course of 1 month, as tolerated. Subjects assigned to receive $CaCO_3$ will receive 648 mg po BID, and then the dose will be titrated up to 1296 mg po q AM and 648 mg po q PM over the course of 1 month, as tolerated.

Study investigators will be blinded to treatment assignment, and the study randomization list will be maintained by Dr. Kunkel. It should be noted that because Metformin has a distinctive odor, it is not possible to fully blind this study. For the purposes of this pilot study, however, we will take advantage of the fact that the dosages and appearance of $CaCO_3$ tablets and metformin tablets are very similar, and because study investigators and data collectors will be blinded to treatment, we believe that the study design is adequately robust.

FDA Regulatory Considerations: Metformin has an excellent long-term safety record and is already FDA approved for utilization in prediabetes. As such, attainment of an FDA IND is not necessary for this study. $CaCO_3$ is being administered in a manner consistent with current guidelines: 1000 mg of elemental calcium are recommended per day for adults, and our dosage delivers 780 mg of elemental calcium daily (https://medlineplus.gov/magazine/issues/winter11/articles/winter11pg12.html).

Lifestyle Intervention: All study participants will be educated in the importance of lifestyle modification in a manner consistent with the Diabetes Prevention Program [122], and all subjects will be expected to follow these instructions throughout the course of the 12 week study period. Specifically, subjects will be asked to attempt to achieve a modest reduction in total calories (500-1000 kcal/d) each day [123], as is consistent with the recommendations of the American Diabetes Association [124]. We will also ask participants to approach the physical activity recommendations of the Diabetes Prevention Program by asking them to walk 10,000 steps per day at least five days per week [125]. This amount of exercise will approximate the 150 minutes of moderately strenuous exercise achieved by participants in the lifestyle intervention arm of the Diabetes Prevention Program.

Study Population: We will recruit potential subjects with prediabetes from the Endocrinology Clinics, from the CTSC participant recruitment service, and from the UNM Prediabetes Cohort Registry. This latter registry is the result of a small CDC-sponsored study performed at UNM a few years ago that identified 104 subjects with prediabetes out of 218 at-risk subjects who were screened (HRRC # 11-422) [126]. These subjects agreed to be contacted in the event of future research involving prediabetes. We will also employ CTSC-sponsored social media to recruit for this study, if necessary. We do not anticipate much difficulty identifying subjects who are interested in participating in this study. We estimate that 50 adults at risk for diabetes will need to be screened to find 30 eligible subjects with prediabetes to enroll in this study.

Table 2: Study Outcome Measures

Study Variable	What It Measures	Metformin Effect
Aim 1: LC3	This test quantitates intracellular autophagosome formation; a marker of autophagy.	Increase
TFEB	This test quantitates via qRT-PCR, changes in gene expression levels of TFEB-regulated genes.	Increase
Aim 2: Total DNA Methylation	This test quantitates the amount of methylated DNA in a 60 ng sample of isolated DNA from PBMCs relative to a methylated control sample.	Increase
Galectin-3	A regulatory molecule acting at various stages along the continuum from acute to chronic inflammation and fibrogenesis.	Increase
Aim 3: A1c	Average glucose concentration over 90 days.	Decrease
BMI	Body mass normalized to height.	Decrease
HOMA-IR Index	Insulin sensitivity using fasting glucose & insulin.	Decrease

Data Analysis: For continuous variables (i.e.- A1c, Body Mass Index, HOMA-IR index, Body Composition, LC3 Score, TFEB Score, Total DNA Methylation, and Galectin-3 concentrations), we will analyze the change from baseline (Week 0) at Week 12 using a two-tailed unpaired *t-test*. Repeated Measures Analysis of Variance will also be performed using the 0, 4, and 12 week data with study drug as the grouping factor and study variable as the repeated factor. We will assume a moderate correlation of 0.7 for laboratory values obtained within subjects over time, and we will again compare responses in the subjects who received metformin with those who received placebo. Descriptive statistics comparing baseline characteristics of the study participants will employ the two-tailed unpaired *t-test* for continuous variables, or the *Chi-Square* or Fisher Exact test for frequency data, as appropriate.

Sample Size Determination and Power: Primary outcome variables for this study are "increase in LC3 Score from baseline" for Aim 1, "increase in total DNA methylation" for Aim 2, and "decrease in A1c" from baseline" for Aim 3. Secondary outcome variables (not powered) are TFEB score for Aim 1, Galectin-3 concentrations for Aim 2, and BMI and HOMA-IR index (using fasting glucose and insulin) for Aim 3. These outcome variables are summarized in **Table 2**. We will enroll 30 subjects into this study and assume a dropout rate of 20%, leaving 12 subjects per group for data analysis. Sensitivity analysis will also be performed with an "intention to treat" analysis using the last value carried forward for subjects who dropped out.

For Aim 1, with 12 subjects per group, a difference in change in LC3 Score from baseline of 8±4 Dots Per Cell (DPC) for Metformin vs. 3±4 DPC for Placebo can be detected in PBMCs using a two-tailed, unpaired *t-test* with $\alpha = 0.05$ and $\beta = 0.83$. Baseline LC3 score is assumed to be 2±2 DPC and not different between groups. This hypothesized difference is typical of those observed in Dr. Deretic's previous *in vitro* studies.

LC3 is our primary outcome variable for Aim 1. During the process of autophagy, autophagosomes engulf cytoplasmic components and concomitantly, the cytosolic form of LC3 (LC3-I) is conjugated to phosphatidyl ethanolamine, resulting in the autophagosomal membrane-bound form (LC3-II). LC3-II is a widely used marker to monitor autophagosome formation by quantitation of the number of LC3-labeled puncta (autophagosomes, or "dots") per cell detected by fluorescence microscopy. An increase in LC3 puncta formation denotes an increase in autophagic activity. The Deretic Lab has employed this technique in many different cell types, including human monocyte-derived macrophage cells (MDMs). Here, MDMs from human subject donors will be cultured and adhered in full media in 96 well plates and then immunostained to detect endogenous LC3 using Anti-LC3 polyclonal antibody (MBL P036) and a secondary fluorescent antibody. Plates will then be imaged using a Cellomics HCS scanner. This device will automatically capture cell images while identifying and quantifying LC3 puncta according to pre-set parameters in the HCS Studio software. This approach eliminates the problems of user subjectivity inherent in conventional microscopy studies while generating sufficient data from many hundreds of cells for robust and reproducible studies.

For Aim 2, we will employ the Imprint Methylated DNA Quantification Kit (Sigma-Aldrich, St. Louis, MO). This kit quantitates the amount of methylated DNA in a 60 ng sample of isolated DNA from PBMCs relative to a methylated control sample. Gomes and colleagues described a study comparing the total DNA methylation of young (26.5 years) and older (70.8 years) healthy subjects. They found a pattern of relative DNA hypo-methylation among the older subjects: 18.2±1.6% in the older subjects versus 21.9±3.0% in the younger subjects [127]. With 12 subjects per group, we can detect a difference of this magnitude with $\alpha = 0.05$ and $\beta = 0.82$.

For Aim 3, with 12 subjects per group, a difference in change in A1c from baseline of 0.7±0.4% for Metformin vs. 0.2±0.4% for Placebo can be detected with using a two-tailed unpaired *t-test* with $\alpha = 0.05$ and $\beta = 0.83$. Baseline A1c is assumed to be 6.1±0.4% and not different between groups.

Potential Challenges: If adherence to lifestyle modification is significantly different between groups, this could become an important confounder. If this occurs, we will perform a

Repeated Measures ANCOVA using “steps per day” and “change in body mass” as covariates.

3.2. Describe blinding, if applicable

Study investigators will be blinded as to study assignment of those 30 individuals enrolled, and the study randomization list will be maintained by UNM Hospital Research Pharmacist Susan Kunkel, Pharm.D. Randomization list will be devised and maintained by Dr. Kunkel.

4. Inclusion and Exclusion Criteria

4.1. Describe how individuals will be screened for eligibility.

Screening will occur at the UNM CTSC Clinical Research Unit.
Inclusion criteria will comprise individuals aged 30-70 years (inclusive) with prediabetes (defined as an A1c of 5.7-6.4%) and a BMI between 27 and 40 kg/m^2 (inclusive).
Exclusion criteria will include prior treatment with metformin or other diabetes medications, pregnancy, the presence of significant renal dysfunction (Serum Creatinine > 1.3 mg/dl for women, > 1.4 mg/dl for men), severe hepatic dysfunction (AST or ALT > 3 times the upper limit of normal), ongoing alcohol or substance abuse, inflammatory bowel disease, ongoing glucocorticoid therapy, or inability to render informed consent.

4.2. Describe the criteria that define who will be included or excluded in your final study sample.

Inclusion criteria will comprise individuals aged 30-70 years (inclusive) with prediabetes (defined as an A1c of 5.7-6.4%) and a BMI between 27 and 40 kg/m^2 (inclusive).

Exclusion criteria will include prior treatment with metformin or other diabetes medications, pregnancy, the presence of significant renal dysfunction (Serum Creatinine > 1.3 mg/dl for women, > 1.4 mg/dl for men), severe hepatic dysfunction (AST or ALT > 3 times the upper limit of normal), ongoing alcohol or substance abuse, inflammatory bowel disease, ongoing glucocorticoid therapy, or inability to render informed consent.

During the screening assessments, we will employ the AUDIT questionnaire to determine whether the participant’s behaviors constitute alcohol abuse. The AUDIT questionnaire (attached) has been added to the “Supporting Documents” section of the proposal. A score of 13 or more in a female, or 15 or more in a male, will constitute an exclusionary criterion for the study.

For the purposes of excluding other drugs of abuse, we will simply ask the participant the following question: “Have you used heroin, cocaine, or methamphetamine, or misused other narcotic drugs, over the past 30 days?” A yes response will constitute an exclusionary criterion for the study.

For women of child-bearing age, exclusion criteria include current pregnancy. Further, they must agree to not get pregnant during this study and to use an effective method of birth control during the course of the study.

4.3. Indicate specifically whether you will include each of the following special populations: (You may not include members of the above populations as subjects in your research unless you indicate this in your inclusion criteria.)

- *Adults unable to consent*
- *Individuals who are not yet adults (infants, children, teenagers)*
- *Pregnant women*
- *Prisoners*

We will not include adults unable to consent, individuals who are not yet adults, pregnant women or prisoners in this study.

