Study Protocol: Metformin and Muscle in Insulin-Resistant Older Veterans
NCT01804049
PI: Dr Robert Klein
Document Date: 08-16-2016

Portland VA Medical Center
Oregon Health & Science University
RESEARCH PROTOCOL

Protocol Title:
Metformin and Muscle in Insulin-Resistant Older Veterans

Principal Investigator:
Robert Klein, MD

Sponsor:
VA Clinical Sciences Research & Development (CSRD)

Specific Aims:
To determine if metformin can decrease the loss of muscle mass and physical performance in insulin-resistant older adults and to investigate the mechanistic pathways involved.

Background and Significance:

Significance

The proposed clinical trial may identify potential new targets for the prevention and treatment of sarcopenia. Sarcopenia is present in up to 24% of adults between the ages of 65 and 70 years and over 50% of adults over the age of 80 in the United States.[1] It is associated with the clinical consequences of lower physical performance, decreased strength, impaired activities of daily living, greater disability, and more falls and fractures.[1-3] As a result, the healthcare costs of sarcopenia were estimated at $18.5 billion for the US population in 2000.[4] With adverse health, lifestyle and economic implications associated with sarcopenia, discovery of therapies to prevent or treat sarcopenia are important, given the growing number of veterans over the age of 65.

Our preliminary data demonstrate a greater likelihood for muscle loss for older insulin-resistant men that may be attenuated with the use of metformin. These results strongly suggest that metformin has beneficial effects on muscle, and that agents acting like metformin may provide a new approach for the prevention or treatment of sarcopenia. To more definitively examine these effects, we will enroll older, insulin-resistant adults into a randomized clinical trial to determine whether metformin can slow the decline in muscle mass and physical performance. Furthermore, histologic measures from muscle biopsies in this clinical study will be used to understand the mechanism by which metformin may prevent sarcopenia. If the clinical trial supports our preliminary data, it will provide an exciting opportunity to develop new approaches to prevent or treat sarcopenia. This research is novel for its combined use of epidemiologic, clinical and translational approaches to understand the loss of muscle mass and function and for its investigation of potential mechanisms for the prevention or therapy of sarcopenia.

Given the poor understanding of mechanisms for sarcopenia and lack of therapies for treating or preventing sarcopenia, there is a great need to train clinician investigators to become sarcopenia researchers. The proposed training plan builds on my background in endocrinology, metabolism, diabetes and epidemiology to add additional experience in clinical trials and translational research skills for studying muscle. The training proposed in this application will give me the ability to conduct epidemiologic, clinical and translational studies to understand sarcopenia in older veterans, thus allowing me to fill a much needed role for the field of sarcopenia research.

Background

Relevance of Sarcopenia to Disability in U.S. veterans

Older U.S. veterans have a high prevalence of poor physical health and functioning.[5, 6] Over half of U.S. veterans reported difficulty with one or more activities of daily living (ADL) and instrumental activities of daily living (IADL).[7, 8] The Vietnam War cohort surveyed in 2001 had equal or higher rates of ADL and IADL disability compared to WWII veterans despite being younger.[9] As the Vietnam veteran cohort enters older age, prevention of sarcopenia will be needed to help keep them independent and functional.

Sarcopenia, defined as low appendicular lean mass, exists in up to 24% of adults under 70 years old and over 50% in those over the age of 80 and is associated with self-reported disability.[1] However, objective measures of physical performance have also been shown to predict incident disability.[10-12] While low muscle mass is associated with disability and functional decline, it cannot completely explain the greater loss in strength or function that occurs in older adults.[13, 14] Moreover, therapies that improve muscle mass may not necessarily improve muscle strength or function, and thus have limited clinical relevance.[15] Future studies on the prevention of disability must consider the both prevention of sarcopenia and the loss of physical performance.

Population at Risk for Sarcopenia

The prevalence of impaired fasting glucose, impaired glucose tolerance, and diabetes increases with aging.[16] Within the Veterans Affairs system, diabetes was reported as the third most common diagnosis and was more common in adults over the age of 65 years.[17, 18] The presence of insulin resistance is associated with an increased risk of sarcopenia development.[19] Older adults with type 2 diabetes have a greater loss in muscle mass and function than adults without diabetes.[20, 21] After exclusion of diabetics, studies still show that insulin resistance is associated with lower muscle strength and increased risk of frailty for older adults.[22, 23]

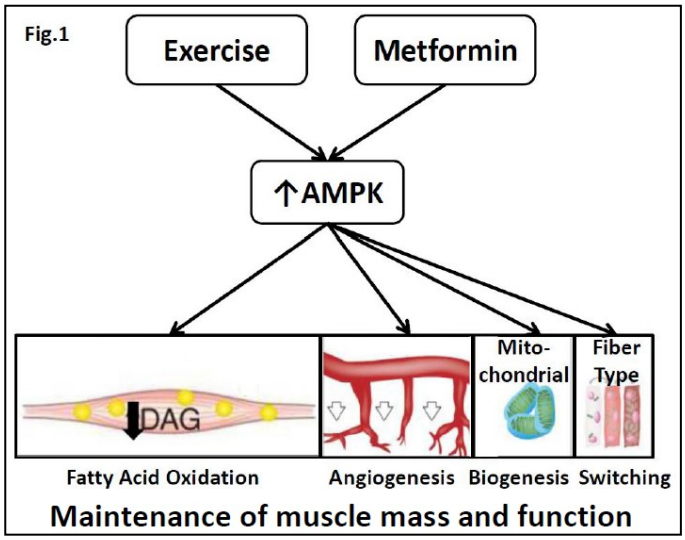
Treatments for Sarcopenia

To date, interventional studies have examined the use of testosterone, growth hormone and exercise to treat sarcopenia, with only exercise training demonstrating an increase in both muscle mass and strength for older adults without adverse effects.[15, 24] Our preliminary data described below suggest that metformin may prevent the loss of lean mass in insulin-resistant, older men.[25] However, there have been no interventional studies to determine the effects of metformin on preventing the loss in muscle mass and muscle function.

Potential Mechanism for Sarcopenia Prevention with Metformin

The skeletal muscle of aging adults differs from young adults with lower 5' adenosine monophosphate-activated protein kinase (AMPK) activity, a selective decrease in type II muscle fibers, accumulation of intramyocellular lipid, lower capillary-to-muscle fiber ratio, and fewer mitochondria.[26-30] Both metformin and exercise can stimulate the phosphorylation of AMPK, an enzyme that can regulate the transcription of genes responsible for switching muscle fiber types from glycolytic to more oxidative, fatigue-resistant fibers, lowering muscle lipid accumulation, stimulating angiogenesis and increasing mitochondrial biogenesis (Fig 1).[31-34]

Aerobic exercise can maintain or improve muscle mass and function in older adults.[35] Histologic changes in muscle accompanying exercise training in older adults include decreased diacylglycerol and ceramide content, increased capillary density, increased mitochondrial content and activity and a switch from type IIB to more oxidative IIA fibers.[36-38] Similar studies to evaluate the effects of metformin on muscle mass, function and histology have not yet been performed in older adults.



However, animal studies suggest that pharmacologic activation of AMPK results in histologic changes in muscle similar to exercise. Treatment of animals with metformin or another AMPK agonist, 5-

aminoimidazole-4-carboxamide ribofuranoside (AICAR), increased fatty acid oxidation and decreased diacylglycerol and ceramide content in skeletal muscle.[39-43] Increased expression of vascular endothelial growth factor and skeletal muscle angiogenesis also occur in mice treated with AICAR.[44, 45] Increased activity of mitochondrial enzymes and expression of mitochondrial protein in muscle with AICAR and increased mitochondrial density with another AMPK activator, β-guanadinopropionic acid, may be mediated through downstream activation of peroxisome proliferator-activated receptor-gamma coactivator-1alpha (PGC1-α).[34, 46-49] AICAR-treated rats appeared to have a switch from type IIB fibers to more oxidative type IIX fibers similar to endurance trained rats.[50] While AICAR and metformin in animals can lead to the histologic changes in muscle in our proposed mechanistic framework (Fig. 1), the effect of metformin on human muscle composition remains to be confirmed.

AMPK activation in basic studies also supports an improvement in muscle mass and function. While AMPK is thought to increase atrophy through inhibition of mTOR from cell-based studies,[51, 52] in vivo studies instead demonstrate increased muscle fiber area and weight with AICAR[53] and increased protein synthesis with metformin.[54] Furthermore, the treatment of sedentary mice with AICAR improved endurance and extended running distance.[55] In contrast, AMPKβ2 knockout mice have decreased muscle fiber size, decreased exercise capacity and endurance.[56]

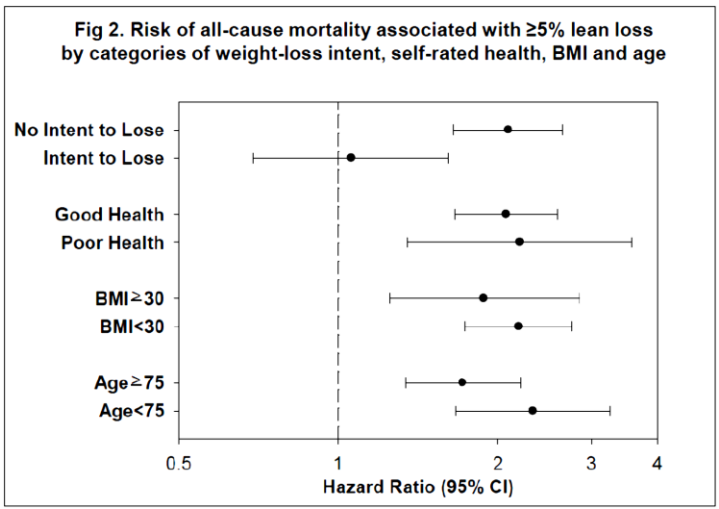
These studies suggest the potential for metformin to positively affect muscle, and provide a compelling rationale for the positive associations between metformin use and muscle mass and function that we describe in our preliminary studies below. As an AMPK activator, metformin is also expected to result in histologic changes for decreasing intramyocellular lipid and increasing capillary density, mitochondrial density and oxidative fibers. If our proposed study provides evidence to support this mechanistic framework, future studies of signaling intermediates for these histologic changes will be pursued with stored tissue specimens. Therefore, our proposed study of metformin treatment in insulin-resistant older adults will provide further insight into its mechanisms for preventing sarcopenia and to identify other therapeutic targets for this condition.

Preliminary Studies / Progress Report:

The Osteoporotic Fractures in Men (MrOS) Study is a prospective cohort study of 5994 ambulatory, community-dwelling men aged 65 and older (mean age 73.7 years, range 64-100) who were enrolled initially March 2000-April 2002 from six U.S. clinical sites (Portland, OR; Palo Alto, CA; San Diego, CA; Minneapolis, MN; Birmingham, AL; and Pittsburgh, PA) for the purpose of studying osteoporotic fracture determinants.[57] Participants returned for follow-up visits in 2005-2006 and 2007-2009 and continue to participate in ongoing follow-up for multiple health outcomes and assessment of vital status. The Study of Osteoporotic Fractures (SOF) is a prospective cohort of 9,704 Caucasian women aged 65 years and older who were enrolled between 1966-1988 from Portland, OR; Minneapolis, MN; Baltimore, MD; and the Monongahela Valley near Pittsburgh, PA for the study of osteoporosis and fractures.[58] They were studied at baseline and returned every 2 years for a clinic visit. Information collected in both of these cohort studies includes dual x-ray absorptiometry scans (DXA) for body composition measurements (in MrOS), biochemical measurements, neuromuscular function, and questionnaires for medical problems, medications, demographic information, and lifestyle factors. These are unique and rich resources for studying longitudinal changes in older men and women. From these studies, the following preliminary data have been generated.

