

Original Article

# Antecedent Metabolic Health and Metformin (ANTHEM) Aging Study: Rationale and Study Design for a Randomized Controlled Trial

Santosh Kumari, PhD,<sup>1,2,†</sup> Matthew T. Bubak, PhD,<sup>3,†</sup> Hayden M. Schoenberg, BS,<sup>1,2</sup> Arik Davidyan, PhD,<sup>3,⊙</sup> Christian J. Elliehausen, BS,<sup>1,2</sup> Katrin G. Kuhn, PhD,<sup>4</sup> Timothy M. VanWagoner, PhD,<sup>5</sup> Rowan Karaman, MD,<sup>6,7</sup> Robert Hal Scofield, MD,<sup>8,9,10,11</sup> Benjamin F. Miller, PhD,<sup>3,11,12,†,⊙</sup> and Adam R. Konopka, PhD<sup>1,2,\* ,†,⊙</sup>

<sup>1</sup>Division of Geriatrics and Gerontology, Department of Medicine, University of Wisconsin–Madison, Madison, Wisconsin, USA. <sup>2</sup>Geriatric Research Education and Clinical Center, William S. Middleton Memorial Veterans Hospital, Madison, Wisconsin, USA. <sup>3</sup>Aging and Metabolism Research Program, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma, USA. <sup>4</sup>Hudson College of Public Health, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, USA. <sup>5</sup>Oklahoma Shared Clinical and Translational Resources, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, USA. <sup>6</sup>Division of Endocrinology, Diabetes, and Metabolism, Department of Medicine, University of Wisconsin–Madison, Madison, Wisconsin, USA. <sup>7</sup>Division of Endocrinology, William S. Middleton Memorial Veterans Hospital, Madison, Wisconsin, USA. <sup>8</sup>Department of Veterans Affairs Medical Center, Oklahoma City, Oklahoma, USA. <sup>9</sup>Arthritis & Clinical Immunology Program, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma, USA. <sup>10</sup>Endocrinology, Diabetes and Metabolism Section, Department of Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, USA. <sup>11</sup>Harold Hamm Diabetes Center, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, USA. <sup>12</sup>Oklahoma Center for Geroscience and Healthy Brain Aging, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, USA.

<sup>†</sup>Denotes equal contribution.

\*Address correspondence to: Adam R. Konopka, PhD, Division of Geriatrics and Gerontology, Department of Medicine, University of Wisconsin–Madison and Geriatric Research Education and Clinical Center, William S. Middleton Memorial Veterans Hospital, Madison, WI 53705, USA. E-mail: [akonopka@medicine.wisc.edu](mailto:akonopka@medicine.wisc.edu)

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## Abstract

The antidiabetic medication metformin has been proposed to be the first drug tested to target aging and extend healthspan in humans. While there is extensive epidemiological support for the health benefits of metformin in patient populations, it is not clear if these protective effects apply to those free of age-related disease. Our previous data in older adults without diabetes suggest a dichotomous change in insulin sensitivity and skeletal muscle mitochondrial adaptations after metformin treatment when co-prescribed with exercise. Those who entered the study as insulin-sensitive had no change to detrimental effects while those who were insulin-resistant had positive changes. The objective of this clinical trial is to determine if (a) antecedent metabolic health and (b) skeletal muscle mitochondrial remodeling and function mediate the positive or detrimental effects of metformin monotherapy, independent of exercise, on the metabolism and biology of aging. In a randomized, double-blind clinical trial, adults free of chronic disease ( $n = 148$ , 40–75 years old) are stratified as either insulin-sensitive or resistant based on homeostatic model assessment of insulin resistance ( $\leq 2.2$  or  $\geq 2.5$ ) and take 1 500 mg/day of metformin or placebo for 12 weeks. Hyperinsulinemic-euglycemic clamps and skeletal muscle biopsies are performed before and after 12 weeks to assess primary outcomes of peripheral insulin sensitivity and mitochondrial remodeling and function. Findings from this trial will identify clinical characteristics and cellular mechanisms involved in modulating the effectiveness of metformin treatment to target aging that could inform larger Phase 3 clinical trials aimed at testing aging as a treatment indication for metformin.

