Effects of chickpea *(Cicer arietinum)* on metabolic dysfunction by modulation of gut microbiota in diet-induced obese mice

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Abstract

The high prevalence of obesity is a major public health issue in modern societies. Visceral obesity has been shown to be closely associated with chronic inflammation, which may result in insulin resistance, type two diabetes, cardiovascular diseases, and fatty liver diseases. Food enters the gastrointestinal (GI) tract and interact with the microbiota therein. Recent studies have shown that an imbalance of the intestinal microbiota contributes to inflammation, obesity, and several metabolic disorders. The health benefits of healthy food have received great attention in recent years. Chickpea *(Cicer arietinum*) is reported to be a healthy ready-to-eat food, which is rich in fiber and bioactive compounds. Therefore, an animal model of metabolic syndrome, mice with a high-fat diet (HFD), was used in this study to examine the effect of chickpea as healthy supplement on modulation of gut microbiota. Our results indicated that chickpea (CP) inhibited hyperlipidemia in HFD-induced obese mice. In addition, the CP supplementation attenuated hepatic lipid accumulation and showed the effect on improving kidney function. By analysis of gut microbiota, the β diversity and NMDS revealed a distinct clustering of microbiota composition for each treatment group. CP mice were more abundant in *Clostridium saccharolyticum* and *Butyricicoccus pullicaecorum*,

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and the genus level of Coprococcus and Butyricicoccus, compared with the HFD group and these bacteria were reported to a depletion in obese individuals and even possessed the anti-inflammatory capabilities. We also used the Sorenson index (Sørensen–Dice index) to measure the similarity between microbiota at different diet, and showed the similarity of gut microbiota composition between each groups varied greatly. These results indicate that CP has promising bioactivity in regulating hyperlipidemia, hepatic steatosis and kidney function by modulating the composition of the gut microbiota.

Keywords: chickpea; hyperglycemia; fatty liver disease; kidney function; gut microbiota

1. Introduction

The high prevalence of obesity is a major public health issue in modern societies. Visceral obesity has been shown to be closely associated with chronic inflammation, which may result in insulin resistance, type two diabetes, cardiovascular diseases, and fatty liver diseases[1]. Food enters the gastrointestinal (GI) tract and interact with the microbiota therein. Recent studies have shown that an imbalance of the intestinal microbiota contributes to inflammation, obesity, and several metabolic disorders[2]. Microbial dysbiosis may compromise the integrity of the colonic barrier and release liposaccharide (LPS) from the surface of Gram-negative bacteria, causing inflammation and disorders. The health benefits of healthy food have received great attention in recent years. Healthy food might reduce the risks of lifestyle related disorders, such as urinary disorders, dysentery and diarrhea, cerebral problems, hypertension, and liver diseases[3]. Therefore, an animal model of metabolic syndrome, mice with a high-fat diet (HFD), was used in this study. After an HFD, the mice show abnormalities in lipid and glucose metabolism, as well as significant dyslipidemia and markers of hepatic steatosis.In recent years, pulses have drawn attention of public as they provide plant-based protein and are

more environment friendly in terms of lower carbon footprint in cultivation [4]. Consumption of pulses is associated with lower risks of cardiovascular disease [5]. The beneficial effects of pulses maybe associated with their nutritions especially polysaccharides and gut microbiota [6]. We determined whether the Chickpea (Cicer arietinum) ameliorates the diet-induced metabolic disorders and whether the microbiota is modulated by the diet treatment.

Here, we used an animal model to investigate the mechanisms responsible for the in vivo effects of Chickpea (Cicer arietinum) on lipid metabolism. In in vivo experiments, high fat diet mice, which show dyslipidemia similar to that seen in patients with obesity, were used as a model to study the pathogenesis and treatment of dyslipidemia.

2. Experimental Section

Animals. One hundred and twenty 5-week-old male C57BL/6J mice were obtained from BioLASCO Taiwan Co, Ltd. All mice were individually housed under a constant temperature (24˚C) and 12-h light/dark cycle at the Animal Center of the National Yang-Ming University, Taipei, Taiwan and subsequently divided randomly into 3 groups (n=8 for each group). One group was fed a normal diet (ND, n = 8). 2 groups were fed with high fat diet for 12 weeks. During the 12 weeks, the 2 groups were fed with high-fat diet group (HFD, n = 8, 45% fat), or HFD with 10% (weight for weight) Chickpea (Cicer arietinum) samples (for each group, n = 8). All experimental diets were prepared every week and stored at 24˚C . At the end of the experimental period, all mice were anesthetized with ether after a 12-h fast. Blood was taken from the inferior vena cava to determine the glucose, plasma lipid, and enzyme concentrations. The liver and adipose tissue were removed, rinsed with physiological saline, weighed, immediately frozen in liquid nitrogen, and stored at -70˚C until analysis. The use of animals for this research was approved by the Animal Research Committee of the National Yang-Ming University and all procedures followed The Guide for the Care and Use of Laboratory Animals (NIH publication, 85-23, revised 1996) and the guidelines of the Animal Welfare Act, Taiwan.

