Xu *et al. Applied Biological Chemistry* (2024) 67:52
https://doi.org/10.1186/s13765-024-00905-7

KSABC
The Korean Society for Applied Biological Chemistry

ARTICLE Open Access

Akkermansia muciniphila postbiotic administration mitigates choline-induced plasma Trimethylamine-N-Oxide production in mice

Hongjuan Xu[1], Xiaoyun Bian[1*], Hongxing Wang[1], Lin Huang[1] and Xiaoxi Chen[1]

Abstract

Background Trimethylamine-N-Oxide (TMAO) is believed to be linked to increased likelihood of cardiovascular disease. While probiotics have shown limited effectiveness in reducing TMAO levels, the potential of postbiotics remains underexplored. This study aimed to evaluate the impact of *Akkermansia muciniphila* (*A. muciniphila*) postbiotic administration on choline-induced TMAO production in mice by modifying the gut microbiota.

Methods Female C57BL/6J mice were divided into six groups, including a control group, high-choline diet group, live *A. muciniphila* probiotic group, pasteurized *A. muciniphila* postbiotic group, sodium butyrate group, and sodium propionate group. Various measurements and analyses were conducted, including TMAO and TMA levels in serum, urine, and cecal contents, as well as the expression of FXR and FMO3 in liver tissues. Additionally, metabolic parameters, body weight, serum lipid profile, hepatic protein expression (FMO3, FXR, CutC, and CutD), and gut microbiota composition were assessed.

Results Administration of *A. muciniphila* postbiotic significantly reduced choline-induced plasma TMAO levels in mice. Furthermore, improvements in serum lipid profiles and liver enzyme levels suggested potential enhancements in lipid metabolism and liver function. The study also observed modulation of specific proteins related to TMAO production and metabolism, including CutC and CutD.

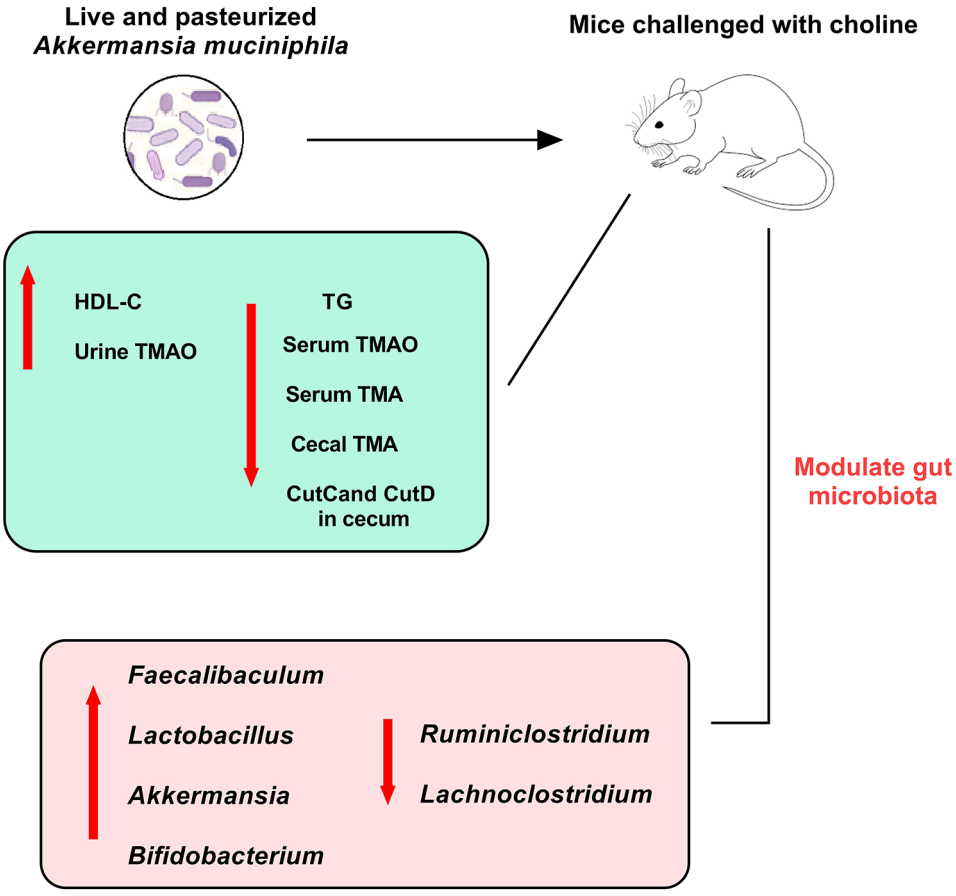
Conclusion The findings highlight the potential of *A. muciniphila* postbiotics as a dietary strategy for mitigating cardiovascular disease risk by modulating the gut-TMAO axis. Postbiotics, particularly *A. muciniphila*, offer advantages over probiotics and warrant further investigation for their therapeutic applications in gastrointestinal and metabolic disorders.

Keywords *Akkermansia muciniphila*, Short-chain fatty acids, Choline, Gut microbiota, Postbiotics

*Correspondence:
Xiaoyun Bian
jsbxy126@163.com

Full list of author information is available at the end of the article

Graphical Abstract



Introduction

The complex community of microorganisms inhabiting the human digestive system, usually referred to as the gut microbiota, plays a crucial role in preserving overall well-being and modulating diverse facets of human physiology, spanning metabolism, immunity, and susceptibility to various ailments [1]. Among the variety of metabolites produced by this complex microbial community, Trimethylamine-N-oxide (TMAO) has appeared as an important contributor in the gut-heart axis, linking dietary habits and intestinal microbial composition to cardiovascular disease (CVD) risk [2, 3]. The TMAO, a metabolite derived from dietary components like carnitine, phosphatidylcholine, and choline by gut microbes, can be further converted by the liver enzyme flavin-containing monooxygenase 3 (FMO3) into a compound that promotes inflammation and atherosclerosis [4]. Previous studies identified a gene cluster in human gut bacteria, named the "cut" cluster, enabling anaerobic choline metabolism. This cluster encodes two key proteins: CutC, a catalytic enzyme, and CutD, a regulatory polypeptide, both originating from the cutC/D genes. These genes are widely present across diverse gut bacteria and are linked to their ability to utilize choline and convert it to trimethylamine (TMA) through an enzymatic process called choline TMA-lyase activity [5–7]. Given the strong association between TMAO levels and CVD risk, researchers have explored various strategies to modulate gut microbiota composition and thereby lower TMAO production.

Probiotics, well-defined as beneficial live microorganisms that can exert beneficial effects when consumed in adequate amounts, have been extensively studied in this regard.8 Extensive research has explored probiotics, defined as beneficial live microorganisms with health benefits when consumed in adequate amounts, to alter gut microbiota composition, reduce TMAO-producing bacteria, and lower plasma TMAO levels [4, 6]. However, limitations like continuous consumption, potential side effects, and variable efficacy between strains hinder their effectiveness [8].