**Clinical Trials Registration Number:** NCT04264897.

Keywords: Geroscience, Healthspan, Insulin sensitivity, Mitochondria, Skeletal muscle

## Rationale

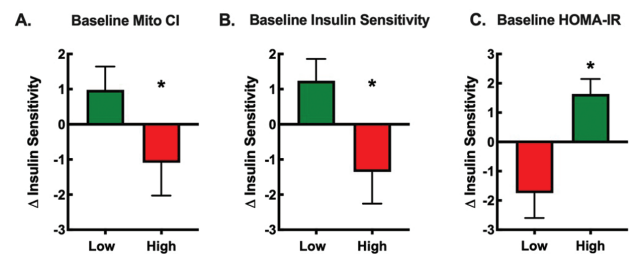
The dramatic demographic shift due to worldwide population aging is one of the most critical societal challenges of our time (1). Within the next 30 years, the number of people older than the age of 65 is estimated to reach 2 billion (2). Increased longevity is accompanied by a parallel increase in the incidence of chronic diseases (2). Age is a primary risk factor for most chronic conditions, including type 2 diabetes (T2D), cardiovascular disease (CVD), arthritis, sarcopenia, dementia, and cancer (3,4). Additionally, ~63% of the population 65 years or older has 2 or more age-related chronic conditions which is nearly double that of people aged 45–64 years (5). A recent framework has divided life span into a time of life free of disease (healthspan) followed by the accumulation of one or more overt age-associated diseases and disabilities (4). There is an urgent need to develop strategies that can simultaneously decrease the risk of age-related comorbidities. Such strategies that delay the onset of age-related chronic conditions to improve healthspan must start before the onset of age-related disease (6). It is now critical to test new or repurposed existing drugs for healthspan extension in people who are currently free of chronic disease.

Metformin is a candidate to be the first pharmaceutical treatment to slow aging and extend healthspan (7). Metformin is the most widely prescribed oral antihyperglycemic medication and has 60 years of safety documentation, low cost, and is associated with decreased risk of mortality and diseases such as T2D, CVD, dementia, and cancer (8–12). While these findings provide rationale and fuel the excitement for metformin to treat aging, the completed studies were conducted in participants with T2D and none were in relatively healthy participants, absent of disease. Thus, the efficacy of metformin to improve outcomes associated with the aging process in human participants free of chronic disease remains incompletely understood.

While the pleiotropic effects of metformin treatment could potentially be advantageous for targeting the basic biology of aging, the health- and lifespan-extending effects of metformin are equivocal across multiple models, including *Caenorhabditis elegans*, mice, rats, and humans. Metformin increases mean life span when started in young *C. elegans* (13,14), yet the opposite is true in aged *C. elegans* where metformin decreases life span (15). However, insulin-resistant *daf-2(e1370)* mutant *C. elegans* were impervious to the decrease in life span by metformin (15). Dietary metformin (1 000 ppm) increased life span and indices of healthspan in male C57BL6 mice (16) but not in male and female UMHET3 mice (17). Metformin can even shorten life span in C57BL6 mice when used at higher dietary doses (10 000 ppm) (16). Collectively, these studies in model systems highlight that the positive effects of metformin are not universal.

