Journal of Functional Foods 41 (2018) 127–134

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Modulation of gut microbiota and gut-generated metabolites by bitter melon results in improvement in the metabolic status in high fat diet-induced obese rats

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ARTICLE INFO

Keywords:
Bitter melon
Obesity
Gut microbiota
Metabolomics

ABSTRACT

The anti-obesity property of bitter melon powder (BMP) was investigated using a high-fat diet (HFD) obesity model. Obese rats were fed a HFD and treated with BMP for 8 weeks. We observed that BMP could effectively retard the body weight gain and improve the insulin sensitivity compared to the HFD group ($p < .05$). BMP also showed reduced endotoxin load as lipopolysaccharide-binding protein (LBP) level in the system. 16S rRNA sequencing indicated that BMP dramaticlly increased the relative abundance of *Blautia* and *Allobaculum* at the genus level. Metabolomic data revealed that dietary BMP changed metabolic profiles, including the metabolism of organic acids, amino acids and sugars. Additionally, some significant correlations was observed between specific gut microbiota at the genus level and effective biomarkers. These results reveal that the inhibition of insulin resistance and anti-inflammatory effect of BMP in obesity are illustrated with the restoration of gut microbiota and gut-generated metabolites.

1. Introduction

In recent years, obesity and its metabolic disorders have emerged as major health concerns worldwide, which are attributable to a combination of genetics, unhealthy diet and lifestyle (Hoyt, Burnette, & Auster-Gussman, 2014). Body weight of diet-induced obese subjects was greatly increased, with evidence of systemic inflammation, insulin resistance, fatty liver and so on.

Recent studies have demonstrated that the indigenous gut microbiota plays a fundamental role in the development of a chronic low-grade inflammatory state in the host, which contributes to the occurrence of chronic metabolic diseases such as insulin resistance, fatty liver and dyslipidemia (Cani, Osto, Geurts, & Everard, 2012). A complex community of microorganisms inhabit throughout the gut, especially in the colon (Blaut et al., 2002). Indeed, it seems that the gut microbiota functions much like a metabolic "organ", participating in nutrient acquisition, energy homeostasis, and, ultimately, the control of body weight. Several studies identified some key species-level phylotypes that are linked with obesity phenotypes in rodents, for example, protective bacteria such as *Bifidobacterium spp*. and a species-level phylotype in the sulphate-reducing family *Desulfovibrionaceae* (Zhang et al., 2010, 2012). What's more, the metabolites in the fecal water resulting from fermentation by the gut microbiota also play a considerable role in both human health and disease (Velagapudi et al., 2010). On one hand, microbial metabolism serves as an important source of energy for the gut wall by fermentation of carbohydrates to organic acids, mainly butyrate (Cani et al., 2008). On the other hand, there are some types of toxic metabolites in the fecal water, such as secondary bile acid, nitro compounds, amines and endotoxins produced by gram-negative bacterial cell walls (Cani et al., 2007; Pearson, Gill, & Rowland, 2009; Wu, Cheng, & Chen, 2011). These toxin contents in fecal water have proved to hold the biological activity of cytotoxicity and genotoxicity to the epithelial cells (Adebola, Corcoran, & Morgan, 2013; Chen, Lin, & Wang, 2010).

The application of metabolomics to feces has been a relatively new omics technique to assess host–microbiome interactions and the functional status of the gastrointestinal system. Nuclear magnetic resonance (NMR) spectroscopy is an attractive technique in metabolomics studies because of its advantages, including simple sample preparation, rapid analysis and high reproducibility. Recently, NMR-based metabolomic technique has been used in the identification of new targets or biomarkers during the dietary treatment (Ogegbo et al., 2012; Regueiro, Vallverdú-Queralt, Simal-Gándara, Estruch, & Lamuela-Raventós, 2014; Zheng et al., 2016). Fecal metabolites are originated from gut microbiota, microbial metabolism, intestinal epithelial cells, and host-microbial co-metabolisms while the dominant are mostly derived from gut

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https://doi.org/10.1016/j.jff.2017.12.050
Received 26 March 2017; Received in revised form 19 November 2017; Accepted 18 December 2017

microbial metabolism of indigestible dietary substances. Nowadays, integrated analysis of the fecal metabonomic and microbiomic phenotypes has been a feasible way to define the changes of fecal metabolic profiles and their correlations with gut microbiota during obesity development (Zhao et al., 2013).

Bitter melon (*Momordica charantia* L.), a traditional Chinese medicinal plant, has been reported to exert potential effects in hypoglycemic, hypolipidemic and improving insulin resistance in the obese (Fuangchan et al., 2011). Related metabolomic studies have found that the anti-obesity property of bitter melon is linked to its effects on metabolic profile and metabolic pathways. Gong, Zhang, and Xu (2016) found that the inhibition of insulin resistance effect of bitter melon in obesity was illustrated with the restoration of free fatty acids and eicosanoids in the serums by using gas and liquid chromatography-mass spectrometry. Bian et al. (2016) revealed that bitter melon changed the metabolic pathways, including pantothenate and Coenzyme A biosynthesis, starch and sucrose metabolism and citrate (TCA) cycle pathway in the urine by NMR-based metab-olomic technique.

However, in obesity, the effects of bitter melon on metabolic profile and its relationship with gut microbiota in the colon are not fully clear. Based on these premises, the present study was undertaken to gain a better understanding of how bitter melon alters the colonic microbiome and the metabolome to improve the metabolic status in HFD-induced obese rats.