3. Results

3.1. The effect of Chickpea sample treatments on serum triglyceride (TG) and serum total cholesterol (TC) in C57BL/6J mice fed an HFD.

Changes in triglycerides (TG) were shown in Figure 3. The results showed that 10% Chickpea significantly reduced the serum level of TG compared with the HFD group (Figure 1).

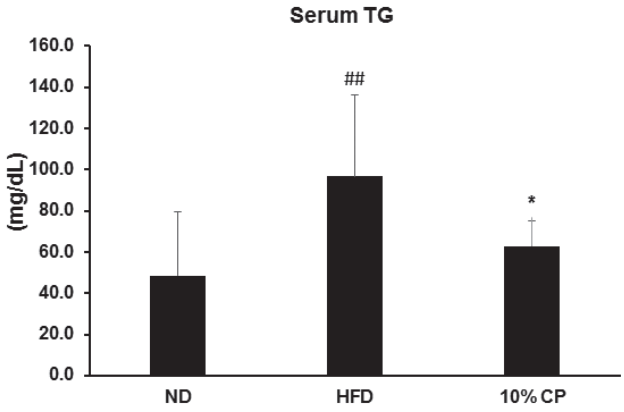

Figure 1.The effect of Chickpea treatments on triglycerides (TG) in C57BL/6J mice fed an HFD. Data are shown as means ± SD. Samples vs. HFD: *P < 0.05; **P < 0.01; ***P <0.001 in decreased level. Samples vs. HFD: #P < 0.05; ##P < 0.01; ###P <0.001 in increased level.

3.2. The effect of Chickpea sample treatments on hepatic triglyceride in C57BL/6J mice fed an HFD.

Changes in hepatic TG was shown in Figure 2. The results showed that 10% Chickpea significantly reduced the hepatic TG compared with the HFD group (Figure 2).

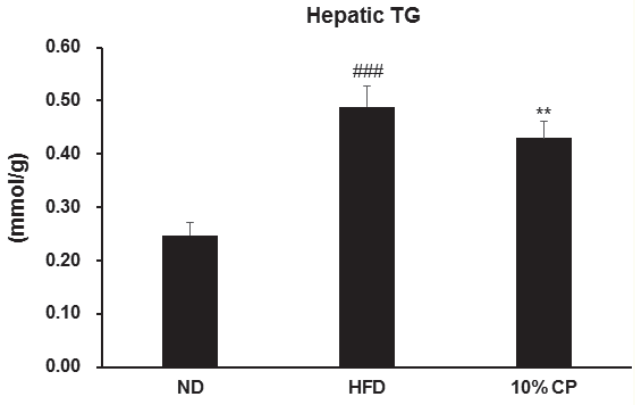

Figure 2. The effect of Chickpea treatments on liver TG and liver cholesterol in C57BL/6J mice fed an HFD. Data are shown as means ± SD. Samples vs. HFD: *P < 0.05; **P < 0.01; ***P <0.001 in decreased level. Samples vs. HFD: #P < 0.05; ##P < 0.01; ###P <0.001 in increased level.

3.3. The histological analysis of liver and adipose tissue in C57BL/6J mice fed an HFD.

The histological analysis of liver tissue was shown in Figure 3. Mice adipose tissue biopsy was observed and shown in Figure 4. The results showed that HFD group contained many lipid droplets in mice liver. 10% Chickpea treatment significantly reduced the lipid droplets compared with the HFD group (Figure 3). On the other hand, the mice receiving dietary 10% Chickpea showed reduction in the EAT adipocyte diameters compared with the HFD group (Figure 4).

