# Diet-induced responses in the heart and the liver of aged mice is sex-specific

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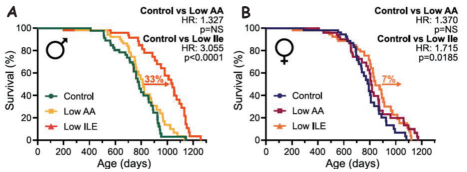
## Summary

Isoleucine is an essential dietary protein. Our lab has found that by reducing isoleucine intake by 2/3 (**Low Ile**), mice exhibit tremendous improvements in metabolism and lifespan. This project studies the effects of Low Ile in mice starting at 20-months-old, the mice-equivalent age of 60 human years.

- Even at this advanced age, Low Ile remains effective in improving metabolism.
- We found significant rejuvenation of the heart structure and function by Low Ile. This benefit was specific to females.
- In the female heart, Low Ile suppressed the group of lipids named phosphatidylglycerols, a mitochondrial component that increases with age.
- In the liver, the two sexes exhibited mostly different set of aged-induced differentially expressed genes. Notably, the female liver had more than twice of the age-driven gene changes as males, and most of these were sensitive to Low Ile treatment.

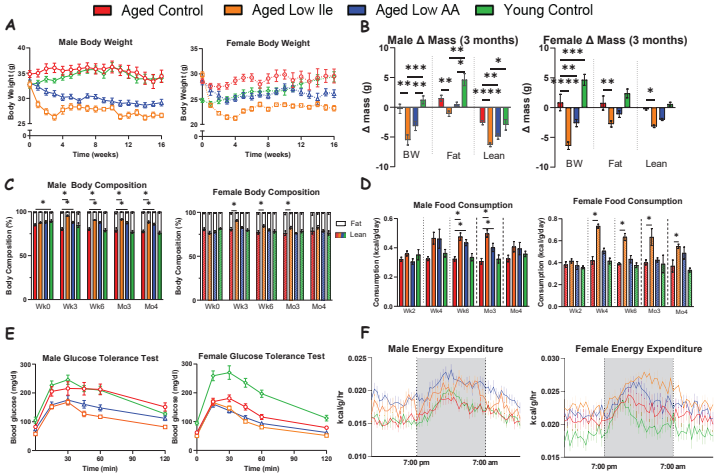
These results demonstrate that Low Ile can have profound influences on metabolism, cardiac health, and liver function even in aged mice, suggesting that dietary interventions are translationally promising for promoting healthy aging even in older people.

## Low Ile Diet Increases Lifespan in UM-HET3 mice (Green et al. 2023)



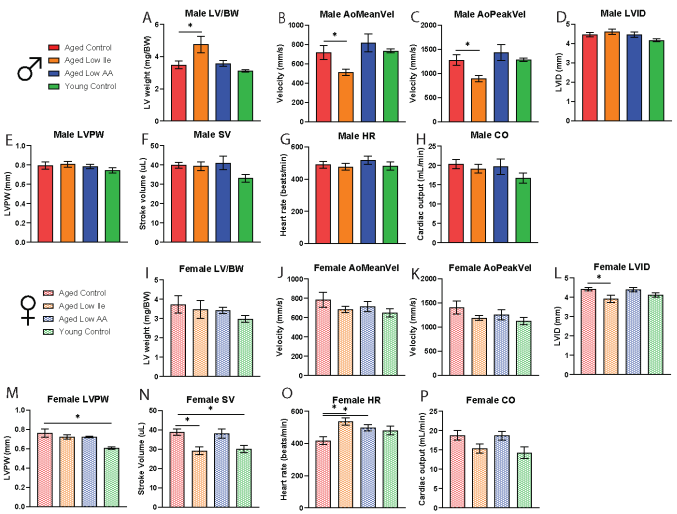

**A-B)** When starting at 6 months of age, reduction of dietary isoleucine by 66% significantly increases the lifespan of genetically diverse UM-HET3 male mice by 33% and females by 7%. (Green et al. 2023, BioRxiv)

