

COVER PAGE FOR PROTOCOL AND STATISTICAL ANALYSIS PLAN

Official Study Title: Acarbose Anti-aging Effects in Geriatric Subjects
(Substudy B & C)

NCT number: NCT 02865499

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Unique Protocol ID: HSC20120304H

Protocol Template Form

Item 1 UTHSCSA Tracking Number	HSC20120304H
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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.
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Purpose/Objectives: Several drugs, including rapamycin (RAPA), an inhibitor of the mTOR pathway, and acarbose (ACA), a metabolic modulator, have been shown to extend the lifespan in laboratory rodents and also delay or reverse certain age-associated functional deficiencies. However, the effects in older animals often differ from those in a younger cohort. In order to determine whether these drugs will enhance healthy aging in older human subjects, a series of pilot studies is proposed where the underlying mechanisms and outcomes will be assessed with the focus being on physiological systems known to decline with aging.

Research Design/Plan:

Sub-study A Rapamycin Alone, or
Sub-study B Acarbose followed by Rapamycin, or
Sub-study C Acarbose Alone, or
Sub-study D Rapamycin Alone – Cardiovascular Effects

The effects of RAPA and/or ACA on cognitive, immune, physical, and other physiological systems known to be impacted with age will be investigated in a pilot study in a small number of elderly subjects. In the sub-studies using RAPA, a control group, matched for age and ethnicity, will be given a placebo (Sub-studies A&B; Sub-study D RAPA/no placebo). The study is designed such that a given subject will be enrolled in one of four sub-studies shown above as Sub-study A-D.

Methods: Subjects who meet the inclusion criteria and choose to participate in one of the sub-studies will be consented and will then undergo a set of standard tests to obtain baseline data on their current health status.

For Sub-study A Rapamycin Alone

Subjects will receive Rapamycin or placebo for approximately two months. We will assess many parameters to assess changes due to the drug (RAPA), particularly in physiological systems known to change with aging, and will also measure indicators of health to ensure safe delivery of RAPA in the elderly cohort. Specifically, the tests to be run include the following: immune response to challenge with the trivalent seasonal influenza vaccine effects on the gut microbiome, perception of quality of life and depression, grip strength, timed walk, cognitive assessment, blood chemistry, immune and endocrine health, and other assessments of safe RAPA delivery. Some subjects may elect to participate in an optional imaging procedure to evaluate cerebral blood flow and metabolic rate of glucose using PET and MRI.

For Sub-study B Acarbose followed by Rapamycin

Subjects will receive ACA orally for approximately 2 months. The battery of tests outlined above (except immunization) will be performed at three time points (pre-ACA, after 2 months on ACA, and 1 month post-ACA termination), to measure the effects of ACA on the gut microbiome, basic blood metabolic markers, blood cell distributions, physical function parameters (including walking speed and grip strength), cognitive abilities, depression, cytokine levels, and blood cell function. ACA will be terminated and all subjects will be rested for approximately 1 month and will then enter the RAPA phase of study; they will be randomly assigned to either RAPA or placebo and will be maintained on the appropriate drug for a total of approximately 2 months. During that time, we will assess response to vaccine challenges and to other physiological measures, including those listed above. Subjects may also elect to participate in optional imaging procedures to evaluate cerebral blood flow and metabolic rate of glucose using PET and MRI.

For Sub-study C Acarbose alone

Subjects will be followed as in substudy B, but the tests will also be performed 1 month after termination of ACA.

For Sub-study D Rapamycin Alone – Cardiovascular Effects

Subjects will receive Rapamycin for approximately two months. We will assess many parameters to assess changes due to the drug (RAPA), particularly in physiological systems known to change with aging, and will also measure indicators of health to ensure safe delivery of RAPA in the elderly cohort. Specifically, the tests to be run include the following: aortic pulse wave velocity to assess aortic compliance and laser-Doppler flowmetry to measure endothelial function; perception of quality of life and depression; cognitive assessment; grip strength, timed walk, blood chemistry and immune health, and other assessments of safe RAPA delivery. Subjects will also participate in cardiac and brain imaging procedures to evaluate cardiac function and cerebral blood flow using MRI. Subjects’ physical activity levels will be monitored by a wearable activity monitor throughout the study.

Clinical Relevance: Our long-term goal is to define the beneficial and detrimental effects of mTOR inhibition and/or metabolic modulation in the elderly.



Item 3 Background	
<p><i>Describe past experimental and/or clinical findings leading to the formulation of your study.</i></p> <p><i>For research involving unapproved drugs, describe animal and human studies.</i></p> <p><i>For research that involves approved drugs or devices, describe the FDA approved uses of this drug/device in relation to your protocol.</i></p>	<p><u>Background on rapamycin (RAPA)</u></p> <p>mTOR inhibition by RAPA was first shown to alter immune function in rodent models of induced autoimmunity. It was speculated that RAPA inhibited T cell proliferative responses. However, recent studies suggest that the drug’s effects are more complex, affecting both innate and adaptive immunity. The effects of mTOR inhibition on immune regulation and the control of T cell differentiation, migration, and expansion have been well studied. One of the most striking findings is an expansion of viral specific memory CD8 T cells. In addition, mTOR inhibition by RAPA appears to skew the CD4+ T cell compartment, with an expansion of suppressive regulatory (T_{REG}) cells and a decrease in the pro-inflammatory T_H1 and T_H17 subsets. This is consistent with the reported efficacy of mTOR inhibition in suppressing autoimmunity in rodents and in humans (type I diabetes, multiple sclerosis, etc.). Although mTOR inhibition by RAPA has been extensively tested in mice as a single agent, there are relatively few such studies in humans. Instead, most RAPA studies in humans have been conducted in solid-organ transplant patients taking multiple immunosuppressives (cyclosporine, tacrolimus, prednisone, etc) that complicate identifying mTOR inhibition by RAPA specific effects. Despite this complication, some surprising effects of mTOR have been reported. For example, although mTOR inhibition by RAPA is considered immunosuppressive, increased susceptibility to infection has <u>not</u> been found and immunity to certain pathogens, like CMV, may even be augmented by mTOR inhibition. Improved responses to other viral challenges have also been reported in transplant recipients.</p> <p>Similarly, no increases in neoplastic disorders have been reported with mTOR inhibition by RAPA, suggesting that tumor immunity is not further reduced and may even be improved by RAPA. Indeed, mTOR inhibition by RAPA appears to suppress or even regress skin cancer in humans. These results are consistent with the well-documented enhancement of viral-specific memory CD8+ T cell immunity in animals. Positive effects of mTOR inhibition by RAPA on autophagy, dendritic cells, and antigen processing also appear to enhance responses to intracellular bacteria. Where tested, humoral immunity is maintained during mTOR inhibition by RAPA, i.e., the addition of RAPA standard immunosuppressive therapy did not reduce the efficacy of influenza vaccination in solid organ</p>

transplant patients. Moreover, addition of RAPA correlated with improved responses in a lung transplant cohort and with broader antibody reactivity in a study of liver and kidney recipients.

Significance It has been suggested that mTOR inhibition by RAPA may slow the progression of aging even if treatment were initiated relatively late in life. However, concerns about RAPA-induced immune alterations remain as there are no human studies that directly assess cognitive, immune, and physiological effects in elderly individuals who are not being treated with other immunosuppressive or anti-proliferative drugs. If successful, this study will provide insights and critical pilot data to facilitate future grant applications to support a large-scale clinical study.

PRELIMINARY DATA

Thus far in our studies, no unanticipated clinically significant effects have occurred. Importantly, the oral glucose tolerance tests (OGTTs) revealed no RAPA-induced diabetogenic effects. Similarly, no changes were noted in any other clinical laboratory, immune, cognitive, physical performance, or self-perceived health status measures. Based on these substudy findings, RAPA can be used safely in older persons who are otherwise healthy.