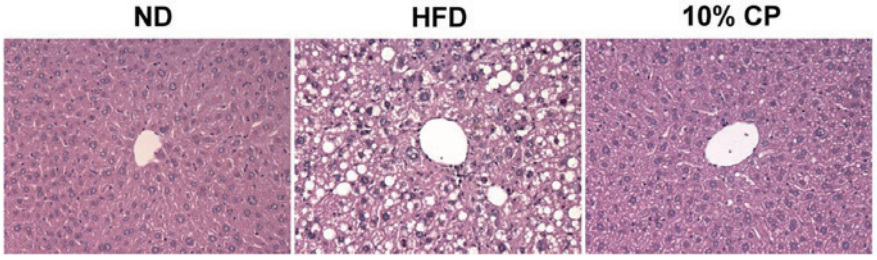

Figure 3. The histological analysis of liver tissue in C57BL/6J mice fed an HFD.

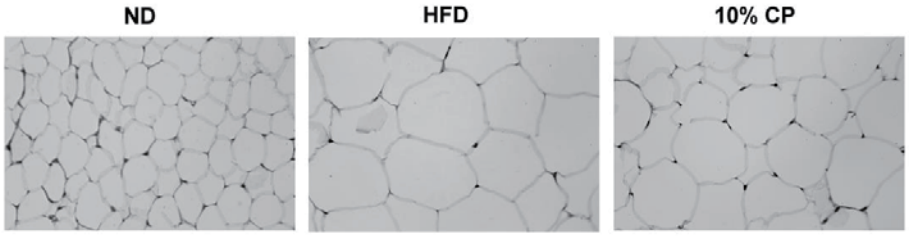

Figure 4. The histological analysis of adipose tissue C57BL/6J mice fed an HFD.

3.4. The effect of 10% Chickpea on alternation of gut microbiota in C57BL/6J mice fed an HFD.

To further confirm the reason why 10% Chickpea inhibited obesity and NAFLD, we used mouse feces to analyze the composition of gut microbiota in mice. The result of heatmap showed the correlations and alternations between the relative abundance of bacterial taxa within mice feces for three different types of diet (Fig. 5). The heatmap analysis showed the amounts of 1 bacterial species were significantly altered by 10% Chickpea respectively in the comparison with HFD mice, indicating that different diets altered the distribution of gut microbiota. The α diversity showed no significant

difference of richness among groups (Fig. 5B), but the β diversity and NMDS analysis revealed a distinct clustering of microbiota composition for each treatment group (Fig. 5C and D). Furthermore, we found the 10% 10% Chickpea have increased abundance sequences representing Coprococcus, saccharolyticum, Butyricicoccus, and pullicaecorum from the LDA analysis. (Fig. 5E). The metastats analysis demonstrated that the similarity of gut microbiota composition between each groups varied greatly (Fig. 5F).

