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Rice bran phenolic extract supplementation ameliorates impaired lipid metabolism in high-fat-diet fed mice through AMPK activation in liver

Ruifen Zhang[1], Qin Ma[1], Xin Tong, Lei Liu, Lihong Dong, Fei Huang, Yuanyuan Deng, Xuchao Jia, Jianwei Chi, Mingwei Zhang*

Sericultural & Agri-Food Research Institute Guangdong Academy of Agricultural Sciences/Key Laboratory of Functional Foods, Ministry of Agriculture and Rural Affairs/ Guangdong Key Laboratory of Agricultural Products Processing, Guangzhou 510610, China

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ABSTRACT

Rice bran is a by-product of rice milling, which contains variety of nutrients, and has been reported to possess various activities. Here, we used high fat (HF) diet induced mice to investigate the effects of rice bran phenolic extracts (RBPE) on lipid metabolism. Sixteen-week intake of RBPE (200 mg/kg/d and 400 mg/kg/d) significantly improved the serum lipid profiles of HF diet-fed mice and ameliorated HF diet-induced liver steatosis. Further study showed that RBPE might regulate lipid metabolism through increasing the levels of serum adiponectin, and thereby promoting the activation of 5′ adenosine monophosphate-activated protein kinase α (AMPKα) in liver. Taken together, RBPE might regulate lipid metabolism through adiponectin-mediated AMPK activation and the subsequent reduction of lipid biosynthesis together with the increase of fatty acid oxidation in liver. These results provide us new evidence of the health benefits of rice bran.

1. Introduction

Hyperlipidemia is defined as pathologically increased plasma levels of triglycerides (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-c), together with decreased level of high-density lipoprotein cholesterol (HDL-c). Changes in energy intake and lifestyle over the past decades have resulted in a dramatic increase in the prevalence of hyperlipidemia (Saklayen, 2018). The pooled estimate of hyperlipidemia prevalence among Chinese adults reaches up to 41.9% (Huang, Gao, Xie, & Tan, 2014). Although hyperlipidemia by itself usually does not cause any symptoms, it may greatly contribute to the development of many chronic diseases, including diabetes mellitus and cardiovascular diseases (Pillarisetti, 2016), which are the leading causes of death worldwide (Barquera et al., 2015; Tao, Shi, & Zhao, 2015). Despite the utilization of various lipid lowering drugs, dietary intervention is still considered to be a safer and more cost-effective preventive and alleviative approach to hyperlipidemia and related chronic metabolic diseases. A 12-week whole-grain based diet, compared to refined grains, was found to reduce postprandial triglycerides responses of the intervened individuals with metabolic syndrome (Giacco et al., 2014). Compared to refined grain, whole grain is less processed and retains the nutrients in the bran layer. Therefore, it is plausible that the whole grain brans might play a role in the regulation of lipid metabolism.

Rice bran is a by-product of rice milling, and has been reported to possess hypolipidemic activity, which is always attributed to its major ingredients rice bran oil and the bioactive component γ-oryzanol (Ausman, Rong, & Nicolosi, 2005; Berger et al., 2005). Besides, rice bran is rich in phenolic compounds, in which the predominant compounds are phenolic acids. Recent studies have indicated that rice bran phenolic acid extract lowered plasma total cholesterol in mice (Jung, Ran Kim, Hwang, & Youl Ha, 2007; Park, Park, Kim, & Chung, 2014). Justo and his colleagues found rice bran enzymatic extract whose main components were protein and fat, attenuated dyslipidemia, hypertension and insulin resistance in obese Zucker rats (Justo et al., 2013). However, phenolic extracts in these studies were used in low purities, which is hard to confirm the effect of phenolic compounds in rice bran on hypercholesterolemia. Moreover, the molecular mechanism is unknown. Previous HPLC analysis has showed brown rice contains p-coumaric acid, chlorogenic acid, vanillic acid, ferulic acid, protocatechuic acid, ferulic acid, epicatechin and sinapic acid (Sukhonthara, Kaewka, & Theerakulkait, 2016; Ti et al., 2015). Evidences showed that these phenolic compounds could modulate lipid metabolism through its antioxidant or anti-inflammatory properties (Cho et al., 2010; Ibitoye &

* Corresponding author.
E-mail address: mwzhh@vip.tom.com (M. Zhang).
[1] Authors with equal contribution.

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Ajiboye, 2018; Wang et al., 2018; Zych, Kaczmarczyksedlak, Wojnar, Folwarczna, & Longevity, 2018). Besides, *p*-coumaric acid and rutin could attenuate lipid accumulation *via* AMPK activation in skeletal muscle (Yoon et al., 2013) and HepG2 cells (Wu et al., 2011), respectively. Recent reports suggest that phenolic extract from rice bran may regulate lipid metabolism via activating AMPK. Therefore, we prepared the rice bran phenolic extract (RBPE), analyzed its phenolic compounds composition and investigated its effects on lipid metabolism in high-fat (HF) diet-induced C57BL/6J mice, and the potential mechanism related to AMPK pathway was also explored.

