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Dietary mung bean protein reduces high-fat diet-induced weight gain by modulating host bile acid metabolism in a gut microbiota-dependent manner

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ABSTRACT

The 8-globulin-rich mung bean protein (MPI) suppresses hepatic lipogenesis in rodent models and reduces fasting plasma glucose and insulin levels in obese adults. However, its effects on mitigating high fat diet (HFD)-induced obesity and the mechanism underlying these effects remain to be elucidated. Herein, we examined the metabolic phenotype, intestinal bile acid (BA) pool, and gut microbiota of conventionally raised (CONV-R) male C57BL/6 mice and germ-free (GF) mice that were randomized to receive either regular HFD or HFD containing mung bean protein isolate (MPI) instead of the dairy protein present in regular HFD. MPI intake significantly reduced HFD-induced weight gain and adipose tissue accumulation, and attenuated hepatic steatosis. Enhancement in the secretion of intestinal glucagon-like peptide-1 (GLP-1) and an enlarged cecal and fecal BA pool of dramatically elevated secondary/primary BA ratio were observed in mice that had consumed MPI. These effects were abolished in GF mice, indicating that the effects were dependent upon the presence of the microbiota. As revealed by 16S rRNA gene sequence analysis, MPI intake also elicited dramatic changes in the gut microbiome, such as an expansion of taxa belonging to the phylum Bacteroidetes along with a reduced abundance of the Firmicutes.

1. Introduction

The growing prevalence of overweight and obesity significantly accounts for the increasing incidence of a number of cardiometabolic diseases (e.g. type II diabetes mellitus and nonalcoholic fatty liver disease) with serious implications for social welfare. The etiology of obesity is thought to involve a complex interplay between genetic susceptibility, and demographic and lifestyle factors. Dietary modification, in the context of lifestyle changes, is regarded as an essential aspect of the prevention and therapeutics of obesity and related conditions.

Mung bean protein isolate (MPI) is mainly composed of 8S globulins, which exhibit high sequence homology and structural similarities to β-conglycinin, a major component of soybean protein [1]. Many physiologically beneficial effects of β-conglycinin have been reported, including reduced food intake, body weight, body fat, insulin resistance, plasma and liver lipids, plasma glucose, and

Abbreviations: HFD, high fat diet; MPI, mung bean protein isolate; CONV-R, conventionally raised; BA, bile acid; GLP-1, glucagon-like peptide-1; GF, germ free; PYY, peptide YY.

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lipogenesis [2–5]. Similarly, MPI has also been indicated to suppress hepatic lipogenesis in rodent models [6] and reduce fasting plasma glucose and insulin levels in obese adults [7,8]. However, the effects of MPI on mitigating high-fat diet (HFD)-induced obesity and the detailed mechanism underlying these effects remain to be elucidated. Furthermore, soybean protein has been frequently reported to exert hypocholesterolemic effects by binding with and hence improving the fecal excretion of intestinal bile acids (BAs), which drives the hepatic uptake of plasma cholesterols and BA biosynthesis [9], and to elicit alterations in the gut microbiota [10]. BA metabolism is reciprocally modulated by gut microbiota, affecting the biotransformation, reabsorption, and excretion of BAs by catalyzing a range of biochemical reactions [11]. Considering the structural similarities between soybean-derived β-conglycinin and the 8S globulins of MPI, we aimed to (1) investigate if replacing diary protein with MPI in an HFD would attenuate the HFD-induced obesity and (2) determine whether dietary protein-induced changes in the gut microbiota and BA metabolism play a role in the physiological functions of MPI.

2. Materials and methods

2.1. Mice, diet and experimental design

Male C57BL/6 mice were purchased from Japan SLC (Shizuoka, Japan) and maintained under a strict 12 h light/dark cycle and controlled atmospheric conditions (23 °C) with free access to food and water. Mice were acclimated to the laboratory conditions on CLEA Rodent Diet (CE-2, CLEA Japan, Inc.) for 1 week prior to the treatment. Male GF ICR mice were maintained in vinyl isolators under a 12-h light-dark cycle. The CONV-R mice (8-week-old) were randomly assigned to high-fat diet (HFD) (n = 10) or MPI-containing HFD (HFD-MPI) (n = 10) for 4 weeks. The GF mice (8-week-old) were also placed on a HFD (50 kGy irradiated) or HFD-MPI (50 kGy irradiated) for 4 weeks (n = 7–8). The compositions of the diets are given in Supplemental Table 1. Body weight was measured weekly. Fecal droppings were collected at the end of treatment. All mice were then sacrificed under deep isoflurane-induced anesthesia. Liver, cecum, epididymal, perirenal and subcutaneous adipose tissues were harvested and weighted. Blood was collected from the inferior vena cava and plasma was separated following immediate centrifugation (7000 g, 5 min, 4 °C). All tissues, feces and plasma were stored at −80 °C until further processing. All experimental procedures involving mice were in accordance with the guidelines of the Committee on the Ethics of Animal Experiments of the Tokyo University of Agriculture and Technology (permit number: 28–87).

