Quantification of healthspan in aging mice: Introducing FAMY and GRAIL

Dudley W. Lamming[1,2]

[1]Department of Medicine, University of Wisconsin-Madison, Madison, WI 53705, USA
[2]William S. Middleton Memorial Veterans Hospital, Madison, WI 53705, USA

Correspondence and Lead Contact

Dudley W. Lamming, PhD
Associate Professor of Medicine
University of Wisconsin-Madison
1685 Highland Ave
MFCB Rm 4147
Madison, WI 53705, USA
dlamming@medicine.wisc.edu
Tel: 608-262-7341
Fax: 608-263-9983

Abstract

The population around the world is graying, and as many of these individuals will spend years suffering from the burdens of age associated diseases, understanding how to increase healthspan, defined as the period of life free from disease and disability, is an urgent priority of geroscience research. The lack of agreed-upon quantitative metrics for measuring healthspan in aging mice has slowed progress in identifying interventions that do not simply increase lifespan, but also healthspan. Here, we define FAMY (Frailty-Adjusted Mouse Years) and GRAIL (Gauging Robust Aging when Increasing Lifespan) as new summary statistics for quantifying healthspan in mice. FAMY is based entirely on a widely utilized clinical frailty index, while GRAIL incorporates frailty, widely utilized healthspan assays, and information about the hallmarks of aging. Both metrics are conceptually similar to quality-adjusted life years (QALY), a widely-utilized measure of disease burden in humans, and can be readily calculated from data acquired during longitudinal and cross-sectional studies of mouse aging. We find that interventions generally thought to promote health, including calorie restriction, robustly improve healthspan as measured by FAMY and GRAIL. Finally, we show that use of GRAIL provides new insights, and identify dietary restriction of protein or isoleucine as an intervention that promotes healthspan but not longevity in female HET3 mice. We suggest that the routine integration of these measures into studies of aging in mice will allow the identification and development of interventions that promote healthy aging even in the absence of increased lifespan.

Keywords: healthspan, frailty, lifespan, calorie restriction, isoleucine restriction

Introduction

As the global population ages, addressing the challenge of age-related diseases has become increasingly urgent. While the field of geroscience has traditionally focused on extending lifespan, there is limited interest in extending the portion of one's life where an individual is frail, unable to perform activities of daily living, and may be in pain. Instead, the key priority of geroscience should be to increase healthspan, defined as "the period of life spent in good health, free from the chronic diseases and disabilities of aging" (1).

The scientific literature has become replete with discussion of interventions that promote healthspan, increasing exponentially from 0-1 references in PubMed each year from 2000-2004 to 251 references in the year 2022. Many of these references can be found in papers, primarily utilizing mice, stating that various geroprotective interventions increase, improve or extend the healthspan of mice. However, as pointed out by a piece in *Geroscience* as well as in recent online discussions (1), there are no quantitative metrics for measuring healthspan in mice, and thus demonstrating that interventions have a statistically significant benefit to healthspan is not formally possible. This unfortunate gap limits our ability to compare the benefits of different interventions on healthspan – and may lead us to entirely miss interventions that promote healthy aging but do not extend lifespan.

While quantitatively determining healthspan has not been rigorously pursued, two complimentary approaches to assaying healthspan in mice have been developed. The first of these approaches is focused on frailty, defined as an accumulation of multiple deficits and a loss of resilience to adverse events. A clinical frailty index (FI) for mice developed by Dr. Susan Howlett and colleagues almost ten years ago has become widely utilized as a robust way of measuring frailty in mice that tracks similar clinical frailty indexes in humans (2) and is strongly associated with mortality (3). Calore restriction (CR), the gold standard for lifespan-extending interventions and preventing or delaying many age-related diseases, clearly reduces frailty as assessed using the FI (4). An important component of the FI approach is that it is an average of multiple deficits, and thus is robust to the exclusion of specific measurements for logistical or scientific reasons, as well as to the inclusion of additional deficits as they are developed (5). Further, tracking frailty does not generally require specialized equipment and can be performed in a completely non-invasive manner, allowing frailty to be measured repeatedly throughout the lifespan. While these are compelling reasons to measure frailty, there is as yet no standard methodology to integrate longitudinal measurements of FI and lifespan.

The second approach, which has been adopted by a variety of leading aging laboratories, is to assess multiple parameters of healthspan using standardized approaches. A recently published consensus toolbox of protocols to assess healthspan in aging mice includes protocols to assess cardiac function, muscle strength and neuromuscular function, metabolic health, and cognitive function (6). The specific assays selected were chosen based on clinical relevance, reproducibility, a low level of invasiveness or stress for the animal, and a low level of technical difficulty. However, as many of these assays still require injections, blood collection, or anesthesia, and/or involve stress, and they will most often be performed only a few times, or perhaps even only once. For these reasons, these assays are best performed on a cohort of mice that will be sacrificed cross-sectionally rather than used for lifespan determination.