In recent years, Postbiotics, defined as non-viable microbial products or components of microbial cells, have emerged as a promising alternative to probiotics for modulating gut microbiota and promoting health and

previous studies conclude that *Lactobacillus* and *Bifidobacterium* were the furthermost proficient strains in decreasing TMAO level in both humans and animals [9, 10]. Unlike probiotics, Postbiotics offer the advantages of stability, extended shelf life, and reduced risk of side effects. Studies have shown that Postbiotics can effectively modulate gut microbiota composition, enhance barrier function, and modulate host immune responses, suggesting their potential as therapeutic agents for various gastrointestinal and metabolic disorders [11]. Some studies testing the effect of Postbiotics in reducing TMAO levels [10, 11]. Recent research has point out that *Akkermansia muciniphila* (*A. muciniphila*) holds great ability as a probiotic agent [12]. *A. muciniphila* is a Gram-negative, obligatory anaerobic, oval-shaped bacterium that breaks down mucus. It produces propionate and acetate as byproducts, which serve as food for other bacteria and the host [13].

This study aimed to examine whether A. muciniphila Postbiotics could reduce choline-induced plasma TMAO production by modulating gut microbiota in mice. We hypothesized that A. muciniphila would reduce the production of TMA and subsequently lower plasma TMAO levels, thereby evaluating its potential as a dietary strategy for mitigating CVD risk.

Materials and methods

Bacterial culture conditions and postbiotic preparation

Akkermansia muciniphila (ATCC BAA-835) obtained from a commercial source was cultured under anaerobic conditions in brain heart infusion broth supplemented with specific additives and 0.05% mucin type III at 37 °C. The cultures were centrifuged at 16,000 × g for 10 min, with the resulting pellet washed three times with phosphate-buffered saline (PBS). To prepare the *A. muciniphila* postbiotic, the live bacteria were pasteurized at 70 °C for 30 min. Subsequently, both live and pasteurized bacteria were suspended in PBS under a low-oxygen environment, adjusting the concentration to 1×10^9 colony-forming units (CFU)/mL. Fresh bacterial suspensions, live or pasteurized, were prepared daily for mouse treatment.

Experimental animals

Female C57BL/6J mice (6–7 weeks old, 25–30 g) were obtained from SLAC Inc (Shanghai, China). Mice were housed in pairs per standard cage with free access to food and water under controlled conditions: 12-hour light/dark cycle, 25 °C±2 °C temperature, and 50% ± 5% humidity.

The study was conducted in accordance with the Declaration of Helsinki, and the animal study protocol was approved by the Ethics Committee of Affiliated Matern & Child Care Hospital of Nantong University (protocol code: MCCH-2023-052). After an adaptation period of one week, thirty-six mice were randomly divided into six groups (control, high-choline diet (HCD), probiotic (live *A. muciniphila*), postbiotic (pasteurized *A. muciniphila)*, Butyrate, and Propionate) ($n=6$). To gain deeper insights into the potential of *A. muciniphila* postbiotic and other short-chain fatty acids (SCFAs), we included butyrate and propionate groups in our study. We aimed to evaluate whether SCFA supplementation, including these two key metabolites, could influence the TMA-TMAO pathway. By incorporating these groups, we sought to understand the combined effects on TMAO production and gut microbiota modulation. By employing this comprehensive approach, we hope to first unveil novel strategies for preventing and managing various health conditions, and secondly to foster a clear understanding of our findings and their potential applications in human health promotion.

The mice of control group received standard chow diet and distilled water orally. The second-to-sixth-groups received a high-choline diet (with 1.3% choline chloride), as well as 0.2 ml of distilled water, probiotic (1×10^9 cfu/mL), postbiotic (1×10^9 cfu/mL), butyrate (sodium butyrate, 400 mg/kg), and propionate (sodium propionate, 200 mmol/L), respectively. In addition to drinking water, each mouse in each treatment group received 0.2 mL of a probiotic, postbiotic, or a combination of butyrate and propionate daily by oral gavage throughout the experiment. The body weight of each mouse was measured weekly during the animal experiment. All treatments were maintained daily for twenty-eight consecutive days [14].

Collection of biological samples

At 28th days of experiment, the animals were placed separately in metabolic cages and the 24 hours' urine was collected. Mice were allowed to eat and drink freely during urine collection. After urine collection, after 8 h fasting, blood samples were obtained by direct cardiac puncture under ether anesthesia. The urine and blood samples were centrifuged at 4000 rpm for 10 min at 4 °C, the supernatant was separated, and stored at −80 °C for biochemical analysis. After scarifying of animals by diethyl ether, the liver was removed, suspended in PBS, and then stored at −80 °C for protein expression analysis. Immediately upon collection, the intestinal contents from the cecum were frozen and stored at a temperature of −80 °C until needed.

Measurement of serum lipids and liver enzymes in mice

The serum levels of triglycerides (TG), total cholesterol (TC), HDL cholesterol, LDL cholesterol alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were determined according to commercial kits instructions.

Measurement of TMAO and TMA levels in serum, urine, and cecal contents

For protein precipitation, serum or urine samples were mixed with acetonitrile (with a ratio of 1: 4). The samples were then spiked with 2.5 μM of d9-TMAO (Sigma, USA). Afterward, the mixtures were fully mixed and incubated at -80 °C for 2 h, centrifuged at 12,000 × g at 4 °C for 15 min, and the supernatant stored at -80 °C. For measuring TMAO and TMA in cecum, the cecal content placid in germ-free pipes, spiked with 2.5 μM of d9-TMAO, and homogenized with a mixture of water, methanol, and acetonitrile (20: 40: 40, v/v/v). Next, the samples were centrifuged as above mentioned, and stored at -80 °C. Next, the TMAO and TMA levels in serum, urine, and cecal content were determined using UHPLC-MS/MS, following the method described by Ramireddy et al. [14] Briefly, 1 μL of each sample was injected into the Waters ACQUITY UPLC system, and then analyzed using the Waters Xevo TQ Mass Spec. The positive ion electrospray ionization (ESI) mode was employed for the analysis. The precursor product ion pairs operated in multiple reaction monitoring (MRM) mode were: m/z146→118 for TMA and d9TMA, as well as m/z 76→59 for TMAO and d9TMAO, respectively.

Expression of FXR and FMO3 in liver tissues

The protein levels of liver Farnesoid X receptor (FXR) (EKU04022-96T; Biomatik USA, LLC, Wilmington, DE) and flavin-containing monooxygenase 3 (FMO3) were determined using commercial ELISA kits obtained from Mybiosource Inc. (San Diego, CA, USA).

Expression of CutC and CutD proteins in cecal content

The protein levels of cecum CutC (choline trimethylamine-lyase) which produces TMA and CutD (a radical enzyme that serves as an activate for choline TMA-lyase) were determined using commercial ELISA kits obtained from Mosak Biotechnology Co., Ltd. (Wuhan, China).

