Official Study Title: Metformin for Preventing Frailty in High-risk Older Adults

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Protocol Template Form

Item 1 UTHSCSA Tracking Number	HSC20150237H

Item 2 Abstract / Project Summary	Provide a succinct and accurate description of the proposed research. State the purpose/aims. Describe concisely the research design and methods for achieving the stated goals. This section should be understandable to all members of the IRB, scientific and non-scientific. **DO NOT EXCEED THE SPACE PROVIDED**.

Purpose/Objectives: Frailty leads to poor health outcomes, such as falls, disability, hospitalization, institutionalization, and death. Diabetes is a significant predictor of the progression of frailty (e.g., gaining one or more frailty characteristic) in community-dwelling older adults (Espinoza et al., 2012). **Approximately 25-30% of older adults have diabetes and another 25-30% have glucose intolerance, a pre-diabetic state** (Kirkman et al., 2012). The major goal of this study to test a novel intervention strategy that could be used in the prevention of frailty in a sub-population of older adults who are at increased risk for becoming frail (those with pre-diabetes).

Aim 1: To determine whether the administration of metformin to glucose intolerant older adults reduces the onset or worsening of frailty.

Aim 2: To determine if the administration of metformin to glucose intolerant older adults reduces inflammation and improves insulin resistance.

Research Design/Plan: Randomized, double-blinded controlled trial of metformin vs. placebo for frailty prevention in older adults with pre-diabetes. This is a pilot study expected to be funded by the NIH-funded Older American Independence Center

Methods: Community-dwelling older adults ≥65 years will be randomized to metformin vs. placebo over a period of 2 years.

Clinical Relevance: The knowledge gained from this proposal will be the first to study a potential intervention targeted toward a central mechanism involved in the etiology of frailty. The results of this study could influence future clinical practice by suggesting that older pre-diabetic adults who are prescribed metformin will be less likely to become frail.

Item 3 Background	
Describe past experimental and/or clinical findings leading to the formulation of your study. *For research involving unapproved drugs, describe animal and human studies.* *For research that involves approved drugs or devices, describe the FDA approved uses of this drug/device in relation to your protocol.*	Insert background: **BACKGROUND AND SIGNIFICANCE** **Description of Frailty**: Frailty is recognized by clinicians who care for older adults as a syndrome of progressive physical decline with age, even after taking common age-associated diseases and conditions into consideration (Fried and Walston, 1998). Frailty has been defined and validated as a medical syndrome of **weakness, slowness, low physical activity, exhaustion and weight loss** (Fried et al., 2001). Individuals with ≥ 3 of these 5 characteristics are categorized as **frail**, individuals with 1 or 2 are categorized as **pre-frail**, and individuals with 0 are classified as **non-frail**. These criteria were developed and were modeled in the Cardiovascular Health Study (Fried et al., 2001). This study showed that subjects identified as frail were significantly more likely to be disabled, have medical comorbidity, and were at increased risk of hospitalization, falling, disability, and death, even after adjustment for potential confounding factors (Fried et al., 2001). The Cardiovascular Health Study also showed that the increased risk for these adverse outcomes followed a step-wise pattern of increasing risk by frailty categorization, i.e. pre-frail conferred higher risk than non-frail and frail conferred higher risk than pre-frail. Although other screening criteria for frailty exist (Rockwood et al., 2005; Studenski et al., 2004), the model proposed by Fried et al. (2001) is the most extensively studied and has been cross-validated in several other cohorts (Bandeen-Roche et al., 2006;

Woods et al., 2005) including our work in the San Antonio Longitudinal Study of Aging (SALSA) (Espinoza and Hazuda, 2008).

Epidemiology of Diabetes in Older Adults: Approximately 25-30% of older adults in the U.S. have diabetes and another 25-30% have glucose intolerance (impaired glucose tolerance [IGT]), a pre-diabetic state (Centers for Disease Control and Prevention, 2014; Kirkman et al., 2012). The Baltimore Longitudinal Study of Aging demonstrated a progressive decline in glucose tolerance from the third through the ninth decade of life (Shimokata et al., 1991). The fasting plasma glucose increases on average 1 mg/dl per decade, and 2 hour glucose after an oral glucose load increased 5.3 mg/dl per decade. This decline in glucose tolerance was also found in data from the National Health and Nutrition Examination Survey (NHANES) III, which showed that in subjects aged 40-49 years the percentage of physician-diagnosed diabetes (fasting glucose ≥ 126 mg/dl) is 3.9%, whereas in subjects ≥ 75 years of age, prevalence increases to 13.2% (Gu et al., 1998). The percentage of subjects with undiagnosed diabetes also increases from 7.1% to 14.1% within these age groups. The cause for the high prevalence of pre-diabetes and diabetes in the aging population is not clear. However, age-dependent decreases in β cell function and insulin sensitivity are thought to play important roles in the deterioration of glucose homeostasis that occurs with advancing age (Donath and Shoelson, 2011). Insulin resistance in aging is thought to occur as a result of increased adiposity, sarcopenia, and decreasing physical activity (Amati et al., 2009). Insulin secretion has been shown to decline with age, at a rate of 0.7% per year in individuals with normal glucose tolerance, and this decrease in β cell function is accelerated approximately 2-fold in individuals with IGT (Szoke et al., 2008). Aging is also known to be associated with decreased β cell proliferation (Rankin and Kushner, 2009) and increased β cell susceptibility to apoptosis (Maedler et al., 2006). Therefore, diabetes and pre-diabetes are highly prevalent in the aging population, which increases risk for adverse aging outcomes including frailty, which ultimately leads to disability and inability to maintain independence.

Role of Diabetes in Frailty: Several studies have demonstrated a strong association between diabetes and frailty. Blaum et al. (2005) found in a study of 599 community-dwelling women that diabetes was significantly associated with pre-frailty (odds ratio [OR] = 2.56, 95% CI: 1.38-4.78) and frailty (OR = 3.92, 95% CI: 1.49-10.34) (Blaum et al., 2005). Kalyani et al. found that post glucose load serum glucose levels were higher in frail compared to non-frail subjects (Kalyani et al., 2012). This group also found that mean oral glucose tolerance test glucose levels were higher at 60, 120, and 180 minutes in frail compared to non-frail older adults (Kalyani et al., 2012). Woods et al. (2005) found that diabetes was predictive of incident frailty, with diabetics having 40% increased risk of becoming frail over the follow-up period (OR = 1.40, 95% CI: 1.11-1.76) (Woods et al., 2005). We have demonstrated that diabetes is predictive of onset of any one of the five frailty characteristics from baseline to follow-up in the SALSA cohort (Espinoza et al., 2012). In line with these findings, insulin resistance is emerging as an important factor in frailty development. For example, Barzilay et al. (2007) found that insulin resistance was associated with incident frailty (OR = 1.15, 95% CI: 1.02-1.31) (Barzilay et al., 2007). Similarly, Kalyani et al. (2010) recently found that elevated hemoglobin A1c (HbA1c) level was associated with lower scores in the Short Physical Performance Battery (SPPB) which includes gait speed, chair rise, and tandem stand (Kalyani et al., 2010). This group found that category of HbA1c (<5.5%, 5.5-5.9%, 6.0-6.4%, 6.5-7.9%, and >8.0%) was significantly associated with increased probability of developing walking difficulty (P=.049) and reduced lower extremity function (P=.001). HbA1c over 8% (compared to <5.5%) was significantly associated with incident frailty (Hazard Ratio [HR] = 3.33, 95% confidence interval [CI] = 1.24-8.93) and with development of slow walking speed, a component of frailty (HR=2.82, 95% CI: 1.19-6.71). Therefore, both insulin resistance and diabetes are associated with frailty and predict its onset.

Effect of Diabetes in Muscle Quality, Function, and Disability: Diabetes is a major cause of physical disability in older adults. Gregg et al. demonstrated that diabetes is associated with a 2-3 times increased risk of disability in basic and instrumental activities of daily living in older adults (Gregg et al., 2000). Kalyani et al. (2010) recently found that 74% of individuals with diabetes in the NHANES study had disability with general physical activities, 52% had disability in lower extremity mobility, 37% had disability in activities of daily living, and 34% had disability in leisure and social activities (Kalyani et al., 2010).

Diabetes (e.g. hyperglycemia) is thought to have a direct, detrimental effect on aging muscle, reducing muscle strength and impairing physical function. Goodpaster et al. found that older adults in the Health, Aging, and Body Composition (Health ABC) Study had poorer muscle strength and quality compared to those without diabetes (Goodpaster et al., 2006). Poor glycemic control defined as HbA1c >8% and duration of diabetes longer than 6 years was associated with even poorer muscle quality. Longitudinal analyses by this group further demonstrated that older adults with diabetes suffered excessive loss of appendicular lean mass compared with non-diabetic older adults over 5 years of follow-up, even after adjustment for sociodemographics and change in body weight (Park et al., 2009). This group also found that older adults with diabetes had greater decline in grip and knee extensor strength over 3 years of follow-up compared to those without diabetes (Park et al., 2007).

More recent studies suggest that even pre-diabetes has similar harmful effects on aging muscle. Barzilay et al. found that quadriceps strength was inversely associated with homeostasis model assessment of insulin resistance (HOMA-IR) in older adults without diabetes, demonstrating that insulin resistance, a pre-diabetic state, is associated with decreased muscle strength (Barzilay et al., 2009). Kuo et al. similarly demonstrated that insulin resistance (also measured by HOMA-IR) was associated with slow walking speed, such that each standard deviation increment in HOMA-IR was associated with a 0.04 m/sec decrease in gait speed (Kuo et al., 2009). Recently Lee et al. demonstrated that men with pre-diabetes or diabetes had a greater loss in total or appendicular lean muscle mass compared to normoglycemic men in a longitudinal study of 3,752 men over 3.5 years (Lee et al., 2011). Further, men with diabetes in this study who were treated with insulin sensitizers (such as metformin or thiazolidinediones) experienced significantly less decline in muscle mass over the period of the study compared to those with pre-diabetes or diabetes who did not have treatment with insulin sensitizers. Therefore, several studies have shown that diabetes and pre-diabetes have effects on aging muscle, but currently there is evidence demonstrating that an insulin sensitizer such as metformin can attenuate this accelerated muscle loss associated with pre-diabetes and diabetes.

