The effects of branched-chain amino acid restriction on Hutchinson-Gilford Progeria Syndrome

Elizabeth Konon, Alexis Mitchell, Colin Boyle, Nicole Cummings, Dudley Lamming

Department of Medicine, University of Wisconsin, and William S. Middleton Memorial Veterans Hospital, Madison WI

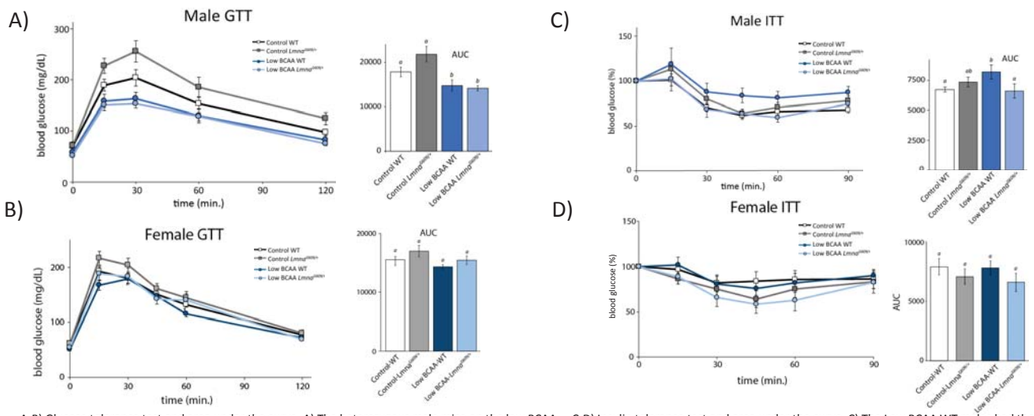
Introduction

- Hutchinson-Gilford progeria syndrome (HGPS) is a rare genetic disorder caused by a splicing mutation in the Lamin A/C gene that leads to the production of an abnormal, truncated Lamin A protein called progerin (Gabriel *et al.,* 2016). Humans with HGPS usually die prematurely as a result of heart attack or stroke.
- HGPS has been associated with defective autophagy and increased signaling of mammalian Target of Rapamycin Complex 1 (mTORC1) (Cao *et al.*, 2011), a protein kinase implicated in aging and many age-related diseases.
- Caloric restriction (CR) is the strongest nutritional intervention known to extend lifespan and improve metabolic health, but is unsustainable for the general population. Dietary regimens that mimic CR but are easier to follow are desirable.
- Our lab recently discovered that specifically reducing dietary levels of the three branched chained amino acids (BCAAs; leucine, isoleucine and valine), which are potent agonists of the mechanistic Target of Rapamycin Complex 1 (mTORC1) protein kinase, has beneficial effects on the metabolic health in mice.
- As inhibition of mTORC1 by the immunosuppressant rapamycin can extend the lifespan of some mouse models of HGPS, we hypothesized that a reduced BCAA diet might be a translatable, food-as-medicine based approach for the treatment of HGPS.
- We used a recently developed mouse model of HGPS, with the same mutation (*Lmna G609G*) found in many humans with HGPS, to test the effect of a reduced BCAA diet on the progression of HGPS.
- *In vivo* glucose tolerance (GTT) and insulin tolerance (ITT) tests were performed to test glucoregulatory control.
- Baseline weight and body composition were measured via MRI scans and mice were also placed in metabolic chambers to measure food intake, activity levels, heat production and respiratory exchange ratio (RER).
- Rotarod and grip strength tests were performed to determine the physical performance of the mice, aiding in determination of frailty.
- **By modifying diets in the form of BCAA restriction, we hope to extend the lifespan and health span of mice with the HGPS mutation. Our research will inform aging research as well as expand possibilities for potential therapeutics for HGPSP and other diseases of aging.**

Methods

- Subjects were 36 male mice and 51 female mice at 7 weeks of age on average.
- Mice were weaned and randomly assigned to either Control diet (21% amino acids) or Low BCAA diet (21% amino acids, with BCAAs reduced by two-thirds).
- The mice were genotyped to determine whether they were wild type or heterozygous for alleles containing the specific human mutation for HGPS.
- For all tests, groups were compared using 2-way ANOVA with a 2x2 factorial. Significance was determined at $\alpha<0.05$.

