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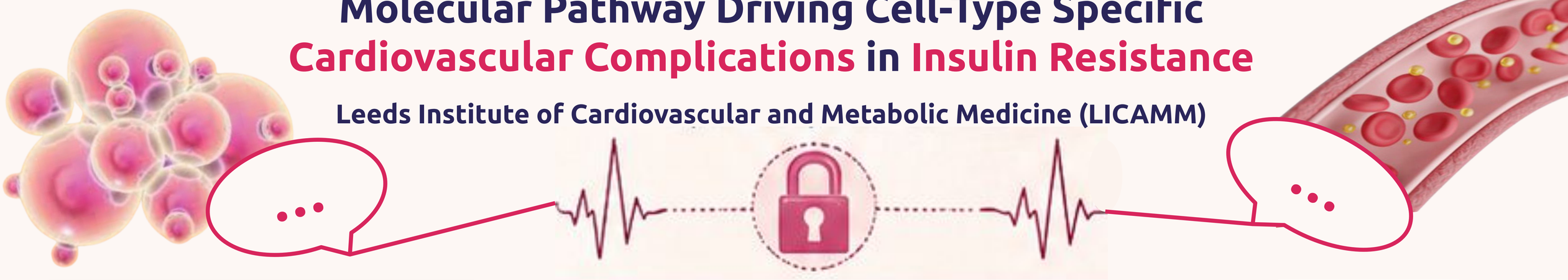
Decoding Cryptic Conversation: Discovery of a Novel Molecular Pathway Driving Cell-Type Specific Cardiovascular Complications in Insulin Resistance

Leeds Institute of Cardiovascular and Metabolic Medicine (LICAMM)



1 Error! Molecular Pathway Not Found

- Cardiovascular disease (CVD) remains the leading cause of death worldwide, accounting for 47% and 39% of deaths in women and men respectively (European Society of Cardiology, 2024).
- Type 2 diabetes mellitus (T2DM) is characterised by insulin resistance and chronic inflammation, with endothelial dysfunction representing an early consequence potentially contributing to later cardiovascular complications.
- Previous research has not fully explored these pathways in the vascular endothelium, highlighting a gap in understanding vascular metabolic dysfunction.

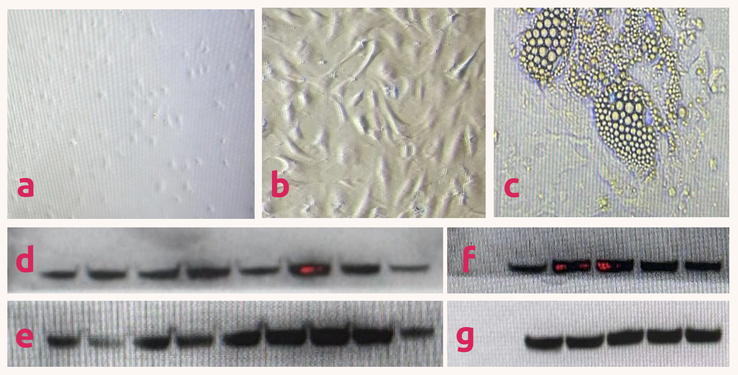
2 SEARCH QUERY: Locate Pathway

Investigate adipose-endothelial intercellular signalling under insulin-resistant conditions.

Generate and analyse experimental data on mechanisms driving diabetic cardiovascular disease.

Explore METRNL and inflammatory miRNAs as potential therapeutic targets.

