HHS Public Access

Author manuscript

Mech Ageing Dev. Author manuscript; available in PMC 2020 June 22.

Published in final edited form as:

Mech Ageing Dev. 2019 June ; 180: 42–48. doi:10.1016/j.mad.2019.03.005.

Frailty index as a biomarker of lifespan and healthspan: focus on pharmacological interventions

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Abstract

Although survival has been the focus of aging research for many years, the field is rapidly evolving towards incorporating healthspan and health indices in studies that explore aging-related outcomes. Frailty is one such measure that is tightly correlated with human aging. Several frailty measures have been developed that focus on phenotypes of aging, including physical, cognitive and metabolic health that define healthspan. The extent at which cumulative deficits associated with frailty predict functional characteristics of healthy aging and longevity is currently unknown. A growing consensus for the use of animal models has emerged to evaluate a composite measure of frailty that provides a translational basis to understanding human frailty. In this review, we will focus on the impact of several anti-aging interventions, some of which have been characterized as caloric restriction (CR) mimetics such as metformin, rapamycin, resveratrol as well as more novel approaches that are emerging in the field - nicotinamide adenine dinucleotide precursors, small molecule activators of sirtuins, and senolytics - on a number of frailty measurements associated with aging-related outcomes in mice and discuss the translatability of such measures to human frailty.

Keywords

Aging; frailty; mouse models; interventions

1. INTRODUCTION

Frailty is classified as one of the most important risk factors for mortality in older adults and is defined as a ‘state of high vulnerability’, correlated with advancing age and detrimental health outcomes when compared to individuals who are not frail [1]. A significant proportion (~ 25–50%) of individuals who are above the age of 85 and other vulnerable populations, such as ethnic minorities and women, are disproportionally living with frailty [2] [3], a condition that is associated with comorbidity and disability [4]. Alas, the etiology

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of frailty remains poorly understood [5]. Studying the burden of frailty in humans has been challenging – particularly due to the ethical, logistical, and biological complications associated with working with older adults [6]. As human life expectancy is increasing by 2 to 3 months every year [7], age-related disorders and disabilities invariably bring with them significant social, medical, and economic challenges that need to be addressed urgently.

Animal models and interventions conducted on them provide an opportunity to study the biological basis of aging and potential ways to delay the onset of aging-related phenotypes [8]. Such studies are also inherently cross-translational in nature as knowledge gathered from animal models can be directly applied to human aging and vice versa. A frailty index score that encompasses various parameters of frailty allows to assess whether an individual is frail or not [1]. Parks and colleagues first implemented a frailty index in mice [9], which has garnered great interest in the aging field for the last several years. Factors related to integument, physical/musculoskeletal, vestibulocochlear/auditory, ocular/nasal, digestive/urogenital, respiratory, and aspects of physical discomfort are used to assess the frailty index in mice (Figure 1). Similar to the human frailty index, a higher index score is associated with lower survivability in mice [10]. These main categories of frailty assessment are further subdivided into characteristics in order to calculate a comprehensive score for each category. These characteristics are as follows: 1) Integument evaluates alopecia, loss of fur color, amount of dermatitis, loss of whiskers, and the general condition of the coat; 2) physical/musculoskeletal parameters examine tumors, distended abdomen, kyphosis (abnormal spinal curvature), gait disorders, tremor, forelimb grip strength, and overall body condition score; 3) the vestibulocochlear/auditory section of the frailty index reflects on vestibular disturbance and hearing loss; 4) ocular/nasal segment explores cataracts, corneal opacity, eye discharge, microphthalmia, vision loss, menace reflex, and nasal discharge; 5) digestive/urogenital section evaluates any malocclusions, rectal prolapse, diarrhea, and vaginal/uterine or penile prolapse; 6) breathing rate/depth is evaluated; and also 7) any physical discomfort as evidenced by the mouse grimace scale and piloerection. Along with these indices, mouse weight and body temperature are also scored.

The overarching goal of developing a mouse frailty index is to justify its incorporation into intervention studies aimed at evaluating age-related phenotypic outcomes with direct translatability into human frailty in the elderly. This article reviews and evaluates the effectiveness of few proposed anti-aging interventions aiming to extend healthy lifespan to specific frailty-related measures. Many of these small molecules were first developed as CR and fasting mimetics [11]. While CR has also been shown to be associated with improvement in frailty in mice [6, 12] in several studies, these will not be discussed in detail in this review. We focused on mouse intervention studies with small molecules that have utilized frailty indices as a measure of efficacy in combatting aging-related phenotypes. A summary of these studies can be found in Table 1.