4.4. Indicate if you excluding any particular populations (e.g., women, children, persons not fluent in English, a particular racial or ethnic group, etc.) and provide justification.

For the purposes of this study we will recruit only adults. No gender, racial or ethnic groups will be excluded. No Spanish language consent form is currently being prepared, but we will consider adding this if there is sufficient demand.

5. Number of Subjects

5.1. If this is a multicenter study, indicate the total number of subjects to be accrued across all sites.

This study will be conducted at only one site, the University of New Mexico Health Sciences Center, Albuquerque, New Mexico.

5.2. Indicate the number of subjects to be recruited at this site.

We estimate that 50 adults at risk for diabetes will need to be screened to find 30 eligible subjects with prediabetes to enroll in this study.

5.3. Provide sample size justification

For Aim 1, with 12 subjects per group, a difference in change in LC3 Score from baseline of 8±4 Dots Per Cell (DPC) for Metformin vs. 3±4 DPC for Placebo can be detected in PBMCs using a two-tailed, unpaired *t-test* with $\alpha = 0.05$ and $\beta = 0.83$. Baseline LC3 score is assumed to be 2±2 DPC and not different between groups. This hypothesized difference is typical of those observed in Dr. Deretic's previous *in vitro* studies.

LC3 is our primary outcome variable for Aim 1. During the process of autophagy, autophagosomes engulf cytoplasmic components and concomitantly, the cytosolic form of LC3 (LC3-I) is conjugated to phosphatidyl ethanolamine, resulting in the autophagosomal membrane-bound form (LC3-II). LC3-II is a widely used marker to monitor autophagosome formation by quantitation of the number of LC3-labeled puncta (autophagosomes, or "dots") per cell detected by fluorescence microscopy. An increase in LC3 puncta formation denotes an increase in autophagic activity. The Deretic Lab has employed this technique in many different cell types, including human monocyte-derived macrophage cells (MDMs). Here, MDMs from human subject donors will be cultured and adhered in full media in 96 well plates and then immunostained to detect endogenous LC3 using Anti-LC3 polyclonal antibody (MBL P036) and a secondary fluorescent antibody. Plates will then be imaged using a Cellomics HCS scanner. This device will automatically capture cell images while identifying and quantifying LC3 puncta according to pre-set parameters in the HCS Studio software. This approach eliminates the problems of user subjectivity inherent in conventional microscopy studies while generating sufficient data from many hundreds of cells for robust and reproducible studies.

For Aim 2, we will employ the Imprint Methylated DNA Quantification Kit (Sigma-Aldrich, St. Louis, MO). This kit quantitates the amount of methylated DNA in a 60 ng sample of isolated DNA from PBMCs relative to a methylated control sample. Gomes and colleagues described a study comparing the total DNA methylation of young (26.5 years) and older (70.8 years) healthy subjects. They found a pattern of relative DNA hypo-methylation among the older subjects: 18.2±1.6% in the older subjects versus 21.9±3.0% in the younger subjects [127]. With 12 subjects per group, we can detect a difference of this magnitude with $\alpha = 0.05$ and $\beta = 0.82$.

For Aim 3, with 12 subjects per group, a difference in change in A1c from baseline of 0.7±0.4% for Metformin vs. 0.2±0.4% for Placebo can be detected with using a two-tailed unpaired *t-test* with $\alpha = 0.05$ and $\beta = 0.83$. Baseline A1c is assumed to be 6.1±0.4% and not different between groups.

6. Study Timelines

6.1. Describe:

- *The duration of an individual subject's participation in the research*
- *The duration anticipated to enroll all subjects*
- *The expected duration for the investigators to complete the study (complete analysis)*

Participation in this study by research subjects will take a total of approximately 6-10 hours over a period of 12 weeks, and 4 visits to the UNM HSC Clinical Research Unit will be required (including the initial screening visit). Total time to complete sample processing and analysis is estimated to be approximately 3 months subsequent to the end of data collection. The entire study, from the beginning of funding and enrollment to the end of the funding cycle is estimated to be one year.

7. Study Endpoints

7.1. Describe the primary and secondary study endpoints.

AIM 1: Demonstrate that Metformin therapy will increase cellular autophagy as an inverse correlate of aging as measured by increases in LC3 and TFEB scores.
Hypothesis 1: In addition to beneficial effects on glycemia, body weight, and body composition, Metformin therapy exerts beneficial effects on surrogate measures of autophagy and aging.
Primary outcome: Increased levels of LC3 in leukocytes**.**

AIM 2: Demonstrate that Metformin therapy will mitigate evidence of aging as measured by increased DNA methylation and decreased serum Galectin 3 among adults with prediabetes.
Hypothesis 2: Metformin therapy exerts beneficial effects on DNA methylation as measures of aging
Primary outcome: Increased DNA methylation in leukocytes.

AIM 3: Monitor the beneficial metabolic effects of Metformin in adults with prediabetes. Hypothesis 3: Metformin therapy will elicit the expected beneficial effects on glycemia, body weight, insulin resistance, and body composition demonstrated in previous studies.

Primary outcome: Decreased A1c.

7.2. Describe any primary or secondary safety endpoints.

TFEB, scores, Galectin-3 levels, BMI, HOMA-IR, fasting glucose, step-counts, pill-counts, body composition, serum creatinine, history and physical exam.

7.3. Describe any exploratory endpoints.

None.

8. Research Setting

8.1. Describe the sites or locations where your research team will conduct the research.

Recruitment, screenings, health questionnaires, subject measurements and phlebotomy will be conducted at the CTSC Clinical Research Unit.

Cell purification and culturing from blood will be conducted at the CTSC T1 laboratory, while LC3 cell treatment and analysis will be conducted in the laboratories of Dr. Vojo Deretic, in the Department of Molecular Genetics and Microbiology, Fitz Hall, Rooms 355, 357 and 371.

8.2. Identify where your research team will identify and recruit potential subjects.

We will recruit potential subjects with prediabetes from the Endocrinology Clinics, from the CTSC participant recruitment service, and from the UNM Prediabetes Cohort Registry (HRRC # 11-422). These latter subjects agreed to be contacted in the event of future research involving prediabetes. We will also employ CTSC-sponsored social media to recruit for this study, if necessary. We do not anticipate having difficulty identifying subjects who are interested in participating in this study. We estimate that 50 adults at risk for diabetes will need to be screened to find 30 eligible subjects with prediabetes to enroll in this study.

8.3. Identify where research procedures will be performed including any laboratory analytics

Study subject interviews, body measurements and phlebotomy will be conducted at the CTSC Clinical Research Unit.

Measurements of A1c, creatinine, fasting blood glucose, electrolytes, and insulin will be conducted in the CTSC Clinical Research Unit using the CTSC Clinical Laboratory or Tricore Laboratory.

Cell purification and culturing from subject's blood draw will be conducted utilizing dedicated bench space in the CTSC T1 laboratory. Cell purification will carry along no personal identifiers, only the blinded numeric assignment, and utilize Ficoll gradient separation for isolation of the mononuclear layer. The services of the T1 Lab will also be used for subsequent RNA purifications, cDNA production, qRT-PCR, DNA Methylation measurements, and assay quantifications.

Cultured cells labeled with the blinded numeric assigned will be transferred to the laboratories of Dr. Vojo Deretic, in Fitz Hall for cell treatment and analysis. Cells undergoing autophagy can be identified by visualizing fluorescently-labeled LC3 puncta and/or the co-localization of fluorescently labeled LC3 and lysosomal markers. LC3 measurements will be quantified using our Cellomics ArrayScan® VTI HCS Reader with a fully automated microscope featuring Zeiss optics for HTS high-content screening of 96

well plates utilizing iDev Intelligent Assay Development workflow software essential for high content analyses studies by trained personnel.

The expression of 4 autophagy genes known to be regulated by TFEB and a control will be measured and analyzed via qRT-PCR.

8.4. Describe the composition and involvement of any community advisory board

A community advisory board will not be utilized.

8.5. For research conducted outside of UNM HSC and its affiliates describe:

- *Site-specific regulations or customs affecting the research*
- *Local scientific and ethical review structure/requirements (Note: include any approvals (IRB, facility, or other) with your submission)*

There will be no research conducted outside of UNM HSC and its affiliates.

9. Resources Available

9.1. Describe the qualifications of the PI and study staff (e.g., training, experience, oversight) as required to perform the research. When applicable describe their knowledge of the local study sites, culture, and society.

Mark Burge, M.D., A faculty Endocrinologist who is widely experienced in the performance of clinical trials.

Vojo Deretic, Ph.D., Chair of Molecular Genetics and Microbiology and a world authority on autophagy.

Lindsey VanDyke, D.O., Endocrinology Fellow in Training.

9.2. When applicable, describe which licensed physicians/providers will be responsible for medical decision-making and ordering and evaluation of necessary diagnostics and therapeutics.

Mark Burge, MD, and Lindsey VanDyke, DO, are licensed physicians who will assume responsibility for medical decision making in this study.

9.3. Describe other resources available to conduct the research: For example, as appropriate:

- *Justify the feasibility of recruiting the required number of suitable subjects within the agreed recruitment period. For example, how many potential subjects do you have access to? What percentage of those potential subjects do you need to recruit?*
- *Describe the time that will be devoted to conducting and completing the research.*
- *Describe the facilities available to conduct the research.*
- *Describe the availability of medical or psychological resources that subjects might need as a result of an anticipated consequences of the human research.*
- *Describe the process to ensure that all persons assisting with the research are adequately informed about the protocol, the research procedures, and their duties and functions.*

We will recruit potential subjects with prediabetes from the Endocrinology Clinics, from the CTSC participant recruitment service, and from the UNM Prediabetes Cohort

Registry. This latter registry is the result of a small CDC-sponsored study performed at UNM a few years ago that identified 104 subjects with prediabetes out of 218 at-risk subjects who were screened (HRRC # 11-422).

These subjects agreed to be contacted in the event of future research involving prediabetes. We will also employ CTSC-sponsored social media to recruit for this study, if necessary. We do not anticipate having difficulty identifying subjects who are interested in participating in this study. We estimate that 50 adults at risk for diabetes will need to be screened to find 30 eligible subjects with prediabetes to enroll in this study.

As this is a Pilot Study, our timeline, is to recruit subjects, conduct the research and collect data, and analyze the data within a one year time frame.