2.2. Plasma biochemical analyses

Plasma glucose was assessed using a portable glucometer with compatible glucose test strips (OneTouch® Ultra®, LifeScan, Milpitas, CA). Plasma cholesterol (LabAssay™ Cholesterol, Wako), non-esterified fatty acids (NEFA) (LabAssay™ NEFA, Wako), insulin [Insulin ELISA KIT (RTU), Shibayagi], peptide YY (PYY) (Mouse/Rat PYY ELISA Kit, Wako), and triglyceride and hepatic triglyceride (LabAssay™ Triglyceride, Wako, Tokyo, Japan) levels were measured using commercial assay kits following manufacturer's instructions. Plasma levels of GLP-1 were determined by enzyme-linked immunosorbent assay (ELISA) [GLP-1 (Active) ELISA KIT, Shibayagi, Gunma, Japan] following treatment with dipeptidyl peptidase IV (DPP-IV) inhibitor (Merck Millipore, Darmstadt, Germany), which prevents the degradation of active GLP-1.

2.3. Hepatic histology

Livers were embedded in OCT compounds (SAKURA Finetek Japan) and sectioned at 7 μm. All slices were stained with hematoxylin and eosin (HE) for microscopic examination.

2.4. Quantification of short-chain fatty acids

Short-chain fatty acids (SCFAs) in feces were determined following a modified protocol as previously described [12].

2.5. Quantification of bile acids

BA standards were purchased from Rikaken (Tokyo, Japan). Lyophilized feces and cecal content were finely ground and well mixed with 0.2 M NaOH (1 mL). The mixtures were then purified from lipids by extraction with hexane (1 mL). The extraction was repeated three times and the aqueous phases along with the solids were combined and incubated at 80 °C for 20 min. The samples were centrifuged (20,000 g, 15 min, 4 °C) and the supernatants were further cleaned up with Oasis PRiME HLB 1 cc cartridges (Waters) that had been conditioned with methanol (1 mL) followed by ultra-pure water (3 mL). The loaded cartridges were washed with ultra-pure water (500 μL) and the analytes were eluted with methanol-acetonitrile (1:1, v/v, 1 mL) for LC-MS analysis. BAs were analyzed on an Acquity UPLC system coupled to a Waters Xevo TQ-S MS (Waters, Tokyo, Japan). The separation was achieved using an Acquity HSS T3 (2.1 3100 mm, 1.7 mm) column (Waters) and gradient elution with 1% acetic acid aqueous solution and acetonitrile-isopropanol (9:1, v/v) as mobile phases. Analytes were detected by multiple reaction monitoring (MRM) in negative ion electrospray mode with source temperature and desolvation temperature set at 150 and 600 °C, respectively. The MRM method parameters were determined by using IntelliStart™ (Waters). Analytes were quantified using external standards. Calibrators were prepared in methanol-acetonitrile (1:1, v/v) over the range of 0.001–1.0 μg/mL, with quality controls at 0.1 and 1.0 μg/mL.

2.6. Analysis of fecal microbiota

Fecal DNA was extracted using FastDNA® SPIN Kit for Feces (MP Biomedicals, Santa Ana, CA). The V4 region of the 16S rRNA gene was amplified using dual-indexed primers. The amplicons were sequenced using an Illumina MiSeq with a MiSeq Reagent kit V3 (Illumina, USA). Paired-end sequencing was carried out using Illumina MiSeq platform. Processing and quality filtering of reads were performed with Quantitative Insights into Microbial Ecology (QIIME) (v1.9.1) and the chimera-free sequences were aligned with the Greengenes database at 97% identity.

To quantify the abundance of Clostridium cluster XIVa, Blautia coccoides JCM1395T were obtained from Japan Collection of Microorganisms of RIKEN BRC and used as standards specifically for the DNA-based determination of fecal bacterial counts. Bacterial DNA was isolated using MonoFas Bacterial Genomic Kit IV (GLC science, Tokyo, Japan). Quantitative real-time-PCR analysis was performed with using SYBR Premix Ex *Taq*II (TaKaRa, Shiga, Japan) on a StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster City, CA). Bacterial primer sequences for Clostridium cluster XIVa are 5′-CGGTACCTGACTAAGAAGC-3’ (forward) and 5′-AGTTTYATTCTTGCGAACG-3’ (reverse) [13].

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