Alginate oligosaccharide improves lipid metabolism and inflammation by modulating gut microbiota in high-fat diet fed mice

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Abstract
Alginate oligosaccharides are associated with some beneficial health effects. Gut microbiota is one of the most recently identified factors in the development of several metabolic diseases induced by high-fat diet. Our objective was to evaluate how alginate oligosaccharides impact on high-fat diet-induced features of metabolic disorders and whether this impact is related to modulations in the modulation of the gut microbiota. C57BL/6J mice were fed with chow diet, high-fat diet, or high-fat diet supplemented with alginate oligosaccharides for 10 weeks. Alginate oligosaccharide treatment improved lipid metabolism, such as reducing levels of TG and LDL-C and inhibiting expression of lipogenesis genes. Alginate oligosaccharide administration reduced the levels of fasting blood glucose and increased the levels of serum insulin. Alginate oligosaccharide treatment was found to lower the expression of markers of inflammation, including IL1β and CD11c. Alginate oligosaccharide treatment modulated gut microbial communities and markedly prompted the growth of Akkermansia muciniphila, Lactobacillus reuteri, and Lactobacillus gasseri. Additionally, alginate oligosaccharide intervention significantly increased concentrations of short-chain fatty acids, such as acetic acid, propionic acid, and butyric acid, as well as decreased levels of endotoxin. Alginate oligosaccharides exert beneficial effects via alleviating metabolic metrics induced by high-fat diet, which is associated with increase in A. muciniphila, L. reuteri, and L. gasseri, as well as the release of microbiota-dependent short-chain fatty acids and inhibition of endotoxin levels.

Keywords Alginate oligosaccharides · Gut microbiota · Lipid metabolism · Short-chain fatty acids (SCFAs)

Introduction
Our gut is populated by an immense and diverse population of bacteria, which is known as the microbiome. Diet is not only essential to maintain host health and growth, but it also affects and supports the symbiotic bacterial communities, and so the mutualistic symbiosis relationship was established between gut microbiota and host. Low intake of dietary fibers and the increased amounts of fat, such as western-style diet, altered gut microbiota composition, and functions, affecting host-microbe interactions, which may lead to dysfunctions, contributing to the increase in the development of intestinal bowel disease, allergies, autoimmune disease, and obesity and its related pathologies (Chakraborti 2015; Yin et al. 2018). These diseases might be prevented by dietary fiber, which impacts gut microbial ecology and host physiology.

Alginate, naturally present in brown seaweed cell walls, is composed of β-D-mannuronic acid (M) and α-L-guluronic...
acid (G) residues. Alginate oligosaccharide, depolymerized from alginate, is widely used in the food and pharmaceutical industry due to its water maintenance, gelling, and viscosifying characteristics. Alginate oligosaccharide has received much attention because of its beneficial effects on host, such as reduction of the risks of cardiovascular disease (Terakado et al. 2012; Ueno et al. 2012) and anti-inflammation (Zhou et al. 2015). Moreover, it has been reported that alginate oligosaccharide fermented by pig fecal microbiota in vitro regulated intestinal bacteria of Atlantic salmon (Gupta et al. 2019; Han et al. 2019). The main objective of this study was to investigate the metabolic impacts of alginate oligosaccharide on high-fat diet-fed mice and to evaluate whether its potential benefits are associated with regulation of the gut microbiota. Revealing the mechanism behind the health-promoting effects of alginate oligosaccharide might guide us to exploit them in the prevention of metabolic-related diseases.

Materials and methods

Materials

Alginate oligosaccharides (AO, degree of polymerization = 1–4, Figs. S1 and S2) were purchased from Nanjing Junlan Biotechnology Company (Jiangsu, China). Six-week-old C57BL/6J male mice (n = 30) were purchased from Experimental Animal Breeding Company of Jinan Pengyue (Shandong, China).