2. FRAILTY IN MOUSE AGING INTERVENTION STUDIES

A) Metformin

Studies in humans have shown that diabetic patients are more likely to have poor muscle health and to develop neurodegeneration as well as loss of executive function [13]. These

impediments beg the question whether metformin, a widely prescribed oral anti-diabetic drug, might be useful in improving such outcomes in people with type 2 diabetes and improve aging in general. The recent plans for establishing the clinical trial known as 'Targeting Aging with Metformin' (TAME; American Federation for Aging Research initiative) reflects this interest [14]. The ability of metformin to improve lifespan and health in humans has led to the conduct of animal studies to determine dose effectiveness and safety, physiological/metabolic effects, and mechanisms of action of this biguanide [15] [16]. Martin-Montalvo and colleagues evaluated mouse healthspan and endpoints related to frailty by assessing the impact of long-term diet supplementation with metformin in C57BL/6 mice [15]. Middle-aged mice treated with metformin performed significantly better on rotarod and treadmill tests and exhibited significantly greater open field test locomotor activity than the control group [17]. Overall health was improved in response to metformin treatment as evidenced by an increase in insulin sensitivity and mitochondrial function, and reduction in chronic inflammation and cataract progression rate [15]. In a second study, 12-month-old C57BL/6 mice on HFD supplemented with 1% metformin performed significantly better on the rotarod test compared to mice on HFD alone [16]. Moreover, chronic, intermittent treatment with 1% metformin significantly improved the average hanging test performance in mice while removal of the drug from the diet led to a weakened functional ability vis-à-vis elevated cage top or accelerating rotarod tests [18]. However, other studies did not substantiate these findings and, instead, suggested that metformin may exert harmful effects by reducing visual acuity of aged male mice upon addition of metformin in drinking water [19]. Other studies in mice fed a HFD also reveal adverse effects of co-treatment with metformin – exacerbation of damage caused to sciatic nerve fibers [20] and incidence of porcelain gallbladder [21]. The exact conditions under which beneficial effects of metformin far exceed the risks is currently an area of scientific investigation. In particular, the doses of metformin used in these animal studies generally far exceed the recommended daily metformin therapy in patients with type 2 diabetes, putting into question the translatability of these findings. Hence, future studies should include more frailty-related outcomes in response to clinically-relevant doses of metformin and dosing schedules.

B) Rapamycin

Rapamycin is currently being used as an immunosuppressant drug during organ transplant. It inhibits the cellular mTOR complex, which acts as a master regulator of protein synthesis and redox sensing. The beneficial effects of rapamycin in improving healthspan and lifespan in mice [22] [23] [24] come with some undesirable effects [25]. Yet, rapamycin has shown promise in several mouse longevity studies that have utilized frailty indices as endpoints. Intraperitoneal daily injection of rapamycin for 90 days improved parameters of muscle function in middle-aged (20–21 mo-old) C57BL/6Nia mice, accompanied by significantly better scores on both grip strength and rotarod tests than control mice [26]. Additionally, improvements in stride length and rotarod performance were found in C57BL/6Nia mice of both sexes with rapamycin supplementation in the same study. The increased frequency of exercise in the treated group suggests that rapamycin may provide greater endurance and resistance to muscle fatigue. A significant reduction of the kyphosis index was observed when 24-month-old C57BL/6J mice were treated with an oral formulation of

microencapsulated rapamycin for a 3-month period [27]. However, these findings are not universal as rapamycin treatment has been found to elicit deleterious effects on some frailty-related outcomes. Rapamycin dose-dependently promoted higher occurrence of cataracts and sex-cell/testicular degeneration in male mice after a 9-month treatment [23]. In the same study, however, the authors observed that the age-associated decline in spontaneous in-cage activity was mitigated by rapamycin in a sex-dependent fashion, with male mice being affected at a lower dose of the drug than females.

