Title

Early or Late-Life Treatment With Acarbose or Rapamycin Improves Physical Performance and Affects Cardiac Structure in Aging Mice

Journal

The Journals of Gerontology Series A, 78(3)

ISSN

1079-5006

Authors

Herrera, Jonathan J

Pifer, Kaitlyn

Louzon, Sean

et al.

Publication Date

2023-03-01

DOI

10.1093/gerona/glac221

Peer reviewed

THE GERONTOLOGICAL SOCIETY OF AMERICA®

Journals of Gerontology: Biological Sciences
cite as: *J Gerontol A Biol Sci Med Sci*, 2023, Vol. 78, No. 3, 397–406
https://doi.org/10.1093/gerona/glac221
Advance Access publication November 7, 2022

OXFORD

Original Article

Early or Late-Life Treatment With Acarbose or Rapamycin Improves Physical Performance and Affects Cardiac Structure in Aging Mice

Jonathan J. Herrera, PhD,[1] Kaitlyn Pifer, BS,[2] Sean Louzon, BS,[1] Danielle Leander, BS,[2] Oliver Fiehn, PhD,[3,•] Sharlene M. Day, MD,[4] Richard A. Miller, MD, PhD,[2,5] and Michael Garratt, PhD[6,*]

[1]Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, Michigan, USA. [2]Department of Pathology, University of Michigan Medical School, Ann Arbor, Michigan, USA. [3]Genome Center, University of California Davis, Davis, California, USA. [4]Department of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA. [5]Geriatrics Center, University of Michigan, Ann Arbor, Michigan, USA. [6]Department of Anatomy, School of Biomedical Sciences, University of Otago, Dunedin, New Zealand.

*Address correspondence to: Michael Garratt, Department of Anatomy, School of Biomedical Sciences, University of Otago, PO Box 56, Dunedin 9054, New Zealand. E-mail: Mike.Garratt@otago.ac.nz

Received: March 8, 2022; Editorial Decision Date: October 18, 2022

Decision Editor: Rozalyn M. Anderson, PhD, FGSA

Abstract

Pharmacological treatments can extend the life span of mice. For optimal translation in humans, treatments should improve health during aging, and demonstrate efficacy when started later in life. Acarbose (ACA) and rapamycin (RAP) extend life span in mice when treatment is started early or later in life. Both drugs can also improve some indices of healthy aging, although there has been little systematic study of whether health benefits accrue differently depending on the age at which treatment is started. Here we compare the effects of early (4 months) versus late (16 months) onset ACA or RAP treatment on physical function and cardiac structure in genetically heterogeneous aged mice. ACA or RAP treatment improve rotarod acceleration and endurance capacity compared to controls, with effects that are largely similar in mice starting treatment from early or late in life. Compared to controls, cardiac hypertrophy is reduced by ACA or RAP in both sexes regardless of age at treatment onset. ACA has a greater effect on the cardiac lipidome than RAP, and the effects of early-life treatment are recapitulated by late-life treatment. These results indicate that late-life treatment with these drugs provide at least some of the benefits of life long treatment, although some of the benefits occur only in males, which could lead to sex differences in health outcomes later in life.

Keywords: Antiaging, Mice, Rapamycin, Acarbose

Aging is an independent risk factor for several clinically significant conditions in humans, including cardiovascular disease, atrial fibrillation, and heart failure (1). Accumulation of diseases and physical deficits with age can decrease life span, diminish healthspan, and reduce the period of life over which an organism remains healthy and free of age-related disease (2,3).

Targeted antiaging interventions, including changes in diet, exercise, and drugs, can increase life span (4) and maintain health into older ages (5) in mice. Two drugs that have repeatedly been shown to extend mouse life span in both sexes across multiple institutions and at varying doses are acarbose (ACA) and rapamycin (RAP). Both treatments have also been shown to improve several indices of healthy aging in animal models, including aspects of physical function and the underlying cardiac system. The starting points for ACA and RAP treatment initiation, however, have varied in previous studies, and there have been few direct, controlled comparisons of the relative effects of treatments when started at different periods of life.

ACA is a glucosidase inhibitor that slows carbohydrate digestion and reduces postprandial blood glucose concentration (6). ACA treatment (1 000 ppm) started at 8 months of age extends male UM-HET3 life span by 16%–17%, and female life span by

5% (7), with similar changes in median life span also observed at a higher treatment dose (2 500 ppm) (8). An additional study has shown that increased life span can be achieved with ACA treatment started in midlife at 16 months of age, although effects are smaller, with male life-span extended by 6% and female by 2% (9). ACA has also been reported to have a range of functional health benefits in mice during aging. Harrison et al. reported that rotarod acceleration performance was improved after training in 22-month-old female mice treated with ACA from 8 months, although performance was not improved significantly in males (8). In a subsequent article, Herrera et al. reported that ACA treatment started at 4 months of age improved rotarod acceleration, rotarod endurance, and grip strength in mice of both sexes when tested at 22 months (10). Thus, there are some discrepancies in how ACA treatment influences physical function in old mice, in particular, the effects on rotarod testing capacity, and whether these health effects differ depending on when treatment begins. ACA treatment beginning at 4 months of age prevents age-related ventricular hypertrophy, alters cardiac lipid metabolism, and ameliorates age-associated changes in certain cardiac lipid species, including lysophospholipids, phospholipids, and triacylglycerides (TAGs) (10). It has been suggested that changes in lipid handling and cardiac function could contribute to improved health and life-span extension (10).

RAP is an inhibitor of the mechanistic target of rapamycin (mTOR) complex, an intracellular signaling complex that has a central role in the regulation of growth and aging. Treatment with RAP at 42 ppm started at 20 months of age increases mean life span by 9% in male mice and 14% in female UM-HET3 mice (11). The same dose of RAP (42 ppm) started at 9 months of age increased the median life span by 23% in male and 26% in female UM-HET3 mice (12). Tests of RAP's effects on physical function during aging have produced varying results, although different mouse strains and treatment periods have been used in different studies. When male C57BL/6J mice were provided RAP treatment (42 ppm) at 4 months, 13 months, or 20–22 months of age for a 1 year period, no improvement in physical function on the accelerating rotarod was observed, nor were age-associated cardiac structural or functional changes seen with RAP treatment (13). In contrast, other studies of late-life RAP treatment beginning at 19 months of age have shown improvements in physical function in C57BL/6NIA mice when tested 6–12 months later, including improved rotarod acceleration capacity but not grip strength (14). Studies of late-life RAP treatment have also focused specifically on cardiac structure and function, showing reversal and attenuation of age-related structural changes in the heart of female mice when treated with RAP for 2–3 months from 2 years of age (15,16). This was associated with a major change and reversal of aging of the heart proteome (17), although changes in the cardiac lipidome with RAP, as observed with ACA treatment, have not previously been assessed.

