Benefits of the soluble and insoluble fractions of bitter gourd in mice fed a high-fat diet

Jie Xu, Ke Cao, Zhihui Feng⁎, Jiankang Liu⁎

Center for Mitochondrial Biology and Medicine, The Key Laboratory of Biomedical Information Engineering of Ministry of Education, School of Life Science and Technology and Frontier Institute of Science and Technology, Xi'an Jiaotong University, Xi'an 710049, China

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ABSTRACT

Background: Bitter gourd (BG) fruit powder was previously showed to prevent high-fat diet (HFD)-induced metabolic disorders in mice. In current study, we investigated the beneficial difference of the water soluble (S-BG) and insoluble fractions (IS-BG) of bitter gourd.

Results: Compared to normal chow diet, the HFD induced greater body weight gain and increased fat mass accompanied by impaired insulin sensitivity, elevated serum lipids and inflammation. Supplementation of BG, S-BG, and IS-BG showed similar beneficial effects and restored the metabolic changes in mice. Interestingly, BG showed no effect on fat mass, which was decreased by both S-BG and IS-BG. Additionally, S-BG and IS-BG inhibited the SREBP-1/FAS pathway, thereby decreasing liver cholesterol accumulation, while only BG showed improvement on mitochondrial activity.

Conclusion: These results further support the position that bitter gourd has multiple active ingredients and that both fractions have beneficial effects on metabolic disorders.

1. Introduction

Metabolic syndrome (MS) is growing into a major public health burden, leading to enormous losses of life quality in both developed and developing nations (Ruiz-Nunez, Dijck-Brouwer, & Muskiet, 2016). MS is characterized by the clustering of risk factors, including insulin resistance, obesity and dyslipidemia (Grundy et al., 2004; O’Neill and O’Driscoll, 2015). As these conditions are among the leading causes of deaths worldwide, preventing metabolic syndrome development is of critical importance (Grundy et al., 2005). Since the development of MS has been largely attributed to a suboptimal lifestyle, including excessive caloric intake, unbalanced diet, chronic stress, and physical inactivity (Danaei et al., 2009; Egger & Dixon, 2011; Ruiz-Nunez, Pruimboom, Dijck-Brouwer, & Muskiet, 2013), nutritional and physical interventions are still considered to be effective strategies to improve metabolic health.

Dietary recommendations have been proposed to prevent or reduce the development of MS in the general population. Momordica charantia L., known as bitter gourd (BG), is a common edible vegetable in Asia. Its anti-diabetic, anti-bacterial, antiviral and anticancer activities have been scientifically demonstrated over previous decades (Grover & Yadav, 2004; Klomann, Mueller, Pallauf, & Krawinkel, 2010; Krawinkel & Keding, 2006). In addition, animal studies have also indicated the effects of BG supplementation in regulating weight gain and lipid metabolism (Gadang et al., 2010). Some pharmacological and safety studies of this herb have been carried out (Fernandes, Lagishetty, Panda, & Naik, 2007). Many studies have reported the effects of bitter gourd extracts on insulin resistance (Shih, Lin, Lin, & Wu, 2009; Wang et al., 2011). Aqueous, chloroform and methanol extracts of BG treatment decreased blood glucose levels in type 1 diabetic and normal rats (Virdi et al., 2003). Aqueous extracts of BG fruit have been demonstrated to reduce VLDL levels and decrease blood glucose levels in normal rats (Uebanso et al., 2007). Previous studies have reported that BG inhibits the development of obesity-associated fatty liver (Xu et al., 2014); however, currently, little is known about the soluble and insoluble components of bitter gourd. Given the diversity of bitter gourd consumption, of which juice is the primary one, we intend to provide more support regarding the benefits of soluble and insoluble bitter gourd, to improve the efficiency of bitter gourd consumption and expand options of bitter gourd related product. Therefore, in the present study, we compared the effects of different parts of bitter gourd extracts on metabolic syndrome and mitochondrial function in HFD induced obese mice.
2. Materials and methods

2.1. Materials

Antibodies against β-actin were purchased from Sigma (St. Louis, MO, USA). Antibody against SREBP-1c was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies against GAPDH and fatty acid synthase (FAS) were purchased from Cell Signaling Technology (Danvers, MA, USA). Antibodies against complexes I, II, III, IV and V were purchased from Invitrogen (Carlsbad, CA, USA). Bitter gourd (BG) powder was prepared by Guangxi Zhennong Seed Industry Co., Led. (Guilin, China).

