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Procyanidin attenuates weight gain and modifies the gut microbiota in high fat diet induced obese mice

Shujuan Zheng[a], Kunlun Huang[a,b], Changhui Zhao[c], Wentao Xu[a,b], Yao Sheng[a,b], Yunbo Luo[a,b], Xiaoyun He[a,b,*]

[a] Beijing Advanced Innovation Center for Food Nutrition and Human Health, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China
[b] Special Food Research Center, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China
[c] Department of Food Quality and Safety, College of Food Science and Engineering, Jilin University, Changchun 130062, China

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ABSTRACT

Procyanidins are a group of flavonoids that can be found in many plants. The objective of the current study was to investigate the anti-obesity effect of procyanidin on high fat diet induced obese C57BL/6J mice. The mice were administrated with 100 mg/kg procyanidin for 12 weeks. The results showed that consumption of procyanidin significantly ameliorated high fat diet induced obesity and its associated risk factors, including reduction of body weight gain, lipid profile improvement and increase of energy expenditure. Moreover, microbial 16S rRNA gene sequencing of the feces demonstrated that procyanidin administration markedly increased intestinal microbiota β diversity and Bacteroidetes quantities but decreased the Firmicutes/Bacteroidetes ratio. We conclude that supplementation with procyanidin prevents obesity and modifies the gut microbiota composition.

1. Introduction

Obesity has become one of the main public health problems in recent decades because it is associated with an increased risk of chronic diseases such as type II diabetes and coronary heart diseases (Haslam & James, 2005; Weyer, Foley, Bogardus, Tataranni, & Pratley, 2000). Obesity is a complicated metabolic disorder featuring an imbalance between calorie intake and expenditure, which is manifested as an increase in adipocyte number (hyperplasia) and size (hypertrophy) (Arner & Spalding, 2010; Hirsch & Batchelor, 1976; Suzuki et al., 2011). Current therapeutic treatments for obesity usually have adverse effects or high rates of secondary failure (Derosa & Maffioli, 2012; Mayer, Hocht, Puyó, & Taira, 2009), and thus investigating potential natural products to prevent obesity has increasingly attracted the attention of the scientific community (Yun, 2010).

In recent years, many natural pigments from vegetables and fruits have shown beneficial health functions (Hsu & Yen, 2008; Kim & Park, 2011; Prior et al., 2009). For example, procyanidins were shown to be more effective than resveratrols or ascorbic acids in scavenging free radicals (Maldonado, Rivero-Cruz, Mata, & Pedraza-Chaverrí, 2005). Procyanidins from the peanut skin decreased the production of inflammatory cytokines, tumor necrosis factor-α, and interleukin-6 in cultured human monocytic THP-1 cells in response to lipopolysaccharides (Tatsuno et al., 2012). Sugiyama et al. (2007) found that the procyanidin was the main component that contributed to the beneficial effect of the apple polyphenol extract on inhibiting TG absorption in mice and humans. However, the evidence of the anti-obesity effects of procyanidin by regulating energy balance is not sufficient.

Dysbiosis in gut microbiota is a critical factor that is closely related to obesity and metabolic syndrome. Reduction of body weight in germ-free mice demonstrated that the gut microbiota affects host energy metabolism and nutrient acquisition (Turnbaugh et al., 2006). For example, the relative abundance of Firmicutes in genetically obese ob/ob mice was increased in comparison with that of the lean (ob/+ and +/+) mice, while the relative abundance of Bacteroidetes was decreased (Ley et al., 2005). In a human study, the subjects consuming the fat- or carbohydrate-restricted low caloric diet had increased abundance of Bacteroidetes compared to their counterparts (Ley, Turnbaugh, Klein, & Gordon, 2006). Therefore, application of the functional foods or bioactive components that can greatly improve the gut microbiota is a promising strategy for preventing and/or treating metabolic diseases.

The majority of dietary procyanidins bypasses the small intestine to reach the microbiome where they are biotransformed into their

* Corresponding author at: Special Food Research Center, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China.
E-mail address: raininghe@163.com (X. He).

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Table 1
Composition of the low fat and high fat diets (%).

	LFD	HFD
Casein	18.958	25.845
Cystine	0.284	0.388
Corn starch	29.859	0.000
Maltodextrin	3.318	16.153
Sucrose	33.177	8.891
Cellulose	4.740	6.461
Bean oil	2.370	3.231
Lard	1.896	31.660
M1002	0.948	1.292
DCP	1.232	1.680
Calcium carbonate	0.521	0.711
Potassium citrate monohydrate	1.564	2.132
V1001	0.948	1.292
Choline bitartrate	0.190	0.258
Edible dye	0.005	0.007
Total	100.010	100.001

metabolites before absorption (Ou & Gu, 2014). Therefore, it's necessary to understand the difference in procyanidin metabolism and biotransformation during intestinal fermentation between the subjects with normal weight and those obese ones. In the present study, we intended to investigate the effects of a type of procyanidin on weight gain, energy expenditure, the gut microbiota and the associated metabolic risk factors in C57BL/6J mice fed with high fat diet (HFD).