Here, we utilize these approaches to define two new quantitative summary statistics to measure healthspan in mice. The first of these, FAMY (Frailty-Adjusted Mouse Years), defines

health as a function of frailty, and integrates health over the lifespan in a matter akin to the widely-used quality-adjusted life year (QALY) in humans (7). The second of these measures, GRAIL (Gauging Robust Aging when Increasing Lifespan), moves beyond frailty as the sole measure of health by also incorportating information from healthspan assays and the hallmarks of aging. We show that FAMY and GRAIL can be readily calculated from data collected during longitudinal mouse aging studies, and that calorie restriction (CR) – widely stated in the literature to promote healthspan – increases both FAMY and GRAIL. We further show that the consideration of FAMY and GRAIL can lead to conclusions that are not immediately apparent from the consideration of lifespan, identifying protein restriction as an intervention that increases healthspan without increasing lifespan, highlighting the potential utility of these indexes in identifying interventions that may be able to improve healthspan.

Results

The Frailty-Adjusted Mouse Year (FAMY) demonstrates the cost of missing health.

In humans, the quality-adjusted life year (QALY) is the gold standard for measuring the effects, positive or negative, on the effects of medical treatments on patients' lives, and serves as a fundamental component of the cost-effectiveness of medical interventions. One quality-adjusted life year (QALY) is equal to 1 year of life in perfect health. Thus, the total QALY for an individual can be calculated as the area under the curve (AUC) of a graph where quality (utility) of life, a unitless value varying between 0 and 1, is plotted on the y-axis and the survival in years in plotted on the x-axis (7).

We considered that by plotting 1-FI over time, we would essentially be able to plot a heathspan curve for each individual mouse in an aging study. In order to calculate the AUC for each animal, we made three simplifying assumptions. First, as aging studies using wild-type mice do not start at birth – many studies start in adult mice and many in mid-life – and frailty assessment may not begin until mid-life or later, we made the simplifying assumption that mice at the beginning of the lifespan study had a FI of 0. Second, we made a simplifying assumption that mice at the end of their life, on the date they are found dead or euthanized due to reaching endpoint criteria, have a FI of 1. Third, in any lifespan study a number of animals are likely to be censored (e.g., when animals are removed for cross-sectional analysis, or when removal from the study results from an error on the part of the experimenter or animal care staff). As the frailty of censored animals after the date of censoring cannot be known, FAMY cannot be calculated for censored animals.

In order to "test-drive" the FAMY, we analyzed publicly available lifespan and frailty data from a recent study of C57BL/6J.Nia conducted by experts in the biology of aging and frailty (8). The mice in this study were appropriately long-lived, with a median lifespan of 954 days (**Fig. 1A**), and frailty in this population of mice increased with age as expected (**Fig. 1B**). We calculated the FAMY for each mouse; the FAMY values primarily grouped between 1.5 and 2.5, with a median of 2.19 (**Fig. 1C**). As expected, FAMY was highly correlated (R^2=0.9337) with lifespan (**Fig. 1D**).

A few points are apparent; first, while the median lifespan of these mice was 2.6 years, the FAMY of 2.19 reflects that the overall population lost 16.2% of its possible healthspan. Second, the longest-lived mouse at 3.32 years had the highest FAMY, 2.67, while the maximum possible FAMY for this mouse was 3.32 (**Fig. 1C**); thus, this animal lost 19.6% of its possible

healthspan. Finally, while the correlation between FAMY and lifespan is very tight, there is variation, with multiple mice surviving for longer than expected with a poorer healthspan (**Fig. 1D, red**), as well as mice that died sooner than expected with good healthspan (**Fig. 1D, blue**).

Calorie restriction quantitatively increases healthspan

CR is widely acknowledged as the gold-standard for geroprotective interventions. CR extends the lifespan of a wide range of species, ranging from yeast, rotifer, worms and flies to mice, rats, dogs, and non-human primates, and has shown favorable effects in short-term human trials (9-16). However, the level of CR that optimally extends lifespan may vary by sex and genetic background, and it has been reported that in some strains 40% CR shortens lifespan (17, 18). While a negative effect of CR on the lifespan of certain mouse strains has only been partially replicated (19), these findings have led some to question both the safety of dietary interventions like CR particularly as applied to humans in the real world beyond the confines of the laboratory (20).

To begin to address this question, we analyzed the healthspan of mice in a recently completed CR study (21). As shown in **Fig. 2**, the approximately 40% CR regimen utilized here results in significant, 16% increase in the median lifespan of male C57BL/6J mice along with a decrease in frailty as the mice aged (**Fig. 2A-B**). A similar but larger effect on longevity was seen in females, with a 39% increase in median lifespan and a dramatic separation of frailty curves between *ad libitum* (AL) fed controls and mice maintained on a CR diet (**Fig. 2C-2D**).

As above, we calculated healthspan using FAMY. In accordance with the general belief that CR is a healthspan-extending intervention, we find that CR significantly increases FAMY in both male and female mice (**Fig. 2E**). Interestingly, while the average healthspan of male mice is increased 15.2% by CR, similar to the effect on lifespan, the healthspan of female mice increases only 31.1%, less than the effect of CR on lifespan. To get a better idea of whether this represented a real difference in the effect of CR on male and female healthspan, we examined the correlation between FAMY and lifespan for each group of mice (**Fig. 2F**). As we expected, the correlation between FAMY and lifespan for each group was quite high, with an R of 0.9 or greater. We plotted regression lines for each group, and we noted that while there was no statistically significant difference in slopes or intercepts between any of the groups, the CR groups were consistently higher than the AL fed groups. As there was no effect of sex, we pooled the sexes, and found that the y-intercept of CR-fed mice was 0.065 FAMY higher ($p=0.035$) than that of AL-fed mice. Thus, it seems that at least in this particular experiment, CR increased healthspan slightly more than it increased lifespan, regardless of sex.