Frailty in mice has also been utilized to better gauge biological age. A physiological frailty index was developed to estimate the biological age of a cohort of mice following treatment with rapamycin as an anti-aging drug. The micellar nanoformulation of rapamycin, rapatar, has been found to significantly improve this frailty index only in HFD-fed male mice [28]. In the HFD male mice, the frailty index score reduced from ~0.3 to ~0.25 with rapatar treatment. In HFD females, the baseline frailty score was higher and rapatar treatment did not result in a change in score. Similar “sex-specific” effects of rapamycin have been previously reported when chronic rapamycin treatment was initiated in 4-mo-old male and female C57BL/6J mice for the duration of their life [29]. In this study, no significant differences in rotarod performance among females were reported while males on rapamycin performed poorly. Moreover, the rapamycin-treated females displayed higher grip strength at all ages with greater spontaneous activity in the older females compared to age-matched controls. No such benefits were noted in males on rapamycin. Regardless of sex, rapamycin did not alter cochlear neurons, outer hair cells, or inner hair cells, and no difference in stride length was observed.

To gain further insights into the cellular mechanisms underlying the physiological manifestations of age-associated frailty, one should consider assessing the effects of molecules such as rapamycin in longevity studies aimed at preventing or delaying the development of frailty. Rapamycin significantly improves neuromuscular coordination without reduction in inflammaging in a mouse model with genetically enhanced NF-κB activity [30]. NF-κB is a redox-sensitive transcription factor that increases expression of pro-inflammatory factors implicated in age-dependent muscle loss, a strong risk factor for frailty [31]. Whether such observations stem from crosstalk between mTOR and signaling pathways implicated in inflammation is currently not known. Yet, we surmise that the molecular mechanisms regulating frailty can be elucidated through the use of small molecules such as rapamycin. In addition to mTOR and NF-κB, there are other age-associated signaling pathway targets, such as Nrf2 and AMPK, that can be studied using pharmacologic and nonpharmacologic strategies.

Overall, results from these animal studies show the ability of rapamycin to promote lifespan and healthspan extension by mitigating frailty-related end points. Despite these advances, more studies are required to better understand the mechanisms of action of molecules like rapamycin and the sex-specific effects that this compound has on frailty.

C) Resveratrol

Resveratrol (RSV) is a naturally-occurring compound with antioxidative properties, found in plants, particularly in grapes. In humans, several studies have suggested its beneficial effects

on lifespan as well as on frailty [32]. The role of this small biomolecule in extending lifespan has also been reported in lower organisms such as *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, and *Drosophila melanogaster* [33]. A number of studies have also been conducted with RSV in rodents, with promising results. One-year-old male C57BL/6Nia mice fed daily a high-calorie diet supplemented with RSV exhibited longer lifespan and performed equally well as their standard diet (SD)-fed littermates on rotarod test [34]. In this study, RSV treatment of 21–24-month-old mice on SD displayed better balance and motor coordination than their age-matched controls [35]. A delayed onset of age-related cataracts was also reported in RSV-treated mice at 30 months of age compared to controls [35]. Several frailty parameters were assessed in 18-month old C57BL/6J mice under caloric restriction and after a 6-month diet supplementation with RSV [6]. Significant improvement in overall frailty index scores (~0.2 in control compared to ~0.18 in RSV) was observed in C57BL/6J mice treated with RSV compared to their SD controls. Whether and how small molecules such as RSV can be successfully combined with other interventions that have shown promising results in aging studies, such as dietary restriction, metformin, rapamycin, and others (see below) is an area that warrants further research.

The impact of RSV treatment on physical performance was also investigated in aged male ICR mice. A 21-day RSV treatment improved time to exhaustion in a swimming exercise performance test, and it also dose-dependently increased grip strength compared to sedentary control mice [36]. In this study, RSV improved exercise-induced fatigue together with a reduction in blood lactate, ammonia, and glucose through molecular mechanisms that are not well understood. In another study, 16-month old C57BL/6J mice that were on a diet supplemented with 25 mg/kg RSV for 4 weeks showed enhanced performance in forelimb grip strength and endurance swimming test when combined with exercise, compared to the no RSV control groups [37]. Future studies should aim at determining a selective dose-response effectiveness of RSV in order to provide beneficial physiological effects during physical activity. Although the translational impact of longevity studies in lower organisms is tantalizing, it is important to implement new studies in animal models that recapitulate human aging and associated frailty outcomes.