2. Materials and methods

2.1. Material

All chemicals and reagents used in the current study were obtained from Sigma Chemical Co. (St. Louis, MO) unless specified otherwise. All solvents and chemicals were of analytical grade. The diets for mice were prepared by Beijing HFK Bioscience co., LID. The detailed composition of the diets were shown in Table 1. The procyanidin (95% purity) in the experiment was purchased from Jianglai Company (Fig. 1).

2.2. Animals and experimental design.

Male C57BL/6J mice (3–4 weeks old) were purchased from Vital River Laboratories (Beijing) and housed (3 animals/cage) under controlled conditions (22 ± 2 °C; 55% ± 10% humidity; 12 h light-dark cycle) in an specific pathogen free (SPF) animal room in the Supervision, Inspection and Testing Center for Genetically Modified Organisms of the Ministry of Agriculture (Beijing, China; license number SYXK Beijing 2015–0045). Animals were acclimated to the environment for 5 days. The mice were then had *ad libitum* access to specified diets: low fat diet (10% calories from fat) or high fat diet (60% calories from fat). Both diets contained proteins that provided 20% of the total calories.

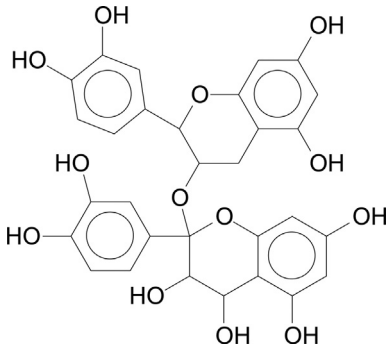

Fig. 1. The structure of procyanidin.

The diet induced obesity (DIO) model was generated for 8 weeks. We marked the mice fed with low fat diet as vehicle control group (LFD, low fat diet, n = 6) and divided the HFD mice into two other groups: negative control group (HFD, 60% high fat diet, n = 6) and HFD + P group (HFD + procyanidin, high fat diet supplemented with procyanidin, n = 6).

All the mice had *ad libitum* access to sterile water throughout the experiment. The mice were fed with the respective diets for additional 12 weeks. In the HFD + P group, mice were given an aqueous solution of procyanidin at 100 mg/kg BW by oral gavage each day for 12 weeks. The mice in LFD group and HFD group received an equal volume of distilled water. Body weights were recorded weekly and the clinical behavior was observed daily. All animal diets were kept at 4 °C for storage, and the thawed food was dispensed to animals every 2–3 days to limit phytochemical degradation in food matrix.

The experimental design was approved by Animal Ethics Committee of China Agricultural University, Beijing, and the approval ID of this study is KY20150017.

2.3. Rectal temperature and energy expenditure

The mice were housed as one animal per cage with free access to food and water. The respiratory quotient (RQ) was measured for 24 h after one day's acclimation in an oxylet system (Panlab; OXYLET). The cold-induced thermogenesis was evaluated in a cold room (4 °C) by an anal temperature measuring instrument (Shenzhen Zhongyidapeng; AT210) and a handheld infrared camera (FLIR T600).

2.4. Preparation of blood samples and biochemical characterization

Blood samples were collected from the orbital venous plexus of mice under anesthesia. The serum was prepared by centrifugation (5000 rpm, 10 min) and stored at −80 °C for further analysis. The mice were killed by decapitation. The abdominal adipose tissues, epididymal adipose tissues and interscapular brown adipose tissues (BAT) were collected and weighted. The interscapular BAT was visible at the level of the shoulder blades and directly picked up when the back skin was removed.

2.5. Gut microbiota analysis

Feces from each mouse were collected on week 12th and stored at −80 °C until analysis. The microbial genomic DNA was extracted (0.2 g) by a previously described method (Guo et al., 2014). The V3 and V4 region of the 16S rRNA was amplified by PCR and sequenced by a MiSeq platform (Illumina, San Diego, USA). After data filtering, the operational taxonomic unit (OTU) - based taxonomy cluster was performed at 97% identity using QIIME pipeline (Caporaso et al., 2010), and the taxonomy annotation was conducted using RDP classifier (Wang, Garrity, Tiedje, & Cole, 2007) and GreenGene database (Desantis et al., 2006). The α diversity and β diversity of each group were calculated based on OTU data using the software PAST version 2.17 (Hammer, 2001). ANOSIM was performed based on OTU data using multivar/one-way-ANOSIM of PAST with the distance measure set as Bray-Curtis. To identify the key genus profiles of the treated and untreated groups, LEfSe analysis was performed using the default parameters (Segata et al., 2011).

2.6. Statistical analysis

Student's *t*-test was used to compare the data between groups by using Microsoft Office Excel 2013. Graphs were plotted using GraphPad Prism 5. All data were presented as means ± SEM. Differences were considered significant when $P < 0.05$.