Restriction of isoleucine quantitatively increases male healthspan as assessed by FAMY

There is a growing awareness among both scientists and the public that a calorie is not “just a calorie,” and that calories from different macronutrient sources may have effects on metabolism and healthy aging that are not simply related to their energy content (22). For example, restriction of dietary protein or of methionine extends the lifespan of rodents (23-26), and lifelong restriction of the three branched-chain amino acids (BCAAs; leucine, isoleucine, and valine) extends lifespan and reduces frailty in male C57BL/6J mice (27).

Building on our recent discovery that restriction of isoleucine alone is sufficient to improve metabolic health and necessary for the benefits of protein restriction in C57BL/6J male mice (28), we decided to test the effects of isoleucine restriction (IleR) on healthy aging. Initiating IleR in 6-

month-old genetically heterogeneous mice of both sexes, we recently found that IleR had profound effects on longevity vs. an amino acid-defined Control diet, extending median lifespan of male by 33% and females by 7%, and extending the maximum lifespan of male mice (**Figs. 3A, B**)(29). In contrast, neither male nor female mice fed an amino acid defined protein restricted diet (PR) in parallel lived longer than Control-fed mice. Male mice fed the PR or IleR diets had reduced frailty vs. Control-fed at multiple time points starting at 24 months of age, while female PR and IleR-fed mice had reduced frailty only at 24 months of age (**Figs. 3C, D**).

To assess the effects of these diets on healthspan, we calculated the FAMY for each mouse in the lifespan study. We observed an overall effect of diet, as well as a sex-diet interaction, on FAMY. IleR increased FAMY in males, but did not significantly increase FAMY in IleR-fed females (**Fig. 3E**). The effect of IleR on median FAMY (+25%) was lower than the effect of IleR on median lifespan (29%), suggesting that as we observed with CR, IleR increases healthspan in males slightly more than it increased lifespan.

Integrating measures of healthspan and the hallmarks of aging

Key advantages of frailty measurements is that most of the measures included in the clinical frailty index for mice generally non-invasive (exceptions include rotarod performance and rectal temperature, and we omit these from longitudinal frailty assessment in our laboratory). However, there are many other procedures to assess the health of aging mice; for example, a toolbox recently published by the MouseAGE international consortium includes detailed procedures for common assays of metabolic health, physical and cognitive performance, and cardiovascular function (6). Twelve hallmarks of aging were recently described; assays to examine the hallmarks of aging typically require blood or tissue samples, typically from euthanized mice (30).

While these healthspan assays and the hallmarks of aging provide valuable information about the health of mice, these assays are often physically stressful, sometimes require injections, blood collection, anesthesia or euthanasia, and for scientific and logistical reasons cannot be performed frequently across the lifespan. We thus decided to construct a new measure, the GRAIL (Gauging Robust Aging when Increasing Lifespan) to integrate information about both frailty and the health/hallmarks.

We decided to calculate GRAIL as the sum of two components as outlined in **Figure 4A**. Similar to the FAMY, we calculated the GRAIL frailty component (GRAIL-FC) by determining the Area Of the Curve for each individual mouse of a graph of 1-FI, but with the FI on the day of death set equal to 0.7, as the highest FI score observed for an individual wild-type mouse does not exceed 0.7 (personnel communication, Dr. Susan Howlett). Thus, GRAIL-FC closely approximates 70% of the FAMY.

The GRAIL health/hallmarks component (GRAIL-HH) was calculated not for individual animals but for specific groups of animals that are being compared (e.g., WT CR males in **Fig. 2**). We take the using an index approach to determinate a health and hallmarks index (HHI) for the average of the list of 22 items in **Table 1**. If the value of the index is comparable to that value for the wild-type untreated control group, we will score the value as 1.0, with 0 indicating an impairment in the health/hallmark component relative to the control group, and 2.0 indicating an improvement. In the event that impairment or improvement in an item is for only a portion of the lifespan or is considered only mild, 0.5 or 1.5 may be scored, respectively. Items for which no information is available – for example, telomere length was not examined in the PR/IleR study

discussed in Figure 3 – are simply omitted. Finally, GRAIL-HH for an individual mouse is calculated by multiplying the average HHI across all scored items by both 0.3 and the lifespan of the lifespan of the animal (**Fig. 4A**). **Figure 4A** illustrates the area of GRAIL-HH for an animal in the control group with a HHI of 1.

Table 1: Items scored in the GRAIL health/hallmarks component. A value of 1.0 indicates the health is comparable to the value of an untreated wild-type control group; 0 indicates an impairment in the health/hallmark, while 2.0 indicates an improvement. In the event of impairment or improvement for only a portion of the lifespan, 0.5 or 1.5 may be scored, respectively.

