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Momordica charantia alleviates the lipid metabolism disorder of mice on a high-fat diet via down-regulating ACACA and FASN

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As a common vegetable, *Momordica charantia (M. charantia)*, also known as bitter melon, has the pharmacological activity of reducing blood sugar and body fat. This experiment explored the effect of *M. charantia* on the mice liver by adding freeze-dried *M. charantia* to the feed intake of high-fat diet mice. Among the three groups of normal diet mice (Control), high-fat diet mice fed with lyophilized bitter melon powder (BM) and single high-fat diet mice (HFD), it was seen that the body weight of BM mice did not change apparently ($p>0.05$), while the body weight of HFD mice increased significantly ($p<0.01$). Serum triglycerides, total cholesterol, high density lipoprotein cholesterol (HDL-C), Low density lipoprotein cholesterol (LDL-C) of HFD mice was significantly higher than those of Control and BM mice ($p<0.01$). The results of RT-PCR and Western blot showed that the transcription and protein expression levels of acetyl-CoA carboxylases alpha (ACACA) and fatty acid synthase (FASN) genes in HFD mice were significantly increased ($p<0.01$), while BM administration could reduce the transcription and expression of these two genes ($p<0.01$). It is concluded that *M. charantia* powder alleviates the lipid metabolism disorder of liver in HFD mice via downregulating ACACA and FASN both in the transcription and protein expression levels, and thus exhibits protective effects for the liver.

1. Introduction

Momordica charantia L., also known as bitter melon (BM), a member of the Cucurbitaceae family, is widely distributed in tropical and subtropical regions of the world, and has been widely utilized as Chinese herbal medicine to treat diseases for a long history. The whole plant especially its seeds and fruits, has multiple pharmacological actions, including anti-diabetic, abortive, deworming, contraceptive, anti-malarial, and laxative effects. Thus, it is used to treat dysmenorrhea, eczema, gout, jaundice, leprosy, hemorrhoids, pneumonia, psoriasis, and rheumatism. In the past ten years, there have been many studies focusing on BM, the fruit of the *M. charantia* plant. Several experiments show that BM has anti-hyperglycemic, anti-bacterial, anti-viral, anti-tumor, immune regulatory, anti-oxidant, anti-diabetic, deworming, anti-mutagenic, anti-lipolytic, anti-fertility, liver protective, and anti-hepatitis effects (Dandawate et al. 2016; Jia et al. 2017; Schepetkin and Quinn 2006).

High-fat diet (HFD) can cause disorders of body lipid metabolism. Acetyl-CoA carboxylases alpha (ACACA) and fatty acid synthase (FASN) are important enzymes that control lipid metabolism. The ACACA gene is a key gene regulating fatty acid synthesis. The encoding protein product of ACACA gene is the acetyl-Coenzyme A carboxylase 1 (ACC1). ACC1 is fused and expressed in all tissues of *Homo sapiens* and other animal species, mainly in the liver and mammary glands in the milk secretion phase. In the case of excessive ATP in the cell, the biotin carboxylase (BC) first transports CO_2 from bicarbonate to the reduction of phytochemicals. It is then catalyzed by the trans catalytic enzyme to transfer to the acetylated coenzyme A (acetyl-CoA) to form malonyl coenzyme A (malonyl-CoA), which is the first step in the synthesis of long-carbon chain fatty acids (Tong 2005). The FASN gene encodes synthetic fatty acid synthase (FAS). FAS is a key enzyme in the *de novo* fatty acid synthesis pathway. Its products serve as substrates to produce long-chain fatty acids, which can act on various important cellular activities including cell division (Chu et al. 2021). Among all body structures, the liver is the main organ to produce fat and FASN is expressed at the highest level in liver tissues (Jayakumar et al. 1995). FASN controls the synthesis of fatty acids and facilitates the formation of cell membranes and organelle membranes, therefore has a certain relationship with cell membrane damage. Most humans and animals will preferentially use new structural lipids synthesized by lipids in their diet. Thus, the synthesis of nascent fatty acids is usually deactivated. The expression of FASN in tissues is generally maintained at a low level, and the expression level of FASN in normal cells is also relatively weak. Nevertheless, FASN expression will increase significantly when cells or tissues are damaged (Bian 2015).

BM has been reported to occupy a significant effect in preventing non-alcoholic fatty liver and improving liver lipid metabolism (Noguchi et al. 2001; Lu et al. 2014). This experiment will aim to further explore the effect of BM on the expression of ACACA and FASN in the liver, thus uncover the detailed activity and molecular mechanism of *Momordica charantia*'s alleviation on the lipid metabolism disorder.

