Reducing dietary branched-chain amino acids improves the metabolic health and reduces cellular senescence in the liver of male C57Bl/6J mice

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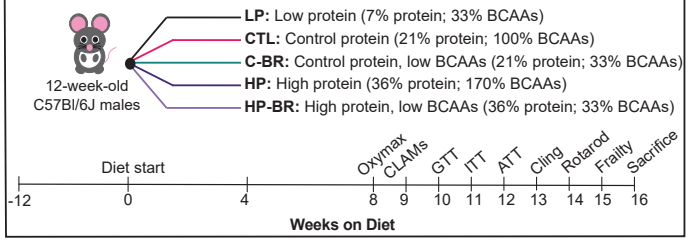
Introduction

Diet has been shown to have a profound impact on aging. Others have previously shown that protein restriction (PR) can increase lifespan and increased protein consumption leads to increased senescence gene expression in the liver of male mice. The Lamming lab has shown that the metabolic benefits of PR are partly due to decreased branched-chain amino acids (BCAAs). We have also shown that BCAA restriction improves metabolic health and extends lifespan in male mice with others showing the converse shortens lifespan. In this study, we set out to comprehensively determine the role of total protein and BCAAs in high-protein-induced senescence and its effects on metabolic health and physical fitness after 16 weeks on diet. We hypothesized that higher protein consumption will lead to increased metabolic dysfunction and increased cellular senescence in mice, which will be rescued by reducing BCAAs from the diet down to level of a low protein diet.

Study Design



Diets low in BCAAs result in lower body composition regardless of protein consumption

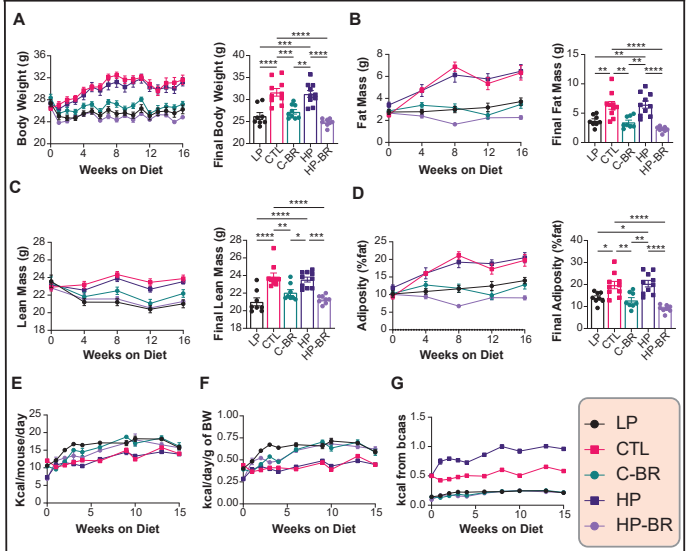


Figure 1. Diets low in BCAAs decrease body weight and body composition in young male C57Bl/6J mice without malnutrition.

(A) Body weight over time and final body weight shows that LP, C-BR and HP-BR fed animals do not gain weight over 16 weeks of dieting compared to the CTL and HP fed animals. (B) Fat mass over time and final fat mass suggests that this attenuation of body weight gain is primarily due to the prevention of fat mass accumulation. (C) Lean mass over time and final lean mass also suggest an attenuation of lean mass gain over time. (D) Percentage of adiposity over time and final adiposity. (E) Food consumption as measured by Kcal per mouse per day over time suggests weight loss is not due to manutrition as diets low in BCAAs display increased kcal consumption. (F) Food consumption per gram of BW suggests animals fed diets low in BCAAs consume higher amounts of food for their body weight. (G) Kcal from BCAAs over time confirm that consumption of BCAAs is reduced in the LP, C-BR and HP-BR diets as compared to CTL and HP. (A-D) one-way ANOVA, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Data represented as mean ± SEM.