Measurement of FMO3 activity in liver tissues

The activity of FMO3 in liver tissue was measured using a previously established method. In brief, liver samples were homogenized in RIPA buffer containing 1 mM PMSF to inhibit protease activity. The protein concentration was then determined using a commercially available BCA assay following the manufacturer's instructions. An equal amount of protein homogenate (1 mg) was added to a reaction mixture containing 100 μM NADPH, 100 μM deuterated TMA (d9-TMA), and 10 mM HEPES buffer (pH 7.4). The final reaction volume was 250 μL. Following incubation at 37 °C for 8 h, the reaction was stopped with 0.2 N formic acid. The mixture was then filtered through a 0.22 μm microfilter, transferred to an injection tube, with the amount of d9-TMAO formed quantified using ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (uHPLC-MS/MS).

Metagenomic DNA extraction and gut microbiota analysis

To quantify the composition of gut microbiota community in fecal samples, real-time quantitative PCR (qPCR) was done according to an earlier described procedure. Metagenomic DNA was extracted from the cecal samples of mice using a FastDNA® SPIN kit for feces and the FastPrep® system (MP Biomedicals, Santa Ana, CA, USA). The extracted total DNA was used as template to amplify the V4 domain of the bacterial 16 S rRNA gene (16 S V4). The amplicon libraries (a mixed of purified PCR products) were used for sequencing by an Illumina MiSeq platform (New England Biolabs, Ipswich, MA, USA). The sequence data of 16 S rDNA were analyzed by the free online Platform of Majorbio I-Sanger Cloud (https://www.i-sanger.com).

Statistical analysis

All data are expressed as Mean ± SD. Statistical analysis was implemented with one-way ANOVA with Tukey post-hoc test using SPSS 22.0 (Chicago, IL, USA). P values < 0.05 were considered to show significant differences for all comparisons. Graphs were drawn using GraphPad Prism v10.0.0, (GraphPad software, Inc, La Jolla, California, USA).

Results

Effect of live or pasteurized A. muciniphila and SCFAs supplementation on body weight in mice challenged with choline

To explore the effect of different supplementations on the body weight of mice tested with choline, the weight of each mouse was measured weekly during the animal experiment, with the ANOVA test utilized to compare the weights between groups on a particular day. The results revealed that the body weight of mice greater than before steadily during the study and HCD, probiotic, postbiotic, butyrate, and propionate groups group had growth curves comparable to that of the control group. As displayed in Fig. 1., live or pasteurized *A. muciniphila* and SCFAs (butyrate and propionate) administration had no statically significant effect on the body weight trend ($p = 0.156$).

Effect of A. muciniphila and SCFAs supplementation on serum lipid profile in mice challenged with choline

Figure 1. indicates the results of serum lipid profile in the tested mice. No significant differences in serum lipid profile (TG, TC, LDL-C, and HDL-C) were detected between the HCD and control groups. As depicted in Fig. 2, oral supplementation of choline challenged

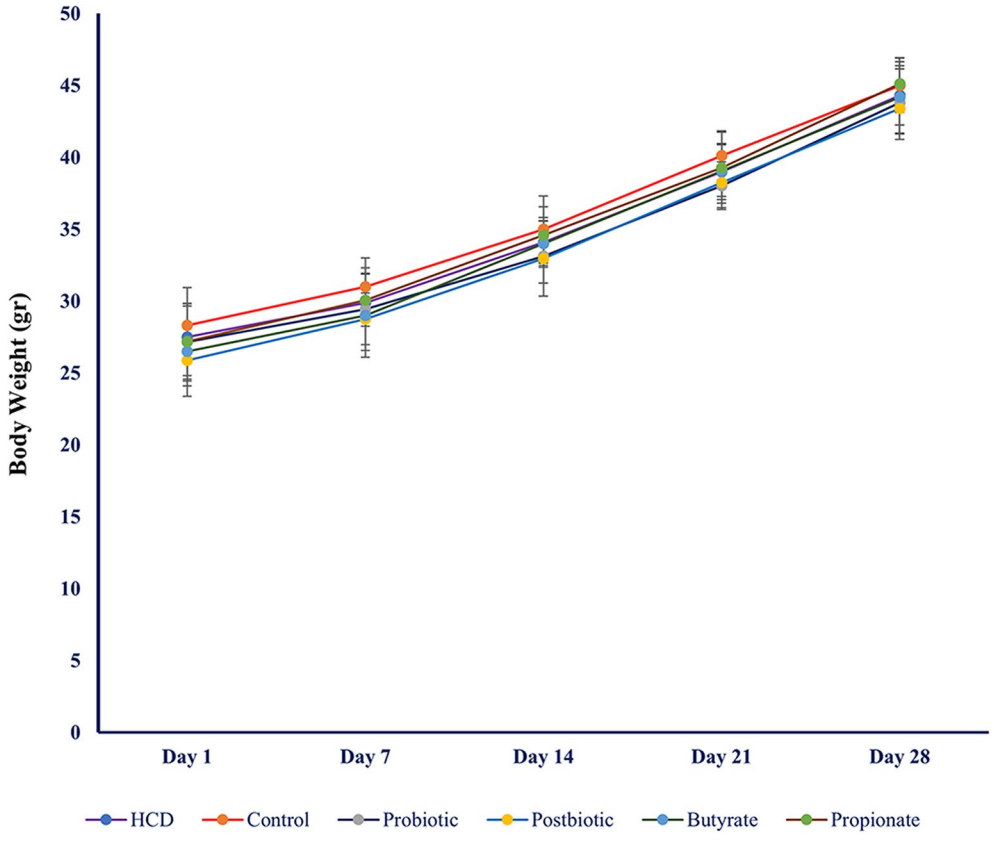


Fig. 1 The effect of *A. muciniphila* and SCFAs supplementation on the body weight in choline challenged C57BL/6J mice ($n=6$). Experimental conditions were as described in methods. All values are presented as mean ± SD. Data were analyzed using one-way ANOVA test

mice with probiotic or postbiotic of *A. muciniphila* significantly elevated and lowered the serum levels of TG and HDL-C, respectively. The results also indicated no noticeable difference in the liver enzymes (ALT and AST) levels between the HCD and control groups, sugegsting that HCD did not damage the liver.

Also, our results revealed that oral supplementation of choline challenged mice with live or died *A. muciniphila*, as well as, butyrate and propionate did not significantly alter ALT and AST enzyme levels (Fig. 3a-b).

Effect of A. muciniphila and SCFAs supplementation on TMAO levels

To examine the effect of *A. muciniphila* and SCFAs supplementation on TMAO levels, we measured TMAO, TMA levels in the serum, TMAO in the urine, and TMA in the cecal content using UHPLC-MS/MS system. As demonstrated in Fig. 4a-d, on the 28th days of experiment, the serum levels of TMAO in the HCD group were meaningfully greater compared to the control group. Compared to the HCD group, live and pasteurized *A. muciniphila* supplementation significantly reduced the serum levels of TMAO. The effect of the killed *A. muciniphila* (postbiotic) on the reduction of serum TMAO level was greater than that of the live *A. muciniphila* (probiotic). Supplementation of choline challenged mice with short chain fatty acids propionate and butyrate also reduced the serum level of TMAO compared to the HCD group, but this effect was less than that of probiotic or postbiotic supplementation. The serum level of TMA was also considerably greater in the HCD group than that in the control group. Compared to the HCD group, supplementation of choline challenged mice with live or pasteurized *A. muciniphila* meaningfully lowered the serum levels of TMA. Oral supplementation of mice with propionate and butyrate also reduced the serum levels of TMA, though this change was not statistically significant. TMAO is mostly excreted through the kidney, and measurement of TMAO in urine showed that the urine levels of TMAO in the HCD group were statically significantly higher than in the control group. As shown in Fig. 4d, supplementation of choline-challenged mice with live or pasteurized *A. muciniphila* did not reduce the urine levels of TMAO. Furthermore, the cecal TMA levels of HCD group were significantly higher than in the control group. Compared with the HCD group, live *A. muciniphila*, pasteurized *A. muciniphila*, propionate, and butyrate significantly lowered cecal TMA levels. Among them, the pasteurized *A. muciniphila* had the greatest effect on reducing cecal TMA.

