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Dietary pomegranate extract and inulin affect gut microbiome differentially in mice fed an obesogenic diet

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ABSTRACT

Growing evidence suggests that dysbiosis of gut microbiota is associated with pathogenesis of a variety of human diseases. Using dietary intervention to shape the composition and metabolism of the gut microbiota is increasingly recognized. In the present study, we investigated the effects of polysaccharide inulin and polyphenol-rich pomegranate extract (PomX) alone or in combination on the cecal microbiota composition and function in a diet induced obesity mouse model. Male C57BL/6 mice were randomly divided into four experimental groups and consumed either high-fat/high-sucrose [HF/HS (32% energy from fat, 25% energy from sucrose, 17% energy from protein)] diet, HF/HS diet supplemented with PomX (0.25%), or inulin (9%) or PomX and inulin in combination for 4 weeks. In mice fed the PomX-diet the proportion of *Turicibacteraceae* and *Ruminococcaceae* was significantly increased compared to the control HF/HS diet. Supplementation with inulin alone and inulin + PomX combination significantly increased the proportion of *Verrucomicrobiaceae (A. muciniphila)* and decreased *Clostridiaceae*. Only mice fed the inulin diet experienced an increase in serum lipopolysaccharide (LPS) and monocyte chemoattractant protein 1 (MCP-1), which was reversed when feeding the inulin + PomX diet. Feeding the inulin + PomX diet was associated with a significant increase in *Bifidobacteriaceae* and *Rikenellaceae*, which may have contributed to the reduction of endotoxemia markers. Inulin supplementation showed lower species richness of gut microbiota compared to mice fed with HF/HS or HF/HS/PomX, and the reduction was reversed by the addition of PomX. Inulin alone and in combination with PomX had distinct microbial clusters determined by both weighted and unweighted UniFrac Beta-Diversity principle coordinate analysis. A total of 19 KEGG biological pathways were significantly regulated in the gut microbiota with PomX and inulin alone or combined treatment. Inulin significantly enhanced KEGG infectious disease-related pathway associated with increase of serum LPS and MCP-1. No changes in gene expression of ileal proinflammatory cytokine and tight junction genes were observed in mice treated with PomX and inulin. Our results demonstrated that the gut microbiota and their biological pathways were differentially effected by dietary PomX and inulin fed combined or alone. It is therefore very important to consider the interaction among bioactive components of food when evaluating potential prebiotic effects.

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1. Introduction

It is estimated that there are 100 trillion bacteria in the gut of a healthy human, approximately 10 times that of total cells in human body [1]. The gut microbiota of humans and other mammals, considered as an endocrine organ, plays critical roles in biological processes such as nutrient utilization, resistance against infections, maturation of the immune system and host metabolism [2,3]. Disturbance of the delicate balance of gut microbiota is associated with the increased risk of pathogenic processes, such as obesity, diabetes, cancers, allergies, autism, asthma and inflammatory bowel diseases (IBD) [2–4]. Generally, higher level of microbial richness and diversity tend to be associated with a healthy state, while dysbiosis and loss of microbial diversity seems to correlate

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with disease. Today over 25 diseases or syndromes have been linked to an altered intestinal microbiome [5,6]. Therefore, more and more research is interested in restoring the healthy composition of gut microbiota in different scenarios.

Increased research efforts are ongoing in developing gut microbiota-manipulating strategies to improve human health. The most common strategies to manipulate the gut microbiota include: 1) Prebiotics: complex carbohydrates, such as polysaccharides (e.g. inulin), oligosaccharides (e.g. oligofructose), short-chain fructooligosaccharides (scFOS) and *trans*-galactooligosaccharides (TOS) etc [7,8]. Both animal and human studies have demonstrated the health benefits of prebiotics, likely regulated through promoting the growth of good bacteria and improve gut endocrine, barrier and immune functions [9–11]. 2) Probiotics: live microbial supplement which affect the host animal by improving its intestinal microbial balance [8]. The commonly used probiotics belong to the genera *Lactobacilli* and *Bifidobacteria*. 3) Diet: high intake of fruits, vegetable and complex carbohydrates providing fiber and polyphenols; and 4) Fecal microbiota transplantation transfer fecal microbiota from healthy donors into individuals with intestinal dysbiosis due to diseases or antibiotic treatment, and recovering the healthy gut microbiota [6,12].

Dietary fibers and polyphenols are the two most studied dietary intervention strategies in modulating gut microbiome composition and function [13,14]. Previous studies have reported health benefits associated with intakes of dietary fibers or polyphenol enriched foods, such as lowering the risk of atherosclerosis [15,16]. Most studies have investigated the individual effect of dietary fibers and polyphenols on the gut microbiome. Some studies showed that dietary fibers and polyphenols differentially regulate stool output and fat and protein digestibility [16]. However, food is a complex matrix. Phenolic compounds are commonly mixed with different macromolecules including complex carbohydrate fibers. It was reported that dietary fiber has profound effects in regulating the release, digestion, diffusion, transportation as well as bioavailability of polyphenols in GI tract [17]. Therefore, it was the goal of our study to determine the effect of a combination of two classes of nutrients, fiber and polyphenol on changes in the intestinal microbiome and markers of inflammation.