2. Material and methods

2.1. Materials and regents

Fresh rice bran was purchased from Guangzhou Liquan Food Co. LTD in Conghua, Guangdong, China. HPD-300 resin was obtained from Cangzhou Baoen Absorption Materials Technology Co. Ltd. (Cangzhou, Hebei, China). Assay kit for TC, TG, LDL-c, HDL-c, free fatty acid (FFA), aminotransferase (AST) and alanine aminotransferase (ALT) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China). ELISA kit for adiponectin was obtained from Crystal Chem (Zaandam, Netherlands). The antibodies against liver X receptor α (LXRα), 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (HMGR), stearoyl-CoA desaturase (SCD) and carnitine palmitoyl transferase 1a (CPT1a) were obtained from abcam biotech (San Francisco, CA, USA); antibodies against 5′ adenosine monophosphate (AMP)-activated protein kinase α (AMPKα), phospho-AMPKα, Sterol regulatory element-binding protein 1c (SREBP1c), peroxisome proliferator activated receptors α (PPARα), acetyl CoA carboxylase (ACC), and phospho-ACC were purchased from Cell Signaling Technology Inc. (Danvers, MA, USA); the antibody against Cholesterol 7α-hydroxylase gene (CYP7A1) was obtained from Millipore Sigma.

2.2. RBPE preparation

RBPE was prepared as we reported previously (Xiao et al., 2019). The rice bran was defatted using supercritical CO_2 fluid technique. The defatted rice bran was homogenized in cold 80% aqueous acetone to extract phenoics. The concentrated phenolic extracts were purified by loading on an HPD-300 macroporous resin column. The fraction eluted with 70% ethanol were then concentrated and freezing-dried to obtain the RBPE. The content of total phenolic compounds was determined by Folin–Ciocalteu colorimetric method. The phenolic composition was analyzed by UHPLC-MS and the contents of phenolic compounds were measured by HPLC-DAD as previously described (Gómez-Caravaca, Segura-Carretero, Fernández-Gutiérrez, & Caboni, 2011). The total phenolic content of RBPE was 82.5 ± 3.26%. Para-coumaric acid, rutin, caffeic, ferulic and sinapic acids are detected as five most abundant compounds, whose contents were 86.18 ± 3.16, 81.75 ± 1.38, 81.87 ± 1.12, 68.74 ± 3.15, and 58.36 ± 2.44 mg/g, respectively, which was described in our previous study (Xiao et al., 2019).

2.3. Animals and diets

Eight-week-old specific pathogen-free male C57BL/6J mice were obtained from Guangdong Medical Laboratory Animal Center (Foshan, Guangdong, China). All the mice were housed in a controlled environment (23 ± 2°C, 50% ± 10% humidity, 12 h light/dark cycle). After 1 week of acclimatization, mice were randomly divided into 4 groups of 10 animals each. The mice in the normal control group (NC) were fed with the purified D12450H diet (10% energy from fat), while those in the other 3 groups were fed with the purified HF diet D12451 (45% energy from fat) *ad libitum* to induce hyperlipidemia. All the diets were obtained from Research Diets, Inc. (New Brunswick, NJ, USA). RBPE dissolved in distilled water was administered intragastrically to the mice in HF + LP and HF + HP groups daily at the dose of 200 mg/kg/d and 400 mg/kg/d, respectively. The animals in the NC and HF groups were given distilled water intragastrically.

After 16 weeks' treatment, the animals were fasted for 12 h and then anesthetized with ether. Blood samples were collected *via* orbital vein and centrifuged at 3000 *g* for 15 min to obtain serum. The liver was separated. After being weighed, a portion of liver was fixed in 4% paraformaldehyde, and the left was cut and flash-frozen in liquid nitrogen. The serum and flash-frozen liver samples were kept at −80°C until further analysis.

All the procedures involving animals followed the guidelines of the national standards outlined in "Laboratory Animal Requirements of Environment and Housing Facilities" (GB 14925–2010).

2.4. Serum biochemistry profiles

Whole blood samples were centrifuged to get serum samples. The serum levels of TC, TG, LDL-c, HDL-c, FFA were determined by commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The activities of AST and ALT in serum were measured according to the manufacturer's protocols (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.5. Histological analysis

For Hematoxylin and Eosin (H&E) staining, freshly collected tissues were fixed in 4% paraformaldehyde at 4°C for 24 h and then stored in 75% ethanol. After hydration using ethanol-water solution, the tissues were embedded in paraffin and cut into slices (4 μm). After dewaxing and rehydration, the slices were stained with hematoxylin and eosin. For red oil O staining, frozen liver tissue was cut (5–10 μm) and stained in oil red O solution. Images were acquired using a microscopy (Leica DMI 4000B, Heidelberger, Baden-Württemberg, Germany).

2.6. Quantification of gene expression in the liver with RT-qPCR

Total RNA was isolated using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA) according to manufacturer's instruction and then reverse transcribed into cDNA. qPCR was performed on a Real-Time PCR system 7500 (Applied Biosystems, Carlsbad, CA) with SYBR

Table 1
Effects of RBPE on the lipid and hormone profiles in serum of the HF-diet-fed mice.

	NC	HF	HF + LP	HF + HP
TC (mmol/L)	2.84 ± 0.08a	3.67 ± 0.10c	3.20 ± 0.11b	2.91 ± 0.11a
TG (mmol/L)	0.76 ± 0.07a	1.38 ± 0.06c	1.13 ± 0.09b	0.82 ± 0.04a
LDL-c (mmol/L)	0.72 ± 0.04a	1.25 ± 0.06c	0.98 ± 0.06b	0.89 ± 0.05b
HDL-c (mmol/L)	1.10 ± 0.07c	0.70 ± 0.11a	0.88 ± 0.09b	1.07 ± 0.07c
FFA (μmol/L)	372.01 ± 17.36a	565.07 ± 14.87d	505.99 ± 15.72c	415.39 ± 12.34b
Adiponectin (μg/mL)	10.58 ± 1.59b	8.77 ± 0.84a	8.22 ± 0.82a	9.79 ± 1.01b

Data are represented as means ± SD.
Values with no letter in common in the same line are significantly different, $p < 0.05$. $n = 10$.