Given the variation in the onset of ACA and RAP treatment regimes and different findings associated with each of the previous paradigms, the primary aim of our study was to compare to untreated animals the effects of ACA (1 000 ppm) or RAP (42 ppm) treatment, with mice beginning these drug treatments at either 4 months or 16 months of age. All mice were assessed at 22 months of age, with mice beginning treatment at 4 months thus undergoing 18 months of treatment, while those beginning at 16 months receiving a 6-month treatment period. We also included both male and female mice in this analysis, as is strongly required in preclinical research and required by the National Institutes of Health, to secondarily determine whether the effects of these treatments were consistent across both sexes. UM-HET3 mice were from a single large cohort and were randomized to the different time course and drug treatment groups, allowing for direct contrasts of time course and treatment effects. We evaluated physical function at 22 months of age, particularly focusing on rotarod acceleration and endurance measures, because these tests assess exercise capacity and global motor function in different contexts (18,19) and have previously been shown to be influenced by both drug treatments. We also assessed changes in cardiac structure and underlying metabolic parameters, given the importance of the cardiac system to functional capacity and healthy aging (20)

Method

Animals and Diets

The mice used in this study were UM-HET3 mice, the progeny of CByB6F1/J (JAX stock number 100009) dams and C3D2F1/J (JAX stock number 100004) sires (21). Breeding mice received a Purina 5008 mouse diet. Weaned mice from these breeders were subsequently fed Purina 5LG6. Mice were housed as previously described (12) in ventilated plastic cages with metal tops and corn cob bedding (Bed O'Cobs, Andersons Lab Bedding), and transferred to fresh cages every 14 days. Mice were provided ad libitum access to food and water. Housing room temperature was maintained within a range between 21°C and 23°C.

Mice in this study were part of a large cohort that were produced under the University of Michigan Glenn Center Funding initiative to provide tissues for investigators interested in understanding cellular changes that underlie life-span extension in response to drug treatment. All diets were prepared and obtained from TestDiet Inc. (Purina Mills, Gray Summit, MO). ACA was obtained from Spectrum Chemical Mfg. Corp. and used at a concentration of 1 000 mg of ACA per kg of diet (1 000 ppm). Encapsulated RAP was obtained from Emtora Biosciences and used at a concentration of 42 mg per kg of diet (42 ppm). The doses used in the ACA and RAP diets have been previously demonstrated to extend the life span of UM-HET3 mice (22,23). Mice received either ACA or RAP diet starting at 2 different ages. Group housed mice from different cages were randomly assigned at weaning to early-life ACA (n = 24 per sex), late-life ACA (n = 12 per sex), early-life RAP (n = 24 per sex), late-life RAP (n =12 per sex), or to the control group (n = 48 per sex). An additional cage of male animals was allocated to the late-life RAP treatment group, giving this group a total sample size of 15. Animals were randomly assigned to groups, although a larger number of animals were allocated to control and early-life treatment groups because it was expected that tissues from these groups would be requested more frequently by researchers (eg, researchers would always need tissues from control animals but may be only interested in comparing the effects of early-life RAP treatment, the most commonly studied antiaging drug intervention).

At 4 months of age, early-life treatment groups were switched to a diet containing either ACA or encapsulated RAP added to 5LG6. At 16 months of age, late-life treatment groups were switched to a diet containing ACA or encapsulated RAP added to 5LG6. Controls remained on an 5LG6 diet. Prior to sampling at the end of the study (at 22 months of age), some mice died (control females: 2; control males: 7; RAP early life males: 2; RAP early life females: 1; RAP late life males: 2). Some mice were also removed from the study due to fighting or because they were used by other investigators (male controls: 24;

female controls: 10; male 4-month ACA: 7; male 16-month ACA: 1; male 4-month RAP: 7; male 16-month RAP: 2; female 4-month ACA: 6; female 16-month ACA: 2; female 4-month RAP: 9; and female 16-month RAP: 0). The final sample size available for testing at 22 months in each group was: male controls (n = 17), male 4-month ACA (n = 17), male 16-month ACA (n = 11), male 4-month RAP (n = 15), male 16-month RAP (n = 13), female controls (n = 36), female 4-month ACA (n = 18), female 16-month ACA (n = 10), female 4-month RAP (n = 14), and female 16-month RAP (n = 12).

Physical Function Capacity Tests

Rotarod physical function testing for each cohort occurred on 2 separate days, with a rest day in between the acceleration and endurance tests. The testing was conducted by trained individuals who were blinded to group assignments. All animals available for each treatment group were tested with sample sizes provided in Figure 1. Rotarod testing was performed using a Ugo Basile 47650 Rota-Rod NG (Ugo Basile Gemonio, Italy) as previously described (24). Mice were randomly assigned to lane position on the rotarod. For acceleration capacity testing, the initial speed was set to 5 rpm, and the rotarod was accelerated at 0.1 rpm/s. Fall latency was recorded over 3 trials with a 1 minute rest interval between each trial. Average fall latency over the 3 trials was used for analysis. For endurance capacity testing, the rotarod speed began at 5 rpm and was accelerated at 0.1 rpm/s over 15 minutes until the speed reached the group-specific average speed achieved during acceleration testing (16 rpm). From this point, mice could then continue for an additional 15 minutes for a total potential run time of 30 minutes. Mice were allowed up to 10 falls within the first 15 minutes and up to 2 falls in the remaining 15 minutes. Fall latency was scored once the mouse fell more than the permitted criteria.

Animal Dissections, Body Weights, and Heart Weights

Mice were dissected between the hours of 0900–1200 following 18 hours of fasting. Following carbon dioxide euthanasia, each mouse was weighed. A cardiac puncture was then performed to extract whole blood. Whole blood was transferred into a Ethylenediaminetetraacetic acid (EDTA)-lined serum collection tube, after which the sample was spun in a 4°C centrifuge at 1000 RPM. Plasma was then removed and stored at −70°C. Hearts were cleaned in a series of phosphate buffered saline baths to remove excess blood and then weighed using an analytical balance. Heart weight data were collected for as many animals from each treatment group as possible, with exact sample sizes provided in Figure 2. Hearts were then dissected into component left ventricles (LV) and right ventricles (RV; septum included in LV). These specimens were weighed, frozen in liquid nitrogen, and then stored in Eppendorf tubes at −70°C. The LV was used for the lipidomics analysis.

Lipidomics

The LV was sent to the NIH West Coast Metabolomics Center at the University of California at Davis for lipidomics processing and analysis, as previously described (10). An untargeted analysis for complex lipids via charged surface hybrid column–quadrupole time of flight mass spectrometer (CSH-QTOF MS/MS) was conducted at the center. For lipid analysis of heart tissue, we included 8 samples per sex per treatment.

Plasma Assays

TAG and glucose concentrations were measured using FujiFilm Wako Chemical LabAssay kits for mouse plasma (Chesterfield County, VA). Insulin concentration was measured using a Crystal Chem Ultrasensitive Mouse Insulin ELISA Kit (Elk Grove Village, IL). Insulin-like growth factor 1 (IGF1) concentrations were measured using Mouse/Rat ALPCO Elisa kit (Boston, MA). Approximately 10 samples per sex, per treatment group were assessed for plasma parameters, with exact sample sizes provided in Figure 5.

Statistics

A 2-way analysis of variance (ANOVA) model was used for each main analysis (using IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY: IBM Corp, SPSS Inc., Chicago, IL), including a 5-group treatment factor (Early-life ACA, late-life ACA, Early-life RAP, late-life RAP, control), a factor for sex (male or female), and an interaction term between treatment and sex. Including sex as the main effect in this analysis allowed for any overall differences between males and females in the variable to be accounted for (ie, males have a bigger heart weight). Including the interaction term also allowed for any differing responses to treatments according to sex (25). We further included 4 planned contrasts of specific treatment group comparisons, contrasting each treatment group to control animals, using the planned contrast function in SPSS. If a significant interaction between treatment and sex was detected, we then conducted further analysis between each treatment group and the control group to explore whether a specific treatment response was contributing to the presence of a sex-specific effect. We then also conducted 2 additional exploratory tests (1 within males and 1 within females) with a 2-tailed Student's t test, to determine if a specific treatment effect within 1 sex was the cause of the interaction effect. For lipidomic data, raw p values from 2-way ANOVAs on each lipid species were adjusted with the Benjamini-Hochberg False Discovery Rate (FDR) method (26), as previously conducted in the context of other drug treatments with sex-specific life-span effects (27). Data were inspected to ensure that it conformed to parametric assumptions and logarithmically transformed when necessary. Lipidomic data were transformed logarithmically before analysis.

