Lifelong valine restriction has sex-specific benefits on health and lifespan of mice

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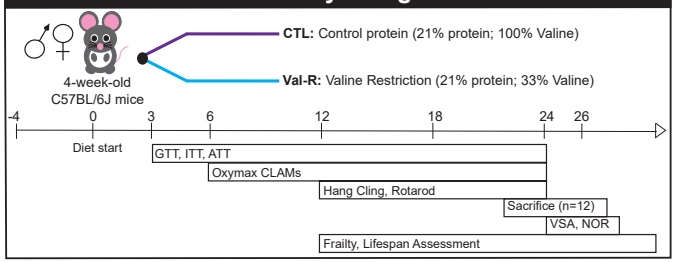
Introduction

Despite prevailing dietary advice, higher protein consumption is associated with increased rates of cancer, diabetes, and diabetes mortality. In contrast, protein restriction (PR) promotes metabolic health in humans, and in rodents, improves glycemic control and promotes leanness. The Lamming lab has previously shown that many of the benefits of PR are the result of a reduced consumption of dietary branched-chain amino acids (BCAAs; leucine, valine and isoleucine). The restriction of BCAAs extends health span and lifespan in mice. The metabolic benefits of BCAA restriction are mediated by isoleucine and valine, with isoleucine restriction being sufficient to extend lifespan in mice. However, it remains unknown whether valine restriction improves lifelong health and extends lifespan. In this study, we utilized male and female mice and placed them on a control (CTL; 21% protein, 100% valine) or valine-restricted (Val-R; 21% protein, 33% valine) diet starting at 4 weeks of age. Every 6 months, we performed metabolic phenotyping assessments until 24 months of age and assessed frailty and body composition longitudinally.

Study Design



Valine restriction prevents body weight accretion

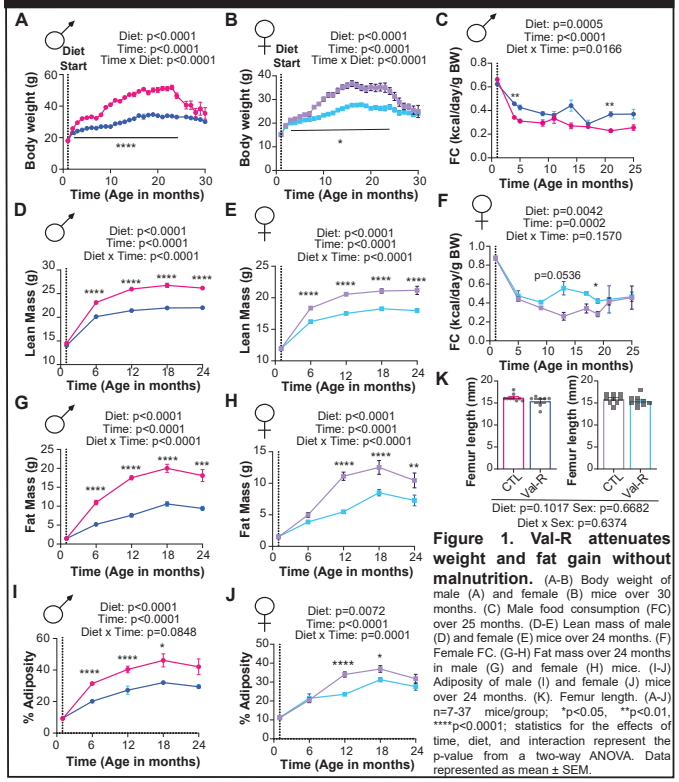


Figure 1. Val-R attenuates weight and fat gain without malnutrition. (A-B) Body weight of male (A) and female (B) mice over 30 months. (C) Male food consumption (FC) over 25 months. (D-E) Lean mass of male (D) and female (E) mice over 24 months. (F) Female FC. (G-H) Fat mass over 24 months in male (G) and female (H) mice. (I-J) Adiposity of male (I) and female (J) mice over 24 months. (K). Femur length. (A-J) n=7-37 mice/group; *p<0.05, **p<0.01, ****p<0.0001; statistics for the effects of time, diet, and interaction represent the p-value from a two-way ANOVA. Data represented as mean ± SEM.