Premix Ex Taq™ II (TaKaRa, Beijing, China). The primers were designed by Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China) and the nucleotide sequences of the primers are listed in Table 1. The relative quantities of mRNA were normalized to GAPDH.

2.7. Western blot analysis

Cytoplasmic protein and nuclear protein in the liver of each group mice were extracted by homogenizing the liver samples in cold extraction buffer, using the respective commercial kits (Beyotime Biotechnology, Shanghai, China). After quantification and denaturation, the protein extracts containing 30 μg of protein were electrophoresed in SDS-PAGE gel and transferred to a PVDF membrane. Membranes were then incubated with antibodies and visualized. Band intensities were quantified by Image J software and normalized to β-actin (whole protein) and histone (nuclear protein).

2.8. Statistical analysis

Results were represented as the means ± SD. Statistical comparisons were evaluated with a one-way analysis of variance by SPSS 18.0 software. Duncan post hoc test was used to analyze the differences between groups. Differences were considered significant at $p < 0.05$.

3. Results

3.1. RBPE improved serum lipid and hormone profiles in HF-diet-fed mice

The concentrations of TC, TG, LDL-c and FFA in the serum of HF group were significantly higher, while the concentration of HDL-c was significantly lower than those of NC group ($p < 0.05$, Table 2), suggesting that HF diet caused hyperlipidemia in mice. RBPE administration significantly alleviated the increase of TC, TG, LDL-c and FFA levels together with the decrease of HDL-c level in serum induced by HF diet ($p < 0.05$, Table 2). Furthermore, the serum lipid profiles of HF + HP group showed better amelioration than those of HF + LP group ($p < 0.05$), except that the LDL-c levels of these 2 groups were similar ($p > 0.05$). The results indicated that RBPE administration could alleviate HF diet induced hyperlipidemia in the mice dose-dependently. We also determined the serum levels of adiponectin, which plays important role in the regulation of lipid metabolism and blood lipids. As shown in Table 2, HF diet intake resulted in a significant reduction of adiponectin, which was reversed in HF + HP but not HF + LP group.

3.2. RBPE alleviated hepatic lipid accumulation and hepatic injury in HF-diet-fed mice

H&E staining of the liver showed that HF diet promoted lipid deposition in hepatocytes exhibiting that many lipid vacuoles diffusely distributed in the hepatic cytoplasm of HF group (Fig. 1A). Consequently, the microstructure of the liver lobule was found disarranged. Meanwhile, the aggregation of orange dye in oil-red O-stained liver sections also indicated obvious hepatic steatosis in HF group mice (Fig. 1B). In contrast to the HF group, fewer and smaller lipid droplets were observed in both RBPE-administered groups, with much less oil-red O dye observed in HF-HP than HF-LP group. Accordingly, the disarrangement of liver lobules was also reversed in the HF-LP and HF-HP groups as shown in H&E-stained sections (Fig. 1A, B).

The results of hepatic TC and TG contents were consistent with the histopathological changes in the liver as shown in Fig. 2A. HF diet intake resulted in obvious increases of TC and TG in the liver from 7.50 and 7.90 μmol/g to 14.36 and 12.46 μmol/g, respectively. Mice treated with low and high dose of RBPE showed significant alleviation of the accumulation of TC by 13.9% and 36.1%, and the accumulation of TG by 26.8% and 36.7%, respectively. The levels of serum transaminases AST and ALT were determined in the present study. As shown in Fig. 2B, the activities of AST and ALT in HF group were 1.59-fold and 1.89-fold higher than those in NC group ($p < 0.05$). RBPE treatment reduced AST and ALT activities in serum dose-dependently by 10.6% and 19.5% at 200 mg/kg bw and by 33.3% and 28.8% at 400 mg/kg bw, respectively. AST level of HF + HP group reduced to the equivalent level to that of NC group ($p > 0.05$). These results indicated that RBPE could alleviate HF diet-induced hepatic lipid accumulation and liver injury in mice.

3.3. RBPE inhibited hepatic SREBP-1c translocation and promoted PPARα activation in HF-diet-fed mice

SREBP 1c, PPARα and LXRα are important nuclear transcriptional factors that regulate lipid metabolism (Sato, 2010; Shimano, 2001). HF diet significantly increased level of SREBP 1c in nuclear protein (Fig. 3A, C); however, there is no significant difference between NC and HF group in the protein levels of LXRα and PPARα (Fig. 3A, B and D). High dose of RBPE treatment could significantly inhibit SREBP1c nuclear protein translocation and promote PPARα activation (Fig. 3A, B and C).

3.4. RBPE regulated enzymes activities related to lipogenesis and lipolysis

HMGR is a rate-controlling enzyme of cholesterol biosynthesis, which can catalyze the reduction of HMG-CoA to mevalonic acid. qPCR analysis showed that exposure to HF diet promoted gene expression of *HMGR* ($p < 0.05$, Fig. 4A). The gene expression of *HMGR* was significantly inhibited in RBPE supplemented mice. Western bolt result also confirmed the inhibitory effects of RBPE on HMGR expression ($p < 0.05$, Fig. 4D, E), which was consistent with the decreased TC

Table 2
Nucleotide sequences of primers.