2. Investigations and results

2.1. Effect of BM freeze-dried powder on the weight of mice

Freeze-dried BM powder used in this study contained 4.2% water, 39.1% dietary fiber, 7.1% minerals, 4.4% crude protein and 2.5% crude fat. The weight of mice was evaluated in BM treated and single HFD mice. The ration formula is shown in Table 1. The weight changes of the mice were detected weekly. Mice were dissected at the tenth week; the blood and the liver of each mouse were collected. According to the mouse body weight change curve

Table 1: Diet administration for normal diet mice and high-fat diet mice

Ingredients	Basal diet	High-fat diet
	Content (%)	
Wheat	38	79.6
Corn	29	
Soybean meal	18	
Fish meal	10	
Wheat bran	5	
Soybean oil	3	
Maltose	2	
Alfalfa	2	
Yeast	1	
Trace elements	1	
Sodium Cholate	0	0.2
Propylthiouracil	0	0.2
Cholesterol	0	1
Lard	0	10
Milk powder	0	4
Egg yolk powder	0	5
Nutrition	Content (%)	
Carbohydrates	69	54.5
Protein	20	19.5
Fat	10	24

from week 1 to week 10, the body weight growth rate of HFD mice was faster than that of mice in the Control group and the BM group (Fig. 1A). After ten weeks feeding, the body weight of mice in HFD group increased significantly ($p<0.01$), and BM freeze-dried powder could remarkably reduce the weight of HFD mice ($p<0.01$) (Fig. 1B). Similarly, HFD could significantly increase liver weight in mice ($p<0.01$), and BM freeze-dried powder enormously attenuated the increase in liver weight caused by HFD ($p<0.01$) (Fig. 1C).

2.2. Effect of BM freeze-dried powder on biochemical indexes of HFD mice

Considering the whole body and liver weight results of the three groups of mice, typical biochemical indexes that had intimate relationship with body weight and fat lipid metabolism such as TG, TC, HDL-C and LDL-C were detected. As shown in Fig.1D, 1E and 1G, HFD could significantly increase serum TG ($p<0.05$), TC ($p<0.01$) and LDL-C ($p<0.01$) levels in mice (Fig. 1D,1E and 1G). However, BM significantly reduced the increase in TG ($p<0.01$), TC ($p<0.05$) and LDL-C ($p<0.01$) levels in mice caused by HFD. Besides, HFD caused a significant decrease in mouse HDL-C ($p<0.01$), and BM reversely increased the mouse HDL-C amount to a normal level comparable to the Control mice ($p<0.05$) (Fig. 1F).

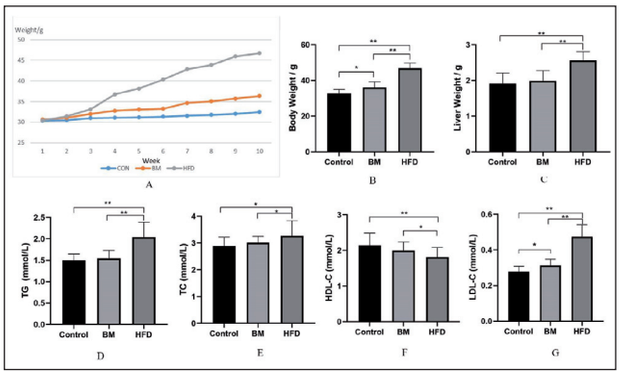

Fig. 1: Body weight and biochemical indexes of mice in Control (CON), BM, and HFD groups. (A) Mouse body weight change curves from week 1 to week 10. (B) Body weight comparison of mice in 3 groups. (C) Comparison of liver weight in mice in 3 groups. (D) Serum Triglyceride content of mice in each group. (E) Serum total cholesterol content of mice in each group. (F) Serum HDL-C content of mice in each group. (G) Serum LDL-C content of mice in each group. N=20; *p<0.05 between two groups, **p<0.01 between two groups.

2.3. Effect of BM freeze-dried powder on the morphology of liver tissue in HFD mice

Figure 2 shows liver sections of three groups of mice. The liver tissue of the control group was regularly clear and the cells were tightly arranged (Fig. 2, left panel). The liver tissue of the mice in the BM group was still clearly visible, the intercellular space was larger than that in the Control group, and there were just a few vacuoles (Fig. 2, middle panel). However, In the HFD group, several vacuoles were observed in the liver, and the liver tissue was incomplete (Fig. 2, right panel), which suggested that the liver morphology was considerably changed and liver injury occurred. Shortly, BM administration significantly rescued the liver injury in morphology and exerted a protective effect.