Results

Early Life or Late-Life Treatment With ACA or RAP Improve Functional Capacity in Mice

ACA and RAP treatment can extend life span in mice and improve indices of physical function that decline with age. We tested whether physical function would differ with ACA or RAP treatment when mice were tested at 22 months of age and evaluated whether the outcomes depended on whether the treatment was started in early adult life (at 4 months of age) versus middle age (at 16 months of age). We first evaluated rotarod acceleration capacity, which tests the ability of a mouse to remain on a continuously accelerating rotarod. This test assesses exercise capacity, global motor function, balance, and motivation (18,19) over a short duration (up to 6 minutes), and we have previously shown the duration that mice can stay on the rotarod declines by 22 months of age (10). There was an overall significant difference between the 5 treatment groups (main effect of treatment in a 2-way ANOVA with 5 treatment groups: $p < .001$; including an additional factor for sex: $p = .062$ and an interaction between sex and treatment: $p = .084$). ACA treatment beginning at either 4 months of age (planned contrast to control group: $p < .001$) or 16 months of age (planned contrast to control group: $p < .001$) resulted in a significant increase in rotarod acceleration capacity compared to controls

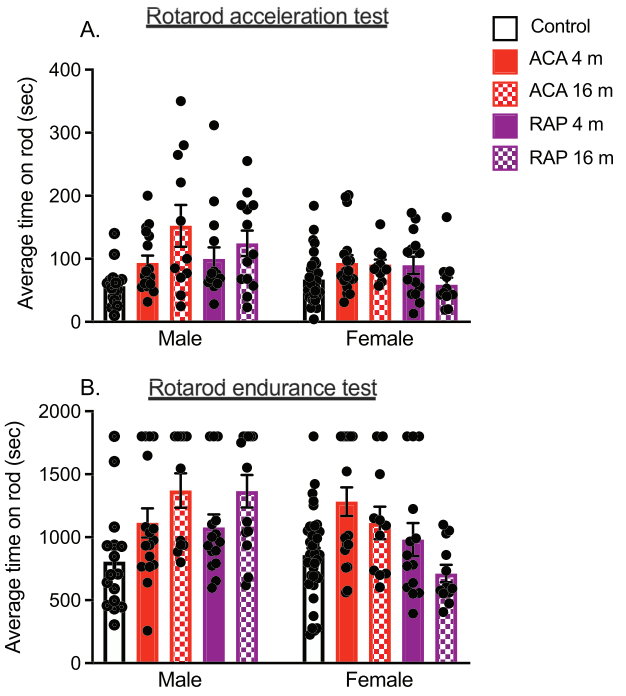

Figure 1. Effects of early and late-life ACA and RAP treatment on physical function capacity. The capacity to remain on a continuously accelerating rotarod (Acceleration Capacity: **A**), and the capacity to remain on a rotarod at a fixed, submaximal velocity (Endurance Capacity: **B**), were assessed in UM-HET3 mice at 22 months of age following the treatment period. Data are presented as mean ± SEM. Group numbers: male controls (n = 17), male 4-month ACA (n = 17), male 16-month ACA (n = 11), male 4-month RAP (n = 15), male 16-month RAP (n = 13), female controls (n = 36), female 4-month ACA (n = 18), female 16-month ACA (n = 10), female 4-month RAP (n = 14), and female 16-month RAP (n = 12). ACA = acarbose; RAP = rapamycin; SEM = Standard Error of the Mean.

(Figure 1A; Supplementary Table S1). Treatment with RAP beginning at 4 months of age also significantly increased rotarod acceleration capacity (contrast to control group: p = .007) and a similar but nonsignificant trend (contrast to control group: p = .051) was observed in mice treated with RAP beginning at 16 months of age (Figure 1A; Supplementary Table S1).

As an additional measure of physical function, we tested whether early or late-life ACA or RAP treatment improved rotarod endurance capacity. Rotarod endurance capacity is a separate functional test that assesses endurance by evaluating the ability of a mouse to remain on a rotarod moving at a continuous, submaximal velocity (10,19) for an established duration of time (up to 30 minutes). Endurance capacity measured in this test also declines with age in mice (19). There was an overall significant difference between the 5 treatment groups (p < .001). There was also a significant interaction between sex and the 5-group treatment factor (interaction between sex and treatment: p = .003). Exploratory follow-up analysis suggested that a sex-specific response to RAP when it began at 16 months of age contributed to this overall interaction effect. When comparing just control animals to those treated with RAP from 16 months of age in a 2-way ANOVA there was a significant interaction between sex and treatment (p < .001), with males showing a significant improvement in rotarod endurance capacity, and females showing no difference from control mice (Figure 1D). There was no evidence of a statistically significant interaction between sex and treatment for ACA beginning at either timepoint, or RAP starting at 4 months of age, in equivalent analysis, and compared to controls, each of these drug treatments led to an improvement in endurance capacity (Supplementary Table S2).

Early Life or Late-Life ACA or RAP Treatment Reduces Cardiac Hypertrophy in Aging Mice

ACA and RAP have previously been shown to provide therapeutic benefits for the aging heart in mice, including a reduction of age-related cardiac hypertrophy (10,16). To evaluate age-related cardiac hypertrophy, we measured postmortem heart weight following early life and late-life treatment by ACA and RAP. We have previously observed that the total weight of the heart increases with age in male mice, which was attributed to an increase in LV weight as observed by echocardiography (10). There was an overall effect of treatment on postmortem heart weight (p < .001). Planned contrasts to the control group showed that all of the 4 treatment groups had significantly lighter hearts at postmortem (p < .05 in each case; Supplementary Table S3). There was also an overall effect of treatment on postmortem LV weight (p < .001), while RV weight did not differ significantly between treatment groups (p = .25). Planned contrasts to the control group for LV weight showed that RAP treatment from either 4 or 16 months significantly reduced LV weight at dissection, while this cardiac parameter was not significantly changed by ACA treatment beginning at either timepoint.

Because the heart weight of an animal will be influenced by their body weight at the time of assessment, we conducted an additional set of exploratory models to determine whether the reductions in heart weight with ACA and RAP treatment were accounted for by a similarly scaled change in body weight with these treatments. We included body weight as a continuous covariate in an analysis of covariance (ANCOVA), with sex and the 5-group treatment factor included as 2 independent variables.

Body weight at the time of dissection was significantly correlated with heart weight (p < .001), and there was an overall effect of the treatment group on heart weight (p < .001) when body weight was included as a continuous covariate. Planned contrasts of each drug treatment group to controls showed that the effect of RAP on heart weight, whether started from either early in life or late life, remained significant when including body weight as a continuous covariate (contrast for 4-month RAP: p < .001; contrast for 16-month RAP: p = .037; Figure 2H; Supplementary Table S3) as a continuous covariate. In contrast, the reduction in whole heart weight with ACA treatment paralleled the reduction in total body weight, and the effect of ACA treatment on heart weight was no longer significant after adjustment for body weight (Figure 2G). The effect of RAP on LV weight was also significantly different from controls when accounting for body weight with both treatment onsets (Supplementary Figure S1; 4-month RAP effect: p < .001; 16-month RAP effect: p = .035).