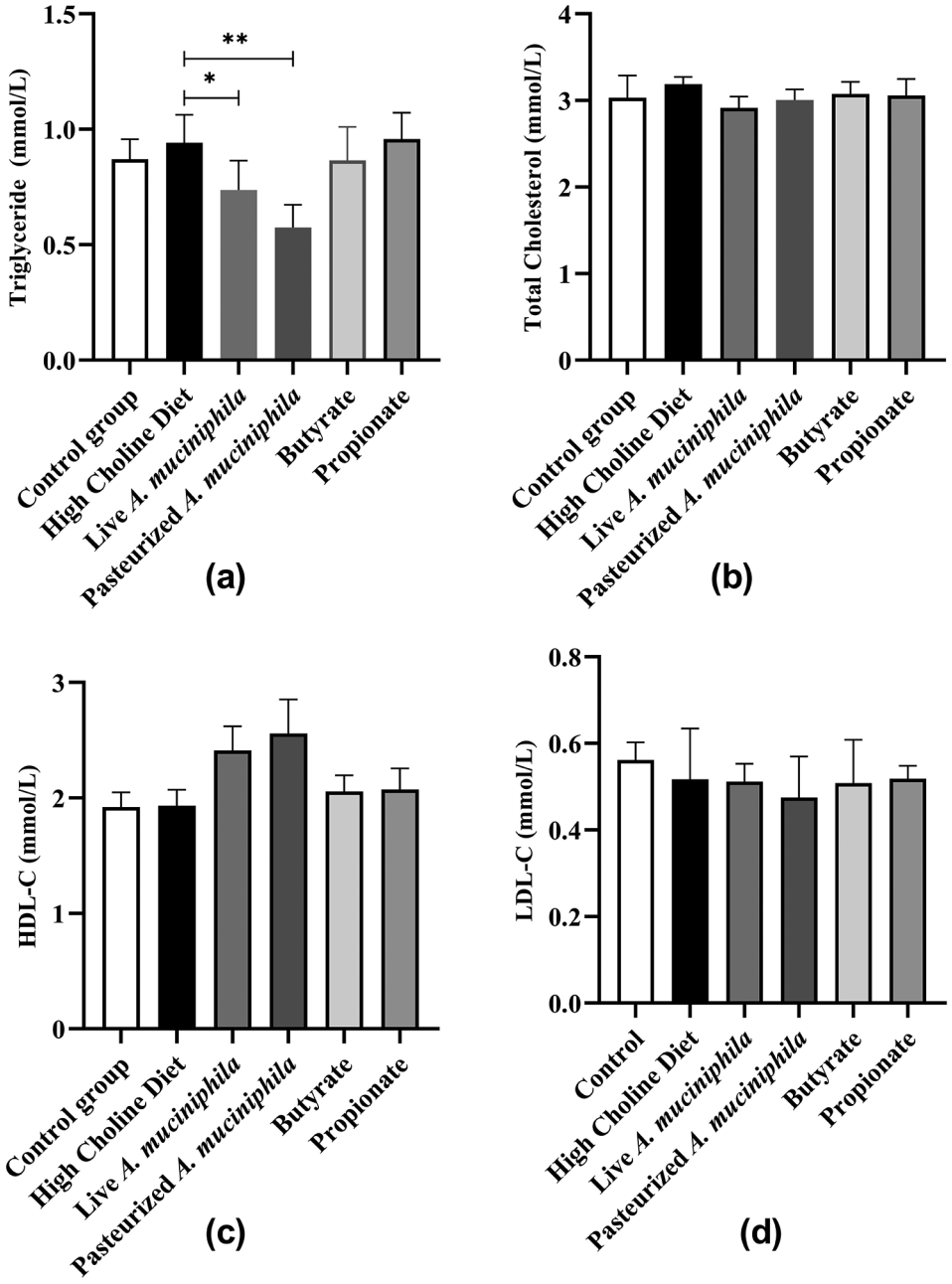


Fig. 2 The effects of *A. muciniphila* and SCFAs on serum levels of triglyceride (**a**), total cholesterol (**b**), HDL-C (**c**), and LDL-C (**d**) levels in choline challenged C57BL/6J mice ($n=6$). Experimental conditions were as described in methods. All values are presented as mean ± SD. Data were analyzed using one-way ANOVA test, * $p<0.05$ and ** $p<0.01$

Effect of A. muciniphila and SCFAs supplementation on FXR and FMO3 protein expression in choline challenged mice

Hepatic FMO3 enzyme plays a central role in the conversion of TMA to TMAO. Overexpression of this enzyme in the liver significantly increases serum levels of TMAO, and its expression is influenced by diet, bile acids, containing bile acid-activated nuclear FXR. As shown in the Fig. 5a-b, no significant difference in FMO3 and FXR protein expression was observed between the HCD and control groups. Furthermore, supplementation of choline challenged mice with *A. muciniphila* (live or pasteurized bacteria) and as well as propionate and butyrate short chain fatty acids did not significantly change the expression of FMO3 and FXR proteins in the liver of rats treated with choline.

