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Therapeutic potential of buckwheat hull flavonoids in *db/db* mice, a model of type 2 diabetes

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ABSTRACT

In this study, the therapeutic effects of buckwheat hull flavonoids (BHFs) on type 2 diabetes *db/db* mice were investigated. After administration by BHFs nine weeks, body weight, visceral index, biochemical indicators in serum and in liver were detected. The liver pathological changes were observed under microscopic observations. Real-time quantitative PCR was used to detect *IRS-1, PI3kα, AKT2, SREBP-1c, FAS, ACC1* mRNA expression in liver. The results showed that insulin resistance significantly reduced after the BHFs treatment. BHFs could reduce the TG, TC, vLDL-c, FFA levels in serum and TG, TC levels in liver and potentiate insulin resistance-related *PI3k/AKT2* signal pathway and down-regulated SREBP-1c, FAS, ACC1 mRNA in the liver tissue of *db/db* mice. Moreover, BHFs could significantly alleviate the structure disorder of liver. The protective effect of BHFs on T2DM may be mediated by facilitating PI3k/AKT signal pathway and down-regulating SREBP-1c, FAS and ACC1 mRNA expression to reduce lipid synthesis.

1. Introduction

Public health data show that 425 million people worldwide are suffering from diabetes (International Diabetes Federation, 2017) making it the most common epidemic metabolic disorder and global health problem in the world (Wang et al., 2013). Of the diabetes patients, the 90% of diabetes patients have been diagnosed as type 2 mellitus (T2DM) (Carnagarin, Dharmarajan, & Dass, 2015). Moreover, most cases of T2DM are associated with obesity and high blood lipid profiles that can lead to disorder in lipid metabolism (Malone & Hansen 2018) and it is suggested the altered lipid metabolism may lead to the insulin resistance (Bjornstad & Eckel, 2018; Morita et al., 2017). Insulin resistance is an important risk factor for T2DM, and the *IRS-1/PI3k/AKT* pathway plays a key role in insulin receptor signal transduction (Nie et al., 2016). Therefore, T2DM improvement may concerned with IRS-1/PI3k/AKT signaling pathway and the expression of fat metabolism related genes, such as sterol regulatory element binding protein-1c (SREBP-1c) and fatty acid synthase (FAS) to reduce hepatic insulin resistance.

Flavonoids, which are well-known and widespread compounds in nature have attracted much attention due to their antioxidant (Krych & Gebicka, 2013), anticancer (Ragab, Yahya, El-Naa, & Arafa, 2014), anti-cardiovascular disease (Tian et al., 2014) and anti-diabetes activities (Ramachandran & Xu, 2015; Zhang et al., 2015). Buckwheat contains multiple types of flavonoids such as vitexin, hyperin and rutin (Zhang, Zhu, Liu, Bao, & Qin, 2017), which have been reported to have positive effects on reducing the risk of diabetes by reducing the expression of transcripts of lipogenic genes and energy intake (Hosaka et al., 2014; Stringer, Taylor, Appah, Blewett, & Zahradka, 2013). Therefore, buckwheat has more received increasing attention as an important resource that can be developed into functional foods, particularly for diabetes therapy.

Buckwheat hulls, by-products of buckwheat seed processing, have mainly been used as pillow fillers (Mackėla, Andriekus, & Venskutonis, 2017). Flavonoids in buckwheat hulls exhibit strong antioxidant activity (Dziadek et al., 2016; Mukoda, Sun, & Ishiguro, 2001; Watanabe, Ohshita, & Tsushida, 1997). Our early studies have shown that hulls have a higher content of hyperoside (Zhao et al., 2018) than rutin which is generally considered to be the main flavonoid of buckwheat and we also have confirmed that flavonoids extracts of buckwheat hulls decrease blood glucose as well as improve insulin resistance in a rat the model of type 2 diabetes rats induced by streptozotocin (Li et al., 2017).

Based on previous researches, in this study, we further evaluated the impact of buckwheat hull flavonoids (BHFs) purified by macroporous resins for improving insulin resistance and lipid metabolism disorders on type 2 diabetes C57BLKs/J *db/db* mice and explored the possible

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metabolic pathways.