Role of Inflammation in Frailty: Several studies have shown an association between frailty and inflammation, and it has been proposed that low-grade inflammation is responsible for poor stress tolerance and lack of physiologic resilience (Collerton et al., 2012; Leng et al., 2007; Walston et al., 2002). The initial studies demonstrating this association were obtained primarily with serum or plasma markers of inflammation, such as interleukin-6 (IL-6) and C-reactive protein (CRP). Walston et al. (2002) found in 4735 participants of the Cardiovascular Health Study that frail individuals had increased mean levels of serum CRP compared to non-frail individuals (5.5 ±9.8 vs. 2.7 ±4.0 mg/L) (Walston et al., 2002). Subsequently, Leng et al. (2002) found in 30 community-dwelling older adults that serum IL-6 concentration was elevated in frail compared to non-frail older adults (4.4 ±2.9 vs. 2.8 ±1.6 pg/mL) (Leng et al., 2002). These peripheral blood markers persisted after exclusion of individuals with cardiovascular disease and diabetes, as well as after adjustment for age, sex, and race. These findings have been replicated by others (Barzilay et al., 2007).

Recently, more mechanistic studies have attempted to determine what immune system changes may play a role in the increased inflammation in frailty. In a study of 32

community-dwelling older adults, Qu et al. (2009) found that monocytic expression of a potent inflammatory chemokine, CXCL-10, was increased in frail compared to non-frail (1.05 ± 0.88 versus 0.53 ± 0.39, P = 0.04) and there was high correlation between monocytic CXCL-10 expression and serum IL-6 levels (r = 0.93, P < 0.0001) (Qu et al., 2009b). This same group analyzed gene array analyses of 367 inflammatory pathway genes in lipopolysaccharide (LPS)-challenged monocytes in 32 older adults. They found that frail individuals had 2-fold increased expression of inflammatory genes compared to non-frail (116 vs. 85 genes) and that frail older adults had higher expression of 7 stress-response genes compared to non-frail. These observations were validated with real time polymerase chain reaction (RT-PCR) (Qu et al., 2009a). Therefore, several studies demonstrate that frailty is associated with increased inflammation, over and above what would be expected with usual aging.

Role of Inflammation in Diabetes: Type 2 diabetes is known to be an inflammatory condition, associated with increased IL-6 and tumor necrosis factor (TNF) -α in plasma (Pickup, 2004) and enhanced activity of the transcription factor nuclear factor (NF)κB, a master regulator of inflammatory responses (Tantiwong et al., 2010). Pre-diabetes also is associated with increased inflammation and the presence of inflammation in the pre-diabetic state predicts the onset of diabetes (Haffner, 2003; Pradhan et al., 2001). The mechanism linking impaired insulin secretion and function of type 2 diabetes with inflammation is thought to be a result of excess glucose and free fatty acid induced stress to insulin-sensitive tissues, particularly adipose and liver, leading to local production of inflammatory cytokines such as TNFα, IL-1 β, and CC-chemokine (CCC) ligand 2, CCC 3, and CCC 8 (Donath and Shoelson, 2011). The local release of cytokines promotes inflammation in other tissues, including the pancreatic islet cells (Donath and Shoelson, 2011). Concentrations of circulating IL-6 and CRP (the hepatic expression of which is triggered by IL-6) are known to be increased in obesity and predict the incidence of type 2 diabetes (Pradhan et al., 2001). Therefore, inflammation plays a major role in the pathogenesis of type 2 diabetes.

Although a general consensus has emerged that inflammation plays a role in the etiology of frailty, the molecular basis for the increased inflammation that leads to frailty is unknown. Because diabetes is a major predictor of frailty based on our own work (Espinoza et al., 2012) and that of others (Kalyani et al., 2012; Woods et al., 2005), we hypothesize that the inflammation observed in insulin resistance and hyperglycemia are in part responsible for the inflammation observed in frailty.

INTERVENTIONS AGAINST FRAILTY

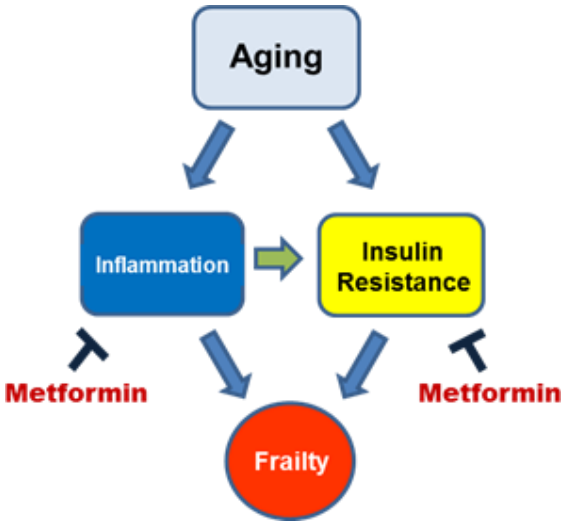
While the importance of frailty and its impact on an aging U.S. society have been widely recognized and there is urgency within the field to address the detrimental effects of frailty, to date there is no agreed upon intervention for frailty (Walston et al., 2006). Perhaps one of the most well-known trials of physical activity in older adults is the Lifestyle Interventions and Independence for Elders Pilot (LIFE-P) Study (LIFE Study et al., 2006). In this study, the investigators examined the effect of a physical activity intervention comprised of a combination of aerobic, strength, balance, and flexibility exercises (versus control) on lower extremity function over a period of one year. Significant improvements in short physical performance battery, (which measures balance, chair rise, and gait speed), and gait speed over 400 meters were observed at 6 and 12 months. However, when examined 2 years after study completion, while individuals in the physical activity group reported spending more time engaged in physical activity, there was no sustained improvements in short physical performance battery or 400-meter gait speed (Rejeski et al., 2009). A subsequent study which examined the effect of the intervention on frailty specifically found that the LIFE-P study intervention led to reduced number of frailty characteristics over at 12 months; however, these improvements were only observed in more robust (less frail) individuals at the baseline of

the study (Cesari et al., 2015). Further, it is not known whether these effects were sustained beyond the period of the study. Therefore, while it is accepted acknowledged that exercise is an important recommendation for older adults, there remains concern about the feasibility of implementing these interventions broadly in a clinical setting, and whether the effects of exercise are sustainable beyond the period of training (Liu and Fielding, 2011).

Recently there has been a growing interest in pharmacologic therapies to improve healthspan and prevent or delay geriatric syndromes such as frailty (Kirkland, 2013). This rising interest is likely largely due to a landmark study conducted at our institution demonstrating that rapamycin extends lifespan in rodents (Harrison et al., 2009). Kirkland proposed possible paradigms for translating interventions from scientific findings in the basic biology of aging field into the clinical setting, which included amelioration of age-related diseases (such as cancer, dementia, atherosclerosis, or diabetes), and disease-specific interventions (such as JAK1/2 inhibitors for myelofibrosis or senolytics for bone fracture non-union). With this research, we propose that metformin treatment in pre-diabetic older adults will prevent frailty. Therefore, we focus on the amelioration of an age-related disease, diabetes, which has been demonstrated to be a major risk factor for frailty and also shares the same underlying pathophysiology of inflammation and insulin resistance.

Role of Metformin in Preventing Diabetes, Reducing Inflammation, and Promoting Longevity and Healthspan: Metformin, a biguanide, is well-tolerated and widely used for the treatment of type 2 diabetes, and is generally recommended for first-line treatment of this disease (American Diabetes Association, 2014b). Early initiation at the time of diagnosis when the HbA1c is not significantly elevated has been associated with improved glycemic control over time and decreased long-term complications (Colaguiri et al., 2002). Metformin has been shown to be highly effective at reducing the onset of diabetes by 31% in 3,234 pre-diabetic adults over a period of 2.8 years in the Diabetes Prevention Program study (Diabetes Prevention Program Research Group, 2002), and at reducing systemic inflammation (Carter et al., 2005; Diabetes Prevention Program Research Group, 2005). More recently, animal studies suggest that metformin has life extension properties, and improves healthspan (Martin-Montalvo A. et al., 2013). Based on landmark work by Dr. Musi (Co-Investigator) we know that metformin lowers glucose primarily via activating AMP-activated protein kinase (AMPK), a major cellular regulator of lipid and glucose metabolism. Although it is not entirely clear whether the potential life extension properties of metformin are directly related to its AMPK activating properties, we do know that the AMPK pathway is highly interrelated to the mammalian target of rapamycin (mTOR) pathway (Barzilai et al., 2012); and, inhibition of mTOR with rapamycin has been shown to extend lifespan in mice based on work conducted at our institution (Harrison et al., 2009).

Figure 1. Conceptual model of the contribution of inflammation and insulin resistance to frailty and metformin action on these.



Significance: The population of older adults in the U.S. is expected to grow exponentially such that the population over the age of 65 is expected to double and the population over the age of 85 is expected to quadruple. In 2030, when the last baby boomer turns 65, one out of every 5 Americans (about 72 million people) will be 65 years or older (Centers for Disease Control, 2013) . While ideally aging would occur in such a way that individuals can maintain function and a high quality of life late into their years, resulting in what has been termed "compression of morbidity;" (Fries, 1980) unfortunately, many older adults will suffer from multiple chronic diseases, disability, and frailty as they age. Over the past decade the geriatrics community has recognized frailty as a geriatric syndrome that dramatically affects the quality of life and increases health care costs, e.g., in 2010 the estimated the cost of frailty was over $18 billion (Janssen et al., 2004). Since Fried's initial description of frailty, numerous investigators have characterized the incidence and progression of frailty in various populations and studies have focused on identifying risk factors and potential physiological markers of frailty. The research described in this proposal will test a pharmacological intervention (metformin) that could potentially be used to delay/reduce the development of frailty in older adults. In this initial study we will study glucose intolerant subjects, a population which encompasses approximately 1/3 of older adults, and is most likely to benefit from metformin (Diabetes Prevention Program Research Group, 2002). The knowledge gained from this research will be the first to study a potential intervention targeted toward a central mechanism involved in the etiology of frailty. Because of the enormous costs associated with frailty (both personal and economic), a treatment that prevents or delays frailty, even in a sub-population of older adults, would have a major positive impact in our society.