Gene name	Gene bank ID	Primers
GADPH	NM_008084.2	GGAGAAACCTGCCAAGTATGATGAC GAGACAACCTGGTCCTCAGTGTA
AMPKα	NM_001013367.3	CCGTCGCCTACCACCTCATCATA TGGTCGTCCAGGAAAGAGTCAGG
PPARα	NM_001113418.1	GATGAAGAGGGCTGAGCGTAGGT CTGCCGTTGTCTGTCACTGTCTG
SREBP1c	NM_011480.3	CGCAAGGCCATCGACTACATCC CTCCACTGCCACAAGCTGACAC
LXRα	NM_001177730.1	GAGGGAGGAGTGTGTGCTGTCA TGGCAGGACTTGAGGAGGTGAG
HMGR	NM_008255.2	CCAACCTTCTACCTCAGCAAGCC CCAGCCATCACAGTGCCACATAC
ACC	AY451393.1	ACAGACCGTGGTAGTTGGCAGAG GCTTCAGAATCCAGGTTCGCAGG
SCD	NM_009127.4	GCAAGCTCTACACCTGCCTCTTC AGCCGTGCCTTGTAAGTTCTGTG
CPT1a	NM_013495.2	CCGCCACCTCTTCTGCCTCTAT AGGAGTCTGGCTCGTGGACAAC
CYP7A1	NM_007824.2	AGGCTGGAGGTGATGTTGAGTGT TGCGGCAAGTTGGCTATGCTAG

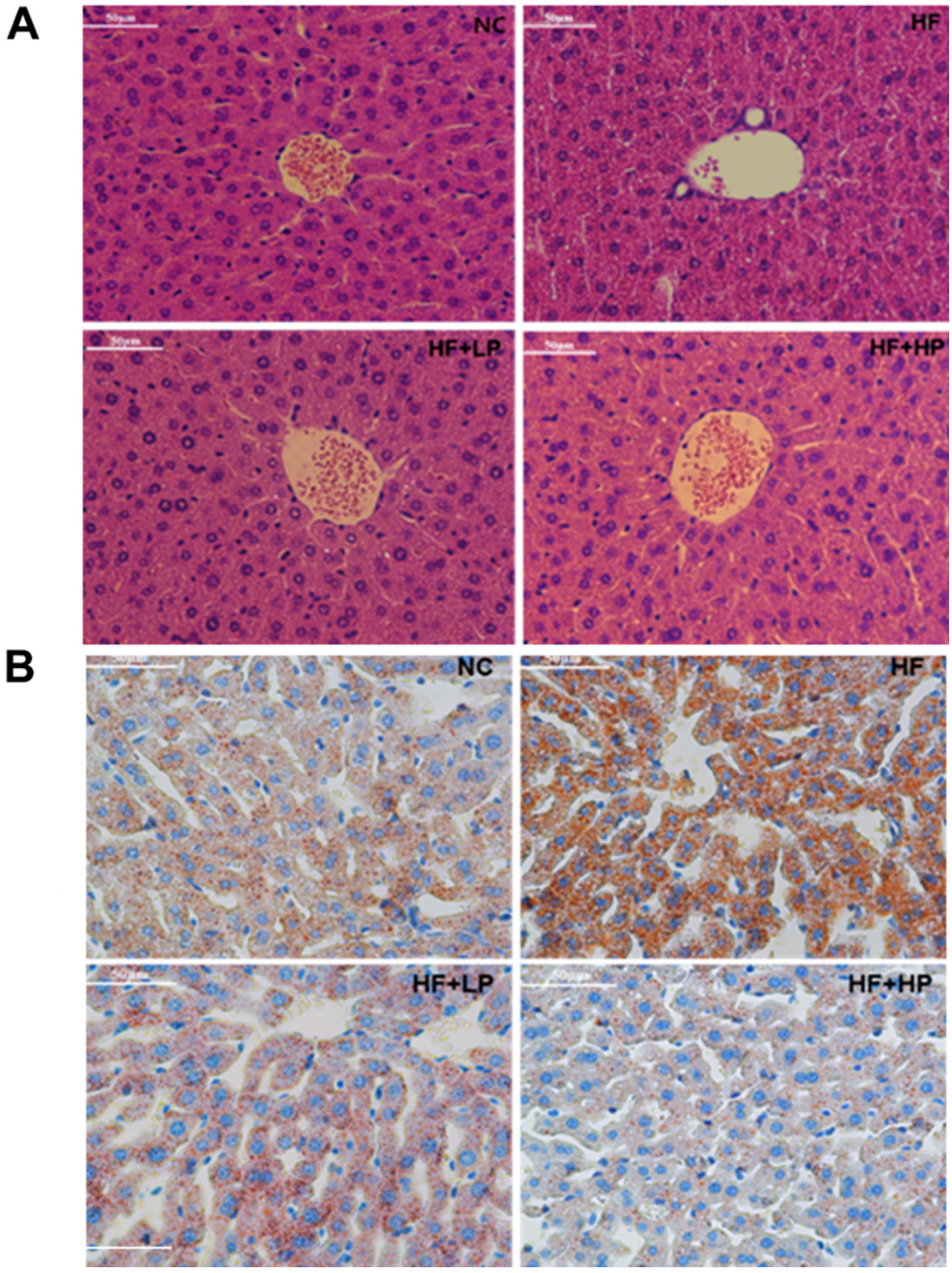

Fig. 1. RBPE alleviated lipid accumulation in liver tissue. (A) H&E staining (×200) and (B) Oil Red O staining (×200) of liver tissue.

level in RBPE-treated mice. ACC, and SCD are downstream target lipogenic genes of SREBP 1c (Eberle, Hegarty, Bossard, Ferré, & Foufelle, 2004). Consistent with its inhibition effect on SREBP 1c, RBPE also significantly attenuated the increased expression of hepatic SCD and inhibited ACC activation by promoting phosphorylation of ACC in HF fed mice (Fig. 4). These results indicated that RBPE could inhibit lipid synthesis through regulating the expression of lipogenic enzymes.