Effects of ACA and RAP on Cardiac Lipid Species

Our previous investigation has shown that ACA treatment beginning from early life significantly alters the lipidomic profile of the heart, in part by reducing some changes in lipid abundance that occur during aging (10). RAP and mTOR inhibition can have major effects on lipid metabolism (28), although changes to the cardiac lipidome have not been explored. We contrasted the cardiac lipidomic profile of each treatment group to that

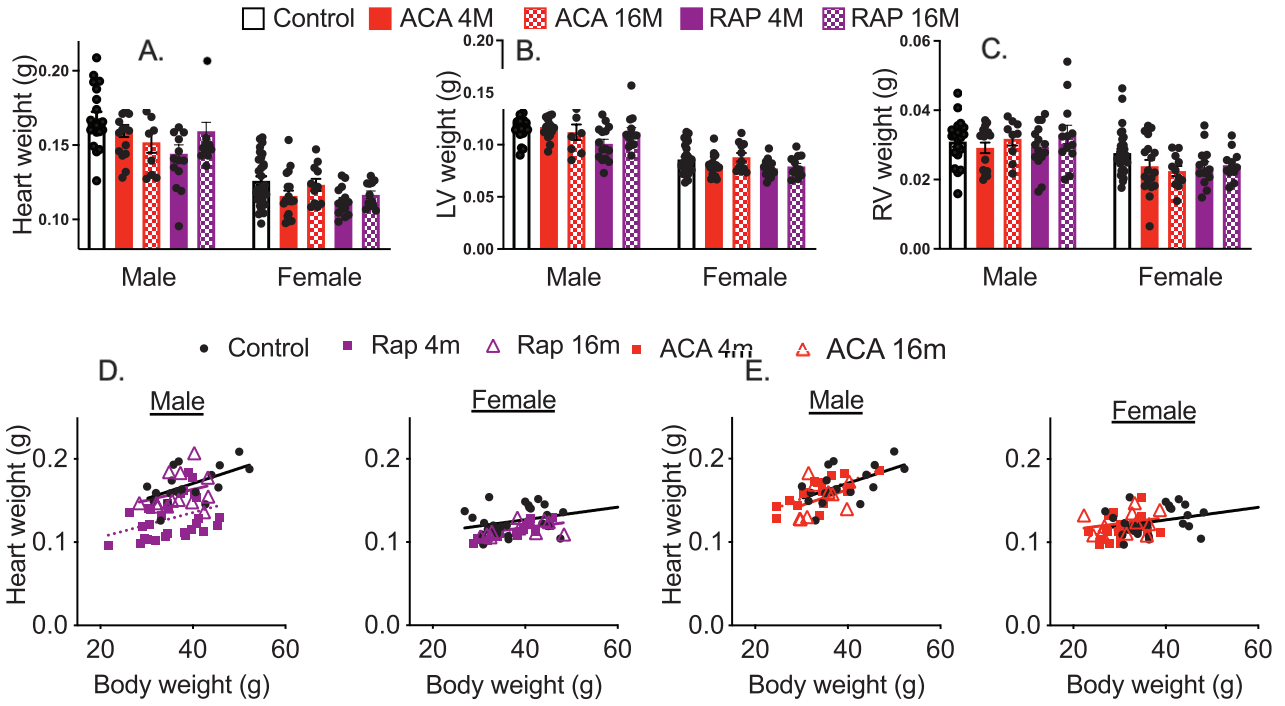

Figure 2. Evaluation of early and late-life ACA and RAP treatment on age-related cardiac structural measures. At 22 months of age following the ACA or RAP treatment period, UM-HET3 male and female hearts were harvested. Whole hearts were weighed (**A**), and then micro dissected into component parts including left ventricles (LV) and right ventricles (RV) . (**B** and **C**). The change in early or late-life RAP-treated heart weights compared to controls remains significant after adjusting for body weight (**D**), while ACA treatment changes in heart weight are explained by changes in body weight (**E**). Data are presented as mean ± SEM. Group numbers: male controls ($n = 20$), male 4-month ACA ($n = 17$), male 16-month ACA ($n = 9$), male 4-month RAP ($n = 15$), male 16-month RAP ($n = 13$), female controls ($n = 30$), female 4-month ACA ($n = 18$), female 16-month ACA ($n = 11$), female 4-month RAP ($n = 14$), and female 16-month RAP ($n = 12$). ACA = acarbose; RAP = rapamycin; SEM = Standard Error of the Mean.

observed in control animals. This provided an opportunity to explore whether the changes that occur with early-life ACA treatment (from 4 months) are similarly observed with late-life ACA treatment (from 16 months) and further evaluate lipidomic alterations that may occur with early or late-life RAP treatment. After correction for FDR, ACA treatment beginning at 4 months of age significantly influenced the abundance of 87 lipid species. These metabolite changes were largely recapitulated by ACA treatment beginning at 16 months of age, as 74 of the metabolites also significantly changed in abundance in the same direction with treatment beginning at 4 months of age (Figure 3A). In addition, 57 other metabolites were significantly altered in abundance by late-life ACA treatment only, with similar effects in both sexes. The heatmap of standardized abundance (Figure 3B) shows all metabolites that were significantly changed with either early or late-life ACA treatment, and illustrates the largely similar change in abundance of lipid species with ACA treatment beginning at 16 months compared to the effects of treatment beginning at 4 months of age (see Supplementary Data Set for details of specific lipid species within each class and p values for each test). TAGs were one of the main lipid classes for which some species were significantly affected by late-life treatment but not early-life treatment (Figure 4A). This appears to occur largely because males treated with ACA from 4 months of age show a larger variation in their TAG responses to treatment (Figure 3A), with several males having similar values to controls, while effects are more consistently different from controls in males treated from 16 months of age.

RAP treatment had a smaller effect on the cardiac lipidome, with 6 phosphatidylcholine (PC) species significantly changed in abundance with early-life treatment compared to controls, and 8 TAGs changed in abundance with late-life treatment (Figure 3B, Supplementary Data Set). There were no metabolites that showed a significant change with both early and late-life treatment (Figure 4A). The heatmap of standardized abundance (Figure 3D) demonstrates a greater similarity to control values in early or late-life RAP treatment when compared to ACA treatment.

ACA and RAP Treatment Effects on Plasma TAGs, Glucose, Insulin, and IGF1

ACA and RAP have been shown to modify circulating levels of plasma TAGs, glucose, insulin, and IGF1 (8,29–31), which can influence health with aging (32–34). We tested whether early or late-life treatment with ACA and RAP would alter these metabolic parameters in aged mice. For plasma TAGs there was a significant effect of the treatment group ($p < .001$). In contrast to the significant alteration in cardiac TAGs, ACA did not influence the concentration of plasma TAGs, regardless of whether treatment began at 4 months or 16 months of age (planned contrast to control animals for ACA beginning at 4 months: $p = .86$; for ACA beginning at 16 months: $p = .62$; Figure 5A; Supplementary Table S4). Compared to control animals, RAP treatment increased plasma TAGs, an effect that occurred equally with early or late-life treatment and similarly between sexes (planned contrast to control animals for RAP beginning at 4 months: $p < .001$; for