Fig. 1. Effects of BG fractions on body. Body weight curve (A), final body weight (B), and body weight gain (C) in male C57BL/6 mice fed a control diet or a high-fat diet supplemented with BG, S-BG, or IS-BG for 16 wk. The data are the mean ± SEM, n = 8. *P < .05, **P < .01 vs. relative control. BG, bitter gourd; S-BG, soluble fraction of BG; IS-BG, insoluble fraction of BG; HFD, high-fat diet.

Fig. 2. Effects of BG fractions on tissue weights. The weight ratio of heart (A), liver (B), epididymal fat mass (C), and perirenal fat mass (D), and HE staining of inguinal adipose tissue (E) in male C57BL/6 mice fed a control diet or a high-fat diet supplemented with BG, S-BG, or IS-BG for 16 wk. The data are the mean ± SEM, n = 8. *P < .05, **P < .01 vs. relative control. BG, bitter gourd; S-BG, soluble fraction of BG; IS-BG, insoluble fraction of BG; HFD, high-fat diet.

2.2. Animals and treatments

Four-week-old male C57BL/6 mice were purchased from the SLAC laboratory Animal Co. Ltd. (Shanghai, China) and were housed in a temperature (22–28 °C)- and humidity (60%)-controlled animal room on a 12 h light/12 h dark cycle (light from 8:00 to 20:00) with food and water provided during the experiments. After 1 week of acclimatization, mice were randomly divided into five groups (n = 10 in each group): mice fed a normal diet (Control, 12% kcal fat content), mice fed...
a high-fat diet (HFD, 45% kcal fat content), mice fed a high-fat diet with daily oral gavage of low-dose BG (0.5 g/kg/day, HFD + BG), mice fed a high-fat diet with daily oral gavage of soluble BG (0.235 g/kg/day, HFD + S-BG), and mice fed a high-fat diet with daily oral gavage of insoluble BG (0.225 g/kg/day, HFD + IS-BG). After 16 weeks, mice were fasted overnight and sacrificed. All of the procedures were performed in accordance with the United States Public Health Services Guide for the Care and Use of Laboratory Animals, and all possible efforts were made to minimize the suffering and number of animals utilized in this study.

2.3. Sample preparation

After the mice were sacrificed, the liver, heart, and visceral fat pads (including the perirenal and epididymal fat pads) were removed, weighed and stored under −80 °C until further analysis. Blood samples were obtained by cardiac puncture, and serum was separated by centrifugation (3000 rpm for 10 min). The triglyceride (TG), total cholesterol (TC), and HDL cholesterol levels were analyzed on an automated biochemistry analyzer (Hitachi Ltd., Tokyo, Japan). The serum levels of TNF-α, free fatty acids, insulin, leptin, CRP, IL-1β, INF-γ and IL-6 were measured using commercial kits according to the manufacturer’s standards and protocols (R&D Systems, Shanghai, China).

2.4. HE staining

Tissues were dissected and fixed in 4% paraformaldehyde for 48 h. Paraffin processing, embedding, and sectioning were performed following standard protocol. Slides were stained with HE staining kits following manufacture guidance (Jiancheng, Jiangsu, China). Slides were visualized by confocal microscopy (Zeiss, Jena, Germany).

2.5. Oral glucose tolerance test (OGTT)

An OGTT was performed after 16 wk of feeding and gavage. All mice were fasted overnight before the test. Blood was taken from the retrobulbar vein both before and 15, 30, 60, 120 min after glucose gavage (1 g/kg body weight). The plasma glucose concentration was determined using the glucose oxidation method.

2.6. Analysis of hepatic cholesterols

Liver tissues were collected and homogenized in ice-cold PBS after centrifugation (1000g, 10 min), and the supernatant was collected for analysis. The concentrations of liver total cholesterol were analyzed using commercial clinical diagnosis kits according to the manufacturer’s protocol (Jiancheng, Jiangsu, China).

