Probiotics and Antimicrobial Proteins (2025) 17:4525–4535
https://doi.org/10.1007/s12602-024-10395-0

RESEARCH

Synbiotic Effects of *Lacticaseibacillus paracasei* K56 and Prebiotics on the Intestinal Microecology of Children with Obesity

Pengwei Zhang[1] · Xianhui Dong[1] · Yijun Zeng[2] · Junkui Chen[3] · Sijia Yang[2] · Peipei Yu[2] · Chunhong Ye[2] · Wei-Lian Hung[4,5] · Qiuyue Jiang[4,5] · Wen Zhao[4,5] · Zhaozhong Zeng[4,5] · Jinjun Li[6] · Li Li[1,2]

Accepted: 28 October 2024 / Published online: 6 November 2024

Abstract

Lacticaseibacillus paracasei K56 (*L. paracasei* K56) is a probiotic with weight-loss effects. However, symbiosis research on the combined effects of *Lacticaseibacillus paracasei* K56 and prebiotics is lacking. Therefore, the aim of this study was to investigate the effects of *L. paracasei* K56, xylooligosaccharide (XOS), galactooligosaccharide (GOS), polyglucose (PG), and their synbiotic combinations (XOS + K56, GOS + K56, and PG + K56) on metabolism and gut composition in children with obesity, using an in vitro fermentation model. Fecal samples were collected from 14 children with obesity for in vitro fermentation, and the effects of the various treatments in gas production and short chain fatty acid synthesis (SCFAs) were assessed. Treatment with probiotics, prebiotics, and synbiotics regulated gut microbiota and metabolites in children with obesity. GOS and XOS had higher degradation rates than PG + K56 synbiotics in the gut microbiota of children with obesity. Moreover, treatment with XOS, GOS, and their synbiotic combinations, (XOS + K56) and (GOS + K56), significantly reduced the production of gas, propionic acid, and butyric acid compared with PG + K56 treatment. Treatments with GOS + K56 and XOS + K56 altered the composition of the gut microbiota, improved the abundance of *Bifidobacteria* and *Lactobacilli*, and reduced the abundance of *Escherichia/Shigella*. Overall, this study provides a theoretical foundation for the use of K56-based synbiotics.

Keywords *Lacticaseibacillus paracasei* K56 · Galactooligosaccharide · Xylooligosaccharide · Polyglucose · Synbiotics · Childhood obesity

Introduction

Global statistics published in 2016 and 2017 indicate an increase in the incidence of obesity, with the number of obese people overtaking the number of underweight individuals [1, 2]. During the 40 years from 1975 to 2016, the incidence of obesity among children and adolescents worldwide has increased from 0.7 to 5.6% for girls and from 0.9 to 7.8% for boys [2], which is equivalent to 340 million overweight or obese children and adolescents [3]. Obesity poses a serious threat to human health, and is a risk factor for diabetes, cardiovascular diseases, certain cancers, and other chronic diseases [4]. The harmful effects of childhood obesity cannot be underestimated as children with obesity are at an increased risk for metabolic diseases, as well as cardiovascular maladies and adulthood cancers [5, 6]. Obesity is usually accompanied by chronic inflammation, insulin resistance, lipid metabolism disorders, and other health-related issues [7], including imbalanced gut microbiota [8]. Most

Pengwei Zhang and Xianhui Dong contributed equally to this work.

✉ Jinjun Li
lijijun@zaas.ac.cn

✉ Li Li
lislucy@163.com

1 The Affiliated Hospital of Hangzhou Normal University, Hangzhou Normal University, Hangzhou 310015, China

2 Hangzhou Normal University, Hangzhou 310015, China

3 Hangzhou Hailu Medical Technology Co., Ltd., Hangzhou 310015, China

4 Inner Mongolia Yili Industrial Group Co., Ltd., Hohhot 010000, China

5 Inner Mongolia Dairy Technology Research Institute Co., Ltd., Hohhot 010000, China

6 Institute of Food Science, Zhejiang Academy of Agricultural Sciences, Hangzhou 30021, China

current treatments for obesity center on lifestyle interventions, which are influenced by several factors, resulting in limited clinical effects [9].

