Scientific Meeting
AuPS | ASB
HOBART · 2022

Abstract: 30O

Ceramide metabolism in skeletal muscle – questioning previous models

Sarah Turpin-Nolan[1], Michael Mah[1], Dovile Anderson[1], Allen Barry[1], Anthony Don[2], Mark Febbraio[1]

[1] *Cellular and Molecular Metabolism Laboratory, Monash Institute of Pharmaceutical Science, Monash University, Parkville, Australia*

[2] *Lipid Metabolism and Neurochemistry Laboratory, Centenary Institute and Sydney University Medical School, Sydney, Australia*

Ceramides are increased in metabolically relevant tissues during obesity and contribute to the development of insulin resistance. However, ceramides vary in acyl-chain lengths from $C_{12:0\text{-}C30:0}$ which are regulated by the (dihydro)-ceramide synthases 1-6 (CerS). Both CerS5 and 6 generate $C_{16:0}$ ceramides whilst CerS1 produces $C_{18:0}$ ceramides. CerS1 is the highest expressed in skeletal muscle which produces $C_{18:0}$ ceramides[1]. High fat diet fed *CerS1*$^{\Delta SkM}$ mice that selectively reduced $C_{18:0}$ ceramides, showed significantly improved insulin and glucose tolerance, but this could not be attributed to previously described mechanisms of skeletal muscle ceramide inhibition of the insulin signalling pathway. The aim of this study was to identify if $C_{18:0}$ ceramides induce changes to the insulin signalling cascade to contribute to insulin resistance.

Methods: C2C12 murine skeletal myoblasts and myotubes were treated with 0.2mM palmitate, 0.2mM stearate and a 1:1 palmitate:stearate combination coupled to 2% fatty acid free BSA for 24 hours for RNA, protein and lipidomic quantification of sphingolipids. Quadriceps and gastrocnemius muscles were isolated from C57Bl6 mice, one muscle was snap frozen and the other underwent myoblast isolation for the measurement of sphingolipids by lipidomic analysis.

Results: Cultured C2C12 myotubes have different ceramide profiles compared to skeletal muscles of mice. Specifically, $C_{16:0}$ and $C_{24:0}$ ceramides were the predominate ceramide species in C2C12 myotubes not $C_{18:0}$ ceramides like mouse quadriceps. Incubations of different fatty acid substrates and combinations to selectively generate a more physiologically relevant ceramide profile failed in C2C12 myotubes revealing that they are not a valid model to study how $C_{18:0}$ ceramides regulate insulin signalling in skeletal muscle. Further experiments using primary cultured myotubes derived from mice demonstrated skeletal muscle myotubes and myoblasts undergo a ceramide synthesis switch from $C_{18:0}$ ceramides to $C_{16:0}$ ceramides during the culturing procedure.

Conclusion: We have determined that studies delineating the role of ceramides in skeletal muscle metabolism performed in myotube and myoblast models, do not reflect the signalling mechanisms that occur in the skeletal muscles of animals and humans. The ceramide profiles from immortalised and primary muscle culture systems are completely different to those *in vivo* and specific ceramide species cannot be selectively produced by manipulating fatty acid substrate availability.