2. Materials and methods

2.1. Materials and reagents

Buckwheat (*Fagopyrum esculentum* Moench) was purchased from ChiFeng, Inner Mongolia province. The kits for insulin, C-peptide, free fatty acid (FFA) and very-low density lipoprotein cholesterol (vLDL-c) were purchased from Shanghai Hengyuan Biological Technology (Shanghai, China). The kits for total cholesterol (TC), triacylglycerol (TG), low density lipoprotein cholesterol (LDL-c), high density lipoprotein cholesterol (HDL-c), glucose, total protein, hepatic glycogen were obtained from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). RNAiso Plus reagent, the PrimeScript™ II 1st Strand cDNA Synthesis Kit and the SYBR® Premix Ex Taq™ II (Tli RNaseH Plus) Kit were purchased from TaKara (Japan). All experiments were performed according to the manufacturer's protocols. Other chemicals used in this study were of analytical grade.

2.2. Preparation of BHFs and determination of total flavonoids

Buckwheat hull extracts (BHEs) were prepared by Zhao's methods (2018) with modification. Briefly, 500 g of buckwheat hull powder (sifted through a 0.25 mm screen) were extracted twice with deionized water (1:20, m/v) at 121 °C for 20 min. The extract was filtered and evaporated under reduced pressure at 70 °C (RE52CS, Yarong, ShangHai, China), then, the powder was lyophilized. Furthermore, BHEs were purified using D101 macroporous resins (Dong Hong chemical company, Shanghai, China) by column chromatography (60 mm × 400 mm). A total of 700 mL of the BHE solution (6 mg/mL) was loaded on the D101 macroporous resins, which had a wet-packed volume of 500 cm^3 with at a flow rate 4 mL/min. Then unabsorbed compounds were washed with 2000 mL of distilled water. Finally, the column was eluted with 70% (v/v) alcohol at a flow rate of 6 mL/min until no color was detected. The freeze-dried eluate was used as BHFs sample. The content of total flavonoids content of BHFs was measured using colorimetric method (Huang & Ho, 2014) and it was 518.4 mg/g hyperoside or 740.5 mg/g rutin. The BHFs was consist of hyperoside, vitexin, isoorientin and a small amount of rutin (Zhao et al., 2018).

2.3. Animals and experimental design

Five-week-old male C57BLKs/J-*db/db* mice (*db/db* mice) were purchased from Nanjing university-Nanjing medical institute (N000180, Nanjing, China). All mice were housed under a controlled condition in individual cages at 22 ± 3 °C and 60–70% relative humidity with a 12 h dark/light cycle in a specific pathogen-free environment and were allowed free access to food and water. After one week of acclimation, they were randomly divided into four groups. The groups consisted of the control group (Con, water, 10 mg/kg/d, n = 8), low-dose BHFs group (BHFs-L, BHFs 10 mg/kg/d, n = 8), middle-dose BHFs group (BHFs-M, BHFs 30 mg/kg/d, n = 8), and a high-dose BHFs group (BHFs-H, BHFs 60 mg/kg/d, n = 8). All mice were given a single oral gavage daily at 3 pm, and were feed a normal diet and water. The weight of experimental animals in each group was measured weekly during the nine-week intervention. All animal experiments were approved by the Institutional Animal Care and Use Committees of Jilin Agricultural University, following the National Research Council Guidelines.

2.4. Serum biochemical measurements

Mice were starved for 12 h at the end of 9th week, and blood was collected from the orbital venous plexus. Serum was obtained by centrifugation of blood samples at 3000*g* for 20 min at 4 °C (3 k15, Sigma, Germany); then, the upper serum was placed in an EP tube and used for the detection of serological indicators. The fasting blood glucose (FBG), fasting serum insulin (FINS), C-peptide, FFA and vLDL-c levels were assayed using commercial test kits. Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was used to evaluate insulin resistance and was calculated with the following formula: HOMA-IR = FINS (mIU/L) × FBG (mmol/L)/22.5.

2.5. Liver biochemical measurements

After collecting blood from sacrificed *db/db* mice, heart, spleen, liver, kidney and abdominal fat tissues were separated and weighed to calculate the organ index. Among these tissues, liver was cut into two specimens. One specimen was washed with cold normal saline and immediately frozen in liquid nitrogen and stored at −80 °C until analysis. The other specimen was blocked in Tissue-Tek OCT (Optimal Cutting Temperature) compound (Sakura Finetek USA, Inc., Torrance, CA 90501 U.S.A) in aluminum foil to prevent tissue damage and immediately placed in liquid nitrogen and stored at −80 °C for histological examinations as described below.

TC, TG, LDL-C, HDL-C, total protein, and hepatic glycogen in the liver were measured according to the instructions of each kit.