CPT1a and CYP7A1 are rate-limiting enzymes of regulating mitochondrial fatty acid β-oxidation and the conversion of cholesterol to bile acids (Qi et al., 2015). HF diet could decrease CPT1a mRNA level, which could be slightly but not significantly increased by RBPE administration (Fig. 5A). Western blot result, however, showed a significantly increased expression of CPT1a in HF-HP group compared to the HF group (Fig. 5C, D). However, RBPE exhibited little effect on the mRNA and protein expression of CYP7A1 (Fig. 5B, C, and E), indicating bile acid biosynthesis might not be the pathway by which RBPE lowered the TC level in HF diet fed mice.

3.5. RBPE promoted AMPKα activation

AMPK is an important protein kinase regulating energy homeostasis (Ferre, Azzout-Marniche, & Foufelle, 2003; Viollet et al., 2006). AMPK activation could switch off the anabolic processes, such as lipogenesis, while switch on the catabolic processes, such fatty acid oxidation (Steinberg & Kemp, 2009). HF diet significantly repressed the phosphorylation of AMPKα, while RBPE treatment greatly promoted the activation of AMPKα (Fig. 6), which is consistent with its regulating effects on SREBP 1c and HMGR expression. These results indicated that RBPE might regulate the hepatic injury and lipid accumulation through AMPK-mediated SREBP and HMGR signaling pathway.

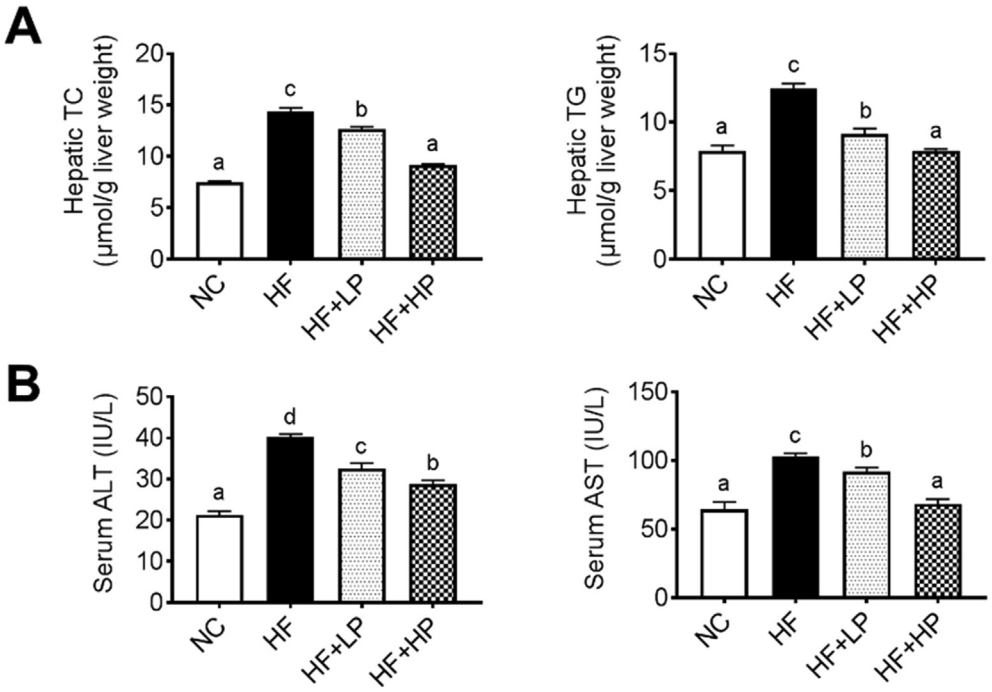

Fig. 2. RBPE alleviated hepatic lipid accumulation and hepatic injury in HF-diet induced mice. (A) Level of hepatic TC (left) and TG (right); (B) Levels of ALT (left) and AST (right) in serum. Data are represented as the means ± SD. Values with no letter in common in the same line are significantly different, $p < 0.05$. $n = 10$ per group.

4. Discussion

Rice bran has been reported to decrease the TC,TG and LDL-c level and increase HDL-c level *in vivo* (Gong, Gong, & Zhang, 2014; Maria Luisa Justo et al., 2016), which was always attributed to its bioactive ingredients, such as fatty acids, protein and γ-oryzanol (Sharif, Butt, Anjum, & Khan, 2014; Sohail, Rakha, Butt, Iqbal, & Rashid, 2017) rather than phenolics. In this study, we used HF-diet-induced mice to investigate the effect of RBPE on hyperlipidemia. Our results showed that 16-week intake of RBPE could significantly improve the serum lipid profiles of HF-diet fed mice. Consistent with our study, Jung et al. reported that phenolic acids extract from rice bran could significantly decrease plasma TC and LDL-c concentrations in C57BL/KsJ-db/db mice (Jung et al., 2007). Ferulic acid, the predominant phenolic acid in rice bran, could improve the lipid profile and glucose metabolism in hypertensive rats (Ohsaki, Shirakawa, Koseki, & Komai, 2008). Our and previous studies suggest that phenolic compounds in rice bran could contribute to the anti-hyperlipidmic effect of rice bran.

