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EFFECTS OF TOCOTRIENOL-RICH FRACTION (TRF) ON FARNESOID-X-RECEPTOR (FXR) GENE AND ITS SIGNALLING PATHWAYS IN THE HEART OF HIGH FAT DIET-FED MICE

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ABSTRACT

Cardiovascular Disease (CVD) is the number one cause of mortality globally of non-communicable disease. High fat diet (HFD) as a risk factor for CVD development. FXR and its target genes (*shp* and *stat3*) are expressed in the heart. The activation of FXR and its target genes in the heart demonstrated anti-inflammatory and anti-atherosclerotic effects, improved cardiac remodelling, and reduced blood pressure. Similarly, tocotrienol-rich fraction (TRF) is known to possess cardioprotective effects such as anti-atherosclerosis, anti-inflammatory, anti-oxidant activities, anti-thromboembolic and anti-adipogenic effects. However, whether TRF activates FXR and its target genes in the HFD animal model is not known. A total of 28 male Jax mice leptin-knockout B6.Cg-LepOb/J strain were used. All mice were given HFD consisting 60% energy of from fat, 20% from carbohydrate and 20% of protein. One group was given HFD without intervention, another three groups were fed with HFD mixed with palm kernel olein (PKO), tocotrienol-rich fraction with long-chain triglyceride (TRF-LCT) and tocotrienol-rich fraction with medium-`chain triglyceride (TRF-MCT) at 200mg/kg/day via oral route. After 6 weeks of treatment, anthropometric indices and random blood glucose were assessed and the heart was collected for measurement of *fxr, shp, stat3, sod1, sod2* and *gpx1* gene expression by real-time quantitative polymerase chain reaction (RT-qPCR), STAT3 Total protein expression and STAT3 pY705 by enzyme linked immunosorbent assay (ELISA). Serum samples were collected for untargeted metabolomic profiling from all mice. The result showed a significant increment of 3.18-fold in *fxr* expression for the HFD+TRF-MCT group (3.18±0.61) compared to HFD. Meanwhile, a significant 10-fold increment in *fxr* expression was seen in the HFD+PKO group (10.0±2.05) compared to HFD. No significant difference was seen in the *shp, sod1* and *sod2* expression between HFD+TRF-MCT and the HFD group. A significant decrease of *stat3* expression was seen in both HFD+PKO (0.49±0.048) and HFD+TRF-MCT (0.53±0.053) groups. A significant increment of 1.79-fold higher *gpx1* expression was observed in HFD+TRF-MCT group (1.789±0.164). No significant differences for STAT3 Total and STAT3 pY705 protein expression were seen in all groups. In metabolomic profiling, 31248 peaks were generated in positive mode, and 15204 peaks were detected in negative mode analysis when comparing HFD+TRF-LCT group against HFD group. Metabolites involving bile acids secretion, biotin metabolism and cholesterol metabolism were found to be significantly involved ($p<0.05$).TRF supplementation in high fat diet-fed mice confers its protective effects via the regulation of cardiac fxr and its target genes, protection against inflammation and oxidative stress through multiple biochemical signalling pathways

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CHAPTER 1
INTRODUCTION

1.1 Research Background

Obesity is defined as excessive fat accumulation in the abdomen and trunks that increases health risk. It is a global health problem in both developing and developed countries which has become a leading health burden in non-communicable diseases (NCD). In 2022, over 890 million adults in the world are now living in obesity and the prevalence of obesity tripled among women (6.6% to 18.5%) and quadrupled in men (3%-14%) (Phelps et al., 2024). Obesity patients are predisposed to metabolic syndrome (MetS) which is characterized by insulin resistance and dyslipidemia. MetS has been recognized as an independent risk factor for cardiovascular diseases (CVDs). CVDs are the number one cause of death of NCD. In 2016, it was estimated that 17.9 million people died from CVDs, representing 31% of all global deaths which 85% are due to heart attack and stroke (World Health Organization, 2018b). In modern lifestyle societies, increased intake of high fat diet contributes to the rise of obesity, promotes metabolic alterations such as insulin resistance, hypertriglyceridemia and high low-density lipoprotein (LDL) cholesterol, which have been recognized as an independent risk factor for CVDs (Keleher et al., 2018a). This obesity-induced metabolic derangement will induce oxidative damage through the bile acids (BAs) metabolism. BAs are involved in multiple signalling pathways such as the farnesoid X receptor (FXR) pathway and the Takeda G-protein-coupled receptor (TGR5) pathway (Repa & Mangelsdorf, 1999) . Among the most specific BAs signalling pathways, FXR has been shown to be expressed in the cardiovascular tissue (Bishop-Bailey et al., 2004a). Activation of FXR in the vasculature improves lipid profile and vascular tension which results in an anti-atherosclerotic effect. Its activation also alters gene expression and has been associated with the regulation of inflammatory processes. Studies have shown that tocotrienol-rich fraction (TRF) is a cardioprotective supplement (Ramanathan et al., 2018). It possesses potent anti-oxidant activities, anti-inflammatory and anti-angiogenic activities (Wong et al., 2017a). These activities could play important roles in reducing the injury to cardiac tissue, thus reducing the risk of CVDs. There are preliminary data from other studies showing that TRF upregulates the synthesis of primary bile acids and