2.6. RNA extraction and quantitative real-time reverse transcriptase polymerase chain reaction (RT-qPCR) analysis

Total RNA was isolated from liver samples using RNAiso Plus Reagents. RNA was then reverse transcribed into cDNA using the PrimeScript™II 1st Strand cDNA Synthesis Kit. Then mRNA expression of *IRS-1*, *PI3kα*, *AKT2*, *SREBP-1c, FAS, ACC1* and *β-actin* in the liver was quantified by real-time PCR using SYBR® Premix Ex Taq™ II (Tli RNaseH Plus, TaKara, Japan) Analysis was performed using qPCR3.2 software (Toptical 96, Jene, Germany) The PCR primer sequences in this study were as follows: *IRS-1* (Fw: 5′-GCT CTC CTT CAG TTC GAT GT-3′, Rv: 5′-CTC AAC TCC ACC ACT CTC CT-3′), *PI3kα* (Fw: 5′-CGC GCC TTT GCC TTT CAA TC-3′; Rv: 5′-ATG CGG TAC AGG CCA GAG AT-3′), *AKT2* (Fw: 5′-GGA TGA AGT CGC CCA CAC AG-3′; Rv: 5′-CCC GAT CCT CCG TGA AGA CT-3′), *β-actin*: (Fw: 5′-AAA TCG TGC GTG ACA TCA AA-3′, Rv: 5′-AAG GAA GGC TGG AAA AGA GC-3′) *SREBP-1c* (Fw: 5′- GAT GTG CGA ACT GGA CAC AG -3′; Rv: 5′- CAT AGG GGG CGT CAA ACAG'), *FAS* (Fw: 5′- GGA GGT GGT GAT AGC CGG TAT -3′; Rv: 5′- TGG GTA ATC CAT AGA GCC CAG-3′), *ACC1* (Fw: 5′-ATG GGC GGA ATG GTC TCT TTC-3′; Rv: 5′-TGGGGACCTTGTCTTCATCAT-3′). Gene expression was calculated using the $2^{-\triangle\triangle CT}$ method after normalization to the reference gene *β-actin*.

2.7. Histological analysis

Slices of that were 10–15 μm in thickness were prepared from frozen section of the liver (LeiKa1850, Switzerland). After incubation at room temperature for 10 min, the slices were stained using an oil red O staining Kit (Xiangsheng, ShangHai, China). Finally, the sections were mounted with resinous mounting medium for microscopic observations (LeiKa, Switzerland).

2.8. Statistical analysis

All experiments were performed in triplicate. The values are presented as the mean ± SD. Charts were made by with Graph Pad Prism 5.0 and Excel Software 2007. Significant differences among the groups were assessed using one-way ANOVA followed by LSD analysis. A p value of less than 0.05 was considered statistically significant.

Table 1
Effect of BHFs on body weight in *db/db* mice.

Groups	Weight (g)				
	1 week	3 weeks	5 weeks	7 weeks	9 weeks
Control	41.11 ± 0.13^{a}	40.61 ± 0.26^{ab}	39.52 ± 0.35^{a}	38.68 ± 0.68^{a}	38.48 ± 0.49^{a}
BHFs-L	41.40 ± 0.23^{a}	40.50 ± 0.52^{ab}	39.83 ± 0.34^{a}	38.13 ± 0.45^{b}	38.85 ± 0.25^{b}
BHFs-M	41.46 ± 0.27^{a}	40.30 ± 0.60^{a}	39.40 ± 0.29^{a}	37.10 ± 0.21^{c}	36.77 ± 0.26^{c}
BHFs-H	41.42 ± 0.47^{a}	40.88 ± 0.42^{b}	38.67 ± 0.72^{c}	34.52 ± 0.32^{d}	34.16 ± 0.46^{d}

Each value is expressed as the mean ± SD (n = 8/group). The results were statistically analyzed with one-way ANOVA. Different letters indicate significantly different values ($p < 0.05$).

3. Results

3.1. Effect of BHFs on body weight in db/db mice

The body weight of *db/db* mice was measured weekly during the experiment. During the first five weeks, the body weight of mice from all treatment groups had no statistically significant difference and declined gradually. The body weight of mice from the BHFs-H group dramatically decreased compared with that of mice from the control group ($p < 0.05$) since administration of five weeks. The body weight of mice from all of the BHFs groups also significantly declined from the 7th week to the 9th week compared with that of mice from the control group ($p < 0.05$). After nine weeks of continuous treated, mice from the BHFs-L, BHFs-M and BHFs-H groups had decreased in weight by 6.16%, 11.3%, 17.52%, respectively, compared with that at the 1st week. (Table 1). However, there was no significant difference in food intake between the mice with BHFs intervention and the control group, the effect of BHFs on food intake was almost insignificant in mice, as shown in Fig. S1.