D) Nicotinamide adenine dinucleotide (NAD) precursors

NAD primarily acts as a co-enzyme in redox reactions and functions as a mediator in glucose and lipid metabolism and mitochondrial function, interacting especially with sirtuins, poly(ADP-ribose) polymerases (PARPs), and the cyclic ADP-ribose synthases (CD38, CD157) [38] [39]. The decline in NAD levels during aging is triggered by decreased synthesis and/or increased NAD^+ consumption [40], and the supplementation with nicotinamide (NAM), a NAD^+ precursor, has been shown to improve healthspan in adult mice, although lifespan was not affected [41]. Given the involvement of NAD in aging and health, pharmacological approaches that rely on small molecules that alter NAD and NADH levels have been recently tested in aging intervention studies with promising but variable results. Within these studies, there is some evidence to suggest that NAD might be involved in altering frailty measures in mice. For example, hearing loss is associated with decreased levels of NAD [42] and administration of nicotinamide riboside, a NAD^+ precursor, prevented noise-induced hearing loss by reducing neurite degeneration through SIRT3

pathway [43]. Age-related sarcopenia, characterized by the loss of muscle mass and function, is associated with decreased physical performance. Muscle-specific deletion of the gene encoding nicotinamide phosphoribosyltransferase (Nampt), a major enzyme involved in NAD^+ synthesis and metabolism, causes age-associated muscle decline, which can be improved by NR supplementation [44]. Furthermore, an age-dependent drop in NAD^+ levels has been associated with vision loss. Supplementation with nicotinamide mononucleotide (NMN), a second NAD precursor, restored vision in wild type and *Nampt* knockout mice [45]. Collectively, intervention by small molecules that alter NAD levels represents an active area of research and future studies should specifically explore how frailty indices are affected by them.

E) Synthetic sirtuin activators

Sirtuins are NAD^+-dependent protein deacetylases that have recently garnered great interest in the aging field. Interestingly, serum sirtuin levels have been recently proposed as protein markers of age-related frailty in humans [46]. Mice overexpressing SIRT1 have an increased healthspan, as evidenced by improved glucose metabolism and lower senescence but without lifespan extension [47]. Long-term diet supplementation with the small molecule SIRT1 activator SRT2104 in male C57BL/6J mice leads to higher endurance on the treadmill and motor performance on the rotarod without impacting spontaneous/voluntary activity [48]. These phenotypic changes coupled with molecular changes suggest the suppression of inflammatory pathways and preservation of muscle and bone mass. Interestingly, a number of SIRT1 activators have been tested in clinical trials (e.g., NCT00937326, NCT00933062, NCT01018017) against a range of diseases, including type 2 diabetes, and are now completed, highlighting the potential clinical significance of this class of compounds. A second small allosteric activator for SIRT1, known as SRT1720, significantly increased motor coordination and balance in male C57BL/6J mice on standard diet at both 13 and 18 months of age, with a positive trend at 24 months [49]. In the same study, a significant reduction in cataract formation was also observed in SRT1720-treated mice at 105 weeks of age [49]. The effects of sirtuins within the context of other indices of frailty have also been reported. For instance, SIRT1-deficient mice exhibit impaired ocular morphogenesis and retinal development [50], and upregulation of SIRT1 confers protection against various ocular diseases such as age-related cataracts [51]. Yet, the beneficial effects of these sirtuin activators might be dependent on factors such as age and exposure to external stressors.

F) Senolytics

In normal aging and age-related diseases, cellular senescence imposes irreversible growth arrest on cells while triggering a biologically-specific phenotype marked by enhanced $p16^{INK4a}$ expression, higher senescence-associated beta galactosidase expression as well as a senescence-associated secretory phenotype (SASP). The ensuing secretion of cytokines, chemokines, and proteases contributes to create an inflammatory milieu associated with tissue aging and age-related diseases such as frailty [52]. Genetic manipulation that targets senescent cells improves age-related phenotypes and frailty [53] while the combination of two senolytic drugs, dasatinib and quercetin, was found to improve the physical capacity and total work performance of mice previously irradiated to induce senescence [54]. The same senolytic combination was recently reported to slow physical dysfunction with regard to

walking speed, hanging endurance, grip strength, treadmill endurance, and daily activity caused by the transplantation of senescent cells in young and old mice [55]. In another study, AP20187 and a combination of dasatinib and quercetin were shown to improve running time in bleomycin-treated mice that exhibited lung fibrosis through alleviation of senescence [56].