Animal treatments

All animal experiments were carried out in Binzhou Medical University and were approved by the animal committee of Binzhou Medical University. Mice were housed under controlled conditions of temperature (22–24 °C) and relative humidity (40–60%) with a 12 h:12 h light:dark cycle. After acclimation for 1 week, the mice were randomly divided into three groups (n = 10 per group) fed with three different diets: normal chow diet (the NCD group), high-fat diet (the HFD group), and high-fat diet with AO (containing 5 g AO per 100 g mice feed, the AO group) for 10 weeks. Feed formulas of NCD (D12450B) and HFD (D12492) are shown in Table S1. Body weight gains were recorded once a week.

Determination of fasting blood glucose

Determination of fasting blood glucose was carried out at the end of week 9 as previously described (Wang and Bao 2017). Mice were fasted for one night (15 h) and blood was collected from the tail vein. Fasting blood glucose was determined using a blood glucometer (Andon Health, Co. Ltd., Tianjin, China).

Collection of samples

Fecal samples were collected at the beginning of week 10. The collected samples were snap-frozen in liquid nitrogen and then transferred to −80 °C refrigerator for subsequent analysis. At the end of the experiment, mice were euthanized by ether induction. As soon as euthanasia was carried out, their blood was collected. Liver, epididymal fat, and abdominal fat from each mouse were separated and weighed. Livers and epididymal fats from three randomly selected mice in each group were harvested and fixed in 4% paraformaldehyde (Biosharp Life Sciences, Anhui, China) at 4 °C for 24 h.

Serum biochemical indicator quantification

Serum samples were separated by centrifuging the blood samples at 1500 rpm for 10 min at 4 °C. Serum biochemical indices including triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were determined by an automatic biochemical analyzer (Roche Diagnostics, cobas c311, Switzerland).

Serum insulin and endotoxin determination

Levels of serum insulin and endotoxin were determined according to the manufacturer’s instructions using ELISA kits purchased from Mbio Company (Shanghai, China) and Yanbixin Company (Beijing, China), respectively.

Histochemical staining

Livers and epididymal fats were first embedded in paraffin and stained with hematoxylin and eosin (HE). The remaining parts of the liver tissue and epididymal fat sections were stained with the Oil Red O working solution. The images were captured by an optical microscope at a magnification of × 400. Adipocyte size was processed by Image J software which was developed by the United States National Institutes of Health.

mRNA levels of lipogenesis genes and pro-inflammatory cytokines

The expression of lipogenesis genes including Fasn, ACC1, SIRT1, Srebpl, and PGC1α and the pro-inflammatory cytokines including IL6, IL1β, CD11c, TNFα, TLR4, and IKKε in epididymal adipose tissues were determined. The total RNA of epididymal adipose tissues was extracted using trizol (TaKaRa, Japan) according to the manufacturer’s protocol. Complementary DNA (cDNA) was prepared using the
Results

AO treatment improved lipid metabolism

Bodyweight gain, liver weight, and epididymal and abdominal fat weight in the HFD group were significantly higher than that of the NCD group (Fig. 1a, b; \( p < 0.05 \)). High-fat diet significantly increased TG, TC, and HDL-C levels compared with the NCD group (Fig. 1c; \( p < 0.05 \)). AO intervention significantly reduced TG and LDL-C concentrations compared with the HFD group (Fig. 1c; \( p < 0.05 \)). RT-PCR analysis further showed that the lipogenesis markers, including Fasn, ACC1, SIRT1, Srebp1, and PGClα, were highly expressed in the HFD group compared with that in the NCD group (Fig. 1d), and these lipogenesis markers were all significantly suppressed by AO treatment (Fig. 1d). Furthermore, concentrations of fasting blood glucose were observed to be significantly higher in the HFD group compared with those in the NCD group (Fig. 1e; \( p < 0.05 \)). AO treatment significantly improved the levels of fasting blood glucose and serum insulin (Fig. 1e, f; \( p < 0.05 \)).