GRAIL - HHI	
Healthspan	
Cardiac Function	Echocardiography
Neuromuscular Function	Grid Hanging Test (Inverted Cling)
	Grip Strength
	Hanging Bar
	Rotarod
Metabolic Health	Body composition
	Energy balance
	Glucose tolerance test
	Insulin tolerance test
Cognitive function	Novel Object Recognition
Hallmarks	
Primary	Genomic instability
	Telomere attrition
	Epigenetic alterations
	Loss of proteostasis
	Disabled macroautophagy
Antagonistic	Deregulated nutrient-sensing
	Mitochondrial dysfunction
	Cellular senescence
Integrative	Stem cell exhaustion
	Altered intracellular communication
	Chronic inflammation
	Dysbiosis

We calculated the GRAIL for HET3 mice fed either PR or IleR diets starting at 6 months of age, determining the GRAIL-FI for each individual animal and then adding to it the GRAIL-HH for each individual animal (**Fig. 4B**). Similarly to the FAMY, we determined that there was a significant effect of diet and a diet-sex interaction; however, we also observed a significant effect of sex. While the effects of PR and IleR on male mice was similar between FAMY and GRAIL – with only IleR-fed males showing improved healthspan – the result for females was quite different. Using the GRAIL calculation, both PR and IleR diets were shown to promote healthspan in females, despite the minimal effect of these diets on lifespan. These findings agree generally with the trend of both PR and IleR to promote healthspan in female HET3 mice. Surprisingly though, a PR diet did not improve healthspan in PR-fed male mice.

Discussion

While it is widely stated that CR and other geroprotective interventions improve healthspan, the effect of these diets and drugs has not been quantitatively or statistically demonstrated due to lack of integrative healthspan metrics. This has likely slowed progress in geroscience, as the lack of quantitative metrics limits our ability to compare the effect of different interventions on healthspan. Further, it will allow us to settle long-standing debates about the effect of widely studied interventions including CR and rapamycin on the healthspan of mice (17, 18, 31, 32). Finally, the use of FAMY and GRAIL may permit us to use advanced techniques, such as genetic mapping, to identify quantitative trait loci and specific gene alleles that contribute to variation in the effect of interventions on not just lifespan, but healthspan, between different strains and sexes of mice.

Here, we have established FAMY and GRAIL as new quantitative summary statistics of healthspan. We have demonstrated that at least one specific CR regimen in C57BL/6J mice extends not only lifespan, but also healthspan as measured by FAMY, in both males and females. We have also shown that an IleR diet increases both lifespan and healthspan in male HET3 mice as assessed using both FAMY and GRAIL. Further, use of GRAIL reveals that both IleR and PR diets extend healthspan in female HET3 mice. Importantly, a PR diet in female HET3 mice increases healthspan by over 25% despite not increasing lifespan by a statistically significant amount. This not only demonstrates that PR can uncouple healthspan from lifespan, but shows that these summary statistics can help identify interventions that improve healthspan and add healthy years of life, despite not increasing median lifespan.

FAMY and GRAIL provide insight into the cost of frailty to healthy aging. Here, we find that frailty shortens the healthspan of mice by approximately 16-20% from the maximum possible healthspan given a fixed maximum lifespan. This percentage of time is similar to the concept of the “lost decade” in human health – the idea that people typically lose 10-20 years of high quality life due to disability and frailty (33). While the NIA Interventions Testing Program (ITP) as well as other groups have over the past two decades have identified many agents that extend lifespan, going forward tracking healthspan may allow the identification of agents that are just as effective (or more so) at adding healthy years to life. Once such intervention might be metformin, which did not extend lifespan in HET3 mice when tested by the ITP, but has been shown to extend healthspan in assays by others (34, 35).

The FAMY and GRAIL healthspan indices designed here limitations that may need to be designed around in future versions of these statistics (e.g., FAMY2 or GRAIL2). First, for both FAMY and GRAIL-FI, we need longitudinal frailty data from across the lifespan, which may not always be available. Secondly, while we have utilized a subset of the 31 original frailty items (2), removing specific items that we examine in healthspan assays, these and additional frailty measures could be incorporated here as well. These could include recently developed laboratory measure of frailty, including blood pressure, blood chemistry, and inflammatory markers (5). Similarly, additional healthspan assays or hallmarks of aging could be incorporated into GRAIL. The individual tests utilized in GRAIL could also potentially be weighted differently. Finally, mice which are censored from log-rank analysis – for example, because they were sacrificed for cross-sectional examination of tissues or molecular signaling – cannot be included in the FAMY or GRAIL calculation. Despite these and other limitations, 90% of respondents to a survey conducted at the 2023 Masoro-Barshop Conference on Aging following a presentation about these new

summary statistics agreed with the statement that “we can quantitatively measure healthspan using FAMY and/or GRAIL.”

In conclusion, we have developed two new summary statistics, FAMY and GRAIL, for the quantification of healthspan in studies of aging mice. These metrics allow the integration of lifespan and frailty data (FAMY) as well as information from healthspan assays and the hallmarks of aging (GRAIL). We have utilized these new metrics to show that CR promotes healthspan in C57BL/6J mice of both sexes, largely due to the benefits to longevity. Further, PR and IleR can promote not only lifespan, but also healthspan; and in females, GRAIL shows that improvements in healthspan can be uncoupled from increased longevity. Additional research remains to be conducted to determine the quantitative effects of other geroprotective interventions, and to identify previously unrecognized geroprotective interventions that improve healthspan without extending lifespan. While additional research will be required, and future versions of FAMY or GRAIL may enhance our ability to quantitatively assess healthspan, our results suggest that FAMY and GRAIL will be widely useful in quantifying the effects of interventions on healthspan.

