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Flaxseed gum reduces body weight by regulating gut microbiota

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ABSTRACT

The function of flaxseed gum (FG) on blood glucose control makes it possible for body weight loss. This experiment was to investigate the anti-obesity effect of FG and the alteration of gut microbiota. Diets with high, middle and low doses of FG were applied to feed obese rats for 5 weeks. The body weights, serum biochemical indices, body fats, short-chain fatty acid (SCFA) contents and metagenomic information of gut microbiota were analyzed. The results showed the FG diet reduced body weights, body fats and total triacylglycerols, and reshaped rat's cecal microbial compositions. The anti-obesity effect of FG could be achieved by appetite suppression by reducing the relative abundance of Firmicutes and/or the Firmicutes/Bacteroidetes ratio and regulating some specific bacteria. The genus *Clostridium* might be the key one for the degradation of FG and production of SCFAs. SCFAs may not be involved in this weight-loss effect.

1. Introduction

Flax (*Linum usitatissiumum* L.) is a blue flowering annual herb. Flax produces small flat seeds, also named as flaxseed. Human have been consuming flaxseed since ancient times. Flaxseed is a valuable and important edible oil source (Cunnane, & Thompson, 1995). It is cultivated in more than 50 countries including Canada, India, China, United States, and Ethiopia, etc. Flax has been cultivated for fiber as well as for medicinal purposes and as nutritional product (Tolkachev, & Zhuchenko, 2000). Recently, new interest in flaxseed is due to its health benefits. Its functional components include lignans (secoiso-laricir-esinol diglucoside (SDG) being the predominant form), α-linolenic acid, and soluble flaxseed gum (FG, also named as Flax mucilage) (Hall Iii, Tulbek, & Xu, 2006).

FG accounts for 2–10% (w/w) in flaxseed. It is extracted mainly from the layer of flaxseed hull with water. FG can be further separated into neutral (NFG) and acidic (AFG) fraction using ion exchange chromatography (IEC). The neutral fraction constitutes L-arabinose, D-xylose, D-galactose and arabinoxylan; acidic fractioncontains L-rhamnose, L-fucose, L-galactose and D-galactouronic acid (Wanasundara & Shahidi, 1994).

FG *in vitro* exhibited a high bile acid binding capacity and generated high amount of acetate and propionate, which indicates that FG may lower serum cholesterol (Fodje, Chang, & Leterme, 2009; Denis, Barbara, & Dominique, 2007; Theuwissen, & Mensink, 2008). Alzuetaetal (2003) have also reported that flaxseed gum could selectively stimulate the growth of *Lactobacilli in vivo*. These reports indicate that FG is a potential prebiotics. However, there is no further report on FG inducing body weight loss via regulating gut microbiota. This study aimed to investigate the anti-obesity effect of FG *in vivo* and the alteration of gut microbiota.

2. Materials and methods

2.1. Animal, diets and sample preparation

The animal experiments were approved by the Institutional Animal Care and Use Committee of Jinan University. Totally 54 male Sprague Dawley rats (4 weeks of age), were bought from Guangdong Medical Laboratory Animal Center (GDMLAC). Forty-eight of the 54 rats were used to build obese model. In brief, after a 10-days adaption with a standard diet (Li, et al., 2015), rats were used to build obese models by feeding them a high-fat diet for 5 weeks. The 50% of the obese model rats with higher body weights were further randomly divided into four groups (6 rats for each) for the following experiment. The four groups individually caged for another 5-weeks trial with (1) a high-flaxseed gum diet (containing 30% flaxseed gum) (Group FG_H), (2) a medium-flaxseed gum diet (containing 20% flaxseed gum) (Group FG_M), (3) a low-flaxseed gum diet (containing 10% flaxseed gum) (Group FG_L) and the standard diet (Group Con). Six of the 54 rats were fed the standard

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Table 1
Diet recipe (%).

Ingredients	High-fat diet (D12492)	Standard diet (AIN-93 M)	Flaxseed gum diet		
			Low	Medium	High
Flaxseed gum	0.00	0.00	10.00	20.00	30.00
Corn starch	0.00	46.57	36.57	26.57	16.57
Dextrin	16.35	15.50	15.50	15.50	15.50
Casein	26.17	14.00	14.00	14.00	14.00
Sucrose	9.00	10.00	10.00	10.00	10.00
Cellulose	6.54	5.00	5.00	5.00	5.00
Soybean oil	3.27	4.00	4.00	4.00	4.00
Lard	32.06	0.00	0.00	0.00	0.00
Mineral mix AIN-93	4.58	3.50	3.50	3.50	3.50
Vitamin mix AIN-93	1.31	1.00	1.00	1.00	1.00
L-cystine	0.39	0.18	0.18	0.18	0.18
Cholinebitartrate	0.33	0.25	0.25	0.25	0.25

diet during the whole experimental period (Group Blank). The flaxseed gum used here was purchased from Shandong Zhongkai Ltd. Co., China. The standard diet and high-fat diet served in this experiment were AIN-93 M (Reeves, Nielsen, & Fahey, 1993) and D12492 (Gajda, Pellizzon, Ricci, & Ulman, 2007), respectively; three diets of flaxseed gum were made by replacing corn starch in the standard diet with equivalent flaxseed gum. All recipes of diet were listed in Table 1. Cecal content and serum samples from each group were immediately collected and stored in liquid nitrogen after the rats were sacrificed, and then transferred into a -80 °C refrigerator. Abdominal and epididymal fat were individually weighed after animals were dissected.