Histology analysis

HE and Oil staining of the liver showed that the hepatocytes were amplified in size, consisted of several fat vacuoles, and obvious inflammatory infiltrates were present around the hepatocyte in the HFD group compared with the NCD group (Fig. 2a, b). AO treatment reduced fat accumulation and inflammation in the liver compared with the HFD group. HE and Oil staining of adipose demonstrated that adipocytes were larger in size in the HFD group than in the NCD group (Fig. 2c, d), and this result was quantified by the analysis of adipocyte size that adipocyte area was increased to 2.78-fold by high-fat diet, and this increase was significantly decreased by AO intervention (Fig. 2e; \( p < 0.05 \)).

AO treatment inhibited inflammation and endotoxin levels

Our results demonstrated that the expressions of inflammatory cytokines including IL6 and CD11c were significantly higher in the HFD group than in the NCD group (Fig. 3a-f; \( p < 0.05 \)). AO treatment significantly reduced the expression of IL1β and CD11c when compared with the HFD group (Fig. 3a-f; \( p < 0.05 \)). Serum endotoxin exhibited higher levels (more than two-fold) in the HFD group than in the NCD group, and AO intervention significantly reduced endotoxin levels (Fig. 3g; \( p < 0.05 \)).

AO treatment enhanced the production of SCFAs

In comparison with the NCD group, the HFD group had about 23%, 61%, 46%, 38%, 19%, and 15% reductions of acetic acid, propionic acid, butyric acid, isobutyric acid, pentanoic acid, isopentanoic acid, and hexanoic acid were measured. SCFAs were determined by gas chromatography coupled with mass spectrometry detection (GCMS-QP2010 Ultra, Shimadzu Corporation, Japan). The DB-FFAP column (1010-67162 InertCap WAX, Shimadzu Corporation, Japan) and hydrogen flame detector FID were used for the measurement. GC oven temperature was initially 50 °C for 1.0 min, raised to 120 °C at 15 °C / min, raised to 170 °C at 5 °C/min, and raised to 240 °C at 15 °C/min with a final hold at this temperature for 3 min. The injector and detector temperatures were 250 °C and 270 °C, respectively.

Statistical analysis

SPSS software (version 24.0; SPSS Inc., IL, USA) was selected to determine the differences. The one-way analysis of variance (ANOVA) was used to compare the difference between the HFD and NCD groups and the AO and HFD groups. \( p < 0.05 \) was regarded as statistically significant for all tests.
propionic acid, butyric acid, isobutyric acid, isopentanoic acid, and hexanoic acid, respectively (Fig. 4). Compared with the HFD group, AO treatment dramatically stimulated acetic acid, propionic acid, butyric acid, isobutyric acid, pentanoic acid, and isopentanoic acid concentrations to 3.31-fold, 19.40-fold, 3.52-fold, 2.93-fold, 2.04-fold, and 2.23-fold, respectively.

AO treatment selectively modulated gut microbiota

Non-metric dimensional scaling (NMDS) analysis demonstrated an obvious separation among the HFD, NCD, and AO groups (Fig. 5a-c). ANOSIM analysis further proved that AO intervention significantly modulated gut microbial communities (Fig. 5c; p = 0.001). The dominant two phyla of gut microbiota in the three groups were Firmicutes and Bacteroides (Fig. 5d). The abundance of Deferrribacteres phylum was increased to 3.15-fold by HFD compared with the NCD group, and AO treatment reversed this increase (Fig. 5d). Elevated levels of Bacteroidaceae, Ruminococcaceae, Lachnospiraceae, Rikenellaceae, and Streptococcaceae families were observed in the HFD group when compared with those in the NCD group (Fig. 5e), and the abundance of the last three families were reduced 83%, 76%, and 96% by AO intervention compared with the HFD group (Fig. 5e). Furthermore, high-fat diet decreased the abundance of the Erysipelotrichaceae family, which was increased 32-fold by AO intervention. The dominant four bacterial species in the AO group were Bacteroides acidifaciens, Lactobacillus gasseri, Lactobacillus reuteri, and Akkermansia
Fig. 2 Effects of AO intervention on changes of histology (magnification, ×400, n = 3). a HE staining of liver; b Oil red O staining of liver; c HE staining of adipose; d Oil red O staining of adipose; and e adipose size. *p < 0.05, **p < 0.01, comparisons between the HFD and NCD groups; *p < 0.05, **p < 0.01, comparisons between the AO and HFD groups. NCD, normal chow diet; HFD, high-fat diet; AO, high-fat diet with alginate oligosaccharides.
Inflammatory cytokines