The JAK/STAT pathway is involved in cytokine production and signaling and contributes to the transcription of genes implicated in SASP and age-related tissue dysfunction. Administration of a JAK1/2 inhibitor effectively improved physical activity, rearing, ambulation counts, hanging endurance, and grip strength in 24-months-old mice [57]. However, the molecular pathways that contribute to senescence are not well understood and represents an area of active research. A recent screening assay for identification of potential senotherapeutic drugs found two inhibitors of the molecular chaperone HSP90 as having senolytic activity *in vitro* and *in vivo*. Oral administration of the HSP90 inhibitor, 17-DMAG, which is also a water-soluble analog of Geldanamycin, extended healthspan in a mouse model of a human progeroid syndrome when started at 6 weeks of age, with improvement in kyphosis, dystonia, tremor, forelimb grip strength, coat condition, ataxia, and gait compared to age-matched controls [58]. Given that many of the senolytic molecules that have been identified thus far may have pleiotropic mechanisms of action, they could potentially be utilized to address a plethora of complexities associated with aging-related phenotypes, including frailty. However, well-designed and well-executed pre-clinical studies, in mice and in other model organisms, are imperative for promising senolytics to be advanced to the clinic.

G) Other interventions

Although the aforementioned lifespan/healthspan extension interventions have specifically looked at frailty indices, there are also other pharmacological agents that may offer promising effects against frailty. To this end, some drugs that have been in clinical use for several decades have been specifically evaluated for their effects on frailty. Lifelong treatment with the angiotensin-converting enzyme (ACE) inhibitor ramipril extends longevity in hypertensive rats, matching that of the normotensive control group [59]. In another study, chronic treatment with enalapril, another ACE inhibitor, for 4 months delayed the onset of age-related frailty in middle-aged and old female C57BL/6J mice while conferring benefits only in the oldest group (25 mo.) of males [10]. For example, enalapril lowered the fraily index from ~0.2 to ~0.15 in middle aged female mice, but not in middle aged males. Lower overall frailty scores were reported in middle-aged females after 3–4 months of treatment with enalapril compared to age-matched controls, an effect not observed in male mice [10]. A reduction in vision loss was also apparent in 21-month-old females on enalapril. Administration of the antihypertensive drug, losartan, for 4 months was associated with an increase in locomotor activity in 18-month-old C57BL/6 mice as measured by treadmill time, standing and travelling activity, but without improvement in grip strength [60]. The antidiabetic drug, acarbose, has well validated anti-aging effects in mice [61], and it has been determined that acarbose treatment significantly attenuates psoriasis-like inflammation in BALB/c mice [62]. It appears, however, that rapamycin, but

not acarbose treatment, attenuates outer hair cell loss in 9 −10-month-old UM-HET3 mice of both sexes [63].

The National Institute on Aging Intervention Testing Program (NIA ITP) reported that 17-α estradiol increases median lifespan in male but not female HET3 mice by 12% [64]. However, specific frailty parameters were not evaluated in the study. Another estrogen, 17-β estradiol, promotes the regrowth of normally pigmented hair shafts after chemotherapy-induced alopecia [65] while conferring better visual acuity in rats with high intraocular pressure [66]. However, the use of estrogens for the improvement of age-related diseases has been widely debated, especially with regard to 17-β estradiol due to its carcinogenicity [67]. Small molecules of natural origin have also been studied within the context of frailty in animal models. For example, Icariin, a natural flavonoid, increased neuromuscular coordination and is accompanied by improvement in rotarod performance [68]. The potential of natural compounds to promote overall health benefits highlights the need to conduct further studies on how plant extracts can be formulated for medical and aging research [69].

3. CONCLUSIONS

Utilizing small molecules to delay the aging process and improve aging-related outcomes is currently a flourishing area of research. Evaluation of the recent literature suggests that there is immense value in incorporating the mouse frailty index in these research efforts. Frailty measurements can potentially be utilized as reliable markers of aging. When frailty-related endpoints are coupled with data on survival, metabolism, and molecular signatures, they can be collectively used as predictors of lifespan and overall health. Several different mouse frailty indices that focus on different endpoints have been proposed [70] [71]. Establishing detailed and consistent protocols on animal frailty assessment that are uniform across institutions and personnel, is a key factor that needs to be addressed in frailty research. Furthermore, implementing a more specific frailty index tailored to different mouse sexes and strains will allow for better accuracy. We recommend greater use of the frailty index in mouse studies that test novel small molecules and novel combinations in aging interventions. It is imperative that the mechanisms of actions of these compounds and druggable molecular targets be identified, where applicable. Enhancement of wellbeing and quality of life can have a profound impact on health objectives of societies. In order to successfully tackle the challenges that prevent us from achieving this, well-designed and thoughtfully executed animal research studies that encompass indices of human aging, such as frailty, are required.