Probiotics and prebiotics improve obesity by adjusting gut microbiota [10] and are more advantageous compared to lifestyle interventions. Bäckhed et al. [11] reported that germ-free mice of normal weight showed weight gains and fat accumulation following the transplantation of gut microbiota obtained from obese mice. Based on this finding, the association between obesity and gut microbiota has been attracting considerable research attention. Children with obesity have a lower gut microbiota diversity than normal children, with significant differences in the relative abundance of specific gut microbiota between both groups [12, 13]. Recent findings indicate obesity-associated dysbiosis as well as dysfunction in host immune response and energy metabolism can be corrected via reasonable supplementation with probiotics, prebiotics, synbiotics, and other dietary supplements [14], which may help reverse obesity. Probiotics are active microorganisms that benefit the host by colonizing the body and altering the composition of the host microbiota. Prebiotics are organic compounds that are neither digested nor absorbed by the host; instead, they specifically promote the metabolism and proliferation of beneficial bacteria in the body, thereby improving the health of the host [15]. A synbiotic is defined as a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms [16], which can exert positive effects on the health of hosts. Synbiotics are believed to confer greater benefits than either prebiotics or probiotics alone [17].

Lactobacillus is a probiotic widely used for the treatment of obesity [18]. *Lacticaseibacillus paracasei* K56 (*L. paracasei* K56), a probiotic isolated from the intestines of children, is resistance against gastric acid and intestinal fluids. Notably, *L. paracasei* K56 can regulate immunity, alleviate intestinal inflammation, and balance gut microbiota, among other functions, showing potential for application in fermented milk, solid drinks, and health food. Moreover, animal experiments have demonstrated that *L. paracasei* K56 effectively mitigates weight gain, reduces fat accumulation, alleviates insulin resistance, and restores pancreatic β-cell function by modulating the gut microbiota [19, 20]. However, studies on the compatibility of synbiotics based on *L. paracasei* K56 are lacking. To explore the suitability of *L. paracasei* K56 for use with prebiotics, this study selected three prebiotics with beneficial effects on obesity: xylooligosaccharide (XOS), galactooligosaccharide (GOS), and polyglucose (PG). This study was aimed at investigating the effects of *L. paracasei* K56, XOS, GOS, PG, and their synbiotic combinations (XOS + K56, GOS + K56, and PG + K56) on metabolism and gut composition in children with obesity. Specifically, we measured the degradation rate, gas production, and short-chain fatty acid (SCFA) output of synbiotics, and analyzed the composition of the microbiota to identify prebiotics with beneficial effects that could be suitable for the growth of *L. paracasei* K56. Our findings indicated that the abundances of *Bifidobacterium* and *Lacticaseibacillus* are increased in the gut microbiota of the GOS, GOS + K56, XOS, and XOS + K56 groups, and that these prebiotics and synbiotics may inhibit the production of propionic and butyric acids, thereby enabling weight loss. The findings of this study may provide a theoretical basis for the formulation and use of a novel *L. paracasei* K56-based synbiotic.

Materials and Methods

Sample Collection and Participants

Fourteen children with obesity (boys, $n = 6$; girls, $n = 8$) aged 9 years were recruited for this study. These volunteers consumed Chinese modern dietary pattern (with high intake of wheat, processed meat and fast food) [21], and none were vegetarians. They had not received antibiotics, probiotics, or prebiotics for at least 3 months prior to sample collection. This research was approved by the Ethics Committee of Hangzhou Normal University (No. 20190061). The participants provided their written informed consent to participate in this study. Fecal samples, collected from the 14 volunteers, were placed in sterile collection tubes and transported to the laboratory within 4 h under low temperature for further analysis.