PRELIMINARY DATA

Validation of the frailty screening criteria in SALSA: We have classified frailty using the Fried criteria (Fried et al., 2001) in the San Antonio Longitudinal Study of Aging (SALSA). SALSA participants were examined at the baseline of the study (1992-1996) and during three follow-up examinations 18 months apart starting in 2000 (2000-2004). At the baseline and follow-up examinations, subjects participated in comprehensive performance-based assessments that included all frailty information required to classify frailty. Because of the unique bi-ethnic nature of the cohort, comprised of approximately equal proportions of Mexican Americans and European Americans, our work was the first to directly compare frailty prevalence and incidence among Mexican Americans and European Americans (Espinoza and Hazuda, 2008; Espinoza et al., 2010).

Individuals were characterized as frail if they have 3 of 5 characteristics: slow walking speed, weak grip strength, low energy expenditure, self-reported exhaustion, and weight loss. Individuals with 1 or 2 of these characteristics were considered pre-frail, and those with none of these were considered non-frail. 701 individuals had complete information for frailty classification at baseline. To determine validity of the phenotype, trends in activities of daily living (ADLs) disability, instrumental activities of daily living (IADLs), number of hospitalized days per year as a measure of health status, and mortality were examined by frailty classification. We hypothesized that frailty would associated with disability, hospitalization days per year, and mortality, and that frailty would predict disability and death.

Baseline characteristics: At baseline, the prevalence of frailty was 9.8%, and the prevalence of pre-frailty and non-frail was 53.1% and 37.1%, respectively (Espinoza and Hazuda, 2008). Frailty prevalence was 18.4% at the first follow-up 6 years later (Espinoza et al., 2010). At baseline, frail individuals had a higher prevalence of diabetes, stroke, hypertension, and arthritis compared with non-frail individuals ($P < 0.05$), but no difference was observed in the prevalence of myocardial infarction, angina, or cancer. Frail individuals were found to have lower socioeconomic status (SES) compared with non-frail individuals, as measured by monthly household income and level of education ($P < 0.05$).

Disability and Dependence: There was a step-wise increase in the degree of disability in activities of daily living (ADLs) ($P < 0.01$) and instrumental activities of daily living (IADLs) ($P < 0.05$) (defined as self-reported difficulty in performing the tasks) with frailty status, as measured by the Structured Assessment of Independent Living Skills (SAILS) scale (higher score indicating increased disability) at baseline and at the first follow-up visit (Table 1).

Table 1: Correlates of frailty status at baseline and 1st follow-up in SALSA.

		ADL Disability*	IADL Disability†	Hospitalized days/year‡
Baseline	n	Mean ± SD	Mean ± SD	Mean ± SD
Not Frail	259	17.9 ± 6.9	39.4 ± 13.4	0.5 ± 2.2
Pre-Frail	370	19.7 ± 8.0	42.8 ± 15.1	1.5 ± 4.6
Frail	64	25.1 ± 8.2	50.9 ± 16.4	4.8 ± 8.4
Follow-up (7 years)				
Not Frail	188	22.8 ± 8.7	60.8 ± 18.4	0.5 ± 1.7
Pre-Frail	239	24.6 ± 8.7	65.8 ± 19.7	2.2 ± 7.4
Frail	28	28.2 ± 9.0	75.5 ± 20.6	2.0 ± 5.4

*P-value for significance (analysis of variance) <0.001 at baseline and <0.01 at follow-up.
†P-value for significance (analysis of variance) <0.05 at baseline and follow-up.
‡P-value for significance (analysis of variance) <0.001 at baseline and not significant at follow-up.

Frailty remained significantly associated with ADL and IADL disability in multivariable linear regression analyses adjusting for sociodemographics and comorbid medical

conditions, such that pre-frail individuals scored 1.3 points higher (P =.03) and frail individuals scored 5.0 points higher (P <.001) on the ADL scale compared to non-frail, indicating higher disability. For IADL disability, pre-frail individuals scored 0.8 points higher (P =.028) and frail individuals scored 3.5 points higher (P <.001).

In longitudinal analyses, frailty was predictive of both ADL and IADL dependence (requiring the assistance of other persons or equipment to perform the task) in the SALSA cohort. Individuals who were classified as frail at the baseline were more than 4 times more likely to develop dependence in ADLs over the follow-up period of the study (10-12 years) compared to those who were non-frail, with odds ratio (OR) of 4.09, 95% confidence interval (CI) of 2.05-8.18, and P-value of <0.001. Similarly, frail individuals were more than 2.5 times more likely to be dependent in IADLs (OR = 2.84, 95% CI: 1.42-5.68, P =0.003) (Espinoza & Hazuda, unpublished, manuscript in preparation).

Health Care Utilization: Frailty was also associated with healthcare utilization as measured by number of hospitalized days within one year at the SALS baseline exam (Table 1). In multivariate analyses, the number of frailty characteristics (0-5) was associated with hospitalization independently of sociodemographic factors (age, sex, ethnic group, and socioeconomic status) and comorbid diseases. Frailty score was independently associated with odds of hospitalization (OR=1.35, 95%CI: 1.08-1.68, P =.008). Thus, with each 1-point increase in frailty score the odds of hospitalization increased by 35% (Espinoza & Hazuda, unpublished).

Mortality: Baseline frailty status was strongly predictive of mortality at 10 to 12 years follow-up. Mortality significantly differed by frailty status; 56.1% (n=37) of frail individuals (n=66) were deceased at 10-12 years of follow-up, compared with 35.0% (n=125) of pre-frail (n=357) and 22.1% (n=55) of non-frail (n=249) individuals (P <.001). We also found that mortality increased similarly with frailty score ranging from 0 to 5 (Espinoza et al., 2012). In adjusted models using Cox proportional hazards models, frailty was shown to predict mortality after adjustment for age, sex, ethnic group, diabetes status, and socioeconomic status (as indicated by years of education and monthly household income) with a hazard ratio for frail individuals of 1.78 (95%CI: 1.16-2.73, P =.008).

Obesity, pre-diabetes and diabetes association with frailty in SALSA: While frailty prevalence is higher in Mexican Americans compared to European Americans (11% vs. 7%, P<.05), there was no significant ethnic difference in frailty prevalence after adjustment for sociodemographic and disease factors, primarily diabetes prevalence which is almost 3-fold higher in Mexican Americans compared to European Americans (33.5% vs. 11.9%, P <.001) (Espinoza et al., 2010). Diabetes was associated with almost three times greater odds of frailty at the baseline of the study (OR = 2.74, 95% CI: 1.51-5.00, P <.001) in multivariable analysis adjusting for sociodemographics (age, sex, socioeconomic status) and other medical comorbid conditions. Frailty was also associated with increasing body mass index and waist circumference at the baseline of the study, as shown in Table 2. We also found a step-wise increase in diabetes prevalence across frailty category, also shown in Table 2.

Table 2. Association of frailty with body mass index and waist circumference

	Non-frail N = 249	Pre-frail N = 356	Frail N = 66	P-value
Body mass index, kg/m^2	27.6 ±4.2	28.7 ±5.6	30.1 ±6.8	.0018
Waist circumference, cm	97.5 ±11.7	99.8 ±14.3	104.3 ±16.0	.0013

More recently, we demonstrated that diabetes was predictive of progression in any one of the five frailty characteristics, i.e. the onset of any of the five frailty characteristics from baseline to follow-up in SALSA (OR = 1.84, 95% CI: 1.02-3.33, P =.04) (Espinoza et al.,

2012). Therefore, individuals who have diabetes and medical complications related to their diabetes have an 84% increased risk of attaining at least one of the five frailty characteristics over time. In another study, we similarly found that better glycemic control (HbA1c <7%) in SALSA participants with diabetes predicted long-term maintenance of lower extremity physical function as measured by the Short Physical Performance Battery (SPPB) (Wang and Hazuda, 2011). The SPPB measures gait speed, balance, and chair rise, and higher scores indicate better lower extremity function. The total SPPB score which combines all three assessments was 0.62 ±0.2 in the better glycemic control group compared to 0.42 ±0.2 in the poorer glycemic control group (P <.05).

Pre-frailty predicts frailty: In our analyses of predictors of incident frailty, we found that pre-frailty is a high risk group, such that pre-frail older adults are over three times more likely to become frail over approximately 7 year follow-up period (OR = 3.19, 95% CI: 1.86-5.47, p<.001). This was the most significant predictor of frailty in multivariable generalized estimating equations model adjusting for sociodemographics, diabetes, and comorbid disease (Espinoza et al., 2010). These findings have been corroborated by others (Woods et al., 2005).

Table 3: Glycoproteins found to differ at least 2-fold in pre-frail compared to non-frail community-dwelling older adults.

