

Sphingolipid Metabolism Marks Early Metabolic Changes in the Genetically Inducible Murine Model Developing Sarcopenia Linked with Pathological Aging

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INTRODUCTION: Frailty syndrome is an inflammation-induced aging pathology coupled with physical disability and muscle weight loss. Lipid metabolism scaffolds acute and chronic inflammatory conditions, however, whether it impacts frailty syndrome and associated sarcopenia is unknown. Therefore, we aimed at deciphering lipid and metabolic reprogramming in the early onset of frailty.

METHODS: The previously validated murine model of frailty syndrome, based on the genetically inducible interleukin-6 expression followed by inflammation-triggered development of frailty-like phenotype, involves gastrocnemius (but not soleus) wasting [1]. The 5-day food-based administration of doxycycline (30 ppm) induced chronic inflammation in the frail model. Using LC-MS/MS, we performed discovery and quantitative metabolomics and lipidomics in plasma, skeletal muscles, and subcutaneous adipose tissue (SAT), in the early frail C57BL/6 mice and the adult littermates. All experiments followed the guidelines and approval of the Institutional Animal Care and Use Committee at the University of Arizona.

RESULTS: In gastrocnemius, but not soleus, untargeted lipidomics indicated 2-fold changes in ceramides and di- and triglycerides, in early frail compared to adult animals (Figure 1A). This was followed by a 2.5-fold increase of ceramide and glucosylceramide levels in plasma, and a reduction of glucosylceramides in SAT. In the early frail animals, di- and triglycerides tended to be downregulated in plasma and SAT, indicative of metabolic inflexibility. Quantitative analyses confirmed distinctive sphingolipid profiles between early frail and adult mice. The C16-, C18-, C24-, and C24:1-ceramides were higher in gastrocnemius and plasma, but decreased in soleus and SAT in the frail group. Accumulation of hexosylceramides in gastrocnemius and plasma, but not soleus, was driven by C16-, C18-, C24-, C24:1-glucosyl-, and C16-, C18- and C24:1-lactosyl features only in the frail group. All hexosylceramides were reduced in the SAT of frail animals. Quantitative enrichment analyses confirmed sphingolipid metabolism as 3rd most enriched pathway in the gastrocnemius of frail animals [1], short- and medium-chain acyl-carnitines, responsible for fatty acid transport into mitochondria, were found to accrue in the gastrocnemius in the frail group, upon quantitative analysis.

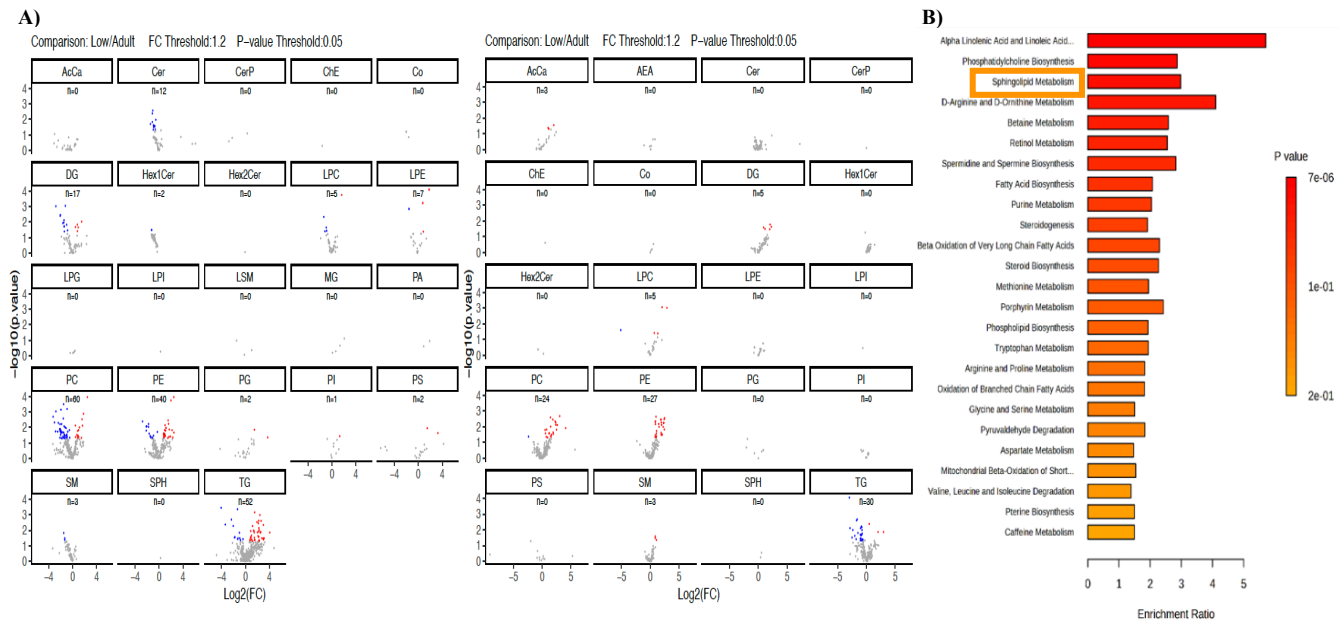
DISCUSSION: We propose an overarching mechanism of the intertissue crosstalk and myocellular metabolic shifts promoting frailty phenotype in the condition of sustained inflammation. Intramyocellular lipid accumulation in gastrocnemius, in the condition of metabolic inflexibility, underpins the early onset of frailty and associated sarcopenia, promoted by the mobilization of lipotoxic species from SAT to the skeletal muscle. The increase in circulating hexosylceramides is consistent with aggravated inflammatory burden and mitochondrial adaptive response [1], possibly led by exosomes upon mitophagy.

SIGNIFICANCE/CLINICAL RELEVANCE: Accumulation of hexosylceramides coupled with aberrant mitochondrial metabolism in gastrocnemius, but not soleus, might be a lipotoxic mechanism underlying frailty-linked sarcopenia, similar to what is already proposed in muscular dystrophy, and age-related sarcopenia. Sphingolipid nodes might serve as actionable targets and pharmacological inhibition of hexosylceramide synthase activity should be tested in sarcopenic conditions.

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REFERENCES: [1] Jergović M, et al. "IL-6 can singlehandedly drive many features of frailty in mice." *Geroscience* 43 (2021): 539-549.

Figure 1. Lipidomic and metabolomic shifts indicate upregulation of sphingolipid synthesis in inflammation-triggered frailty-related sarcopenia.



A. The distribution of main lipid sub-classes in gastrocnemius (left) and soleus (right) in the early frail group (denoted as Low) compared with adult mice. Deconstructed volcano plots are separated based on lipid classes. The red/blue dots represent features up/downregulated, above both thresholds of fold change($x \geq 1.2$) and t -tests($y \geq 0.05$). AcCa, acylcarnitines; AEA, acylethanolamine; Cer, ceramides; ChE, cholesterol ester; Co, coenzyme; MG/DG/TG, mono/di/tri-glycerides; HexCer, hexosylceramides; L, lyso-; PC/PE/PG/PI/PS, phosphatidyl-choline/ethanolamine/glyceride/inositol/serine; PA, phosphatidic acid; SM, sphingomyelin; SPH, sphingosine. B. Quantitative enrichment analyses showing upregulated metabolic pathways in plasma from early frail in comparison with adult animals.