The landmark Diabetes Prevention Program trial examined the progression of participants with prediabetes to T2D (18). Over the 3 years of study, the metformin treatment group (1 700 mg/day;  $n = 1 073$ ) had significantly less progression to T2D compared to controls. However, the effect of metformin was minimal in those with a lower body mass index and lower fasting glucose and tended to be less effective in older versus younger adults (18,19). Therefore, it remains unclear if metformin will be an effective treatment to improve aging processes in people free of chronic disease. These findings are in line with our recently completed randomized, double-blinded clinical trial investigating the impact

of metformin on the healthspan-extending effects of exercise (20). Sedentary older participants ( $n = 53$ ,  $62 \pm 2$  years) completed 12 weeks of aerobic exercise training while taking either placebo or metformin (1 500–2 000 mg/day). On average, metformin prevented the expected exercise-induced increase in whole-body insulin sensitivity and mitochondrial complex I-linked respiration. However, there was a wide range of intersubject heterogeneity specific to the metformin group, but not the placebo group, where some participants demonstrated the expected exercise-induced improvement in insulin sensitivity while others had no improvement or a decrement in insulin sensitivity (20). Retrospectively, we used baseline subject characteristics of the metformin-treated group and rank sorted the participants to determine if any baseline characteristics were associated with the change in insulin sensitivity after exercise training and metformin treatment (Figure 1). There were significant differences in the change in insulin sensitivity associated with baseline mitochondrial complex I respiration, insulin sensitivity, and HOMA-IR, when participants were ranked and divided into 2 groups representing the upper 50% (high) and lower 50% (low). In other words, negative responders were participants who began the study with greater mitochondrial complex I respiration and insulin sensitivity and lower HOMA-IR. We interpreted these secondary analyses to suggest that participants who entered the study in a metabolically healthy state had detrimental changes in insulin sensitivity when taking metformin with exercise, while those that were relatively less metabolically healthy had positive changes. We do not yet know if these detrimental or positive responses happen with metformin monotherapy in the absence of exercise training. Therefore, if metformin is to be recommended to slow the onset of age-related disease, we need to further understand the characteristics of participants who do or do not benefit from metformin treatment.



**Figure 1.** Retrospective analysis of baseline subject characteristics on the change in insulin sensitivity after metformin plus exercise. Participants ( $n = 25$ –27) from our previous study (21) were divided into 2 groups representing the upper 50% (high) and lower 50% (low) for baseline (A) skeletal muscle mitochondrial complex I (CI)-linked respiration, (B) insulin sensitivity, and (C) HOMA-IR. The negative responders, noted in the red bars, were the participants who began the study with greater mitochondrial CI-linked respiration, higher insulin sensitivity, and lower insulin resistance. The positive responders, noted in green bars, were the participants who began the study with lower mitochondrial CI-linked respiration, lower insulin sensitivity, and greater insulin resistance. These data suggest those who were metabolically healthy had a negative response while those who were relatively less healthy had a positive response to metformin adjuvant to exercise. As previously described (21), we performed skeletal muscle biopsies to assess skeletal muscle mitochondrial respiration in permeabilized fibers, a 75 g oral glucose tolerance test to determine insulin sensitivity via the Matsuda Index, and fasting insulin and glucose values to determine insulin resistance via HOMA-IR. \* $p < .05$  vs. Low.

Skeletal muscle is the largest organ in the body and is critical for maintaining physical function, energy balance, and metabolic health across the life span. With increasing age, there is an association between skeletal muscle mitochondrial respiration and walking speed (22), aerobic capacity (21,23), muscle strength (23), and insulin sensitivity (21). However, as previously reviewed (6,24), studies evaluating the effects of metformin on skeletal muscle mitochondria are limited with nearly an equal number of studies supporting or refuting the idea that metformin inhibits complex I activity. Studies that suggest an inhibitory effect of metformin on skeletal muscle mitochondria have used suprapharmacological doses (25–27). Our data were the first to show that a clinically relevant dose of metformin prevented the expected increase in complex I-linked mitochondrial respiration after aerobic exercise training (20). Whether metformin acts directly on the mitochondria or indirectly through mitochondrial protein remodeling remains unclear. We have previously shown that adding metformin to rapamycin in young UMHE3 mice decreases protein synthesis rates of complexes within the electron transport system, including proteins belonging to complex I (28). Therefore, it remains to be reconciled if complex I protein remodeling is a mechanism by which metformin monotherapy modulates skeletal muscle respiration and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) emissions.