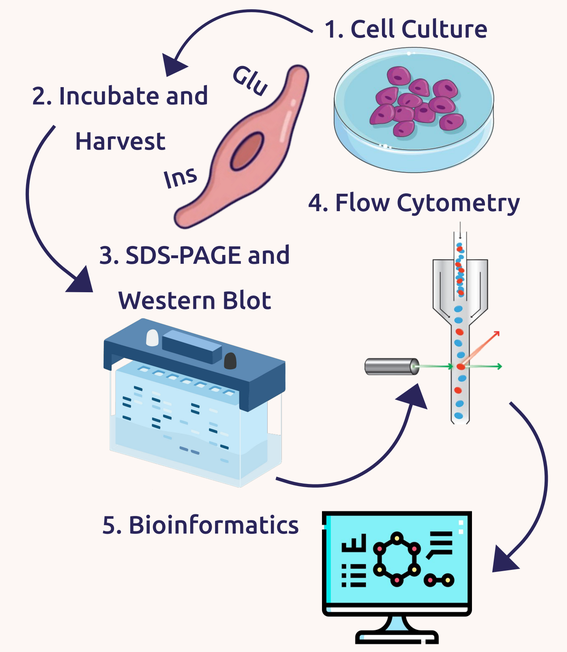
Fig. 1: Microscopy and Densitometry



(a) SVECs. (b) Undifferentiated human adipocytes. (c) Differentiated human adipocytes. (d) Akt-p Blot (SVECs). (e) Actin Blot (SVECs). (f) Akt-p Blot (Adip). (g) Actin Blot (Adip).

3 Loading...

1. Cell Culture: Model vascular endothelial cells in vitro.
2. Incubate and Harvest: Model insulin-resistant conditions in cultured cells and human adipocytes by treating them with insulin and glucose at varying doses for 24 hours.
3. SDS-PAGE → Western Blot: Quantify proteins linked to insulin signalling and endothelial function via densitometric imaging.
4. Flow Cytometry: Characterise cellular responses at single-cell resolution to examine cellular phenotypes and biomarkers associated with metabolic dysfunction.
5. Bioinformatics analysis using miRNet to prioritise next steps.



4 Signal Detected!

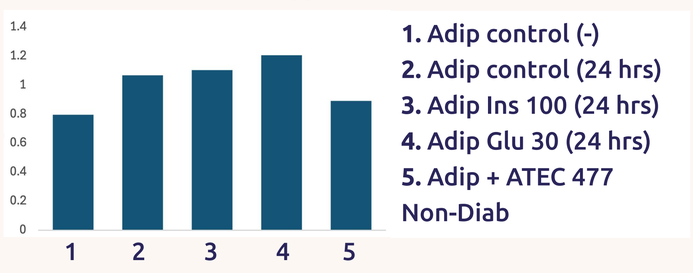


Fig. 2: Western blot analysis of Akt phosphorylation (Akt-p) normalized to Actin in adipocytes following metabolic and endothelial-derived treatments.

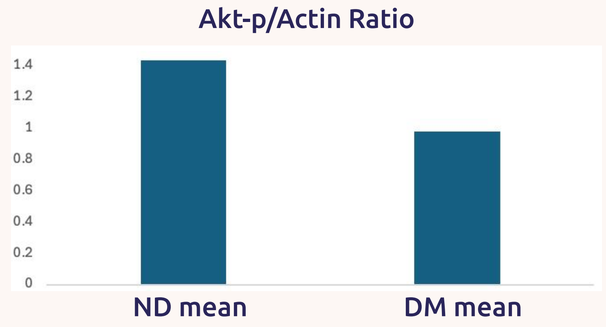


Fig. 3: Western blot analysis of Akt phosphorylation (Akt-p) normalized to Actin in non-diabetic (ND) versus diabetic (DM) SVECs.

Pathway	Adjusted P-value
TNF signalling	1.4e-12
Insulin resistance	7.8e-9
Insulin signalling	1.45e-8
PI3K-Akt signalling	1.48e-8
AMPK signalling	8.02e-8
Actin cytoskeleton regulation	0.0000121
TGF-β signalling	0.0000831
Toll-like receptor signalling	0.000143
Adipocytokine metabolism	0.000171
Fatty acid metabolism	0.000781
Glycolysis/ gluconeogenesis	0.00194
Type 2 diabetes mellitus	0.00293
Vascular smooth muscle contraction	0.00736
Adipocyte lipolysis	0.0113
Tight junction	0.0113
JAK–STAT signalling	0.0208

Table 1: Significant inflammatory and metabolic pathways identified by KEGG enrichment analysis via miRNet.

