**Uncoupling Mitochondrial Dysfunction from Insulin Resistance: Ceramide as a Metabolic Gatekeeper**

**INTRODUCTION**: Skeletal muscle insulin resistance (IR) is a defining feature of type 2 diabetes and a key node in cardiometabolic disease. Mitochondrial dysfunction and lipotoxicity have long been implicated in IR pathogenesis, yet their causal interplay remains unclear.

**AIM**: To determine whether mitochondrial dysfunction per se is sufficient to drive skeletal muscle IR and to elucidate the role of lipids in this process.

**METHODS**: We generated skeletal muscle-specific MIPEP knockout (MIPEP-mKO) mice to induce mitochondrial dysfunction through impaired processing of mitochondrial precursor proteins. Mitochondrial function, lipid species, and insulin sensitivity were assessed using proteomics, targeted lipidomic, in vivo glucose uptake, and GLUT4 translocation in isolated muscle fibres under chow and high-fat diet (HFD) conditions.

**RESULTS**: MIPEP-mKO mice exhibited severe mitochondrial dysfunction, including depletion of respiratory complexes, mitochondrial fragmentation, mitochondrial depolarisation, and oxidative stress. This was accompanied by significant accumulation of lipotoxic intermediates, including diacylglycerols and acylcarnitines. Surprisingly, despite these defects, insulin-stimulated glucose uptake and GLUT4 translocation remained intact under chow-fed conditions. Targeted lipidomics revealed a selective depletion of C18:0 ceramide—a known inhibitor of insulin sensitivity—driven by impaired serine metabolism. When challenged with HFD, MIPEP-mKO mice displayed exacerbated skeletal muscle IR, indicating increased vulnerability to nutrient-induced metabolic stress despite elevated systemic FGF21 levels.

**CONCLUSION**: Mitochondrial dysfunction and lipid accumulation alone are insufficient to impair insulin sensitivity in skeletal muscle. Instead, our data point to a protective role for reduced ceramide biosynthesis under basal conditions, and highlight ceramides as critical modulators of insulin action, both upstream and downstream of mitochondrial dysfunction. These findings redefine the metabolic hierarchy in muscle IR and underscore the importance of nutrient context in determining insulin responsiveness.