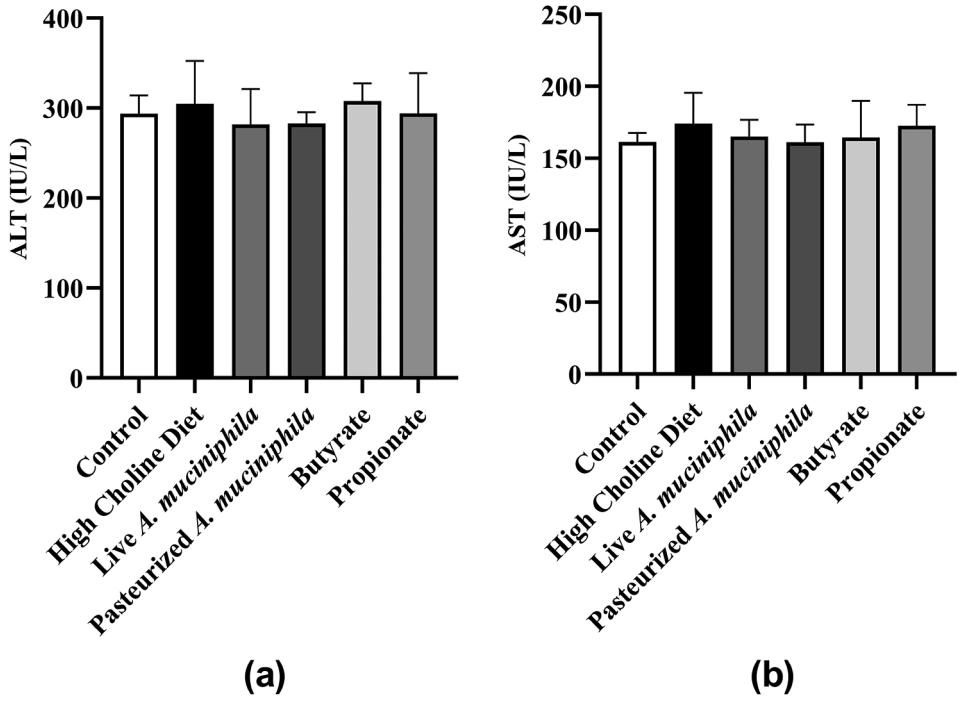


Fig. 3 The effects of *A. muciniphila* and SCFAs on serum levels of ALT (**a**) and AST (**b**) in choline challenged C57BL/6J mice ($n=6$). Experimental conditions were as described in methods section. All values are presented as mean ± SD. Data were analyzed using one-way ANOVA test

Effect of A. muciniphila and SCFAs supplementation on hepatic FMO3 activity in choline challenged mice

As illustrated in Fig. 5c, no statically significant differences in hepatic FMO3 enzymatic activities were detected among the HCD and control groups. As compared with the HCD group, *A. muciniphila* supplementation a little affected FMO3 activities, but the variances were not satanically significant.

Effect of A. muciniphila and SCFAs supplementation on protein levels of CutC and CutD in Cecum of choline challenged mice

As shown in Fig. 6a-b, the cecum levels of CutC and CutD proteins in the HCD group was significantly higher than that in the control group. In compression to HCD group, supplementation of choline challenged mice with *A. muciniphila* significantly reduced the protein levels of CutC.and CutD in cecal content. Orally supplementation of mice with propionate and butyrate also reduced cecum level of CutC.and CutD proteins compared to HCD group, but this effect was less than *A. muciniphila* supplementation.

Effect of A. muciniphila and SCFAs supplementation on gut microbiota composition in choline challenged mice

Figure 7 demonstrates that the presence of *Akkermansia* and *Bifidobacterium* was lower in the HCD group compared to the control group. In contrast, compared to the control group, the relative proportion of *Ruminiclostridium* and *Lachnoclostridium* were enhanced significantly in HCD group. Furthermore, *A. muciniphila* treated mice had a significant higher proportion of *Faecalibaculum* and *Lactobacillus* compared to HCD group (Fig. 7c-e).

Discussion

The present study represents a significant advance in our understanding of the potential therapeutic effects of *A. muciniphila* postbiotic supplementation on choline-induced TMAO production in mice. To the best of our knowledge, this is the first study to inspect the impact of postbiotic on TMAO levels in the context of *A. muciniphila* supplementation. The findings revealed that both *A. muciniphila* and short-chain fatty acids (SCFAs) supplementation resulted in a significant reduction in TMAO levels in various biological samples, including serum, cecum, and urine. Interestingly, the effect was more pronounced with pasteurized A. muciniphila, suggesting that the cell components of *A. muciniphila* may play a crucial role in mediating these observed effects. In addition to TMAO reduction, supplementation with *A. muciniphila* postbiotic also demonstrated an impact on the serum lipid profile in choline-challenged mice. Specifically, it led to elevated serum HDL-C levels and lowered TG levels, indicating a potential beneficial effect on lipid metabolism.

Furthermore, our study revealed that supplementation with butyrate, one of the SCFAs, also influenced

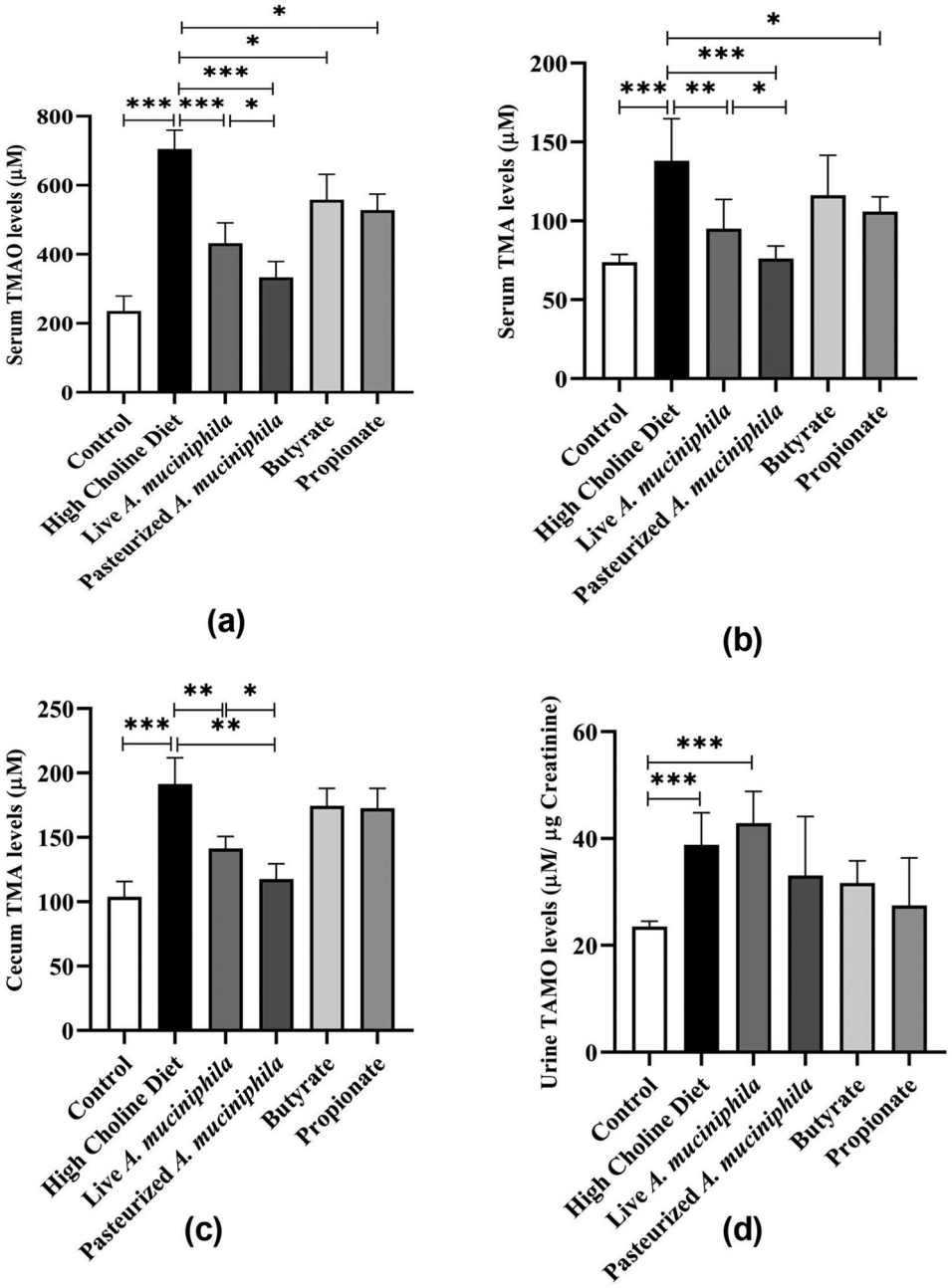


Fig. 4 The effects of *A. muciniphila* and SCFAs on serum TMAO (**a**), serum TMA (**b**), cecum TMA (**c**), and urine TMAO (**d**) levels in choline challenged C57BL/6J mice ($n=6$). Experimental conditions were as described in methods. All values are presented as mean ± SD. Data were analyzed using one-way ANOVA test, * $p<0.05$, ** $p<0.01$, and *** $p<0.001$

