**Overexpression of ACAD10 alters the insulin signaling pathway and activates aspects of the mitochondrial integrated stress response (ISRmt**).

Aims: Acyl-CoA dehydrogenase family member 10 (ACAD10) is a mitochondrial protein purported to be involved in the fatty acid beta-oxidation pathway. Variants in ACAD10 are associated with type 2 diabetes, insulin resistance and lipid oxidation in Pima Indians1[. However, metabolic phenotyping](https://pubmed.ncbi.nlm.nih.gov/20390405/) following deletion of ACAD10 revealed no changes in body composition, energy expenditure, glucose homeostasis, mitochondrial function or metformin action2. Currently, it is unknown whether upregulation of ACAD10 alters metabolic function.

Methods: To characterise the effect of ACAD10 overexpression on metabolic pathways, we overexpressed ACAD10 using an adenovirus in HepG2 liver cells. Western blotting and qPCR were used to examine insulin signaling and related pathways while mitochondrial function was assessed via a Seahorse bioanalyser assay.

Results: Overexpression of ACAD10 in HepG2 cells, did not alter mitochondrial function but did alter components related to the insulin signalling pathways such as increasing the phosphorylation of AKT and the phosphorylation of GSK-3β, ERK and p70 S6K. Profiler PCR Gene Array of 84 genes related to insulin pathways identified 18 genes that were significantly different with ACAD10 overexpression, 15 of which we validated via qPCR analysis. Of these, the two genes most upregulated with ACAD10 overexpression were Serpine1 (PAI-1) and FOS (cFOS) which were also elevated at the protein level. As cFOS is a Fibroblast growth factor 21 (Fgf21) target gene, we assessed markers of the integrated stress response (ISR) with ACAD10 overexpression. While Fgf21 was unchanged, several other genes related to the ISR such as Gdf-15, Asns and Gadd34 were elevated.

Conclusion: Overexpression of ACAD10 alters the mRNA and protein expression of components related to the insulin signalling pathway and the ISR, a finding warranting further investigation in in vivo models.

References:

1. Bian L, et al, Diabetologia, 2010, PMID: 20390405.
2. Yew MJ, et al, Diabetes Obesity and Metabolism, 2024, PMID: 38351663.