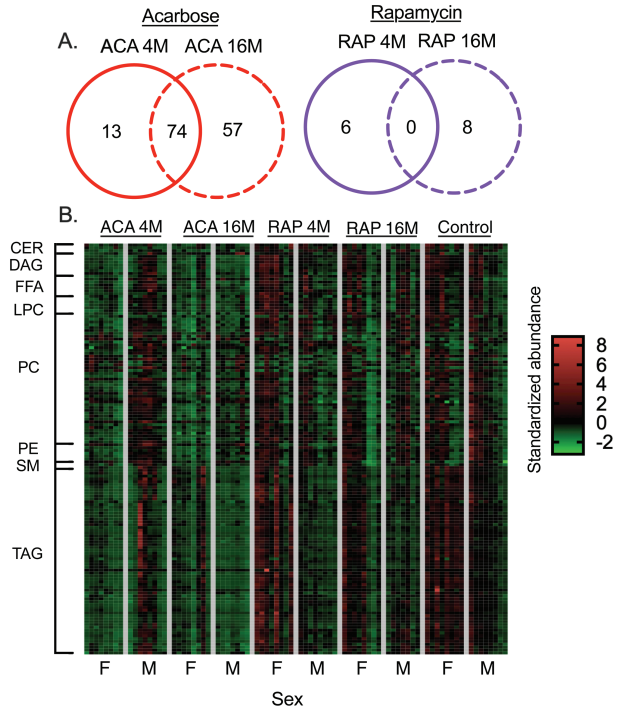

Figure 3. Cardiac lipidomic response to ACA and RAP treatment. The number of lipid species that were significantly altered in abundance with ACA or RAP treatment are shown in Venn diagrams (**A**), when each treatment was started in either early life (4 months of age) or late life (16 months of age). Heatmap showing relative abundance for 145 lipid species, those which showed a significant change with ACA or RAP treatment from at least 1 treatment onset (see Supplementary Data Set for statistics for individual lipid species). Values for each species have been standardized prior to plotting, showing the number of standard deviations that each observation falls above or below the mean. Standardized abundance is shown for controls or ACA treated animals starting at 4 or 16 months of age and RAP from the same timepoints (**B**). Each column represents abundance values for an individual mouse, with samples organized according to treatment group and sex. Group numbers: $n = 8$ per treatment group. ACA = acarbose; RAP = rapamycin; TAG = triacylglyceride; PC = phosphatidylcholine CER = Ceramide; DAG = Diacylglyeride; FFA = Free Fatty Acid; LPC = Lysophosphatidylcholine; PE = Phosphatidylethanolamine; SM = Sphingomyelin.

ACA beginning at 16 months: $p < .001$; Figure 5A; Supplementary Table S4). This lack of time course-specific effect differs from a previous study assessing the metabolic effects of RAP in younger genetically heterogeneous mice, where 2 weeks of injectable RAP treatment increased plasma TAGS, but this effect was diminished after 20 weeks (31). Neither fasting glucose nor fasting insulin concentrations were significantly influenced by the treatment group (overall effect of treatment for fasting glucose: $p = .41$; for fasting insulin: $p = .18$; Figure 5B and C; Supplementary Tables S5 and S6). This contrasts with previous work that demonstrated elevated fasting plasma glucose levels and reduced plasma insulin when ACA-treated animals were sampled at younger ages (7,30). We observed that plasma IGF1 concentration differed significantly by treatment group ($p < .001$; Figure 5D). As previously reported (7,30), IGF1 concentration was reduced by ACA treatment beginning at 4 months of age (planned contrast to control group: $p = .034$; Figure 5D; Supplementary Table S7), an effect that was also observed with treatment beginning at 16 months of age (planned contrast to control group: $p = .012$; Figure 5D; Supplementary Table S7). Early or late-life RAP treatment did not affect plasma IGF1 concentration compared to controls (Figure 5D).

Discussion

In this study, we conducted a controlled comparison of the effects of ACA and RAP treatment on rotarod functional capacity, cardiac hypertrophy, and lipidomic changes, specifically comparing early and late treatment onset to see whether they induce similar effects when animals are assessed at 22 months of age. Mostly, early or late-life treatment onset of either drug had very similar effects when compared in old animals, both in measures of physical function and metabolomics in the heart. Thus, treatment beginning later in life recapitulates health benefits that are observed with treatment started in young adults, which is an important consideration for drugs to have translational potential in humans (35).

Our analysis of physical function was restricted to 2 rotarod tests, one of which assesses acceleration capacity and the other endurance capacity. The ability of a mouse to remain on a rotarod is expected to be influenced by a range of factors, including exercise capacity and motivation, as well as neurologic, motor, and vestibular function (19). Treatment with either ACA or RAP from 4 months of age improved the functional performance in both these tests when animals are assessed at 22 months of age. For ACA, treatment starting at 16 months of age recapitulated this effect and provided a robust improvement in capacity. For RAP treatment beginning at 16 months of age, there appeared to be a sex-specific effect, only benefiting males, with females showing no significant improvement compared to controls. This suggests that RAP treatment from earlier in life is required to improve female acceleration and endurance capacity on a rotarod. This effect is not consistent with the changes seen in life span under RAP treatment in UM-HET3 mice, because late-life treatment increases life span in both sexes, with a greater increase in median life span in females (11,12,14). One previous study has observed male-specific life-span extension with transient, late-life RAP treatment (C57BL/6JNia strain) (36), without a significant improvement in female survival, similarly hinting that some late-life treatments may be particularly beneficial for males.

On an anatomical and physiological level, our study focused on cardiac structural and lipidomic changes, because previous comprehensive analyses showed the heart to be a critical organ that is altered by both ACA or RAP treatment (10,16,17). With ACA treatment, we have previously observed that the cardiac lipidome shows a dramatic restructuring, partly reversing changes that occur with aging (10). Evidence from our current study suggests that some of these changes are recapitulated with treatment beginning at 16 months of age. The lipids that differed in abundance to control animals at 22 months of age were very similar in animals that began treatment at 4 or 16 months. The effects and implications of these lipidomic responses are still to be further evaluated, but might help to explain the improved protection from cardiovascular disease and events seen in some humans treated with ACA (37,38).

Comprehensive lipidomic remodeling has also been observed in the livers and intestines of mice treated with ACA from early ages (39,40) suggesting widespread effects on lipid handling. These changes could conceivably be important in helping to improve survival, as well as enhancing physical function and cardiac health in mice as they age. It has been previously observed that reduced