Lectin	Up-regulated	Down-regulated
Concanavalin A	Transferrin	
	Haptoglobin	
Jacalin	Transferrin	Hemopexin precursor
	Kininogen-1 variant (isoform)	Kininogen-1 variant
		Fibrinogen isoform
		Apolipoprotein E
		Leucine rich alpha-2 Glycoprotein 1

Inflammation and frailty: Based on our data (Espinoza et al., 2012) and those of others showing that pre-frailty is an important risk factor for future frailty, we sought a translational approach to determine if pre-frailty is associated with physiologic dysregulation as measured by changes in the serum glycoprotein profile. Because glycoproteins are secreted from cells/tissues in the body, they provide valuable insight into the overall health state of a cell and have been useful as disease markers, i.e., "fingerprints" for specific disease states which are likely to have alterations in post-translational modification and glycoprotein expression. For this study, we recruited and characterized frailty using validated criteria (Espinoza and Hazuda, 2008; Fried et al., 2001) in community-dwelling older adults from the independent living community at the Air Force Villages retirement community in San Antonio, TX. Using lectin-affinity chromatography, 2-dimensional (2-D) gel electrophoresis, our group developed a protocol to screen the glycoproteome of the plasma from these participants (Shamsi et al., 2012). This is the first study to use a proteomic screen to identify proteins that would be predictors of frailty. Subjects for proteomic comparative analysis were age- and sex-matched non-frail (*n* = 4) and pre-frail (*n* = 4) community-dwelling older adults (mean age 81, 50% female). The data in Table 3 lists the proteins that differ at least 2-fold in levels on the 2-D gels in pre-frail compared to non-frail community-dwelling adults. Many of the glycoproteins that were found to differ by frailty status are related to inflammation,

Table 4. Plasma protein concentrations by frailty category

	Non-Frail n = 22 Mean ± SD or n (%)	**Pre-Frail** n = 31 Mean ± SD or n (%)	**Frail** n = 12 Mean ± SD or n (%)	**P-value for Frailty Difference** or n (%)
Transferrin, ng/mL	43.4 ± 11.4	54.3 ± 11.9	58.3 ± 10.2	<.001
Fibrinogen, g/L	40.6 ± 9.3	51.2 ± 19.5	70.4 ± 17.5	<.0001
Haptoglobin, mg/mL	1.1 ± .6	1.3 ± .6	1.3 ± .6	.51
Interleukin-6, pg/mL	0*	0.13 ± 0.33	0.60 ± 0.98	.0035

* All samples in the non-frail group were found to be below the detectable level for Interleukin-6

including transferrin, haptoglobin, and fibrinogen. We conducted a larger study in the same population using enzyme linked immunosorbent assay (ELISA) to measure plasma concentration of transferrin, fibrinogen, and haptoglobin in 65 older community-dwelling adults. We found that transferrin, fibrinogen, and IL-6 plasma concentration increased step-wise with increasing frailty category (non-frail, pre-frail, and frail), as shown in Table 4 (Darvin et al., 2014).

Metformin prevents frailty in older Veterans with diabetes: In a study in which we used an administrative definition of frailty (presence of falls, fracture, gait disorder, electrolyte disturbance, coagulopathy, anemia or weight loss) we recently found that metformin (compared to sulfonylurea) reduced onset of frailty in 3,194 adult veteran patients with diabetes (OR = 0.66, 95% CI: 0.61-0.71, P <.001) (Wang et al., 2014). Metformin usage was also associated with a reduction in mortality as compared to older Veterans being treated with sulfonylureas (hazard ratio [HR] = 0.69, 95% CI: 0.60-0.79, P <.001).

RESEARCH DESIGN AND METHODS

Study Design. Aims 1 and 2 will be carried out simultaneously.

Specific Aim 1: To determine whether the administration of metformin to pre-diabetic (specifically, glucose intolerant) older adults reduces the onset or worsening of frailty. This Aim will test the hypothesis that older glucose intolerant subjects receiving metformin will have a lower conversion rate from non-frail to pre-frail and pre-frail to frail.

Specific Aim 2: To determine if the administration of metformin to pre-diabetic (specifically, glucose intolerant) older adults reduces inflammation and improves insulin resistance. This Aim will test the hypothesis metformin will reduce systemic and cellular (muscle) inflammation and improve insulin sensitivity and that these improvements in inflammation and insulin action will predict the anti-frailty effect of metformin.

Aim 1. We propose a double blind randomized control study to evaluate the effect of metformin on frailty progression. Assuming a 4-5% drop out rate we will enroll 600 subjects with the goal of studying 120 completers. Subjects will be aged ≥65 years and higher and community-dwelling. In this study we will enroll subjects with impaired glucose intolerance because this group encompasses approximately 1/3rd of the older population, are at increased risk for developing diabetes, and are the most likely to benefit from a potential anti-inflammatory and insulin-sensitizing intervention. Subjects will be non-frail or pre-frail based on Fried criteria. Individuals with renal disease (glomerular filtration rate < 45 mL/min) and residents of nursing home and assisted living facilities will be excluded.

Glucose intolerance, which is considered a pre-diabetic state, will be determined according to American Diabetes Association criteria (American Diabetes Association, 2014a) based on oral glucose tolerance test (OGTT) with 2 hour values of 140 – 199 mg/dL after an oral glucose load, and no diagnosis of diabetes in the past 12 months According to the CDC estimates, 35% of the U.S. population is pre-diabetic (Centers for Disease Control and Prevention, 2014). Yet, this is a conservative estimate, as this figure is based on all U.S. adults, whereas the rate of pre-diabetes is considerably higher in older adults as insulin resistance increases with age. Based on the data from the CDC statistics (Centers for Disease Control and Prevention, 2014), among individuals of age 65 years or older, 26% have diabetes and 51% have pre-diabetes.

Based on a recent data query at the Audie L. Murphy VA hospital (ALMVAH), we identified 7,015 patients of 65+ years old with diabetes. From this data, we project that there will be 13,760 (=7015*51/26) individuals with pre-diabetes of age 65 years or older in ALMVAH. In addition, according to Kalyani et al (Kalyani et al., 2012), among older

patients with normal fasting glucose status, 48% had impaired glucose tolerance when screened by oral glucose tolerance test, as planned in this study. Therefore, if we were to screen all older Veterans without diabetes at ALMVAH, we are likely to identify a total of 31,1627 individuals with pre-diabetes (=13760+7015*(1-26%-51%)/26%*48%). Based on our work in SALSA, 10% older adults will be frail (53% will be identified as pre-frail and 37% will be identified as frail), and would be excluded from this study. Thus, we would potentially have a pool of were either non-frail or pre-frail. Thus we expect to have approximately 12,384-280,464 patients at ALMVAH who meet the pre-diabetes and frailty (must not be frail at study entry) eligibility criteria for this study. Drs. Espinoza and Musi (Co-Investigator) have extensive experience conducting large human studies with multiple study procedures (ASPREE Investigator Group, 2013; DeFronzo et al., 2011); therefore, this recruitment goal is realistic and achievable.

Based on our work and others, we project that 47-58% (n =56-70) will worsen in frailty status over the period of the study (Espinoza et al., 2012; Gill et al., 2006). This is likely an under-estimate given the fact that all subjects in this study will be pre-diabetic and will be at increased risk for developing diabetes as compared to this prior work.

Aim 2. The goal of this Aim is to gain insight into the mechanism(s) underlying the effect of metformin on frailty. In Aim 2 we will test two potential mechanisms:

(1) A reduction in insulin resistance (i.e., increased insulin sensitivity). Because metformin is an insulin sensitizer used to treat type-2 diabetes, metformin could reduce/delay the development of frailty through its action on the natural course of diabetes development.

(2) A reduction in chronic inflammation. As noted above, increased inflammation is believed to be fundamental in the etiology of frailty. Because metformin has been reported to reduce inflammation in subjects with diabetes (Carter et al., 2005), metformin could reduce/delay the development of frailty by reducing the inflammation in pre-diabetic subjects.

To test these two potential mechanisms we will measure:

1) insulin sensitivity at baseline, at year 1, and year 2 (at the end of the study). However, if a participant converts to diabetes based on the oral glucose tolerance test which will be conducted every 6 months, insulin sensitivity will be measured immediately without waiting until the 1 or 2 year time point

2) markers of tissue inflammation (muscle) at baseline, year 1, and year 2 (end of the study)

3) markers of systemic inflammation (plasma) at baseline and every 6 months thereafter during the two years of treatment with metformin (or placebo)

Insulin sensitivity will be quantitated with the euglycemic hyperinsulinemic clamp technique which is the most accurate method to assess insulin sensitivity (DeFronzo, 1979). To assess the inflammatory status of each subject we will measure markers of both systemic inflammation (e.g., plasma levels of inflammatory proteins) and tissue inflammation in skeletal muscle (e.g., NF-kappa B and MAPK signaling, and mRNA levels of inflammatory cytokines). These experiments will allow us to thoroughly assess the inflammatory status of the subjects because the changes in inflammatory mediators in tissues are often greater than changes observed in the blood. We have chosen to measure cellular inflammation in skeletal muscle because it makes up a major percentage of body mass, sarcopenia is a central feature of aging (Fried et al., 2001), and loss of muscle has been associated with increased systemic inflammation in older adults (Ferrucci et al., 2002). It should be noted that a novel aspect of this application is that it will be the first study on frailty to measure physiological parameters in tissues of the subjects; all previous studies, which measured biological/biochemical processes, have