Materials and methods

The lifespan and frailty data analyzed here were taken from Yu *et al.*, 2019, *Cell Reports*; Schultz *et al.*, 2020, *Nature Communications*; and Green *et al.*, 2023, *Cell Metabolism*. Data was analyzed using Excel, Prism Graphpad, and R.

Declaration of Interests

DWL has received funding from, and is a scientific advisory board member of, Aeovian Pharmaceuticals, which seeks to develop novel, selective mTOR inhibitors for the treatment of various diseases.

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Figure 1

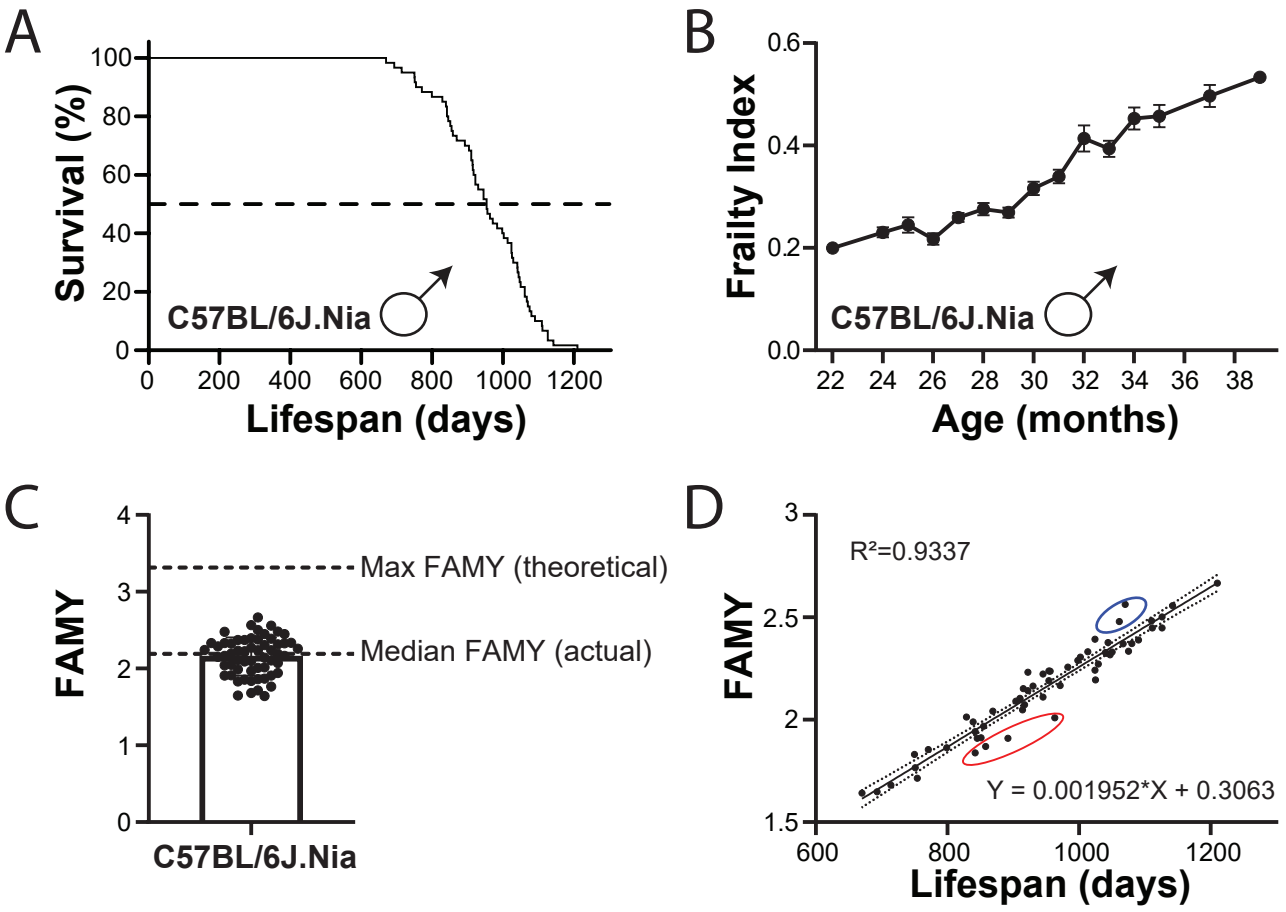

A
Survival (%)
100
80
60
40
20
0
0
200
400
600
800
1000
1200
Lifespan (days)
C57BL/6J.Nia
B
Frailty Index
0.6
0.4
0.2
0.0
22
24
26
28
30
32
34
36
38
Age (months)
C57BL/6J.Nia
C
FAMY
4
3
2
1
0
Max FAMY (theoretical)
Median FAMY (actual)
C57BL/6J.Nia
D
FAMY
3
2.5
2
1.5
R²=0.9337
Y = 0.001952*X + 0.3063
600
800
1000
1200
Lifespan (days)

Figure Legends

Figure 1. The Frailty-Adjusted Mouse Year (FAMY) as a measure of mouse healthspan.