2.2. MiSeq sequencing of the V3 region of 16S rRNA genes

Bacterial genomic DNA in rats' cecal contents was extracted by using TIANamp Stool DNA kit (Tiangen, Beijing, China) according to manufacturer's instructions. The primers, P1 and P2 (ACTCCTACGGG AGGCAGCAG and GGACTACHVGGGTWTCTAAT) corresponding to positions 338F to 806R in bacterial 16SrRNA gene, were used to amplify the V3-V4 region of each sample by PCR. PCR reactions were performed in triplicate 20 μL mixture solution containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, and 10 ng of template DNA. PCR reactions were run in a thermocycler PCR system (ABI GeneAmp® 9700, USA) using the following programme: 3 min of denaturation at 95 °C followed by 27 cycles of 30 sec at 94 °C, 30 sec at 55 °C and 45 sec at 72 °C, with a final extension at 72 °C for 10 min. Amplicons were extracted from 2% agarose gels and purified with the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) according to the manufacturer's instructions and quantified using QuantiFluor™ -ST (Promega, USA). Purified amplicons were pooled in equimolar amounts and paired-endsequenced (2 × 250) on an IlluminaMiSeq platform according to the standard protocols.

2.3. Bioinformatics of sequencing data

Raw fastq files were demultiplexed, quality-filtered using QIIME (version 1.9.1) with the following criteria: (i) The 300 bp reads were truncated at any site receiving an average quality score < 20 over a 50 bp sliding window, discarding the truncated reads that were shorter than 50 bp. (ii) Exact barcode matching, 2 nucleotide mismatch in primer matching, reads containing ambiguous characters were removed. (iii) Only sequences that overlap longer than 10 bp were assembled according to their overlap sequence. Reads which could not be assembled were discarded.

Operational Taxonomic Units (OTUs) were clustered with 97% similarity cutoff using Usearch (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 (SSU123) 16S rRNA database using confidence threshold of 70% (Amato et al., 2013).

Hierarchical clustering (Hcluster) analysis was performed according to the data matrix of unweighted pair group method with arithmetic mean (UPGMA), and a tree-like structure was built to express and compare the similarity and difference between communities. The distance matrix was calculated by the Bray-Curtis method (Jiang et al., 2013):

$$D_{Bray-Curtis} = 1-2\frac{\sum \min(S_{A,i}, S_{B,i})}{\sum S_{A,i} + \sum S_{B,i}}$$

Among them, $S_{A,i}$ means the amount of sequences in No. i OTU of Sample A; $S_{B,i}$ means the amount of sequences in No. i OTU of Sample B.

2.4. Determination of SCFAs

The concentrations of SCFAs in cecal contents were measured with the method described by Campbell, Fahey, and Wolf (1997).

2.5. Serum biochemical analysis

Blood samples were collected from the tail vein after overnight fasting and centrifuged at 12,000 rpm for 30 min to pellet blood cells, and the serum was stored at −80 °C until further analysis. The analysis of serum total cholesterol (TC), total triacylglycerols (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) was performed via UV–vis spectrophotometer using Konelab 20XTi (Thermo Fisher Scientific, USA).

2.6. Statistical analysis

Results are expressed as mean values and standard deviations. The statistical analysis was performed with SPSS 20.0 software (SPSS Inc., Chicago, Ill., USA). The analysis was conducted by two-tailed *t*-test or one way ANOVA followed by Tukey test. Statistical significance was set at a $P < 0.05$ and highly significance was $P < 0.01$.

3. Results

3.1. Body weight and body weight gains

The body weight gain (BWG) of Group Blank was significantly higher at week 2 ($P < 0.05$) and remained similar since then ($P > 0.05$). Different from Group Blank, the BWGs of Group FG_H, FG_M and FG_L had lower BWG since week 3 ($P < 0.05$). Specifically, Group FG_H and FG_M showed a negative growth of BWG at week 3 while Group FG_L started to loss weight at week 4. The BWG of Group FG_M and FG_L remained negative ($P > 0.05$) while a significant increment of BWG was observed in Group FG_H at week 5 ($P < 0.05$). As for Group Con, its BWG kept increasing from the beginning to the end (Table 2).

3.2. Serum biochemical indices and weights of body fat

The value of total cholesterol (TC) in Group FG_H was significantly lower when compared with that in Con ($P < 0.05$). No significant difference of TC value was found in Group FG_M, FG_L and Blank compared with Group Con (Table 3).

The values of total triacylglycerols (TG) in Group FG_L, FG_M and FG_H were significantly lower than that in Con ($P < 0.05$ or $P < 0.01$), while Group Blank had similar value of TG to that in Group Con ($P > 0.05$) (Table 3).

However, except for the value of low-density lipoprotein cholesterol (LDL-C) in FG_L and Blank, which was significantly higher than that in