	only studied serum/blood. By studying tissues it will be possible to gain further insight into the molecular/biochemical mechanisms responsible for the loss of musculoskeletal function characteristic of frailty. Moreover, in the future we could conduct studies to determine the effect of metformin on the transcriptome and metabolome, and relate those findings with the data obtained in this study regarding expression of inflammatory mediators and measurements of NF-kappa B and MAPK signaling.
Item 4 Purpose and rationale *Insert purpose, objectives and research questions/hypotheses here.* *If you cut and paste from another document, make sure the excerpted material answers the question*	Insert purpose: The **major goal** of this study to test a novel intervention strategy that could be used in the prevention of frailty in a sub-population of older adults who are at increased risk for becoming frail. **Overall Rationale** The rationale for this study comes from data generated in three areas. **First**, research showing that type 2 diabetes is a major risk factor for frailty. Numerous groups, including ours, have shown that diabetes increases the risk of becoming frail by 40% (Woods et al., 2005) and the risk of gaining any one frailty characteristic by 80% (Espinoza et al., 2012). **Second**, accumulating evidence suggests that inflammation plays a role in the etiology of frailty. For example, markers of inflammation increase in frail compared to non-frail subjects (Darvin et al., 2014; Shamsi et al., 2012; Walston et al., 2002) and diabetes and insulin resistance (major risk factors for frailty) are considered inflammatory states (Haffner, 2003; Pickup, 2004; Pickup et al., 2000). **Third**, our recent data showing that metformin, an insulin sensitizer that has been shown to decrease inflammation as well as decrease the onset of diabetes, significantly reduces (34%) the risk of older patients with type 2 diabetes becoming frail as they age. **Based on these data we hypothesize that metformin will reduce the development of frailty in older adults through its ability to reduce inflammation and improve insulin sensitivity.** We have chosen to use metformin as the intervention for the following reasons: 1) it is well tolerated and widely used, 2) our epidemiologic data showing that prevalence of frailty is lower in diabetic subjects that use metformin compared with subjects that use other antidiabetic agent, 3) several studies suggesting that metformin might have aging-modulating properties e.g., it appears to have life-extending (Martin-Montalvo A. et al., 2013) and anti-cancer properties (Berstein, 2012), which may be related to its AMPK-activating effect(Zhou et al., 2001); and 4) it is recommended by the American Diabetes Association as a treatment option for pre-diabetes (American Diabetes Association, 2014b).

Item 5 Study Population(s) Being Recruited In your recruitment plan, how many different populations of prospective subjects do you plan to target? Provide number: 1 *e.g., a population can be individuals with type 2 diabetes controlled with diet and/or a population of healthy controls. Or a population can be individuals attending an education program, etc.* <u>List each different population</u> on a separate row and provide a short descriptive **label**: Older community-dwelling adults (age ≥ 65 years) who are pre-diabetic. *(e.g., normal-healthy, diabetics, parents, children, etc.)* *To add rows use copy & paste*	Identify the criteria for **inclusion**:	Identify the criteria for **exclusion**:
Older community-dwelling adults (age ≥ 65 years) who are pre-diabetic	**Inclusion criteria:** 1) Men and women 2) All ethnic groups 3) Age 65 and older 4) Community-dwelling 5) Pre-diabetic based on oral glucose tolerance test (OGTT) with 2 hour values of 140 – 199 mg/dL after an oral glucose load, and no diagnosis of diabetes in the past 12 months 6) Subjects must have the following laboratory values: Hematocrit ≥ 33%, aspartate aminotransferase (AST) < 2 X upper limit of normal, alanine aminotransferase (ALT) < 2 X upper limit of normal, alkaline phosphatase < 2 X upper limit of normal, normal urinalysis (no clinically significant white blood cells, red blood cells, or bacteria), platelets ≥100,000, PT < 15 seconds and PTT < 40 seconds, glomerular filtration rate [GFR] ≥ 45	**Exclusion criteria:** 1) Characterized as frail, defined as the presence of 3 or more of: 1) weak hand grip strength, 2) slow walking speed, 3) low physical activity, 4) unintentional weight loss of ≥ 10lbs over the past year, 5) self-reported exhaustion 2) Resident of nursing home or long-term care facility 3) Subjects with diabetes with range fasting glucose in diabetes range (≥ 126 mg/dL), or 2-hour glucose within diabetes range on OGTT (≥ 200 mg/dL), 4) Subjects taking drugs known to affect glucose homeostasis 5) Untreated depression or Geriatric Depression Scale (GDS) score on 15-item scale >7 6) Diagnosis of any disabling neurologic disease such as Parkinson's Disease, Amyotrophic Lateral Sclerosis, multiple sclerosis, cerebrovascular accident with

	mL/min, and urine protein ≤ 100 mg/dL by lab urinalysis.	residual deficits (muscle weakness or gait disorder), severe neuropathy, diagnosis of dementia or Mini-mental State Exam (MMSE) score <24, cognitive impairment due to any reason such that the patient is unable to provide informed consent. 7) History of moderate-severe heart disease (New York Heart Classification greater than grade II) or pulmonary disease (dyspnea on exertion upon climbing one flight of stairs or less; abnormal breath sounds on auscultation) 8) Poorly controlled hypertension (systolic >160 mmHg, diastolic >100 mmHg) 9) Peripheral arterial disease (history of claudication) 10) Moderate to severe valvular heart disease 11) Subjects who have been treated with long term (>30 days) systemic steroids, anabolic steroids, growth hormone or immunosuppresants within the last 6 months. Males with a medical history of testosterone deficiency who are on a stable dose of testosterone replacement (for ≥ 3 months) are allowed. 12) Subjects who have been treated with short term (<30 days) systemic steroids, anabolic steroids, growth hormone or immunosuppressants within the last 1 month. 13) Chronic inflammatory condition, autoimmune disease, or infectious processes (e.g., active tuberculosis, HIV, rheumatoid arthritis, systemic lupus erythematosus, acute or chronic hepatitis B or C)

		14) Active tobacco use (within 6 months) 15) Illicit drug use 16) Active malignancy, non-skin 17) Disease or condition likely to cause death within 5 years 18) Hypersensitivity to metformin or pioglitazone 19) Any disease or condition considered to be exclusionary based on the clinical opinion and discretion of the PI

Item 6
Research Plan / Description of the Research Methods *a.* *Provide a **comprehensive narrative** describing the **research methods**. Provide the **plan for data analysis** (include as applicable the **sample size calculation**).*

Step-by-Step Methods:

Experimental

Screening Tests

a) Telephone Screening Potential participants will be asked screening questions over the phone. Only those without exclusion criteria will be scheduled for an in-person screening visit.

b) History & physical examination will be performed by Dr. Espinoza (board-certified Internist and Geriatrician) to document medical history and physical exam findings according to key inclusion/exclusion criteria (see Human Subjects). The history & physical examination will also include screening questions for cognition, quality of life, and depression. Human studies will be conducted in the Bartter Research Unit (BRU) of the ALMVAH and at the MARC on the 3rd Floor; however, the Visits 2, 8 and 14 which include the Insulin Clamp and Muscle Biopsy will continue to be done at the ALMVAH BRU. Waist circumference using the World Health Organization protocol will also be measured as part of the history & physical examination.

c) Frailty assessment: Individuals identified as frail (approximately 10% of the community-dwelling older adult population) (Espinoza and Hazuda, 2008) will be excluded. Pre-frailty is defined as the presence of 1 or 2 of: 1) self-reported unintentional weight loss of 10 lbs or more in last year, 2) self-reported exhaustion, 3) low physical activity based on the Minnesota Leisure Time Physical Activity Questionnaire, 4) weak hand grip, 5) and slowness on 10-foot walk at usual pace. These measures have been standardized in SALSA, and will be used in this study as described (Espinoza and Hazuda, 2008). Non-frailty is defined as the presence of 0 characteristics. Frailty will be assessed every 6 months using the Fried criteria. If a participant's frailty category or score worsens based on Fried criteria (Fried et al., 2001), it will be repeated in 3 months and then at the 6 months intervals as per the protocol thereafter. At the baseline, self-reported unintentional weight loss will be used as criteria for weight loss as stated above. However, at all subsequent visits, individuals who have lost ≥5% of their baseline weight will be considered to have met the criteria for weight loss. As a measure of lower extremity function, we will use the Short Physical Performance Battery (SPPB) (Guralnik et al., 1994) and the 6-Minute Walk Test (6-MWT) (Harada et al., 1999). Both the SPPB and the 6-MWT are assessments of physical function commonly used in geriatrics studies. Lower extremity strength will be measured every 6 months with a knee extension dynamometer chair. The participants will be positioned in an upright position, with the ankle to a force or torque transducer at the knee angle of 90°. After 3 warm-up trials, 3 trials will be conducted to measure maximal voluntary contraction force of the knee extension muscle. We will also screen for depression using the Geriatric Depression Scale (Yesavage et al., 1982) and cognitive

impairment using the Mini Mental State Examination (Folstein et al., 1975) as part of the frailty assessment.

d) Laboratory tests. Complete blood count (CBC), urinalysis, comprehensive metabolic panel with liver function tests and ECG will be performed. Creatinine clearance (mL/min) will be estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula. For women with a plasma creatinine _≤0.7, (plasma creatinine/0.7)$^{-0.329}$ x (0.993)^{age} (x 166 if black; x 144 if white or other); for women with a plasma creatinine >_0.7, (plasma creatinine/0.7)_$^{-1.209}$ _ (0.993)^{age} (x 166 if black; x 144 if white or other); for men with a plasma creatinine ≤ 0.9; (plasma creatinine/0.9)$^{-0.411}$ x (0.993)^{age} (x 163 if black; _x 141 if white or other); for men with a plasma creatinine >_0.9, (plasma creatinine/0.9)$^{-1.209}$ _ (0.993)age (x 166 if black; x 144 if white or other). This will be done using the on line resource from the National Kidney Foundation: http://www.kidney.org/professionals/KDOQI/gfr_calculator. Other blood tests include lipid panel, Hemoglobin A1c (HbA1c), and coagulation tests (PT and PTT) and B12.

e) Oral glucose tolerance test (OGTT): Baseline (fasting) samples for determination of glucose, insulin, and free fatty acid (FFA) concentrations will be drawn at -30, -15, and 0 min (these basal values are averaged). At time zero, each subject will ingest 75 g of glucose. Glucose, insulin and FFA are determined every 30 min for 2 h following glucose ingestion. The Matsuda index of insulin sensitivity will be calculated from OGTT results, as described (Matsuda and DeFronzo, 1999). In subjects that qualify based on the screening tests, the OGTT will be performed every 6 months to monitor for conversion to diabetes.

