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Quercetin ameliorates HFD-induced NAFLD by promoting hepatic VLDL assembly and lipophagy via the IRE1a/XBP1s pathway

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ABSTRACT

The consumption of a quercetin-rich diet has been well-established as a feasible method against non-alcoholic fatty liver disease (NAFLD); however, the molecular mechanisms underlying the progression of NAFLD and its intervention by quercetin remain largely obscure. Male Sprague-Dawley rats fed high-fat diet (HFD), and HepG2 cells stimulated with free fatty acid, were treated with quercetin and various pharmacological reagents to explore the effect of signaling pathways involved in endoplasmic reticulum stress on very low-density lipoprotein (VLDL) assembly and lipophagy. Quercetin intake decreased hepatic TG content by 39%, with a 1.5-fold increase in VLDL, and up-regulated spliced X-box binding protein 1 (XBP1s) expression compared with the HFD group. Thapsigargin or STF-083010 (an IRE1α endonuclease inhibitor) decreased VLDL content in a dose-dependent manner, partially counteracting the protective effects of quercetin, 4-PBA or APY-29 (an IRE1α endonuclease activator). Additionally, microsomal TG-transfer protein complex expression was increased with quercetin-treated and down-regulated by STF-083010. Moreover, quercetin increased co-localization of lysosomes with lipid droplets (LDs) accompanied by decreased p62 accumulation. STF-083010 partially abolished the effect of quercetin on LDs autophagy in an mTOR-independent manner. Collectively, these findings demonstrate that hepatic VLDL assembly and lipophagy are the main targets of quercetin against NAFLD via the IRE1a/XBP1s pathway.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD), a hallmark of metabolic syndromes frequently associated with obesity, hyperlipidemia, and type 2 diabetes mellitus, is an emerging common problem with a heavy health burden in both developed and developing countries. Hepatic steatosis ensues when the rate of fatty acid (FA) esterification into triacylglycerol (TG) exceeds the rate of FA output through lipolysis and mitochondrial fat acid oxidation or very low-density lipoprotein (VLDL) secretion (Kawano and Cohen, 2013). NAFLD ranges from simple steatosis to steatohepatitis with progressive fibrosis and ultimately can lead to cirrhosis. NAFLD has been proposed to be a major preventable cause of cirrhosis worldwide.

Recently, a large body of epidemiological research has demonstrated that consumption of vegetables and fruits associated with a reduced risk of all-cause mortality protects against NAFLD, type 2 diabetes, and cardiovascular disease (Zelber-Sagi et al., 2017). Two main classes of dietary phytochemicals contained in fruits and vegetables, namely, polyphenols and carotenoids, have attracted widespread attention in investigations of the beneficial effects of a diet rich in vegetables and fruits (Zelber-Sagi et al., 2017). Quercetin (3, 3′, 4′, 5, 7-pentahydroxyflavone), the most widely distributed, is flavonoid

Abbreviations: ERs, endoplasmic reticulum stress; FFA, free fatty acid; HFD, high fat diet; IRE1α, inositol-requiring transmembrane kinase/endoribonuclease 1α; LDs, lipid droplets; LMP, lysosomal membrane permeabilization; NAFLD, non-alcoholic fatty liver disease; TG, triacylglycerol; Tg, thapsigargin; VLDL, very low density lipoprotein; XB1s, spiced X-box binding protein 1; 4-PBA, 4-phenylbutyric acid

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polyphenols ubiquitously present in the plant kingdom, and exhibits extensive bioactivity, such as antioxidant, anti-inflammatory, and immunomodulatory activity (Aguirre et al., 2014; Kawabata et al., 2015; Pisonero-Vaquero et al., 2015; Li et al., 2013; Vidyashankar et al., 2013; Wang et al., 2013; Ying et al., 2013). Emerging evidence has shown that quercetin is effective in reversing the symptoms of NAFLD by suppressing lipid accumulation and ameliorating the lipidemic profile *in vivo* and *in vitro* (Gnoni et al., 2009; Seiva et al., 2012; Ulasova et al., 2013). Nevertheless, the exact mechanism underlying the protective effects of quercetin in hepatic steatosis is still poorly understood, although a few signaling pathways and targets have been explored (Casaschi et al., 2002; Shimizu et al., 2015).

Hepatic TG content is controlled by multiple dietary, hormonal, and genetic factors that regulate the balance among fatty acid uptake, synthesis, oxidation, and export via secretion of TG-rich VLDL (Cheng et al., 2016). Endoplasmic reticulum (ER) crucial for the formation of lipid droplets (LDs) is pivotal for VLDL assembly and progression of hepatic steatosis (Fu et al., 2012; Song et al., 2017; Wu et al., 2015). ER homeostasis is maintained through an adaptive mechanism termed the unfolded protein response, which is mediated through inositol-requiring transmembrane kinase/endoribonuclease 1 α (IRE1α), protein kinase R–like ER kinase (PERK), and activating transcription factor 6 α (ATF6α) (Walter and Ron, 2011). Interestingly, emerging evidence has illustrated the effects of quercetin on alleviating ER stress (Hayakawa et al., 2015; Liu et al., 2017; Suganya et al., 2014). Our previous study showed that quercetin attenuates ER stress and inflammation after intense exercise in mice through phosphoinositide 3-kinase and nuclear factor-kappa B (NF- κB) (Tang et al., 2016). Kimchi methanol extract containing quercetin has been reported to ameliorate hepatic steatosis and ER stress induced by a high cholesterol diet (Woo et al., 2017). Based on a fluorescence quenching (FQ)-based screening strategy *in vitro*, Wiseman et al. (2010) identified quercetin as a compound that activates IRE1α endonuclease activity in yeast, which is responsible for producing spliced X-box binding protein 1 (XBP1s). However, the mechanisms underlying the effects of quercetin on ER stress and VLDL assembly in hepatic lipid homeostasis are largely unknown.