## Late-life Low Ile Diet Induces Beneficial Metabolic Adaptations



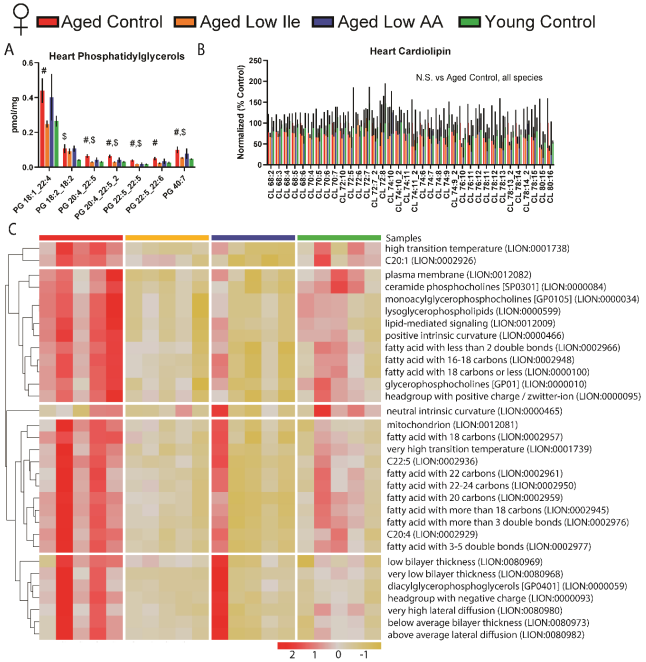

**A-B)** When Low Ile or Low AA diet starts at 20 months of age in C57BL/6J mice, mice of both sexes loses body weight, with the Low Ile diet being the most effective over Low AA.
**C)** Body composition percentile of these animals show that mice on the Low Ile diet achieve a lean body type.
**D)** Food consumption monitoring revealed overeating by both groups of dietary intervention animals.
**E)** Both Low Ile and Low AA diet effectively improves the glucose tolerance of mice at 21 months of age.
**F)** Metabolic chamber experiments demonstrate that both dietary interventions are effective in increasing the energy expenditure of aged mice.

## Low Ile Rejuvenates Aged Female Heart Functions



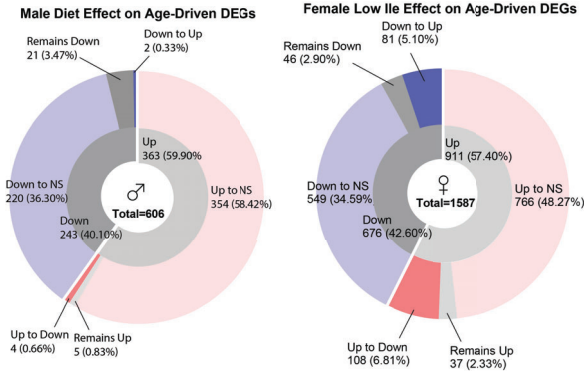

**A-H)** Echocardiogram of male mice at 25 months of age. Presented in order A) body weight-normalized left ventricle mass, B) mean aortic flow velocity, C) peak aortic velocity, D) left ventricle inner diameter, E) left ventricle posterior wall diameter, F) stroke volume, G) heart rate, H) cardiac output.
**I-J)** Echocardiogram of female mice at 25 months of age, presented in the same order as males.

## Dietary Interventions Rejuvenates the Heart Lipid Profile



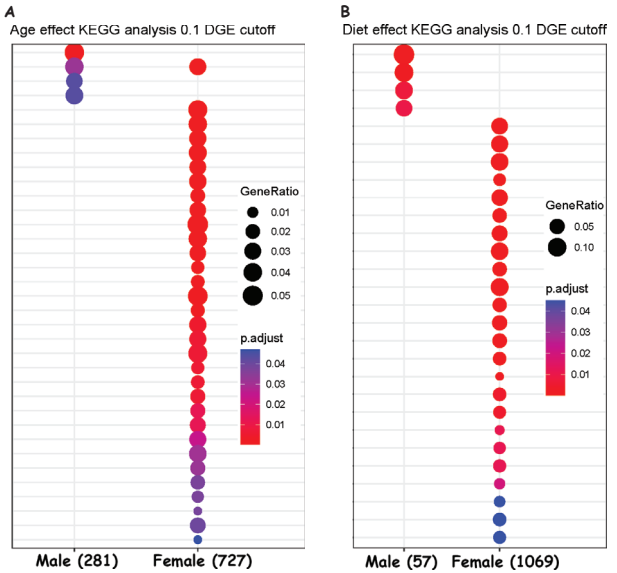

**A-B)** Statistically significant phosphatidylglycerols (A) and all cardiolipins (B), which were not significant, in female mice heart at 24 months of age after 4 months of dietary intervention.
**C)** LION lipid ontology analysis of significantly altered lipid species in the female mice heart.

## Gene Expression Analysis of the Low Ile Liver in Aged Mice




Liver transcriptomic analysis found a large number of age-driven differentially expressed genes (DEGs) which were sensitive to the Low Ile dietary intervention. Colored outer ring = diet-sensitive age-driven genes. The number of expression changes is more than doubled in the females.

## Changes in Gene Expression Pathways are Sex-Dependent




**A)** Pathway enrichment analysis of the differentially expressed genes induced by aging.
**B)** Pathway enrichment analysis of the differentially expressed genes induced by Low Ile.

**In conclusion**: A diet with 2/3 reduced isoleucine content (Low Ile) is effective in improving metabolic health in aged mice and we found rejuvenation effects of the lipid profile in the heart. But, various changes were highly sex-dependent and follow-up studies will highlight the specific effects of this diet and their consequences.

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