5 Translating...

- Pathway enrichment analysis identified multiple significantly enriched pathways associated with insulin resistance and cardiovascular disease (**Table 1**).
- Akt phosphorylation (Akt-p) was 32% lower in diabetic SVECs (0.98) compared with non-diabetic SVECs (1.44) (**Fig. 3**), suggesting reduced Akt pathway activity in diabetic endothelial cells.
- High-glucose exposure increased Akt-p in adipocytes despite the insulin-resistant conditions, suggesting activation of a compensatory metabolic signalling response.
- Treatment with non-diabetic endothelial-derived factor ATEC 477 reduced Akt-p relative to the 24-hour controls (**Fig. 2**), which suggests endothelial-adipocyte communication modulates metabolic signalling pathways beyond the canonical insulin-Akt axis (**Fig. 4**).

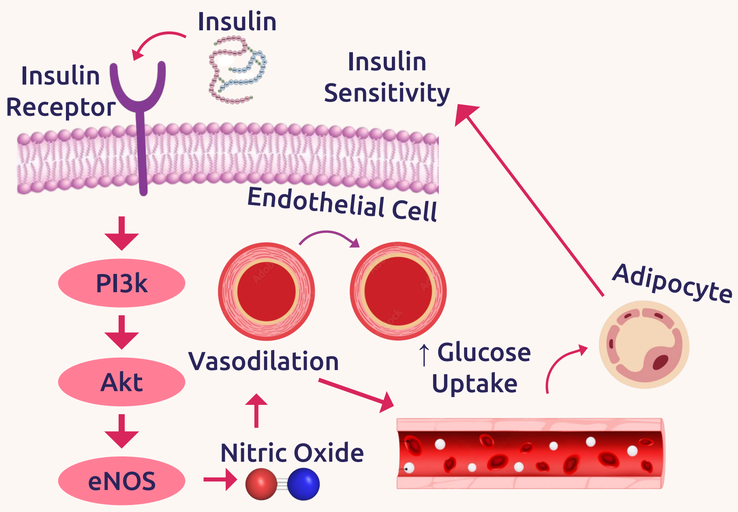


Fig. 4: Canonical insulin-Akt axis

6 Warning!

- More metabolic levels should be investigated along with Akt-p (I.e. eNOS activation) to determine the precise mechanism
- Endothelial cells and adipose tissue were cultured separately, hence the intercellular crosstalk was not experimentally validated.

Scan for the full bibliography!

7 DEVELOPER UPDATE: Directions for Future Research

- These findings support the concept of endothelial-adipocyte crosstalk in the regulation of metabolic homeostasis and may play an important role during the development of insulin resistance.
- The differential effects of glucose and ATEC 477 suggest that endothelial-derived signals may influence adipocyte function through alternative or compensatory signalling mechanisms.

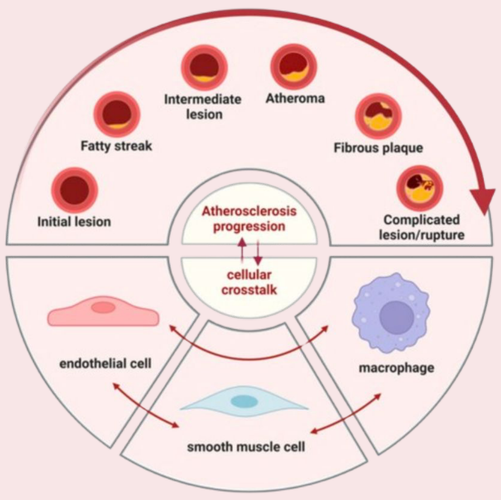


Fig. 5: International Journal of Molecular Sciences, *Metabolic dysfunction and heart disease*, 2025.

Further studies to investigate this may include...

Confocal Microscopy Investigate subcellular localisation of Akt-p in non-diabetic and diabetic HUVECs.	**Co-localisation** Dual immunofluorescence staining of Akt-p and METRNL to explore shared role in endothelial insulin resistance.
VE-cadherin Staining Structural changes observed via super-resolution microscopy may reveal influence of vascular phenotype on metabolic dysfunction	**Fluorescent Labelling and Live-cell Imaging:** Investigate endothelial-adipocyte crosstalk by visualising uptake of endothelial-derived factors by adipocytes.

Scan me!

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