Effects of mTOR inhibition by RAPA on humoral immunity An age-associated decrease in humoral (antibody) responses has been reported for many different antigens in both humans and mice. As we published, this loss of immune competence is particularly acute when the antigen has never before been encountered by the responding individual. To assess whether mTOR inhibition by RAPA could reverse the age-associated loss of immune competence, mice were fed diets containing RAPA or control without RAPA for 7 months; they were then immunized with bacteria. The mice were bled 2 weeks later and ELISAs performed to measure anti-bacterial antibody titers. As shown in Figure 1, RAPA had no effect on antibody titers elicited in young mice. However, in older animals, where the immune responses were expectedly lower, some RAPA treated mice showed higher responses. **These data provide support for our hypothesis that mTOR inhibition will improve vaccine efficacy in the elderly.**

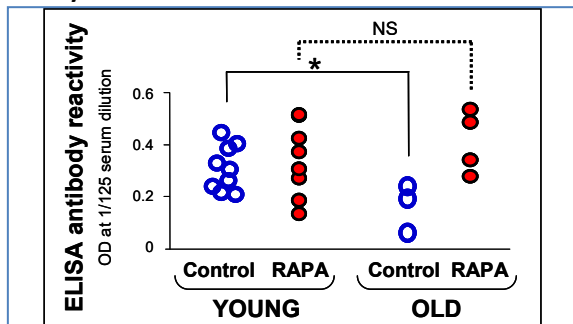


FIG 1. RAPA enhances the antibody response to bacterial challenge, but only in old mice. Young (2 month) and old (20 month) mice were placed on diets containing encapsulated rapa (RAPA, ●) or control (capsular material, ○) for 7 months and were then immunized with bacteria. The mice were bled 2 weeks post challenge and ELISAs were performed to measure anti-bacterial antibody titers. [Data from Benavides & Kraig]

Effects of mTOR inhibition by RAPA on parameters of immune health in mice: Since inhibitors of mTOR are pleiotropic, affecting many immune parameters, we opted to focus on biomarkers that are also implicated in aging. In order to ask whether RAPA reverses or exacerbates phenotypes associated with immunosenescence, studies have already been undertaken in mice through the auspices of the NIA-funded GO grant (RC2 AG 036613-01; A. Richardson, PI) by Drs. Curiel and Kraig. These data are summarized in Table 1. Of particular interest are markers (like baseline inflammation) which increase in response to one condition (aging) while decreasing in response to the other (RAPA). The ratio of naïve to memory T cells is also affected in opposite directions by aging and RAPA. **These murine data provide the rationale for parameters to be studied in Aim III.**

Immune parameter	Effect of aging	Effect of RAPA	Expected outcome
INNATE IMMUNITY			
Baseline inflammation	↑	↓	Could reverse age-associated inflammation
ADAPTIVE IMMUNITY			
Naive humoral response	↓	Varies with age & antigen	Has not been tested in elderly humans
Ratio of naïve/memory T cells	↓	↑	Could enhance responses in elderly
T cell activation by antigen	↓	No change seen	
T _{REG} cells	Mixed results	No major change	No rapa-induced immune suppression
T _H 1 and T _H 17	T _H 1 ↓ and T _H 17 ↑	increased changes in the same direction for both	↓ Th1 will reduce inflammation ↑ Th17 good for tumor immune surveillance
PD-1 (negative signaling)	↑mice; humans unclear	↓	Would improve beneficial T cell functions

TABLE 1. Summary of immune parameters impacted by aging and/or RAPA; as studied in the Curiel and Kraig labs

Discoveries typically begin at the bench with basic research and are then translated to the clinical level. A major unanswered question is whether mTOR inhibition by RAPA would be harmful or beneficial in humans. Studies of a variety of physiological functions in rodents have found no evidence for negative effects of mTOR inhibition by RAPA or related rapalogs agents, and in a few cases, e.g., grip strength and cognition, mTOR inhibition by RAPA may have actually improved function.

The age-related decline in physical function by loss of muscle strength negatively impacts health outcomes in older people by increasing the risk of falls, hospitalization, disability, morbidity, and mortality all of which carry significant health-care cost. An association between general health status and muscle function in relation to muscle mass and has been noted in healthy older adults. Interventions that improve muscle function provide benefits to both physical and emotional well-being of older individuals. Can mTOR inhibition by RAPA do the same? Furthermore, mTOR inhibition has shown promise against memory impairment and β -amyloid deposition in transgenic mouse models of Alzheimer's disease. Can mTOR inhibition by RAPA or rapalogs do the same in humans?

It was recently reported that 16 weeks of RAPA treatment in symptomatic AD mice increased cerebral blood flow (CBF) through nitric oxide (NO) dependent mechanisms. RAPA treatment also restored brain vascular density, reduced cerebral amyloid angiopathy and microhemorrhages, decreased amyloid burden, and improved cognitive function. Based on these findings, RAPA appears to be a promising agent to ameliorate cognitive dysfunction in elderly humans.

As noted above, pre-clinical studies in animals show that RAPA has beneficial effects on lifespan and health span. More importantly, RAPA increases CBF in laboratory animals and improves cognitive dysfunction in AD animal models. Despite these findings, the potential for adverse reactions to RAPA has limited investigations in healthy human subjects such that our current knowledge remains incomplete, especially for clinical application in older humans. Most knowledge of mTOR antagonist use in humans is derived from studies of patients with serious chronic medical problems such as solid organ transplant patients or cancer patients who also receive multiple agents that confounding results. We have found oral RAPA to be safe in humans who are in the 'oldest old' (over 85) age group; a group with the greatest burden of age-related disease. If RAPA ameliorates age-related disease, this age group is likely to benefit more than younger age groups. We will administer RAPA to healthy elderly (70-95 year old) persons who in good health, with all medical conditions well controlled and clinically stable.

While it will not be possible to directly evaluate the long-term anti-aging (lifespan, healthspan, physical performance) actions of RAPA because subjects will be treated for 4 months (Substudy D is 2-3 months), we have designed this study as an initial step to generate information concerning potential beneficial and adverse effects of RAPA. We have preliminary data that suggests physical performance (walking speed) and cognition are improved in the 'oldest old' with RAPA. We propose to expand our studies of oral RAPA treatment to a larger group of healthy older adults and to explore the vascular effects of RAPA on brain and peripheral vascular beds. The findings from this study will provide important preliminary data for a dedicated clinical trial of RAPA in older subjects.

Background on acarbose (ACA)

The NIH-funded Interventions Testing Program (ITP) was developed to assess the potential of drugs, delivered as dietary supplements, to extend lifespan in mice. In 2009, the ITP reported that rapamycin (RAPA) was efficacious in enhancing longevity, even if treatment was initiated late in life. Since RAPA is an immune modulator whose effects can vary with age, we undertook a preliminary clinical trial in healthy elderly human subjects. Our early pilot data suggested improvements in cognition, physical function, and immunity [Kellogg, Kraig, Chiodo, Curiel]. However, given scientific concerns about the potential immunosuppressive side effects of RAPA, we were intrigued by a recent publication in which the ITP showed that another drug, acarbose (ACA), was even more efficacious at extending lifespan, at least in male mice. ACA is an FDA-approved α -glucosidase inhibitor with a long history of clinical use in management of diabetes with an excellent safety profile that is far superior to RAPA.

To investigate ACA as a potential anti-aging therapy for use in humans, we propose a pilot study of ACA in elderly subjects. Our goals are to: i) obtain preliminary data to better estimate power for a larger trial, ii) establish a safe dosing strategy for ACA in non-diabetic elderly humans, iii) determine whether positive ACA outcomes seen in diabetics (i.e., improved endothelial function) are also seen in older non-diabetic subjects, iv) assess whether ACA can reverse age-associated deficits in cognition, physical function, and immunity, and v) determine whether ACA alters the gut microbiome as such changes could impact longevity and immune competence. For ACA, such a translation into geriatric medicine would be relatively straight-forward as it is FDA approved with a low incidence of side effects.

Our current understanding of ACA's effects primarily derives from patient-based trials in diabetics that report reductions in weight, improved cardiovascular function, and other health benefits. Only a few trials of ACA have been done in non-diabetics and these report beneficial weight reductions. In elderly diabetics (average age \approx 70) ACA was found to be safe and moderately effective in reducing hyper-glycemia. These elderly patients experienced ACA side effects similar to younger groups: primarily flatulence. We propose to assess ACA safety and potential anti-aging benefits in elderly non-diabetics.

