**Abstract title:**

Systems genetics links *Ets1* suppression to enhanced glucose uptake in insulin-resistant adipocytes

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Aim: White adipose tissue (WAT) is critical in systemic metabolic homeostasis given the link between obesity and metabolic disease. However, some individuals develop obesity yet retain normal metabolic profiles, suggesting that metabolic homeostasis is regulated by genetic and environmental factors. This study aims to leverage the genetic heterogeneity of Diversity Outbred in Australia (DOz) mice, a mouse population mirroring human genetic diversity, to uncover regulators of WAT glucose metabolism.

Methods: Utilising a novel assay to accurately quantify glucose uptake (GU) into WAT *ex vivo*, we measured insulin-stimulated GU in 559 DOz mice fed either chow or a Western-style diet (WD). Quantitative trait locus (QTL) mapping was performed to identify genetic markers associated with GU. The target of interest was knocked down in 3T3-L1 adipocytes, followed by performing cellular GU assay, GLUT4 trafficking assay, and proteomics to evaluate its role.

Result: Haplotype- and SNP-based QTL mapping identified 6 QTLs associated with GU in WAT. Notably, we identified a peak on chromosome 9 which had a strong signal using both approaches. Top SNPs at this peak were located in a region encoding a transcription factor named Protein C-ets-1 (*Ets1*). Stratifying the genotype by diet at this SNP suggested that *Ets1* may only affect GU in WD-fed animals. Remarkably, silencing *Ets1* (shETS1) in 3T3-L1 adipocytes rescued insulin-stimulated GU in insulin-resistant (IR) adipocytes. Proteomics revealed that a greater number of key proteins involved in insulin-stimulated GU, including GLUT4, were downregulated in shETS1 cells in the IR condition. Despite this, GLUT4 translocation remained unaffected. Furthermore, proteomics suggested a shift in heme metabolism in the IR condition, marked by lower Delta-aminolevulinate synthase 1 (ALAS1) and increased heme oxygenase-2 (HMOX2) expression.

Conclusion: Overall, results indicate that ETS1 knockdown may improve GU in IR 3T3-L1 adipocytes by reducing excess heme synthesis and enhancing heme breakdown, thereby alleviating oxidative stress associated with IR.