Funding sources:

Intramural Research Program, NIA/NIH

ABBREVIATIONS

ACE	angiotensin converting enzyme
AL	*ad libidum*
AMPK	5’-AMP activated protein kinase

CR	calorie restriction
DQ	combination of dasatinib and quercetin
FI	frailty index
HFD	high fat diet
NAD	nicotinamide adenine dinucleotide
NAM	nicotinamide
NR	nicotinamide riboside
NMN	nicotinamide mononucleotide
RSV	resveratrol
SD	standard diet
NA	not available

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HIGHLIGHTS

- Frailty is increased during human aging and, therefore, has been evaluated as a target for anti-aging interventions
- Aspects of the mouse frailty index have been utilized to quantify the efficacy of anti-aging interventions *in vivo*
- While studies investigating metformin, rapamycin, resveratrol, NAD+ precursors, sirtuin activators, and senolytics have incorporated some indices of the mouse frailty index, opportunities to expand its use exist

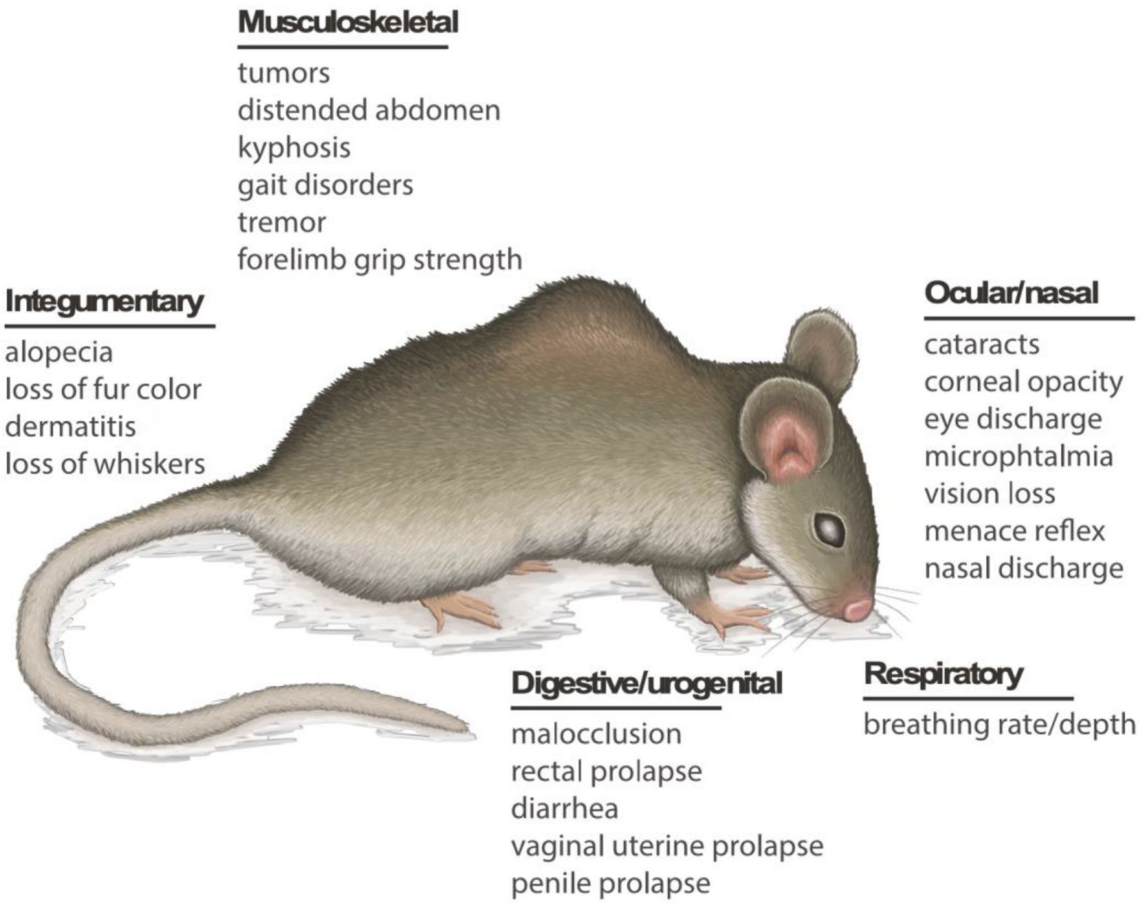

Figure 1.

Systemic effects of frailty in aging mouse. The main categories of frailty assessment are subdivided into characteristics in order to calculate a comprehensive score for each category.