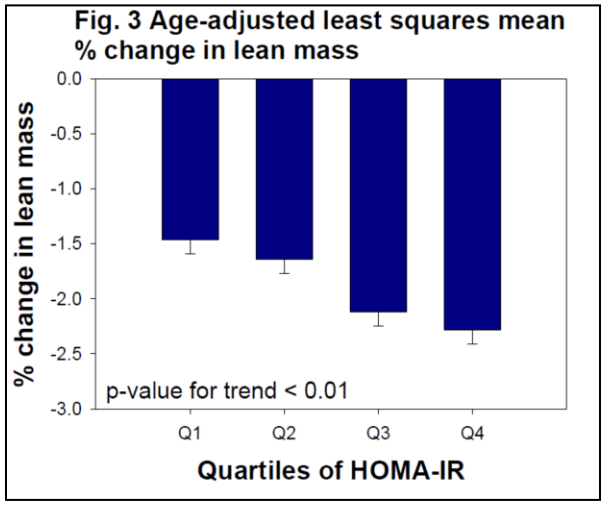
Lean mass loss is associated with increased risk of mortality

To examine the mortality risks of sarcopenia in older men, we used data from participants in the MrOS study who had DXA-based measurements of body composition on average 4.6 (range: 3.5-5.9) years apart. Older men with ≥5% lean mass loss had a 1.8-fold increased risk for all-cause mortality compared to men who maintained lean



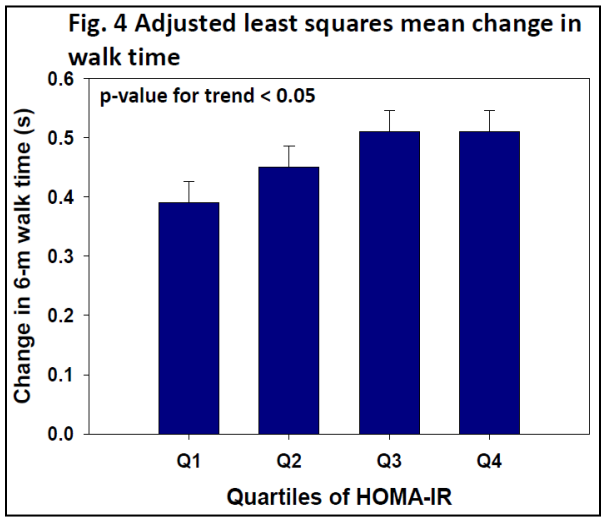
mass. This risk remained significantly elevated even after accounting for baseline lifestyle factors and medical conditions and did not differ by baseline age, BMI or self-rated health (interaction p-value<0.10) (Fig. 2). However, the risk of all-cause mortality associated with ≥5% total body lean mass loss was higher for men who lost weight unintentionally (HR 2.09, 95% CI 1.65, 2.65) compared to men who lost weight intentionally in whom there was no increase in mortality risk (HR 1.06, 95% CI 0.69, 1.62). Overall these findings indicated that involuntary lean mass loss doubled the risk of all-cause mortality in older men. While other studies have shown increased morbidities with sarcopenia, this study showed that there is also an increased risk of mortality. These results were presented and honored with a Presidential Poster Award at the 2010 Annual American Geriatrics Society Meeting and were published in the Journal of the American Geriatrics Society.[59]

Insulin resistance is associated with lean mass loss



Baseline insulin resistance was predictive of future loss in lean mass in older, non-diabetic men. Insulin resistance was measured by the homeostasis model assessment of insulin resistance (HOMA-IR) in non-diabetic men in MrOS at baseline. There was a significant trend towards greater % loss in lean mass with increasing insulin resistance (Fig. 3). This association was unaltered by further adjustment for baseline physical activity and changes in physical activity during this period of time. The loss of lean mass associated with greater insulin resistance also existed independent of other metabolic co-morbidities like hypertension, body weight and dyslipidemia. These data were presented at the Gerontology Society of America meeting in November 2010 and a manuscript describing these results was published in the Journal of the American Geriatrics Society.[60]

Insulin resistance is associated with increasing 6-meter walk time



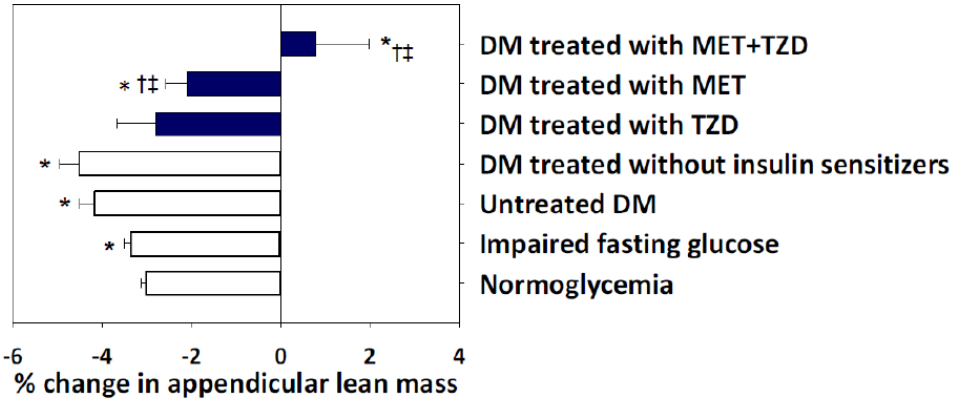
The associations between baseline insulin resistance and the longitudinal change in 6-meter walk time was also examined in 3256 MrOS participants without diabetes who had these measurements taken 4.7 years apart. There was a significant trend towards increased 6-meter walk time ($p<0.05$) with greater insulin resistance as measured by the homeostasis model assessment of insulin resistance (HOMA-IR) in models accounting for age, clinic site, self-rated health and baseline walk time, physical activity, hypertension and the change in lean mass (Fig. 4). Compared to the most insulin-sensitive men in the lowest quartile of HOMA-IR, insulin-resistant men in the highest two quartiles of HOMA-IR had a significantly greater increase in walk time in fully-adjusted models (p-values<0.05). Therefore, greater insulin resistance in older men without diabetes is also associated with an accelerated loss in gait speed (data not yet published).

Metformin use may attenuate lean mass loss for older men with diabetes

To further assess the association between insulin resistance and changes in lean mass, the loss in appendicular lean mass over 3.5 years was evaluated in older men who were grouped into the following categories of glycemic status at baseline: normoglycemia (n=1853), impaired fasting glucose (n=1403), untreated diabetes mellitus

(n=234), diabetes mellitus treated with insulin-sensitizers (n=151) and diabetes mellitus treated without insulin-sensitizers (n=111). Even after adjustment for baseline demographics, lifestyle factors and medical comorbidities, the loss in appendicular lean mass was significantly attenuated in diabetic men on insulin-sensitizers compared to men with normoglycemia, impaired fasting glucose, untreated diabetes mellitus or diabetic men not on insulin-sensitizers, $p<0.001$ for all comparisons (Fig. 4). Among diabetic men on insulin-sensitizers, metformin use was associated with significantly less appendicular lean mass loss (-0.41 Kg) compared to men with normoglycemia (-0.75 Kg), $p<0.01$; whereas the amount of appendicular lean mass loss for men on thiazolidinediones (-0.65 Kg) did not differ significantly from men with normoglycemia, $p=0.67$. These data suggest that use of insulin-sensitizers, particularly metformin, may attenuate the loss of appendicular lean mass for older men with diabetes. Additional sensitivity analyses were performed, and these results were unchanged even after exclusion of 42 men on insulin. These findings have recently been published in Diabetes Care.[25]

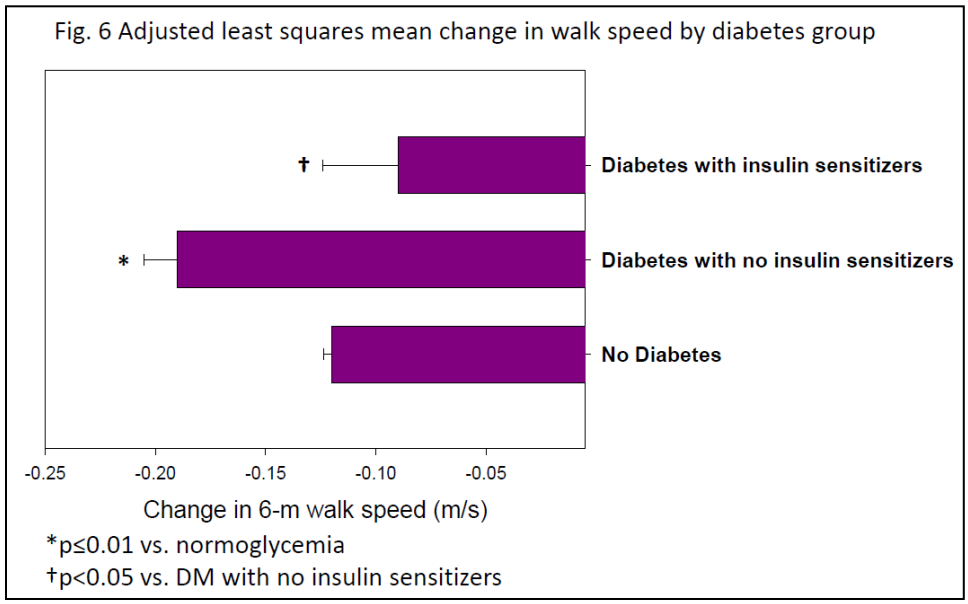
Fig. 5



***p≤0.05 vs normoglycemia**
†p≤0.01 vs Untreated DM, ‡p≤0.01 vs DM treated without insulin sensitizers
Models were adjusted for age, race, clinic site, smoking status, education, alcohol use, physical activity, baseline total fat mass, baseline total lean mass, self-rated health, cardiac disease, hypertension, dyslipidemia, ACE inhibitor and β-blocker use

Metformin may attenuate the loss in usual gait speed for older women with diabetes

We hypothesized that insulin sensitizer (metformin/thiazolidinediones) use would also be associated with attenuated loss in physical performance in older diabetic women. In the Study of Osteoporotic Fractures, a multi-center, longitudinal cohort study, 2826 women aged ≥70 years had physical performance measures assessed between 1997-1998 and 4.9±0.6 years later. Least squares mean change in 6-meter walk speed was estimated in multivariate linear regression models for women with no diabetes (n=2711), diabetes mellitus with no insulin-sensitizers (n=162) and diabetes mellitus with insulin-sensitizers (n=30). After accounting for baseline age, race, BMI, education, self-rated health, hypertension, walk speed, and use of estrogen and statins, the decline in walk speed was significantly greater in women with diabetes who were not using insulin-sensitizers (-0.18 m/s, 95% CI -0.21, -0.15) than in women with no diabetes (-0.12 m/s, 95% CI -0.12, -0.11); whereas the loss in gait speed for women with diabetes using insulin-sensitizers (-0.09 m/s, 95% CI -0.17, -0.04) did not differ to the loss seen in women with no diabetes. These results were unchanged after excluding women using insulin. These findings also remained unchanged after excluding 2 women on thiazolidinediones, suggesting that metformin use in older diabetic women may attenuate the loss in walk speed (data not yet published).