![Graphs showing cytokine levels](image)

**Fig. 3** AO intervention alleviated inflammation and endotoxemia. **a** IL6; **b** IL1β; **c** CD11c; **d** TNFα; **e** TLR4; **f** IKKε in adipose, and **g** serum endotoxin. Data are represented as mean ± SEM (n = 7). *p < 0.05, **p < 0.01, comparisons between the HFD and NCD groups; *p < 0.05, **p < 0.01, comparisons between the AO and HFD groups. NCD, normal chow diet; HFD, high-fat diet; AO, high-fat diet with alginate oligosaccharides

**Statistical analysis of gut microbiota**

An evaluation of the top thirty-five OTUs of different genera in three groups further revealed that AO intervention significantly modulated specific gut microbiota (Fig. 5f). Furthermore, AO significantly enriched *A. muciniphila, L. reuteri, and L. gasseri* population (Fig. 5g-i).

**Fig. 5** AO intervention enriched *A. muciniphila*.

**Serum endotoxin**

![Graph showing serum endotoxin levels](image)

**Fig. 6** AO intervention restored the gut microbiota disturbed by high-fat diet through enhancing the abundance of specific gut microbiota.

**Spearman correlation analysis**

Correlation analysis between gut microbiota family and TG and LDL-C indicated that unidentified *Melainabacteria, Peptostreptococcaceae, Atopobiaceae, Burkholderiaceae, Akkermansiaceae, Erysipelotrichaceae*, and *Lactobacillaceae* genera were observed in the HFD group than in the other two groups (Fig. 6a), which indicated that AO treatment restored the gut microbiota disturbed by high-fat diet through enhancing the abundance of specific gut microbiota.
demonstrated negative relationships with TG (Fig. 6b; \( p < 0.05 \)) and unidentified Melainabacteria, Burkholderiaceae, Akkermansiaceae, Prevotellaceae, and Bacteroidaceae demonstrated negative relationships with LDL-C (Fig. 6b; \( p < 0.05 \)). And Erysipelotrichaceae and Lactobacillaceae demonstrated negative relationships with fasting blood glucose (Fig. 6b; \( p < 0.05 \)). Moreover, Bifidobacteriaceae, Peptostreptococcaceae, Eggerthellaceae, Erysipelotrichaceae, and Lactobacillaceae had negative correlations with endotoxin (Fig. 6b; \( p < 0.05 \)). Akkermansiaceae, Prevotellaceae, and Bacteroidaceae had significantly positive correlations with at least three kinds of SCFAs (Fig. 6b; \( p < 0.05 \)).

Moreover, correlation analysis indicated that Parasutterella, Akkermansia, Faecalibaculum, Dubosiella, and Lactobacillus were all negatively associated with TG (Fig. 6c; \( p < 0.05 \)), and Parasutterella, Akkermansia, Alloprevotella, and Bacteroides had negative correlations with LDL-C (Fig. 6c; \( p < 0.05 \)). Additionally, Anaerotruncus, Tyzzerella, Acetatifactor, and Intestinimonas demonstrated positive relationships with fasting blood glucose or negative relationships with serum insulin (Fig. 6c; \( p < 0.05 \)). And Parasutterella and Lactobacillus demonstrated negative relationships with fasting blood glucose (Fig. 6c; \( p < 0.05 \)). And Anaerotruncus, Odoribacter, Gemella, Oscillibacter, Blautia, and Mucispirillum had positive
relationships with endotoxin (Fig. 6c; \( p < 0.05 \)). Moreover, Faecalibaculum, Dubosiella, and Lactobacillus had negative correlations with endotoxin. Correlation analysis between gut microbiota genus and SCFAs indicated that Parasutterella, Akkermansia, Alloprevotella, Allobaculum, and Bacteroides had significantly positive correlations with at least three kinds of SCFAs (Fig. 6c; \( p < 0.05 \)).