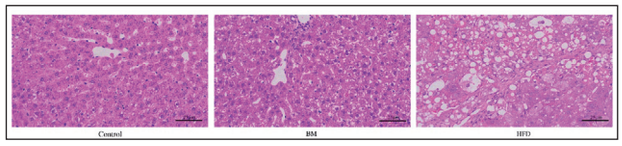

Fig. 2: Effect of BM freeze-dried powder on the morphology of liver tissue in HFD mice. Left panel: Control group; middle panel: BM group; right panel: HFD group. Scare bar: 25 μm.

2.4. FASN and ACACA genes were detected by qRT-PCR

To further explore the mechanism regulating fat lipid metabolism in the liver, the expression levels of FASN and ACACA genes were detected by qRT-PCR. As shown in Table 2, specific primers of FASN and ACACA were designed by Primer-BLAST at National Center for Biotechnology Information (NCBI). According to the results of qRT-PCR in Fig. 3, HFD could significantly upregulate FASN and ACACA in mouse liver ($p<0.01$). In BM groups while mice intaking BM freeze-dried powder, FASN transcription was also significantly lower than that in HFD mice ($p<0.01$) (Fig. 3A); and liver ACACA transcription was also lower than that in HFD mice ($p<0.05$) (Fig. 3B).

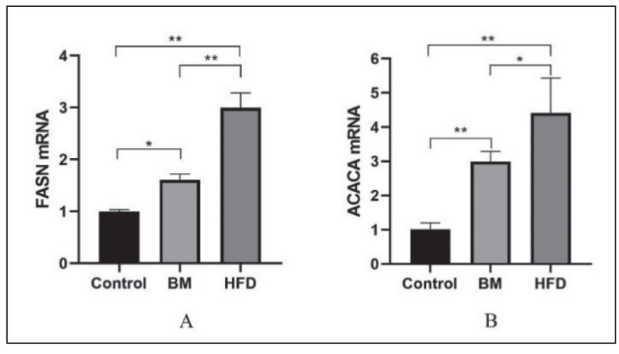

Fig. 3: Effect of BM freeze-dried powder on FASN and ACACA gene transcription in HFD mouse liver. (A) FASN mRNA levels in mouse liver. (B) ACACA mRNA levels in mouse liver. N=3; *p<0.05 between two groups, **p<0.01 between two groups.

Table 2: Primer sequence of FASN and ACACA used in the experiment

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
β-actin	CCATAAACGATGCCGGA	CACCACCCATAGAATCAAGA
FASN	AGCACTGCCTTCGGTTCAGTA	AAGAGCTGTGGAGGCCACTTG
ACACA	CCTCCGTCAGCTCAGATACA	TTTACTAGGTGCAAGCCAGACA

2.5. FASN and ACACA were detected by Western blot

The protein expression levels of FASN and ACACA were also investigated. Western blot results showed that HFD could significantly increase the expression of ACACA and FASN in mouse liver ($p<0.01$). The expression of ACACA (Fig. 4A, 4B) and FASN (Fig. 4A, 4C) in the liver of BM mice was higher than that of the Control group, but significantly lower than that of the HFD group ($p<0.01$) (Fig. 4).

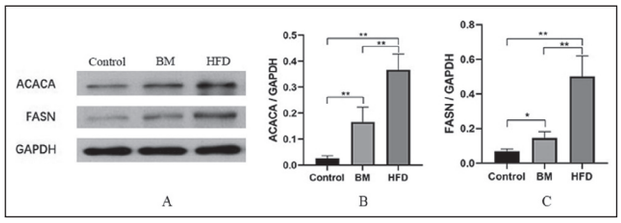

Fig. 4: Effect of BM freeze-dried powder on the expression of ACACA and FASN proteins in the liver tissues of HFD mice. (A) Western blot results of ACACA and FASN proteins in mouse liver tissues. (B, C) Statistical results of Western blot of ACACA and FASN protein levels were normalized to GAPDH and the quantitative analysis of ACACA/GAPDH ratio and FASN/GAPDH ratio were shown in (B) and (C), respectively. N=3; *p<0.05 between two groups, **p<0.01 between two groups.