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jff.2018.11.011.

3.2. Effect of BHFs on the organ indexes in db/db mice

After BHFs intervention, the abdominal fat index of *db/db* mice decreased gradually, with mice from the BHFs-H group showing the most significant decline of 15.2% ($p < 0.05$) compared with mice from the control group. However, the BHFs had no influence on the heart, spleen, kidney and liver indexes in *db/db* mice (Table 2).

3.3. Effect of BHFs on the serum biochemical indexes in db/db mice

The serum biochemical index results in *db/db* mice after nine weeks of BHFs intervention (Fig. 1) showed a decreasing trend the FBG content of each mice from BHFs intervention group compared with mice from the control group, but there was no significant difference (Fig. 1A). However, the levels of fasting insulin in both mice from the BHFs-M group (7.19 ± 0.97 μIU/mL) and the BHFs-H group (5.51 ± 0.31 μIU/mL) were significantly lower than that of mice from control group (9.00 ± 0.80 μIU/mL, $P < 0.05$, Fig. 1B). Particularly, this trend was consistent with the changes in the serum C-peptide content (Fig. 1C), namely, the significant decrease of C-peptide in mice from the BHFs-M (377.42 ± 37.34 pg/mL) and BHFs-H groups (327.96 ± 62.84 pg/mL) compared with that of mice from the control group ($p < 0.05$). Next, the HOMA-IR indices were calculated to evaluate the insulin resistance of *db/db* mice (Fig. 1D), and the results showed that BHFs significantly improved the insulin resistance of *db/db* mice. In addition, a significant reduction of HOMA-IR in mice from the BHFs-M and BHFs-H groups was observed compared with that of mice from the control group (36.62% and 44.02%, $p < 0.05$).

Furthermore, the levels of TG and TC in mice from the experimental groups also decreased compared with that of mice from the control group. In particular, mice from the BHFs-H group showed prominent performance, that decreased by 30.40% and 30.97% for the TG and TC level, respectively ($p < 0.05$) (Fig. 1E and F). All mice from the BHFs groups ameliorated the low HDL-c level (Fig. 1G) and showed vLDL-c (Fig. 1H). Meanwhile, mice from the BHFs-M and BHFs-H groups had a significantly reduced FFA level ($p < 0.05$, Fig. 1I)

3.4. Effect of BHFs on biochemical content in the db/db mouse liver

Based on liver the biochemical indicators of the livers of *db/db* mice, BHFs had decreased the hepatic TG level, but had no effect on the TC level (Fig. 2A and B). The live TG level in mice from the BHFs-H group was significantly reduced compared with that of mice from the control group ($p < 0.05$). As shown in Fig. 2C, no change of liver in glycogen content was determinate in comparison between the mice from the BHFs-L, BHFs-M groups and control group (8.13 mg/g); the liver glycogen content of mice from the BHFs-H group, however, was significantly increased (64.0%) compared with that of mice from the control group (13.33 mg/g) ($p < 0.05$).

3.5. Effect of BHFs on insulin resistant and lipid metabolism pathway gene expression in liver

To further examine the role of BHFs in insulin resistance, the expression of *IRS-1*, *PI3Kα, AKT2* in the liver was measured. The expression of *IRS-1* in both the mice from the BHFs-M and the BHFs-H

Table 2
Effect of BHFs on the organ index in *db/db* mice.

Groups	Index (mg/g)				
	Heart	Spleen	Kidney	Liver	Abdomen fat
Control	4.55 ± 1.33^{a}	1.58 ± 0.44^{a}	11.46 ± 1.34^{a}	61.95 ± 8.11^{a}	57.95 ± 6.06^{a}
BHFs-L	4.63 ± 0.82^{a}	2.43 ± 0.95^{a}	12.87 ± 1.12^{a}	61.38 ± 11.64^{a}	54.01 ± 8.63^{ab}
BHFs-M	4.01 ± 0.64^{a}	1.92 ± 0.49^{a}	12.56 ± 2.85^{a}	56.11 ± 4.94^{a}	50.64 ± 9.52^{ab}
BHFs-H	4.62 ± 1.25^{a}	1.46 ± 0.35^{a}	13.01 ± 3.35^{a}	65.87 ± 10.45^{a}	49.13 ± 4.52^{b}

Each value is expressed as the mean ± SD (n = 8/group). The results were statistically analyzed with one-way ANOVA. Different letters indicate significantly different values ($p < 0.05$).
Organ index = Organ weight/Final body weight. Abdomen fat index = Abdomen fat weight/Final body weight.