Although the hypolipidemic effect of phenolics in rice bran has been reported in the previous study (Jung et al., 2007), related mechanism

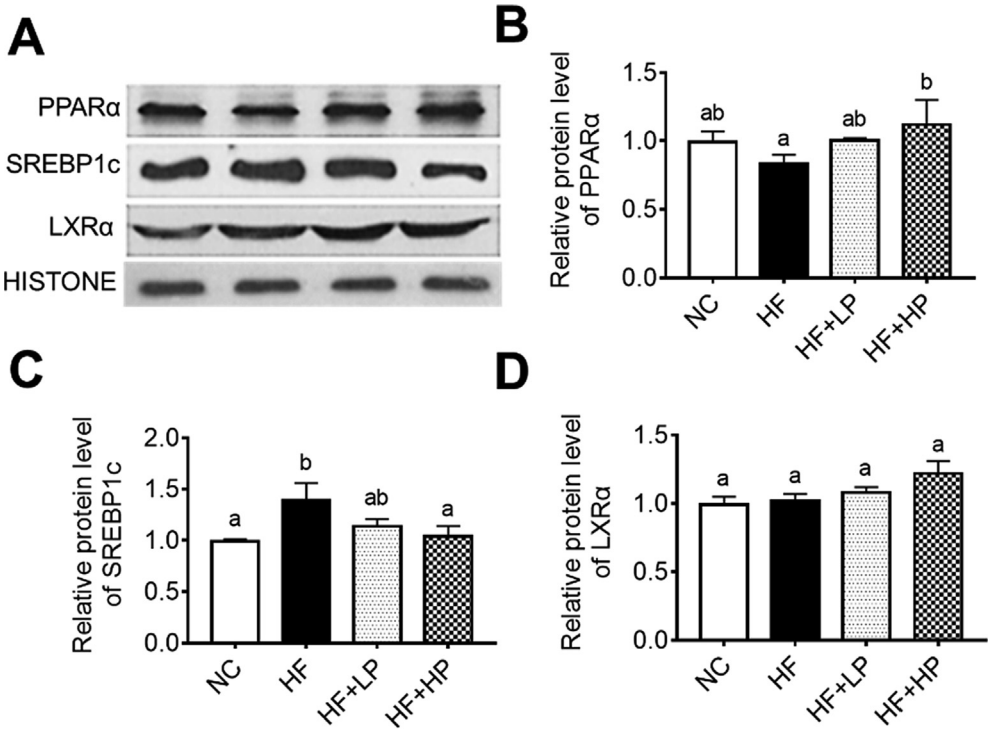

Fig. 3. Effect of RBPE on translocation of nuclear transcriptional factors. (A) Representative bands of western blot; Relative protein levels of (B) PPARα, (C) SREBP 1c, and (D) LXRα in nuclear protein.

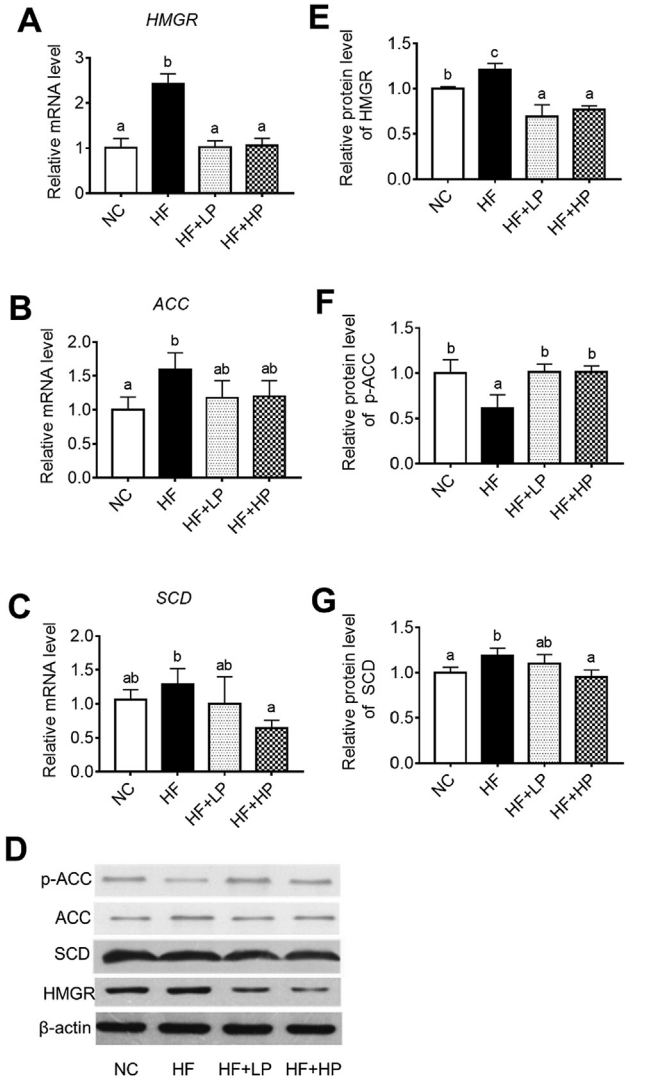

Fig. 4. Effects of RBPE on lipogenesis. Relative mRNA level of (A) *HMGR,* (B) *ACC* and (C) *SCD*; (D) Representative bands of immunoblot; Relative protein levels of (E) HMGR, (F) phosphorylated ACC and (G) SCD.

was not involved. We further investigated the possible mechanism by which RBPE improves the lipid metabolism. Liver plays a central role in lipid metabolism, where lipid can be *de novo* synthesized or oxidized (Nguyen et al., 2008). It is reported that 70–80% of nonalcoholic fatty liver disease patients suffer from hyperlipidemia (Miura & Ohnishi, 2012). Excessive energy intake may impair liver function and induce hepatic steatosis (Musso, Gambino, & Cassader, 2009). In our study, we found that HF diet administration could promote lipid accumulation in liver and induce hepatic injury. Levels of serum ALT and AST were also significantly increased in HF group indicating injury of hepatocytes in the liver. RBPE treatment could significantly counteract the effect of HF diet. These results was consistent with our previous finding that RBPE alleviated ethanol-induced liver injury through improving liver lipid profiles (Xiao et al., 2019).