TABLE 1:

Major frailty-related findings from aging interventions using Metformin, Rapamycin, Resveratrol, NAD precursors, SIRT1 activators, and Senolytics

Ref.	Strain	Age of Onset	Sex	Intervention	Dose, Route of Exposure, Duration	Frailty Index Measures
(18)	C57BL/6	104-weeks-old	M	Metformin	AIN-93G diet supplemented with 1% metformin every other week (EOW) or for 2 consecutive weeks each month (2WM) for the remainder of their lives	Performance on the rotarod and forelimb grip strength measures were undistinguishable compared to their SD controls. Tests were performed at 17 weeks of intermittent metformin treatment. Longitudinal measures are needed to determine effects on healthspan.
(16)	C57BL/6	12-month-old	M	Metformin	HFD based on AIN-93 G supplemented with 1.0% metformin for 6 months	Attenuated the decline in motor performance
(17)	C57BL/6	54-week-old	M	Metformin	Daily treatment with 0.1% metformin in diet until natural death	Improved physical performance Decreased incidence of cataract
(41)	C57BL/6J	1-year-old	M	Nicotinamide (NAM)	62-week NAM supplementation (0.5 and 1.0 g/kg of diet) in SD or HFD for the remainder of their lives	HFD-fed mice with supplemen tation of NAM, improved healthspan parameters, specifically: Motor coordination via rotarod testing and locomotor activity (both total distance and average speed) during open field testing
(45)	Nampt$^{rod/-rod}$ mice backcrossed onto C57BL/6J	6-week-old	N/A	NMN	NMN was administered via daily i.p. injection (150 mg/kg) for 4 weeks	Vision loss was rescued via significant recovery of scotopic and photopic retinal function
(44)	*Nampt* knockout (mNKO)	3-month-old	M & F	NR & NAM	NR chloride and NAM were dissolved weekly in drinking water (12mM) and provided ad libitum	Intervention restored exercise performance and endurance in KO mice
(43)	Wld^{S}, BalbC, and CBA mice on C57BL/6J background	8–10-week-old	N/A	NR	NR was administered by i.p. twice daily for a period of 5 days prior to noise exposure and then 14 days thereafter	NR treatment provided protection against hearing loss in all tested mouse strains
(29)	C57BL/6J	4-month-old	M & F	Rapamycin	Microencapsulated rapamycin (14ppm) continued throughout life	No differences in stride length or age-related hearing loss Worsening of motor function in males Increased grip strength in females Spontaneous activity greater in females
(24)	C57BL/6Nia	19-month-old	M & F	Rapamycin	Rapamycin supplemented in diet (14 ppm)	Stride length improved at 32 mo. of age Better motor function for both males and females No effect on grip strength regardless of sex
(27)	C57BL/6J	24-month-old	F	Rapamycin	Oral delivery of microencapsulated rapamycin (14 ppm in diet) for 3 months	Significant reduction in kyphosis Index at 27-months of age Rapamycin increased resistance to muscle fatigue
(23)	UM-HET3	20–22-month-old	M & F	Rapamycin	Mice exposed to rapamycin at doses of 4.7, 14, or 42 ppm for 9 months	Higher incidence of testicular degeneration (13% in the controls and 83% in all rapamycin-treated groups) and cataracts in treated males