Our early preliminary data demonstrate that RAPA at 1 mg/day is well tolerated by elderly humans and hypothesized adverse events (diabetes, immunosuppression, etc.) have not been observed. While these results indicate that RAPA use in healthy elderly persons is not problematic, we have found suggestions of improved cognitive, immune, and physical function. *This leads us to seek objectively measurable outcomes likely to show RAPA benefit. We have concluded that the cardiovascular parameters of aortic compliance and endothelial function answer our quest.*

SubStudy D - Background and Significance – Rapamycin Alone – Cardiovascular Effects

RAPA as a modulator of age-related cardiovascular dysfunction. Aging is associated with reduced compliance of large elastic arteries and endothelial dysfunction due to reduced nitric oxide (NO) bioavailability. Recent work by Lesniewski, et al (10) showed that 6-8 weeks of RAPA treatment improved compliance in elastic vessels and NO-dependent endothelial function in old (30mo) mice. RAPA treatment improved aortic compliance in old mice through decreasing collagen and advanced glycosylation end-products (AGEs) in the aorta without changes in intima-media thickness or aortic elastin content while improved endothelial function was effected by improved NO bioavailability

through decreased arterial oxidative stress. Their results are consistent with our unpublished findings that topical RAPA decreases collagen levels in keloids and improves NO-dependent endothelial function in older humans. The foregoing results suggest that increased mTOR activation may underlie reductions in large elastic artery compliance, structural adaptations in the vessel wall, and endothelial dysfunction due to vascular oxidative stress with advancing age. The finding that RAPA treatment reversed these deleterious cardiovascular changes suggests that such treatment holds promise for the treatment of large arterial as well as arteriolar aging and thus the potential prevention of age-associated CVD in humans. We will explore the role of mTOR in these age-associated vascular dysfunctions, by examining the impact of mTOR antagonism with RAPA on aortic PWV and NO-dependent endothelial function in cutaneous arterioles in elderly humans.

With aging, systemic inflammation increases and likely contributes to the development of age-associated pathologies including cardiovascular diseases (CVDs) (3, 7, 14). CVDs are primarily diseases of the arterial system and are associated with reduced large artery compliance and reduced endothelial function. Recent work in old (30mo) mice showed that both large vessel compliance and nitric oxide (NO) dependent endothelial function improved with 6-8 weeks of RAPA treatment (10). Subjects will undergo imaging procedures to evaluate cardiac function and cerebral blood flow using MRI.

To test whether the same RAPA effects occur in elderly humans (age 70-95), we propose to add noninvasive measurements of the following cardiovascular and physical activity variables::

- Aim D-1 aortic pulse wave velocity (an index of large vessel compliance)
- Aim D-2 heat induced arteriolar vasodilation (an index of NO-dependent endothelial function)
- Aim D-3 cardiac and brain MRI to measure diastolic function and cerebral blood flow
- Aim D-4 physical activity monitoring (an index of changes in activity, sleep, and calorie expenditure) *Importantly, this would be beneficial through validation of the CV measures in human subjects.*

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

Purpose and Aims for Sub-study A & B – Rapamycin

Rapamycin (RAPA), an inhibitor of the mTOR pathway, is used clinically as an immunosuppressive drug in preventing transplant rejection and with drug eluting stents to limit coronary restenosis. When tested in mice, RAPA was shown to extend lifespan, even if treatment was initiated relatively late in life. The drug was shown to delay, but not alter, the range of illnesses at death and also attenuated the age-related decline of spontaneous activity suggesting that it increased healthspan. Rapamycin (RAPA) extends lifespan in laboratory animals and has been shown to delay the onset of several age-related diseases; however, clinical administration of RAPA has been reported to effect hyperglycemia and potentially new onset diabetes in human solid organ transplant patients. Given that the human population has a high prevalence of obesity and age-related insulin resistance, extrapolation of this observation to the human population in general has raised concerns as to whether RAPA administration in healthy humans could lead to diabetes. Human clinical studies that report RAPA-induced hyperglycemia have focused on transplant patients with pre-existing conditions with multiple medical problems who receive complex pharmacological immunosuppressive treatments. No long-term studies of the effects of RAPA in non-transplant patients who are otherwise healthy have been done, although in our experience RAPA treatment of up to 4 months does not lead to hyperglycemia in healthy older persons age 80-95. Given that it is unclear whether the previously mentioned transplantation-associated hyperglycemic risks of RAPA are directly to RAPA per se or confounding factors in transplant patients, we propose to add oral glucose tolerance tests (OGTT) to our ongoing RAPA trial in healthy older persons to clarify this issue. Our long-term goal is to define the beneficial and detrimental effects of mTOR inhibition in the elderly. Using subjects (70-95 years old) available through the Geriatric Evaluation and Management Clinic (GEM) at the STVHCS (South Texas Veterans Health Care System), we are uniquely positioned to assess the effects of mTOR inhibition in vivo on health status, vaccine efficacy, and on parameters of immune health. We hypothesize that RAPA will improve immunity by reducing the immune dysfunction associated with increasing age. Expected outcomes are decreased inflammation and enhanced vaccine-mediated immunity.

Aim I. Assess mTOR inhibition safety in elderly human subjects, 70-95 years of age.

Prior human studies of mTOR inhibition by RAPA have focused on subjects with underlying health issues (transplant recipients, patients with cancer or autoimmunity, etc.) so RAPA was often used in combination with other immunosuppressive or anti-proliferative drugs. Thus, we propose to assess whether RAPA can be safely delivered to an otherwise healthy, but elderly test population. Towards this end, subjects will be placed on mTOR inhibition by RAPA (Rapamune, Wyeth/Pfizer) and an equal number on placebo. Their health status will be assessed by: i) physical examination, ii) clinical blood work iii) weekly self-reporting of complications to the clinical nurse, and iv) use of both a depression scale and the SF36 questionnaire. Participants will remain on drug therapy (either Rapa or placebo) for the entire ~2 months of the RAPA study.

Aim II. Assess the effects of mTOR inhibition on vaccine efficacy in the elderly.

Humoral immune responses decline with advancing age in humans as evidenced by decreased vaccine efficacy and increased susceptibility to infection. Thus, to determine whether mTOR inhibition by RAPA alters the age-associated loss in immune competence, study participants will be immunized with the seasonal flu vaccine (subjects will not have had prior exposure to the composition used). Both humoral (antibody titer) and cellular (T cell proliferation) responses will be assessed. In addition, the subjects will receive a second immunization to test effects on memory B (antibody) and T cell responses.

Aim III. Determine whether mTOR inhibition can improve general immune features in the aged

mTOR inhibition's effects on immune parameters characteristically altered by aging will be assessed. These include: i) serum cytokine levels (IL-6, TGF- β , etc.) as indicators of increased inflammation, ii) the naive to memory T cell ratio that decreases with age due to thymus involution and memory expansion; and iii) T cell functional subsets as assessed by fluorescence-activated cell sorting (FACS) for cell surface markers and cytokine measures.

Aim IV. Determine whether mTOR inhibition can improve muscle strength and cognitive function

Measurements of handgrip strength, timed walking, and cognition will be assessed. The objective of this sub-study is to assess the short-term effect of rapamycin in healthy age 70-95 subjects on parameters that assess physical function and allow us to assess whether this agent has anti-aging, health-span promoting effects in humans. Our limited goal would be to test the hypothesis that mTOR inhibition improves muscle

strength and cognition, consistent with improving health span. It was reported that 16 weeks of RAPA treatment in symptomatic AD mice increased cerebral blood flow (CBF); RAPA also restored brain vascular density, reduced cerebral amyloid angiopathy and microhemorrhages, decreased amyloid burden, and improved cognitive function. Based on these findings, RAPA appears to be a promising agent to ameliorate cognitive dysfunction in elderly humans.