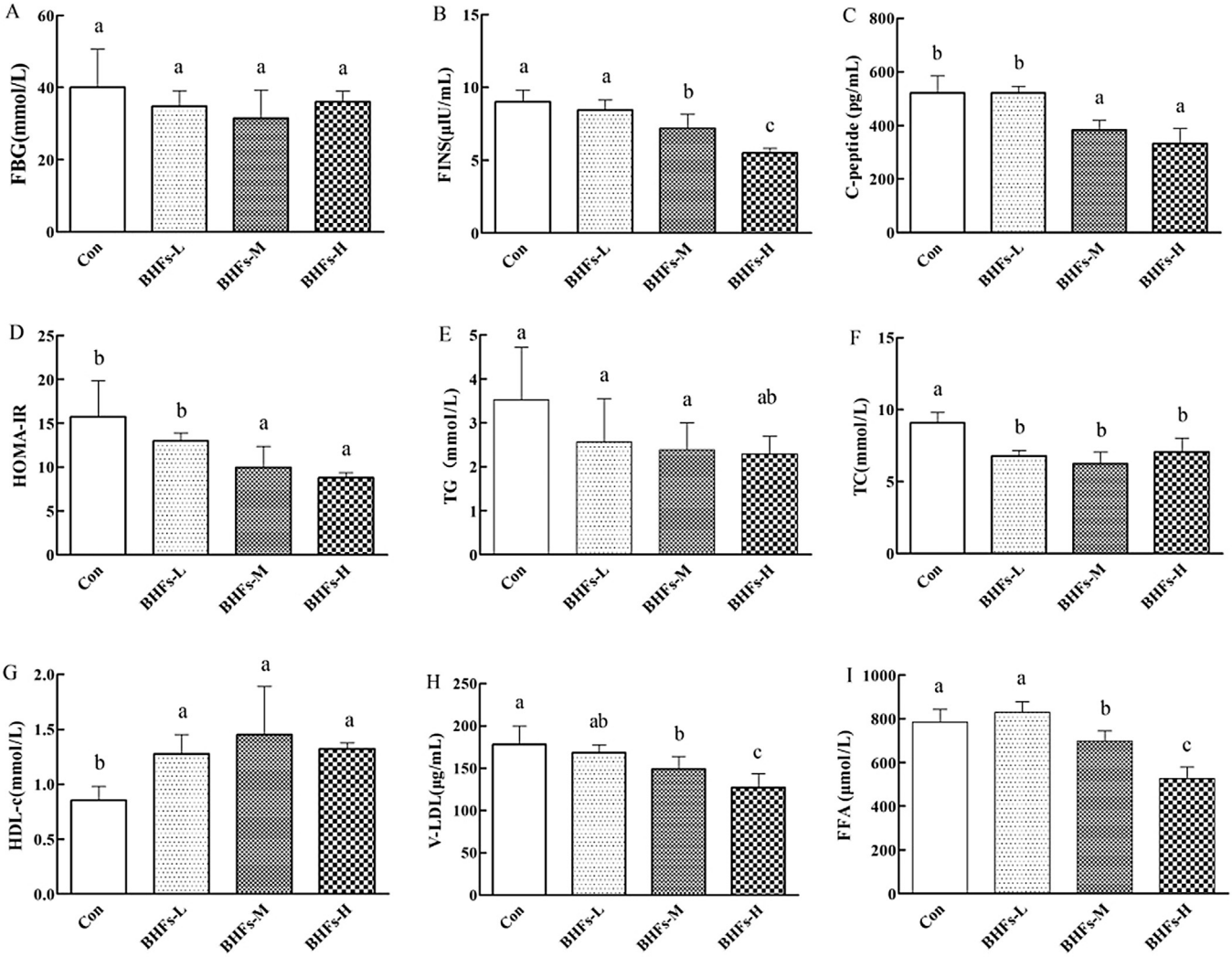

Fig. 1. Effect of BHFs on the biochemical indexes in serum of *db/db* mice after nine weeks of intervention. (A) FBG, (B) FINS, (C) C-peptide, (D) HOMA-IR, (E) TG, (F) TC, (G) HDL-c, (H) vLDL-c, (I) FFA. Each value is expressed as the mean ± SD (n = 8/group). The results were statistically analyzed with one-way ANOVA. Different letters indicate significantly different values ($p < 0.05$).