LC3 measurements will be quantified using our Cellomics ArrayScan® VTI HCS Reader with a fully automated microscope featuring Zeiss optics for HTS high-content screening of 96 well plates utilizing iDev Intelligent Assay Development workflow software essential for high content analyses studies by trained personnel in the Deretic Lab, located in Fitz Hall, Room 355. Other instrumentation utilized is located in the CTSC T1 laboratories.

The UNM CTSC will supply study coordinator services for this study.

- *If CTSC resources are being accessed, the signed CTSC resources attachment must be uploaded on the CTSC Submission page in Click.*

  A signed CTSC Resources Attachment has been uploaded on the CTSC submission page in Click.

10. Prior Approvals

10.1. Describe any approvals that will be obtained prior to commencing the research. (e.g., school, external site. funding agency, laboratory, radiation safety, or biosafety approval.)

None.

10.2. Upload the required Departmental Review Form signed by your Department Chair (or authorized designee if the PI is the Department Chair) into Click under "supporting documents."

Done.

10.3. If a study includes ionizing radiation, the Radiation Safety Attachment (HUS-FORM_1) must be uploaded (attached) in Click with your submission. The consent should include radiation exposure information in the Risks section.

Not applicable.

10.4. If applicable to the study, include the signed "Biological Specimens" and/or "Drug Attachment" in Click with your submission.

Done.

11. Multi-Site Research

Not applicable.

11.1. If this is a multi-site study where the UNM HSC PI is the lead investigator, or UNM HSC is the coordinating site, describe the processes to ensure communication among sites, such as:

- *All sites have the most current version of the protocol, consent document, and HIPAA authorization.*
- *All required approvals have been obtained at each site (including approval by the site's IRB of record).*
- *All modifications have been communicated to sites, and approved (including approval by the site's IRB of record) before the modification is implemented.*
- *All engaged participating sites will safeguard data as required by local information security policies.*
- *All local site investigators will conduct the study appropriately.*
- *All non-compliance with the study protocol or applicable requirements will reported in accordance with local policy.*

11.2. Describe the method for communicating to engaged participating sites:

- *Adverse events*
- *Problems*
- *Interim results*
- *Data and safety monitoring reports*
- *The closure of a study*

11.3. If the UNM HSC investigator is serving as the "sponsor-investigator" of a FDA-regulated trial, describe how sponsor responsibilities will be fulfilled, including, but not limited to:

- *Trial Monitoring*
- *Investigational Product Accountability*
- *Safety and other interim reporting to investigators and FDA*
- *Unanticipated Problem reporting to investigators, IRBs, and FDA*

12. Study Procedures

12.1. Describe, in chronological order, all research procedures and interventions being performed and when they are performed. Include:

- *Each specific intervention, procedure, examination, imaging, laboratory test, etc. that subjects will undergo for the purposes of the research and the purpose of it.*

At the initial screening visit, subjects will receive a standard medical history and physical examination, and medical records and current medication lists will be reviewed by study personnel. They will be asked to complete a short health-history questionnaire. If female of child-bearing age, they will receive a urine pregnancy test to ensure that they are not pregnant. They will be asked to submit to a small blood draw

to determine A1c and test that kidney and liver function are normal. It is expected that this visit will take 1 or 2 hours to complete.

After enrollment, at Baseline Week 0 visit, Week 4 and Week 12 visits, subjects will have the following measured, including a draw of 12 teaspoons of blood:

- Medical history and physical exam, health history questionnaire.
- Body composition (percent lean, percent fat), by Bioelectrical Impedance.
- Height, weight and blood pressure.
- A1c level (a measure of blood sugar over the past several weeks)
- Fasting blood glucose
- Fasting serum Insulin
- Electrolytes
- Serum Creatinine
- LC3 score (measured via cultured mononuclear cells and LC3 antibody)
- TFEB score (measured via cultured mononuclear cells and qRT-PCR)
- Galectin-3 score (measured via assay)
- DNA Methylation (measured via Imprint Methylated DNA Quantification Kit (Sigma-Aldrich, St. Louis, MO).
- Step diary (measured via pedometer step counts)
- Count of remaining pills of study medication remaining

No specific genetic tests will be performed during this study.

- *Each drug, biologic, device, or other such product used in the research, the purpose, and the regulatory status (e.g., investigational, marketed – on label, marketed – off label, etc.)*

Randomized, double-blind assignment to –

Metformin 500 mg po BID, and then the dose titrated up to 1000 mg po q AM and 500 po q PM over the course of 1 month, or…

$CaCO_3$ Placebo, 648 mg po BID, and then the dose titrated up to 1296 mg po q AM and 648 po q PM over the course of 1 month.

- *Each survey, questionnaire, interview, focus group, etc., that subjects will be asked to complete or participate in for the research and the purpose of it.*

Health History Questionnaire has been uploaded.

Step Diary has been uploaded.

Each data source that will be used to gather information about subjects and the purpose of it (confidentiality will be addressed later.

For UNM patients, participant electronic medical records may be reviewed for inclusion and exclusion criteria, concomitant medical conditions, and medication list.

- *Indicate whether subjects would already be expected to undergo any of the procedures for clinical, diagnostic, or other non-research purposes*

  None.

- *Include all referenced study instruments, such as questionnaires, scripts, diaries, and data collection forms with your submission as separate attachments.*

  Uploaded.

- *For HUDs, provide a description of the device, a summary of how you propose to use the device, including any screening procedures, the HUD procedure, and any patient follow-up visits, tests, or procedures. Note whether the HUD is being used for clinical purposes only or if you are proposing to study the safety or effectiveness of the device.*

  Bioelectrical Impedance will be used to determine body composition. This device is well validated, safe, and is being used for study data purposes.

Visit	Screening: 1-2 hour outpatient visit	Week 0; 1-2 hour outpatient visit	Week 4 (± 7 days): 1-2 hour outpatient visit	Week 12 (± 7 days): 2 hour outpatient visit
Event	Sign consent form. Medical history and physical exam, health history questionnaire. Labs for A1c, blood salts, kidney and liver function. Urine pregnancy test (if appropriate)	Height and weight, body composition. Labs for A1c, blood sugar, insulin, fasting glucose, blood salts, kidney function, TFEB, LC3 score, Galectin 3 and DNA Methylation. Study medication & step-counter provided	Height and weight. Labs for A1c, fasting glucose, insulin, blood salts, kidney function, TFEB, LC3 score, Galectin 3 and DNA Methylation. Study medication and step-diary collected.	Height and weight, body composition. Labs for A1c, fasting glucose, insulin, blood salts, kidney function, TFEB, LC3 score, Galectin 3 and DNA Methylation. Study medication and step-diary collected

13. Data Analysis

13.1. Describe the data analysis plan, including any statistical procedures.

13.2. Provide a power analysis

For Aim 1, with 12 subjects per group, a difference in change in LC3 Score from baseline of 8±4 Dots Per Cell (DPC) for Metformin vs. 3±4 DPC for Placebo can be detected in PBMCs using a two-tailed, unpaired *t-test* with $\alpha = 0.05$ and $\beta = 0.83$. Baseline LC3 score is assumed to be 2±2 DPC and not different between groups. This hypothesized difference is typical of those observed in Dr. Deretic's previous *in vitro* studies.

LC3 is our primary outcome variable for Aim 1. During the process of autophagy, autophagosomes engulf cytoplasmic components and concomitantly, the cytosolic form of LC3 (LC3-I) is conjugated to phosphatidyl ethanolamine, resulting in the autophagosomal membrane-bound form (LC3-II). LC3-II is a widely used marker to monitor autophagosome formation by quantitation of the number of LC3-labeled puncta (autophagosomes, or "dots") per cell detected by fluorescence microscopy. An increase in LC3 puncta formation denotes

an increase in autophagic activity. The Deretic Lab has employed this technique in many different cell types, including human monocyte-derived macrophage cells (MDMs). Here, MDMs from human subject donors will be cultured and adhered in full media in 96 well plates and then immunostained to detect endogenous LC3 using Anti-LC3 polyclonal antibody (MBL P036) and a secondary fluorescent antibody. Plates will then be imaged using a Cellomics HCS scanner. This device will automatically capture cell images while identifying and quantifying LC3 puncta according to pre-set parameters in the HCS Studio software. This approach eliminates the problems of user subjectivity inherent in conventional microscopy studies while generating sufficient data from many hundreds of cells for robust and reproducible studies.

For Aim 2, we will employ the Imprint Methylated DNA Quantification Kit (Sigma-Aldrich, St. Louis, MO). This kit quantitates the amount of methylated DNA in a 60 ng sample of isolated DNA from PBMCs relative to a methylated control sample. Gomes and colleagues described a study comparing the total DNA methylation of young (26.5 years) and older (70.8 years) healthy subjects. They found a pattern of relative DNA hypo-methylation among the older subjects: 18.2±1.6% in the older subjects versus 21.9±3.0% in the younger subjects [127]. With 12 subjects per group, we can detect a difference of this magnitude with $\alpha = 0.05$ and $\beta = 0.82$.

For Aim 3, with 12 subjects per group, a difference in change in A1c from baseline of 0.7±0.4% for Metformin vs. 0.2±0.4% for Placebo can be detected with using a two-tailed unpaired *t-test* with $\alpha = 0.05$ and $\beta = 0.83$. Baseline A1c is assumed to be 6.1±0.4% and not different between groups.

Potential Challenges: If adherence to lifestyle modification is significantly different between groups, this could become an important confounder. If this occurs, we will perform a Repeated Measures ANCOVA using “steps per day” and “change in body mass” as covariates.

14. Provisions to Monitor the Data to Ensure the Safety of Subjects

This section is required when research involves more than Minimal Risk to subjects. Describe:

14.1. The entity (e.g., DMC, DSMB) or individuals (e.g., medical monitor) who will perform data and safety monitoring. Describe whether they are independent of or affiliated with the sponsor or investigator. If a DMC or DSMB is planned, describe the composition of the committee or board. Generally, a DSMB or DMC should be composed of experts in all scientific disciplines needed to analyze and interpret the data (e.g., epidemiologists, biostatisticians, subject matter experts).