2.7. Assays for mitochondrial complex activity

Mitochondria were isolated as previously described (Sun et al., 2010). The activities of reduced nicotinamide adenine dinucleotide (NADH)-ubiquinone reductase (complex I), succinate-CoQ oxidoreductase (complex II), cytochrome C oxidoreductase (complex III) were measured spectrophotometrically using conventional assays as previously described (Li et al., 2008; Sun, Luo, Long, Wei, & Liu, 2006).
2.8. Western blotting

Liver tissues were lysed in Western and IP lysis buffers (Beyotime, Jiangsu, China). The homogenates were centrifuged at 13,000 g for 15 min at 4 °C. The supernatants were collected, and the protein concentrations were determined using a BCA protein assay kit (Thermo Scientific, IL, USA). Equal aliquot (20 μg) of protein was analyzed by Western blotting, and chemiluminescent detection was performed using an ECL Western blotting detection kit and quantified by scanning densitometry.

2.9. Statistical analysis

The results are presented as the mean ± S.E.M. Statistical analyses were performed using one-way ANOVA followed by least significant different post hoc analyses. For all of the analyses, values of p < .05 were considered to be statistically significant.

3. Results

3.1. The effects of the BG fractions on body and tissue weights

Mice were divided into five groups for initiating diet and BG treatment, and the average body weight did not differ within the five groups. During the trial period, the body weight of all groups increased. The HFD group exhibited a steady increase in body weight from the 6th week, while the HFD+BG and HFD+S-BG showed a lower increase compared to the HFD group (Fig. 1A). The final body weight and body weight gain were significantly higher in the HFD group compared to the control group and were lower in the HFD+BG and HFD+S-BG groups (Fig. 1B and C). Although the HFD+IS-BG group trended toward a decreased body weight, no significance difference was observed (Fig. 1A–C). No significant difference was observed on heart weight (ratio to body weight) among five groups (Fig. 2A). A decreased liver weight ratio was observed in the HFD and HFD+BG groups compared to the control group (Fig. 2B), but no difference was observed within the four HFD groups. A dramatic increase of epididymal fat ratio (Fig. 2C) and the perirenal fat mass (Fig. 2D) was found in the HFD group compared to the control group. BG supplementation had no effect on the fat mass, while both HFD+S-BG and HIF+IS-BG showed significantly decreased fat mass/body weight ratio compared to the HFD group (Fig. 2C and D). HE staining of inguinal adipose tissue showed enlarged fat cell in HFD group compared to control, BG and fraction treatment had no obvious effect on adipose hypertrophy compared to HFD group (Fig. 2E).

3.2. The effects of the BG fractions on glucose tolerance

An oral glucose tolerance test was performed during the 16th week of treatment. As shown in Fig. 3, the HFD group displayed significantly lower glucose tolerance compared to the control group, which was efficiently improved in the BG, S-BG, and IS-BG groups (Fig. 3A and B). Consistently, the fasting serum glucose and insulin levels were higher in the HFD group and were significantly reduced by BG, S-BG, and IS-BG supplementation (Fig. 3C and D).

3.3. The effects of the BG fractions on serum cytokines and lipids

Sixteen weeks on a HFD is considered to be long term feeding and will result in a reduction of central leptin sensitivity (Lin, Thomas,
As expected, the HFD group had significantly higher serum leptin levels, which were lower in all of the groups receiving BG supplementation (Fig. 4A). Obesity is also associated with elevated levels of proinflammatory cytokines in the circulation (Tataranni & Ortega, 2005). We found that TNF-α, CRP, INF-γ, IL-1β, and IL-6 levels in the serum were increased in the HFD group, and all of the BG supplements sufficiently reduced these levels (Fig. 4B and C). Hyperlipidemia is commonly associated with obesity. In our animal model, we found increased triglyceride levels in the HFD group as expected, which were lower in the HFD + BG and HFD + S-BG groups (Fig. 5A). Total cholesterol was increased in the HFD group as well and not affected by the BG fractions (Fig. 5B). Interestingly, the HFD group displayed higher HDL-cholesterol levels, which were found to be sufficiently lowered by both S-BG and IS-BG, while BG supplementation had no significant effect (Fig. 5C). The HFD group also had higher concentrations of serum FFAs, which were efficiently decreased by BG and its fractions (Fig. 5D).