In addition to assembly and secretion of VLDL, lysosomal autophagic degradation of intracellular LDs has emerged as a novel mechanism of cellular lipid removal and shuttling to mitochondria for beta-oxidation (Singh et al., 2009). Although the phenomenon of autophagy-mediated degradation of various organelles was confirmed since the early 1960s (Ashford and Porter, 1962), the contribution of autophagy to LD degradation has been identified within the past decade. Since a groundbreaking study by Singh et al. in 2009, which introduced the term “lipophagy”, many studies have emerged to elucidate the onset and progression of NAFLD (Lin et al., 2016; Parafati M et al., 2015; Zubiete-Franco et al., 2016). Actually, hormone-sensitive lipase (HSL) and adipose TG lipase (ATGL) activity are considered the primary mechanisms through which TG contained within LDs is degraded before the discovery of lipophagy (Schulze et al., 2017). Importantly, ER stress has been demonstrated to dynamically interact with autophagy (Shimodaira et al., 2014; Wang et al., 2014; Yang et al., 2010), and blockage of autophagy, including chaperone-mediated autophagy (CMA) can exert a strong inhibitory effect on LDs breakdown (Rodriguez-Navarro et al., 2012; Yang et al., 2010). Quercetin has been presumed to induce protective autophagy and apoptosis of ovarian cancer cells through ER stress (Liu et al., 2017) and in a rat model of Parkinson's disease (El-Horany et al., 2016). However, little attention has been paid to the association between ER stress and lipophagy, and less is known about the effect of quercetin on hepatic lipophagy.

Herein, we hypothesized that quercetin could promote VLDL assembly and lipophagy via the IRE1a/XBP1s pathway to reduce hepatic lipid accumulation. To test this hypothesis and analyze the underlying mechanisms, we administered quercetin to rats fed a high-fat diet and inhibited XBP1s expression in a free fatty acid (FFA) -induced steatosis model in HepG2 cells.

2. Materials and methods

2.1. Chemicals and reagents

Quercetin (purity ≥ 98%, HPLC), oleic acid (OA), palmitic acid (PA), thapsigargin (Tg) and 4-phenylbutyric acid (4-PBA) were purchased from Sigma (USA). The VLDL ELISA kit was provided by Cloud-Clone Corp (USA). GRP78, LAMP2 and MTTP antibodies were obtained from Santa Cruz (USA). IRE1a and XBP1 antibodies were from Abcam (UK). All of the other antibodies were obtained from CST (USA).

2.2. Animal treatment

Male Sprague-Dawley rats weighing 180–200 g were obtained from the Beijing Huafukang Bioscience Co. Inc. (China). Following one week of acclimation, the rats were randomly divided into 4 groups (n = 10 per group): normal control group (C), high-fat diet (HFD) group (H, composed of 52% carbohydrates, 30% fat and 18% protein), high-fat diet with quercetin group (H + Q, quercetin: 100 mg/kg.bw), and quercetin control group (C + Q). Quercetin was administered by gavage. After 8 weeks, the rats were sacrificed after overnight fasting. Serum and hepatic samples were collected rapidly for further biochemical assays.

2.3. Determination of lipid parameters

Serum lipid profiles (total cholesterol/TC, TG), hepatic high-density lipoprotein-C (HDL-C), low-density lipoprotein-C (LDL-C), and VLDL levels were detected according to the manufacturer's instructions. Hepatic or cellular lipids were extracted for cholesterol and triglyceride assays based on procedures developed by Folch et al. (1957) and Li et al. (2013), respectively, using a chloroform–methanol mixture (2:1, v/v). The dried lipid residues were dissolved in ethanol for the cholesterol and triglyceride measurements.

2.4. Histological analysis

Hematoxylin and eosin (H&E) staining was performed on liver sections for histopathological examination. In addition, liver tissues fresh-frozen were stained with Oil red O (ORO) for lipid staining.

2.5. Cell culture and treatment

FFA-induced HepG2 steatosis was developed as previously described (Ricchi et al., 2009). In brief, cells were cultured in 1% BSA medium, with 1 mM FFA (PA: 0.33 mM and OA: 0.66 mM), Tg, 4-PBA, STF-0830100 (Selleckchem, S7771), quercetin and APY-29 (MedChem Express, HY-17537). After 24 h, cells were collected for bioassays.

2.6. AO, LysoTracker Red DND-99 and BODIPY 493/503 staining

After the designated treatments, the cells were incubated with 5 μg/mL acridine orange (AO, Sigma) for 15–30 min at 37 °C. For double labeling, the cells were loaded with 100 nM LysoTracker Red DND-99 (Molecular Probes, USA) for 15–30 min at 37 °C. Subsequently, 1 μg/mL BODIPY 493/503 (Invitrogen, USA) was added for 30 min at 37 °C.

2.7. Western analysis

Cells or tissue samples were lysed at 4 °C in RIPA Lysis Buffer, and immunoblotting was performed according to the manufacturer's guidelines (Bio-Rad, Hercules, CA). The band densities were measured with ImageJ software.