

**UNIVERSITI TEKNOLOGI MARA**

**MOLECULAR MECHANISM OF  
STEVIOSIDE ON TNF-A-INDUCED  
INSULIN RESISTANCE IN 3T3-L1  
ADIPOCYTES BY INTEGRATION OF  
METABOLOMICS, PROTEIN AND  
GENE EXPRESSION**

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## ABSTRACT

The increasing prevalence of insulin resistance (IR) and T2DM provides compelling evidence for the identification of novel biomarkers, molecular targets and the development of effective drugs to prevent and treat the disease. Stevioside (SVS), the main constituent of *Stevia rebaudiana* Bertoni, has several therapeutic effects for metabolic syndrome, including lowering blood pressure, reducing blood glucose levels, potentiating insulin secretion and improving insulin sensitivity. However, the molecular basis underlying the antidiabetic effect of SVS on metabolic changes and pathways of insulin resistance is unknown. Therefore, the aim of this study was to investigate the metabolome response of SVS in a cell culture model of insulin resistance using metabolomics, protein and gene expression analyses. 3T3-L1 adipocyte cells were stimulated with 1.0 ng/mL TNF- $\alpha$  for insulin resistance and treated with SVS or rosiglitazone maleate (RM). The cell lysate was harvested and analysed by liquid chromatography-mass spectrometry-quadrupole time-of-flight analysis (LC/MS-QTOF). Principal component analysis (PCA) was used to identify statistically distinct metabolites for SVS in TNF- $\alpha$  induced insulin resistance 3T3-L1 adipocytes, and metabolomics pathway analysis (MetPA) was used to analyse and visualise the metabolic pathways involved. The expression of proteins and genes in SVS-treated insulin-resistant 3T3-L1 adipocytes was analysed by Western blot and quantitative RT-PCR assay. Metabolomic analysis of TNF- $\alpha$  induced insulin resistance 3T3-L1 adipocytes revealed ten potential biomarkers and metabolic pathways related to amino acid, lipid, cofactors and vitamins, and nucleotide metabolism. Treatment of insulin-resistant 3T3-L1 adipocytes with SVS showed a different spectrum in altering metabolites and metabolic pathways. A total of 24 metabolites were identified as potential biomarkers for SVS treatment in TNF- $\alpha$  induced insulin resistance 3T3-L1 adipocytes. The major metabolic pathways altered by SVS were glycine, serine, and threonine metabolism, arginine and proline metabolism, phenylalanine, tyrosine, and tryptophan metabolism, alanine, aspartate, and glutamate metabolism, glycerophospholipid metabolism, arachidonic acid metabolism, linoleic acid metabolism, pentose and glucuronate interconversions metabolism, retinol metabolism, and thiamine metabolism. The protein expression of IR $\beta$ , AKT, and GLUT4, and the expression of the PPAR $\gamma$  gene were significantly upregulated, and the expression of the NF- $\kappa$ B protein and gene was significantly downregulated by SVS. The increase in insulin sensitivity and glucose uptake by SVS may be due to the effect of SVS on metabolic biomarkers through increased antioxidant defence, reduced pro-inflammatory cytokines, upregulation of the pentose phosphate pathway and glycolysis, and increased membrane fluidity. Thus, our results suggest that SVS may modulate insulin resistance by regulating biometabolic markers and key proteins and genes in the insulin signalling pathway to treat T2DM.

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