Summary

The longitudinal data from MrOS and SOF have helped to establish that insulin-resistant, older adults experience a greater loss in lean mass and physical performance. However, older adults with diabetes who were treated with metformin had a significant attenuation in the loss of lean mass and walk speed. These preliminary data provide the rationale for determining whether metformin can be used to prevent the loss of muscle mass and function. Although these observational results are compelling, validation with a randomized controlled trial is essential. Furthermore, a carefully performed trial will provide critical data about the mechanistic effects of metformin on muscle.

Research Design and Methods

Subjects and Experimental Design:

Overview

Older adults with impaired fasting glucose (IFG) will be randomized to into a double-blinded, placebo-controlled intervention study with metformin. This clinical trial has the primary outcome of change in appendicular lean mass and physical performance assessed between baseline and year 3 based on our prior observational findings above and Power calculations below. The secondary outcome of change in muscle histology over 6 months using muscle biopsies is planned in a substudy of participants given previous studies that show significant biological effects on muscle with metformin treatment during this time interval.[32, 61]

Study Population

Inclusion criteria: 120 randomized, sedentary, weight-stable, ambulatory men and women 65 years or older with pre-diabetes identified with IFG (100 mg/dL≤fasting glucose<126 mg/dL) on no diabetes medications. Participants must demonstrate that they are able to ambulate 400 meters without assistance. Participants must be able to fast for 12 hours prior to blood draw.

Subjects who meet the above inclusion criteria and meet the additional exclusion criteria for the Muscle Biopsy Substudy listed below will be considered for enrollment (32 subjects) in the muscle biopsy substudy.

Exclusion criteria: Participants will be excluded if they plan to move away from the Portland metropolitan area during the 3 year study time frame. Participants with conditions that are relative contraindications to metformin or affect muscle mass or performance measurements will be excluded as outlined below. Specific criteria are delineated below.

Exclusion Criteria

1. Chronic medical conditions affecting muscle mass or function
    a. Active non-skin cancer
    b. Hypogonadism
2. Medications affecting muscle mass or function – See section "Prohibited Medications."
3. Lifestyle factors affecting muscle
    a. Intentional/unintentional weight loss
    b. Vigorous regular exercise
4. Contraindications to metformin
    a. Renal dysfunction defined as creatinine ≥ 1.5 mg/dL for men or ≥ 1.4 mg/dL for women or eGFR< 60 mL/min
    b. Liver dysfunction defined as ALT > 48 U/L, AST > 41 U/L
    c. B12 deficiency defined as B12 level < 180 pg/mL
    d. Congestive heart failure
    e. Known hypersensitivity to metformin
    f. Excessive alcohol intake (average of ≥ 2 alcoholic beverages/day over a month)
    g. Serious medical condition in the opinion of the investigator

Additional Exclusion Criteria for Muscle Biopsy Substudy

1. Conditions that increase bleeding risk
    a. Platelet count < 150 billion/L
    b. INR > 1.2
    c. aPTT > 36 seconds
2. Medications that increase bleeding risk – See section "Prohibited Medications."
3. Allergy to lidocaine

Prohibited Medications:

1. Medications affecting muscle mass or function
    a. Glucocorticoids
    b. Androgen/antiandrogens
2. Medications that increase bleeding risk (for muscle biopsy substudy)
    a. Warfarin
    b. Clopidogrel/ticlopidine
    c. Aggrenox
    d. Dabigatran
    e. Anagrelide

<u>Participant recruitment</u>

We will plan to screen up to 3,000 older adults (including phone screens) from VA primary care clinics (Portland, Vancouver, Metro East, Metro West, and West Linn) to account for those who may not qualify due to the presence of exclusion criteria. We plan to enroll up to 300 participants in order to randomize 120 subjects at the VA Portland Health Care System (VAPORHCS) since we anticipate many participants to screen out of the study based on lab values after having signed a consent form. Advertisement flyers will be posted on approved bulletin areas on the VAPORHCS. VA primary care physicians may refer any interested and potentially eligible subjects to the study team. Mailing recruitment will also occur, using primary care and endocrine patient listings of those

who meet the age criteria, medication and medical history exclusions, and are not diagnosed with diabetes.

The following criteria will be applied to the VISN 20 (Veterans Integrated Services Network) data warehouse to identify potential participants for recruiting mailing: 1) patients age 65 years and greater seen in VA primary care clinics (Portland, Vancouver, Metro East, Metro West and West Linn) in the past year; 2) ICD-9 codes will be used to exclude patients with diabetes and other exclusion diagnoses; and 3) patients will be excluded if they have received one or more prescriptions for diabetes medications or contraindicated medications in the past year. We will adhere to the VAPORHCS policy on recruitment via letters. As we indicated in the Application for a Waiver of Authorization and Informed Consent Process, we will use CPRS to collect names, mailing addresses, birthdates, telephone numbers and social security numbers.

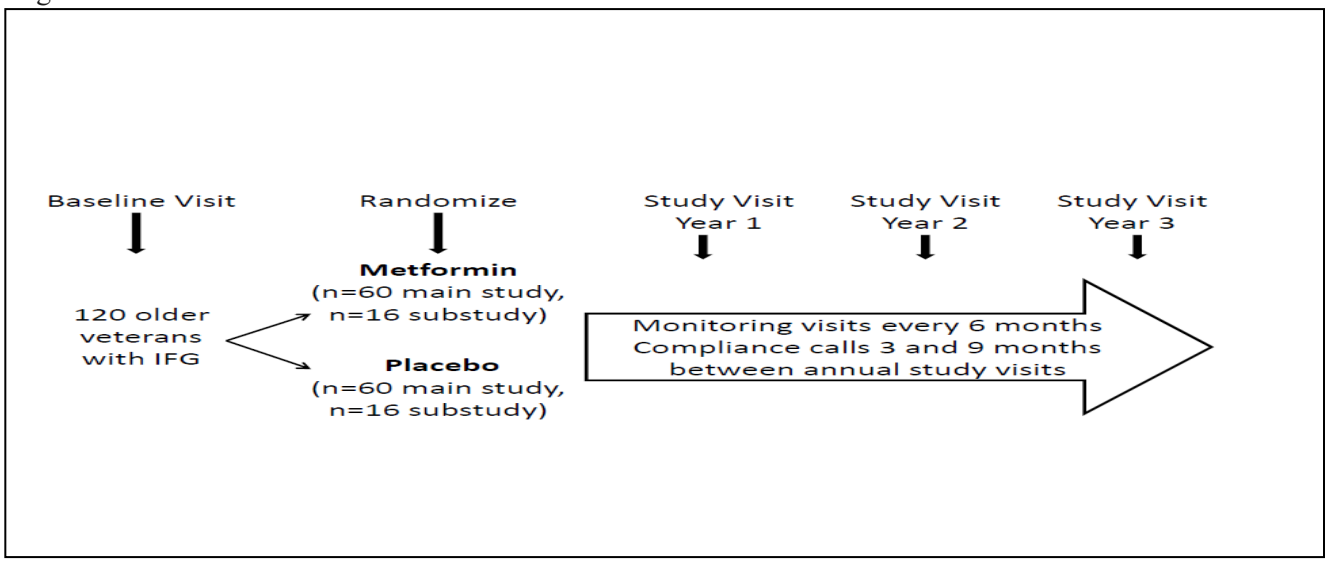
Initial contact with potential subjects will occur in clinic, by phone or by mail. If in clinic, their provider will approach them first to see if they are interested in the study and ask if they agree to be contacted by study personnel. If the patient agrees, they can initiate contact with the study team by phone or have the study coordinator speak to them in clinic or call them later. If initial contact is by phone, potential subjects can contact the study coordinator directly using information on the advertising flyers. If initial contact is by mail, potential subjects will receive a recruitment letter indicating the option to opt out by returning an included letter indicating the wish to not be contacted again. The letter will state that if a response is not received within two weeks, potential subjects may be contacted by phone. Included with this mailing will be an approved informational letter from the Chief of Primary Care.

During the screening telephone call, a study coordinator will discuss the inclusion and exclusion criteria and provide further details on the study. If participants are interested and are potentially eligible, a Screening visit will be scheduled.

Study design

The proposed study duration of 3 years has been selected based on our prior observational findings over 3.5 years.[25] Eligible participants will have an initial Baseline visit, followed by randomization to placebo or metformin (Fig. 7). Between visits, telephone surveying of medication compliance will be obtained and any AEs reviewed. Participants will be seen every 6 months, and have annual study visits for measurements of muscle mass and performance. A substudy of participants will also include muscle biopsies at 2 visits; within 14 days from baseline and at the 6 month visit.

Fig. 7



Primary outcome measures

To test the primary hypothesis that metformin-treated participants will have a slower decline in muscle mass and physical performance than those on placebo, we will be measuring total and appendicular lean mass using dual x-ray absorptiometry and assessing physical performance with the short physical performance battery (SPPB), grip strength and a 400 meter walk. Our preliminary findings suggest that there should be attenuation in the loss in appendicular lean mass and gait speed, and additional performance measures will be explored.

Secondary outcome measures

The secondary hypothesis is that the muscle composition of metformin-treated participants will have a greater increase in number of oxidative fibers, decrease in intramyocellular lipid, increase in capillary density and increase in the number of mitochondria than placebo-treated participants. These outcomes have been chosen based on our hypothetical framework in Figure 1 that was developed from known alterations in these measures in animal models treated with metformin or other AMPK agonists. Quantification of intramyocellular lipid, fiber types, capillary density and mitochondrial content will be obtained through histochemical analyses of muscle biopsy specimens as described in the Muscle Biopsy section.

Study Visits
All visits will be completed at VAPORHCS and/or at OHSU.

Screening Visit

IRB-approved study personnel will have a full discussion with the potential subject, including information on study visits, procedures, risks, and benefits. All participants will be informed of their right to withdraw from the study at any time. The principal investigator will answer any questions from participants at any time. Participants will provide written informed consent prior to any study procedures being performed. A copy of the IRB-approved informed consent and signed HIPAA authorization form will be given to the subject. Demographic information (including age, gender, and race) and lifestyle factors (including physical activity, smoking history and alcohol intake) will be obtained. Medical history (including self-reported physician medical diagnoses), drug allergies/adverse effects and medications will be obtained. Height, weight and blood pressure (BP) will be measured. Criteria for exclusion criteria will be formally reviewed. Participants must demonstrate that they are able to ambulate 400 meters without assistance. Laboratory assessment for B12 levels and liver, kidney and clotting function will be performed. The total amount of blood drawn will be 10 ml. Labs will be drawn for fasting glucose to identify participants with IFG. All labs will be ordered after consent is obtained. Participants will be instructed to remain fasting for 12 hours prior to the study day. This visit could take up to 2 hours. Participants who have IFG will be scheduled to attend a Baseline visit if the Screening visit does not reveal presence of an exclusion criterion.