groups slightly increased (Fig. 2D), with no significant difference ($p > 0.05$). Furthermore the mRNA level of *PI3K* and *AKT2* in mice from the BHFs-H group was significantly increased, showing 1.75 (Fig. 2E) and 1.49-fold change, respectively (Fig. 2F), compared with that of mice from the control group ($p < 0.05$). From expression of *SREBP-1c*, *FAS* and *ACC1* in the liver, the expression of *S SREBP-1c* in the BHFs-H group significantly decreased 75.58%, compared with control group ($p < 0.05$). The expression of *FAS* and *ACC1* in the BHFs-M and the BHFs-H group slight decreased, whereas mice from the BHFs-H group observably decreased by 62.20% and 86.52%, respectively ($p < 0.05$), compared with control group.

3.6. Histomorphological analysis

The altered appearance of histopathological changes of the liver was analyzed. Oil - red O stained live sections showed that the liver of mice from the control group was severely deformed due to excessive fat accumulation, with large amount of vacuoles. By contrast, the BHFs intervention significantly reduced the degree of fat accumulation in the liver according to the intervention dose. Among them, mice from the BHFs-H group showed the most significant improvement effect, with almost no fat accumulation and no vacuole observed (Fig. 3).

4. Discussion

Obesity is directly related to type 2 diabetes (Kumar et al. 2012). *Db/db* mice are a recognized model of obesity and type 2 diabetes that lacks a functional leptin receptor (Herberg & Coleman, 1977; Sullivan, Harcourt, Xu, Forbes, & Gilbert, 2015). Contrary to several studies (Kumar et al. 2012; Lee, Kim, Shin, & Yoon, 2014) that the weight loss of *db/db* mice was related to the reduction of food intake and fat, we found that BHFs not only reduced the weight of *db/db* mice (Table 1), but also significantly decreased the white fat index of the abdomen (Table 2). However there was no significant change in food intake. This may involved with energy consumption about respiratory quotient and the cause remains to be studied. At least, loss of weight and abdomen fat by BHFs had prevented obesity which could reduce risk of diabetes.

FBG is most important indicator for the diagnosis of diabetes. In the early stage of the research, we found that buckwheat hull extracts reduced the FBG level in rats with type 2 diabetes induced by streptozotocin with high fat and sugar contents (Li et al. 2017); however, the results were not consistent in *db/db* mice (Fig. 2A). It is speculated that the cause of this phenomenon is related to the different pathogeneses of the different diabetes models (Yamabe, Kang, Park, Tanaka, & Yokozawa, 2009). Whereas BHFs had effectively improved insulin resistance in *db/db* mice (Fig. 1D) through reducing the levels of insulin