Aim V. Assess the effects of RAPA on the gut microbiome. The microbiota is known to change with aging and this likely contributes to increased inflammation in older individuals. RAPA also likely alters the microbiome, potentially reversing the aberrant effects of age; this will be examined before and after RAPA administration.

[Purpose and Aims for Sub-study B & C – Acarbose](#)

Treatment with acarbose (ACA), an α -glucosidase inhibitor, was shown to extend median lifespan by 22% in male mice. Since ACA is FDA-approved for use in humans and has been extensively employed for the management of diabetic symptoms, largely without adverse outcomes, it has been posited that acarbose could be efficacious as a safe anti-aging therapeutic. However, the effects of ACA treatment could be differentially affected by age, so it is imperative to test ACA directly in older individuals (aged 70-95 years old). Thus, we propose to perform a small pilot study assessing the effects of ACA on systems known to be impacted by aging (endothelial function, cognition, muscular, and immune). In addition, since oral ACA is likely to impact the gut microbiome with downstream effects on inflammation and other aging phenotypes, the microbiota will be assessed in these elderly subjects before, during, and after treatment. *This pilot study will directly test our hypothesis that ACA treatment could be used in elderly humans to slow the progression of increased inflammation and age-associated pathologies due, at least in part, to changes in the gut microbiome.*

Aim VI. Develop a safe protocol for testing ACA in elderly non-diabetic human subjects. Subjects will be recruited from the VA Geriatrics Clinics and placed on a regimen of ACA. Safety of treatment will be assessed by i) physical examination, ii) clinical blood work, iii) self-reporting, and iv) SF36 questionnaires.

Aim VII. Ask whether ACA can ameliorate age-associated functional deficits. Participants will be tested prior to ACA initiation, during treatment, and post drug withdrawal for the following parameters: **physical function** (grip strength and walking speed), **cognition** (EXIT, SLUMS, TAPS), and **immunity** (cytokine profiles, T_{REGS} numbers, the CD4/CD8 T cell ratio, and naïve/memory T cell ratios).

Aim VIII. Assess the effects of ACA on the gut microbiome. The microbiota is known to change with aging and this likely contributes to increased inflammation in older individuals. ACA also likely alters the microbiome, potentially reversing the aberrant effects of age; this will be examined longitudinally.

[Purpose and Aims for Sub-study D – Cardiovascular Function with Rapamycin](#)

With aging, systemic inflammation increases and likely contributes to the development of age-associated pathologies including cardiovascular diseases (CVDs) {Franceschi, 2014 #2611;Howcroft, 2013 #2610;Michaud, 2013 #2612}. CVDs are primarily diseases of the arterial system and are associated with reduced large artery compliance and reduced endothelial function. Recent work in old (30mo) mice showed that both large vessel compliance and nitric oxide (NO) dependent endothelial function improved with 6-8 weeks of RAPA treatment {Lesniewski, 2017 #2605}. RAPA has also been shown to attenuate the age-related decline of spontaneous activity suggesting that it increased healthspan {Miller, 2011 #1350}. Similar effects on activity were reported in dogs treated with RAPA {Urfer, 2017 #2617}.

Subjects will be recruited from the VA Geriatrics Clinics, posted study flyers in community-based private practice offices or senior centers, UT Health Find A Study website, and or newspaper advertisements. Interested candidates will be directed to contact the Barshop Clinical Research Call Center at (210) 450-0020. Potential candidates may be contacted by phone from the study team for phone screening after verbal consent is provided. Once the study team establishes contact with a potential candidate who then understands the basics of the study and if desired, interested subjects may receive a copy of the informed consent form by US Postal Service for review prior to the screening appointment. (Email to VA subjects is prohibited.)

Aim D-1. Test whether RAPA increases aortic compliance in elderly subjects. Aortic pulse wave velocity (PWV) will be measured before and during 8 weeks of RAPA administration. Applanation tonometry will be used to record peripheral waveforms from the carotid and femoral arteries simultaneously with electrocardiogram (ECG) recording to measure PWV. Typically carotid and femoral recordings are performed on the right side of the body, but if necessary may be done on the left.

Aim D-2 Test whether RAPA improves endothelial function in elderly subjects. NO-dependent endothelial function will be measured by monitoring the cutaneous arteriolar vasodilation with laser-Doppler flowmetry (LDF) in response to local warming of the skin from 34°C to 39°C and 42°C. Measurements will be made before, during, and at the end of 8 weeks of RAPA (Table 2).

Aim D-3. Examine whether mTOR inhibition improves diastolic heart function and increases cerebral blood flow in humans.

Does mTOR inhibition improve diastolic function and/or increase cerebral blood flow in 70-95 year olds? This portion of the project will explore whether mTOR inhibition with RAPA increases cerebral blood flow (CBF) in mouse models of Alzheimer's Disease (AD) and improves cognition (1). Also, rapamycin has been shown to improve diastolic heart function in aging animal models {Chiao, 2016 #2623;Urfer, 2017 #2617}. Our preliminary data in 'oldest old' humans (age 89-93) and a published pilot study (1) suggest similar cognitive effects in humans. We will examine these RAPA effects in older humans (age 70-95). Based on the foregoing observations, we hypothesize that RAPA effects on heart function, the CBF as well as cognitive and physical function are beneficial. We will test our hypothesis in 70-95 year old humans treated with RAPA for 8 weeks by addressing whether systemic RAPA increases CBF in elderly humans. MRI will be used to measure EF, SV, LV volumes, peak LV filling rate, and the E/A wave ratios of flow through the mitral valve. Cardiac data will be normalized to BSA. CBF also will be measured by MRI. Measurements will be made before RAPA is administered, as well as after completion of RAPA administration. Each subject will be his own control in this design.

Aim D-4. Examine whether mTOR inhibition increases levels of activity in humans.

In previous mouse studies, rodents (males) treated with RAPA demonstrated increased physical activity. We hypothesize that RAPA will increase activity levels in humans. Activity data will be collected using a Garmin Vivofit3. We will examine effects on overall activity (time, distance, step count, calories burned, total hours of sleep, periods of movement, and periods of restful sleep) {O'Brien, 2015 #2716;Schrack, 2016 #2715;Šimůnek, 2016 #2714}

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<p>Item 5 Study Population(s) Being Recruited</p> <p>In your recruitment plan, how many different populations of prospective subjects do you plan to target? Provide number: 1</p>	<p>Identify the criteria for inclusion:</p>	<p>Identify the criteria for exclusion:</p>
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<p><i>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.</i></p> <p>List each different population on a separate row and provide a short descriptive label: <i>(e.g., normal-healthy, diabetics, parents, children, etc.)</i></p> <p><i>To add rows use copy & paste</i></p>		
<p>normal, healthy 70-95 years old; Veterans and non-veterans (All sub-studies)</p> <p>In Sub-study D we will enroll males only, 70-95 years old, veterans and non-veterans.</p>	<ul style="list-style-type: none"> • Since this is a small pilot study, we have opted to limit subject heterogeneity by focusing on 70-95 years of age. • Subjects will be in good health with all chronic diseases (hypertension, coronary artery disease, etc.) clinically stable. Selected subjects will be in good health (Per the World Health Organization good health will be defined as complete physical, mental, and social well-being and not merely the absence of disease or infirmity. • For our purposes all diseases or infirmities will be clinically stable whether managed by medications or not. • All ethnicities will be included. • For cardiac and brain imaging by MRI, a pre-MRI screening questionnaire will be used to assess MRI safety and neurological health. 	<p>Exclusion criteria include:</p> <ul style="list-style-type: none"> • Received current season influenza vaccine (exclusion for substudy A, B, C*; *NOT used in SubStudy D) • Diabetes, (*Substudy D - with A1c ≥ 6.5 or if treated with medication affecting glucose homeostasis History of skin ulcers or poor wound healing, • Smoking, • Liver disease, • Coumadin anti-coagulation, • treatment with drugs known to affect cytochrome P450 3A (diltiazem, erythromycin, etc) due to its role in RAPA metabolism • Treatment (>30days of therapy or long term) with a systemic immunosuppressant (prednisone, etc.) within the last year. • Patients with history of recent (within 6 months) Myocardial Infarction or active Coronary Disease. • Patients with history of recent (within 6 months) intestinal disorders • Exclusion criteria for MRI scan: known claustrophobia, metal implants in soft tissue of the body including pacemakers, aneurysm clips, ferrous metal fragments not anchored to bone (bullets, BBs, shrapnel, metal shavings), implanted medication pumps, and oral-facial metal appliances that are permanently secured but may result in low image quality. Participants may also be excluded for history of severe head trauma, brain injury, brain surgery, inflammation of the brain, or history of seizures.