(A-B) Survival (A) and Frailty Index (B) of C57BL/6J.Nia male mice from (8). (C) Frailty-Adjusted Mouse Years (FAMY) was calculated using the survival and frailty data plotted in panels A and B. Max FAMY (theoretical) indicates the FAMY score if the longest-lived mouse in this study remained in perfect health (FI=0) for its entire life. (D) FAMY plotted as function of lifespan. The best-fit linear regression line with 95% confidence bands is shown.

Figure 2

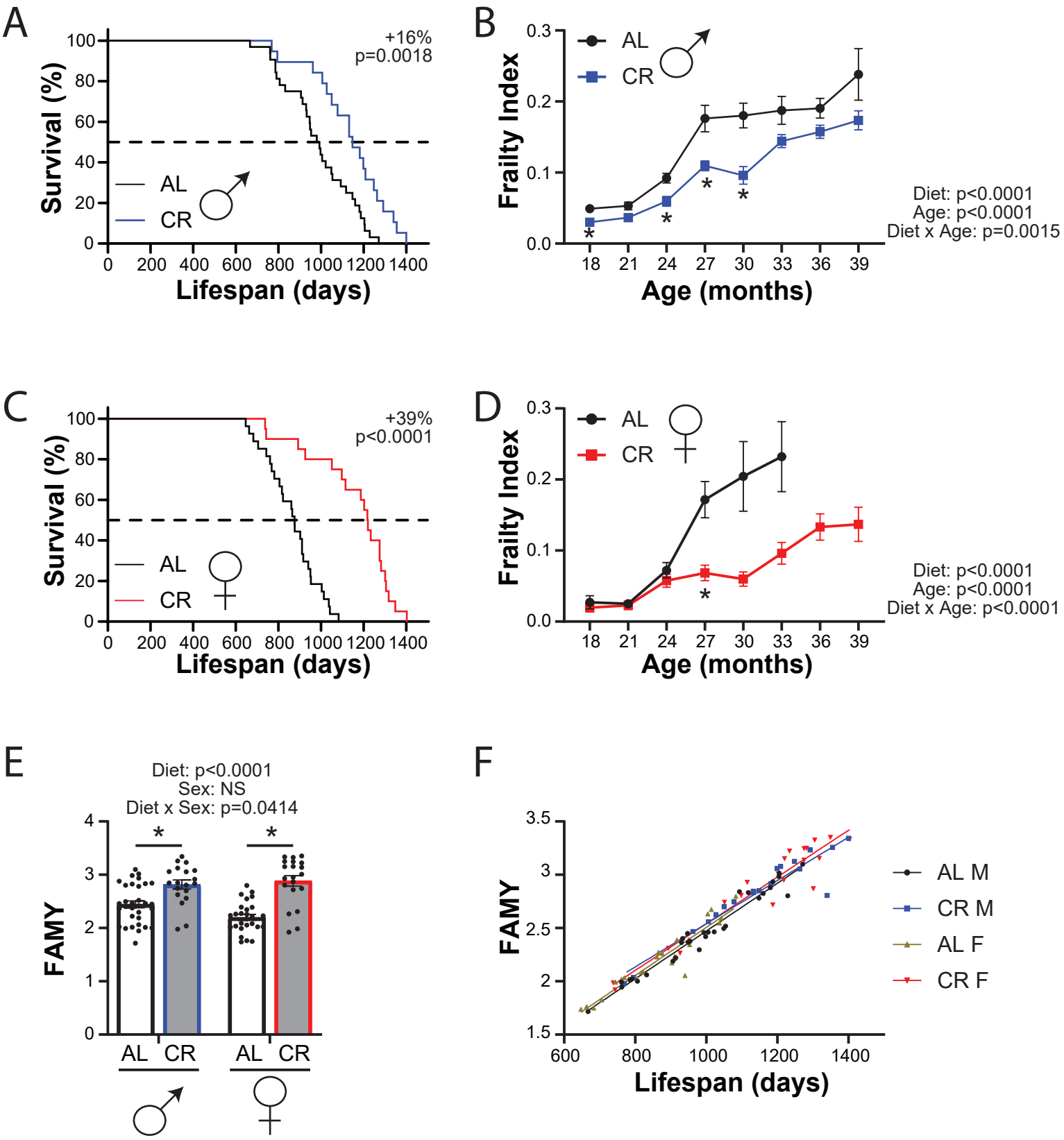

A
Survival (%)
Lifespan (days)
+16%
p=0.0018
AL
CR
B
Frailty Index
Age (months)
AL
CR
Diet: p<0.0001
Age: p<0.0001
Diet x Age: p=0.0015
C
Survival (%)
Lifespan (days)
+39%
p<0.0001
AL
CR
D
Frailty Index
Age (months)
AL
CR
Diet: p<0.0001
Age: p<0.0001
Diet x Age: p<0.0001
E
Diet: p<0.0001
Sex: NS
Diet x Sex: p=0.0414
FAMY
AL
CR
AL
CR
F
FAMY
Lifespan (days)
AL M
CR M
AL F
CR F

Figure 2. Quantifying the effect of CR on the healthspan of inbred mice with FAMY.