Baseline Visit

The Baseline visit will occur in 14 days (+/- 7 days) from Screening. Participants will be instructed to remain fasting for 12 hours prior to the study day. This visit could take up to 3 hours. The study measures will be conducted by the IRB-approved study team who are all blinded to treatment allocation. The participants will first get a fasting blood draw at VAPORHCS. All study subjects will be provided breakfast after their fasting lab draw has been done. A fasting glucose level will be measured. Subjects will proceed with the following study measures: review and completion of questionnaires, medication inventory, physical performance tests, and whole body DXA. For those subjects who signed the consent indicating their interest in participating in the muscle biopsy substudy and are eligible, they will be scheduled for a Baseline muscle biopsy visit within 14 days of their

baseline visit.

Because adults with IFG have a higher risk of developing type 2 diabetes, all study participants will receive guidelines for diet and lifestyle modification consistent with the goals of the Diabetes Prevention Program at baseline and yearly visits.[72] These include intake of < 25% of calories as fat and engaging in ≥150 minutes of physical activity weekly.

Baseline Muscle Biopsy Visit (if in substudy)

Participants enrolled in the muscle biopsy substudy will receive a telephone call within 3 days prior to their biopsy visits to remind them to fast for 12 hours prior to the visit, to remind them to abstain from vigorous physical activity, and to ensure they are not on anticoagulant or antiplatelet medications within 7 days from the biopsy visit with the exception of aspirin or other anti-inflammatory medications which cannot be taken within 3 days of the biopsy visit.

This visit will occur within 14 days from Baseline, and it could take up to 3 hours. It will be completed at OHSU. Eligibility criteria for the muscle biopsy will again be reviewed, and if there are no contraindications, the participant will undergo muscle biopsy. Participants will be given breakfast after muscle biopsy has been obtained.

Phone calls between visits
At 3 and 9 month time points between annual visits, telephone surveying of medication compliance will be obtained. Any adverse events (AE) will be reviewed. A 24-hour phone number will be provided to participants to call in the event of any adverse outcome between these contact points. Additional phone calls may be made if subjects are found to be non-compliant.

All study participants will receive a telephone call to remind them to fast for 12 hours prior to their study visit.

Participants enrolled in the muscle biopsy substudy will receive a telephone call within 3 days prior to their biopsy visits to remind them to fast for 12 hours prior to the visit, to remind them to abstain from vigorous physical activity, and to ensure they are not on anticoagulant or antiplatelet medications within 7 days from the biopsy visit.

Half-Yearly Visits (6 months; Year 1 +6 months; Year 2 +6 months)

Half-yearly visits will occur at 6 Months, Year 1 + 6 Months, and Year 2 + 6 Months. All subjects will go to VAPORHCS for these visits. For those in the substudy, only for the 6 Month visit, they will also go to OHSU after labwork is drawn at VAPORHCS.

This visit could take up to 2 hours for participants (3 hours for participants in the muscle biopsy substudy at the 6 Month visit). At each half-yearly visit, all participants will have fasting labwork drawn, and have questionnaires reviewed and completed. Subjects >80 years old will get labs checked for creatinine and eGFR. Participants in the muscle biopsy substudy will undergo muscle biopsy if their morning STAT labs demonstrate platelet, INR, and aPTT levels in the normal range. Participants will be given breakfast after fasting lab work/muscle biopsy has been obtained.

Annual Visits (Years 1, 2, and 3)

These annual visits could take up to 3 hours. The participants will first get a fasting blood draw. They will then be given breakfast before proceeding with the following study measures: review and completion of questionnaires, medication inventory, physical performance tests, and whole body DXA.

Study Drug

We have chosen to study metformin in this clinical trial due to the observation that metformin use was associated with an attenuated loss of muscle mass and function in our preliminary studies. While both metformin and thiazolidinediones can upregulate AMPK and PGC1α in muscle to increase muscle fatty acid oxidation, angiogenesis, mitochondrial biogenesis and oxidative muscle fibers for healthier-appearing muscle,[32, 34, 68] metformin has additional benefits on mortality, microvascular and macrovascular outcomes in patients with type 2 diabetes;[69] whereas, thiazolidinediones have associated risks of adverse cardiovascular outcomes.[70, 71]

The VA Research Pharmacy will be used to randomize, dispense, and track blinded placebo and metformin tablets in 3-month supplies. A covariate-adaptive randomization scheme will be used to ensure equal age, gender and race distributions. The investigators and study participants will be blinded to the randomization of the study drug assignments. A 90-day supply of study drug will be mailed every 3 months to participants with a pre-paid envelope for returning unused study drugs for pill counts. An additional 4-day buffer may be included to avoid lapses in study drug administration due to unforeseen complications in medication delivery and/or receipt. To minimize self-limiting gastrointestinal side effects, participants randomized to metformin will begin with an oral dose of 850 mg tablets once daily (taken with meals) for 1 month with titration up to 850 mg twice daily for the remainder of the study. We anticipate an effect on muscle using this dosage, since a significant reduction in peripheral insulin resistance occurs even with a metformin daily dosage of 750 mg.[61] Furthermore, this dose was safe and effective in lowering the risk of diabetes for subjects with prediabetes in the Diabetes Prevention Program.[75] Plans for monitoring and preventing risks associated with metformin are further discussed under Human Subjects.

Instances where the study drug is discontinued include the following: development of a contraindication to metformin as described under the section for exclusion criteria; development of lactic acidosis; diagnosis of diabetes; hospitalization; 48 hours prior to procedures or scans that require contrast medium; or development of B12 deficiency. For those who are hospitalized or receive contrast medium, efforts will be made to recheck kidney function within 48 hours after discharge/procedure and the study drug will be restarted if there is no evidence of renal dysfunction. For tests or procedures that require fasting, study drug will be stopped 24 hours prior to test or procedure, and study drug can be resumed after the participant is allowed to eat again. Compliance to study drug will be tracked and accounted for in analyses.

Study Procedures
All study procedures are research-related.

Exam: Exams for annual visits will occur at OHSU after fasting labwork has been drawn at VAPORHCS. At Screening, baseline and yearly exams, height will be measured in meters using a wall-mounted Harpenden stadiometer, body weight will be measured in kilograms using a balance beam scale, waist and hip circumference measured with a tape measure, and measurements of pulse and blood pressure will be taken.

Labs: The lab samples will be collected at VAPORHCS at the Screening (after consent is obtained), Baseline, Half-Yearly Visits and Annual Visits, and processed at VAPORHCS or OHSU. Fasting plasma will be drawn at Screening for ascertainment of IFG (100mg/dL≤fasting glucose<126mg/dL) and exclusion criteria: creatinine ≥ 1.5 mg/dL for men or ≥1.4 mg/dL for women, eGFR<60 mL/min, B12 <180 pg/mL, ALT>48 U/L, or AST > 41 U/L. Whole blood will be assayed for platelet counts, and platelet-poor plasma will be assayed for INR and aPTT. At Baseline, fasting plasma will just be assayed for glucose. At annual follow-up study visits, fasting plasma will be assayed for glucose, insulin, B12 (pg/mL), creatinine (mg/dL), alanine transaminase (U/L), aspartate aminotransferase (U/L), and estimated glomerular filtration rate (mL/min). Exclusion criteria for follow up labs will be identical with the following exception: The FDA guidelines requiring the safe use of metformin have recently changed and the eGFR cutoff as indicated by the FDA is now eGFR < 45 mL/min/1.73 m2 which we will use as our exclusion criteria for continuation of study drug. With the exception of insulin, the blood labs will be analyzed at VAPORHCS. Insulin will be analyzed at OHSU.

A total of 10 ml of blood will be drawn at the Screening Visit. At the Baseline visit, 30 mL of blood will be drawn. At the half-yearly visits, a total of 20 mL of blood will be drawn. At the Annual follow-up visits, 30 mL of blood will be drawn.

Any clinically significant abnormal laboratory findings at the scheduled end of the study should be followed up until satisfactory resolution or diagnosis can be made.

Biorepository

A separate biorepository protocol will be submitted to the IRB for approval. Additional samples of blood and muscle tissue (if in substudy) will be collected and stored for future analyses at the Baseline visit, muscle biopsy visit, half-yearly visits, and annual visits. These are described above in the Lab section and Muscle Biopsy section in this protocol below.

Questionnaires: Prior to all study visits from Baseline to Year 3/End of Study, questionnaires to assess lifestyle, diet, physical activity, physical function, fatigue and medical diagnoses will be mailed to participants in their visit reminder letter to be completed and returned on the study visit day. A #2 pencil will be included with this mailing in order for participants to fill out the scantron portion of the food questionnaire. The baseline questionnaire will also include demographic questions like marital status, living situation, education, employment and socioeconomic status. The follow-up questionnaires will include study drug compliance questions to assess adherence to study drug. Participants will have questionnaires reviewed at the study visit by the study coordinator for completeness and will finish any incomplete sections at the study visit. At 1, 3 and 9 month time points between study visits, adherence to study drugs will be surveyed over the telephone.[67]

Medication Use: Participants will be instructed to bring all prescription and over-the-counter medications to their study visits, and research staff will take an inventory of all medication names and doses. For the convenience of participants, a bag will be mailed along with the visit reminder letter that they may use to bring their medications to the visit. Participants will be given pre-paid envelopes to mail unused study drugs to the research pharmacy every 3 months for pill counts to track compliance.

Physical Performance Tests: Performance tests will occur at VAPORHCS and OHSU. They will be conducted at Screening, Baseline, and the annual visits. At Screening, subjects must demonstrate they are able to walk 400 meters. At Baseline and the annual visits, assessments of grip strength, a 400 meter walk, timed 6 meter walk, all of the short physical performance battery (SPPB) tests, and Instrumented Sway (ISway) test will be conducted. All performance tests will be instrumented with Mobility Lab sensors on bilateral upper and lower extremities, waist and chest using elastic straps. The ISway test consists of 3 trials where the participant stands for 30 seconds without moving with their arms at their side. Each trial will have measures of amplitude, velocity, frequency and jerkiness of postural sway in lateral and anterior-posterior directions with 42 metrics. The SPPB comprises tests of balance, timed walk and chair stands and is predictive of subsequent disability in older adults.[11] For tests of

balance, the ability and/or duration (s) in which a participant can maintain a tandem, semi-tandem and side-by-side stand will be recorded. Participants will be timed in the task of rising from a seated position 5 times in rapid succession without use of their arms. Those who are unable to complete this task without the use of their arms will be considered unable. The participant's time to complete a 6 meter walking course at his/her usual pace will be measured with a stopwatch. Walking speed (meters/second) will be determined from the better of two trials. In addition to the SPPB, tests of endurance and strength will assessed with a 400 meter walk and grip strength. The time to complete a 400 meter walk test will also be measured with a stopwatch. Participants will be considered unable to perform this task if they require more than 15 minutes for the 400 meter walk or if they require use of assistance. Grip strength will be assessed using a Jamar dynamometer in the participant's dominant hand.[73] The maximum effort from two trials will be taken as the measurement of maximum grip strength (kilograms).

Imaging (DXA): DXAs will occur at OHSU. At Baseline and the annual visits, whole body scans will be taken with a Hologic QDR 4500W DXA scanner by a certified DXA operator to determine total body lean mass and appendicular lean mass (kilograms), as previously described.[59, 60] Standard Hologic procedures will be used to define regions on the whole body DXA scans using cut lines. The arms will be delineated by a cut line drawn between the head of the humerus and the scapula at the glenoid fossa and the leg will be delineated by a cut line drawn across the midpoint of the femoral neck. Appendicular lean mass will be calculated as the sum of lean mass in the arms and legs. Hologic whole-body QC phantoms will be scanned daily to monitor longitudinal changes so that correction factors can be applied to measurements to adjust for longitudinal drift.