3. Discussion

With the advancement of social economy, health problems caused by diet have become a research hotspot. Fatty liver, diabetes, hypertension and other diseases caused by HFD threaten human health (Matheus et al. 2017; Cerf 2018; Recena et al. 2019). Long-term HFD can easily induce liver disease in animals. HFD can disrupt the endoplasmic reticulum (ER) calcium homeostasis (Wires et al. 2017), inhibit adenosine 5'-monophosphate-activated protein kinase (AMPK) activation (Wang et al. 2017), downregulate peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α) expression and suppress the nuclear factor kappa-B (NF-κB) pathway (Barroso et al. 2018), which can induce cell damage and liver inflammation. According to our test results, after 10 weeks of HFD, the weight of the mice increased significantly, and the weight of the liver also increased; at the same time, a large amount of steatosis appeared in the liver. Moreover, the biochemical indicators were significantly different from those of mice with normal diet. Our results showed that the HFD we used to establish model mice was successful.

As our experiments revealed, body weight, liver tissue morphology and biochemical indexes of HFD mice that contained BM freeze-dried powder were favorably improved. There are many biologically active compounds in bitter gourd fruit, including polysaccharides, proteins, lipids, triterpenoids (Chen et al. 2008; Zhao et al. 2014; Chang et al. 2008), saponins (Akihisa et al. 2007; Ma et al. 2014; Murakami et al. 2001), peptides, flavonoids, alkaloids, and sterols (Ahmad et al. 2012). These substances can resist inflammation, anti-hyperlipidemia, anti-hypertension, anti-bacterial, immune regulation, and liver protection (Jia et al. 2017). Previous studies have shown that BM has a significant role in combating liver injury and inflammation. It can suppress the alcohol-induced elevation of CYP2E1, SREBP-1, FAS, and ACC protein expression. It has beneficial effects against alcoholic fatty liver, in which they attenuate oxidative stress and inflammatory responses (Lu et al. 2014). Our findings provide additional evidence that BM exerts liver protective effects.

ACACA and FASN are important components of lipid metabolism in the cytoplasm. They are responsible for the synthesis of fatty acids in the cytoplasm. More specifically, ACACA catalyzes the synthesis of fatty acid (FA) by converting acetyl-CoA to malonyl-CoA (Fullerton et al. 2013); FAS is the core enzyme of the *de novo* adipogenesis (DNL) pathway, which catalyzes malonyl and Acetyl-CoA synthesizes palmitate and coenzyme A (Raza et al. 2018). According to our results, BM can significantly reverse the effect of HFD on the expression of ACACA and FASN both in the gene transcription and protein levels. The increased expression of ACACA and FASN can reduce liver steatosis (Kim et al. 2017; Zhang et al. 2019). This is in line with our test results (Figs. 3 and 4). As downstream genes of the AMPK pathway, ACACA and FASN are often regulated by AMPK. Moreover, studies have shown that activation of the AMPK pathway can reduce or prevent non-alcoholic fatty liver caused by HFD, indicating that the activation of AMPK is important for liver lipid metabolism (Cheng et al. 2018; Liu et al. 2019; Liou et al. 2019; Zheng et al. 2018; Manna et al. 2017; Du et al. 2015). Although this study did not explore the expression changes of the AMPK pathway, it can be confirmed that as the downstream genes of AMPK, ACACA and FASN play important roles in liver injury, and the regulation of ACACA and FASN by BM will support the notion that BM alleviates the lipid metabolism disorder of mice on a high-fat diet *via* downregulating ACACA and FASN.

In summary, *Momordica charantia* can improve liver fat metabolism by regulating the expression of ACACA and FASN genes in mouse liver, thereby protecting liver steatosis caused by HFD.

4. Experimental

4.1. Animal experiments

All animal experiments were approved by the Animal Care and Use Committee of Hubei University of Science and Technology (Approval No.: 2021-03-020) strictly in accordance with the Regulations of Hubei Province on Laboratory Animal Management (011043145-029-2013-000009). Briefly, 60 C57BL/6 mice (7 weeks old male mice weighing 30±2 g, Changsheng Biotechnology, Liaoning) were raised in a SPF-level Laboratory Animal Room. The humidity was controlled at 50-75%, and the temperature was controlled at 25 °C. Under the condition of a 12h light-dark cycle, the mice freely took food and water. After a 10-day adaptation period, the mice were randomly divided into three groups with 20 mice in each group: control group (CON), high-fat diet with 5% BM freeze-dried powder (BM) and single high-fat diet group (HFD). The BM administration and single HFD feeding lasted for ten weeks. The body weight was recorded every week for growth curve and the weight of each mouse and livers were calculated at week 10 when experiment terminated and mice were sacrificed.