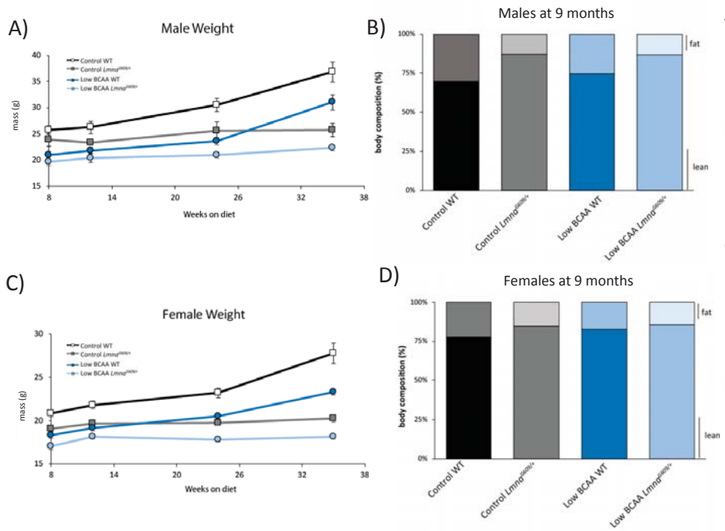
Decreased consumption of BCAAs improves glucose tolerance in males



A-B) Glucose tolerance test and area under the curve. A) The heterozygous male mice on the low BCAA diet had significantly lower blood glucose levels than those on the control diet and had blood glucose levels comparable to the wild type mice on the same diet. B) There were no significant differences between any of the female groups

C-D) Insulin tolerance test and area under the curve. C) The Low BCAA WT males had the highest blood glucose levels signifying the worst glucose tolerance. The Low BCAA heterozygous mice and the Control WT mice had the best glucose tolerance. D) There were no significant differences between any of the female groups. e

Low BCAA diet prevents weight gain by preventing fat gain



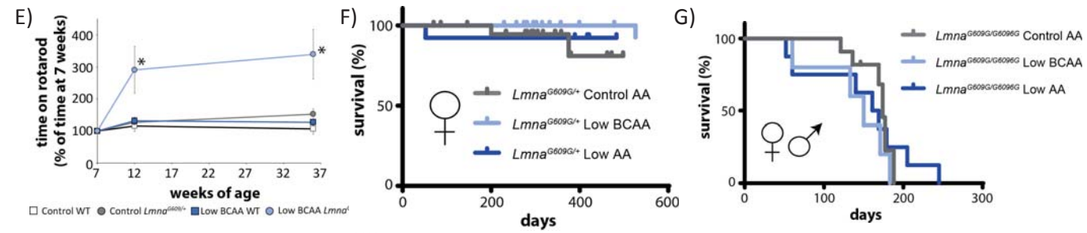
A) Male body weight. The Control WT mice weighed significantly more throughout the study while the Low BCAA *Lmna* $^{G609/+}$ weighed the least. Low BCAA WT gained more weight than the Control *Lmna* $^{G609/+}$.

B) Body composition. The Control WT had the greatest percentage of fat mass, correlating with their weight gain. The Low BCAA WT had a greater percentage of fat than *Lmna* $^{G609/+}$ on both diets.

C) Female body weight. Similar trend as males in terms of weight gained. Control WT weighed the most and Low BCAA *Lmna* $^{G609/+}$ weighed the least. Low BCAA WT gained more weight than Control *Lmna* $^{G609/+}$.

D) Body composition. The Control WT had a greater percentage of fat than the other groups. This could contribute to the weight gain seen in these mice throughout their lifespan.

Low BCAA diet improves rotarod performance and may extend lifespan in *Lmna*$^{G609G/+}$ mice



E) Rotarod Test. The Low BCAA *Lmna* $^{G609/+}$ stayed on the rotarod significantly longer than the other groups.

F) Lifespan curve of *Lmna* $^{G609/+}$. Less Control mice currently alive than other diets (n=27-60/group).

G) Lifespan curve of mutants. Mice on Low AA diet lived longer than mice on Control and Low BCAA diets (n=5-11/group).

Conclusions

- A reduction in BCAA consumption improves glucoregulatory control in *Lmna*$^{G609G/+}$ mice.
- MRI data suggests that Low BCAA diets in *Lmna* $^{G609/+}$ mice reduce weight gain by reducing fat gain.
- A low BCAA diet may increase muscular strength or decrease the decline in muscle strength in *Lmna*$^{G609G/+}$ mice.
- Reducing dietary BCAAs or total AAs may improve health and longevity in mice, and may inform future therapeutic treatments for HGPS and other age related diseases.

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