Muscle Biopsy studies (for those in the substudy): Subjects are asked to participate in the muscle biopsy studies based on their platelet and INR results. There will be 32 subjects in the substudy. Subjects will not enroll in the muscle biopsy studies or have a second muscle biopsy if they take medications that increase bleeding risk, have an allergy to lidocaine, or have platelet count < 150 billion/L, INR > 1.2, or aPTT > 36 seconds.

At the Baseline Muscle Biopsy visit and 6 month visit, fasting muscle biopsy samples will be obtained at OHSU by a physician or certified nurse practitioner from the vastus lateralis muscle, 15 cm above the patella using the modified Bergstrom technique, as described.[36, 74-76] Using a 2% lidocaine for anesthetic, a small, ¼ incision will be made in the skin and fascia to place a 5 mm Bergstrom biopsy needle while suction is applied through the 140 cc syringe, obtaining approximately 150-200 mg of muscle. The incision will be closed with sterile adhesive strips, and a pressure bandage will be applied for a period of 24 hours. This procedure takes approximately 15 minutes, is a relatively painless, and well-tolerated in older adults. Subjects will be instructed not to perform physical exercise 48 hours prior to the muscle biopsy procedure to help prevent acute effects of exercise on intramyocellular lipids (IMCL) (7:8). They will also be given discharge instructions after the procedure for precautions to follow to prevent complications.

All muscle specimens will be processed at OHSU. The specimen (50 mg) for histochemistry will be mounted on a small piece of cork, placed in cooled isopentane for 2-3 minutes and then frozen in liquid nitrogen. Another portion (50 mg) will be placed in a cryovial and flash frozen in liquid nitrogen for analysis of diacylglycerol and ceramide content. The remaining tissue will flash frozen in liquid nitrogen, and stored for future analyses.

All specimens will be stored in a -80 freezer in the biorepository located at the Oregon Clinical & Translational Research Institute (OCTRI) lab until they are analyzed. All muscle specimens will be stored after being assigned code numbers and the information linking these code numbers to the corresponding subjects' identities will be kept in a separate, secure, locked location. This is done to protect research subject's confidentiality. If a research subject decides to withdraw the specimens may continue to be stored as outlined in the approved consent documents. Identifiers will be stored separately from the samples.

During the course of the study, 100 mg of stored muscle biopsy specimens will be prepared for histochemical analyses will be sectioned on a cryostat at -20 C and placed on precleaned glass slides. A longitudinal section will be made and imaged at 36,000 magnification for acquisition of 15-20 micrographs to determine mitochondrial content using digital image analytical software (Metamorph 6.3) as previously described.[77] Succinate

dehydrogenase staining and quantification using image analysis of staining intensity will be assessed as described.[75] Triglyceride content will be determined using Oil Red O staining using established methods.[78] Capillary density (number of capillaries/muscle area) will be determined by visualization using a tetramethylrhodamine isothiocyante (TRITC) excitation filter after thawed and air dried sections are fixed in 0.25% formaldehyde, incubated with lectin and rinsed.[36] The percentage of Type I, IIa and IIx fibers will be determined from manual counting 100-300 fibers in stained sections. The sections will first be incubated overnight at room temperature with antibodies against antihuman myosin heavy chain-7 (type I fibers) and myosin heavy chain-2 (type IIa fibers) and then incubated with secondary antibodies conjugated with fluorescein (type IIa fibers) and rhodamine (type I fibers) . Type IIx fibers will remain unstained. Images will be acquired with an optical microscope.[36, 74] Diacylglycerol and ceramide content will be measured using high-performance liquid chromatography-tandem mass spectrometry from a homogenized (using ice-cold buffer of 250 mM sucrose, 25 mMKCl, 50 mMTris, and 0.5 mM EDTA, pH7.4) liquid nitrogen-frozen sample as previously described.[36]

Meals

After fasting labwork/biopsies have been taken, participants will be provided breakfast by the OCTRI Bionutrition Unit.

Schedule of Study Procedures

	Screening Visit	*Baseline Visit*[a]	*Baseline Muscle Biopsy Visit*[b]	*3 and 9 Months, Year 1 +3 and +9 Months, Year 2 +3 and +9 Months Phone Calls*[c]	*6 Month Visit*[d]	*Years 1, 2, and 3/ End of Study Visit*[d]	*Year 1 + 6 Months, and Year 2 + 6 Months Visits*[d]
Consent	X						
Inclusion/Exclusion	X						
Height	X					X	
Weight, blood pressure	X	X				X	
Demographics	X						
Medical History	X						
Questionnaires		X			X	X	X
Labs		X			X	X	X
Fasting Glucose	X	X				X	
Fasting Insulin		X				X	
ALT	X					X	
AST	X					X	
Creatinine	X				X[f]	X	X[f]
eGFR[e]	X				X[f]	X	X[f]
B12	X					X	
Platelets	X				X[g]		
INR, aPTT	X				X[g]		
Biorepository		X			X	X	X
Physical Performance	X	X				X	
DXA		X				X	
Muscle Biopsy (only for substudy)[h]			X		X		
Concurrent medications	X	X			X	X	X
Medication compliance				X	X	X	X
AE monitoring				X	X	X	X
Meals		X	X		X	X	X

[a]Baseline visit will occur 14 ± 7 days from Screening visit.
[b]For substudy, Baseline Muscle Biopsy visit will occur within 14 days from Baseline visit.
[c]At 3 and 9 month time points between annual visits, telephone surveying will occur.
[d]Half-yearly visits and annual visits will occur with ± 7 day visit window.
[e]eGFR is calculated from the creatinine.
[f]Every 6 months, creatinine and eGFR will be obtained for subjects over 80 years old.
[g]At 6 months, STAT platelets, INR, and aPTT will be obtained for subjects in the substudy before the biopsy.
[h]Muscle biopsy samples are for this study and for biorepository.

Adverse events

Adverse events (AEs) will be evaluated at each visit. Principal Investigator or MD Co-Investigator will determine the relation of the side effect to treatment as: 1) related, 2) possibly related, or 3) not related.

A serious adverse event (SAE) is defined as an AE that results in death, a life-threatening experience, inpatient hospitalization or prolongation of hospitalization (for a patient already hospitalized); persistent or significant disability or incapacity; congenital anomaly and/or birth defects; an event that jeopardizes the subject and may require medical or surgical treatment to prevent one of the preceding outcomes; or death**.**

"Unanticipated" means an event that is new or greater than previously known in terms of nature, severity, or frequency, given the procedures described in protocol-related documents and the characteristics of the study population. Unanticipated problems involving risk (UPRs) are physical, psychological, social, or economic risks to subjects or others, including device and drug effects, and anything reportable to the FDA.

All unanticipated SAEs and UPRs must be reported to the joint IRB within 5 business days of awareness, via OHSU eIRB UP form. SAEs and UPRs will also be reported to the VA Data Monitoring Committee. Copies of the report documents will be kept in the study regulatory binder.

Compensation

Subject compensation will be offered at the end of each study visit, as follows:

- $50 at Baseline, Year 1, Year 2, and Year 3/End of Study visits
- $50 at Baseline Muscle Biopsy visit and at 6 month visit (if in substudy)
- $5 at 6 month; $5 at Year 1 +6 month; and $5 at Year 2 +6 month visits

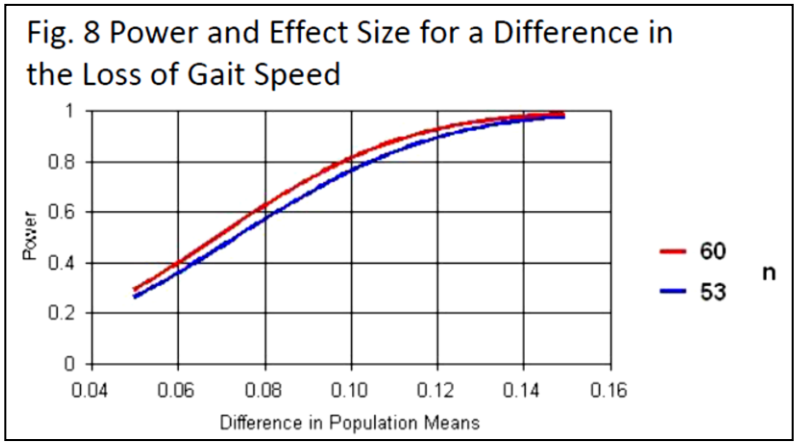
If subject does not complete study, payment will be prorated.

The payments are reasonable and commensurate with the expected contributions of the subject. Each annual study visit is 2-3 hours. Baseline and annual visits include a fasting blood draw, height, weight, blood pressure, questionnaires, performance tests, and DXA. The procedures at every 6 month study visits (in between annual visits) are only fasting labs and questionnaires for all participants. For the muscle biopsy substudy, there will be a separate 1-2 hour visit at baseline and an additional 1 hour added to the 6 month visit.

Payments are fair and appropriate and do not constitute (or appear to constitute) undue pressure or influence on, or coercion of, the prospective research subjects to volunteer for or continue participation in the research study.

Statistical considerations

Sample Size and Power



Based on prior observational findings, there was a significant difference (2.2%, p< 0.01) in the percentage change in appendicular lean mass over 3.5 years between metformin-treated and untreated men. The sample size required for 80% Power to detect this difference and reject the null with SD=4% and α=0.05 for an unpaired, 2-sided t-test is 106 study participants (53 in each group). We plan to enroll 300 participants and, of those, randomize 120 (60 in each group) to allow for potential drop outs due to noncompliance and other reasons (7.5-10%). While 8-11% of participants may progress to diabetes yearly,[72, 79, 80] all efforts will be made to retain these participants in the study and to include them in intent-to-treat analyses. The Power and effect size estimates for gait speed (SD=0.19) for α=0.05 for 60 participants and 53 participants per group are illustrated in the graph (Fig. 8) and based on our preliminary data in older women. The sample size of 60 participants per group will have 73% Power to detect a 0.09 m/s difference in gait speed. Within each group, 16 participants will be randomly selected and consented to undergo muscle biopsies at baseline and 6 months for secondary analyses of muscle histologic changes with metformin vs. placebo treatment. There is an expected change in these histologic measures within 6 months given prior studies that detect changes in AMPK activation, ATP concentration and insulin sensitivity prior to 6 months with use of metformin.[3, 4] Estimated effect sizes of the difference in histologic measures between the two groups after 6 months are shown below in Table 3. While this study is adequately powered for 3 year outcomes in muscle loss, interim analyses may be performed by a Data Monitoring Committee (managed by the VA CSRD), given blinding of the primary investigator to the intervention, and the trial may be terminated early if a significant difference in the primary outcome between metformin- and placebo-treated groups is noted before 3 years.