Valine restriction increases energy expenditure

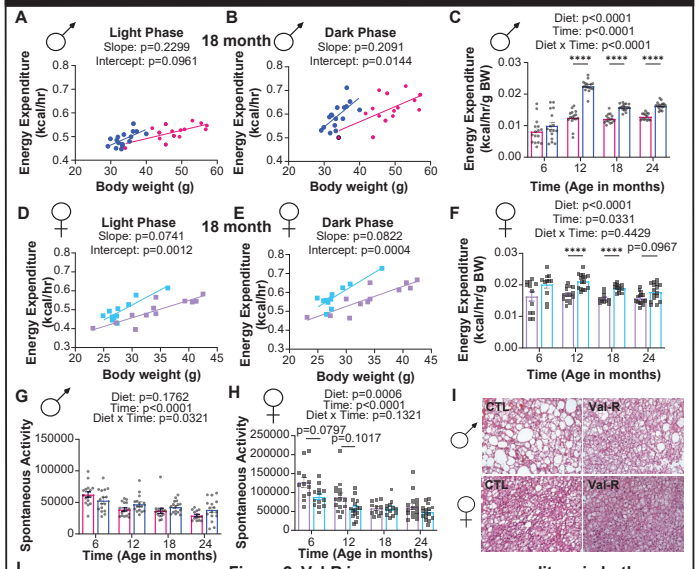


Figure 2. Val-R increases energy expenditure in both sexes. (A-B) Representative male energy expenditure (EE) graphs normalized to body weight, analyzed by ANCOVA, in both the light (A) and dark (B) phase at 18 months of age. (C) EE over time display that Val-R males have increased EE. (D-E) Representative female EE graphs, analyzed by ANCOVA, in both the light (D) and dark (E) phase. (F) EE over time display that Val-R females have increased EE. (G-H) Spontaneous activity was totaled from a period of 24 hours at multiple time points in males (G) and females (H). (I) Representative images of brown adipose tissue (BAT). (J) Quantified monolocular cells in BAT of male and females. (A-B, D-E) n=10-16 mice/group; ANCOVA. (C,F, G-I) n=10-16 mice/group; statistics for the overall effects of time, diet, and the interaction represent the p value from a two-way ANOVA, *p<0.05, **p<0.01, **p<0.001, ****p<0.0001. Data represented as mean ± SEM.

Valine restriciton improves metabolic health

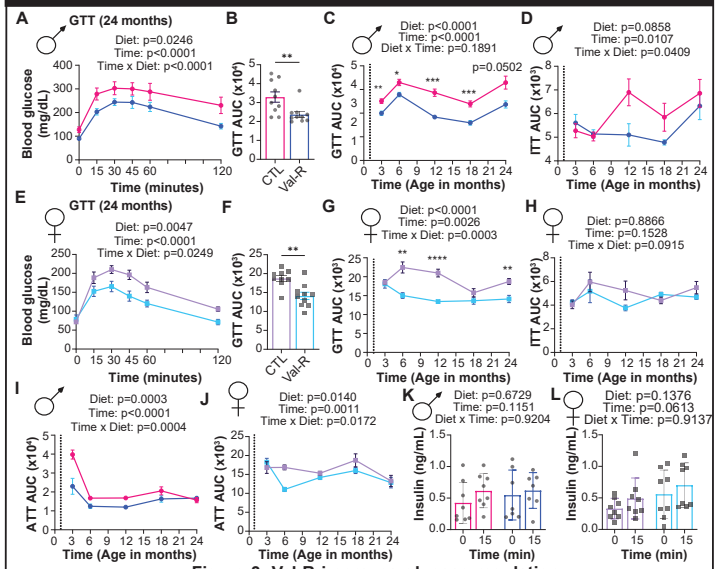


Figure 3. Val-R improves glucose regulation. (A-B) A glucose tolerance test (GTT) was conducted in male mice after a 16-hour fast after 24 months on diet. (C) GTT area under the curve (AUC) was plotted over time. (D) Insulin tolerance test (ITT) in males was conducted after a 4-hour fast. (E-F) A GTT was conducted in female mice at 24 months on diet. (G) Val-R improves glucose tolerance in females. (H) ITT AUC in females. (I-J) Alanine tolerance test (ATT) was conducted after a 16-hour fast and AUC was plotted over time in male (I) and female (J) mice. (K-L) A glucose-stimulated insulin secretion (GSIS) assay was performed at 19 months of age in male (K) and female (L). (M) Prolongevity hormone and metabolic regulator FGF21 was measured in male and female mice at 19 months of age in a fasted state. (A, C-E, G-L) n=9-12 mice/group;*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, statistics for the overall effects of time, diet, and the interaction represent the p value from a two-way ANOVA. (B,F) n=9-12 mice/group; **p<0.01 as analyzed by t-test. Data represented as mean ± SEM.