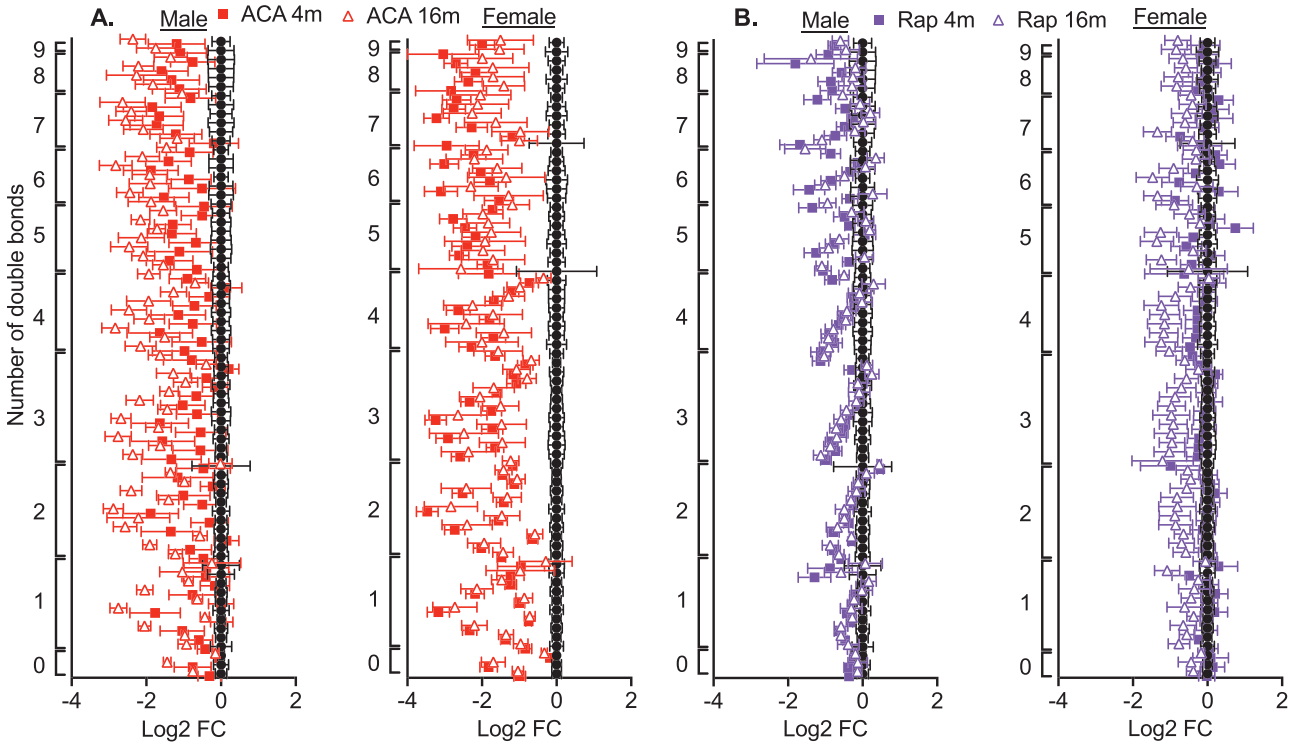

Figure 4. Log2 fold change in TAG species following ACA or RAP treatment. UM-HET3 mice were sampled at 22 months of age after treatment with either ACA (**A**) or RAP (**B**) from 4 months or 16 months of age. Each symbol (squares or triangles) represents the Log2 fold change in abundance (± SEM) for a specific TAG species, calculated as a change from the average abundance of untreated control values (black circles). Data are stratified by sex. TAG species are ordered accorded to number of double bonds, and within each double bond group, species are ordered with higher chain lengths higher on the y-axis (see Supplementary Data Set for changes in specific species). Group numbers: $n = 8$ per treatment group. ACA = acarbose; RAP: rapamycin; TAG = triacylglyceride; SEM = Standard Error of the Mean.

cardiac TAG abundance is associated with improved physical function in mouse models. For example, in swim-trained mice that show improved endurance, TAG abundance is reduced in the heart (41). In mouse models of pathological hypertrophy, TAG abundance can be increased, illustrating a correlation between TAGs and cardiac health. In humans, increases in TAG abundance and changes in the lipidome are observed with obesity, and are detectable before pathologic left ventricular hypertrophy is evident (42). However, while lipid changes and particularly altered TAG abundance correlate with cardiac health, it is yet to be established if they are causally involved in cardiac hypertrophy or changes in exercise capacity. Indeed, in our study RAP treatment also had a consistent effect on the size of the heart regardless of age at treatment onset, reducing age-associated cardiac hypertrophy. But compared to ACA, RAP had a much smaller effect on the heart lipidomic profile, with less than 10 lipids changing significantly in abundance with treatment after correction for FDR. This may suggest that the change in the size of the heart with RAP treatment is not caused by an underlying change in the cardiac lipidome.

Previous research has shown that RAP treatment can cause a major change in the cardiac proteome, reversing many changes in protein abundance that occur with aging (17). In contrast, previous research has failed to detect a similar change in protein abundance with ACA treatment (10), although animals in the ACA study were assessed at a younger point during aging when age-associated changes may have been less pronounced (24 months rather than 28 months). It seems likely that while both ACA and RAP are effective in reducing cardiac hypertrophy, the underlying structural and cellular changes in the heart that cause this are different. Further research that assesses the cardiac histological changes that occur with these treatments, including cardiomyocyte hypertrophy and collagen deposition, would help to establish the anatomical changes that lead to reduced macroscopic cardiac hypertrophy. In addition, future measurement and correction of heart weight by tibial length could be a more effective way of correcting heart weight by body size given that both ACA and RAP can affect body weight and adiposity.

The occurrence of different physiological responses to these treatments is perhaps unsurprising given their different mechanisms of action, with RAP reducing mTOR signaling and influencing a range of cellular processes, including protein translation (43,44). ACA, on the other hand, acts to reduce the breakdown of complex carbohydrates in the intestine (45) and therefore reduces the rate of increase and maximal extent of circulating postprandial glucose (8). Other evidence that these 2 treatments produce different physiological responses was seen with an assessment of plasma TAGs and circulating IGF1. Plasma TAGs were increased with RAP treatment only, and IGF1 was reduced with ACA treatment only, effects that have previously been reported (30,31), and occurred similarly with early and late-life treatment.

There are several limitations that should be noted with our study. First, we focused only on changes in rotarod capacity as a measure of physical function, which has previously been shown to be influenced by these drugs in different contexts. It would be important to determine whether the time-course independent benefits we observed are restricted to tests on a rotarod, or extend to other health assessments, such as grip strength, visual contrast discrimination, learning and memory, hearing, immune function, bone strength, reflex speed,