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:**Methods for Sub-study A & B Rapamycin**

mTOR inhibition: Subjects will be randomized to mTOR inhibition by RAPA or placebo in equal sized groups. Those receiving the drug will be started on 1 mg per day with no loading dose. Dosage will be adjusted for a target whole blood concentration of 10-15 ng/ml and maintained there until the study's conclusion. RAPA blood levels will be determined by mass spec. The initial trough level will be drawn one week after initiation of therapy. When a maintenance dose is adjusted, the new dose will be maintained for 7 days before additional adjustments are made based on concentration monitoring. Reported adverse reactions to RAPA and related agents when used as solitary agents include mucositis, pneumonitis, skin rash, hyperlipidemia, hyperglycemia, thrombocytopenia, and anemia.

Clinical assessment: Subject health will be confirmed initially by history and physical exam, normal laboratory tests (fasting lipid profile, metabolic profile, CBC, and CK), and 12-lead EKG. 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). At each visit, patients will receive a standard history, physical examination, blood chemistry assessment, and be queried in detail for RAPA adverse reactions including infections. A questionnaire (SF-36) designed to assess the benefits of health care interventions and the Geriatric Depression Scale questionnaire will allow us to characterize any adverse or beneficial effects. Between visits, subjects will be contacted weekly by the Clinical Nurse to ensure that no complications have arisen. Blood test for CK will be monitored.

If subjects have previously undergone all or part of screening labs/tests within the past 4 months, the data may be accepted for eligibility determination without screening labs or procedures having to be repeated.

Aim II. Assess the effects of mTOR inhibition on vaccine efficacy in the elderly.

Rationale: One serious consequence of immune senescence is the age-associated loss of responsiveness to immune challenges. When examined in humans, mTOR inhibition by RAPA did not appear to decrease vaccine efficacy, but this is difficult to conclude given that these subjects were on combination therapies for serious underlying health issues (recent transplant or cancer). Thus, using the cohort described in Aim I, we have a unique opportunity to ask whether mTOR inhibition by RAPA will enhance immunity in otherwise clinically stable elderly individuals.

Immunization: We chose a vaccine that is commonly given to the elderly and was previously reported to show small improvements in a study of mTOR inhibition in adults performed by Novartis (Mannick, et al.). The study will be timed such that none of our subjects will have already been immunized with that season's trivalent influenza vaccine although it is likely that they will have been previously immunized with other flu variants and/or previously had flu infections. We will compare pre-immune and post-immune data to control for the heterogeneity in the population. Typically, the seasonal flu vaccine contains three different influenza viruses determined each year by the CDC based on variants predicted to dominate in the US during. A flu shot is considered standard of care for individuals in this age group and the composition used will be the one recommended by the VA; it is approved for humans. Standard adult dosing of Trivalent influenza vaccine will be initiated 1½ months after RAPA treatment begins and it will be given only once, as recommended.

Outcome measures and expected results: Subjects will return to the clinic for blood collection as indicated on Figure 2; both humoral (antibody titer) and cellular (T cell proliferation) responses to influenza antigens will be assessed. Effects of mTOR inhibition by RAPA on B cell responses: Following each immunization (at day 7 and 14), 5 ml of blood will be collected and serum prepared. The titer of anti-flu virus antibodies in these specimens will be determined by ELISAs using standardized kits (Alpha Diagnostics Inc). If our hypothesis were correct, the primary responses would be low in these elderly subjects with higher levels in individuals on RAPA.

Effects of mTOR inhibition by RAPA on influenza virus-specific T cells: Age associated deficiencies are also seen for cellular (T-cell mediated) responses. This is likely due to a loss of naïve T cell production (a consequence of thymus

involution), coupled with an increase in memory T cells due to antigenic exposure. Thus, it is also critical to ask whether RAPA will compensate for losses in T cell immunity with age. Towards this end, 25 ml blood will be collected 7 days after each immunization. Peripheral blood mononuclear cells (PBMCs) will be harvested and stimulated *in vitro* in triplicate with serial dilutions of recombinant flu viral antigens or with control stimuli [PHA or anti-CD3/CD28 (positive controls) and vehicle (negative control)]. Proliferation and cytokine production in response to the test stimulus are measured to determine both the extent of T cell activation and the nature of the T cell response (i.e. T_H1, T_H2, T_H17). The effects of mTOR inhibition on T cell maturation in response to an immune challenge have not been rigorously investigated and have never been tested in elderly humans. If our hypothesis were correct, RAPA would enhance antigen-specific T cell proliferation while suppressing overall proliferation to PHA or anti-CD3/CD28 stimuli.

Aim III. Determine whether mTOR inhibition can improve general immune features in the aged

Rationale The effects of mTOR inhibition are pleiotropic and the consequences of treatment may be different in young immunologically-competent individuals than in elderly subjects with age-associated immune dysfunctions (Fig. 1). Thus, we will examine several parameters of immune health in the mTOR inhibition by RAPA and placebo controlled subjects to assess whether the drug has mitigated the effects of aging. Importantly, we will be able to correlate improved humoral responses (Aim II) with changes in specific immune parameters measure in Aim III.

Immune parameters assessed and expected results We will focus on parameters known to change with age (Table 1) using blood collected after approximately 1, 6, and 8 weeks on RAPA or placebo, the following will be assessed and compared to levels determined prior to initiation of the treatment: i) Serum cytokine levels; Luminex-based assays will be used; these allow simultaneous measurement of 28 different cytokines. It is expected that RAPA will have the beneficial effect of decreasing the levels of pro-inflammatory cytokines (TGF- β , IL-12, IL-6, TNF- α). ii) Naive T cells are lost with aging due to thymus involution and a lifetime of clonal expansion of memory T cells. In mice, RAPA appears to protect these naive T cells which could then allow for a healthier immune response to “new” antigens in older individuals. To assess whether the same is seen in humans, PBMCs will be analyzed by FACS to assess the proportion of memory vs. naive T cells both before and after RAPA treatment. iii) T cell functional subsets will be assessed by FACS for cell surface markers and intracellular cytokines. Although expectations are less clear here, it will be important to assess whether RAPA has effects on human T cell development that are analogous to those seen in rodents. Moreover, these measurements have not previously been ascertained for human subjects in this advanced age group.

Aim IV. Determine whether mTOR inhibition can improve muscle strength and cognitive function

The following tests will be performed to assess RAPA affects on physiological parameters:

- 1) Grip strength (3 consecutive measurements by grip-strength dynamometer): participants will be seated in a chair with the forearm at a 90° elbow bend and 3 consecutive grip strengths will be completed with a standard grip strength dynamometer (in Kg) for the dominant hand. Results will be averaged for analysis.
- 2) Timed 40-foot walk: each participant will perform 3 walks (timed with a stopwatch) at their preferred walking speed over a measured 40-foot path. Results will be averaged for analysis.

Statistical comparisons will be made between measurements made before and after, as well as both within and between mTOR inhibited and placebo groups to test our hypothesis.

Cognitive assessment: All subjects will undergo cognitive assessments within one week of their recruitment (before treatment) and at the end of treatment (approximately 2 months after entering the RAPA phase of the study). The cognitive tests will be selected from a battery of the following validated tests – EXIT, SLUMS, and/or TAPS – with care taken to avoid use of ones employed in the ACA phase (as appropriate) to limit the effects of “learning/memory”.

Results of the foregoing (grip strength, timed walk, and cognitive assessment scores) will be compared between groups.

Aim V. Assess the effects of RAPA on the gut microbiome.