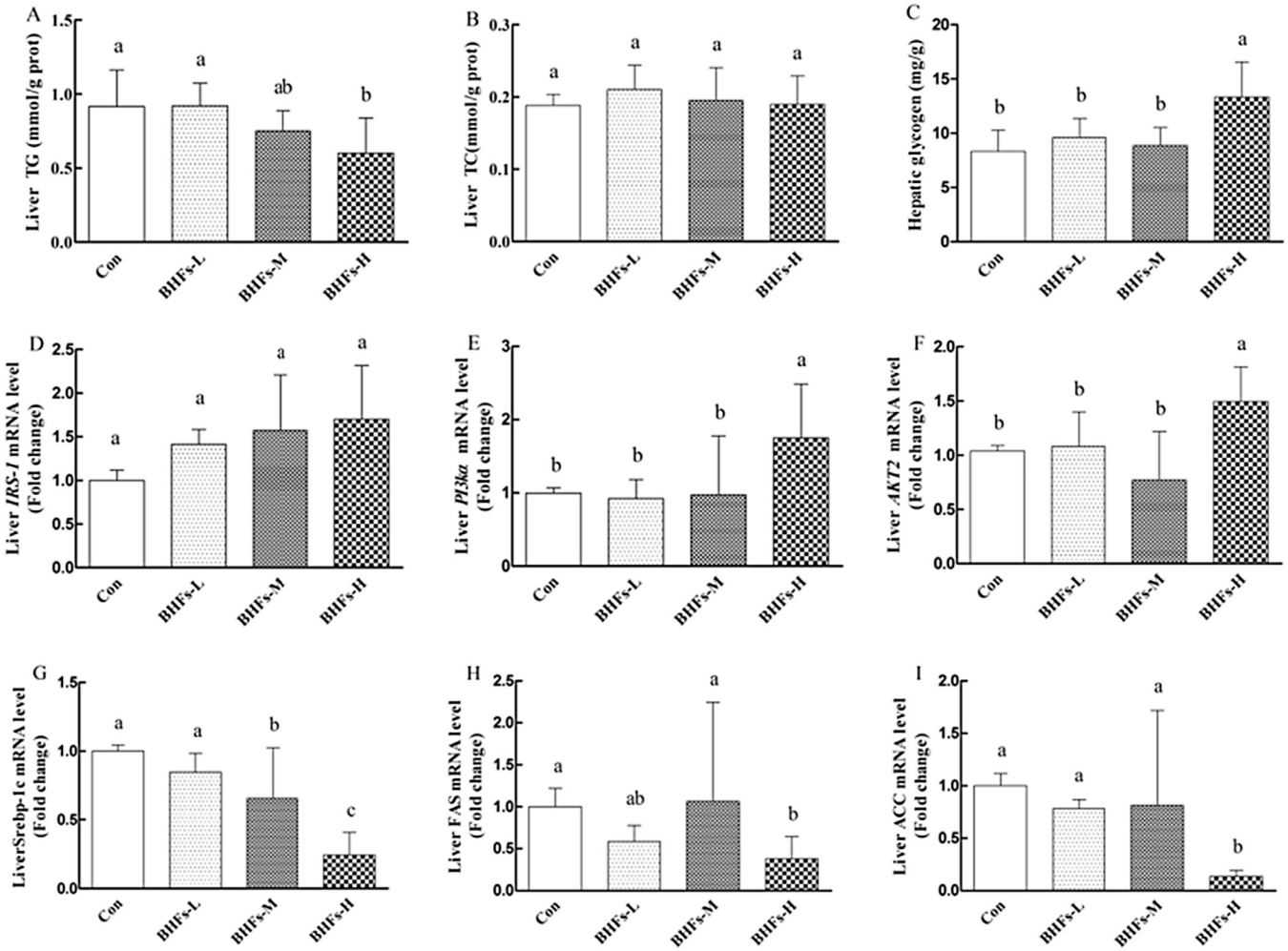

Fig. 2. Effect of BHFs on TG(A), TC(B), Glycogen(C) level and *IRS-1*(D), *I3kα*(E), *AKT2*(F), *SREBP-1c*(G), *FAS*(H), *ACC1*(I) gene expression in the liver of *db/db* mice. Each value is expressed as the mean ± SD (n = 8/group). The results were statistically analyzed with one-way ANOVA. Different letters indicate significantly different values ($p < 0.05$).

and C-peptide (Fig. 1C).

Meanwhile, insulin resistance is a critical component in the development of type 2 diabetes mellitus (Bjornstad & Eckel, 2018; Tripathy et al. 2004). In many cases, insulin resistance in liver is associated with reduced expression of major IRS-1 or IRS-2 (Kerouz, Horsch, Pons, & Kahn, 1997). The IRS/PI3K/AKT signaling pathway is a key pathway to play an important role in glucose metabolism and glycogen synthesis (Molina et al. 2017; Nie et al., 2016). Glycogen is a crucial glycogen metabolic index that is related to the distribution and absorption of glucose and is the main storage form of the glucose in the body. In this study, BHFs enhanced expression of *PI3k* and *AKT* in the liver of *db/db* mice (Fig. 2E and F) and significantly increased the level of hepatic glycogen (Fig. 2C) indicating that BHFs can improve insulin resistance and glycogen synthesis through activation of *PI3k/AKT*.

Another important point is that obesity is often accompanied by abnormal fat levels and lipoprotein metabolism disorders, such as elevated LDL-c and vLDL-c levels (Franssen, Monajemi, Stroes, & Kastelein, 2011; May, Kuklina, & Yoon, 2010). During insulin resistance, the accumulation of body fat and larger fat cells leads to the release of free fatty acids (FFA) into the bloodstream. Excessive the FFA can affect not only fat storage capacity, but can also lead to over deposition non-adipose tissues (pancreas islet, skeletal muscle and liver, etc), which