We will employ Dr. David Schade, MD, to act as an independent monitor in the capacity of our Data Safety and Monitoring Board. Dr. Schade is independent of this study and has no vested interest in its outcome. Treatment for diabetes in this study is consistent with current standard of care, and the risks of Metformin or $CaCO_3$ therapy are generally mild and well characterized. We will track all study-related adverse events and prepare a report for Dr. Schade’s review each year prior to protocol renewal for the HRRC. We will track patients who are unable to tolerate study medication for any reason, including gastrointestinal complaints. We will also track and report all SAEs, as is standard for all clinical trials.

14.2. The safety information that will be collected and monitored.

Serum creatinine.

14.3. The frequency or periodicity of review of data, such as specified points in time or after a specific number of participants have been enrolled.

Safety and efficacy data will be collected at 0, 4 and 12 weeks. DSM reports will be prepared for the DSMB annually at the time of HRRC protocol renewal.

14.4. The plans for review of scientific literature and data from other sources that may inform the safety or conduct of the study.

Literature review was performed by Lindsey VanDyke, DO, and Michal Mudd from Dr. Deretic's lab.

14.5. The procedures for analysis and interpretation of the safety data.

Adverse event rates will be compared following conclusion of the study.

14.6. The conditions that would trigger a suspension or termination of the research (i.e., stopping rules), if appropriate.

An unexpected high rate of SAEs (40% or greater) would prompt unblinding and investigation to see if these events are clustered in one group or another. If that is the case, the DSMB and the IRB would be consulted to see if cessation of the study is warranted.

14.7. The plan for reporting findings to the sponsor, investigators, and HRRC.

AE reporting will be to the HRRC as is standard for clinical trials.

15. Withdrawal of Subjects

15.1. Describe any anticipated circumstances under which subjects may be withdrawn from the research without their consent.

Subjects may be withdrawn from the study for any reason. These typically include cessation of communication with the research team.

15.2. Describe any procedures for orderly termination/safe withdrawal (e.g., tapering of meds, physical exams, laboratory or other tests, etc.).

No tapering of medication is necessary with $CaCO_3$ or Metformin. Study drug will be discontinued at the end of the study or upon subject withdrawal. If desired by the participant, we will communicate with the participant's primary care physician about study treatment, study drug assignment, and results at the conclusion of the study.

15.3. Describe any procedures for partial withdrawal (e.g., from procedures but allowing continued data collection by record review, phone contact, etc.).

We will attempt to schedule a study close-out visit for any participant who is withdrawn from the study. Failing that, we will attempt to conduct an exit interview via telephone.

15.4. Describe the disposition of existing data/specimens when a subject withdraws. Describe any restrictions on a subject's ability to withdraw any already gathered data or specimens (e.g., unable to retrieve because it has been stripped of identifiers and

no code exists to allow re-linking). (Note: FDA requires that existing data be maintained for studies subject to FDA oversight.).

Study data will be maintained for up to 10 years after completion of the study. Thereafter, identified study data will remain available in the Lobo Vault as per NIH data-sharing regulations.

15.5. Describe withdrawal procedures and any limitations in the consent document.

Participants are informed of their right to withdraw in the consent form.

16. Data Management/Confidentiality

16.1. Indicate how the research team is permitted to access any sources of information about the subjects.

Subjects will agree to allow study staff to access and review their UNM electronic medical records. No disclosure will be made outside of study staff. No PHI will be divulged in resultant publications of this research.

16.2. Note whether the research requires the access, use, or disclosure of direct identifiers (e.g., name, medical record number, etc.)

We will maintain patient identifiers in a separate file maintained on a secure UNM computer and in Dr. Burge's folder on the H drive.

16.3. Note whether the research requires the access, use, or disclosure of Protected Health Information.

Subjects will agree to allow study staff to access and review their UNM electronic medical records. No disclosure will be made outside of study staff. No PHI will be divulged in resultant publications of this research.

16.4. Note whether the data includes information that may be considered sensitive or require additional protections such as HIV, genetic test results, mental health information, substance abuse information, criminal records, etc.

Not applicable.

16.5. Indicate whether a Certificate of Confidentiality will be used to protect data from forced release (e.g., subpoena) and whether the certificate is in place or will be applied for once IRB approval is in place. More information on Certificates of Confidentiality is available here: http://grants.nih.gov/grants/policy/coc/index.htm

Not applicable.

16.6. Describe the steps that will be taken secure the data (e.g., training, authorization of access, password protection, encryption, physical controls, certificates of confidentiality, and separation of identifiers and data) during storage, use, transmission and transport.

Subjects will be assigned Unique Identifiers for all study data. Master links to PHI will be maintained in a separate file maintained on a secure UNM computer and in Dr. Burge's folder on the H drive.

16.7. If data will be coded, describe the nature of the code and mechanisms that will be used to protect the code (e.g., secure storage, limited access, separate location from research data).

We will use the HRRC number plus a sequential number for all subjects who sign a consent form: 17-XXX-01, 17-XXX-02, etc.

16.8. Describe any procedures that will be used for quality control of collected data.

Data entered into spreadsheets or into REDCap will be randomly checked with source documents to assure accuracy.

16.9. If data will be transferred or transmitted to outside locations or entities, describe:

- *What information will be included in that data or associated with the specimens?*
- *Where and how data or specimens will be stored?*
- *How long the data or specimens will be stored?*
- *Who will have access to the data or specimens?*
- *Who is responsible for receipt or transmission of the data or specimens?*
- *How data and specimens will be transported?*

Not applicable.

16.10. Describe if data will be collected, transmitted, and/or stored via the internet, the identifiability of the data, and the security measures that will be employed to protect it.

Some data will be stored on the UNM HSC secure H drive, as is consistent with current guidelines. Working data will be entered into REDCap through the UNM CTSC Biomedical Informatics Service.

16.11. Describe if data will be collected by audio or video recording, how the recordings will be secured, whether and when recordings will be transcribed, if the transcription will include identifiers, if, when, and how the recordings will be deleted. Describe if the subjects will have the opportunity to review the recordings and request full or partial deletion. If the recordings may include persons other than the subjects, describe how this will be managed.

Not applicable.

16.12. Describe if the data will include photographs, what will be included in the photographs, and how the photographs will be secured. Describe if subjects will have the opportunity to review the photographs and request destruction. If the photographs may include persons other than the subjects, describe how this will be managed.

Not applicable.

17. Data and Specimen Banking

17.1. If data or specimens will be banked or archived locally for future use, provide the name and IRB number of the repository that they will be deposited into. Describe exactly what data or specimens will be banked and for what purposes, and whether the data or specimens will include identifiers, be coded, or be fully stripped of all identifiers with no code or key that would allow relinking. Be certain to describe the banking in the primary consent. A separate consent and authorization, if applicable, will be necessary for the banking activity itself and is typically provided by the repository. If you need to establish a repository for the purposes of banking or archiving data or specimens, a separate submission for the repository is needed as this is considered to be a distinct research activity under the regulations.

Not applicable.

17.2. If this is a multi-center study, and/or if data or specimens will be banked or archived elsewhere, identify who the holder of the data or specimens will be, exactly what data or specimens will be banked and for what purposes, and whether the data or specimens will include identifiers, be coded, or be fully stripped of identifiers with no code or key that would allow relinking. A Materials Transfer or other agreement may be necessary, please consult with the HSC Sponsored Projects Office at 505-272-6264 or by email at hsc-preaward@salud.unm.edu. Material Transfer Agreement procedures may be found at http://hsc.unm.edu/financialservices/preaward/ancillary-agreements/material-transfer-agreements/procedures.html. Be certain to describe the banking in the consent and authorization, using opt-in procedures, and the procedures for subjects to request withdrawal of their data or specimens and any limitations on their ability to do so.

Not applicable.

18. Risks to Subjects

18.1. List the reasonably foreseeable risks, discomforts, hazards, or inconveniences to the subjects related the subjects' participation in the research. Describe the probability, magnitude, duration, and reversibility of the risks. Consider physical, psychological, social, legal, and economic risks. Note that almost all research includes confidentiality risks.

Study medication risks: Participants may experience some side effects from the study medication. Approximately 20% of patients experience gastrointestinal bloating with Metformin, but these symptoms tend to improve over time. Some of the more common side effects of metformin include abdominal or stomach discomfort, cough or hoarseness, decreased appetite, diarrhea, fast or shallow breathing, fevers or chills, a general feeling of discomfort, lower back or side pain, muscle pain or cramping, painful or difficult urination, and sleepiness. Less common side effects of metformin include anxiety, blurred vision, chest discomfort, cold sweats, coma, confusion, cool or pale skin, depression, difficult or labored breathing, dizziness, fast or irregular or pounding or racing heartbeat, a feeling of warmth, headache, increased hunger, increased sweating, nausea, nervousness, redness of the face or neck or arms or upper chest, seizures, shortness of breath, slurred speech, tightness in the chest, unusual tiredness or weakness, or wheezing. Rare side effects of metformin include a behavior change similar to being drunk, difficulty with concentration, drowsiness, lack or loss of strength, or restless sleep.

There are no known serious side effects to the $CaCO_3$ Placebo therapy. There is a theoretical increased risk of kidney stones in a patient who is predisposed to them (< 1%). CaCO3 may also cause mild gas, bloating, or constipation in up to 30% of participants. CaCO3 may also interfere with the absorption of certain other drugs from your gastrointestinal tract. These medications include certain antibiotics (quinolones, tetracycline), certain osteoporosis medications (alendronate, residronate), heart and blood pressure medications (digoxin, diltiazem, verapamil, amlodipine), thyroid hormone (levothyroxine), and diuretics (hydrochlorothiazide). Additionally, estrogen therapy might increase the absorption of CaCO3 from the gut. For the most part, these effects can be avoided by taking the calcium at different times than the other medications. Subjects will be

instructed to report any side effects they experience while taking part in this study to their study doctor or study personnel.

Blood drawing risks: Drawing blood may cause temporary pain and discomfort from the needle stick. Bruising at the site of the needle insertion, sweating, feeling faint or lightheaded and, in rare cases, infection may also occur. There is also a < 1% chance of passing out briefly during blood draws. If this occurs, subjects will be asked to lie down and rest until they feel better. All blood draws will occur in a clinical setting and will be performed by a phlebotomist who is trained in aseptic technique and in recognizing the signs of distress. After the blood draw, a bandage and care instructions will be provided. In the event that the subject feels faint or shaky after the blood draw, they may be provided with a small snack.