In Vitro Fermentation Test

All samples were subjected to batch culture and fermentation experiments using the method described by Wu et al. [22]. Fresh fecal samples (0.8 g) were treated with 8 mL of 0.1 M anaerobic phosphate-buffered saline (pH 7.0), following which the feces were homogenized and filtered using a HALO-F100 fecal processor (Suzhou Hailu Biotechnology, Jiangsu, China) to obtain a 10% fecal suspension. Thereafter, each sample was inoculated with yeast extract–casein hydrolysate–fatty acid medium (YCFA) modified growth medium containing the following [23]: 10 g/L of tryptone, 2.5 g/L of yeast extract, 10 mg/L of hemin, 1 g/L of$_{L}$-cysteine hydrochloride, 0.9 g/L of NaCl, 0.009 g/L of $MgCl_2 \cdot 6H_2O$, 0.45 g/L of KH_2PO_4, 0.45 g/L of KH_2PO_4, 1 mg/L of resezurin, 1 µg/L of biotin, 1 µg/L of cobalamin, 3 µg/L of p-aminobenzoic acid, 5 µg/L of folic acid, and 15 µg/L of pyridoxamine. In this study, a novel strain of *L. paracasei* K56 sourced from the gut microbiota of healthy infants in China by Inner Mongolia Yili Industrial Group Co., Ltd. was utilized. K56 (*L. paracasei* K56 [3×10^8 CFU/mL]), GOS (4 g/L), XOS (4 g/L), and

PG (4 g/L), obtained from Yuanye Biotechnology Co., Ltd. (Shanghai, China). GOS + K56 (GOS [4 g/L], *L. paracasei* K56 [3×10^8 CFU/mL]), XOS + K56 (XOS [4 g/L], *L. paracasei* K56 [3×10^8 CFU/mL]), and PG + K56 (PG [4 g/L], *L. paracasei* K56 [3×10^8 CFU/mL]) were placed in eight different culture media fermentation flasks, and treated with fecal culture from the YCFA culture medium, as the control. Following inoculation, the culture flasks were placed in a 37 °C incubator and fermented for 24 h. Subsequently, the samples were used for testing and further analysis.

Determination of Degradation Rates and Gas Measurement

The degradation rates of prebiotics and synbiotics were quantified using thin-layer chromatography (TCL Silica gel 60 F254, Merck, Germany) [24], the degradation rate was calculated based on the integration of the gray value of each sample on the chromatography plate.Total gas release and H_2, CO_2, CH_4, and H_2S concentrations during in vitro fermentation were evaluated using a gas analyzer (HL-QT01, Hailu Biotech, Hunan, China) [25].

Detection of SCFAs

The concentration of SCFAs in each fermentation broth was measured using a gas chromatograph (GC-2010 Plus, Shimadzu, Japan) coupled with a DB-FFAP column (0.32 mm * 30 × 0.5 mm) (Agilent Technologies, USA) and an H_2 Flame ionization detector. Crotonic acid (trans-2-butenoic acid) was used as the internal standard for the determination of the concentrations of acetic, propionic, butyric, isobutyric, valeric, and isovaleric acids [22]. The total acid production is the sum of the concentrations of the aforementioned six SCFAs.

16S rRNA Gene Sequencing

Bacterial genomic DNA was extracted from fecal and fermented samples using a QIAamp DNA fecal kit (Qiagen, Germantown, MD, USA). The V3–V4 region (bacterial 16S rRNA gene) of the extracted DNA was amplified using the barcode primers, 341F (5′-CCTAYGGGRBGCASCAG-3′) and 806R (5′-GGACTACNNGGGTATCTAAT-3′) [22]. An Illumina HiSeq 2500 system (Beijing, China) was used for next-generation sequencing, while the QIME (quantitative insight into microbial ecology) pipeline was used to identify the sequence through a barcode Recognition sequence. Sequences with 97% similarity were categorized into operational taxonomic units (OTUs) using Mothur software and annotated using the SILVA database. Sequencing data were analyzed using Genomics Software (Visual Genomics Soft). A sequence from each OTU was selected for representative purposes. The ribosome database project (RDP) classifier technique and SILVA database were used to classify representative sequences. Mothur was used to calculate suitable coverage, α diversity (including the Simpson and Shannon indices), and richness (observed number of OTUs). β-diversity was calculated using weighted principal coordinate analysis (PCoA), while α-diversity was calculated using the Shannon index. The correlation between the species, SCFA, and gases were illustrated using a heatmap based on the Spearman rank correlation coefficient.