TMAO levels in the mice. TMAO has been associated with hypertension risk, and the dose-dependent reduction in plasma TMAO and its metabolite dimethylamine (DMA) observed in our study may be attributed to a decline in gut microbial TMA formation [11, 15]. These findings highlight the potential of *A. muciniphila* postbiotic supplementation in modulating TMAO metabolism and propose that targeting the gut microbiota may offer a novel therapeutic approach for the treatment and prevention of metabolic diseases. Previous gut microbiota-targeted therapies, including probiotics postbiotics, prebiotics, fecal microbiota transplantation, and bacterial metabolite modulation, have shown promising benefits in TMAO modulation [6, 7, 10].

Further, *A. muciniphila* supplementation was found to induce modifications in the composition of the gut microbiota in choline-challenged mice. Specifically, it led to an increase in the abundance of *Faecalibaculum* and

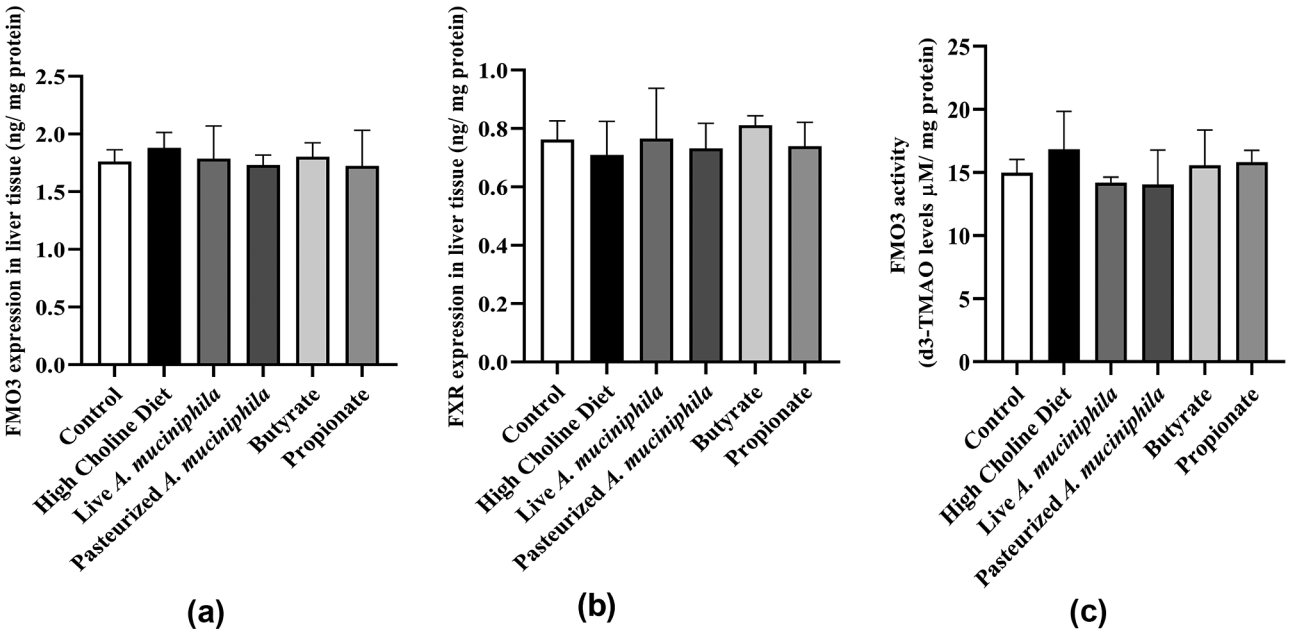


Fig. 5 The effects of *A. muciniphila* and SCFAs on FMO3 and FXR protein expression (**a** and **b** respectively), and FMO3 activity (**c**) in choline challenged C57BL/6J mice ($n=6$). Experimental conditions were as described in methods. All values are presented as mean ± SD. Data were analyzed using one-way ANOVA test

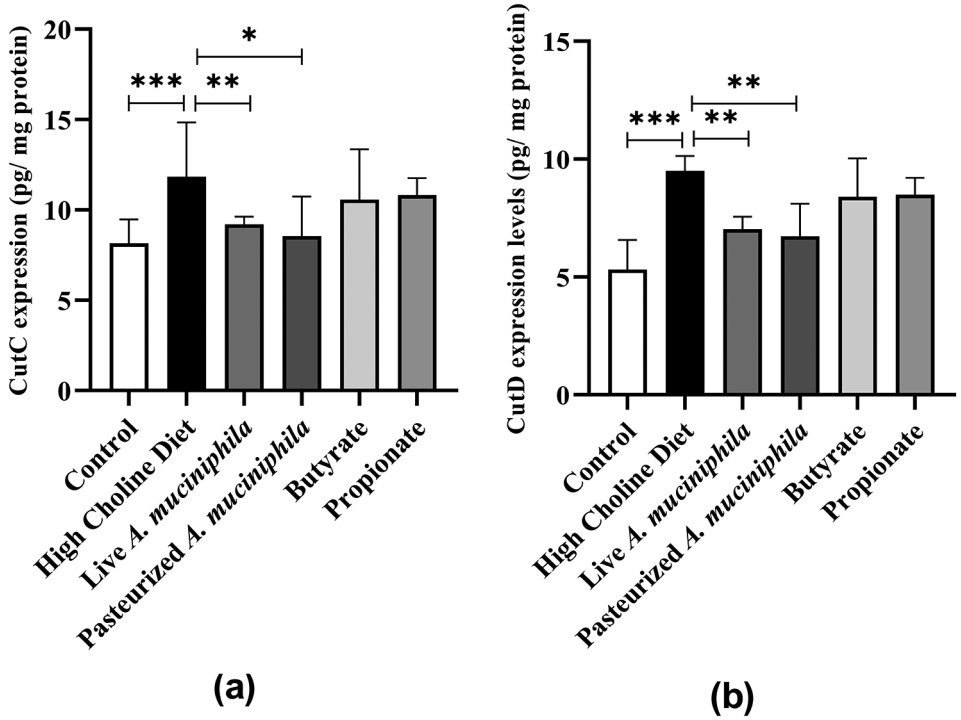


Fig. 6 The effects of *A. muciniphila* and SCFAs on cecum levels of CutC (**a**) and CutD (**b**) in choline challenged C57BL/6J mice ($n=6$). Experimental conditions were as described in methods. All values are presented as mean ± SD. Data were analyzed using one-way ANOVA test and * $p<0.05$, ** $p<0.01$, and *** $p<0.001$