**Functional prediction of gut microbiota**

Insights into the AO impact on metabolic pathways of intestinal microbiota were carried out through the Tax4Fun prediction, which suggested that AO intervention modulated microbial metabolism. Metabolic pathways of replication and repair, membrane transport, translation, nucleotide metabolism, and glycans biosynthesis and metabolism were significantly suppressed by a high-fat diet, which are essential in life activities (Fig. 7a; \( p < 0.05 \)). AO intervention significantly upregulated the microbial carbohydrate metabolism, replication and repair, membrane transport, translation, nucleotide metabolism, glycans biosynthesis and metabolism, folding, sorting, and degradation, as well as enzyme families, whereas AO intervention significantly inhibited the microbial amino acid metabolism, energy metabolism, signal transduction, cell motility, and cardiovascular diseases (Fig. 7b; \( p < 0.05 \)).

**Discussion**

Obesity phenotype, including body weight gain, weight of liver, epididymal fat, and abdominal fat, were exacerbated...
by high-fat diet, and high-fat diet also aggravated metabolic metrics, such as lipid metabolism. There was a trend for body weight gain and liver weight to be decreased with AO intake. And AO intervention significantly improved lipid metabolism, such as reducing levels of TG and LDL-C (Fig. 1c). Importantly, AO intervention inhibited the expression of fatty acid synthase-related genes (Fig. 1d). And the expression of Fasn gene, encoding a core enzyme that is critical for de

**Fig. 6** Statistical and correlation analysis of gut microbiota. a Statistical analysis of top thirty-five different genera in three groups; b Spearman correlations between metabolic traits and bacterial families; and c Spearman correlations between metabolic traits and bacterial genera. \( n = 10 \) each group. Z score value represents the normalized abundance. \( r \) value represents correlation coefficient. * \( p < 0.05 \), ** \( p < 0.01 \). TG, triacylglycerols; LDL-C, low-density lipoprotein cholesterol; FBG, fasting blood glucose; NCD, normal chow diet; HFD, high-fat diet; AO, high-fat diet with alginate oligosaccharides
novo lipogenesis pathway and cellular substrate energy metabolism including the synthesis of long-chain fatty acids from acetyl-CoA, malonyl-CoA, and dihydronicotinamide adenine dinucleotide phosphate (NADPH), was less than one-sixth of that in the HFD group after AO treatment. Furthermore, it has been reported that Fasn gene is evidently and actively involved in the energy homeostasis process and its suppression was linked to a decrease of body and adipose tissue weight, as well as resolution of fatty liver in diet-induced obese mice (Kumar et al. 2002; Thupari et al. 2002). Therefore, the decrease in expressions of fatty acid synthesis-associated genes in this study could explain in part the beneficial effects on lipid metabolism after AO treatment.

High-fat diet is closely related to inflammation in adipose tissue, and our results showed that high-fat diet-induced inflammation based on the expression of pro-inflammatory cytokines and histological analysis. And upregulation of some cytokines, such as IL6 and CD11c, accelerated the progression of a variety of diseases, such as metabolic syndrome and some kinds of cancers (Cox et al. 2015). Compared with the HFD group, AO treatment exhibited reduced levels of IL6, IL1β, CD11c, and TNFα by 48%, 52%, 67%, and 51%, respectively (Fig. 3a-f), and this anti-inflammatory effect was confirmed by histological analysis (Fig. 2). Among these four cytokines, IL6 produced from white adipose tissues, skeletal muscle, and liver is a pleiotropic and a central player in the inflammation regulation and host defense mechanisms (Makki et al. 2013). Inflammatory inhibition response accounts for important roles in reducing the risks of metabolic disorders, such as the etiology of insulin resistance (Rogero and Calder 2018).