Rationale Composition of the gut microbiota is known to change with age and this most likely contributes to increases in inflammation seen in older individuals. Given its broad effects on metabolic regulation and its oral delivery route, RAPA is likely to alter the gut microbiome and may thereby elicit changes in the host immune system (addressed in Aims II and III). In order to address this possibility, we will collect fecal specimens from the RAPA and placebo treated subjects at 4 time points, prior to RAPA treatment and after 1, 6, and 8 weeks on the drug. The gut microbiome will be

characterized initially from the pre-treatment and 8 week treatment samples. The other fecal specimens will be stored for future analysis of the kinetics of interesting changes observed.

Approach The advent of next generation DNA sequencing has revolutionized efforts to characterize the endogenous microbiome. An oral medication, like RAPA, will likely target the gut microbiome. Thus, subjects will self-collect feces in sterile containers and deliver it to the clinic at their regular visits. DNA isolation will be performed by the Genomics Core facility on a fee-for-service basis and the samples will be delivered to the Sequencing Core Facility for analysis. The bacterial rDNA in each fecal sample will be amplified using primers from conserved regions of the 16S ribosomal RNA genes. Amplified DNAs are then used to generate a library which is subsequently subjected to deep (next gen) sequencing.

Sub-study B & C Acarbose

ACA delivery: After baseline data are collected, ACA treatment will be initiated in all subjects. For the first week, the dose will be 50mg once daily at supper-time. This will be increased to 50mg twice daily (breakfast and supper) during the second week and 50mg with each meal (breakfast, lunch, supper) during the third week. By the fourth week, the full dose of 100mg with each meal will be taken. Subjects will be instructed to take their pills at the beginning of a meal.

Clinical assessment: Subject health will be confirmed initially by history and physical exam, normal laboratory tests (fasting lipid profile, fasting blood sugar, complete metabolic profile, CBC), and 12-lead EKG. 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). At each visit, patients will receive a standard history and physical examination and be queried for any adverse reactions. A questionnaire (SF-36) will be administered before and after ACA treatment to assess patient perceived health effects and the Geriatric Depression Scoring system will be used to assess any changes in this parameter. Between visits, subjects will be contacted weekly to ensure that no complications have arisen. If the highest dose were not well tolerated by a given subject, we would then drop down to 50mg ACA three times a day, but they would remain in the study. At this lower dose, gastrointestinal issues have been reported in fewer than 6% of subjects.

If subjects have previously undergone all or part of screening labs/tests within the past several months, the data may be accepted for eligibility determination without screening labs or procedures having to be repeated.

Subjects that have completed taking *placebo* in SubStudy A and pass other necessary screening evaluations may be enrolled to SubStudy C.

Approach Subjects will be studied at 3 time points: pretreatment, after 2 months on ACA, and 1 month after termination of the drug. Thus, each subject will serve as his own control and no placebo group is needed for the ACA phase.

Physical function will be assessed using two independent assays of parameters known to decline with age, handgrip strength and timed 40-foot walk. Performance measures will be done longitudinally at each clinic visit to offer the greatest potential to detect changes due to ACA treatment and subsequent withdrawal.

1) **Grip strength** (3 consecutive measurements by grip-strength dynamometer): participants will be seated in a chair with the forearm at a 90° elbow bend and 3 consecutive grip strengths will be completed with a standard grip strength dynamometer (in Kg) for the dominant hand. Results will be averaged for analysis.

2) **Timed 40-foot walk:** each participant will perform 3 walks (timed with a stopwatch) at their preferred walking speed over a measured 40-foot path. Results will be averaged for analysis.

Cognitive function will be assessed within one week of subject recruitment (before treatment), and at the end of treatment (2 months). These tests will be performed only twice to avoid learning effects that could confound the results. Three independent tests will be employed: EXIT (includes letter fluency) (13), SLUMS (St. Louis University Mental Status exam, which includes a memory test, digit span, and animal fluency) (14), GDS (Geriatr Depression Scale) and TAPS (Texas Assessment of Processing Speed; a digit/symbol coding test available in alternate forms to eliminate learning effects) (15). If significant improvement were seen with one (or more) of the cognitive measures, that test (or

those tests) would be repeated at the end of the withdrawal period to assess longevity of the response. In addition, we will assess depression using the Geriatric Depression Score questionnaire.

CLOX Assessment: clock-drawing tests (CDT) as a screen for cognitive impairment at screening.

Immune function Blood will be collected from each subject before treatment and after two months on ACA. General parameters of immune health that are known to change with age will be assessed, as described below:

1) **Serum cytokine** levels will be determined using Luminex-based assays that allow simultaneous measurement of 27 different cytokines. It is known that aging is associated with an increase in the levels of pro-inflammatory cytokines (IL-12, IL-6, TNF- α), which contributes to the loss of immune regulation with age and increased susceptibility to certain pathogens. ACA reversal of this effect would be highly significant.

2) **T_{REGs}** have been shown by us and others to increase with age. These cells function to suppress ongoing immune responses and their presence in older individuals correlates with diminished immunity (19). To address whether ACA can reverse this increase in T_{REGs}, PBMCs (peripheral blood mononuclear cells) will be analyzed by FACS to assess the proportion and number of CD4⁺CD25⁺foxp3⁺ cells (T_{REGs}). Future experiments (beyond this pilot grant) would then be needed to examine T_{REG} function and expression profiles.

3) **CD4/CD8 T cell ratio** is a parameter known to change with aging and HIV infection. Using flow cytometry of PBMCs, the ratio of CD4⁺ T cells / CD8⁺ T cells will be determined before, during, and after ACA.

4) **Naive T cell numbers** decline with age due to thymus involution and a lifetime of clonal expansion of memory T cells. This contributes to the loss of immune competence leading to decreased efficacy of vaccines and increased susceptibility to infection in the elderly. The loss of naïve T cells among PBMCs will be analyzed by FACS at the 5 time points (before, during and after treatment) for both CD4⁺ helper T cell (T_H) and naïve CD8⁺ cytotoxic T cell (T_c) subsets. ACA induced recovery of naïve T cells would be significant.

Expected Results Statistical comparisons will be made between the pre-treatment time point and the post-treatment point (2 months on ACA). It is our hope that the ACA-induced extension of lifespan in mice will be echoed by improvements in all of the parameters measured which were selected due to their known deficiencies with aging. This would suggest simultaneous effects on three systems: immune, neuromuscular, and cognitive.

Aim VII. Assess the effects of ACA on the gut microbiome.

Rationale Composition of the gut microbiota is known to change with age and this most likely contributes to increases in inflammation seen in older individuals. Given its function in gastrointestinal metabolism, ACA is also likely to alter the gut microbiome; this may contribute to the beneficial effects of ACA on diabetic symptoms since it is known that changes in the resident microbes can impact both immune and endocrine functions. Although there were no searchable publications reporting characterization of the effects of ACA on the gut microbiome, there is a 2012 listing on *ClinicalTrials.gov*, entitled "Efficacy of acarbose on intestinal microbiome and incretins of Type 2 diabetes" from Dr. Ning, Shanghai Jiao Tong University School of Medicine; no results have been posted to-date. Also, an abstract from the University of Alabama, Birmingham (Dr. Brewer) reports on gut microbiome changes in mice treated with ACA [<http://www.uab.edu/medicine/camac/images/5-Abstract.pdf>]. Our hypothesis is that ACA will result in improvement of functions known to decline with age (immune, endothelial, cognitive, and neuromuscular). We further speculate that changes to the microbiome induced by ACA may play a role in mediating these pleiotropic effects. In order to address this possibility, we will collect fecal specimens from the ACA cohort and characterize the gut microbiome at the 2 time points prescribed above [pretreatment and after 2 months on ACA].