Inulin is widely present in plants. The American inulin intake ranges between 1 and 4 g per day. Reduced incidence of overweight was observed in subjects consuming ≥2.3 g/d of inulin [18]. Inulin is known to have great potential for modifying the gut microbiota. Studies have shown that inulin stimulates the growth of Bifidobacteria and intestinal formation of short chain fatty acids (SCFA) in both human and animals [19–21]. Pomegranate (Pom) is one of the most polyphenol-rich fruits and its health benefits have been extensively studied. The most abundant phenolic compounds in pomegranate extract (PomX) are ellagitannins (ETs), which contribute considerably to the health benefits of Pom [22–25]. ETs are hydrolyzed to ellagic acid (EA) and further catabolized into urolithins in the intestine. ETs/EA have very low bioavailability, and therefore interact extensively with gut bacteria. It is quite common that foods containing both of them are consumed together. In this study we compared the modulation of gut microbiota after 4-weeks dietary intervention with PomX and inulin alone or in combination. To investigate the effect of PomX and inulin on the intestinal microbiota we selected an obesity mouse model of feeding a high fat/high sucrose (Western-style) diet. Previous studies demonstrated that feeding an obesogenic diet leads to changes in the intestinal microbiota, intestinal transport and barrier function (leaky gut) leading to an increase in serum concentrations of bacterial lipopolysaccharide (LPS) and low grade systemic inflammation [26]. We therefore also examined serum LPS and MCP-1 levels in mice fed with HF/HS diet, HF/HS diet supplemented with PomX (0.25%), or inulin (9%) or PomX and inulin in combination. We also investigated the effect of PomX and inulin on blood and tissue cholesterol, and demonstrated additive effects of PomX and inulin in lowering blood and liver cholesterol (J Nutr Biochem, in review).

2. Materials and methods

2.1. Chemical reagents and plant materials

All solvents were HPLC grade from Fisher Scientific (Tustin, CA, USA). Pomegranate extract (PomX) was purchased from Pom Wonderful (Los Angeles, CA, USA), and HPLC analysis of the ellagitannin contents of PomX is shown in Supplementary Table 1 [27], providing ETs at 382 mg/g. High performance (HP) inulin with average degree of polymerization (DP) 23 (DP ≥ 5; ≥99.5%) was purchased from ORAFTI active food ingredients.

2.2. Experimental animal and treatment

All mouse procedures were approved by the UCLA Animal Research Committee in compliance with the Association for Assessment and Accreditation of Laboratory Care (AAA-LAC) International. A total of twenty-four C57BL/6J male mice (strain JAX 000664) were received from The Jackson Laboratory at 5–6 weeks of age. After 1 week of acclimatization, the mice were divided into four groups (n = 6) with similar body weight distribution in each group, and fed either with high fat (HF)/high sucrose (HS) diet (D12266B), or HF/HS diets supplemented 0.25% PomX, 9% Inulin, or 0.25% PomX combined with 9% inulin (Supplementary Table 2). The supplements were mix into the HF/HS diet by Research Diets Inc. To compare the mouse dose to human dose we considered the following facts. The mice weighed between 25 and 30 g during the study period and the average food intake was about 2.5–3 g/day. In human, PomX is well tolerated at 1000 mg/day (16.7 mg/kg) [27] and inulin intake is within the range of 25–35 g/day [28], equals to 205 mg/kg and 7.2 g/kg in mice respectively [29]. In our study, mice were fed 0.25% PomX and/or 9% inulin, providing approximate 248 mg/kg PomX and 8.9 g/kg inulin. which is about 20% higher than the doses reported for human consumption, but lower or in accordance with several other studies [22,30,31]. The food and water was free accessible. Body weights were recorded weekly and food consumption three times per week. After 4 weeks of dietary treatments, mice were euthanized. The total gut content was harvested and weighed and stored at - 80 °C for further analysis.

2.3. Miseq sequencing

DNA from cecum was extracted using the commercial extraction system (QIAamp® Stool DNA Extraction Kit, Qiagen, Valencia, CA). The quality and quantity of the DNA was confirmed using a Nanodrop 1000 (Thermo Fisher Scientific, Wilmington, DE). Sequencing was performed at MR DNA (www.mrdnalab.com, Shallowater, TX, USA) on a Miseq (Illumina, San Diego, CA) following the manufacturer's guidelines. Operational taxonomic units (OTUs) were defined by clustering against GreenGenes 13_8 reference sequences at 3% divergence (97% similarity). Final OTUs were taxonomically classified using Green Genes database. Within community diversity (α-diversity) was calculated using Quantitative Insights Into Microbial Ecology (QIIME) software package [32]. β-diversity was measured by calculating the weighted and unweighted UniFrac distances [33] using QIIME default scripts. And UniFrac PCoA biplot was visualized using EMPEROR. Statistical difference between different time points was analyzed by ANOISM using PRIMER-E software (version 7). Linear discriminant analysis (LDA) coupled

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