Metabolic analysis of mouse sarcopenic skeletal muscle identifies new strategies to increase lifespan in *C. elegans*

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Introduction

Sarcopenia is a common age-related condition characterized by the degeneration of skeletal muscle cell, resulting in loss of skeletal muscle tone, mass, and quality. Skeletal muscle is a source of systemic metabolites and growth factors that are important for the central nervous system such as BDNF, NAD, and lactate. These molecules are essential for healthy neuronal aging. We hypothesize that age-related loss of skeletal muscle might result in decreased availability of these nutrients, resulting in reduced neuronal function, and/or increased susceptibility to unhealthy aging and neurodegenerative disease.

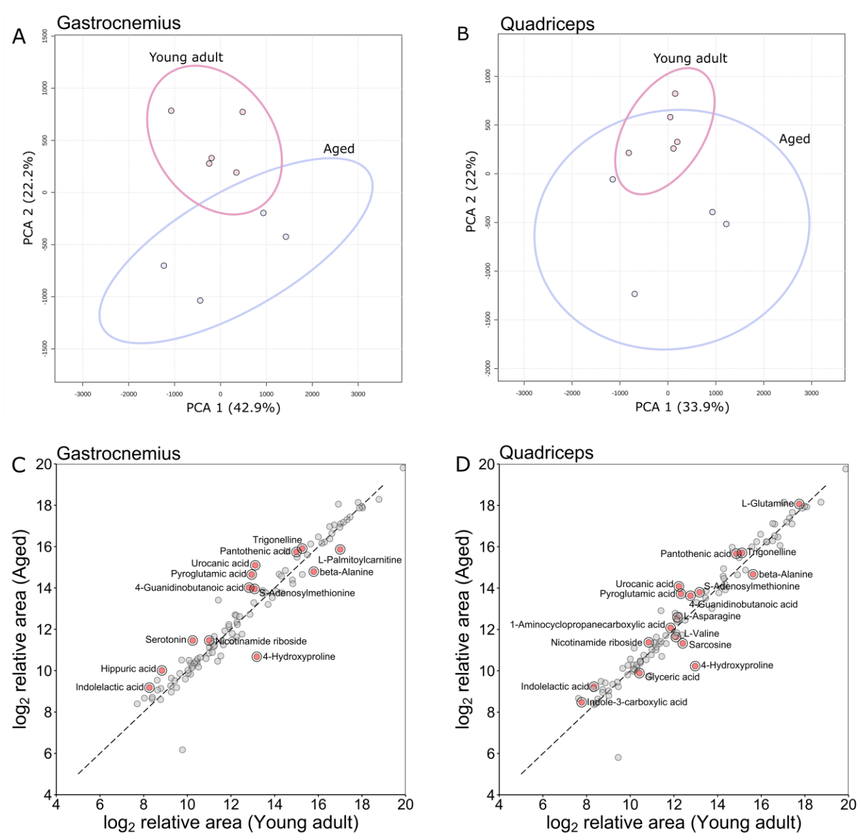
Aim

Identify potential muscle metabolic candidates that regulate healthy aging.

Method

- **C57BL/6J mice** were aged to 4 and 25 months of age
- **Skeletal muscle: gastrocnemius and quadriceps** were isolated and flash-frozen or fixed in paraformaldehyde
- **Small molecular weight metabolomics** was performed on flash-frozen samples by the Swedish Centre of Metabolomics
- **Histopathology** for H&E, mitochondrial markers, and DNA damage was performed on wax sections
- ***C. elegans* lifespan assays** were performed to assess hits from the metabolomics dataset

Mouse skeletal muscle identifies altered metabolism during aging

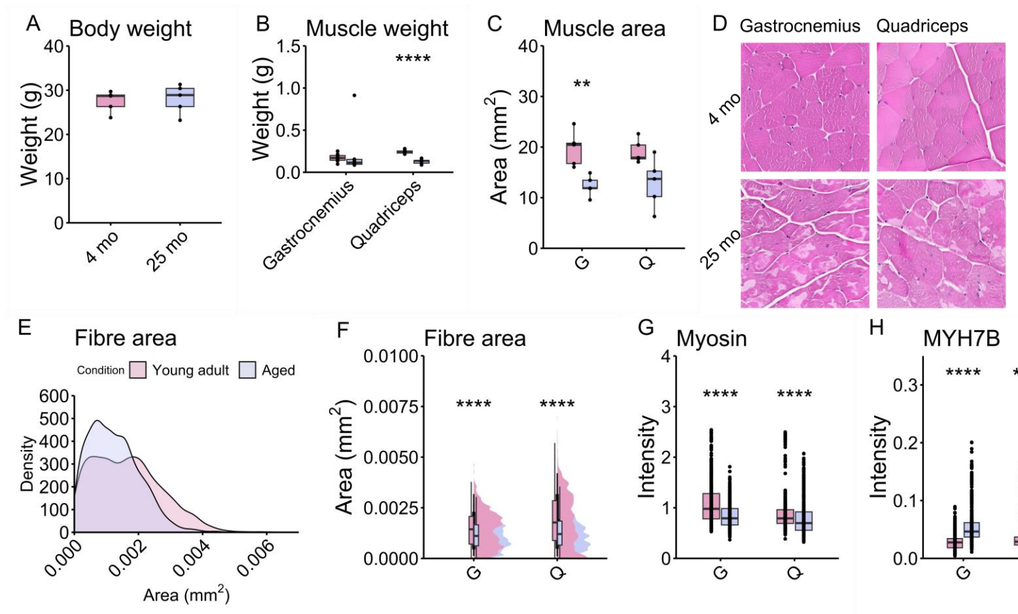


Small molecular weight metabolomics identified 108 enriched out of 502 low weight metabolites screened in young adult and aged mouse skeletal muscle. PCA plots demonstrate two distinct clusters of young adult and aged mice in gastrocnemius **(A)** and quadriceps **(B)**. Plot of gastrocnemius muscle showing 13 significantly altered metabolites during aging (FDR < 0.1) **(C)**. Plot of quadriceps muscle showing 17 significantly altered metabolites during aging (FDR < 0.1) **(D).**