Statistical Analysis

All data are represented as mean ± standard deviation (M ± SD). Significance differences between groups were calculated using one-way analysis of variance (ANOVA), followed by least significance difference (LSD) test. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software (version 23.0; SPSS Inc. Chicago, IL, USA). All charts were constructed using the GraphPad Prism 8 software (GraphPad Software Inc., San Diego, CA, USA).

Results

Prebiotic Degradation Rate

The degradation rates of the three prebiotics and synbiotics fermented in vitro are shown (Fig. 1a). The degradation rates of XOS, GOS, and PG by fecal bacteria from children with obesity were 70.41 ± 22.35, 49.79 ± 20.33, and 68.58 ± 20.33%, respectively. Following the addition of K56, the degradation rates of XOS, GOS, and PG decreased to 64.88 ± 27.69, 40.33 ± 25.3, and 59.03 ± 18.51%, respectively, although this trend was not significant. Notably, GOS and XOS had significantly higher ($p < 0.05$) degradation rates than PG + K56.

Total Gas Production Volume and Percentage of CO_2, CH_4, H_2, and H_2S

Gas production in each group was measured after 24 h of in vitro fermentation (Fig. 1b–f). Gas production was significantly lower ($p < 0.05$) in the GOS + K56, XOS, and XOS + K56 groups than in the PG and PG + K56 groups. Specifically, the volume of gas produced in the XOS, PG, and GOS groups decreased following the addition of K56, although the decrease was not significant. An analysis of the gas fractions showed that CO_2 output was significantly lower ($p < 0.05$) in the GOS + K56 and PG + K56 groups than in the PG group, CH_4 output was significantly lower