Ref.	Strain	Age of Onset	Sex	Intervention	Dose, Route of Exposure, Duration	Frailty Index Measures
						Lower incidence of lesions within uterine lining with highest dose of rapamycin
(26)	C57BL/6JNia	20–21-month-old	M & F	Rapamycin	Daily intraperitoneal (i.p.) injections of 8 mg/kg rapamycin for 90 days.	Significant increase in muscle function
(34)	C57BL/6nia	1-year-old	M	RSV	2 doses of RSV (5.2 ± 0.1 and 22.4 ± 0.4 mg.kg-1/day) added to standard and high-calorie diets for 6 months	Improved endurance/motor skills Increased survival in high-calorie fed mice supplemented with RSV compared to their high calorie only counterparts.
(37)	C57BL/6J	16-month-old	M	RSV	Diet supplementation with 25 mg/kg RSV for 4 weeks	Mice on either RSV or exercise training or the combination of exercise + RSV had greater forelimb grip strength than control group. Mice on exercise alone and exercise + RSV performed significantly better during an endurance swimming test than controls
(36)	ICR	6-week-old	M	RSV	Oral administration of RSV at 25, 50, and 125 mg/kg/day in standard laboratory diet for 21 days	Significantly longer exhaustive swimming time for mice with the lowest RSV dose than vehicle control group
(6)	C57BL/6J	18-month-old	M & F	RSV	RSV (100 mg/kg) was supplemented in diet AIN-93G die fed AL, until they reached 24 month-of-age	6 months of RSV treatment significantly reduced the FI from ~0.2 to ~0.18 in C57BL/6J mice compared to their control counterparts Reported was a significant increase in the proportion of mice with a frailty score greater than 0 for corneal opacity in the SD-fed group (34%) compared to the RSV diet group (0%)
(57)	C57BL/6	24-month-old	M	Ruxolitinib	Treatment of aged mice with JAK inhibitor in diet (60 mg/kg BW) for a duration of 10 weeks	Treatment with JAK 1/2 inhibitor significantly enhanced physical function in aged mice, specifically: Motor coordination, grip strength measures, hanging time, and frequency of rearing
(53)	*BubR1*$^{H/H}$ *and INK-ATTAC.* All mice were on a mixed 129 × C57BL/6 × FVB genetic background	*3-week-old*	F	Senolytic AP20187	Mice were treated with AP20187 (0.2 μg.g^{-1} BW) by i.p. injection every 3rd day for beginning at 3 weeks of age until 8 months old	Treatment significantly delayed onset of cataracts & lordokyphosis Better treadmill exercise performance (both duration and distance travelled) in treated mice, indicative of an attenuation of sarcopenia and preservation of muscle fibers
(53)	*BubR1*$^{H/H}$ *and INK-ATTAC.* All mice were on a mixed 129 × C57BL/6 × FVB genetic background	5-month-old	F	Senolytic AP20187	Mice were treated with AP20187 (0.2 μg/g BW) by i.p. injection	Cataract formation was not reduced in treated mice and remained unchanged Improvement in treadmill exercise testing was still prominent in 10-month-old timepoint
(55)	syngeneic C57BL/6 mice	6-month-old	M	Senolytics	Transplantation of control and doxorubicin-treated preadipocytes isolated from luciferase-expressing transgenic (LUC^{+}) mice into syngeneic mice via i.p. injection. Physical performance tests were evaluated 1 month later	Physical dysfunction was induced as evidenced by significantly lower maximal walking speed on rotarod, grip strength, and hanging endurance compared to controls

Ref.	Strain	Age of Onset	Sex	Intervention	Dose, Route of Exposure, Duration	Frailty Index Measures
(58)	*Ercc1* $^{-/\Delta}$ mice in an f1 background (C57Bl/6:FVB/n)	6 weeks-of age	M & F	Senolytics	17-DMAG (10 mg/kg) was administered 3x/week every 3 weeks (intermittent treatment) beginning at 6-weeks-of-age via oral gavage	Treatment significantly reduced incidence of kyphosis, loss of forelimb grip strength, coat condition, gait disorder, and overall body condition
(56)	Ink-Attac mice on a C57BL/6 × BALB/c background	2.5–8 months of age	M	Senolytics	Mice were treated with PBS or 2 U/kg bleomycin via aerosolized i.p. injection and then randomized. Vehicle, AP20187 (10 mg/kg via i.p. injection), or the combination of Dasatinib (5 mg/kg) + Quercetin (50 mg/kg) delivered via oral gavage, termed DQ) was administered.	Exercise capacity was evaluated on a motorized treadmill. Mice treated with bleomycin alone ran shorter mean and maximal distances as compared to all other groups. Mice on bleomycin + AP20187 and bleomycin + DQ ran further than mice on bleomycin alone even though all three groups' performance was subpar to their vehicle controls
(48)	C57BL/6J	6-month-old	M	SRT2104	Mice were fed AIN-930 diet supplemented with SRT2104 (100 mg/kg/bw) for the remainder of their lives	Treatment correlates with higher endurance on treadmill Increased performance on rotarod
(49)	C57BL/6J	6-month-old	M	SRT1720	Mice were fed either HFD or SD supplemented with SRT1720 (100 mg/kg/bw) for the reminder of their lives	SRT1720 significantly increased motor coordination and balance Significant reduction in cataract formation at 105 weeks-of-age

List of abbreviations: AL, *ad libidum*; CR, calorie restriction; DQ, combination of dasatinib and quercetin; F, females; FI, frailty index; HFD, high fat diet; M, males; NAM, nicotinamide; NR, nicotinamide riboside; NMN, nicotinamide mononucleotide; RSV, resveratrol; SD, standard diet. NA, not available.