Approach The advent of next generation DNA sequencing has revolutionized efforts to characterize the endogenous microbiome. In fact, these genomic-based technologies have revealed many new bacterial species that were missed using microbiologic and serologic approaches. The endogenous microbiota plays a critically important role in generating an efficacious immune system and a healthy functioning gut. Of relevance to this proposal, the microbiome also regulates susceptibility to obesity and diabetes. An oral medication, like ACA, will likely target the gut microbiome. Thus, subjects will self-collect feces in sterile containers and deliver it to the clinic at their regular visits. DNA isolation will be performed by the Genomics Core facility on a fee-for-service basis and the samples will be delivered to the Sequencing Core Facility for analysis. The bacterial rDNA in each fecal sample will be amplified using primers from conserved regions of the 16S ribosomal RNA genes. Amplified DNAs are then used to generate a library which is subsequently subjected to deep (next gen) sequencing.

Expected Results For humans, there have been many reports documenting the bacterial species that dominate in older vs. young individuals (24). Given the ages of our subjects, we would expect to see a profile more similar to the “old” profile. It would be most exciting if ACA were to alter the microbiome, making it more “youthful”.

Sub-study D Rapamycin Alone – Cardiovascular Effects

For Aims D-1, D-2, D-3, and D-4:

Intervention with RAPA will begin at the end of Visit 2 once all screening and baseline measurements have been collected. Subjects will self-administer RAPA once daily for a period of 8 weeks (56 doses) and there will be no placebo group in this Sub-study. We will screen 12 MALE subjects to complete 6. No females will be enrolled in this pilot substudy.

Clinical assessment: See also Sub-study A (except without OGTT). In substudies A, B and C, no detrimental diabetogenic effects of RAPA were found, i.e. OGTT, FBS, HbA_{1c} were unaltered by RAPA.

If subjects have previously undergone all or part of screening labs/tests within the past 4 months, the data may be accepted for eligibility determination without screening labs or procedures having to be repeated.

Physical function will be assessed using two independent assays of parameters known to decline with age, handgrip strength and timed 40-foot walk. Performance measures will be done longitudinally at Visits 2, 3, and 4 to offer the greatest potential to detect changes due to RAPA treatment.

1) **Grip strength** (3 consecutive measurements by grip-strength dynamometer): participants will be seated in a chair with the forearm at a 90° elbow bend and 3 consecutive grip strengths will be completed with a standard grip strength dynamometer (in Kg) for the dominant hand. Results will be averaged for analysis.

2) **Timed 40-foot walk:** each participant will perform 3 walks (timed with a stopwatch) at their preferred walking speed over a measured 40-foot path. Results will be averaged for analysis.

3) **Activity** will be continuously monitored in participants by wearing a Garmin Vivofit3 Activity Monitor. This battery operated, waterproof activity monitor will be worn continuously over the period of enrollment to record participant activity (steps per day, periods of sleep). The Garmin Vivofit and similar devices have been used successfully in other studies and have been found to be acceptable to older volunteer subjects {O’Brien, 2015 #2716;Schrack, 2016 #2715;Šimůnek, 2016 #2714}.

Immune function will be assessed pre-treatment and at the end of treatment (Visits 1 and 4). We will perform tests for serum cytokine profiles, flow cytometry with T cell subsets, and T cell activation potential.

Cognitive function will be assessed before treatment at Visit 2, and at the end of treatment (2 months, Visit 4). These tests will be performed only twice to avoid learning effects that could confound the results. Three independent tests will be employed: EXIT (includes letter fluency) (13), SLUMS (St. Louis University Mental Status exam, which includes a memory test, digit span, and animal fluency) (14), GDS (Geriatric Depression Scale) and TAPS (Texas Assessment of Processing Speed; a digit/symbol coding test available in alternate forms to eliminate learning effects) (15). If significant improvement were seen with one (or more) of the cognitive measures, that test (or those tests) would be repeated at the end of the withdrawal period to assess longevity of the response. In addition, we will assess depression using the Geriatric Depression Score questionnaire.

Aim D-1. Test whether RAPA treatment increases aortic compliance in elderly subjects.

Approach. PWV will be measured before, during, and at the end of RAPA treatment as per Table 2. Measurement of aortic PWV is the simplest way to assess arterial compliance non-invasively in humans and reflects the ability of the arterial system to cope with systolic ejection volume. It also implies that the compliant arterial system’s major function is to relay cardiac contraction during diastole. Indeed, cardiac contraction lasts only one third of a cardiac cycle of the time. Due to the distension of the compliant large arteries during systole, after the end of cardiac contraction, the compliant large arteries return to their initial dimension and thereby help maintain blood flow. This phenomenon is of key physiological importance as diastolic flow in arteries may represent more than half of cardiac output. Large artery compliance represents the dynamic force opposing ejection (also called impedance): if high, it helps in ejection, the lower the compliance, diastolic facilitation of ejection is lessened. Many methods have been proposed to quantify this phenomenon. The simplest non-invasive approach to measure arterial compliance is PWV, i.e., the transit time measurement of the pulse wave from the aortic valve to the terminus of the aorta (1). We will use the SphygmoCor

system, which has been used in successful large clinical trials and population surveys. This device uses a large band piezoelectric probe and allows the arterial pulse recording in succession (carotid then femoral), both signals being synchronized with the same time basis (ECG R wave) (1). We have such a device available and are experienced with its use in humans. To measure PWV, the subject will rest in supine position for at least 5-10 min to reach the physiological baseline conditions, the room will be maintained at a standardized temperature, and the blood pressure will be accurately measured within minutes from the PWV recording times. Serial longitudinal measurements are performed as summarized in Table 2. All PWVs will be done in fasting condition. Overall, the reproducibility of PWV measurements is good (CV in the range of 8-12%) (1).

Expected Results-There are many reports documenting that the PWV is greater in older vs. young humans (1). Given the ages of our subjects, we expect to see a pattern of initially high PWV before RAPA treatment that would be reduced with RAPA. In the placebo group, we expect that their initially high PWV will be unaltered or increase during the study.

Aim D-2. Test whether RAPA treatment improves endothelial function in elderly subjects.

Approach. It is well known that microvascular endothelial dysfunction precedes dysfunction in conduit arteries and is predominantly due to reduced NO bioavailability in both aging and disease states. Choi, et al (2), have developed microvascular function test that reflects primarily NO-dependent vascular function and is thus of significant clinical utility. This test uses rapid local heating of forearm skin from 34°C to 39°C to produce a hyperemic vasodilation (an increase in cutaneous vascular conductance (CVC=LDF/MAP)) that is 80% dependent on NO. This is the most robust test of NO-dependent dilation that can be performed non-invasively in humans, as the multifold increase in conductance from 34°C to 39°C is almost entirely effected by NO. Due to the noninvasiveness and ease of performing the test, we will use rapid local heating to 39°C to assess NO-dependent dilation and thus microvascular function. Rapid local heating to 39°C elicits a plateau which reaches 50% of maximal CVC as effected by local warming to 42°C, which allows for assessment of interventions that may alter maximal vasodilatory mechanisms that are NO-independent. Upon arrival in the laboratory, subjects will be placed in a semi-supine position in a dialysis chair and be instrumented on their right forearm for laser-Doppler flowmetry measurements of skin blood flow (SkBF) with a LDF probe equipped with a local heating unit and thermocouple. A Finapres cuff placed on one finger for blood pressure measurements (MAP). Local skin temperature (Tloc) at the LDF site will be held at 34°C for approximately 5-10 minutes to establish a stable baseline measurement. Tloc will be then increased at a rate of 0.1°C/sec to 39°C and then held constant. After LDF has reached a stable plateau (30–40 min) at Tloc=39°C, Tloc will be raised to 42°C for about 30 min to maximally vasodilate the skin (9, 15, 17). For data analysis, CVC will be calculated. Averages of maximal absolute values of CVC (in mV) with Tloc=34, 39, and 42°C will be calculated and compared both within and between subjects by repeated measures ANOVA. All of the needed equipment is available in Dr. Kellogg's lab.

Expected Results. It is well known that NO-dependent vasodilation is reduced in older vs. young individuals (5, 6). We expect that CVC responses to skin warming to 39°C will increase both during and after RAPA when compared to pre-RAPA responses.