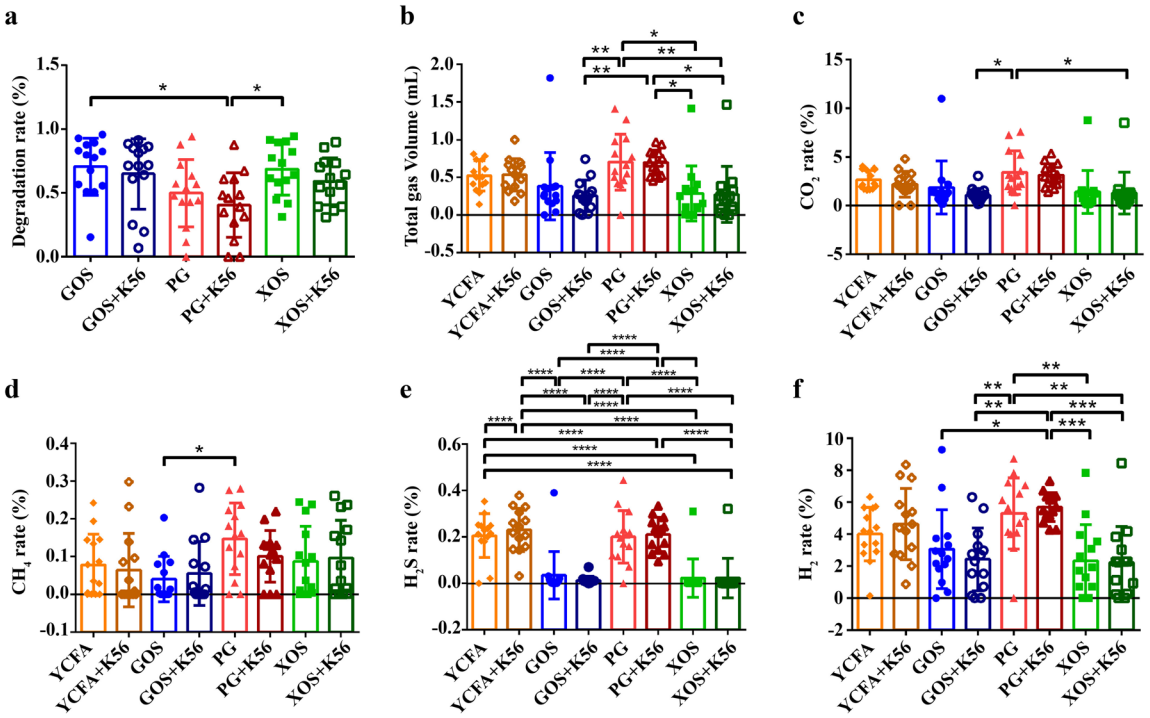

Fig. 1 Degradation rate (**a**), total gas production (**b**), and CO_2 (**c**), CH_4 (**d**), H_2 (**e**), and H_2S (**f**) production ratio in various groups. Statistical significance of the differences between groups was calculated using one-way ANOVA with LSD test, $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$ were considered with significant difference, and no mark means there is no significance

($p<0.05$) in the GOS group than in the PG group, while H_2 output was significantly lower in the XOS + K56 group than in the PG + K56 group. Additionally, H_2S output was significantly lower ($p<0.0001$) in the GOS, GOS + K56, XOS, and XOS + K56 groups than in the YCFA, YCFA + K56, PG, and PG + K56 groups. However, there was no significant difference in H_2S output between the GOS and GOS + K56 groups, as well as between the XOS and XOS + K56 groups, indicating that GOS and XOS were beneficial for reducing H_2S production.

Acid Production Analysis

There were significant differences ($p<0.05$) in the concentrations of different SCFAs (Fig. 2a–f), as well as the total acid production among the eight media. Notably, total acid output was significantly higher ($p<0.05$) the PG group than in XOS + K56 group. Although there was no significant difference in acetic acid output among the eight culture media, there was a significant difference ($p<0.0001$) in the proportions of acetic acid produced. The proportion of acetic acid produced in the XOS, XOS + K56, and GOS + K56 groups was significantly higher than that produced in the PG, PG + K56, YCFA, and YCFA + K56 groups. Additionally, the GOS group produced a higher proportion ($p<0.05$) of acetic acid than the PG and YCFA + K56 groups. Moreover, the GOS, GOS + K56, XOS, and XOS + K56 groups had lower concentrations ($p<0.05$) of propionic and butyric acids than the PG group. Additionally, the GOS + K56 and XOS + K56 groups had lower concentrations ($p<0.05$) of propionic acid than the PG + K56 group.

Microbial Diversity and Principal Component Analysis

There were no significant differences in the number of OTUs and Ace, Chao1, Shannon, and Simpson indices among the groups (Fig. 3a–e), indicating that prebiotics and synbiotics failed to alter the gut microbiota diversity. PCoA indicated that the total genera of gut microbiota in the GOS, GOS + K56, XOS, and XOS + K56 groups were significantly different from those in the YCFA, YCFA + K56, PG, PG + K56 groups (Fig. 3f). Compared with that in the YCFA group, the GOS, GOS + K56, XOS, and XOS + K56 groups had a significantly higher abundance ($p<0.05$) of *Bifidobacterium* (Fig. 4), but a lower abundance ($p<0.05$) of *Escherichia/Shigella*. However, there were no significant differences ($p>0.05$) the abundances both bacterial genera between the K56 and YCFA groups. Notably, *Lacticaseibacillus* spp. was significantly enriched in the GOS + K56, PG + K56, and XOS + K56 groups.