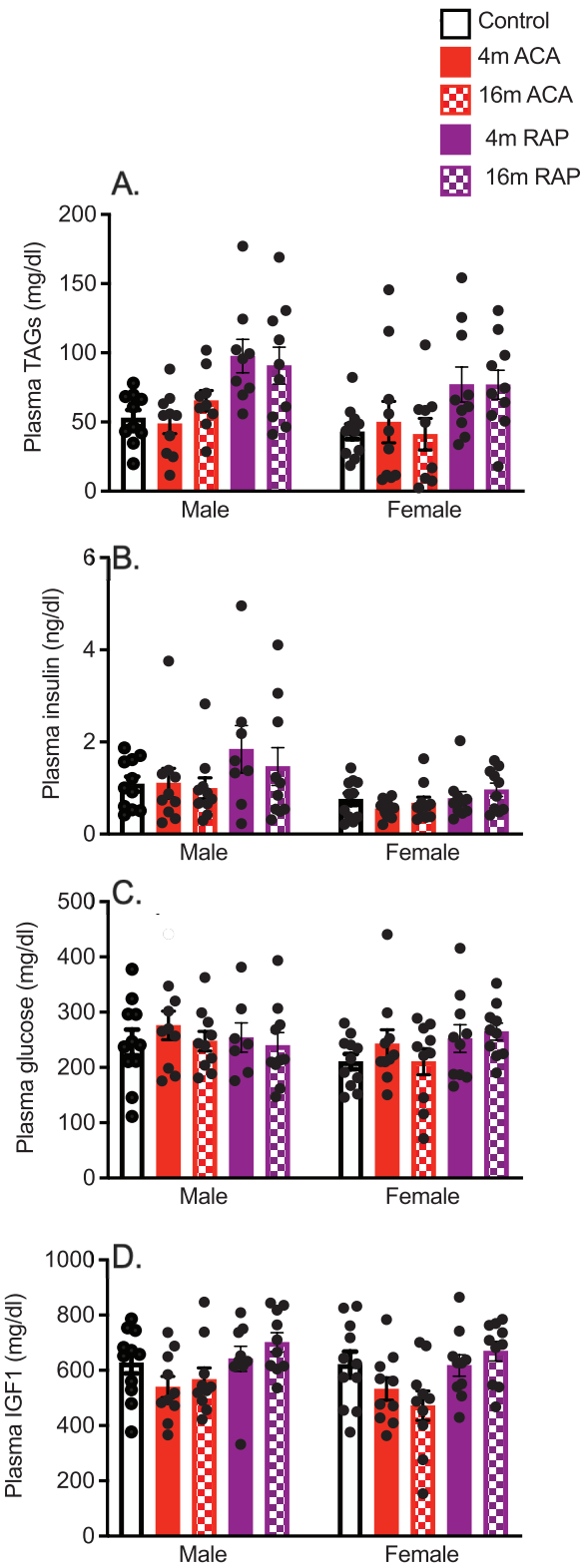

Figure 5. Effects of early and late-life ACA or RAP treatment on plasma metabolites. At 22 months of age, following early or late-life ACA or RAP treatment, plasma was obtained from UM-HET3 mice following a 18 hour fast. (**A**) Triacylglyceride (TAG) levels were significantly elevated in RAP-treated mice, whether treatment started at 4 or 16 months, compared to age matched controls in both male and female mice, but no significant change was detected with ACA treatment. (**B** and **C**) Plasma glucose or insulin levels did not change with either treatment regardless of duration. (**D**) Early and late-life ACA treatment significantly decreased IGF1 levels, an effect not observed with RAP treatment. Data are presented as mean ± SEM. Group numbers: male controls (n = 10), male 4-month ACA (n = 10), male 16-month ACA (n = 9), male 4-month RAP (n = 9), male 16-month RAP (n = 10), female controls (n = 11), female 4-month ACA (n = 10), female 9-month ACA (n = 10), female 4-month RAP (n = 9), and female 16-month RAP (n = 9). ACA = acarbose; RAP = rapamycin; IGF1 = insulin-like growth factor 1; SEM = Standard Error of the Mean.

or other aspects of metabolic health (46). Second, over the course of the treatment period, an uneven number of animals were lost from each treatment group, due to death, fighting, and use by other investigators. While this was anticipated within the study design, and the groups started with relatively large sample sizes for such mouse experiments, factors like fighting that were particularly problematic in the control male group could lead to selective disappearance within a particular treatment group. This is a problem that many mouse aging studies suffer, and could potentially be ameliorated by repeatedly sampling animals across their lifespan to test and control for selective disappearance of certain individuals (eg, if those that balance well on a rotarod are prone to fighting). Third, we also observed the possible presence of sex-specific responses to drug treatments when started at 16 months of age. Understanding the exact nature of these sex-specific responses should benefit from follow-up studies with a larger sample size that specifically designed to detect interactions between sex and treatment and the presence or absence of treatment effects in 1 sex only.

Taken together, and in light of previously published life-span data, treatment with ACA and RAP starting in middle age is largely effective at improving indices of health in mice, when compared to animals that began treatment at 4 months of age. Beginning treatments later in life is likely more translatable to humans and could avoid or minimize unwanted side effects of treatments, including those that may be detrimental to health over longer treatment durations.

Supplementary Material

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

Funding

Work was supported by grants from the National Institutes of Health (AG023122, AG022303, and ES030158), the Paul F. Glenn Center for Biology of Aging Research, and the American Federation for Aging Research (Junior Faculty Grant).

Conflict of Interest

None declared.

Acknowledgments

The authors thank Daniel Michele and Steve Whitesall at the University of Michigan Physiology Phenotyping Core for their technical expertise and assistance with our experiments, as well as Roxann Alonso, Amanda Keedle, Natalie Perry, Kate Szczesniak, Danielle Szczesniak, and Lynn Winkelman for technical assistance with mouse husbandry, surgery, and dissections. J.J.H. acknowledges support from University of Michigan Systems and Integrative Biology T32 Training Grant (GM008322-28,29). M.G. acknowledges support of a fellowship from the Michigan Society of Fellows.

Author Contributions

All authors contributed to the experiments conducted, R.A.M. and S.M.D. supervised the research, M.G. analyzed the data and wrote the manuscript with input from other authors. J.J.H., S.M.D., R.A.M., and M.G. designed research; J.J.H., S.L., K.P., D.L., O.F., and M.G. performed research; M.G. analyzed data.