According to common medical study guidelines, subjects should not donate too much blood at one time, and though the total amount of blood to be obtained during this 12 week study is less than ½ of a typical blood donation, we will instruct the blood donor they should plan not to donate blood until after 8 weeks after the completion of the study.

Allergic reaction: Although allergies to Metformin are rare, there is a risk of allergic reaction with any drug. Subjects will be advised of the symptoms of an allergic reaction that may include, but are not limited to, trouble breathing, fast heart rate, rash, dizziness, itching, and swelling. They will be instructed to contact the study team immediately, or present to the nearest emergency room if they experience any of these symptoms.

Bioelectrical Impedance Analysis: This procedure has been shown to cause mild skin irritation from electrode adhesives, or temporary discomfort, although this is rare. We will not perform this test in people with a lot of metal in their body (such as a metal hip or other joint replacement), those with amputations, those who have received radiographic contrast material within the last 72 hours, or people with coronary artery stents or metallic sutures, since these factors can interfere with the test results.

Reproductive Risks: There are no known reproductive risks in this study, and Metformin has been used safely during pregnancy on many occasions. Nevertheless, we will not recruit subjects in this study who are pregnant. Women of child-bearing age who are enrolled will be requested to employ an acceptable method of birth control (condoms or other barrier method, birth control pills, an IUD, or long-acting implantable contraception) for the duration of the study and to notify study personnel immediately if they become pregnant during the course of this study.

General: There are risks of stress, emotional distress, inconvenience and possible loss of privacy and confidentiality associated with participating in any research study. Subjects will be informed that they can discuss these concerns and any others regarding the study by contacting the study doctor.

18.2. If applicable, indicate which procedures may have risks to the subjects that are currently unforeseeable.

Not applicable.

18.3. If applicable, indicate which procedures may have risks to an embryo or fetus should the subject be or become pregnant. If pregnancy testing or birth control provisions are required, describe these.

Metformin and $CaCO_3$ have been used safely in many pregnancies, but pregnant women will not be allowed to participate in this study.

18.4. If applicable, describe risks to others who are not subjects.

Not applicable.

18.5. Describe the steps being taken to minimize the probability or magnitude of risks.

Metformin (1500 mg daily) and CaCO3 (1944 mg) are being titrated up to full dose slowly.

Note: All risks described here should also be described in the consent document.

19. Potential Benefits to Subjects

19.1. Describe the potential benefits that individual subjects may experience from taking part in the research. Include as may be useful for the IRB's consideration, the probability, magnitude, and duration of the potential benefits.

19.2. Indicate if there is no direct benefit. Do not include benefits to society or others in this section.

Note: All potential benefits described here should also be described in the consent document.

Although some subjects may lose a small amount of weight with Metformin therapy, there may be no direct benefit to them from participating in this study. However, it is hoped that information gained from this study will help in the future understanding and treatment of prediabetes and further, help us better understand whether or not Metformin might result in anti-aging effects on cells.

20. Recruitment Methods

20.1. Describe when, where, and how potential subjects will be recruited.

20.2. Describe the methods that will be used to identify potential subjects (e.g., chart review, referral, etc.).

20.3. Describe materials that will be used to recruit subjects (e.g., emails, scripts, advertisements, brochures, flyers, etc.). Attach draft copies of the documents or audio or video recordings with the application. Once the draft has been approved, the final copy of the printed material, audio or video recording must be submitted for review and approval prior to implementation. Please see Worksheet HRP-315 for information on advertisement standards.

We will recruit potential subjects with prediabetes from the Endocrinology Clinics, from the CTSC participant recruitment service, and from the UNM Prediabetes Cohort Registry. This latter registry is the result of a small CDC-sponsored study performed at UNM a few years ago that identified 104 subjects with prediabetes out of 218 at-risk subjects who were screened (HRRC # 11-422) [126]. These subjects agreed to be contacted in the event of future research involving prediabetes. We will also employ

CTSC-sponsored social media to recruit for this study, if necessary. We do not anticipate much difficulty identifying subjects who are interested in participating in this study. We estimate that 50 adults at risk for diabetes will need to be screened to find 30 eligible subjects with prediabetes to enroll in this study.

21. Provisions to Protect the Privacy Interests of Subjects

21.1. Describe the steps that will be taken to protect subjects' privacy interests. "Privacy" refers to persons and their interest in controlling the access that others have to themselves. For example, based on their privacy interests, people may want to control:

- *The time and place/setting where they are examined or provide information*
- *The nature of the information they provide*
- *The nature of the experiences they are exposed to*
- *Who may observe or have access to information about them*

For example, individuals may not want to be approached for participation, provide responses to a research interview, or undergo a research procedure in a location where they may be seen or overheard.

The CTSC Participant Recruitment Service maintains a "do not call" list, and we will abide by that request. Calls to potential participants will be will be made during working hours. If that proves unsuccessful in contacting patients, one or two calls might be made in the evening hours. Study personnel will describe the study and gauge the subject's interest.

21.2. Describe the steps that will be taken to protect subjects' privacy including privacy protections during recruitment, consent, and data collection. Issues related to data are addressed in the Data Management/Confidentiality Section.

Contact information for subjects contacted through the Participant Recruitment Service who opt not to participate will be destroyed within three days, as per PRS policy.

22. Economic Burden to Subjects

22.1. Describe any costs that subjects may be responsible for because of participation in the research. Clearly stipulate what procedures are standard of care and what procedures are research-related in the table below. Please place an X in the box for the responsible party for each procedure involved.

List any costs to participants (or their 3rd party payer); include any charges for study procedures, visits, or drug/devices.

No costs will be incurred by study participants for this study.

Research Procedures	Number of Samples & Procedures	Responsible Party	
		Study	3rd Party Payer or Participant
_____	_____	☐	☐
_____	_____	☐	☐
_____	_____	☐	☐
_____	_____	☐	☐

_____	_____	☐	☐
_____	_____	☐	☐
_____	_____	☐	☐
_____	_____	☐	☐
Standard of Care Procedures	**Number of Samples/Procedures**	**Responsible Party**	
		Study	**3rd Party Payer or Participant**
_____	_____	☐	☐
_____	_____	☐	☐
_____	_____	☐	☐
_____	_____	☐	☐
_____	_____	☐	☐
_____	_____	☐	☐
_____	_____	☐	☐
_____	_____	☐	☐

22.2. List any other costs to participants not already described above.

Not applicable.

22.3. Indicate whether subjects will be charged for investigational drugs, devices, procedures.

Not applicable.

22.4. Explain who will be responsible for paying for treatment of adverse events.

Study subjects will assume responsibility for the costs related to adverse events, as per HRRC boilerplate.

22.5. Ensure that the cost section of the consent form reflects the cost that are covered by the sponsor and the costs for which the subjects (or 3rd party payers) are responsible.

Done.

23. Compensation

23.1. Describe any plans for compensation or reimbursement for subjects (amounts, methods (e.g., cash card), and payment schedule). Describe why the proposed amount is reasonable and appropriate for the subjects' time and inconvenience. Credit for payment should be prorated and not be contingent upon the participant completing the entire study. Any amount paid as bonus for completion of the entire study should not be so great that it could unduly induce subjects to remain in the study when they otherwise would have withdrawn. Note: Consult with your department official for reporting requirements associated with cash or merchandise cards distributed to research subjects.

In return for time and effort participating in this study, subjects will be compensated up to $120 if they complete the study. Specifically, a cash card worth $30 will be given for each of the four study visits. If they do not complete the study, they will be paid $30 for each visit completed. The method of payment will be via ClinCard administered by the CTSC. We do not feel this is an amount that will unduly influence subjects to

participate or remain in the study when they otherwise would withdraw. If we are having difficulty enrolling patients because of the low rate of reimbursement, we may approach the HRRC to increase the amount being offered.

24. Compensation for Research-Related Injury

24.1. If the research involves more than Minimal Risk to subjects, describe the plan for compensation in the event of research related injury.

24.2. If subjects are responsible for seeking their own form of care for research-related injury, describe how this will be communicated and what options are available to participants.

"No commitment is made by the University of New Mexico Health Sciences Center (UNM HSC) to provide free medical care or money for injuries to participants in this study. If you are injured or become sick as a result of this study, UNM HSC will provide you with emergency treatment at your cost. It is important for you to tell your study doctor immediately if you have been injured or become sick because of taking part in this study. If you have any questions about these issues, or believe that you have been treated carelessly in the study, please contact the Human Research Review Committee (HRRC) at the University of New Mexico Health Sciences Center, Albuquerque, New Mexico 87131, (505) 272-1129 for more information."

25. Consent Process

25.1. Indicate whether you will you be obtaining consent, and if so describe:

25.1.1. Who will be responsible for obtaining consent and their qualifications/training to do so. Be certain to identify which study team members will obtain consent in Click under Project Contacts.

Study personnel or CTSC personnel will obtain informed consent.

25.1.2. Where will the consent process take place and the provisions for privacy.

Informed consent will be obtained at the UNM CTSC Clinical Research Unit during the screening visit. If participants desire more time to think about it, they will be granted that time.

25.1.3. The steps that will be taken to minimize the possibility of coercion or undue influence.

We will make it clear that the study is entirely voluntary.

25.1.4. The waiting period available between reviewing the study and consent with the potential subject and obtaining the consent.

The study design is simple, so we feel that most potential participants will be ready to make a decision after having the study described and reviewing the consent form. For those who require more time, they will be provided a copy of the consent form and allowed to return at their convenience, if desired.

25.1.5. Processes to ensure ongoing consent throughout the study.

Any unhappy participants will be reminded that they have the right to withdraw from the study at any time.

25.1.6. Any steps that will be taken to enhance understanding.

We will use the "teach-back" method to ensure that potential participants understand the study procedures.

25.1.7. Any procedure/testing for ensuring that the consent is understood by the potential subject (e.g., teach back).

We will use the "teach-back" method to ensure that potential participants understand the study procedures.

Subjects not fluent in English

25.1.8. Indicate what language(s) other than English are understood by prospective subjects or representatives.

We do not anticipate enrolling any "Spanish-language-only" patients in this study, but we may revisit this issue if we are having difficulty enrolling subjects.

25.1.9. If you anticipate enrolling subjects who do not understand or have limited fluency in English, describe the process to ensure that the oral and written information provided to those subjects initially and throughout their participation will be in the language they understand (e.g., use of translations and interpreters). Please note that translations of consent documents and subject materials will likely be required once the content of the English-language version is approved.