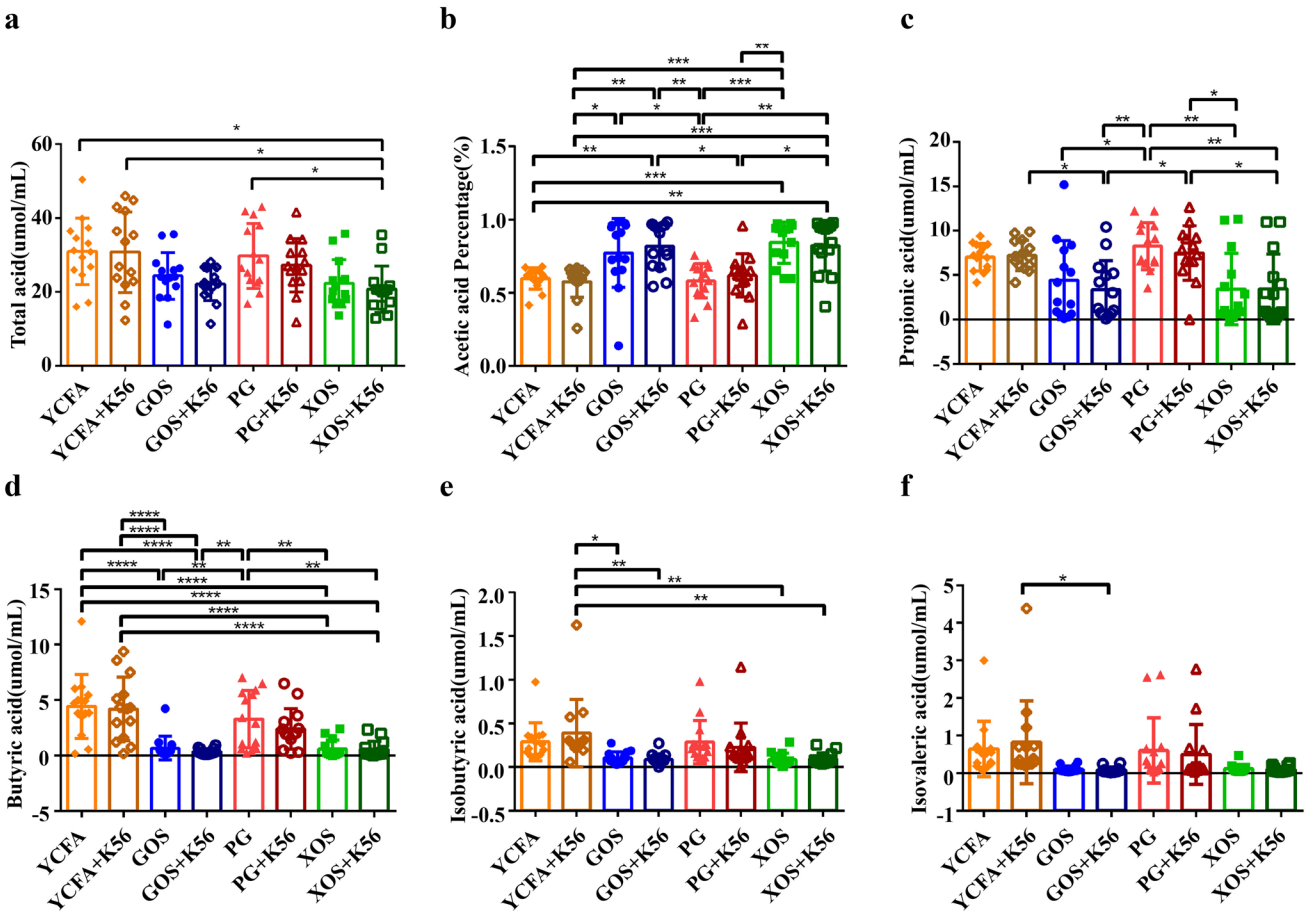

Fig. 2 Total (**a**), acetic (**b**), propionic (**c**), butyric (**d**), isobutyric (**e**), and isovaleric (**f**) acid contents in different groups. Total acid includes the concentrations of acetic, propionic, butyric, isobutyric, valeric, and isovaleric acid. Statistical significance of the differences between groups was calculated using one-way ANOVA with LSD test, $*P<0.05$, $**P<0.01$, and $***P<0.001$ were considered with significant difference, and no mark means there is no significance

Correlation Analysis of Degradation Rate and Gas Production

In the GOS + K56 group, *Bifidobacterium* was correlated with a decrease in CO_2, H_2, and total gas output, *Escherichia/Shigella* were associated with higher CO_2 production (Fig. 5a1), *Megamonas* was correlated with increased propionic acid synthesis, and *Prevotella* was positively correlated with isovaleric acid synthesis but negatively correlated with the total acid content (Fig. 5a2). *Bifidobacteria* were negatively correlated with the production of propionic acid and butyric acid (Fig. 5a2).