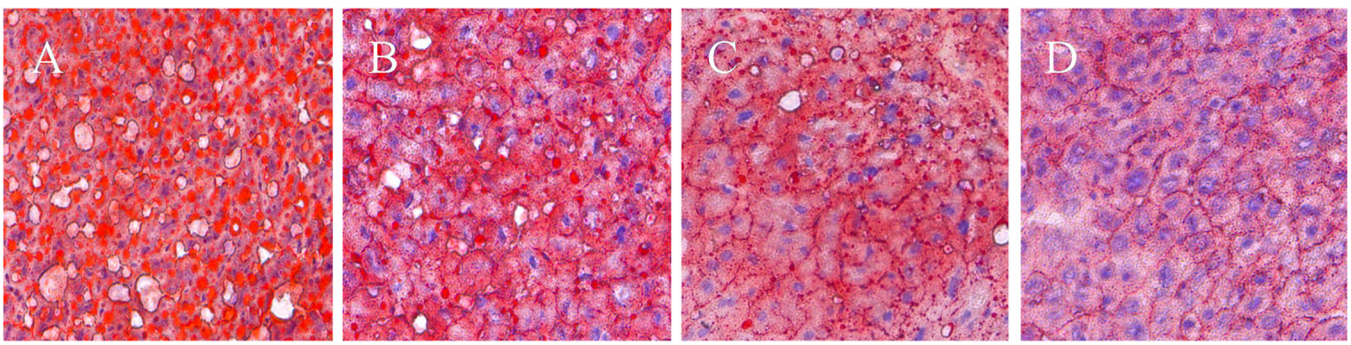

Fig. 3. Histological analysis of liver sections from different groups. Conventional Oil Red O staining was performed. A: Con; B: BHFs-L, C: BHFs-M, D: BHFs-H.

cause tissue damage, and further aggravates insulin resistance (Xia, Morley, & Scherer, 2014). Abnormal cholesterol metabolism is closely related to the HDL-c and LDL-c levels. The protective mechanism of HDL-c for atherosclerosis mainly depends on reversing cholesterol transport, that is, excess cholesterol is transferred from surrounding tissues to the liver to remove it (Simic et al. 2017). Here, we also confirmed that BHFs can reduce the levels of FFA and vLDL-c and increase the level of HDL-c in serum of db/db mice (Fig. 1G–I). In addition, insulin can up-regulate the transcription factor *SREBP-1c* for expression of *ACC1* (Lopez, Bennett, Sanchez, Rosenfeld, & Osborne, 1996) and *FAS* (Bennett, Lopez, Sanchez, & Osborne, 1995) that are important for fatty acid synthesis and lipid biosynthesis. After the intervention of BHFs, the expression levels of *SREBP-1c*, *FAS* and *ACC1* (Fig. 2G–I) in the liver of *db/db* mice were significantly reduced as well as the levels of TG and TC in serum (Fig. 1E and F) and TG in liver (Fig. 2A) and was also correspondingly decreased. The histomorphological analysis of the liver stained by Oil - red O showed that the BHFs intervention significantly reduced the degree of fat accumulation (Fig. 3). It can be indicate that the improvement of lipid metabolic disorder in *db/db* mice by BHFs is closely related to the down-regulation of a series of lipid synthesis genes. Therefore, these results strongly demonstrated that BHFs improve lipid metabolism disorder.

However, significant changes of *IRS-1* in the liver were not observed in this study. Some studies have been reported that the role of either hepatic IRS1/IRS2 for liver is surprisingly less significant. Mice with targeted knockdown of *IRS1/IRS2* in liver by shRNA displayed no alterations of total PI3K or AKT activity and had almost normal glucose tolerance and insulin sensitivity (Taniguchi, Ueki, & Kahn, 2005). Overall, based on the macroscopic and microscopic morphological studies, this study explored the therapeutic effect and action mechanism of BHFs in *db/db* mice at low dose, in which something worth to study. The composition of its components and detailed mechanism of action remain unclear. Its action mechanism may be multi-targets and multi-pathways, thereby further research is needed.

5. Conclusion

In summary, our data suggest that buckwheat hull extract greatly reduces the symptoms of insulin resistance in *db/db* mice and improves lipid metabolism disorders. Therefore, it is reasonable to believe that buckwheat hull can be used as a raw material supplement for diabetes prevention and treatment or that its extract may have a positive therapeutic effect on patients with high cholesterol and a lipid metabolic disorder.

6. Author's contributions

All of the authors listed have made substantial, direct and intellectual contribution to the work and approved it for publication.

7. Ethics statement

a. the material has not been published in whole or in part elsewhere;
b. the paper is not currently being considered for publication elsewhere;
c. all authors have been personally and actively involved in substantive work leading to the report, and will hold themselves jointly and individually responsible for its content;
d. all relevant ethical safeguards have been met in relation to patient or subject protection, or animal experimentation.

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Conflict of interest statement

All authors declare there are no conflicts of interest.

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