(A-B) Survival (A) and Frailty Index (B) of C57BL/6J male mice fed either *ad libitum* or calorie restricted (CR) starting at 10 weeks of age. (C-D) Survival (C) and Frailty Index (D) of C57BL/6J female mice fed either *ad libitum* or calorie restricted (CR) starting at 10 weeks of age. (E) FAMY calculated using the survival and frailty data plotted in panels A-D. (F) FAMY plotted as function of lifespan. (A, C) Log-rank test for AL vs. CR. (B, D) Mixed-effects model (REML) for time and diet with post-hoc Sidak's test, *$p<0.05$. (E) Sidak test following 2-way ANOVA. *$p<0.05$, P-values for the overall effect of Diet, Sex and the interaction represent the significant p-values from the two-way ANOVA. (F) The best-fit linear regression line for each group is shown. Data represented as mean ± SEM.

Figure 3

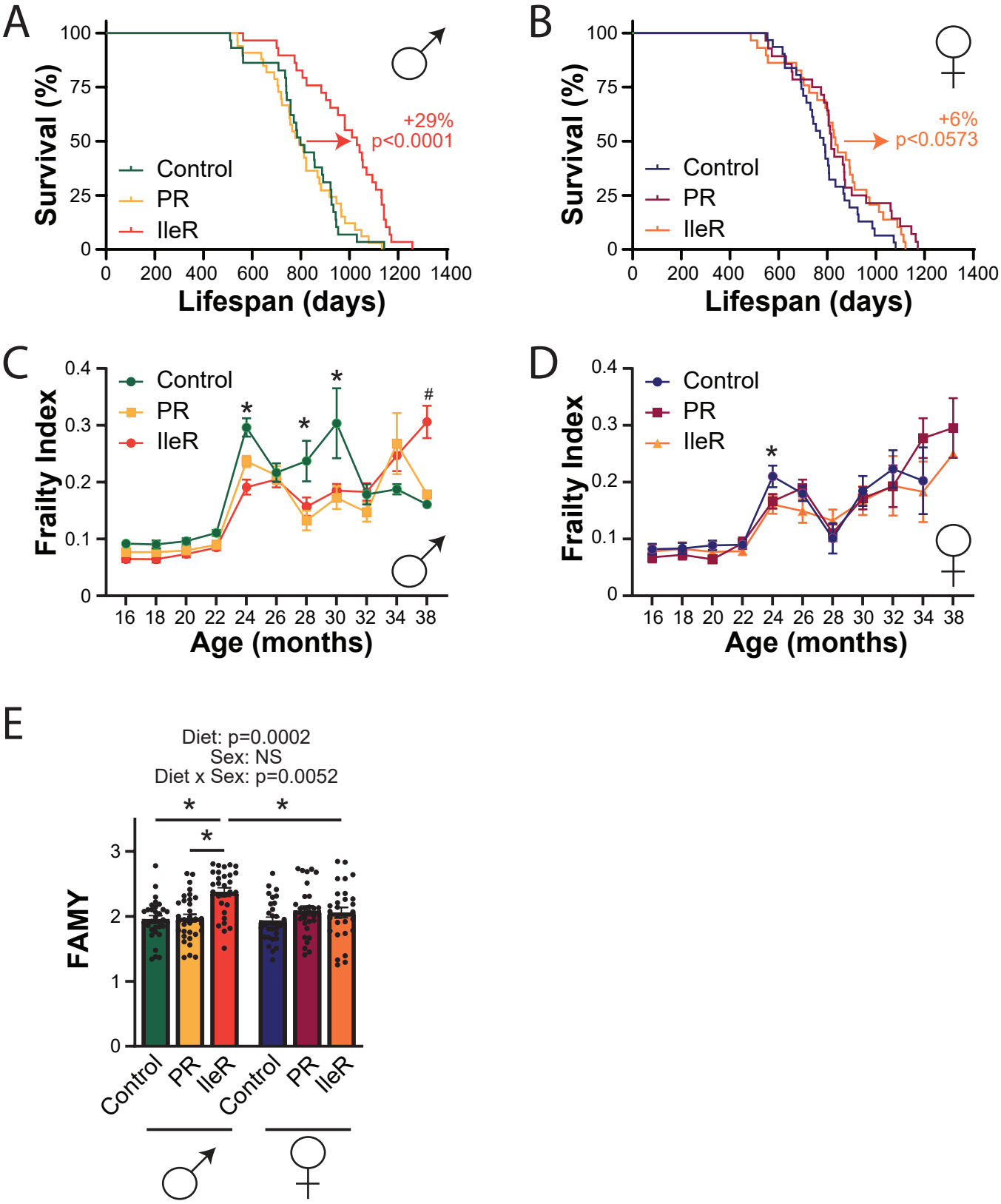
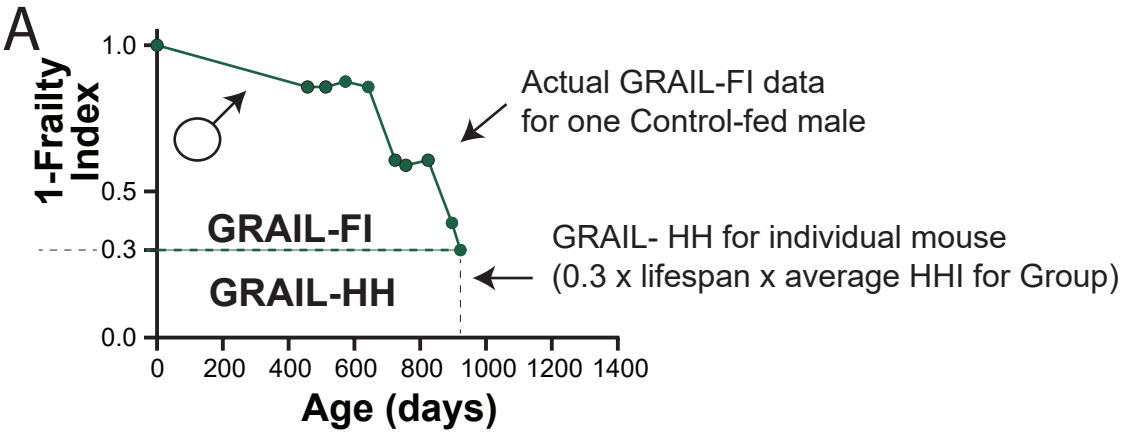

