Abstract: 50O

Metabolomic analysis of mouse skeletal muscle and liver responses to acute exercise and disruption of AMPK-glycogen binding

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Introduction: Exercise is well known to elicit wide metabolic health benefits. However, the breadth of molecular mechanisms underlying these beneficial metabolic effects are not fully known. A key energy-sensing enzyme activated in response to exercise is the AMP-activated protein kinase (AMPK), a master regulator of energy metabolism that binds glycogen, a major energy reserve primarily stored in liver and skeletal muscle. Our group has recently shown that disrupting glycogen binding capacity in AMPK double knock-in (DKI) mice is associated with reduced maximal running speed and impairments in whole-body and tissue metabolic homeostasis. Metabolomic analyses of plasma revealed that DKI mice have increased utilisation of amino acids versus wild type (WT) mice following exercise. However, metabolomic analyses of metabolically active tissues including skeletal muscle and liver are also required to more fully understand the molecular metabolic responses to acute treadmill exercise and potential mechanisms underlying the physiological effects of disrupting AMPK-glycogen binding in mice.

Methods: Gastrocnemius skeletal muscle and liver tissue samples were collected from age-matched male WT and AMPK DKI mice with disrupted AMPK-glycogen binding at rest and immediately following 30-min submaximal treadmill running. An untargeted mass spectrometry-based metabolomic approach was utilized to determine changes in metabolites occurring in response to acute exercise and disrupting AMPK's glycogen binding capacity. Complementary real-time metabolic phenotyping assays using the Seahorse XFe24 analyser and Oroboros O2k respirometer are being performed to compare energy metabolism and substrate utilisation profiles between genotypes in mouse embryonic fibroblast cells and tissue samples obtained from WT and DKI mice.

Results: Metabolomics identified a total of 94 and 151 metabolites in skeletal muscle and liver, respectively. Similar to the plasma metabolite responses observed across genotypes and conditions, metabolomic analyses indicated significant overall metabolite profile shifts between WT and DKI mice at rest, as well as significant metabolite profile differences between the rested and exercised conditions. In contrast to liver, an interaction effect was observed in skeletal muscle, indicating differential muscle metabolite responses to acute exercise between genotypes. Metabolic phenotyping of WT and DKI mouse cells and tissues is currently underway to further interrogate metabolic pathways identified to be affected by AMPK-glycogen binding disruption.

Conclusion: Metabolomics has uncovered concomitant alterations in the plasma, skeletal muscle and liver metabolite profiles between rested and exercised mice in both genotypes, and between genotypes at rest. These mouse tissue metabolomic datasets complement our previous whole-body, tissue and molecular characterisation of WT and DKI mice, revealing potential novel molecular mechanisms in tissues and the circulation that may contribute to exercise's metabolic health benefits and the physiological effects of disrupting AMPK-glycogen binding *in vivo*.