Valine restriction displays a sexual dimorphic effect on health span and lifespan

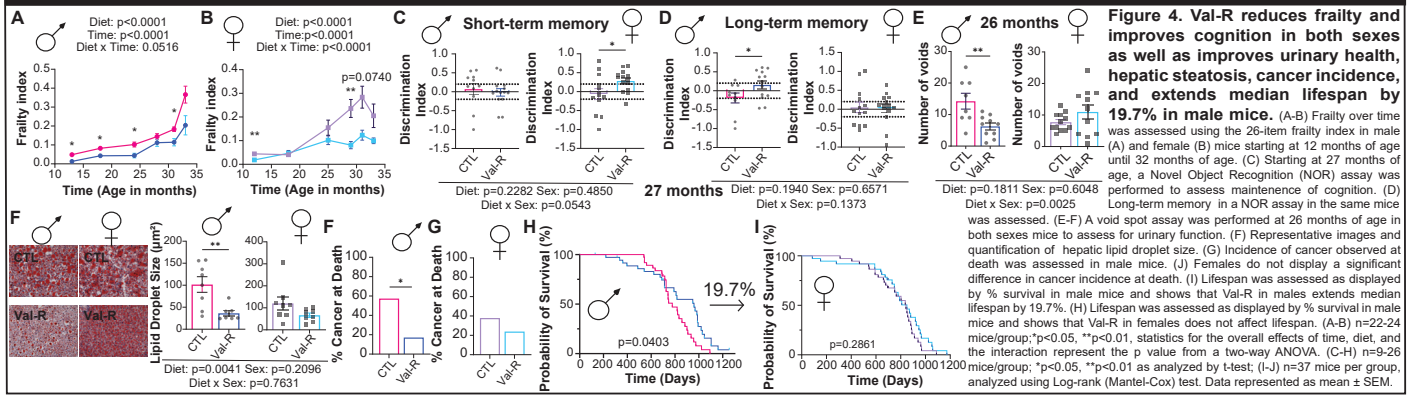


Figure 4. Val-R reduces frailty and improves cognition in both sexes as well as improves urinary health, hepatic steatosis, cancer incidence, and extends median lifespan by 19.7% in male mice. (A-B) Frailty over time was assessed using the 26-item frailty index in male (A) and female (B) mice starting at 12 months of age until 32 months of age. (C) Starting at 27 months of age, a Novel Object Recognition (NOR) assay was performed to assess maintenence of cognition. (D) Long-term memory in a NOR assay in the same mice was assessed. (E-F) A void spot assay was performed at 26 months of age in both sexes mice to assess for urinary function. (F) Representative images and quantification of hepatic lipid droplet size. (G) Incidence of cancer observed at death was assessed in male mice. (J) Females do not display a significant difference in cancer incidence at death. (I) Lifespan was assessed as displayed by % survival in male mice and shows that Val-R in males extends median lifespan by 19.7%. (H) Lifespan was assessed as displayed by % survival in male mice and shows that Val-R in females does not affect lifespan. (A-B) n=22-24 mice/group;*p<0.05, **p<0.01, statistics for the overall effects of time, diet, and the interaction represent the p value from a two-way ANOVA. (C-H) n=9-26 mice/group; *p<0.05, **p<0.01 as analyzed by t-test; (I-J) n=37 mice per group, analyzed using Log-rank (Mantel-Cox) test. Data represented as mean ± SEM.

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Conclusion and Future Directions

- Val-R prevents body weight accretion without malnutrition in both sexes
- Val-R improves energy balance and glucose regulation in both sexes
- Val-R reduces frailty and improves cognitive performance in both sexes
- Val-R improves urinary function, reverses hepatic steatosis, reduces cancer incidence and extends lifespan in male mice
- Future directions include determining the potential pathway these benefits are mediated through