A. muciniphila was decreased by high-fat diet in our results, which was consistent with the findings in genetically ob/ob and diet-induced obese mice (Mehrpouya-Bahrami et al. 2017). AO intervention significantly reshaped the bacterial communities. The beneficial effects of AO on metabolic metrics might be correlated with a robust regulation in the growth of A. muciniphila, which was increased to 275.27-fold after AO intake (Fig. 5g). Everard et al. (2011) also showed that oligofructose dramatically increased the abundance of A. muciniphila in genetically obese mice. Notably, A. muciniphila showed a negative correlation with TG and LDL-C, as well as a positive correlation with serum insulin
Reports documenting potential roles for *A. muciniphila* in metabolic syndrome are on the rise. For example, the improved metabolic features of metformin, a therapeutic drug for type II diabetes, have been also associated with an increase in *A. muciniphila* population (de la Cuesta-Zuluaga et al. 2017). Moreover, *Akkermansia* spp. linked to the prevention of diet-induced obesity, liver steatosis, and insulin resistance in mice supplemented with a polyphenol-rich cranberry extract (Anhê et al. 2015). Additionally, *A. muciniphila* was observed to be negatively associated with TC and LDL-C levels in postmenopausal women with obesity (Chen et al. 2018). Although we have not directly established the causal association between *A. muciniphila* and the improved effects on lipid metabolism and inflammation in the present study, there has been an evidence that *A. muciniphila* intake improves high-fat diet–induced metabolic disorders, including fat mass gain, fasting hyperglycemia, metabolic endotoxemia, and inflammation in mice (Everard et al. 2013). Furthermore, oral administration of *A. muciniphila* reduces the damage of lipotoxicity, oxidative stress, and inflammation in diabetic rats (Zhang et al. 2018). More recently, *A. muciniphila* supplementation improves several metabolic parameters in overweight and obese humans (Depommier et al. 2019).

High-fat diet led to dysbiosis, as suggested by the lower abundance of *Lactobacillus* (Fig. 5e). In line with this, *L. reuteri* and *L. gasseri* proportions were significantly reduced by high-fat diet (Fig. 5h, i). *L. reuteri* and *L. gasseri* were increased by AO intervention and they exhibited multi-correlations with metabolic traits, including negative correlations with TG, fasting blood glucose, and serum endotoxin (Table S3). The casual relationship between *Lactobacillus* population and the improved effects of metabolic syndrome...
was documented by previous studies in mice. Oral *L. reuteri* and *L. gasseri* have been reported to reverse high-fat diet-induced metabolic disorders in murine models (Fak and Backhed 2012; Shi et al. 2013). In the randomized controlled trials, *L. reuteri* and *L. gasseri* intake was reported to increase insulin secretion in glucose-tolerant humans (Simon et al. 2015) and improve the accumulation of abdominal adiposity in adults with obese tendencies (Kadooka et al. 2010), respectively. Furthermore, *L. gasseri* reduced body weight gain and improved glucose tolerance in rats via prompting energy expenditure (Juarez et al. 2013). And *L. reuteri* decreased inflammatory and oxidative damage in mice with endotoxic shock (Juarez et al. 2013). Additionally, *L. reuteri* suppressed TNFα-induced IL8 expression in human intestinal epithelial cells (Ma et al. 2004). Moreover, *L. gasseri* inhibited the ratio of inflammation-type macrophages to anti-inflammatory ones in adipose tissues (Kawano et al. 2016).