A
Survival (%)
Lifespan (days)
Control
PR
IleR
+29%
p<0.0001
B
Survival (%)
Lifespan (days)
Control
PR
IleR
+6%
p<0.0573
C
Frailty Index
Age (months)
Control
PR
IleR
D
Frailty Index
Age (months)
Control
PR
IleR
E
Diet: p=0.0002
Sex: NS
Diet x Sex: p=0.0052
FAMY
Control
PR
IleR
Control
PR
IleR

Figure 3. Quantifying the effect of reduced protein or isoleucine on the healthspan of genetically heterogeneous mice with FAMY.

(A-B) Survival of male (A) and female (B) UM-HET3 mice fed the indicated diets starting at 6 months of age. (C-D) Frailty index of the mice plotted in panels A-B. (E) FAMY calculated using the survival and frailty data plotted in panels A-D. (A-B) Log-rank test for Control vs. IleR. (C-D) Mixed-effects model (REML) for time and diet with post-hoc Tukey's test, *p<0.05 Control vs. PR and Control vs. IleR; #p<0.05 Control vs. IleR only. (E) Tukey test following 2-way ANOVA. *p<0.05, P-values for the overall effect of Diet, Sex and the interaction represent the significant p-values from the two-way ANOVA. Data represented as mean ± SEM.

Figure 4


A
1-Frailty Index
1.0
0.5
0.3
0.0
GRAIL-FI
GRAIL-HH
0
200
400
600
800
1000
1200
1400
Age (days)
Actual GRAIL-FI data
for one Control-fed male
GRAIL- HH for individual mouse
(0.3 x lifespan x average HHI for Group)

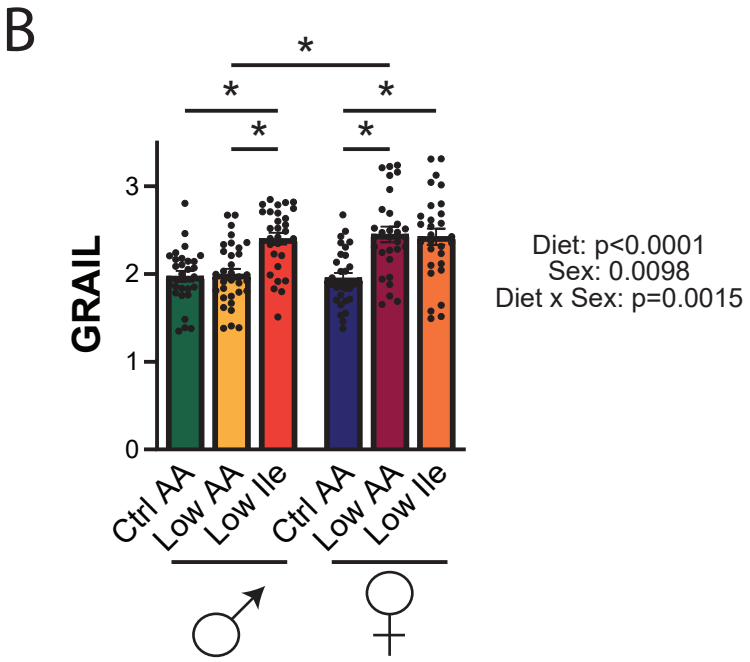

B
GRAIL
3
2
1
0
*
*
*
*
*
Ctrl AA
Low AA
Low Ile
Ctrl AA
Low AA
Low Ile
Diet: p<0.0001
Sex: 0.0098
Diet x Sex: p=0.0015

Figure 4. Integrating frailty, healthspan assays, and the hallmarks of aging with GRAIL (Gauging Robust Aging when Increasing Lifespan).

(A) Illustration of GRAIL calculation. The GRAIL score for an individual mouse combines the area under the curve of 1-frailty of an individual animal (GRAIL-FI), which varies over time, and information about the average effect on healthspan assays and the hallmarks of aging (GRAIL-HH). (B) GRAIL calculated using the survival and frailty data plotted in **Fig. 3 A-D**. Tukey test following 2-way ANOVA. *$p<0.05$, P-values for the overall effect of Diet, Sex and the interaction represent the significant p-values from the two-way ANOVA. Data represented as mean ± SEM.