Not applicable.

25.1.10. Short-form consent documents are available for unanticipated enrollments of persons who don't understand or have limited fluency in English. However, based upon the nature of the research (e.g., clinical trials) subsequent translation of the consent document may be required so that the subject has access to written information about the research in a language they understand.

Not applicable.

Cognitively Impaired Adults/Adults Unable to Consent/Use of a Legally Authorized Representative

25.1.11. The IRB must specifically approve the enrollment of adults unable to consent and adults with cognitive impairment or limited decision-making capacity. Complete the applicable checklist in the Checklists Section of this Protocol Template.

We do not plan to enroll any cognitively disabled individuals into this study.

25.1.12. Describe whether the entire subject population or a portion of it is expected to have limited or no ability to provide legally effective consent.

No.

25.1.13. Describe the process to determine whether an individual is capable of consent.

We will assess their ability to read the consent form and to describe study procedures to the study team.

25.1.14. Describe the process to determine whether a prospective subject is capable of providing consent. Include who will be responsible for determining capacity and how it will be documented.

All study team members who obtain informed consent will make the determination of fitness. In the event of dispute, Dr. Burge will make the final decision. We will assess their ability to read the consent form and to describe study procedures to the study team.

25.1.15. Describe how the participant's decisional capacity will be assessed as the study proceeds in order to evaluate any fluctuation in the participant's level of capacity to consent.

We will assess their ability to read the consent form and to describe study procedures to the study team.

25.1.16. If it can be anticipated that some or all subjects will regain capacity to provide consent, describe the provisions to provide them with information about their participation in the research and to seek their consent for ongoing participation, if applicable.

Not applicable.

25.1.17. For research conducted in New Mexico, review "SOP: Legally Authorized Representatives, Children, and Guardians (HRP-013)" to be aware of which individuals in the state meet the definition of "legally authorized representative."

Not applicable.

25.1.18. For research conducted outside of the New Mexico, provide information that describes which individuals are authorized under applicable law to consent on behalf of a prospective subject to their participation in the procedure(s) involved in this research.

Not applicable.

25.1.19. Describe how the representative's authority to provide consent will be confirmed.

Not applicable.

25.1.20. Describe the process for assent of the subjects. Indicate whether:

- *Assent will be required of all, some, or none of the subjects. If some, indicated, which subjects will be required to assent and which will not.*
- *If assent will not be obtained from some or all subjects, an explanation of why not.*
- *Describe whether assent of the subjects will be documented and the process to document assent.*

Not applicable.

Subjects who are not yet adults (infants, children, teenagers)

25.1.21. Provide the age range of the children anticipated to be enrolled in the research.

25.1.22. Describe the criteria that will be used to determine whether a prospective subject has not attained the legal age for consent to treatments or procedures involved in the research under the applicable law of the jurisdiction in which the research will be conducted.

- *For research conducted in New Mexico, review "SOP: Legally Authorized Representatives, Children, and Guardians (HRP-013)" to be aware of which individuals in the state meet the definition of "children."*
- *For research conducted outside of New Mexico, provide information that describes which persons have not attained the legal age for consent to treatments or procedures involved the research, under the applicable law of the jurisdiction in which research will be conducted.*

25.1.23. Describe whether parental permission will be obtained from:

- *One parent (may be permissible, if the IRB approves, for (1) research not involving greater than minimal risk, or (2) research involving greater than minimal risk but presenting the prospect of direct benefit to individual subjects)*
- *Both parents unless one parent is deceased, unknown, incompetent, or not reasonably available, or when only one parent has legal responsibility for the care and custody of the child. (Permissible for research involving greater than minimal risk and no prospect of direct benefit to individual subjects.)*

25.1.24. Describe whether permission will be obtained from individuals other than parents, and if so, who will be allowed to provide permission. Describe the process used to determine these individuals' authority to consent.

25.1.25. Indicate whether the children to be enrolled in the research should be capable of providing assent.

25.1.26. Indicate if assent will be obtained from all, some, or none of the children and provide justification. If assent will be obtained from some children, indicate which children will be asked for assent.

25.1.27. When assent of children will be obtained describe the proposed assent process and whether and how assent will be documented. The assent process and documentation of assent should be age-appropriate and may consist of different procedures for different age groups.

This section is not applicable to our study.

Waiver or Alteration of Consent Process (consent will not be obtained, required element of consent will not be included, or one or more required elements of consent will be altered)

- *Complete the applicable checklists in the Checklists section of this Protocol Template if you are requesting a waiver or alteration of consent for this research*
- *Consent can be waived for all of some subjects (e.g., the research includes a retrospective cohort)*
- *Consent can be waived in full or in part (e.g., partial waiver for recruitment purposes)*

Not applicable.

26. Documentation of Consent

26.1. Describe if you plan to use a consent form to document consent. Use the UNM HSC consent generator or one of the consent templates available on the HRPO website. Attach consent documents as fully editable Word documents (i.e., please don't submit protected documents or pdfs). Please include page numbers in the footer (e.g., Page 1 of XX).

Consent form attached.

26.2. If the study is collecting and/or storing tissue samples, include a Tissue Banking Consent Form (and Authorization if the specimens will be accompanied by PHI).

Not applicable.

26.3. Describe if you plan to obtain consent but will be using a script, information sheet, or other mechanism. If you will obtain consent verbally, attach a consent script and information sheet, if you will be providing one. If you will be obtaining consent via an on-line survey, please use the survey cover letter consent template on the HRPO website and include your email script with your submission.

Complete the checklist for "Waiver of Documentation of Consent" in the Checklists section of this Protocol Template. If you will be excluding or modifying one or more of the required elements of consent you will also need to request an Alteration of Consent.

We will not use a script. We will describe the study and then let subjects review the consent form. We will then offer to answer any questions the participant has, and we will quiz the subject about a few aspects of the study.

27. Study Test Results/Incidental Findings

*27.1. **Individual Results:** Indicate whether you intend to share study test or procedure results with study participants. If so, describe which results will be shared, whom the results will be shared with (e.g., subjects, parents, primary care physicians), and how the findings will be communicated (e.g., in person consultation, posting in medical record, etc.). If the findings are the results of laboratory tests, indicate whether the tests will be processed in a CLIA-certified lab.*

We will use CLIA and/or CAP-certified laboratories. Participant A1c lab results upon request.

*27.2. **Incidental Findings:** Based upon the nature of the research, and the tests that will be performed, indicate if you anticipate that the research may result in incidental findings (traditionally defined as results that arise that are outside the original purpose for which the test or procedure was conducted (for example, a potential tumor is identified but this is not the reason imaging was obtained). If so, please describe your plans for communication of such results to subjects and their health care providers, if appropriate. If there are limitations on the accepted validity of the results (e.g., test performed in non-CLIA lab, test available in the context of research only), please describe and provide a plan for confirmatory testing or justification for why it is not recommended, not necessary, or not possible. If you do not plan to provide results, provide justification.*

- *Be certain to describe your plans for provision of study results and incidental findings in your consent documents.*

- *For more information on incidental findings, please consult the President's Bioethics Commission Report "Anticipate and Communicate: Ethical Management of Incidental and Secondary Findings in the Clinical, Research, and Direct-to-Consumer Contexts": http://bioethics.gov/sites/default/files/FINALAnticipateCommunicate_PCSBI_0.pdf*
- *For information specific to Whole Genome Sequencing, please consult the President's Bioethics Commission Report "Privacy and Progress in Whole Genome Sequencing": http://bioethics.gov/sites/default/files/PrivacyProgress508_1.pdf*

If any information becomes available that alters the risk/benefit ratio of this short study, we will inform the HRRC and participants by phone, personal interaction in the CTSC, or via telephone, personal interaction, or letter.

28. Sharing Study Progress or Results with Subjects

28.1. Describe whether you intend to provide subjects with a summary of the trial progress while the study remains underway. If so, describe your plans and the mechanisms that you will use (e.g., newsletter, handouts, mailings, etc.). Please note that all written materials that will be provided to subjects need to be reviewed and approved by the IRB prior to use.

28.2. Describe whether you intend to provide subjects with a summary of the study results after the study is complete. If so, indicate if the information will include study arm assignment if the study involved blinding. Please describe your plans for dissemination of results and the mechanisms that you will use. Please note that IRB review of materials may be required, consult with the HRPO prior to distribution.

We do not plan to provide subjects with a summary of the study progress or findings. A copy of publications resulting from this study will be provided to participants who request it.

29. Inclusion of Vulnerable Populations

29.1. If the research involves individuals who are vulnerable to coercion or undue influence, describe who will be included, why their participation is necessary or warranted, and any additional safeguards included to protect their rights and welfare. The following is not intended to serve as a comprehensive list, rather to provide some examples for your consideration.

29.1.1. If the research includes students or employees, describe protections to promote the voluntary nature of participation and minimize the risks associated with access to or use of data by persons in a position of actual or perceived authority.

29.1.2. If the research includes economically disadvantaged persons, describe the mechanisms to promote the voluntary nature of participation and to minimize economic risks associated with participation.

29.1.3. If the research includes educationally disadvantaged persons, describe the mechanisms to ensure that they are provided information and materials that enhance their ability to understand the research initially and throughout their participation in the research.

29.1.4. If the research includes seriously or terminally ill patients, describe the mechanisms to ensure that they understand the true purposes of the research, the risk it entails, and what is known or not understood about the likelihood of individual benefit

29.1.5. If the research involves pregnant women, note this here and complete the Pregnant Women Checklist in the Checklist Section of this Protocol Template.

29.1.6. If the research involves neonates of uncertain viability or non-viable neonates, note this here and complete the applicable checklist in the Checklist Section of this Protocol Template.

Note: For the purposes of the federal research regulations, viability is established shortly after delivery. "Viable, as it pertains to the neonate, means being able, after delivery, to survive (given the benefit of available medical therapy) to the point of independently maintaining heartbeat and respiration." Once a neonate has been determined viable, they are considered a child under the regulations.

29.1.7. If the research involves prisoners, note this here and complete the Prisoners Checklist in the Checklist Section of this Protocol Template.

29.1.8. If the research involves persons who have not attained the legal age for consent to treatments or procedures involved in the research ("children"), note this here and complete the Children Checklist in the Checklist Section of this Protocol Template.