Consumption of lower dietary BCAAs improves metabolic health

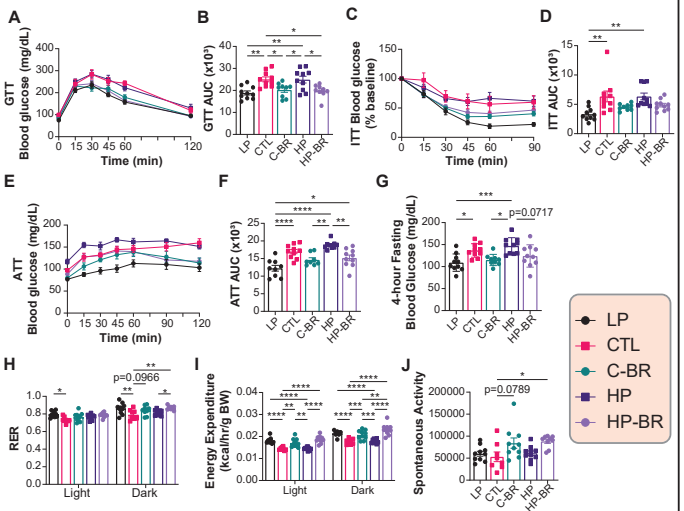
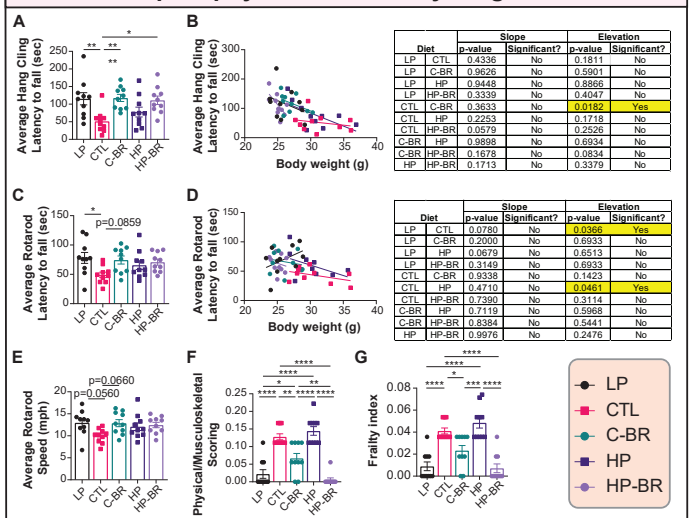


Figure 2. Low BCAA diets improve glucose regulation and increases energy expenditure.

(A-B) A glucose tolerance test (GTT) was conducted after a 16-hour fast after 10 weeks on diet. (C-D) An insulin tolerance test (ITT) was conducted after a 4-hour fast after 11 weeks on diet. (E-F) An alanine tolerance test (ATT) was conducted after a 16-hour fast after 12weeks on diet. (G) 4-hour fasting blood glucose (FBG) at week 11. (H) Respiratory exchange ratio (RER) was measured in the light and dark cycle. (I) Energy expenditure normalized to body weight over time. (J) Spontaneous activity was totaled from a period of 24 hours. (B, D, F, G, J) n=10 mice/group; one-way ANOVA, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. (H-I) n=10 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.

Alterations in protein and BCAA consumption does not impact physical fitness in young mice



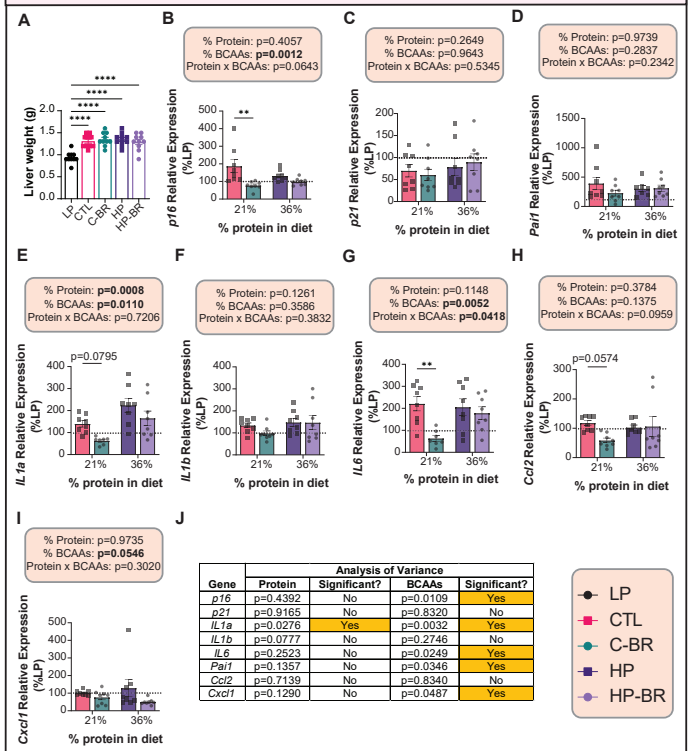
Diet		Slope p-value	Slope Significant?	Elevation p-value	Elevation Significant?
LP	CTL	0.4336	No	0.1811	No
LP	C-BR	0.9626	No	0.5901	No
LP	HP	0.9448	No	0.8866	No
LP	HP-BR	0.3339	No	0.4047	No
CTL	C-BR	0.3633	No	0.0182	Yes
CTL	HP	0.2253	No	0.1718	No
CTL	HP-BR	0.0579	No	0.2526	No
C-BR	HP	0.9898	No	0.6934	No
C-BR	HP-BR	0.1678	No	0.0834	No
HP	HP-BR	0.1713	No	0.3379	No