Adiponectin, an adipocyte-derived hormone, plays important role in the regulation of lipid homeostasis (Musso et al., 2005). Hypoadiponectinemia was found in the populations with metabolic syndrome and nonalcoholic fatty liver disease. Strategies to enhance the synthesis and secretion of adiponectin are considered to be potential effective for metabolic syndrome (Ghadge, Khaire, & Kuvalekar, 2018). Therefore, the decreased adiponectin levels in the HF group mice resulted from the metabolic imbalance induced by HF diet. RBPE administration reversed the above changes indicating that RBPE might exert its anti-hypolipidemic effects via increasing the production of adiponectin.

Adiponectin regulates energy homeostasis through activating PPARα and AMPK in muscles and liver (Ishtiaq, Rashid, Hussain, Arshad, & Khan, 2019; Wolf, 2003; Yamauchi et al., 2001). Furthermore, as the predominant phenolic components in RBPE, *p*-coumaric acid and rutin could activate AMPK to rectify the dysregulation of lipid metabolism in skeletal muscle (Yoon et al., 2013) and HepG2 cells (Wu et al., 2011), respectively. Hence, the phosphorylation of AMPKα in the liver was analyzed in the present study to investigate the effect of RBPE. The increased ratio of phosphorylated to total AMPK protein in HF + LP and HF + HP groups indicated that RBPE administration activated AMPK. Previous studies showed that p-coumaric acid and ferulic acid improved the secretion of adiponection in diabetic rats (Abdel-Moneim, El-Twab, Yousef, Reheim, & Ashour, 2018) and rats with metabolic syndrome (Wang et al., 2015). These results indicated that RBPE might activate AMPK *via* elevating adiponectin secretion, in which individual phenolic compounds such as p-coumaric acid, ferulic acid and rutin, play an important role.

AMPK could phosphorylate SREBP1 and SREBP2, which inhibits the nuclear translocation of these transcription factors responsible for the *de novo* synthesis of fatty acid and cholesterol (Li et al., 2011). ACC and SCD are the lipogenic genes regulated by SREBP1(Eberle et al., 2004). ACC is the rate-limiting enzyme of fatty acid synthesis, whose activity can be inhibited through phosphorylation by AMPK. In this study, the decreased nuclear translocation of SREBP1c and increased phosphorylation of ACC in RBPE treated mice manifested the inhibition effect of RBPE on lipid synthesis. RBPE could also promote lipid combustion by increasing the expression of CPT1a, which might be attributed to the increased serum adiponectin through the adiponectin/PPARα pathway (Park et al., 2016). Although we found lower TG levels in HF-LP group than HF group, the protein levels of PPARα and CPT1a were not significantly different in HF-LP and HF groups. The phosphorylation and inactivation of ACC reduced the production of fatty acid in the HF-LP group. In addition, the increased activity of CPT1a resulted from the inactivation of ACC might also contribute a lot to the decreased TG levels. It is well known that malonyl-CoA, the product catalyzed by ACC, is an allosteric inhibitor of CPT-1a (Raud et al., 2018). Therefore, the decreased production of malonyl-CoA would disinhibit CPT-1a promoting oxidation of fatty acid.

AMPK can also regulate cholesterol homeostasis. HMGR, a rate-controlling enzyme in *de novo* synthesis of cholesterol, can be inhibited by AMPK phosphorylation (Hardie, 2004; Zhang, Zhou, & Li, 2009). AMPK can also inhibit the expression of HMGR through inactivating SREBP2 as mentioned above (Li et al., 2011). RBPE administration significantly inhibited HMGR mRNA and protein expression in HF diet-induced mice, which is consistent with its lowering effect on TC in serum and liver. Degradation to bile acids is the major pathway for catabolism of cholesterol. CYP7A1 is the rate-limiting enzyme responsible for bile acid synthesis in the liver. The expression of CYP7A1 is regulated by LXR (Wang & Tontonoz, 2018). Therefore, we also determined the effects of RBPE on the expression of CYP7A1 and activation of LXRα. Our data showed that RBPE administration did not influence their expression, indicating that the lipid metabolism regulation effect of RBPE might not involve bile acid synthesis.

In summary, RBPE could improve lipid profiles and inhibit hepatic lipid accumulation in HF diet-induced mice. The regulatory effect of RBPE on lipid metabolism was related to adiponectin-mediated AMPK activation and the subsequent reduction of lipid biosynthesis together with the increase of fatty acid oxidation in liver through regulating genes involved in lipid metabolism.