Study Procedures. Subjects who qualify for the study will undergo the following additional studies:

a) Activity monitoring. An activity monitor (Actigraph, LLC) will be used to monitor physical activity over a 10 day period. The monitor will be mailed to the participant approximately 2 weeks prior to a scheduled visit. He or she will place the monitor on the wrist as he or she would a watch, and wear until returning to the next visit or we will provide a postage paid envelope for the participant to mail back to the study team. We will retrieve the monitor at this time. This measurement will be taken prior to randomization and then at 2 time points following randomization (see table of study visits and procedures below).

b) Body composition. Whole body DEXA is performed to measure lean and fat body mass (lean mass values are used to adjust insulin sensitivity values) and to examine the effect of the interventions on body composition.

c) Insulin clamp: Insulin sensitivity will be measured after an overnight fast using a 130-min euglycemic hyperinsulinemic (40mU.m^2) clamp with tritiated glucose as described (DeFronzo, 1979). Indirect calorimetry is performed during the 45 min prior the clamp and 30 min before the end of the insulin clamp to measure fat and carbohydrate oxidation and oxidative and non-oxidative glucose disposal. This procedure will not be performed if subject has donated blood within the last 2 months; subject does not have normal electrolytes (potassium ≥3.3 mmol/L); there is difficulty obtaining IV access; if the patient has had high exposure to radiation in the last year from general medical care (>50 millisieverts) (Hruska & O'Connor, 2015); or for any other concern as determined by the PI.

d) Muscle biopsies: Thirty min before starting the insulin clamp a biopsy of the *vastus lateralis* muscle will be performed for measurements of local (muscle) inflammation as described (Sriwijitkamol et al., 2007). A second muscle biopsy is performed in the contralateral leg before the end of the clamp for measurements of insulin receptor signaling. Occasionally, a small amount of fat tissue is removed along with the muscle tissue. If this occurs, the fat tissue will be stored and analyzed for inflammation markers in similar fashion. This procedure will not be performed if there is difficulty obtaining IV access, if the patient is anticoagulated for any reason and PCP does not feel it is safe for anticoagulant to be ceased for 1 week prior to the biopsy, if the patient does not tolerate a prior biopsy, or for any other concern as determined by the PI

e) Systemic Inflammation & Genetic Markers of Aging: The levels of IL-6, IL-1β, CRP, IL-1RA, TNFs R1 and 2, fibrinogen, transferrin, and TNFα in plasma will be measured using enzyme-linked immunosorbent assay (ELISA) (antibody source: GenWay Biotech, San Diego, CA) as described by Shamsi et al. (2012) (Shamsi et al., 2012). As noted above, a large number of studies have shown that IL-6 and CRP increase in frail older adults compared to non-frail older adults. Plasma levels of IL-1β and TNFα, which are also markers of inflammation, have been shown to increase in diabetic subjects (Pickup et al., 2000), and

metformin has been shown to reduce plasma TNFα concentration (McCoy et al., 2012). The processed blood samples will also be used to evaluate genetic markers associated with aging.

f) Cellular Inflammation: We will assess the level of cellular inflammation by measuring the levels of the mRNA transcripts for IL-1β, IL-6, MCP-1, and TNFα in skeletal muscle before and after metformin treatment. The mRNA expression level of these genes is considered a robust marker of cellular inflammation. Total RNA will be extracted from frozen tissue (100-200 mg) using the RNeasy kit (Qiagen, Valencia), and the mRNA levels will be measured by qRT-PCR with primers for each transcript using SYBR Green PCR Master Mix (Applied Biosystem, Inc.) with a CFX96 real time polymerase chain reaction Touch System (Bio Rad), and the expression of the housekeeping gene, glyceraldehydephosphate dehydrogenase (Gapdh*)* will be used as the control using the 2-deltadelta Ct approach. Measurements of NFκB and MAPK signaling, two key mediators of the inflammation state, will be conducted as described (Hussey et al., 2013).

g) AMPK, insulin signaling and mitochondrial function: The AMPK (AMPK and ACC phosphorylation) and insulin (IRS-1 and Akt phosphorylation) signaling pathways will be evaluated as described (Hussey et al., 2013). The AMPK pathway is being evaluated because it is thought to mediate the beneficial effects of metformin on metabolism (Musi et al., 2002; Zhou et al., 2001), inflammation (Kim and Choi, 2012) and aging (Onken and Driscoll, 2010). IRS-1 and Akt phosphorylation will be measured to establish the relationship between parameters of insulin signaling and frailty. IRS-1, Akt, AS160, mTOR and S6K phosphorylation will be measured to establish the relationship between parameters of insulin/mTOR signaling and frailty. We will also measure mitochondrial function from the muscle tissue using Oroboros equipment to measure ATP production (Lanza and Nair, 2009).

h) Microbiome analysis: Stool will be collected at Visits 2, 8, and 14 for analysis of the microbiome using 16S sequencing. Stool collection containers will be provided to subjects at Visits 1, 7, and 13 with instructions on how to collect the stool and return at the following visit.

i) Pharmacological intervention: After the baseline studies, subjects will be randomized to metformin (titrated up to 1000 mg twice daily, as tolerated) vs. placebo over a period of 6 months. Metformin and placebo are obtained from Major Pharmaceuticals (Livonia, MI). Randomization and drug preparation and dispensing will be performed by the Research Pharmacist of the BRU. Subjects will be initiated at 500 mg daily at Visit 2. Subjects will be seen in person at 1 week, and if tolerating metformin, will be directed to increase to 500 mg twice daily after 2 weeks. Phone calls will be made about every two weeks to titrate medications as tolerated as needed until patient is at maximum dose tolerated with goal dose of 1000 mg twice daily. At 3 months subjects will be seen in-person for a history & physical examination. OGTT will be performed every 6 months to monitor for diabetes conversion. Plasma metformin level will be measured once the participant has reached his/her maximum tolerated dose at either Visit 2, 4, 5, 6, 7, 8, 10, 11, 12, 13 or 14 If participants convert to diabetes and are started on metformin from placebo, an additional metformin level will be measured once the patient has reached his/her maximum tolerated dose at either Visit 2, 4, 5, 6, 7, 8, 10, 11, 12, 13, or 14. All patients will also receive patient education handouts about lifestyle modification to prevent diabetes at Visit 2.

j) Diet and exercise counseling: After the baseline visit, subjects will receive standard diet and exercise advice using resources available from the National Institute on Aging. This will be conducted by study staff member, Beverly Orsak, RN, who is a certified diabetes educator. Resources will be given to subjects, including "Exercise & Physical Activity: Your Everyday Guide from the National Institute on Aging" available from https://www.nia.nih.gov/health/publication/exercise-physical-activity/introduction, and "What's On Your Plate? Smart Food Choices for Healthy Aging" available from https://www.nia.nih.gov/health/publication/whats-your-plate.

k) Management of conversions: Any one of the following will prompt 2-hour OGTT to confirm possible diagnosis of diabetes: fasting glucose ≥ 126 mg/dL, HbA1c ≥ 6.5%, 2-hour OGTT glucose of ≥ 200 mg/dL, or clinical signs and symptoms of diabetes. If any of these occur, OGTT will be conducted within 6 weeks. If the subsequent 2-hour OGTT glucose is ≥ 200 mg/dL, diabetes conversion will be confirmed. If diabetes conversion occurs based on the OGTT, measurements of frailty, insulin sensitivity (clamp), body composition (DEXA), coagulation tests, stool sample, and inflammation (muscle and systemic) will be

performed. If these assessments are conducted within 1 month of the 12 month or 24 month assessments, the 12 or 24 month assessments will not be conducted. After these repeat measurements are performed, the study allocation will be unblinded, and, if receiving placebo, subjects will be initiated on metformin and titrated as above. Medication management will occur by an independent practitioner (MD or NP) on the study staff who is not the PI such that the PI will remain blinded to study treatment. Hemoglobin A1c will be monitored in those who have converted to diabetes, and, if it reaches ≥ 7.5, subjects will be initiated on 15 mg pioglitazone in addition to metformin unless there is a contraindication to pioglitazone. The rationale for adding pioglitazone versus other antidiabetic agents is that the low dose metformin-thiaozolidinedione combination has been shown to have a potent effect to prevent diabetes (Zinman et al., 2010). Pioglitazone will be increased to 30 mg if hemoglobin A1C remains ≥7.5. Pioglitazone will not be initiated in any subject with the following: hematuria, history of bladder cancer, lower extremity edema > 1+,or known history of osteopenia or osteoporosis. Subjects will be seen every 3 months for monitoring of HbA1C. If maximal metformin (1000 mg BID) and pioglitazone (30 mg daily) doses are reached (or if the patient cannot tolerate metformin and/or pioglitazone) and HbA1C remains ≥ 8, subjects will be referred to their primary care physicians for further treatment, but will remain in the study for frailty assessment and all other follow-up studies. Subjects who initially were on metformin will receive pioglitazone 15 mg (in addition to metformin) if Hemoglobin A1c reaches ≥7.5, which will be titrated to 30 mg if necessary as described above.

Follow up visits. History/physical exam will be conducted every 6 months. Fasting glucose measurement and HbA1c will be conducted every three months, and OGTT will be conducted every 6 months. However, if fasting glucose and/or the HbA1c suggest conversion to diabetes based on American Diabetes Association criteria, OGTT will be performed. Assays of systemic inflammation (in plasma) will be conducted every 6 months. Insulin clamp with muscle biopsies will be conducted at 12 and 24 months, such that the treatment effect on tissue inflammation and insulin signaling may be determined. The table below outlines the follow up visits and procedures. A grace period of +/- 3 weeks is provided to complete follow-up visits.