29.1.9. If the research involves cognitively impaired adults, note this here and complete the Cognitively Impaired Adults Checklist in the Checklist Section of this Protocol Template.

We do not plan to recruit or enroll members of vulnerable populations in this study.

30. Community-Based Participatory Research

30.1. Describe involvement of the community in the design and conduct of the research. If members of the community will fulfill key research responsibilities such as recruitment and consent, describe what research activities community members will be responsible for, how they will be trained, and the plan for quality oversight. When relevant, please include information regarding the approval of the research at collaborating sites (e.g., Albuquerque Public Schools).

Note: "Community-based Participatory Research" is a collaborative approach to research that equitably involves all partners in the research process and recognizes the unique strengths that each brings. Community-based Participatory Research begins with a research topic of importance to the community, has the aim of combining knowledge with action and achieving social change to improve health outcomes and eliminate health disparities.

Not applicable.

31. Research Involving American Indian/Native Populations

31.1. Please provide detailed information of the local research context including how the research questions are sensitive to community attitudes and how the PI has

ascertained that the proposed research is acceptable to the local population in terms of tribal regulations, applicable law and standards of professional conduct and practice. Attach any supporting documents from tribal officials or entities addressing the status or requirements for review of the research activity from tribal officials or tribal entities (for example, Indian Health Services, the Navajo Nation IRB).

This research proposal is not specifically geared toward members of any one community, nor will we exclude members of any community. As American Indian/Native populations do experience significant levels of prediabetes, however, and as our local population includes a significant number of Native American persons, we expect that our research recruitment will include subjects from this population and that our findings would be applicable and of interest to them.

32. Transnational Research

32.1. When conducting transnational research, you must ensure that subjects are provided equivalent and appropriate protections for human subjects located outside of the United States. Please refer to the following website for current OHRP interpretations of research standards, equivalent protections, and for a current compilation of international research standards and regulatory agencies. http://www.hhs.gov/ohrp/international/index.html

*32.2. **Location:** Describe the research locale and how and why the setting was chosen. Describe significant cultural norms, local laws, and differences with U.S. culture with respect to autonomy, perception of research, recruitment, consent, age of majority, parental permission, etc.*

*32.3. **Study Personnel:** Describe the qualifications of the researcher and research team to perform research in the community/culture where it will occur. Indicate the research team's ability to speak, read, and write the language of the subjects. Describe the researcher's knowledge of or expertise in local or state laws, culture, and community norms. Indicate if the researcher was invited into the community (provide documentation, if available). If not invited, then describe how the researcher will have culturally appropriate access to the community.*

*32.4. **Consent:** Describe the consenting procedure that you intend to use for the research and why it is appropriate for the community where the research will occur. Describe how you will ensure that potential subjects understand the research, and the voluntariness of their participation.*

*32.5. **Community Consultation:** Describe any plans for community consultation to assess receptiveness to the proposed research and to obtain feedback on how it should be conducted and any limitations or boundaries that should be respected. Describe plans for dissemination of results to subjects and to the community.*

Not applicable.

33. Drugs or Devices

33.1. If the research involves drugs or devices, describe your plans to store, handle, and administer those drugs or devices so that they will be used only on subjects and be used only by authorized investigators.

We will use the UNM Investigational Pharmacy to package, label and deliver study drug. Study drug may be stored in the CTSC pharmacy for 1 or 2 days prior to

participant appointments. After randomization, all subjects will receive study medication in the form of either Metformin or Placebo (i.e.- nearly identical $CaCO_3$) as prepared and labelled by the UNM Hospital research pharmacist, Susan Kunkel, PharmD. Subjects who choose to withdraw from the study will be required to return unused study medications provided to them. Metformin has an excellent long-term safety record and is already FDA approved for utilization in the setting of prediabetes. As such, attainment of an FDA IND is not necessary for this study.

33.2. If the drug is investigational (has an IND), identify the holder of the IND/IDE/Abbreviated IDE.

No IND is necessary. Meformin is currently approved for use in prediabetes. $CaCO_3$ Placebo has no known anti-diabetes activity.

33.3. For research involving drugs, complete and attach a signed "Drug Attachment", available in Click or the HRPO website.

A signed "Drug Attachment" form is attached.

33.4. For research involving devices, complete the "Device Checklist" in the Checklist Section of this template.

Not applicable.

Checklist Section

This section contains checklists to provide information on a variety of topics that require special determinations by the IRB. Please complete all checklists relevant to your research.

I. Waivers or Alterations of Consent, Assent, and HIPAA Authorization

A. Partial Waiver of Consent for Screening/Recruitment

Complete this checklist if you are requesting a partial waiver of consent so that you can review private information to identify potential subjects and/or determine eligibility prior to approaching potential subjects for consent or parental permission.

Not applicable.

1. Describe the data source that you need to review (e.g., medical records):

2. Describe the purpose for the review (e.g., screening):

3. Describe who will conducting the reviews (e.g., investigators, research staff):

4. Do all persons who will be conducting the reviews already have permitted access to the data source?

☐ Yes

☐ No. Explain:

5. Verify that each of the following are true or provide an alternate justification for the underlined regulatory criteria:

   a) The activity involves no more than minimal risk to the subjects because the records review itself is non-invasive and the results of the records review will not be used for any purposes other than those described above.

      ☐ True

      ☐ Other justification:

   b) The waiver or alteration will not adversely affect the rights and welfare of the subjects because eligible subjects will be approached for consent to participate in the research and are free to decline. Further, the information accessed during the records review will not be disclosed to anyone without a legitimate purpose (e.g., verification of eligibility).

      ☐ True

      ☐ Other justification:

   c) The research could not practicably be carried out without the waiver or alteration because there is no other reasonably efficient and effective way to identify who to approach for possible participation in the research.

      ☐ True

      ☐ Other justification:

   d) Whenever appropriate, potentially eligible subjects will be presented with information about the research and asked to consider participation. *(Regulatory criteria: Whenever appropriate, the subjects will be provided with additional pertinent information after participation.)*

      ☐ True

      ☐ Other justification:

Partial Waiver of HIPAA Authorization for Screening/Recruitment
Complete the following additional questions/attestations if the records you will review to identify potential subjects and/or determine eligibility include Protected Health Information (PHI).

6. Will you be recording any PHI when conducting the records review to identify potential subjects and/or determine eligibility?

   ☒ Yes. Describe: *Name, contact information, Medical Record Number.*

☐ No

7. If you answered "Yes" to question 6 above, please describe when you will destroy identifiers (must be the earliest opportunity consistent with the conduct of the research) or provide justification for why they must be retained:

   Identifiers will be destroyed upon closure of the study.

8. The PHI accessed or recorded for identification/screening purposes will not be reused or disclosed to (shared with) any other person or entity, except as required by law, for authorized oversight of the research study, or for other research for which the use or disclosure of the PHI would be permitted under the Privacy Rule.

   ☒ True

   ☐ False

B. Waiver of Documentation of Consent

Complete this checklist if you intend to obtain consent verbally but will not be obtaining signatures from subjects on a consent form to document consent. Waivers of documentation of consent are commonly requested when using scripts, information sheets, or email or survey introductions to present the elements of consent instead of using a traditional consent form.

Not applicable.

1. Are you requesting a waiver of documentation of consent for some or all subjects?

   ☐ All

   ☐ Some. Explain:

2. Provide justification for <u>one</u> of the following:

   a) That the only record linking the subject and the research would be the consent document and the principal risk would be potential harm resulting from a breach of confidentiality. Each subject will be asked whether the subject wants documentation linking the subject with the research, and the subject's wishes will govern.

   b) That the research presents no more than minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context.

3. Do you intend to provide subjects with a written statement regarding the research in lieu of a traditional consent form?

   ☐ Yes. Please attach a copy to your submission in Click.

   ☐ No

C. Alteration of Consent

Complete this checklist if you intend to obtain consent but will be eliminating or altering one or more of the required elements of consent. Alterations of consent are commonly requested for research involving deception or for minimal risk research when an abbreviated consent is desired and one or more of the required element are not relevant to the research.

Not applicable.

Note: FDA-regulated research is not eligible for an alteration of consent.

1. Which element(s) of consent do you wish to eliminate and why?

2. Which element(s) of consent do you wish to alter and why?

3. Provide justification for each of the following regulatory criteria:
   a) The research involves no more than minimal risk to the subjects:

   b) The waiver or alteration will not adversely affect the rights and welfare of the subjects:

   c) The research could not practicably be carried out without the waiver or alteration:

   d) Whenever appropriate, the subjects will be provided with additional pertinent information after participation:

D. Full Waiver of Consent/Parental Permission

Complete this checklist if you are requesting a full waiver of consent for all subjects or certain subject groups (e.g., retrospective cohort). Full waivers of consent are commonly requested when the research does not include any opportunity for interaction with subjects (e.g., chart review).

Note: FDA-regulated research is not eligible for a full waiver of consent using these criteria. If you believe that your FDA-regulated research may be eligible for a waiver under another mechanism, such as planned emergency research, contact the HRPO for assistance in determining what information to provide to the HRRC.

Not applicable.

1. Are you requesting a waiver for some or all subjects?
   - ☐ All
   - ☐ Some. Explain:
2. Provide justification for each of the following regulatory criteria:
   a) The research involves no more than minimal risk to the subjects:

   b) The waiver or alteration will not adversely affect the rights and welfare of the subjects:

   c) The research could not practicably be carried out without the waiver or alteration:

   d) Whenever appropriate, the subjects will be provided with additional pertinent information after participation:

E. Full Waiver of Consent/Parental Permission (Public Benefit or Service Programs)

Complete this checklist if you are requesting a full waiver of consent for all subjects or certain subject groups (e.g., retrospective cohort) and the research involves the evaluation of a public benefit or service program.

1. Are you requesting a waiver for some or all subjects?
   - ☐ All
   - ☐ Some. Explain:
2. Provide justification for each of the following regulatory criteria:
   a) The research or demonstration project is to be conducted by or subject to the approval of state or local government officials and is designed to study, evaluate, or otherwise examine: (i) public benefit or service programs; (ii) procedures for obtaining benefits or services under those programs; (iii) possible changes in or alternatives to those programs or procedures; or (iv) possible changes in methods or levels of payment for benefits or services under those programs:

b) The research could not practicably be carried out without the waiver or alteration.