3.4. The effects of the BG fractions on liver SREBP-1c pathway activation

HE staining of liver tissue showed increased accumulation of lipids in HFD group, while BG and fractions treatment groups showed lower lipids accumulation (Fig. 6A) Consistent with the serum total cholesterol levels, liver cholesterol was significantly increased in the HFD group and was decreased by BG and its fractions (Fig. 6B). The SREBP-1 pathway is the primary cause of lipogenesis and excess lipid accumulation in the liver. We therefore analyzed the SREBP-1c protein levels and found higher SREBP-1c protein levels in the HFD group, which was decreased in both the HFD + S-BG and HFD + IS-BG groups (Fig. 6C and D). FAS, the downstream target of SREBP-1c, showed no significant difference between the HFD and control groups; however, both S-BG and IS-BG supplementation sufficiently reduced FAS protein expression (Fig. 6C and E).

3.5. The effects of the BG fractions on liver mitochondrial function

Mitochondrial activity is another factor that regulates lipid metabolism in the liver. Therefore, we further analyzed the mitochondrial content and complex activities. As shown in Fig. 7, the mitochondrial complex subunit protein levels did not differ among the treatment groups (Fig. 7A); however, the mitochondrial complex I, II, and III activities were found to be lower in the HFD group compared to the control group (Fig. 7B–D). BG supplementation showed significant improvement, but neither S-BG nor IS-BG had improving effects on the complex activities (Fig. 7B–D).

4. Discussion

Obesity is a major risk factor that can be attributed to insulin resistance. Although many insulin-sensitizing agents are widely available, interest has been evoked in dietary adjuvants that possess hypoglycemic properties. More than 400 herbal products have been used to reduce blood glucose, but only a small number of these products have received scientific and medical evaluations to assess their efficacy (Sathishsekar & Subramanian, 2005). Among them, BG has been a popular recourse that has received widespread attention in the scientific community; however, a well-defined mechanism by which BG extract exerts its beneficial effects has not been elucidated. In our previous study, we reported that BG could modulate mitochondrial activity, prevent oxidative stress and inhibit lipid accumulation in obesity-associated fatty liver (Xu et al., 2014). In the present study, we further demonstrate that both the water soluble and insoluble fractions of BG exhibit beneficial effects on HFD-induced obese mice, indicating multi-mechanisms of BG against metabolic disorders.

Our previous study showed that a dose of 0.5 g/kg BG could significantly reduce HFD-induced metabolic abnormalities in mice; therefore, the same dose was chosen as a positive control in the current study (Xu et al., 2014). After 16 weeks of being fed a HFD, male C57BL/6 mice showed increased body weight gain and white adipose tissue mass. Interestingly, BG significantly reducing body weight gain, which was not observed in previous study, and it may potentially attribute to the independently prepared bitter gourd powder for both experiments. Even though, variation on body weight gain was observed at the dose of 0.5 g/kg in both experiments, the overall observations showed consistent indication that bitter gourd has significant benefits improving metabolic parameters in diet-induced obesity model. IS-BG showed no effect on body weight gain, but efficiently reduced white adipose mass. Only S-BG reduced both whole body weight and white adipose mass, suggesting a more consistent benefit from S-BG. Although an increasing number of studies have shown that an increase in abdominal fat mass, either visceral or subcutaneous, is important for the pathogenesis of both insulin resistance and glucose intolerance (Kursawe et al., 2010; Misra & Vikram, 2003; Smith et al., 2001), an increased adipose mass may not fully contribute to metabolic abnormalities and certain states of increased fat mass with preserved metabolic fitness have been referred to as a “healthy” expansion of adipose tissue (Rutkowski, Stern, & Scherer, 2015). Therefore, although the whole BG powder had no significant effect on the adipose mass reduction, it sufficiently reduced fasting insulin and glucose and improved glucose tolerance. Since similar benefits were observed with both S-BG and IS-BG supplementation, we propose that multiple active compounds in BG might contribute to improving adipose function and insulin sensitivity and that either water soluble or insoluble BG would be worthwhile to consume.