Study visits and procedures															
Visit #	1	2	3	4	5	***6	7	8	9	***10	11	***12	13	14	15
Year	1									2					
Month	Screening Visit	0	1	3	6	9	12	12	12	15	18	21	24	24	24
Time Interval		3-21* Days	5-7 Days	2.5 Mo	3 Mo	3 Mo	3 Mo	3-21 Days	5-7 Days	2.5 Mo	3 Mo	3 Mo	3 Mo	3-21 Days	5-7 Days
History & Physical, with Frailty Assessment	X			X	X		X				X		X		
Biodex	*X				*X		*X				*X		*X		
Activity monitor		X							X						X
Quality of life assessment	X			X	X		X				X		X		
EKG	X						X						X		
Urinalysis	X						X						X		
OGTT	X				X		X				X		X		
Safety labs	X			X	X	X	X			X	X	X	X		
HbA1c	X			X	X	X	X			X	X	X	X		
Coagulation tests	X						X						X		
Systemic inflammation		*X			X			*X			X			*X	
DEXA		*X						*X						*X	
Stool collection		*X						*X						*X	
Insulin clamp w/ biopsy		*X						*X						*X	
Examination of biopsy site			**X						**X						**X

*These visits may be done at other approximate visits to reduce the amount of exposure to the participant due to COVID-19.

**Due to COVID-19, the participants can opt to do this visit via phone or in person.
***Due to COVID-19, if the participants do not need to do the Frailty assessment, this visit can be done via phone. Labs may be performed at the Quest Diagnostics laboratory nearest to the participant instead of the research unit.

Urinalysis (UA) will be repeated if needed prior to starting on pioglitazone (only for subjects who convert to diabetes and do not have adequate glycemic control on metformin alone).

Basic metabolic panel (kidney function) will be performed as needed to check kidney function at least 48 hours after any radiologic exam using contrast dye.

DEXA may be performed at Visit 7 instead of Visit 8 and at Visit 13 instead of Visit 14 if participant unable to arrive early to the clinical research unit on Visit 8 and Visit 14.

Potential risks include the following:

The potential patient risks are discussed below:

1 .Phlebotomy. All studies involve the withdrawal of blood. In no instance will this exceed 600 ml within a two month period. Any subject who has donated blood over the two month period prior to study will be excluded from participating. If a subject participates in a study, he/she will be told he/she should not donate any blood for the study duration. Any subject with a hematocrit less than 33% will be excluded from study. Infection is possible with venipuncture but rare (<1%). Venipuncture will be performed by inserting a single, small needle into a vein in the subject's arm after cleaning the local area with isopropyl alcohol. This procedure will be performed by trained staff members with extensive experience in venipuncture. Mild pain during the blood draw is common but temporary. Local hematomas occur in about 1% of subjects.

2. Administration of Insulin and Glucose. Since the glucose infusion is designed to counterbalance the metabolic effects of insulin, hypoglycemia will not occur. No other side effects of insulin and glucose are known. Plasma glucose concentration will be determined at 5 min intervals throughout the period of insulin/glucose administration. There is a possibility that the insulin clamp will not be performed if unable to establish IV access (most common reason) or for any other reason precluding its completion at the discretion of the PI.

3. IV lines. Catheters will be placed in an antecubital vein and a hand vein for the euglycemic clamps. If the euglycemic clamp is not performed, an IV may be placed if a biopsy is performed. Local hematomas occur in about 1% of catheterization. Infection is possible (<1%), but we have not experienced this complication. One instance of thrombophlebitis has been observed (<1%). The hand with the catheter will be placed in a warm (55^{o} C) transparent plastic box to arterialize venous blood. We have observed one instance of skin burning (2nd degree) using these boxes (<0.01%).

4. Muscle biopsy. At the time of biopsy, subjects may feel pain, discomfort, or pressure (variably described by different subjects) for about 5-10 seconds. Pain or discomfort ceases as soon as the cannula is withdrawn. These will be performed by trained, experienced staff, either an MD or Nurse Practitioner. Over the last 20 years, our group has experience with approximately 1300 biopsies. Local hematomas occur in <2% of subjects. One patient experienced a moderately painful hematoma that resolved within 2 weeks (0.1%). About 1 in 50 subjects report non-clinically evident numbness or altered sensation at the biopsy site, which is transient. All subjects will return to the clinical research unit within 7 days to evaluate the biopsy site. On any particular day, the muscle biopsy is performed twice, once in a fasting state, and then lastly after exposure to insulin during the insulin clamp, in order to measure muscle

tissue insulin signaling response. There is the possibility that a future biopsy may not be done at the discretion of the PI in case the subject did not tolerate well a prior biopsy.

5. Frailty assessment, SPPB, and 6-MWT. There is minimal risk to the physical measurements of frailty assessment, which includes measurement of strength (hand grip using handheld dynamometer; knee extension using a seated computerized dynamometer) and 10-foot walking speed at usual pace. The risk associated with grip strength measurement is that some subjects may have some slight muscle or joint soreness that should resolve by itself within hours or a few days. It is possible that some subjects may experience transient fatigue and/or pain in the legs associated with walking 10 feet, which should resolve within minutes to hours. To minimize risk of falls during the 10-foot walk, the study investigator or trained study personnel will walk side-by-side with the subject throughout the walk. Additionally, the study investigators or study personnel have experience working with older persons, and will be able to evaluate the subject's ability to walk independently and safely. If the subject does not appear capable of performing the 10 foot walk due to any reason (dizziness, lower extremity weakness) the procedure will not be performed. If the subject expresses concern and/or anxiety about performing the task, it will not be performed. If participants use an assistive device for ambulation, this may be used during the walking speed assessment. Dr. Espinoza has performed this assessment on approximately 80 subjects from the Air Force Villages retirement community with no adverse event or fall. The procedure is safe. The SPPB is a standardized assessment of lower extremity function, which includes standing balance, 4-meter walk (approximately 13 feet), and repeated standing from a seated position (chair stand). The battery has an excellent safety record. It has been administered to over 20,000 persons in various studies and no serious injuries are known to have occurred. The 6-MWT test is a well-established assessment that will be conducted in an unobstructed hallway utilizing a standardized script. Total distance covered and number and duration of rests and symptoms will be recorded. With this test, there is a risk of the participant losing their balance and falling. We will minimize this risk by: (1) safely escorting participants to chairs located along the walking course should they become unsteady; (2) following the participant at a close distance; and, (3) being nearby should the participant need assistance. Further, if a participant appears to have major difficulty or is unable to complete the shorter walking tests above, the 6-MWT will not be performed. The lower extremity strength testing is low risk to the participant as we are asking participants to extend their leg against resistance. There is possibility of mild discomfort during the test due to muscle exertion, but it is self-limiting and will resolve as soon as the test is stopped or the participant voluntarily reduces the amount of force applied.

6. Questionnaire data collection. Various standardized questionnaires will be used to obtain information relative to these analyses, including some information needed to ascertain frailty (physical activity level and self-reported exhaustion), a depression scale, and screen for cognitive impairment (Tariq et al., 2006). These are minimal risk to the participant but could result in mild psychological distress which should be transient and resolve. To minimize this risk, study personnel will be sure to inform participants that he/she can skip any question he/she prefers not to answer. All of the questions in the interview will be performed by a trained staff member or the PI, who is a geriatrician, to assure risks are minimal as would be the case in standard clinical care.

7. Administration of metformin. The administration of metformin in adults with normal renal function is safe, the inclusion/exclusion criteria have been tailored so as to reduce the possibility of adverse events. The most severe adverse event associated with metformin use is lactic acidosis which is very rare (occurs in < 1%), and is more common in patients with impaired renal function. Therefore, individuals with creatinine clearance <45 mL/min are excluded from this study based on FDA dosing recommendations. More common adverse reactions (occur in > 10%) are diarrhea, nausea, and vomiting. Subjects will be provided with the common side effects as part of informed consent and will be monitored closely throughout the study. The medication will be titrated to the maximum tolerable dose. Subjects who do not tolerate metformin will be disenrolled.

8. Administration of pioglitazone. Only subjects who convert to diabetes and whose hemoglobin A1C is over ≥7.5 despite metformin administration will receive pioglitazone. Subjects with hematuria on urinalysis or history of bladder malignancy will not be administered pioglitazone. Subjects with liver disease and elevated transaminases are excluded from the study. More common adverse reactions (occur in > 10%) are edema and upper respiratory tract infection. Patients will be evaluated with routine history and physical examinations to monitor for adverse reactions, and those with peripheral edema greater than 1+ or any evidence suggestive of heart failure will not receive pioglitazone.

9. Radiation exposure. Subjects will be exposed to a small amount of radiation during the insulin clamp and dual-energy x-ray absorptiometry (DEXA) exam (36.48 mrem), which is approximately 17% of the average amount of natural environmental radiation exposure (620 mrem dose) that each member of the general public receives per year. Tritiated glucose is given during the insulin clamp studies. The radiation exposure from tritiated glucose is well within guidelines (Shreve et al., 1958;. U.S. Department of Commerce National Bureau of Standards Handbook 69, 1969). The cumulative radiation exposure is well within the dose range (1,000 mCi) approved by the University of Texas Health Science Center Radiation Safety Committee. At these low exposures, risk is minimal. If a participant has had exposure to radiation due to medical tests or treatments and the estimated total exposure within one year is expected to be > 1,000 mCi, the insulin clamp and/or DEXA exam will not be performed at the discretion and estimation of the PI.

Data Analysis Plan:

STATISTICAL METHODS & POWER CALCULATIONS

Overview. In all analyses for Aim 1 and Aim 2, primary inference will be drawn based on intention to treat (ITT) analyses. Average complier effect (ACE) will be used to inform the metformin effect under full compliance to treatment assignments (Angrist et al, 1996). Per protocol analyses will be used to inform the metformin effect among completers. All analyses will be carried out unadjusted and with adjustment for covariates (Table 7) and covariate interactions with treatment and time. Corrections for multiple comparisons will be applied as appropriate.