References

1. Steenman M, Lande G. Cardiac aging and heart disease in humans. *Biophys Rev.* 2017;9:131–137. doi:10.1007/s12551-017-0255-9
2. DuGoff EH, Canudas-Romo V, Buttorff C, Leff B, Anderson GF. Multiple chronic conditions and life expectancy: a life table analysis. *Med Care.* 2014;52:688–694. doi:10.1097/MLR.0000000000000166
3. Schoenborn NL, Blackford AL, Joshu CE, Boyd CM, Varadhan R. Life expectancy estimates based on comorbidities and frailty to inform preventive care. *J Am Geriatr Soc.* 2021;70:99–109. doi:10.1111/jgs.17468
4. Longo VD, Antebi A, Bartke A, et al. Interventions to slow aging in humans: are we ready? *Aging Cell.* 2015;14:497–510. doi:10.1111/acel.12338
5. Fontana L, Partridge L, Longo VD. Extending healthy life span–from yeast to humans. *Science.* 2010;328:321–326. doi:10.1126/science.1172539
6. van de Laar FA, Lucassen PL, Akkermans RP, van de Lisdonk EH, Rutten GE, van Weel C. α-Glucosidase inhibitors for patients with type 2 diabetes. *Diabetes Care.* 2005;28:154–163. doi:10.2337/diacare.28.1.154
7. Harrison DE, Strong R, Allison DB, et al. Acarbose, 17-α-estradiol, and nordihydroguaiaretic acid extend mouse lifespan preferentially in males. *Aging Cell.* 2014;13:273–282. doi:10.1111/acel.12170
8. Harrison DE, Strong R, Alavez S, et al. Acarbose improves health and lifespan in aging HET3 mice. *Aging Cell.* 2019;18:e12898. doi:10.1111/acel.12898
9. Strong R, Miller RA, Antebi A, et al. Longer lifespan in male mice treated with a weakly estrogenic agonist, an antioxidant, an α-glucosidase inhibitor or a Nrf2-inducer. *Aging Cell.* 2016;15:872–884. doi:10.1111/acel.12496
10. Herrera JJ, Louzon S, Pifer K, et al. Acarbose has sex-dependent and independent effects on age-related physical function, cardiac health and lipid biology. *JCI Insight.* 2020;5:e137474. doi:10.1172/jci.insight.137474
11. Harrison DE, Strong R, Sharp ZD, et al. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature.* 2009;460:392–395. doi:10.1038/nature08221. http://www.nature.com/nature/journal/v460/n7253/suppinfo/nature08221_S1.html
12. Miller RA, Harrison DE, Astle CM, et al. Rapamycin, but not Resveratrol or Simvastatin, extends life span of genetically heterogeneous mice. *J Gerontol A Biol Sci Med Sci.* 2011;66:191–201. doi:10.1093/gerona/glq178
13. Neff F, Flores-Dominguez D, Ryan DP, et al. Rapamycin extends murine lifespan but has limited effects on aging. *J Clin Invest.* 2013;123:3272–3291. doi:10.1172/JCI67674
14. Zhang Y, Bokov A, Gelfond J, et al. Rapamycin extends life and health in C57BL/6 mice. *J Gerontol A Biol Sci Med Sci.* 2014;69(A):119–130. doi:10.1093/gerona/glt056
15. Flynn JM, O'Leary MN, Zambataro CA, et al. Late-life rapamycin treatment reverses age-related heart dysfunction. *Aging Cell.* 2013;12:851–862. doi:10.1111/acel.12109
16. Quarles E, Basisty N, Chiao YA, et al. Rapamycin persistently improves cardiac function in aged, male and female mice, even following cessation of treatment. *Aging Cell.* 2020;19:e13086. doi:10.1111/acel.13086
17. Dai DF, Karunadharma PP, Chiao YA, et al. Altered proteome turnover and remodeling by short-term caloric restriction or rapamycin rejuvenate the aging heart. *Aging Cell.* 2014;13:529–539. doi:10.1111/acel.12203
18. Graber TG, Maroto R, Fry CS, Brightwell CR, Rasmussen BB. Measuring exercise capacity and physical function in adult and older mice. *J Gerontol A Biol Sci Med Sci.* 2021;76:819–824. doi:10.1093/gerona/glaa205
19. Justice JN, Carter CS, Beck HJ, et al. Battery of behavioral tests in mice that models age-associated changes in human motor function. *AGE.* 2014;36:583–595. doi:10.1007/s11357-013-9589-9
20. Jakovljevic DG. Physical activity and cardiovascular aging: physiological and molecular insights. *Exp Gerontol.* 2018;109:67–74. doi:10.1016/j.exger.2017.05.016
21. Miller RA, Harrison DE, Astle CM, et al. An aging interventions testing program: study design and interim report. *Aging Cell.* 2007;6:565–575. doi:10.1111/j.1474-9726.2007.00311.x
22. Miller RA, Harrison DE, Astle CM, et al. Rapamycin-mediated lifespan increase in mice is dose and sex dependent and metabolically distinct from dietary restriction. *Aging Cell.* 2014;13:469–477. doi:10.1111/acel.12194
23. Harrison DE, Strong R, Alavez S, et al. Acarbose improves health and lifespan in aging HET3 mice. *Aging Cell.* 2019;18:e12898. doi:10.1111/acel.12898
24. Garratt M, Leander D, Pifer K, et al. 17-Alpha estradiol ameliorates age-associated sarcopenia and improves late-life physical function in male mice but not in females or castrated males. *Aging Cell.* 2019;18:e12920. doi:10.1111/acel.12920
25. Garcia-Sifuentes Y, Maney DL. Reporting and misreporting of sex differences in the biological sciences. *eLife.* 2021;10:e70817. doi:10.7554/eLife.70817
26. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Series B (Methodological).* 1995;57:289–300. doi:10.1111/j.2517-6161.1995.tb02031.x
27. Garratt M, Lagerborg K A, Tsai YM, Galecki A, Jain M, Miller RA. Male lifespan extension with 17-α estradiol is linked to a sex-specific metabolomic response modulated by gonadal hormones in mice. *Aging Cell.* 2018;0:e12786. doi:10.1111/acel.12786
28. Kennedy BK, Lamming DW. The mechanistic target of Rapamycin: the grand ConducTOR of metabolism and aging. *Cell Metab.* 2016;23:990–1003. doi:10.1016/j.cmet.2016.05.009
29. Lamming DW, Ye L, Katajisto P, et al. Rapamycin-induced insulin resistance is mediated by mTORC2 loss and uncoupled from longevity. *Science.* 2012;335:1638–1643. doi:10.1126/science.1215135
30. Garratt M, Bower B, Garcia GG, Miller RA. Sex differences in lifespan extension with acarbose and 17-alpha estradiol: gonadal hormones underlie male-specific improvements in glucose tolerance and mTORC2 signaling. *Aging Cell.* 2017;16:1256–1266. doi:10.1111/acel.12656
31. Fang Y, Westbrook R, Hill C, et al. Duration of rapamycin treatment has differential effects on metabolism in mice. *Cell Metab.* 2013;17:456–462. doi:10.1016/j.cmet.2013.02.008
32. Garratt M, Nakagawa S, Simons MJP. Life-span extension with reduced Somatotrophic signaling: moderation of aging effect by signal type, sex, and experimental cohort. *J Gerontol A Biol Sci Med Sci.* 2017;72:1620–1626. doi:10.1093/gerona/glx010
33. Nelson JF, Strong R, Bokov A, Diaz V, Ward W. Probing the relationship between insulin sensitivity and longevity using genetically modified mice. *J Gerontol A Biol Sci Med Sci.* 2012;67:1332–1338. doi:10.1093/gerona/gls199
34. Brewer RA, Gibbs VK, Smith DL, Jr. Targeting glucose metabolism for healthy aging. *Nutr Healthy Aging.* 2016;4:31–46. doi:10.3233/NHA-160007
35. Kirkland JL. Translating the science of aging into therapeutic interventions. *Cold Spring Harb Perspect Med.* 2016;6:a025908–a025908. doi:10.1101/cshperspect.a025908
36. Bitto A, Ito TK, Pineda VV, et al. Transient rapamycin treatment can increase lifespan and healthspan in middle-aged mice. *eLife.* 2016;5:e16351. doi:10.7554/eLife.16351
37. Chiasson J, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M; STOP-NIDDM Trial Research Group. Acarbose treatment and the risk of cardiovascular disease and hypertension in patients with impaired glucose tolerance: the stop-niddm trial. *JAMA.* 2003;290:486–494. doi:10.1001/jama.290.4.486
38. Holman RR. What does the Acarbose Cardiovascular Evaluation (ACE) trial tell us? *J Diabetes.* 2018;10:683–685. doi:10.1111/1753-0407.12770
39. Gibbs VK, Brewer RA, Miyasaki ND, Patki A, Smith DL Jr. Sex-dependent differences in liver and gut Metabolomic profiles with acarbose and

calorie restriction in C57BL/6 mice. *J Gerontol A Biol Sci Med Sci*. 2017;73(2):157–165. doi:10.1093/gerona/glx127

40. Ma S, Yim SH, Lee SG, et al. Organization of the mammalian metabolome according to organ function, lineage specialization, and longevity. *Cell Metab*. 2015;22:332–343. doi:10.1016/j.cmet.2015.07.005
41. Tham YK, Bernardo BC, Huynh K, et al. Lipidomic profiles of the heart and circulation in response to exercise versus cardiac pathology: a resource of potential biomarkers and drug targets. *Cell Rep*. 2018;24:2757–2772. doi:10.1016/j.celrep.2018.08.017
42. Wende AR, Abel ED. Lipotoxicity in the heart. *Biochim Biophys Acta*. 2010;1801:311–319. doi:10.1016/j.bbalip.2009.09.023
43. Laplante M, Sabatini DM. mTOR signaling at a glance. *J Cell Sci*. 2009;122:3589–3594. doi:10.1242/jcs.051011
44. Saxton RA, Sabatini DM. mTOR signaling in growth, metabolism, and disease. *Cell*. 2017;168:960–976. doi:10.1016/j.cell.2017.02.004
45. DiNicolantonio JJ, Bhutani J, Keefe JH. Acarbose: safe and effective for lowering postprandial hyperglycaemia and improving cardiovascular outcomes. *Open Heart*. 2015;2:e000327. doi:10.1136/openhrt-2015-000327
46. Bellantuono I, de Cabo R, Ehninger D, et al. A toolbox for the longitudinal assessment of healthspan in aging mice. *Nat Protoc*. 2020;15:540–574. doi:10.1038/s41596-019-0256-1