F. Full Waiver of HIPAA Authorization

Complete this checklist if you are requesting a full waiver of the requirement to obtain HIPAA authorization for all subjects or certain subject groups (e.g., retrospective cohort). Full waivers of HIPAA authorization are commonly requested when the research does not include any opportunity for interaction with subjects (e.g., chart review).

1. Are you requesting a waiver of authorization for some or all subjects?

   ☐ All

   ☒ Some. Explain: *Medical records at UNM.*

2. Describe your plan to protect health information identifiers from improper use and disclosure:

   A master key to link unique identifiers will be maintained in a separate file on a secure UNM computer and the H drive in Dr. Burge's folder.

3. Describe your plan to destroy identifiers at the earliest opportunity consistent with conduct of the research (absent a health or research justification for retaining them or a legal requirement to do so):

   Identifiers will be destroyed when the study is closed.

4. Describe why the research could not practicably be conducted without the waiver or alteration:

   Retention of identifiers helps make sure participants qualify for the study, allows the study team to make certain of drugs and doses that the patient is receiving, facilitates communication with the participant, and enhances participant safety.

5. The PHI accessed or recorded for identification/screening purposes will not be reused or disclosed to (shared with) any other person or entity, except as required by law, for authorized oversight of the research study, or for other research for which the use or disclosure of the PHI would be permitted under the Privacy Rule.

   ☒ True

   ☐ False

G. Other Waiver Types

If you are seeking another waiver type (e.g., Planned Emergency Research, Waiver of Parental Permission to Protect Child Participants, Enforcement Discretion for In

Vitro Diagnostics, etc. contact the HRPO office for assistance in determining what information to submit for the HRRC's consideration.

Not applicable.

II. Vulnerable Populations

A. Adults with Cognitive Impairments

Complete this checklist if the subject population will include adults with cognitive impairments.

This checklist does not need to be completed if the research doesn't involve interactions or interventions with subjects and will be conducted under a waiver of consent.

1. Describe why the objectives of the study cannot be met without inclusion of adults with cognitive impairments.

2. Describe how capacity to consent will be evaluated.

3. If subjects may regain capacity to consent, or if subjects may have fluctuating capacity to consent, describe your plans to evaluate capacity to consent throughout the research and to obtain consent to continue participation if capacity is regained.

4. Describe your plans, if any, to provide information about the research to subjects and the steps you will take to assess understanding.

5. Describe your plans to obtain assent, including whether assent will be obtained from none, some, or all subjects.

6. Describe why risks to subjects are reasonable in relation to anticipated benefits to the subjects.

7. If this study involves a health or behavioral intervention, describe why the relation of the anticipated benefit to the risk of the research is at least as favorable to the subjects as that presented by alternative procedures.

8. Describe your plans for monitoring the well-being of subjects including any plans to withdraw subjects from the research if they appear to be unduly distressed.

B. Children

Complete this checklist if the subject population will include children.

1. Select the category of research that you believe this research falls within and provide justification for any associated criteria. If there are different assessments for different groups of children or arms (e.g., placebo vs. drug), include a memo to provide an assessment for each group.

☐ Research not involving greater than minimal risk. *(Minimal risk means that the probability and magnitude of harm or discomfort anticipated in the research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests.)*

☐ Research involving greater than minimal risk but presenting the prospect of direct benefit to the individual subjects.

Provide justification for each of the following criteria:

(1) The risk is justified by the anticipated benefit to the subjects:

(2) The relation of the anticipated benefit to the risk is at least as favorable to the subjects as that presented by available alternative approaches:

☐ Research involving greater than minimal risk and no prospect of direct benefit to individual subjects, but likely to yield generalizable knowledge about the subject's disorder or condition.

Provide justification for each of the following criteria:

(1) The risk represents a minor increase over minimal risk:

(2) The intervention or procedure presents experiences to subjects that are reasonably commensurate with those inherent in their actual or expected medical, dental, psychological, social, or educational situations:

(3) The intervention or procedure is likely to yield generalizable knowledge about the subjects' disorder or condition which is of

vital importance for the understanding or amelioration of the subjects' disorder or condition

C. Pregnant Women and Fetuses

Complete this checklist if the subject population will include pregnant women and fetuses.

This checklist does not need to be completed if the research is both minimal risk and is not conducted, funded, or otherwise subject to regulation by DHHS, DOD, EPA, or VA.

Provide justification for each of the following:

1. Where scientifically appropriate, preclinical studies, including studies on pregnant animals, and clinical studies, including studies on non-pregnant women, have been conducted and provide data for assessing potential risks to pregnant women and fetuses.

   Our numbers are too small to provide meaningful data on use of Metformin or $CaCO_3$ in pregnancy.

2. The risk to the fetus is caused solely by interventions or procedures that hold out the prospect of direct benefit for the woman or the fetus; ***or***, if there is no such prospect of benefit, the risk to the fetus is not greater than minimal and the purpose of the research is the development of important biomedical knowledge which cannot be obtained by any other means.

   No pregnant women will be enrolled.

3. Any risk is the least possible for achieving the objectives of the research.

   No pregnant women will be enrolled.

D. Neonates of Uncertain Viability or Nonviable Neonates

Complete this checklist if the subject population will include neonates of uncertain viability.

Provide justification for each of the following:

1. Where scientifically appropriate, preclinical and clinical studies have been conducted and provide data for assessing potential risks to neonates.

2. Each individual providing consent is fully informed regarding the reasonably foreseeable impact of the research on the neonate.

3. Individuals engaged in the research will have no part in determining the viability of a neonate.

4. The research holds out the prospect of enhancing the probability of survival of the neonate to the point of viability, and any risk is the least possible for achieving that objective, ***or***, the purpose of the research is the development of important biomedical knowledge which cannot be obtained by other means and there will be no added risk to the neonate resulting from the research

E. Nonviable Neonates

Complete this checklist if the subject population will include nonviable neonates.

Provide justification for each of the following:

1. Where scientifically appropriate, preclinical and clinical studies have been conducted and provide data for assessing potential risks to neonates.

2. Each individual providing consent is fully informed regarding the reasonably foreseeable impact of the research on the neonate.

3. Individuals engaged in the research will have no part in determining the viability of a neonate.

4. The purpose of the research is the development of important biomedical knowledge that cannot be obtained by other means.

Verify each of the following:

5. Vital functions of the neonate will not be artificially maintained
   - ☐ True
   - ☐ False

6. The research will not terminate the heartbeat or respiration of the neonate
   - ☐ True
   - ☐ False

7. There will be no added risk to the neonate resulting from the research
   - ☐ True
   - ☐ False

F. Biomedical and Behavioral Research Involving Prisoners

Complete this checklist if the subject population will include prisoners.

Note: Minimal risk for research involving prisoners is the probability and magnitude of physical or psychological harm that is normally encountered in the daily lives, or in the routine medical, dental, or psychological examination of healthy persons.

1. Select and justify which allowable category of research involving prisoners this research falls within:

☐ Study of the possible causes, effects, and processes of incarceration, and of criminal behavior, provided that the study presents no more than minimal risk and no more than inconvenience to the subjects

☐ Study of prisons as institutional structures or of prisoners as incarcerated persons, provided that the study presents no more than minimal risk and no more than inconvenience to the subjects

☐ Research on conditions particularly affecting prisoners as a class (for example, vaccine trials and other research on hepatitis which is much more prevalent in prisons than elsewhere; and research on social and psychological problems such as alcoholism, drug addiction, and sexual assaults)

☐ Research on practices, both innovative and accepted, which have the intent and reasonable probability of improving the health or well-being of the subject

☐ Epidemiologic studies in which the sole purpose is to describe the prevalence or incidence of a disease by identifying all cases or to study potential risk factor associations for a disease, the research presents no more than Minimal Risk and no more than inconvenience to the subjects, and Prisoners are not a particular focus of the research.

2. Provide justification for each of the following regulatory criteria:

   a) Any possible advantages accruing to the prisoner through his or her participation in the research, when compared to the general living conditions, medical care, quality of food, amenities and opportunity for earnings in the prison, are not of such a magnitude that his or her ability to weigh the risks of the research against the value of such advantages in the limited choice environment of the prison is impaired

b) The risks involved in the research are commensurate with risks that would be accepted by nonprisoner volunteers

c) Procedures for the selection of subjects within the prison are fair to all prisoners and immune from arbitrary intervention by prison authorities or prisoners. Unless justification is provided, control subjects must be selected randomly from the group of available prisoners who meet the characteristics needed for that particular research project

d) The information is presented in language which is understandable to the subject population

e) Adequate assurance exists that parole boards will not take into account a prisoner's participation in the research in making decisions regarding parole, and each prisoner is clearly informed in advance that participation in the research will have no effect on his or her parole

f) When appropriate, adequate provision has been made for follow up examination or care after research participation, taking into account the varying lengths of individual prisoners' sentences, and for informing participants of this fact

---

III. Medical Devices

Complete this checklist if the research evaluates the safety or effectiveness of a medical device. If more than one medical device is being evaluated, provide the requested information for each.

A. Device Name:

B. Manufacturer:

C. Does the research involve a Significant Risk Device under an IDE?

☐ Yes. Include documentation of the FDA approval of the IDE with your submission. *Acceptable methods of documentation include: (1) FDA letter noting IDE number and approval status; (2) Industry sponsor letter noting IDE number and FDA approval status; or (3) FDA-approved industry sponsor protocol with IDE number noted*

☐ No

D. Is the research IDE-exempt?

☐ Yes. Include a FDA letter with your submission noting the determination that the research is IDE-exempt or a letter from the sponsor (or sponsor-investigator) justifying why they believe the research is IDE-exempt*.

☐ No

E. Does the research involve a Non-Significant Risk (NSR) Device?

☐ Yes. Include a FDA letter with your submission noting the determination that the research is NSR or a letter from the sponsor (or sponsor-investigator) justifying why they believe the research is NSR**.

☐ No

* This FDA guidance includes a description for when a device study is exempt from the IDE requirements:
http://www.fda.gov/downloads/RegulatoryInformation/Guidances/UCM127067.pdf

**This FDA guidance includes information on how to differentiate between Significant Risk and Non-Significant Risk device studies:
http://www.fda.gov/downloads/RegulatoryInformation/Guidances/UCM126418.pdf