Ongoing research

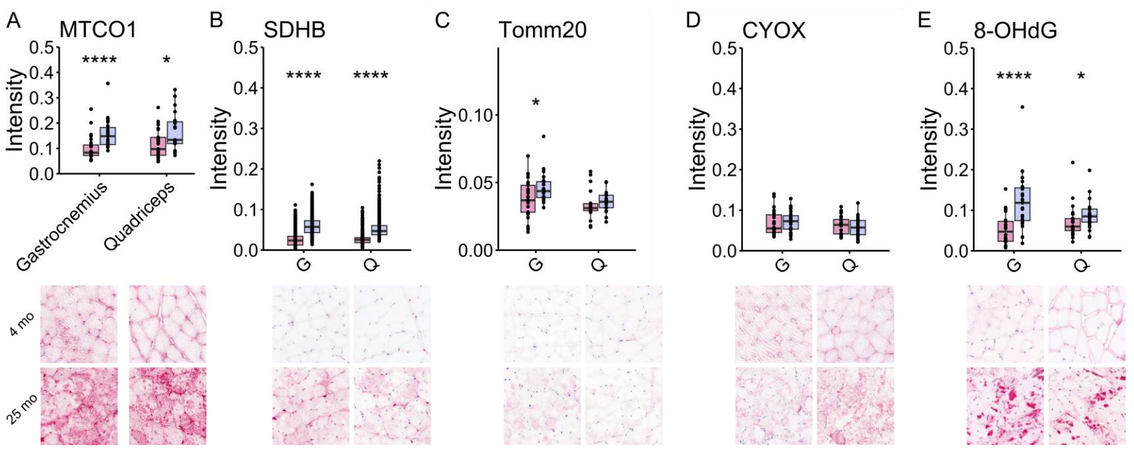
- Lifespan assays using *C. elegans*
- Effect of metabolite supplementation on mitochondria
- Effect of metabolite supplementation in *C. elegans* models of muscle wasting disease

Reduced total muscle size and muscle fibre size during aging



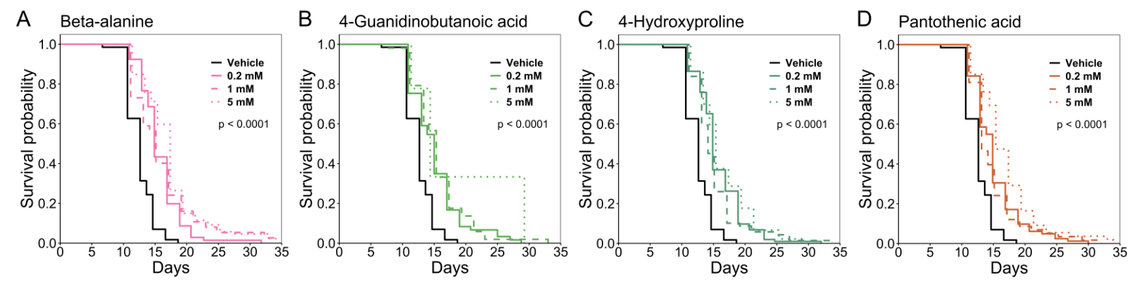
Body weight did not change during aging (**A**), while muscle weight decreased (**B**). Gastrocnemius and quadriceps muscle were stained with haematoxylin and eosin (H&E) to analyse muscle morphology during aging and identified a decrease in muscle area (**C**). H&E staining shows an integrated muscle fibre network in young adult muscle (4 months) and degraded muscle fibres in aged muscle (25 months) (**D**), quantification of individual fibres identified a reduced fibre area (**E, F**) during aging. Muscle specific myosin (**G**) decreased during aging, while MYH7B (**H**, type I fibre specific myosin) increased, suggesting a shift in ratio of type I (oxidative, slow-twitch) and type II (glycolytic, fast-twitch) fibres). * = p < 0.05, ** = p < 0.01, *** = p < 0.001, **** = p < 0.0001.

Shift in mitochondrial processes and increased DNA damage in mouse skeletal muscle during aging



Muscle biopsies were stained for mitochondrial markers. Quantification of representative images of MTCO1 (**A**, component of complex IV), SDHB (**B**, succinate dehydrogenase), Tomm20 (**C**, component of the mitochondrial outer membrane), CYOX (**D**, component of cytochrome c oxidase), 8OHdG (**E**, marker for DNA damage), identified a shift from oxidative respiration to glycolytic, by an increase of MTCO1 and SDHB. We identified DNA damage in aged muscle by an increase of 8OHdG. * = p < 0.05, ** = p < 0.01, *** = p < 0.001, **** = p < 0.0001.

Novel strategies to increase lifespan in *C. elegans*



Compounds identified in our metabolomics dataset were triaged based on a literature- and pathway analysis and effects on lifespan were assessed in a first round of lifespan assays (8 compounds tested in a flooding assay). Based on this, survival assays were performed for β-alanine (**A**), 4-guanidinobutanoic acid (**B**), 4-hydroxyproline (**C**), and pantothenic acid (**D**), showing an increase of lifespan in *C. elegans* after supplementation.

Conclusion

Using aged mouse tissue we can identify metabolic processes during skeletal muscle aging and identify treatments for aging- and mitochondrial-related diseases.

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