CRediT authorship contribution statement

Ruifen Zhang: Conceptualization, Methodology, Project administration, Writing - review & editing. **Qin Ma:** Investigation,

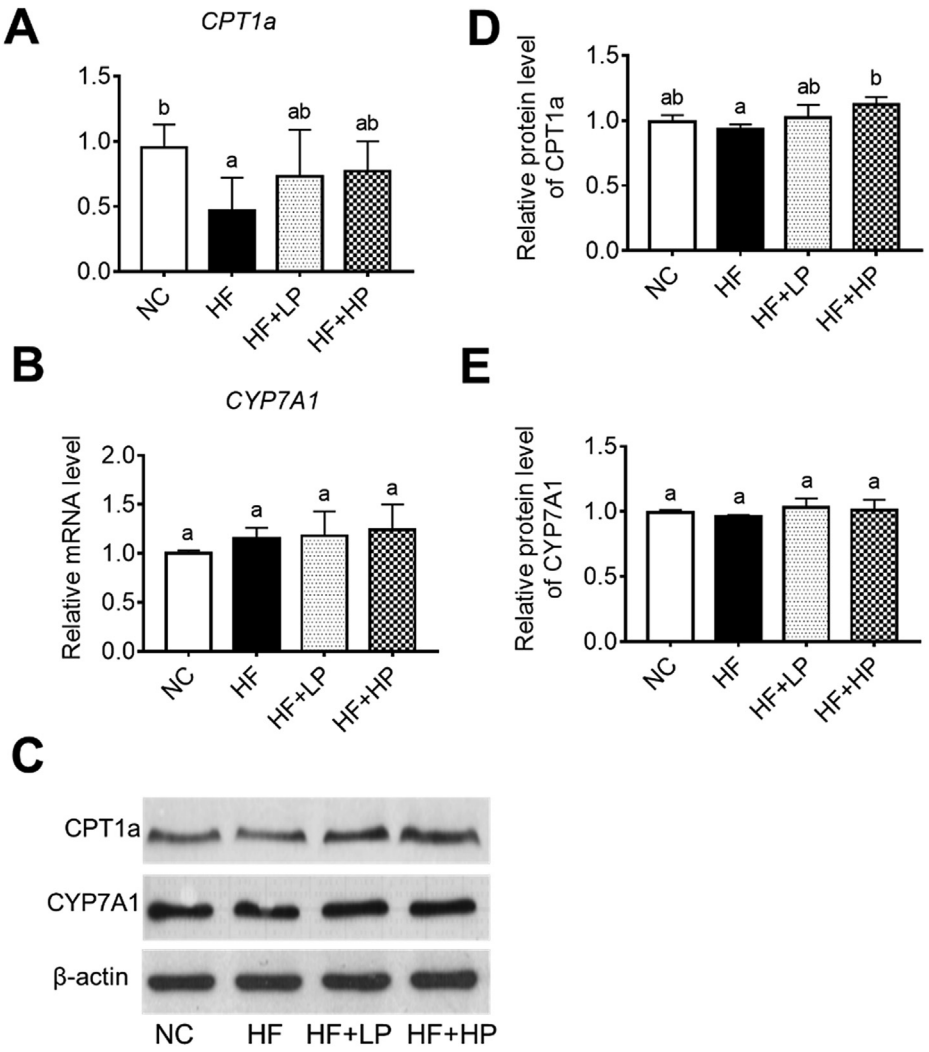

Fig. 5. Effects of RBPE on lipolysis. Relative mRNA levels of (A) *CPT1a,* and (B) *CYP7A1*; (C) representative bands of immunoblot; Relative protein levels of (D) CPT1a and (E) CYP7A1.

Visualization, Data curation, Writing - original draft. **Xin Tong:** Investigation. **Lei Liu:** Investigation. **Lihong Dong:** Resources, Software. **Fei Huang:** Formal analysis. **Yuanyuan Deng:** Formal analysis. **Xuchao Jia:** Investigation. **Jianwei Chi:** Resources, Software, Validation. **Mingwei Zhang:** Supervision, Funding acquisition.

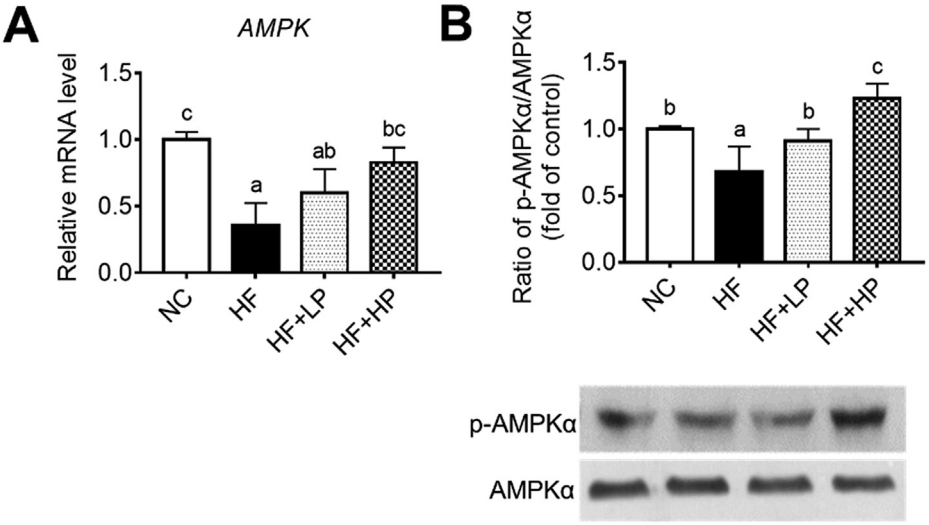

Fig. 6. RBPE enhanced the phosphorylation of AMPK. (A) Relative mRNA level of AMPK; (B) protein expression of AMPK phosphorylation.

Ethical Statement

All the procedures involving animals followed the guidelines of the national standards outlined in "Laboratory Animal Requirements of Environment and Housing Facilities" (GB 14925–2010) and experiments were approved by the Ethics Committee for Experimental Animal Care of Sericultural & Agri-Food Research Institute Guangdong Academy of Agricultural Sciences (Guangzhou, Guangdong, China).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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