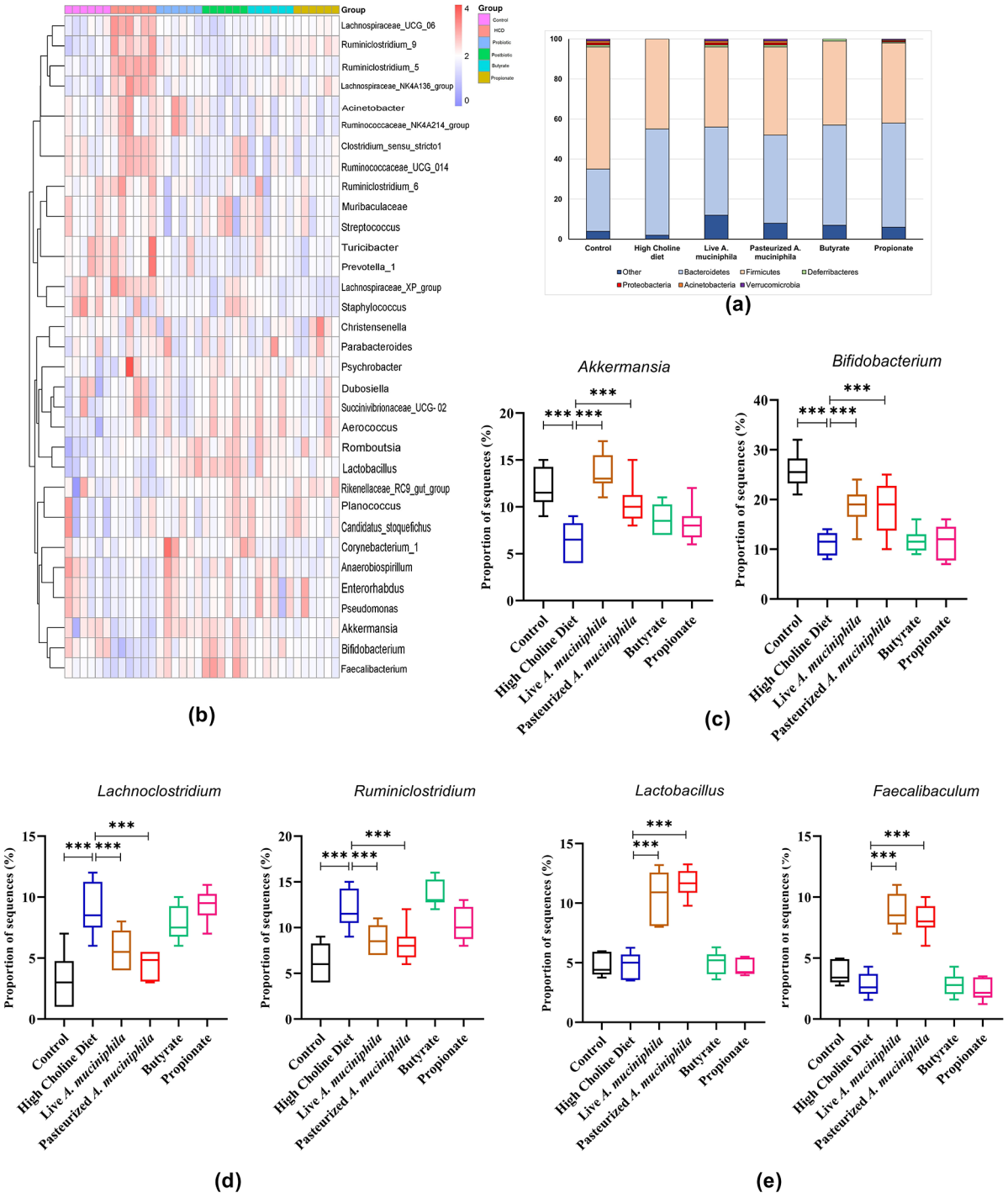


Fig. 7 *Akkermansia muciniphila* altered the composition of gut microbiome ($n = 6$): (**a**) distribution of cecal microbial at phylum level; (**b**) Heatmap of the distribution of the cecal microbial, X-axis represents the groups, Y-axis represents the genus, and the color means the relative abundance; (**c-e**) relative abundance of the genera among six groups. All values are presented as mean ± SD. Data were analyzed using one-way ANOVA test, and *** $p < 0.001$

Lactobacillus, while lowering the abundance of *Ruminiclostridium* and *Lachnoclostridium*. These changes in gut microbiota composition may contribute to reduced production of TMAO by influencing the metabolic activities of the gut microbiota [16]. Previous studies have demonstrated the ability of *A. muciniphila* supplementation to significantly lower TMAO levels in animal models. This remarkable effect is attributed to *A. muciniphila's* ability to orchestrate a shift in the gut microbial community, favoring the growth of bacteria that produce less trimethylamine (TMA), the precursor of TMAO. This shift in microbial composition can be attributed to

A. muciniphila's production of antimicrobial peptides, modulation of bile acid metabolism, and production of short-chain fatty acids, all of which can influence the growth and activity of other gut bacteria [17]. Our findings suggest a potential for *A. muciniphila* postbiotics to influence TMAO metabolism in mice, warranting further research to confirm and understand the mechanisms involved, including potential limitations on urinary excretion.

The underlying mechanisms by which *A. muciniphila* reduces choline-induced TMAO production may involve the modulation of gut microbiota composition and the expression of proteins involved in TMAO synthesis [13, 15]. *A. muciniphila* supplementation was found to reduce the expression of CutC and CutD proteins in the cecum, which contribute to the conversion of choline to TMA [18]. Additionally, SCFA supplementation reduced the expression of FMO3 protein in the liver, which is essential for the conversion of TMA to TMAO [19, 20]. These findings are in line with previous studies demonstrating the efficacy of *A. muciniphila* and SCFAs in modulating gut microbiota composition and reducing TMAO production [21, 22]. The implications of these findings are significant, as they provide insights into potential strategies for preventing cardiovascular disease and other health risks associated with TMAO [23].

Another potentially protective effect of *A. muciniphila* supplementation may involve its influence on hepatic FMO3 activity as well as the expression of FMO3 and FXR proteins in choline-challenged mice [24]. FMO3 activity is crucial in TMAO production, and our study found that *A. muciniphila* supplementation had a modest effect on FMO3 activities [20]. While *A. muciniphila* supplementation showed some influence on FMO3 activity in the liver, the effect was not statistically significant [25]. This suggests that while FXR-mediated FMO3 regulation might modulating gut bacteria composition may play a more substantial role in how A. muciniphila influences TMAO production. This highlights the multifaceted role of *A. muciniphila* in modulating TMAO metabolism, emphasizing its potential as a therapeutic target for cardiovascular health. By understanding the mechanisms by which *A. muciniphila* influences TMAO production, we can gain valuable insights into developing novel strategies to reduce TMAO levels and mitigate the risk of cardiovascular disease.