Table 7. Covariates included in statistical analyses

Categories of covariates	Baseline	Time Varying
Demographics (age, sex)	X	
BMI (obesity), sarcopenia	X	X
Lipids, blood pressure	X	X
Fasting glucose, HbA1c, Insulin Sensitivity from OGTT (Matsuda) and Insulin Clamp	X	X
Inflammation (systemic & muscle), Insulin Signaling (muscle)	X	X
Diabetes conversion		X
Pioglitazone use		X

The impact of metformin treatment on the progression of frailty status (non-frail, pre-frail, frail) over time will be assessed with a repeated measures mixed effects multinomial logistic model that models the log-odds of adjacent frailty statuses in terms of an indicator of metformin use, time, and the metformin use by time interaction. The possibility of non-linear trends of the log-odds of frailty statuses will be assessed by including an additional time factor (e.g., time squared) and its interaction with metformin use. The best fitting model will be used for inference. Secondary outcomes including gait speed, 6 minute walk, SPPB, frailty index constructed from the research data collected according to the Rockwood index (Searle et al., 2008; Rockwood et al., 2007), and body composition will similarly assessed. Under the linear trend model, the beneficial impact of metformin will be revealed by a significant time by treatment interaction associated with the log-odds of frailty status progression, indicating that the rate of increase in the likelihood of frailty progression over time is reduced in the metformin arm relative to the placebo arm. Under the non-linear trend model, the impact of metformin will be revealed by a significant reduction in the log-odds of frailty status progression in the metformin arm versus placebo at certain time points during the follow-up. SAS proc **nlmixed** will be used in the mixed effects model analyses. Proc **nlmixed** is preferred over other mixed effects modeling procedures such as GEE because it is valid under missing at random (MAR) assumption, accommodates linear and non-linear modelling, and uses maximum likelihood estimation.

Of secondary interest, a per protocol analysis will be carried out with restriction to patients who follow the protocol throughout the study, and where those who drop out, violate the protocol or change treatments are excluded. Separately, those who change treatments will be summarized and described. To account for any treatment change, we will also consider principal stratification analysis (which requires categorization of patients based on treatment change patterns) and marginal structural modeling (which obtains the overall metformin effect accounting for treatment change, and time-varying covariates (e.g., diabetes status), and outcomes (e.g. frailty, physical function) history.

Metformin dose will be handled in the statistical analysis by conducting dose-response analysis among metformin users. Several options of metformin dose/exposure variables will be considered in separate analyses: average daily dose, clinical meaningful threshold for average daily dose, total days of supply, and clinical meaningful threshold for total days of supply. Should there be any discrepancy among these dose-response analyses, we will reconcile these results for the final interpretation.

3.D.3. Power Calculation for Aim 1. With 60 completers per arm, this study will achieve a power of 86% to detect an overall odds ratio of 0.20 for a one ordinal category increase in the frailty score in metformin versus placebo based on SAS **proc nlmixed** analyses of 1000 simulated databases with 6 repeated measures per subject, all subjects at frailty score 0 or 1 at baseline, an increasing random pattern in the placebo group, a pattern of lower frailty scores with increasing time on study in the treated group, and 10% of subjects in each arm remaining at baseline levels throughout all subsequent visits, two-sided testing, and a significance level of 5%. [93, 94] At visits 1 through 6 Placebo subjects were assigned a 56% chance of increasing by one level, 22% staying the same, and 22% decreasing by one frailty level, and Treated subjects were assigned a 33% chance of increasing by one level, a 33% chance of staying the same, and a 33% chance of decreasing by one frailty level relative to the previous visit. With these parameter values, at baseline 50% of subjects were at frailty level 0 and 50% at level 1 in each arm and at visit 6 the distributions for Placebo were 0: 25%, 1: 22%, 2: 20%, 3: 18%, 4: 11%, 5: 4% on the average across the 1000 simulations. Distributions for Treated were 0: 50%, 1: 24%, 2: 14%, 3: 11%, 4: 0%, 5: 0% on the average across the 1000 simulations.

3.D.4. Aim 2. To address the question as to whether an anti-frailty effect of metformin is mediated by its insulin sensitizing and anti-inflammation effects, we will employ the unified mediation analysis framework by Vanderweele. Insulin sensitivity (Matsuda index derived from the OGTT) will be measured at baseline, 6, 12, 18 and 24 months, and 8 biomarkers of systemic inflammation (IL-6, CRP, TNFα, IL-1RA, TNFs R1 and 2, fibrinogen, and transferrin), insulin sensitivity (clamp) and multiple biomarkers of muscle inflammation and insulin signaling (NFκB and MAPK signaling, and mRNA levels of inflammatory cytokines) will be measured at baseline, 12 months, and 24 months. First, we will conduct separate repeated measures mixed effects linear model analyses to assess the effect of metformin treatment on each insulin sensitivity and inflammation measure. In each of these analyses, the outcome (or its normal transformation) will be modelled in terms of an indicator of metformin treatment, time, the treatment by time interaction, and covariates. The coefficient associated with the treatment by time interaction will inform the effect of metformin on the temporal change of each biomarker. We will then conduct repeated measures mixed effects multinomial logistic model to assess whether the odds of an increase in the frailty score is associated with the change in insulin sensitivity from baseline to 24 months (ΔIS), and change in inflammation from baseline to 24 months (ΔI) by modelling the log-odds of frailty worsening in terms of metformin treatment, time, ΔIS, ΔI, (metformin) treatment×time, and covariates. The treatment×time×ΔIS and treatment×time×ΔI interactions will be explored to assess whether metformin attenuates the effect of ΔIS and ΔI: this effect will be revealed by a significant coefficient associated with treatment×time×ΔIS or treatment×time×ΔI. If some ΔIS and ΔI measures are collinear, then we will model the odds of frailty worsening in terms of ΔIS and ΔI separately. All of these analyses will be initially carried out for a pair of an inflammation biomarker and an insulin sensitivity marker separately. Aim 2 will be verified in principle if (i) metformin is associated with significant increase in ΔIS and decrease in ΔI; and (ii) frailty worsening is

associated with significant decrease in ΔIS and increase in ΔI; and (iii) the indirect effects of metformin (mediated by ΔI and ΔIS) calculated based on Vanderweele are significant.

3.D.5. Power Calculation for Aim 2. Inflammation. A 3 month clinical trial of insulin sensitizers versus placebo in patients with impaired glucose fasting glucose or diabetes found decreased TNFα in treated patients (effect size =1.5) [96]. Assuming the same or larger decrease in TNFα, 60 completers in each group, 6 repeated measures, autocorrelation 0.5, and overall type 1 error of 0.05, we expect to have power ≥95% to detect a group difference with regard to mean TNFα and power ≥95% power to detect an increase in TNFα and frailty worsening in the combined cohort [PASS Version 11, NCSS Kaysville UT 2011]. Insulin sensitivity. The Diabetes Prevention Program demonstrated improved fasting glucose in metformin treated adults (decrease of 4.1 mg/dL) with pre-diabetes and increased fasting glucose (by 0.4 mg/dL) in placebo group [97]. Assuming the same treatment effect on fasting glucose in this study, and n=60 completers per arm, this study will attain a power of 80% for testing the null hypotheses of no treatment effect, two sided testing, 6 repeated measures, and an autocorrelation of 0.5 [PASS Version 11, NCSS Kaysville UT 2011].

3.D.6. Time to major adverse event. The relation between treatment and time to major adverse outcome will be studied with and without adjustment for covariates and will be described with Kaplan-Meier curves. An adverse outcome will be defined as a) 2 point increase in the frailty score, or b) fall resulting in bone fracture or other serious injury, c) admission to a nursing home or skilled care facility, or d) death. The time to adverse outcome will be defined as the time to first occurrence of any of a), b), c) or d) and will otherwise be considered censored at loss to follow-up or end of study. Without adjustment, treatment groups will be contrasted on time to major adverse outcome with a logrank test and the treatment-specific accumulated incidence of adverse outcome will be described with Kaplan-Meier curves and 95% confidence bands. An adjusted contrast of treatment groups will be carried out with a Cox proportional hazards model in terms of treatment group, age, ethnicity, gender, and baseline values of frailty score. OGTT and inflammatory biomarkers will be entered as time-dependent covariates. Logrank testing, Kaplan-Meier estimates of survival distributions, and covariate adjusted Cox modelling of relative risks accommodate non-informative right censored survival data.

3.D.7. Missing Data. Limited missingness in outcomes and associated covariates is possible due to loss to follow-up. Missing covariates without loss to follow-up may occur due to missed appointments and intercurrent illness. In the primary analyses, model estimates will be derived based on the likelihood of observed data, an approach that gives consistent estimates under the MAR assumption: “missingness” depending upon the observed data but not the unobserved. To further address the possibility of missing data under the MAR assumption, we will conduct all analyses without and with multiple imputation using SAS **proc mianalyze** and its companion procedure **proc mi.** The **mianalyze** procedure combines the results of the analyses of imputations and generates valid statistical inferences and MI creates multiply imputed data sets for incomplete multivariate data.

3.D.8. Randomization. Participants will be randomized to metformin or placebo in a balanced design.

3.3.9. Blinding. This study is double blinded. Only the study pharmacist has knowledge of the medication each participant is taking. As per the requirement by the NIA-appointed DSMB, one study statistician has been and will remain unblinded. The unblinded study statistician will prepare DSMB reports as per the guidelines provided from the National Institute on Aging (https://www.nia.nih.gov/research/dgcg/clinical-research-study-investigators-toolbox/data-and-safety-monitoring). Any unblinded report will be reviewed during a closed DSMB session with only the unblinded statistician present and without any study investigators present.

Item 7 Risks Section: Complete the following table to describe the risks of all **research procedures** listed in Step 2, Institutional Form (items 28-34). *Do not list risks of Routine care procedures here.* ☒ N/A, Risks are described in the informed consent document – do not complete this table.	
Research procedures *example:* • History and physical • Questionnaire • Laboratory tests *Add or delete rows as needed*	**Risks** List the reasonably expected risks under the following categories as appropriate:

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