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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-α for insulin resistance and treated with SVS or rosiglitazone maleate (RM). The cell lysate was harvested and analysed by liquid chromatography-mass spectrometry-quadrapole time-of-flight analysis (LC/MS-QTOF). Principal component analysis (PCA) was used to identify statistically distinct metabolites for SVS in TNF-α 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-α 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-α 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 IRβ, AKT, and GLUT4, and the expression of the PPARγ gene were significantly upregulated, and the expression of the NF-κB 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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CHAPTER ONE
INTRODUCTION

1.1 Research Background

Metabolism is a cellular process that occurs in the body by converting food into energy. Many enzymes and metabolic pathways are involved in this process. Disruption of the normal metabolism of amino acids, carbohydrates or lipids leads to metabolic diseases or disorders. Genetic defects lead to inherited metabolic disorders, while changes in diet, exercise, ageing and other environmental factors contribute to acquire metabolic disorders (Heindel et al., 2017). According to Boelens & Wynn (2017), the global epidemic of obesity and diabetes is linked to metabolic syndrome. This syndrome can be characterised by insulin resistance, abdominal obesity, dyslipidaemia, hypertension and hyperglycaemia. A common metabolic disorder, metabolic syndrome is defined as a risk factor for type 2 diabetes mellitus, cardiovascular disease, cancer, and other related diseases. (Grundy et al., 2004; Alberti et al., 2009).

Diabetes mellitus (DM) is a metabolic disease characterised by persistent hyperglycaemia. The prevalence of diabetes mellitus is increasing rapidly, affecting more than 400 million people worldwide (Khursheed et al., 2019). According to the National Health Morbidity Survey 2015, 17.5% of Malaysians have diabetes mellitus (Institute for Public Health, 2015). Type 1 diabetes mellitus (T1DM) is an autoimmune disease that affects pancreatic cells and reducing or impairs insulin production. In contrast, type 2 DM is caused by an impairment of the pancreatic beta cells, which reduces the person's ability to use insulin. Type 2 diabetes mellitus (T2DM) accounts for 90-95% of diabetes cases. This condition is the result of a disturbance in insulin action called insulin resistance (American Diabetes Association, 2014). According to the International Diabetes Federation (IDF), type 2 currently affects more than 387 million people worldwide DM and is expected to affect more than 592 million people by 2035 (Guariguata et al., 2014). The increasing prevalence of T2DM provides compelling evidence for the identification of novel biomarkers, molecular targets and the development of effective drugs to prevent and treat the disease.