2. Materials and methods

2.1. Preparation of bitter melon powder (BMP)

Fresh bitter melons were collected from Lvjian Agricultural Station (Yangzhong City, China) and authenticated by Jiangsu Academy of Agricultural Science. The process of bitter melon powder preparation was the same as the previous method (Bai, Zhu, & Dong, 2016). And the chemical profile of BMP includes 38.44% dietary fiber, 34.01% carbohydrate, 12.17% protein, 11.75% water, 1.40% ash, 1.05% lipid, 0.63% phenolics, 0.32% flavonoids and 0.21% saponins as previously reported by Bai et al. (2016).

2.2. Animal studies

Male SD rats (*Sprague–Dawley* rats, rattus norregicus) weighing 200 ± 20 g were purchased from the Laboratory Animal Research Center of Jiangsu University (LARC, Zhenjiang, China) with the license number SCXK (SU) 2013–0011 and strictly conducted in accordance with the guidelines for animal care of the National Institute of Health. Rats were maintained in a 12-h light/12-h dark cycle (light on at 8:00 a.m. and off at 8:00 p.m.) at 23 ± 2 °C and given diet and water ad libitum.

The obese rat model was established for 8 weeks according to the previous method (Bai et al., 2016). After designing the model, total 30 experimental rats given normal control diet (NCD; 70% corn, 1.8% bean pulp, 17% fishmeal, 5% grass powder, 2% yeast powder, 1.9% vegetable oil, 0.4% essential amino acid, 0.8% vitamin, 0.7% mineral substance, 0.2% $CaHPO_4$, 0.2% salt) and high fat diet (HFD; containing 73% normal control diet, 12% lard, 10% sucrose, and 5% yolk powder) were divided into three groups (n = 10 each): Group 1, normal rats were given a NCD and 4 mL distilled water/kg body weight (bw); Group 2, obese rats were given a HFD and 4 mL distilled water/kg bw; Group 3, obese rats were given a HFD and 400 mg BMP/kg bw.

During the 8-week experiment, the body weight and food intake were recorded every week. After 8 weeks, the animals were deprived of feed overnight and anesthetized with chloral hydrate. Blood samples were immediately collected by abdominal aortic method and centrifuged at 3500 rpm for 10 min to obtain serum, which was stored at −20 °C for further analysis. The contents in the proximal colon (immediately below the cecum) were collected and stored at −80 °C for 16S rRNA sequencing and NMR spectroscopy analysis.

2.3. Metabolic parameters

Fasting glucose concentration was determined using One Touch Ultra Blood Glucose Meter (Johnson & Johnson Medical Ltd., Shanghai, China). Fasting insulin levels were determined using commercial rat insulin ELISA kits (eBioscience, Inc., San Diego, CA, USA). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as follows: fasting insulin (mU/L) × fasting glucose (mmol/L)/22.5.

We measured serum LBP using an ELISA kit (USCN Life Science and Technology Co., Ltd, Wuhan, China), according to the manufacturer's instructions.

2.4. Fecal DNA extraction and sequencing

Colon contents from the three groups (n = 6 each) were obtained immediately after being anesthetized and transported in the shortest time to −80 °C for storage until the gut microbiota analysis. Total bacterial DNA was extracted using DNA Stool Kit (Omega Bio-tek, Norcross, GA, U.S.) with bead-beating pre-treatment. Referring to other reports (Cook et al., 2016; Rintala et al., 2017), we chose the V4-V5 region of the bacteria 16S ribosomal RNA gene, which were amplified by PCR (95 °C for 2 min, followed by 25 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s and a final extension at 72 °C for 5 min) using primers 515F 5′-barcode- GTGCCAGCMGCCGCGG)-3′ and 907R 5′-CCGTCAATTCMTTTRAGTTT-3′. Purified amplicons with different barcodes were pooled in equimolar and paired-end sequenced (2 × 250) on an Illumina MiSeq Platform (San Diego, CA, USA) according to the standard protocols.

Operational Units (OTUs) were clustered with 97% similarity cutoff using UPARSE (version 7.1 http://drive5.com/uparse/) and chimeric sequences were identified and removed using UCHIME. The taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier (http://rdp.cme.msu.edu/) against the silva (SSU115) 16S rRNA database using confidence threshold of 80% (Amato et al., 2013).

2.5. Sample preparation for ^{1}H NMR spectroscopy

Samples of contents in the proximal colon (immediately below the cecum) from HFD and BMP groups (n = 10 each) were weighted and suspended in 1500 μL purified water. Four seconds on/three seconds off cycling program was used (15 cycles) in the ice water bath for its in-solution ultrasonic extraction process (Sonics VX-130, USA). Samples were centrifuged at 13,000 rpm, 4 °C for 15 min, aqueous layer was transferred to 0.5 mL 3 KDa ultrafiltration filter (Mimacon, USA). Filtrate was collected by centrifuging the sample at 13,000 rpm, 4 °C for 45 min. 450 μL filtrate was transferred to a clean 2 mL centrifuge tube. 50 μL DSS standard solution (Anachro, Canada) was added. Samples were mixed well before transfer to 5 mm NMR tube (Norwell, USA) and analyzed by high-resolution NMR spectroscopy.

2.6. ^{1}H NMR measurement and preprocessing

Spectra were collected at 298 K using a Bruker AV III 600 MHz spectrometer equipped with an inverse cryoprobe. The first increment of a 2D-^{1}H, ^{1}H-NOESY pulse sequence was utilized for the acquisition of ^{1}H NMR data and for suppressing the solvent signal. Experiments used a 100 ms mixing time along with a 990 ms pre-saturation (~80 Hz gammaB1). Spectra were collected at 25 °C, with a total of 128 scans over a period of 15 min.

The collected Free Induction Decay (FID) signal was automatically zero filled and fourier transform in Processing module in Chenomx NMR Suite 8.1. (Chenomx Inc., Edmonton, Canada) The data was then carefully phased and baseline corrected by experienced technician in