****Depending on availability of subjects, shared lab equipment, and personnel resources, the vascular function assessments for baseline measurements of PWV and or LDF may be scheduled and performed at Visit 1 OR Visit 2 (see Table 2). This allows flexibility for the subject and facilitates protocol compliance for the research team.**

MRI Studies of Cardiac function and Cerebral Blood flow

Aim D-3. Examine whether mTOR inhibition improves diastolic function and increases cerebral blood flow in humans. **We** will explore whether mTOR inhibition with RAPA improves cardiac diastolic function and cerebral blood flow (CBF). Diastolic function and CBF will be measured by MRI **pre- and post-treatment with RAPA at Visits 2 and 4.**

Expected Results-There are reports that the cardiac function and CBF are improved in animal models by RAPA treatment {Chiao, 2016 #2623;Urfer, 2017 #2617}. Given the ages of our subjects, we expect to see a pattern of initially impaired diastolic function and CBF before RAPA treatment that would be improved with RAPA.

Aim D-4. Examine whether mTOR inhibition increases physical activity in humans.

Expected Results-RAPA treatment increases physical activity in animal models. {Miller, 2011 #1350;Urfer, 2017 #2617} . We anticipate that similar activity increases will be found in humans with RAPA administration.

Pilot data will be collected on at least 6 subjects to analyze whether treatment with RAPA increases physical activity. Using a Garmin Vivofit3 Activity Monitor [Garmin, Inc.] provided by the study to each participant. we will examine whether RAPA effects overall activity by measuring time, distance, step count, calories burned, total hours of sleep, periods of movement, and periods of restful sleep. Subjects will wear activity monitors continuously for approximately 2 weeks from consent to baseline visit at which we will download pre-intervention activity levels. Subjects continue wearing bracelet-style activity monitors throughout the study duration and upon returning to the clinic (4 weeks and 8 weeks of RAPA treatment), provide data downloads at each of the Visits 3 and 4. Participants will keep the activity tracking devices after the study is complete as a thank-you for their participation {O'Brien, 2015 #2716;Schrack, 2016 #2715;Šimůnek, 2016 #2714}.

Data Analysis Plan:

This is a pilot study designed to produce the data necessary for future larger studies. No data are currently available to guide sample size calculations.

OVERALL EXPERIMENTAL DESIGN The collection of samples and performance of procedures for Substudies A, B and C will typically be performed as indicated below**. Also, V1 may be combined with V0 if subject arrived fasting.

The collection of samples and performance of procedures for Substudy D will typically be performed as indicated in Table 2 on the following page.. However, at the discretion of the PI, some collections may be done at a visit before or after the one shown.

Table 1. Substudy A Rapamycin Only study visits 0, 1, 7-10; **Substudy B ACA+RAPA** study visits 0-10; **Substudy C ACA Only** study visits 0- 6 (as summarized in table below). **TABLE 1 DOES NOT APPLY TO SUBSTUDY D****

Visit number	V0	V1	V2	V3	1V4	1.5V5	2V6	3V7	4V8	5V9	6V10
Visit goal	Consent	Screening	Pre-ACA	ACA – 1 month	ACA – 6-7 wks	ACA ends	Post ACA2 month	termination	7 day RAPA	6 wks RAPA Safety	Imm
Study Phase	A,B,C	A,B,C	B,C	B,C	B,C	B,C	B,C	A,B	A,B	A,B	A,B
Week number (of phase) (time variable between visits 3 and 4)			0	4	6-7	48	12	0 (for RAPA)	1	6	8
Timing (days) – range				25-35 days after V2	38-45 days after V2	50-70 days after V2	25-35 days after V5	25-35 days after visit 2	6-9 days after V7	40-45 days after V7	13-15 days after V9
PROCEDURES											
Consent form	X			X							
EKG		X		X							
Physical exam & history; Safety measures: AE's and problems		X	X	X	X	X	X	X	X	X	X
Clinical blood tests, include:				X			X	X	X	X	X
Complete blood count		X	#			X	X	#	X	X	X
Metabolic profile		X	#			X	X	#	X	X	X
Fasting blood sugar; HbA1c		X	#			X	X	#	X	X	X
Fasting lipid profile		X	#			X	X	#	X	X	X
Urinalysis								x			x
OGTT		X			X		X		x	X	
Acarbose treatment begins			X		X						
Acarbose treatment ends						X	X				
RAPA or placebo begins								X	X		
SF-36 questionnaire			X		X	X	X	X	X		X
RAPA levels									X	X	X
Blood draw for lab, includes:				X			X	X	X	X	X
Cytokine profiles			X	X		X	X	X	X	X	X
Blood cell subsets by flow			X	X		X	X	X	X	X	X
T cell subsets			X	X		X	X	X	X	X	X
T cell proliferation to mitogen			X		X	X	X	X	X	X	X
Collect feces			X		X	X	X	X	X	X	X
Constipation Assessment			X	X	X	X	X	X	X	X	X
Immunizations: flu vaccine										X	X
T cell prolif to flu antigens									X	X	X
Antibody to flu antigens									X	X	X

Hand grip and 40 ft walk test*			X	X		X	X	X	X	X	X
Cognitive tests: EXIT/SLUMS and/or TAPS*			X	X		X	X	X	X		X
GDS (Geriatr Depression Scale)*			X	X		X	X	X	X		X
CLOX Assessment (cognitive assessment)*		X		X							
PET/MRI								O	O		O
Notes: X=scheduled procedure; O=optional procedure (if funding found and subject consents) *May be done over Visit 0 and Visit 1; #=procedure performed if > 2 weeks from prior measure at V1											

Table 2. VISIT SCHEDULE: Sub-study D. Rapamycin Alone – Cardiovascular Effects

VISIT NUMBER	V1	V2	FU1	V3	FU2	V4
Visit goal	Consent/ screen (fasting)	Pre-drug (not fasting)	Phone follow up*	4 wks RAPA	Phone follow up*	8 wks RAPA
Week number		0	1	4		8
PROCEDURES	2-2.5h	3-3.5		2.5-3		4
Consent form and CLOX>10	✓					
SAFETY/AEs/METABOLIC						
Physical exam	✓	✓		✓		✓
ECG	✓					✓
Self reported Adverse Events (AEs)	✓	✓	✓	✓	✓	✓
CBC (blood cell counts)	✓			✓		✓
Fasting blood sugar/CMP	✓			✓		✓
Fasting lipids	✓					✓
HbA1c	✓					✓
Urinalysis	✓					✓
IMMUNE FUNCTION						
Serum for cytokine profiles	✓			✓		✓
Flow cytometry: T cell subsets	✓			✓		✓
T cell activation potential	✓			✓		✓
RAPAMYCIN LEVEL						✓
Meal/snack	✓					✓
COGNITIVE FUNCTION						
Geriatric depression scale		✓				✓
EXIT, SLUMS, TAPS		✓				✓
VR-36 QOL		✓				✓
PHYSICAL FUNCTION						
Hand grip and 40 foot walk		✓		✓		✓
Garmin VivoFit3 Activity Monitor	DISPENSE	BRING*		BRING*		BRING*
DISPENSE STUDY MED		✓		✓		
VASCULAR FUNCTION						
Aortic Pulse Wave Velocity (PWV)	✓**	✓**		✓		✓
Arteriolar Endothelial Function (LDF)	✓**	✓**		✓		✓
CARDIAC/CEREBRAL BLOOD FLOW FUNCTION						
MRI		✓				✓
Schedule MRI - Date						

*Telephone follow up of subjects to assess tolerability and or adverse reactions to RAPA will be conducted at least once between V2 and V3. If tolerating well after 1-2 weeks of RAPA treatment without side effects, no further calls may be necessary. If side effects are reported at any time during RAPA administration, the investigator may choose to modify dosing, if appropriate, and follow by phone on a weekly basis until resolved.

BRING* – At Visits 2, 3 and 4, subjects will bring their Garmin VivoFit3 Activity Monitor so that activity data can be downloaded to a protected server with adequate storage and backup.

**Depending on availability of subjects, shared lab equipment and personnel resources, the vascular function assessments for baseline measurements may be scheduled and performed at Visit 1 OR Visit 2. This allows flexibility and promotes protocol compliance.

Item 7

Risks Section. Risks are described in detail in the Step 2-Institutional Form.