4.2. Chemicals and drugs

BM freeze-dried powder was provided by Quanao biotech (QA202000920, Xi'an China). Barium chloride ($BaCl_2$) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Aminopyridine (AP) was purchased from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan). Other reagents were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd (Shanghai, China) unless special indicated. Potassium chloride (KCl) was supplied by Mingsheng Pharmaceutical Group Co., Ltd (Zhejiang, China). Sodium hydrogen carbonate ($NaHCO_2$), glucose, calcium chloride hexahydrate ($CaCl_2{\cdot}2H_2O$), anhydrous magnesium sulfate ($MgSO_4{\cdot}7H_2O$), and sodium chloride (NaCl) were supplied by Tianjin Damao Chemical Reagent Factory (Tianjing, China).

4.3. Hematoxylin and eosin staining

Histological slides were prepared by fixing the liver samples in 4% paraformaldehyde. Liver samples were dehydrated in ethanol, cleared with xylene, and embedded in paraffin wax. Later, tissue sections were cut into 5 μm thickness, dewaxed with xylene and stained with hematoxylin and eosin for the histological examination. Ultimately, a Olympus BX51 fluorescence microscope (Tokyo, Japan) was used to examine the slides.

4.4. Triglyceride and total cholesterol test

Triglyceride determination kit (A110-1-1, Nanjing, China) was used to detect the serum triglyceride concentration by lipase method; the total cholesterol determination kit (A111-1-1, Nanjing, China) was used to detect the total cholesterol concentration by the cholesterol esterase method.

4.5. HDL-C and LDL-C detection

HDL-C assay kit (A112-1-1, Nanjing Jiancheng, China) and LDL-C assay kit (A113-1-1, Nanjing Jiancheng, China) were used to detect the content of HDL-C and LDL-C in serum samples.

4.6. Quantificational real-time polymerase chain reaction (qRT-PCR)

Trizol (Servicebio, Wuhan, China) was used to isolate total RNA from livers. The RNA was reversely transcribed with cDNA Synthesis kit (Tsingke, Wuhan, China) according to manufacturer's instructions. The quantificational real-time polymerase chain reaction (qRT-PCR) was performed on LightCycler 96® instrument (Roche, Switzerland). The ΔΔCt method was used to normalize and analyze the data. β-Actin was employed as a loading control.

4.7. Western blot

The collected livers were homogenized and lysed with protein lysis solution. Quantified protein with BCA Protein Assay Kit (Abcam, UK). Lysates were boiled in sample buffer for 10 min; 30 μg of cell protein were subjected to immunoblotting analysis. PVDF membranes were blocked with 5% nonfat dry milk in PBS-T with 0.05% Tween20 in 10 mM phosphate-buffered (isotonic) saline at 37 °C for 2 h with constant shaking. The membranes were then probed with monoclonal rabbit ACACA (1:500) and monoclonal rabbit FASN antibodies (1:500) (Bioss, China) at 4 °C overnight, and then incubated with goat anti-rabbit polyclonal antibodies conjugated to horseradish peroxidase. Proteins were detected using enhanced chemiluminescence reagents (Biosharp, China). The amount of protein transferred onto the membranes was normalized by immunoblotting of monoclonal GAPDH antibody, (1:4000) (Abclonal, China).

4.8. Statistical analysis

Result values are presented as mean±standard deviation (SD), and the one-way Analysis of Variance (ANOVA) was used to analyze the multigroup differences. Use IBM SPSS Statistics 25 to perform one-way ANOVA analysis on the data. A t test was used to examine the differences between two groups. GraphPad Prism software 8.0 is used for graph analysis. The difference value of $p<0.05$ is considered statistically significant.

Conflicts of interest: The authors declare that there is no conflict of interest regarding the publication of this paper.

Author contributions: Study design, HP.Z., YX.P., HY.L. and TH.S.; conducting experiments, XH.L., WQ.M., KY.X. and H.L.; statistical analysis and data interpretation, HP.Z., MC.H. and TH.S.; manuscript preparation, MC.H. and TH.S.. All authors gave final approval and agreed to be accountable for all aspects of work ensuring integrity and accuracy.

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