Diet		Slope p-value	Slope Significant?	Elevation p-value	Elevation Significant?
LP	CTL	0.0780	No	0.0366	Yes
LP	C-BR	0.2000	No	0.6933	No
LP	HP	0.0679	No	0.6513	No
LP	HP-BR	0.3149	No	0.6933	No
CTL	C-BR	0.9338	No	0.1423	No
CTL	HP	0.4710	No	0.0461	Yes
CTL	HP-BR	0.7390	No	0.3114	No
C-BR	HP	0.7119	No	0.5968	No
C-BR	HP-BR	0.8384	No	0.5441	No
HP	HP-BR	0.9976	No	0.2476	No

Figure 3. Protein and BCAA consumption does not affect grip strength and balance, but does impact body condition frailty scoring.

(A-B) Hang cling to assess for grip strength was measured and normalized to body weight to account for weight as a factor via ANCOVA. (C-D) Rotarod was performed to measure for coordination and normalized to body weight via ANCOVA. (E) Rotarod speed was measured. (F-G) Physical/musculoskeletal scoring from frailty assays are displayed to display differences in body condition. (A-G) n=10 mice/group; one-way ANOVA, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Data represented as mean ± SEM.

Reducing consumption of dietary BCAAs reduces cellular senescence in the liver



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Gene	Analysis of Variance: Protein	Significant?	BCAAs	Significant?
p16	p=0.4392	No	p=0.0109	Yes
p21	p=0.9165	No	p=0.8320	No
IL1a	p=0.0276	Yes	p=0.0032	Yes
IL1b	p=0.0777	No	p=0.2746	No
IL6	p=0.2523	No	p=0.0249	Yes
Pai1	p=0.1357	No	p=0.0346	Yes
Ccl2	p=0.7139	No	p=0.8340	No
Cxcl1	p=0.1290	No	p=0.0487	Yes

Figure 4. Lowering consumption of BCAAs results in decreased cellular senescence gene expression in the liver of young male mice.

(A) Liver weight at sacrifice at 16 weeks on diet; n=10 mice/group; one-way ANOVA, ****p<0.0001. (B-I) Gene expression of senescence markers *p16*, *p21*, *Pai1*, *IL1a*, *IL1b*, *IL6*, *Ccl2* and *Cxcl1* normalized to LP (relative expression at 100). (J) Multiple linear regression analysis was performed on gene expression to assess for contribution of protein versus BCAAs in differences of expression. (B-I) n=7-8 mice/group; statistics for the overall effects of protein, BCAAs, and the interaction represent the p value from a two-way ANOVA from a Sidak's post-test examining the effect of parameters identified as significant in the 2-way ANOVA. Data represented as mean ± SEM.

Conclusion and Future Directions

- Lowering consumption of BCAAs results in lowered fat mass, improved glucose homeostasis and increased energy expenditure
- Altering protein and BCAA consumption does not impact physical performance tests, but does impact body condition-related frailty measures
- Reduced consumption of BCAAs result in decreased senescence gene expression in the liver of young male mice
- Future directions include looking at the role of the individual BCAAs as well as FGF21 and mTORC1

Acknowlegements

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