Table 3 Estimated detectable difference in muscle histology measures at 80 and 90% Power

	SD	70%	80%	90%
Capillary Density (AU)	0.35	0.32	0.36	0.42
IMCL (AU)	0.50	0.46	0.51	0.59
SDH (AU)	0.50	0.46	0.51	0.59
% Type I Fibers	24.0	21.8	24.6	28.4

SD derived from measures in older insulin-resistant adults[36]

Statistical Analyses

To assess a difference in the primary outcomes of change in muscle mass and performance between metformin- and placebo-treated groups, longitudinal (mixed effects) models will be used to estimate the trajectory of change. These models will account for correlation of outcomes within subjects and allows for those with missing follow-up data to be included in analyses. Four measures of muscle mass and performance (baseline, year 1, 2 and 3) are the outcomes in separate models. These will be modeled as a linear change over time, and other functional changes (squared, cubic and quadratic) will be evaluated. In the best fitting model, an interaction between time and group (metformin vs. placebo) will be included in the model to determine if changes in muscle mass/performance differ between the two groups. For the secondary outcomes of muscle histology, the discrete change in measures of mitochondrial content, muscle capillary density, percentage of fiber types and

intramyocellular lipid content over 6 months will be calculated. The absolute and percentage changes in these measures will serve as the outcome in linear regression models with treatment group as the dichotomous exposure. Nonlinear associations between the exposure and outcomes will be evaluated with higher order terms. For the analyses of primary and secondary outcomes, potential confounders including demographic information, physical activity, diet, lifestyle factors, medical history and medication use are expected to be evenly distributed between the groups in a random fashion; nevertheless, we will evaluate for potential confounding by these factors and include them in multivariable models if they are significantly associated with our exposure and outcome and modify the effect size by >10%. There is no anticipated gender difference in the study outcomes, so the study has not been powered to detect a significant interaction by gender. However, the primary and secondary outcomes will also be stratified by gender to explore if there is a gender-specific muscle response to metformin. To account for missing data, multiple imputation modeling will constructed from participants with complete data. Participants with missing data will have those values imputed at each time point multiple times from these models utilizing available covariate data and then analyzed according to the principles of multiple imputation.[82] Results will be reported with and without imputed values.

Data Integrity

For any protected health information (PHI) used for recruitment purposes (i.e. from patient lists for mailing recruitment letters), the information will strictly be stored on the secure VA server.

Study forms will contain the subject number, and may contain PHI. All study forms with PHI will be stored securely in locked file cabinets in a locked office, or on a secure electronic VA drive. All PHI will be coded prior to release for analysis.

There is a transfer of ownership of data from VAPORHCS to OHSU. The data will be managed at OHSU behind OHSU firewall. The Oregon Clinical & Translational Research Institute's (OCTRI) Biomedical Informatics Program has the resources and expertise – software engineering, research analysts, and project management – available to assist researchers and staff with the storage, retrieval, sharing, and optimal use of biomedical information, data, and knowledge for problem solving and decision-making. The data collection and management tools utilized for this grant are REDCap (Research Electronic Data Capture). The REDCap system provides secure, web-based applications that are flexible enough to be used for a variety of types of research, provide an intuitive interface for users to enter data and have real time validation rules (with automated data type and range checks) at the time of entry. This system offers easy data manipulation with audit trails and reporting for reporting, monitoring and querying patient records, and an automated export mechanism to common statistical packages (SPSS, SAS, Stata, R/S-Plus).

Human Subjects Considerations:

Potential Risks, Protection from Risks, and Risk/Benefit Discussion

Potential Risks

This is an FDA approved drug for an unapproved purpose. However, this drug has been well-studied in and sometimes used for people with impaired fasting glucose to prevent progression to diabetes and risks in this population are no different than those in the FDA approved population.[82, 92] The proposed metformin dose of 850 mg twice daily has been previously studied safely in a population with pre-diabetes for the prevention of diabetes.[75] This study is not intended to be used to support a significant change in the labeling or in the advertising of metformin, and the same route of administration will be used. With these elements, an IND is not necessary.

Risks of Metformin Use

There are risks of gastrointestinal distress, hepatitis, or lactic acidosis. Gastrointestinal distress includes diarrhea, nausea, vomiting, flatulence, indigestion, abdominal discomfort/pain, constipation, and distention of the abdomen. Gastrointestinal symptoms are common during initiation of metformin therapy. Metformin is known to be substantially excreted by the kidney, and the risk of metformin accumulation and lactic acidosis increases with the degree of impairment of renal function. Lactic acidosis is an extremely rare, but serious, metabolic complication that can occur due to metformin accumulation during treatment with metformin. Occurrence of gastrointestinal symptoms could be due to lactic acidosis or other serious disease. A decrease in vitamin B12 levels has been observed in clinical trials with metformin. Signs of lactic acidosis include feeling very weak, tired or uncomfortable, unusual muscle pain, trouble breathing, unusual or unexpected stomach discomfort, feeling cold, feeling dizzy or lightheaded, and suddenly developing a slow or irregular heartbeat. Contrast (in radiologic) studies with iodinated materials can lead to acute alteration of renal function and have been associated with lactic acidoses.

There are other rare risks from metformin use. Acute congestive heart failure, acute myocardial infarction and other conditions characterized by hypoxemia may occur in the setting of lactic acidosis and may also cause prerenal azotemia (a medical condition characterized by abnormally high levels of nitrogen-containing compounds in the blood). Alcohol is known to potentiate the effect of metformin on lactate metabolism. Subjects should be warned against excessive alcohol intake, acute or chronic. Hypoglycemia does not occur in patients receiving metformin alone under usual circumstances of use, but could occur when caloric intake is deficient, when strenuous exercise is not compensated by caloric supplementation, during concomitant use with other glucose-lowering agents (such as sulfonylureas and insulin) or ethanol, or with substantial alcohol use. When a patient stabilized on any diabetic regimen is exposed to stress such as fever, trauma, infection, or surgery, a temporary loss of glycemic control may occur. When starting to take metformin, a metallic taste could occur.

With the exclusion criteria and screening tests, these risks (except gastrointestinal intolerance) would be minimized. See section “Protection Against Risks of Metformin Use.”

Risk of Diagnosis of Diabetes

From participating in this study, there is a risk that diabetes may be detected. Participants and their primary care physician will be notified so that they can seek confirmatory testing and appropriate medical care from their primary care physician.

Risks of Study Procedures

Muscle biopsy: The risks of percutaneous muscle biopsy are bleeding and infection (both rare), bruising (infrequent) and discomfort (likely). With the use of a local anesthetic, the discomfort of muscle biopsy is described as about twice that of intravenous cannulation. Some residual stiffness in the vastus lateralis can persist for 1-2 days (common). An elastic wrap and ice bag are applied post-biopsy to decrease the risk of bruising and for an anesthetic effect. Subjects from similar protocol have not experienced any adverse effects from these procedures other than a small amount of residual localized soreness at the biopsy site. Additional risk includes any unusual reaction to the elastic bandage wrap and ice, i.e. leg numbness which would indicate the elastic bandage had been applied too tightly or the ice left on too long; or any skin redness, irritation, and chafing from the applied antibiotic ointment and/or steri-strips.

DXA scans: There is risk of radiation exposure. There will be multiple scans during the study which will increase exposure to radiation.

Blood collection: There is a risk of pain, bleeding, infection, and bruising. These risks will be minimized by using standard blood draw procedures.

Physical Performance: Participants with neuromuscular dysfunction may have a risk of falling during the

performance exams.

Risk of Breach of Confidentiality

There is a potential risk of breach of confidentiality. This will be minimized by coding the data and samples, using a unique subject code. The link matching the identifiable information to the subject code will be kept only by IRB-approved study personnel designated by the PI, on a password-protected drive behind the secure VA firewall or in locked file cabinets in a locked VA office. Coded samples will be processed in the VAPORHCS and OCTRI laboratories, and then transferred (without any identifiers) to Dr. Goodpastor's lab at Sanford-Burnham Translational Research Institute for Metabolism and Diabetes for muscle biopsy analysis. The signed consent forms and paper study documents will be kept in a locked file cabinet in a locked office or on a password-protected drive behind the secure VA firewall.

Adequacy of Protection from Risk

The study will be reviewed and approved by the joint VAPORHCS-OHSU IRB. The investigators will adhere to the
Data Monitoring Committee plan described in this protocol. Trained and IRB-approved study personnel will perform all data collection and information will be coded with a subject identifier to protect subject confidentiality.

Protection Against Risks of Metformin Use

By enrolling participants without liver or kidney dysfunction or congestive heart failure, the risk of lactic acidosis or hepatitis on metformin will be decreased. Furthermore, we intend to prevent such risks by screening liver and kidney function every year in all participants and quarterly in adults aged 80 or greater and stopping metformin in participants who develop evidence of liver/kidney dysfunction for their safety. Per 2016 FDA guidelines, metformin use will be discontinued if the eGFR drops below 45 mL/min/1.73 m2. For those who are hospitalized or receive contrast medium, the drug will be held and efforts will be made to recheck kidney function within 48 hours after discharge/procedure. The study drug will be restarted only if there is no evidence of renal dysfunction. For tests or procedures that require fasting, study drug will be stopped 24 hours prior to test or procedure, and study drug can be resumed after the participant is allowed to eat again. Participants with B12 deficiency will not be enrolled in the study, and B12 levels will be monitored throughout the study. If B12 deficiency is found, the participant will discontinue study drug. Initial weight loss due to gastrointestinal symptoms is usually self-limiting and continued metformin use is associated with weight stability.[4] We have chosen a slow titration up of metformin dosage from 850 mg qd to 850 mg bid to allow for minimization of self-limiting gastrointestinal distress and plan to carefully monitor weight and dietary intake at each study visit. If participants report symptoms of lactic acidosis during screening for adverse events, laboratory confirmation will be sought and study drug cessation if found.

Protection Against Risks of Diagnosis - From labs at Screening and study visits, diabetes may be diagnosed (defined by fasting plasma glucose≥126 mg/dL). Participants will be notified and referred to their primary care physician.

Protection Against Risks of Study Procedures

*Muscle biopsy***:** The exclusion of participants on warfarin, clopidogrel, Aggrenox, ticlopidine, dabigatran or anagrelide will minimize serious bleeding with the procedure. We will also ensure that participants are not on these medications within a week of their second muscle biopsy. Furthermore, participants will have screening of INR, aPTT and platelets and will not be enrolled in the muscle biopsy substudy if there is any abnormality in clotting function to avoid serious bleeding. They will also have reassessment of these indices prior to the second

biopsy and will not undergo the second biopsy unless these labs are normal. Local anesthesia with 2% lidocaine will be used to reduce pain associated with the muscle biopsy. To ensure safety with the anesthetic, no participants with an allergy to lidocaine will be enrolled. To minimize the risk of infection from muscle biopsy, the procedure will be performed using sterile techniques. Appropriate pressure, hemostasis and use of an elastic wrap and ice bag after the biopsy will decrease the risk of bruising and aid in an anesthetic effect. Participants will be given instructions on care of the biopsy incision site to prevent bleeding and infection after the procedure.

DXA scans: The radiation exposure from one whole body DXA scan is 3.6 (4.6) μSv of radiation which is less than the accumulated daily background radiation dose to the whole body.[3] Therefore even the amount of radiation from four DXA scans is nominal and should not raise the risk for adverse health effects.

Blood collection: A sterile technique will be used prior to venipuncture or intravenous access and pressure will be applied for appropriate hemostasis after withdrawal of the needle/catheter.

Physical Performance: To prevent harm from falls during the exam, neuromuscular tests will be administered by two staff members if the participant has a history of falling or is so frail that a fall might occur.