SCFAs, the major products of dietary fiber fermentation, act as communication molecules between gut microbiota and host metabolism. Alginate oligosaccharide fermented by pig fecal microbiota in vitro was reported to increase SCFA levels, especially butyric acid, and alter the microbiota compositions (Han et al. 2019). Moreover, the beneficial properties of oligosaccharides are dependent on the release of SCFAs by microbial fermentation (Long et al. 2018; Yan et al. 2019), and mannan oligosaccharide (100 mg kg⁻¹ day⁻¹) was reported to increase serum butyrate to more than six-fold compared with the HFD group (Yan et al. 2019). Potential roles of SCFAs in inflammation modulation have been documented in reports. For example, butyrate alleviated inflammation in fish with parasite infection (Piazzon et al. 2017), and it might also execute beneficial effects on LPS-induced intestinal inflammation in pig (Melo et al. 2016). Furthermore, our previous study indicated that oral butyrate suppressed high-fat diet-induced inflammation (Zhai et al. 2019). The beneficial roles of SCFAs in preventing vascular inflammation and relevant diseases by activation of G protein-coupled receptor 41/43 (GPR41/43) and inhibition of histone deacetylases (HDACs) (Liu et al. 2018). Moreover, propionic acid has been reported to reduce fatty acid levels in the liver and plasma, decreases food intake, and increases glucose uptake (Al-Lahham et al. 2010). Furthermore, dietary butyrate supplementation has been shown to reduce total body weight, liver weight, and epididymal fat pad weight in mice fed with high-fat diet, and it also decreased serum insulin, leptin, and fasting glucose concentrations (McNabney and Henagan 2017). Butyrate was reported to inhibit high-fat diet-induced obesity through the activation of adiponectin-mediated pathway and AMPK in liver and muscle tissue (den Besten et al. 2015; Hong et al. 2016). In line with the raise of SCFA levels after AO treatment in our study, SCFA-producing bacteria, such as *Akkermansia*, *Alloprevotella*, *Parasutterella*, and *Allobaculum*, were also enriched (Han et al. 2018; Shimizu et al. 2018; Wang et al. 2018; Yin et al. 2018).

Endotoxin, also namely lipopolysaccharide (LPS), plays critical roles in the onset and progression of inflammation and related metabolic diseases (Anhê et al. 2015). Injection of LPS for 4 weeks induced low-grade inflammation, insulin resistance, and weight gain and LPS might directly trigger chronic inflammation in obese individuals (Schachter et al. 2018). LPS might stimulate the expression of pro-inflammatory cytokines through activating Toll-like receptor 4 (TLR 4) via the nuclear factor-kappa B (NF-kB) pathway. AO intervention significantly reduced endotoxin levels; this could be explained in part by the anti-inflammation effects of AO (Fig. 8). Notably, LPS-containing *Desulfovibrionaceae* family showed positive correlations with endotoxin (Fig. 6b), which was also decreased by AO treatment (Fig. 5e).

In conclusion, we found that AO treatment protects from high-fat diet-induced metabolic disorders and significantly modulates gut microbial communities. Our study further suggests that the ability of AO intervention to raise the relative proportion of *A. muciniphila*, *L. reuteri*, and *L. gasseri* is
playing a key role in the protective effect. Importantly, the beneficial properties of AO are dependent on the release of microbiota-dependent SCFAs and the decrease of endotoxin. Therefore, our study suggests that AO intake may be a novel strategy in the prevention of metabolic syndrome.

**Author contributions**  YW, LL, and SQ designed the research. YW and JY were responsible for the execution of the study, data collection, and analysis. YW, LL, and SQ interpreted the data. YW, LL, and CY played major roles in drafting, writing, and revising this manuscript. All authors have known and agreed to this final manuscript.

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**Compliance with ethical standards**

**Conflict of interest**  The authors declare that they have no conflict of interest.

**Ethical approval**  Permissions for this animal procedure had been requested and approved from the Institutional Animal Care and Use Committee of Binzhou Medical University.

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