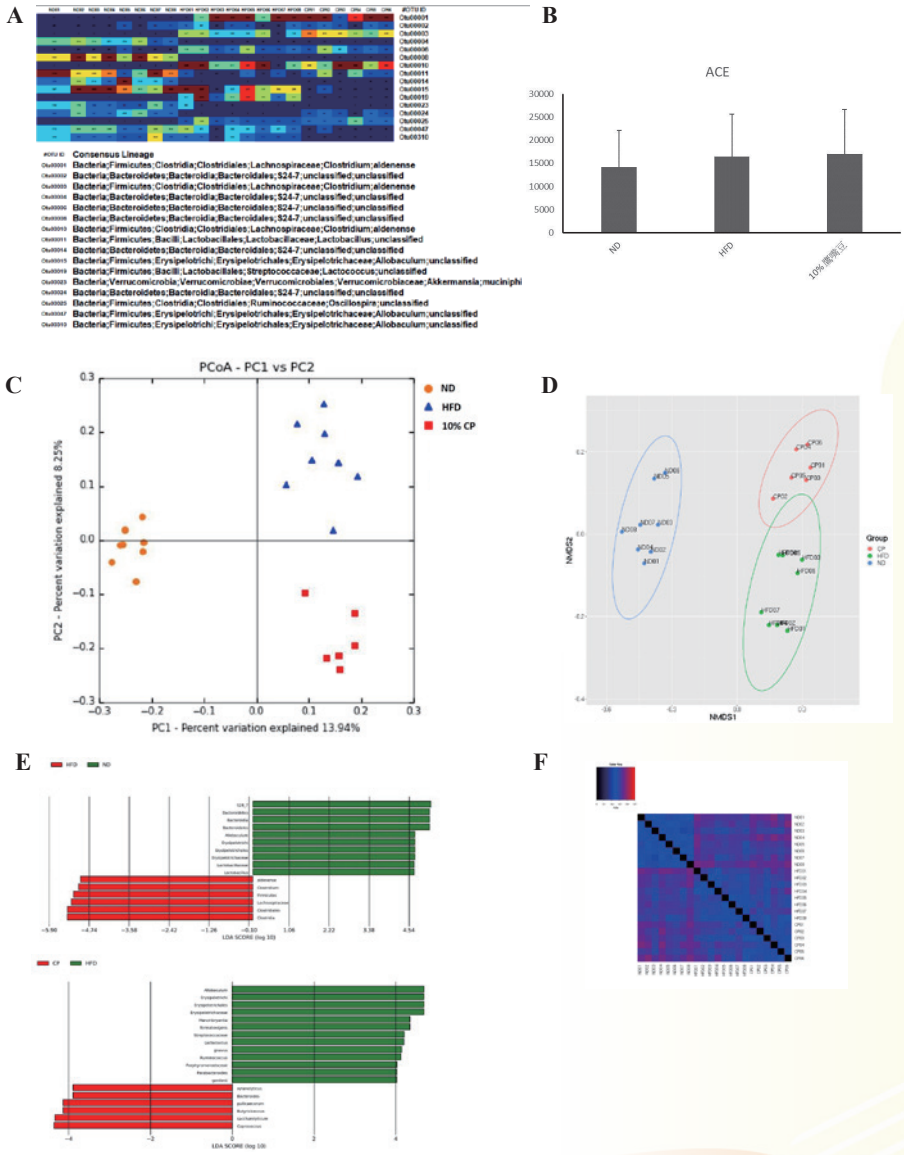

Figure 5. The effect of 10% Chickpea on alternation of gut microbiota in C57BL/6J mice fed an HFD.

4. Discussion

Obesity has been a worldwide issue due to the dramatic global increase in obesity in the recent decades, and higher risk of obese complications than people without obesity. As one of the obese consequences, Nonalcoholic fatty liver disease (NAFLD) is gradually becoming one of the most common chronic liver disease, and as long as it is ignored, it may develop into end-stage of liver disease, such as nonalcoholic steatohepatitis (NASH), liver cirrhosis, and hepatocellular carcinoma (HCC). Currently, no pharmacotherapy or treatment guideline has been approved for the treatment of NAFLD, emphasizing the need for development of novel treatments for NAFLD. However, improvement of lifestyle based on exercise and a balanced diet are considered the cornerstone of NAFLD management. Studies have reported a higher intake of plants not only decreases oxidative stress, relieves inflammation, and reduces lipid accumulation, but also regulates the diversity of gut microbiota, to be significantly associated with lower risk of NAFLD. Therefore, preventing NAFLD through dietary intervention may be an important and feasible strategy.

We found that 10% Chickpea treatment had the obvious inhibitory effect on obesity and NAFLD. The 10% Chickpea treatment showed lower hyperlipidemia. Further, we used mouse feces to analyze the composition of gut microbiota in mice. Although the alpha diversity showed no significant difference among groups, UniFrac-based principal coordinates analysis (PCoA) and Non-metric Multidimensional Scaling (NMDS) revealed a distinct clustering of microbiota composition for each treatment group, which presenting different diets altered the composition of gut microbiota. Using the LDA score analysis demonstrated that significantly differed in relative abundance between ND and HFD, different herbal diet and HFD, respectively. Compared with the HFD group, 10% Chickpea were more abundant in Clostridium saccharolyticum and Butyricicoccus pullicaecorum, and the genus level of Coprococcus and Butyricicoccus, and these bacteria were reported to a depletion in obese individuals and even possessed the anti-inflammatory capabilities. We also used the Sorenson index (Sørensen–Dice index) to measure the similarity between microbiota at different diet,

and showed the similarity of gut microbiota composition between each groups varied greatly.

5. Conclusion

In this study, we studied the detailed mechanisms associated with the anti-obesity action and NAFLD, with Chickpea samples based on the integration of the lipid profile and the phenotype biomarkers from the liver and adipose tissue. Our study was designed to elucidate the metabolic actions after Chickpea samples supplementation in C57BL/6J mice with diet-induced obesity (DIO), in particularly focusing on its role of ameliorating obesity-associated hepatic steatosis. Our data provides novel insights into the effect of Chickpea samples on the interplay between the liver, adipose tissue and gut microbiota.

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