Protection Against Risk of Breach of Confidentiality

Subject confidentiality will be maintained in accordance with HIPAA regulations. The recruitment database will be protected by current network security in place in the VAPORHCS and all data collected prior to consent will be stored at VAPORHCS. After consent is signed, data will be maintained in the OCTRI/OHSU REDCap database. In addition, the database will be password protected, and only project personnel approved by Dr. Klein will be given access to them.

Potential Benefits of Research

Potential benefits to participants include the detection and potentially earlier treatment of diabetes mellitus. Participants in both metformin and placebo groups will benefit from routine lifestyle modification counseling at yearly visits for prevention of diabetes.

Others may benefit from a greater understanding of the effect of metformin on muscle mass and function in insulin-resistant older adults.

VA Data Monitoring Committee (DMC)

A data monitoring committee will be provided by VA CSR&D. With DMC's experience in the conduct and monitoring of clinical trials, it will assist with ensuring the overall safety of study participants, assessing safety and efficacy of the study intervention, monitoring the overall conduct of the clinical trial including recommendations on recruitment and retention of participants and improvement of study drug adherence, and providing recommendations on stopping or continuation of the trial. SAEs and UPRs will be reported to the DMC. For further details, see the "Instructions for the Review of New (uninitiated) Protocols by DMC Members."

Inclusion of Women and Children

Because sarcopenia is a condition of older age, children will not be enrolled in this study. Women will be included in a proportion reflective of their gender distribution at the VAPORHCS.

Criteria for Discontinuation

Subjects will be withdrawn from further study drug administration in the event of any of the following:

- Diagnosis of diabetes

- If hospitalized for any reason and after 48 hours of discharge, there is evidence of renal dysfunction
- If they require contrast medium and after 48 hours of procedure, there is evidence of renal dysfunction
- Developed lactic acidosis
- Developed renal insufficiency at the level indicated by the FDA (eGFR < 45 mL/min/1.73 m^2).
- Development of B12 deficiency
- Excessive alcohol intake
- Subject withdraws consent
- Significant deviation from the protocol or eligibility criteria in the opinion of the investigator
- Any other reason that in the opinion of the investigator, would justify removing the subject from the study

There are no pre-defined stopping rules for termination of the study.

VA Research:

The following are the components of the study which will be conducted at the VAPORHCS:

- Advertising
- Recruitment
- Study visit activities at Screening, Baseline, half-yearly visits, and annual visits
    - Consent
    - Height, weight
    - Blood pressure
    - Demographics
    - Medical history
    - Study questionnaires
    - Blood draw for labs
    - Physical performance test
    - Concurrent medications
    - AE monitoring
    - Meals
- Lab analysis for fasting glucose, ALT, AST, creatinine, eGFR, B12, platelets, INR and aPTT
- Study drug - storage, dispensing, randomization, tracking, and pill counts

OHSU Research:

The following are the components of the study which will be conducted at OHSU:

- Study visit activities at Baseline, Baseline Muscle Biopsy Visit (if in substudy), 6 Month Visit (if in substudy) and annual visits
    - Height, weight
    - Blood pressure
    - Study questionnaires
    - Physical performance tests
    - DXA
    - Muscle biopsy
    - Concurrent medications
    - AE monitoring
    - Meals
- Lab analysis for fasting insulin
- Study database (REDCap)
- Statistical analyses

- Biorepository for blood and muscle biopsy samples

Literature Cited:

1. Lee CG, Boyko EJ, Strotmeyer ES, et al. Association between insulin resistance and lean mass loss and fat mass gain in older men without diabetes mellitus. J Am Geriatr Soc 2011;59(7):1217-1224.
2. Lee CG, Boyko EJ, Barrett-Connor E, et al. Insulin Sensitizers May Attenuate Lean Mass Loss in Older Men With Diabetes. Diabetes Care 2011;34(11):2381-2386.
3. Musi N, Hirshman MF, Nygren J, et al. Metformin increases AMP-activated protein kinase activity in skeletal muscle of subjects with type 2 diabetes. Diabetes 2002;51(7):2074-2081.
4. Tamura Y, Watada H, Sato F, et al. Effects of metformin on peripheral insulin sensitivity and intracellular lipid contents in muscle and liver of overweight Japanese subjects. Diabetes Obes Metab 2008;10(9):733-738.
5. Baumgartner RN, Koehler KM, Gallagher D, et al. Epidemiology of sarcopenia among the elderly in New Mexico. Am J Epidemiol 1998;147(8):755-763.
6. Janssen I, Heymsfield SB, Ross R. Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. J Am Geriatr Soc 2002;50(5):889-896.
7. Melton LJ, 3rd, Khosla S, Crowson CS, et al. Epidemiology of sarcopenia. J Am Geriatr Soc 2000;48(6):625-630.
8. Rabasa-Lhoret R, Bastard JP, Jan V, et al. Modified quantitative insulin sensitivity check index is better correlated to hyperinsulinemic glucose clamp than other fasting-based index of insulin sensitivity in different insulin-resistant states. J Clin Endocrinol Metab 2003;88(10):4917-4923.
9. Festa A, D'Agostino R, Jr., Hanley AJ, et al. Differences in insulin resistance in nondiabetic subjects with isolated impaired glucose tolerance or isolated impaired fasting glucose. Diabetes 2004;53(6):1549-1555.
10. Tripathy D, Carlsson M, Almgren P, et al. Insulin secretion and insulin sensitivity in relation to glucose tolerance: lessons from the Botnia Study. Diabetes 2000;49(6):975-980.
11. Cowie CC, Rust KF, Ford ES, et al. Full accounting of diabetes and pre-diabetes in the U.S. population in 1988-1994 and 2005-2006. Diabetes Care 2009;32(2):287-294.
12. Park SW, Goodpaster BH, Lee JS, et al. Excessive loss of skeletal muscle mass in older adults with type 2 diabetes. Diabetes Care 2009;32(11):1993-1997.
13. Suwa M, Egashira T, Nakano H, Sasaki H, Kumagai S. Metformin increases the PGC-1alpha protein and oxidative enzyme activities possibly via AMPK phosphorylation in skeletal muscle in vivo. J Appl Physiol 2006;101(6):1685-1692.
14. Janssen I, Shepard DS, Katzmarzyk PT, Roubenoff R. The healthcare costs of sarcopenia in the United States. J Am Geriatr Soc 2004;52(1):80-85.
15. Rogers WH, Kazis LE, Miller DR, et al. Comparing the health status of VA and non-VA ambulatory patients: the veterans' health and medical outcomes studies. J Ambul Care Manage 2004;27(3):249-262.
16. Selim AJ, Berlowitz DR, Fincke G, et al. The health status of elderly veteran enrollees in the Veterans Health Administration. J Am Geriatr Soc 2004;52(8):1271-1276.
17. Villa VM, Harada ND, Washington D, Damron-Rodriguez J. The health and functional status of US veterans aged 65+: implications for VA health programs serving an elderly, diverse veteran population. Am J Med Qual 2003;18(3):108-116.
18. Crimmins EM, Saito Y, Reynolds SL. Further evidence on recent trends in the prevalence and incidence of disability among older Americans from two sources: the LSOA and the NHIS. J Gerontol B Psychol Sci Soc Sci 1997;52(2):S59-71.
19. Villa VM, Harada ND, Washington D, Damron-Rodriguez J. Health and functioning among four war eras of U.S. veterans: examining the impact of war cohort membership, socioeconomic status, mental health, and disease prevalence. Mil Med 2002;167(9):783-789.
20. Onder G, Penninx BW, Ferrucci L, et al. Measures of physical performance and risk for progressive and catastrophic disability: results from the Women's Health and Aging Study. J Gerontol A Biol Sci Med Sci 2005;60(1):74-79.
21. Guralnik JM, Ferrucci L, Simonsick EM, Salive ME, Wallace RB. Lower-extremity function in persons

over the age of 70 years as a predictor of subsequent disability. N Engl J Med 1995;332(9):556-561.
22. Guralnik JM, Ferrucci L, Pieper CF, et al. Lower extremity function and subsequent disability: consistency across studies, predictive models, and value of gait speed alone compared with the short physical performance battery. J Gerontol A Biol Sci Med Sci 2000;55(4):M221-231.
23. Goodpaster BH, Park SW, Harris TB, et al. The loss of skeletal muscle strength, mass, and quality in older adults: the health, aging and body composition study. J Gerontol A Biol Sci Med Sci 2006;61(10):1059-1064.
24. Visser M, Goodpaster BH, Kritchevsky SB, et al. Muscle mass, muscle strength, and muscle fat infiltration as predictors of incident mobility limitations in well-functioning older persons. J Gerontol A Biol Sci Med Sci 2005;60(3):324-333.
25. Borst SE. Interventions for sarcopenia and muscle weakness in older people. Age Ageing 2004;33(6):548-555.
26. Hayward R. *VA QUERI Diabetes Mellitus Report.* Washington, D.C.2002.
27. Miller DR, Safford MM, Pogach LM. Who has diabetes? Best estimates of diabetes prevalence in the Department of Veterans Affairs based on computerized patient data. Diabetes Care 2004;27 Suppl 2:B10-21.
28. Fink RI, Kolterman OG, Griffin J, Olefsky JM. Mechanisms of insulin resistance in aging. J Clin Invest 1983;71(6):1523-1535.
29. Park SW, Goodpaster BH, Strotmeyer ES, et al. Accelerated loss of skeletal muscle strength in older adults with type 2 diabetes: the health, aging, and body composition study. Diabetes Care 2007;30(6):1507-1512.
30. Barzilay JI, Blaum C, Moore T, et al. Insulin resistance and inflammation as precursors of frailty: the Cardiovascular Health Study. Arch Intern Med 2007;167(7):635-641.
31. Barzilay JI, Cotsonis GA, Walston J, et al. Insulin resistance is associated with decreased quadriceps muscle strength in nondiabetic adults aged >or=70 years. Diabetes Care 2009;32(4):736-738.
32. Lang T, Streeper T, Cawthon P, et al. Sarcopenia: etiology, clinical consequences, intervention, and assessment. Osteoporos Int 2010;21(4):543-559.
33. Lexell J. Human aging, muscle mass, and fiber type composition. J Gerontol A Biol Sci Med Sci 1995;50 Spec No:11-16.
34. Goodpaster BH, Carlson CL, Visser M, et al. Attenuation of skeletal muscle and strength in the elderly: The Health ABC Study. J Appl Physiol 2001;90(6):2157-2165.
35. Ryan NA, Zwetsloot KA, Westerkamp LM, et al. Lower skeletal muscle capillarization and VEGF expression in aged vs. young men. J Appl Physiol 2006;100(1):178-185.
36. Crane JD, Devries MC, Safdar A, Hamadeh MJ, Tarnopolsky MA. The effect of aging on human skeletal muscle mitochondrial and intramyocellular lipid ultrastructure. J Gerontol A Biol Sci Med Sci 2010;65(2):119-128.
37. Mortensen B, Poulsen P, Wegner L, et al. Genetic and metabolic effects on skeletal muscle AMPK in young and older twins. Am J Physiol Endocrinol Metab 2009;297(4):E956-964.
38. Lira VA, Benton CR, Yan Z, Bonen A. PGC-1alpha regulation by exercise training and its influences on muscle function and insulin sensitivity. Am J Physiol Endocrinol Metab 2010;299(2):E145-161.
39. Stephens TJ, Chen ZP, Canny BJ, et al. Progressive increase in human skeletal muscle AMPKalpha2 activity and ACC phosphorylation during exercise. Am J Physiol Endocrinol Metab 2002;282(3):E688-694.
40. Lovell DI, Cuneo R, Gass GC. Can aerobic training improve muscle strength and power in older men? J Aging Phys Act 2010;18(1):14-26.
41. Dube JJ, Amati F, Stefanovic-Racic M, et al. Exercise-induced alterations in intramyocellular lipids and insulin resistance: the athlete's paradox revisited. Am J Physiol Endocrinol Metab 2008;294(5):E882-888.
42. Coggan AR, Spina RJ, King DS, et al. Skeletal muscle adaptations to endurance training in 60- to 70-yr-old men and women. J Appl Physiol 1992;72(5):1780-1786.
43. Menshikova EV, Ritov VB, Fairfull L, et al. Effects of exercise on mitochondrial content and function in aging human skeletal muscle. J Gerontol A Biol Sci Med Sci 2006;61(6):534-540.