This clinical trial will address 2 unresolved questions: (a) Does antecedent metabolic health influence healthspan-related responses to metformin monotherapy? (b) Does metformin treatment lead to mitochondrial remodeling in skeletal muscle that changes mitochondrial function? We hypothesize that metformin treatment will

improve insulin sensitivity and glucoregulation in insulin-resistant individuals, but will decrease or not improve insulin sensitivity and glucoregulation in insulin-sensitive individuals. We also hypothesize that metformin treatment will remodel skeletal muscle mitochondria in a way that improves mitochondrial function in participants who are insulin-resistant but decreases mitochondrial function in participants who are insulin-sensitive. We will explore if proposed biomarkers of aging (29) predict or correlate with changes in insulin sensitivity and mitochondrial metabolism in disease-free individuals. This is the first human clinical trial aimed at identifying the clinical and cellular characteristics that modulate the effectiveness of metformin on the metabolism and biology of aging in individuals free of disease.

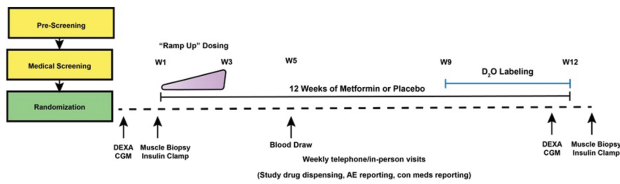
## Study Overview

We have started a 12-week randomized, double-blind, placebo-controlled clinical trial at 2 sites: (a) Oklahoma Medical Research Foundation/University of Oklahoma Health Sciences Center (collectively referred to as OKC) led by B. F. Miller and (b) the University of Wisconsin–Madison (UWM) led by A. R. Konopka. The ongoing trial is registered at clinicaltrials.gov (NCT04264897). We are recruiting 148 participants, including men and women of all races and ethnic backgrounds between the ages of 40 and 75 who are not being treated with glucose-lowering drugs nor have overt chronic diseases (Table 1). Participants are eligible if they have impaired fasting glucose, obesity, hypertension, hyperlipidemia, or are physically inactive or on medications treating these CVD risk factors. Fifty percent of participants will be recruited at each site (OKC and UWM), where half of the participants are stratified as insulin-sensitive ( $n = 74$ ) and half as insulin-resistant

**Table 1.** Study Inclusion and Exclusion Criteria

Inclusion criteria
<ul style="list-style-type: none"> <li>• Between the ages of 40 and 75 years</li> <li>• No chronic illnesses</li> <li>• Comprehension of the protocol as indicated by an ability to respond to questions about the study after reading the consent form</li> <li>• Be able to use and be reached by phone</li> <li>• Self-mobility</li> <li>• HOMA-IR <math>\leq 2.2</math> or <math>\geq 2.5</math></li> </ul>
Exclusion criteria
<ul style="list-style-type: none"> <li>• Pregnancy</li> <li>• Heart disease (history, abnormal ECG, abnormal stress ECG)</li> <li>• Cerebrovascular disease (history)</li> <li>• Cancer (history)</li> <li>• Chronic respiratory disease (history, FEV1/forced vital capacity &lt;70, FEV1 &lt;80% predicted)</li> <li>• Chronic liver disease (history, abnormal blood liver panel defined as outside normal reference range for alanine aminotransferase: &gt;52 units per liter (U/L))</li> <li>• Diabetes (history, HbA1C <math>\geq 6.5</math>, fasting blood glucose <math>\geq 126</math> mg/dL)</li> <li>• Alzheimer's (history)</li> <li>• Chronic kidney disease (history, abnormal blood kidney panel including serum creatinine &gt;1.4 and glomerular filtration rate &lt;45 mL/min/1.73<sup>2</sup>)</li> <li>• Problems with bleeding, on medication that prolongs bleeding time</li> <li>• Those on glucose-lowering drugs</li> <li>• Vitamin B12 deficiency (&lt;193 pg/mL)</li> <li>• Those planning to have imaging that requires intravenous contrast dye (within 6 weeks) or is on medications contraindicated with the use of metformin: Dofetilide, Lamotrigine, Pegvisomant, Somatropin, Trimethoprim, Trospium, Gatifloxacin, Cephalexin, Cimetidine, and Dalfampridine</li> <li>• Tobacco use</li> <li>• Allergies to lidocaine or metformin</li> <li>• Alcohol use that is equal to or greater than the following guidelines of greater than 3 drinks on any single day or no more than 7 drinks a week for women, and no more than 4 drinks on a single day or greater than 14 drinks a week for men</li> </ul>