Along with body weight and adipose mass gains, HFD-fed mice in
the present study displayed increased plasma leptin, which increases in proportion to the fat mass and promotes cholesterol ester synthesis in macrophages in a hyperglycemic environment (Xu et al., 2003). Increases in leptin may function as an acute pro-inflammatory response to prevent excessive cellular stress, which was further confirmed by the increased TNF-α, CRP, INF-γ, IL-1β, and IL-6 levels in HFD-fed mice. All of the BG supplements significantly reduced the serum leptin levels as well as the inflammation markers, further highlighting the beneficial effects of both BG fractions against inflammation. Obesity is a known cause of dyslipidemia that is characterized by increased TG and decreased HDL-C concentrations (Rashid, Watanabe, Sakaue, & Lewis, 2003). Dietary fat is not usually absorbed from the intestine unless it has been subjected to the action of pancreatic lipase during digestion. Therefore, inhibition of the hydrolysis of dietary fat may decrease intestinal fat absorption, leading to a reduction in obesity and hyperlipidemia (Paccaud, Schlüter-Fasmeyer, Wietlisbach, & Bovet, 2000). Even though low plasma HDL-C level has been well identified as a metabolic risk factor in high-risk individuals, while higher HDL-C is usually considered beneficial, the change of HDL-C level in HFD-induced animal models has been controversial. Previous study showed that high fat diet could increases HDL levels through increasing the transport rates and decreasing the fractional catabolic rates of HDL-C ester, and the rise in HDL-C was considered defensive response of animals (Hayek et al., 1993). In current study, HDL-C level was increased in HFD group compared to the control group. Even though a decrease was observed in HFD+S-BG and HFD+IS-BG group compared to HFD group, the levels of HDL-C in both groups didn’t show significant decrease compare to the control group, which indicates both S-BG and IS-BG could normalize the metabolic response of mice to HFD feeding. Obesity-associated dyslipidemia is always accompanied by increased non-esterified fatty acids (NEFA), also known as free fatty acids (FFA), which can mediate many adverse metabolic effects (Karpe, Dickmann, & Frayn, 2011). Although whole BG powder and BG fractions showed inconsistent effects on the serum TG and CHO levels, the FFA levels were significantly reduced by all BG treatments, which may contribute to a reduced inflammatory response and improved insulin sensitivity.

Obesity is associated with an increased risk of nonalcoholic fatty liver disease (NAFLD) (Saadeh, 2007). HFD induces excess cholesterol accumulation in the liver in mice, which represents an imbalance between complex interactions of metabolic events (Fabbrini, Sullivan, & Klein, 2010). All of the BG supplements reduced lipid accumulation. We found that the key lipogenesis regulator, SREBP-1c, was activated in the HFD group and suppressed by S-BG and IS-BG. A similar suppression was also observed for FAS, which contributes to lower lipid. These data suggest that the benefit of whole BG on liver lipid accumulation is independent of the lipogenesis pathway. Mitochondria play central roles in ATP production and energy expenditure, and excessive energy substrates lead to mitochondrial dysfunction, with consequential effects on lipid and glucose metabolism (Bournat & Brown, 2010; Rogge, 2009). We therefore analyzed the effect of BG supplements on the liver mitochondrial function and determined that only whole BG could efficiently improve the mitochondrial complex activities that were impaired by HFD feeding, while neither S-BG nor IS-BG had such effects. Therefore, we propose that whole BG and BG fractions could reduce liver lipid accumulation through different mechanisms. Given the fact that BG contains hundreds of ingredients, only dozen of which have been identified as active nutrients that have metabolic beneficial, the majority of BG compounds remain unstudied. It’s unclear why the whole powder exerted differently with its fractions on liver analysis, further investigations should be exercised to identify the active components in different BG fractions and explore potential counteracting effects among active ingredients.
In conclusion, the data presented here demonstrated that whole BG powder, S-BG, and IS-BG can reduce HFD-induced hyperlipidemia, hyperglycemia, fatty liver and improve glucose tolerance in mice. The potential mechanism for the beneficial effects of S-BG and IS-BG on fatty liver involve reducing inflammation and down-regulating the SREBP-1c/FAS pathway, while whole BG powder may work through improving mitochondrial function. Together, these findings further support the position that bitter gourd has multiple active ingredients and demonstrate that both water soluble and insoluble fractions have beneficial effects on metabolic disorders. Further research is warranted to explore the major active components of different BG fractions to help establish their clinical applications.

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Completing interests

The authors declare that they have no conflicts of interest.


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