44. Smith AC, Mullen KL, Junkin KA, et al. Metformin and exercise reduce muscle FAT/CD36 and lipid accumulation and blunt the progression of high-fat diet-induced hyperglycemia. Am J Physiol Endocrinol Metab 2007;293(1):E172-181.
45. Smith AC, Bruce CR, Dyck DJ. AMP kinase activation with AICAR simultaneously increases fatty acid and glucose oxidation in resting rat soleus muscle. J Physiol 2005;565(Pt 2):537-546.
46. Lee WJ, Kim M, Park HS, et al. AMPK activation increases fatty acid oxidation in skeletal muscle by activating PPARalpha and PGC-1. Biochem Biophys Res Commun 2006;340(1):291-295.
47. Smith AC, Bruce CR, Dyck DJ. AMP kinase activation with AICAR further increases fatty acid oxidation and blunts triacylglycerol hydrolysis in contracting rat soleus muscle. J Physiol 2005;565(Pt 2):547-553.
48. Kaushik VK, Young ME, Dean DJ, et al. Regulation of fatty acid oxidation and glucose metabolism in rat soleus muscle: effects of AICAR. Am J Physiol Endocrinol Metab 2001;281(2):E335-340.
49. Ouchi N, Shibata R, Walsh K. AMP-activated protein kinase signaling stimulates VEGF expression and angiogenesis in skeletal muscle. Circ Res 2005;96(8):838-846.
50. Zwetsloot KA, Westerkamp LM, Holmes BF, Gavin TP. AMPK regulates basal skeletal muscle capillarization and VEGF expression, but is not necessary for the angiogenic response to exercise. J Physiol 2008;586(Pt 24):6021-6035.
51. Winder WW, Holmes BF, Rubink DS, et al. Activation of AMP-activated protein kinase increases mitochondrial enzymes in skeletal muscle. J Appl Physiol 2000;88(6):2219-2226.
52. Jorgensen SB, Treebak JT, Viollet B, et al. Role of AMPKalpha2 in basal, training-, and AICAR-induced GLUT4, hexokinase II, and mitochondrial protein expression in mouse muscle. Am J Physiol Endocrinol Metab 2007;292(1):E331-339.
53. Reznick RM, Shulman GI. The role of AMP-activated protein kinase in mitochondrial biogenesis. J Physiol 2006;574(Pt 1):33-39.
54. Irrcher I, Ljubicic V, Kirwan AF, Hood DA. AMP-activated protein kinase-regulated activation of the PGC-1alpha promoter in skeletal muscle cells. PLoS One 2008;3(10):e3614.
55. Suwa M, Nakano H, Kumagai S. Effects of chronic AICAR treatment on fiber composition, enzyme activity, UCP3, and PGC-1 in rat muscles. J Appl Physiol 2003;95(3):960-968.
56. Nakashima K, Yakabe Y. AMPK activation stimulates myofibrillar protein degradation and expression of atrophy-related ubiquitin ligases by increasing FOXO transcription factors in C2C12 myotubes. Biosci Biotechnol Biochem 2007;71(7):1650-1656.
57. Krawiec BJ, Nystrom GJ, Frost RA, Jefferson LS, Lang CH. AMP-activated protein kinase agonists increase mRNA content of the muscle-specific ubiquitin ligases MAFbx and MuRF1 in C2C12 cells. Am J Physiol Endocrinol Metab 2007;292(6):E1555-1567.
58. Drake JC, Alway SE, Hollander JM, Williamson DL. AICAR treatment for 14 days normalizes obesityinduced
dysregulation of TORC1 signaling and translational capacity in fasted skeletal muscle. Am J Physiol Regul Integr Comp Physiol 2010;299(6):R1546-1554.
59. Gore DC, Wolf SE, Sanford A, Herndon DN, Wolfe RR. Influence of metformin on glucose intolerance and muscle catabolism following severe burn injury. Ann Surg 2005;241(2):334-342.
60. Narkar VA, Downes M, Yu RT, et al. AMPK and PPARdelta agonists are exercise mimetics. Cell 2008;134(3):405-415.
61. Steinberg GR, O'Neill HM, Dzamko NL, et al. Whole body deletion of AMP-activated protein kinase {beta}2 reduces muscle AMPK activity and exercise capacity. J Biol Chem 2010;285(48):37198-37209.
62. Orwoll E, Blank JB, Barrett-Connor E, et al. Design and baseline characteristics of the osteoporotic fractures in men (MrOS) study--a large observational study of the determinants of fracture in older men. Contemp Clin Trials 2005;26(5):569-585.
63. Cummings SR, Black DM, Nevitt MC, et al. Appendicular bone density and age predict hip fracture in women. The Study of Osteoporotic Fractures Research Group. Jama 1990;263(5):665-668.
64. Lee CG, Boyko EJ, Nielson CM, et al. Mortality risk in older men associated with changes in weight, lean mass, and fat mass. J Am Geriatr Soc 2011;59(2):233-240.
U.S. Census Bureau: State and County QuickFacts. Data derived from Population Estimates, American Community Survey, Census of Population and Housing, County Business Ownders, Building Permits,

Consolidated Federal Funds Report, Census of Governments. 2010
66. Blank JB, Cawthon PM, Carrion-Petersen ML, et al. Overview of recruitment for the osteoporotic fractures in men study (MrOS). Contemp Clin Trials 2005;26(5):557-568.
67. Subar AF, Thompson FE, Kipnis V, et al. Comparative validation of the Block, Willett, and National Cancer Institute food frequency questionnaires : the Eating at America's Table Study. Am J Epidemiol 2001;154(12):1089-1099.
68. Boucher B, Cotterchio M, Kreiger N, et al. Validity and reliability of the Block98 food-frequency questionnaire in a sample of Canadian women. Public Health Nutr 2006;9(1):84-93.
69. Washburn RA, Smith KW, Jette AM, Janney CA. The Physical Activity Scale for the Elderly (PASE): development and evaluation. J Clin Epidemiol 1993;46(2):153-162.
70. Svarstad BL, Chewning BA, Sleath BL, Claesson C. The Brief Medication Questionnaire: a tool for screening patient adherence and barriers to adherence. Patient Educ Couns 1999;37(2):113-124.
Coletta DK, Sriwijitkamol A, Wajcberg E, et al. Pioglitazone stimulates AMP-activated protein kinase signalling and increases the expression of genes involved in adiponectin signalling, mitochondrial function and fat oxidation in human skeletal muscle in vivo: a randomised trial. Diabetologia 2009;52(4):723-732.
72. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. N Engl J Med 2008;359(15):1577-1589.
73. Juurlink DN, Gomes T, Lipscombe LL, et al. Adverse cardiovascular events during treatment with pioglitazone and rosiglitazone: population based cohort study. BMJ 2009;339:b2942.
74. Lipscombe LL, Gomes T, Levesque LE, et al. Thiazolidinediones and cardiovascular outcomes in older patients with diabetes. Jama 2007;298(22):2634-2643.
75. Knowler WC, Barrett-Connor E, Fowler SE, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 2002;346(6):393-403.
76. Harkonen R, Harju R, Alaranta H. Accuracy of the Jamar dynamometer. J Hand Ther 1993;6(4):259-262.
77. Amati F, Dube JJ, Carnero EA, et al. Skeletal-Muscle Triglycerides, Diacylglycerols, and Ceramides in Insulin Resistance: Another Paradox in Endurance-Trained Athletes? Diabetes 2011
78. Pruchnic R, Katsiaras A, He J, et al. Exercise training increases intramyocellular lipid and oxidative capacity in older adults. Am J Physiol Endocrinol Metab 2004;287(5):E857-862.
79. Evans WJ, Phinney SD, Young VR. Suction applied to a muscle biopsy maximizes sample size. Med Sci Sports Exerc 1982;14(1):101-102.
80. Chomentowski P, Coen PM, Radikova Z, Goodpaster BH, Toledo FG. Skeletal muscle mitochondria in insulin resistance: differences in intermyofibrillar versus subsarcolemmal subpopulations and relationship to metabolic flexibility. J Clin Endocrinol Metab 2011;96(2):494-503.
81. He J, Goodpaster BH, Kelley DE. Effects of weight loss and physical activity on muscle lipid content and droplet size. Obes Res 2004;12(5):761-769.
82. Nathan DM, Davidson MB, DeFronzo RA, et al. Impaired fasting glucose and impaired glucose tolerance: implications for care. Diabetes Care 2007;30(3):753-759.
83. Ratner RE. An update on the Diabetes Prevention Program. Endocr Pract 2006;12 Suppl 1:20-24.
84. Bajaj M, Baig R, Suraamornkul S, et al. Effects of pioglitazone on intramyocellular fat metabolism in patients with type 2 diabetes mellitus. J Clin Endocrinol Metab 2010;95(4):1916-1923.
85. Groenwold RH, Donders AR, Roes KC, Harrell FE, Jr., Moons KG. Dealing with missing outcome data in randomized trials and observational studies. Am J Epidemiol 2012;175(3):210-217.
86. Goodpaster BH, Kelley DE, Thaete FL, He J, Ross R. Skeletal muscle attenuation determined by computed tomography is associated with skeletal muscle lipid content. J Appl Physiol 2000;89(1):104-110.
87. Goodpaster BH, He J, Watkins S, Kelley DE. Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes. J Clin Endocrinol Metab 2001;86(12):5755-5761.
88. Lee CG, Carr MC, Murdoch SJ, et al. Adipokines, inflammation, and visceral adiposity across the menopausal transition: a prospective study. J Clin Endocrinol Metab 2009;94(4):1104-1110.
Lee CG, Fujimoto WY, Brunzell JD, et al. Intra-abdominal fat accumulation is greatest at younger ages

in Japanese-American adults. Diabetes Res Clin Pract 2010;89(1):58-64.
90. National Center for Veterans Analysis and Statistics: Veterans by State, Age Group, Period, Gender [internet]. 2007; http://www.va.gov/vetdata/Veteran_Population.asp.
91. Lewis MK, Blake GM, Fogelman I. Patient dose in dual x-ray absorptiometry. Osteoporos Int 1994;4(1):11-15.
92. Diabetes Care 2012 Jan; 35 Suppl 1: S11-63. Standards of medical care in diabetes – 2012.