In conclusion, our study suggests a potential for *A. muciniphila* postbiotic and SCFAs to reduce choline-induced TMAO production in mice, but further investigation is required to confirm and understand the mechanisms involved, including potential limitations on urinary excretion. These effects are associated with alterations in gut microbiota composition and modulation of proteins involved in TMAO synthesis. Additionally, *A. muciniphila* supplementation revealed potential benefits in improving lipid metabolism, as evidenced by augmented HDL-C levels and lowered TG levels. These findings highlight the therapeutic potential of *A. muciniphila* postbiotics in mitigating TMAO-related health risks and suggest that targeting the gut microbiota and its metabolites could be a promising approach for managing metabolic diseases. Further research is needed to elucidate the underlying mechanisms and to validate these findings in human studies.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13765-024-00905-7.

Supplementary Material 1

Author contributions

Conceptualization: Hongjuan Xu, Xiaoxi Chen. Methodology: Hongjuan Xu. Validation: Hongxing Wang, Xiaoxi Chen. Formal Analysis: Xiaoxi Chen. Investigation: Hongjuan XuResources: Data Curation: Xiaoxi Chen. Writing—Original Draft Preparation: Hongjuan Xu, Xiaoyun Bian, Writing—Review and Editing: Hongjuan Xu, Xiaoyun Bian, Hongxing Wang, Lin Huang, Xiaoxi Chen. Visualization: Xiaoxi Chen. Supervision: Xiaoxi Chen. Project Administration: Hongjuan Xu, Xiaoyun Bian. Funding Acquisition: Xiaoxi Chen.

Funding

Not available.

Data availability

All data generated and analyzed during this study are included in the manuscript. The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Declarations

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki, and the animal study protocol was approved by the Ethics Committee of Affiliated Matern & Child Care Hospital of Nantong University (protocol code: MCCH-2023-052).

Consent for publication

All authors support the submission to this journal.

Conflict of interest

The authors declare that they have no conflicts of interest.

Author details

[1]Department of Internal Medicine, Affiliated Matern & Child Care Hospital of Nantong University, No. 399, Century Avenue, Chongchuan District, Nantong, Jiangsu Province 226001, China

Received: 30 December 2023 / Accepted: 13 May 2024
Published online: 30 May 2024

References

1. Alonso VR, Guarner F (2013) Linking the gut microbiota to human health. Br J Nutr 109(S2):S21–S26
2. Witkowski M, Weeks TL, Hazen SL (2020) Gut microbiota and cardiovascular disease. Circ Res 127(4):553–570

3. Moludi J et al (2018) New insights on atherosclerosis: a cross-talk between endocannabinoid systems with gut microbiota. J Cardiovasc Thorac Res 10(3):129
4. Moludi J et al (2020) Metabolic endotoxemia and cardiovascular disease: a systematic review about potential roles of prebiotics and probiotics. Clin Exp Pharmacol Physiol 47(6):927–939
5. Moludi J et al (2019) The efficacy and safety of probiotics intervention in attenuating cardiac remodeling following myocardial infraction: literature review and study protocol for a randomized, double-blinded, placebo controlled trial. Contemp Clin Trials 15:100364
6. Din AU et al (2019) Amelioration of TMAO through probiotics and its potential role in atherosclerosis. Appl Microbiol Biotechnol 103:9217–9228
7. Smits LP et al (2018) Effect of vegan fecal microbiota transplantation on carnitine-and choline-derived trimethylamine-N-oxide production and vascular inflammation in patients with metabolic syndrome. J Am Heart Assoc 7(7):e008342
8. Marteau P, Shanahan F (2003) Basic aspects and pharmacology of probiotics: an overview of pharmacokinetics, mechanisms of action and side-effects. Best Pract Res Clin Gastroenterol 17(5):725–740
9. Vinderola G, Sanders ME, Salminen S (2022) The concept of postbiotics. Foods 11(8):1077
10. Tain Y-L et al (2023) Reprogramming effects of Postbiotic Butyrate and Propionate on maternal high-fructose Diet-Induced offspring hypertension. Nutrients 15(7):1682
11. Cuevas-González P, Liceaga A, Aguilar-Toalá J (2020) Postbiotics and paraprobiotics: from concepts to applications. Food Res Int 136:109502
12. Zhang T et al (2019) Akkermansia muciniphila is a promising probiotic. Microbiol Biotechnol 12(6):1109–1125
13. Cheng D, Xie M (2021) A review of a potential and promising probiotic candidate—Akkermansia muciniphila. J Appl Microbiol 130(6):1813–1822
14. Ramireddy L et al (2021) Molecular identification and selection of probiotic strains able to reduce the serum TMAO level in mice challenged with Choline. Foods 10(12):2931
15. Kechagia M et al (2013) *Health benefits of probiotics: a review* Int Sch Res Notices, 2013
16. Wu Y-T et al (2022) Dietary plant and animal protein sources oppositely modulate fecal Bilophila and Lachnoclostridium in vegetarians and omnivores. Microbiol Spectr 10(2):e02047–e02021
17. Ghaffari S et al (2023) Akkermansia muciniphila: from its critical role in human health to strategies for promoting its abundance in human gut microbiome. Crit Rev Food Sci Nutr 63(25):7357–7377
18. Dalla Via A et al (2019) Urinary TMAO levels are associated with the taxonomic composition of the gut microbiota and with the choline TMA-lyase gene (cutC) harbored by enterobacteriaceae. Nutrients 12(1):62
19. Gatarek P, Kaluzna-Czaplinska J (2021) Trimethylamine N-oxide (TMAO) in human health. EXCLI J 20:301
20. Fennema D, Phillips IR, Shephard EA (2016) Trimethylamine and trimethylamine N-oxide, a flavin-containing monooxygenase 3 (FMO3)-mediated host-microbiome metabolic axis implicated in health and disease. Drug Metab Dispos 44(11):1839–1850
21. Shen J et al (2023) Akkermansia muciniphila attenuated lipopolysaccharide-induced acute lung injury by modulating the gut microbiota and SCFAs in mice, vol 14. Food & Function, pp 10401–10417. 23
22. Ma J et al (2023) Gut microbiota remodeling improves natural aging-related disorders through Akkermansia muciniphila and its derived acetic acid. Pharmacol Res 189:106687
23. Kanitsoraphan C et al (2018) Trimethylamine N-oxide and risk of cardiovascular disease and mortality. Curr Nutr Rep 7:207–213
24. Yan J, Sheng L, Li H (2021) Akkermansia muciniphila: is it the Holy Grail for ameliorating metabolic diseases? Gut Microbes 13(1):1984104
25. Xu Y et al (2020) Function of Akkermansia muciniphila in obesity: interactions with lipid metabolism, immune response and gut systems. Front Microbiol 11:219

Publisher's Note