Note: ECG = electrocardiogram; FEV = forced expiratory volume.



**Figure 2.** Study design schematic. To understand the translational potential of metformin to slow aging, this ongoing 12-week, double-blinded, randomized control trial aims to determine how antecedent metabolic health (insulin resistant vs insulin sensitive) and the relationship between skeletal muscle mitochondrial remodeling and function affect the potential healthspan-extending effects of metformin. After screening, eligible participants will be randomized to 12 weeks of placebo or metformin (1 500 mg/day) by the study statistician. Pre- and postintervention assessments include dual-energy x-ray absorptiometry (DEXA) to assess body composition, continuous glucose monitoring (CGM) to determine gluco-regulation, proposed blood-based biomarkers of aging, hyperinsulinemic-euglycemic clamp to measure peripheral insulin sensitivity, and muscle biopsy samples to evaluate mitochondrial function by high-resolution fluoro-spirometry. We will also use deuterium oxide (D<sub>2</sub>O) stable isotope labeling during the last 4 weeks of the intervention to determine individual and complex-specific mitochondrial remodeling from postmuscle biopsy samples. W = Week; con meds = concomitant medications.

( $n = 74$ ) based on baseline HOMA-IR ( $\leq 2.2$  or  $\geq 2.5$ ). As illustrated in Figure 2, eligible participants will complete a dual-energy x-ray absorptiometry scan to assess body composition, continuous glucose monitor wear period to evaluate ambulant glucose behavior, a skeletal muscle biopsy to determine mitochondrial respiration, hydrogen peroxide emissions, and protein remodeling, and a 3-hour hyperinsulinemic-euglycemic clamp to measure peripheral insulin sensitivity. Details of the study design, participants, and methodology are given in Supplementary Material.

## Conclusions

There is an urgent need to develop new therapeutic options to prevent or delay the deleterious changes of aging that contribute to morbidity among older adults. As we have illustrated, there are contradictory findings about chronic metformin treatment, and the effects of metformin treatment in those free of disease are largely unknown. Although there are studies using metformin to delay morbidity in rodent models, there is a noted lack of investigations examining metformin use in a middle-to-older-aged human population absent of metabolic disease. Importantly, within a population of disease-free individuals, there is a range of metabolic health and insulin sensitivity, which makes some more at risk for chronic age-related disease than others. With the geroscience goal of preventing or delaying morbidity and mortality, it is implied that treatment should begin before the accumulation of age-related morbidities. Therefore, our study will close the knowledge gap in understanding how metformin may affect aging-related processes in participants free of disease and identify the mechanisms through which metformin has potentially varying effects on aging-related outcomes. By the conclusion of this study, we will have provided insight into which participants could benefit from metformin treatment and thereby inform the design of a larger clinical trial aimed at using metformin to target aging.

## Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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## Conflict of Interest

None declared.

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## Author Contributions

A.R.K. is the Co-I for the project and PI for the UWM site. B.F.M. is the PI for the project and OKC site. A.R.K. and B.F.M. are responsible for study design, study execution, data collection, data analysis, and data interpretation. S.K., M.B., A.D., C.J.E., and T.M.V. are responsible for data collection and analysis. K.G.K. is a biostatistician and is responsible for randomization and data analysis. R.K. and R.H.S. are study physicians and provide medical oversight.

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