In the XOS + K56 group, *Bifidobacterium* was correlated with a decrease in CO_2 and CH_4 production, *Lacticaseibacillus* was negatively correlated with CO_2 and its degradation rate (Fig. 5b1), *Lactobacillus* was negatively correlated with propionic acid production, *Escherichia* and *Shigella* were associated with butyric acid synthesis, *Megamonas* was associated with propionic acid production, and *Enterococcus* was positively associated with isobutyric acid output (Fig. 5b2).

In the PG + K56 group, *Bacteroides* was positively correlated with CH_4 output, *Escherichia/Shigella* were negatively correlated with CH_4 production, while the degradation rate of *Lacticaseibacillus* was negatively correlated with CH_4 (Fig. 5c1). In SCFA metabolism, *Bacteroides* was positively correlated with acetic, butyric, and total acid contents. *Prevotella* increased the content of isobutyric acid, which is related to the synthesis of propionic acid, *Megamonas* was correlated with propionic acid synthesis, *Enterococcus* was negatively correlated with valeric acid output, *Lacticaseibacillus* was positively correlated with valeric acid, while *Limosilactobacilli* were negatively correlated with valeric and isovaleric acids synthesis (Fig. 5c2).

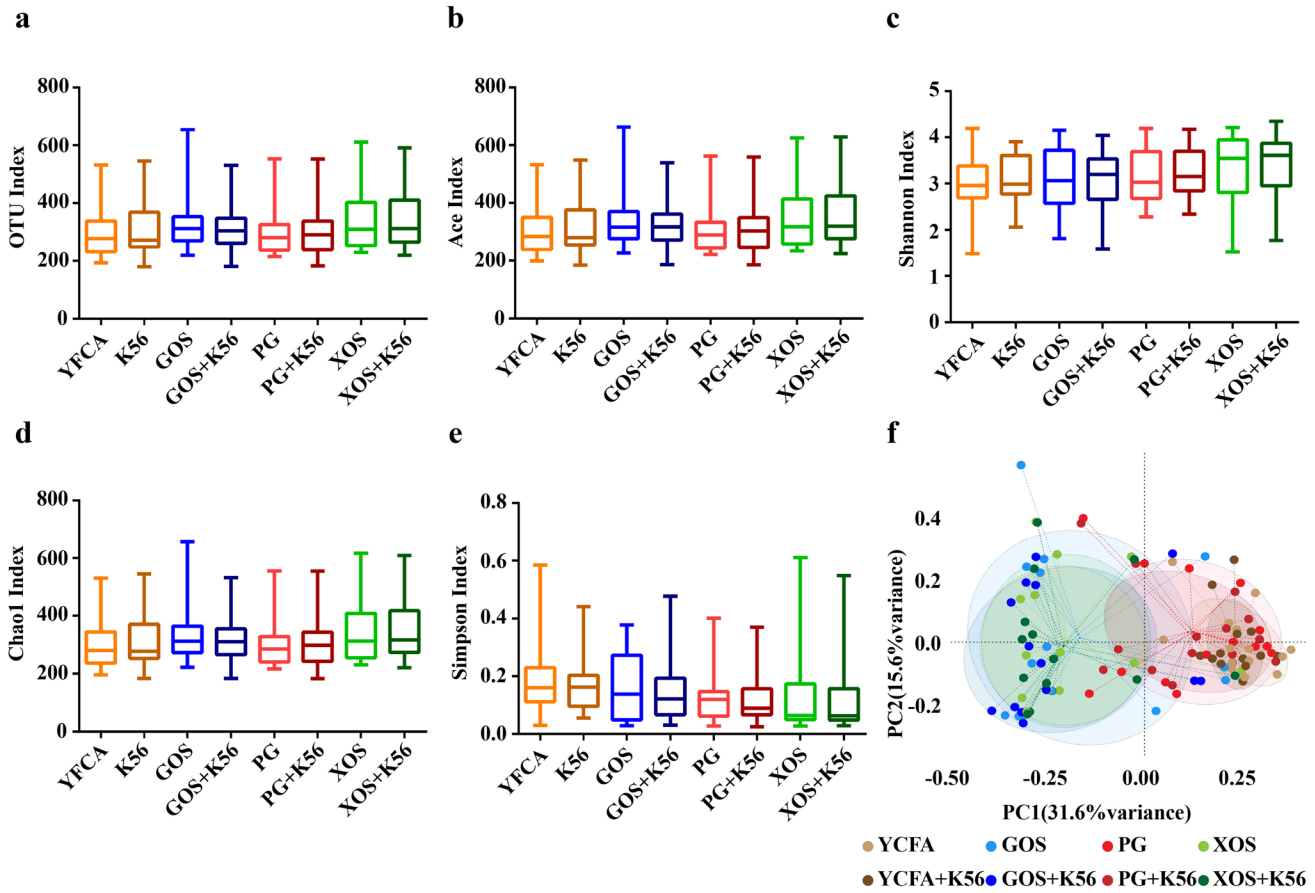

Fig. 3 PCoA plots for α-diversity indices in different groups, including the OUT (**a**), Ace (**b**), Shannon (**c**), Chao1 (**d**), and Simpson index (**e**), and β-diversity analysis (**f**)

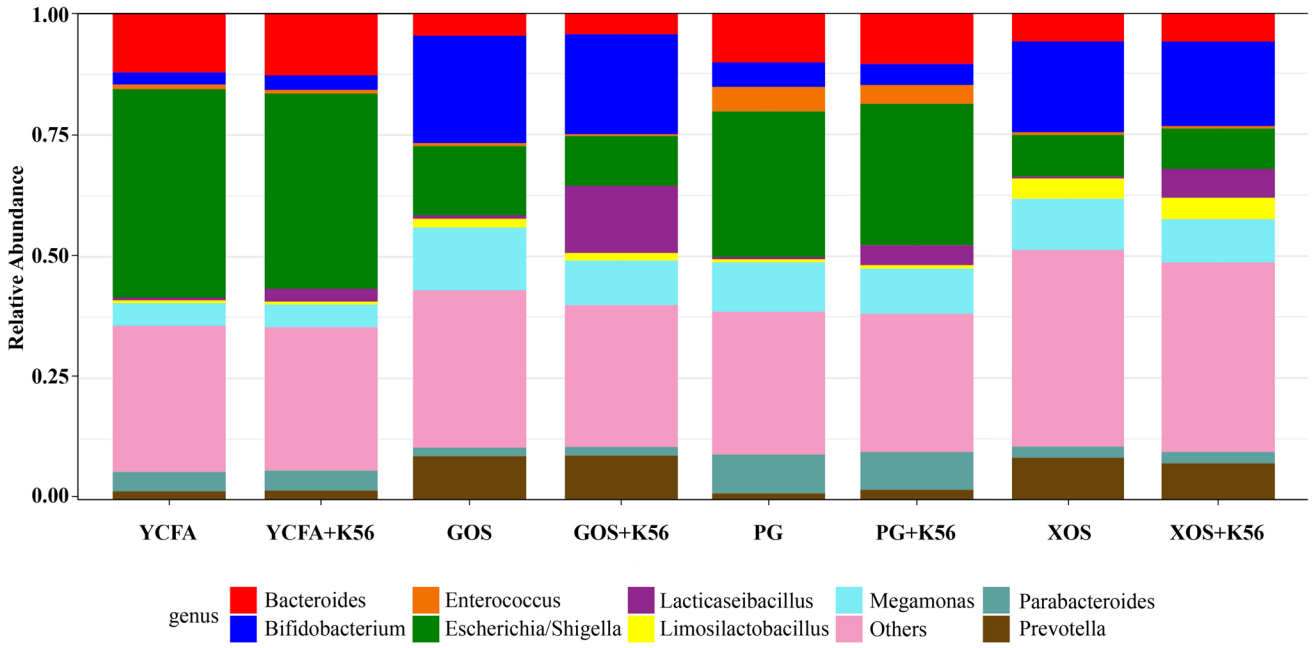

Fig. 4 Histogram of species composition at the genus level and relative abundance of dominant species in different groups

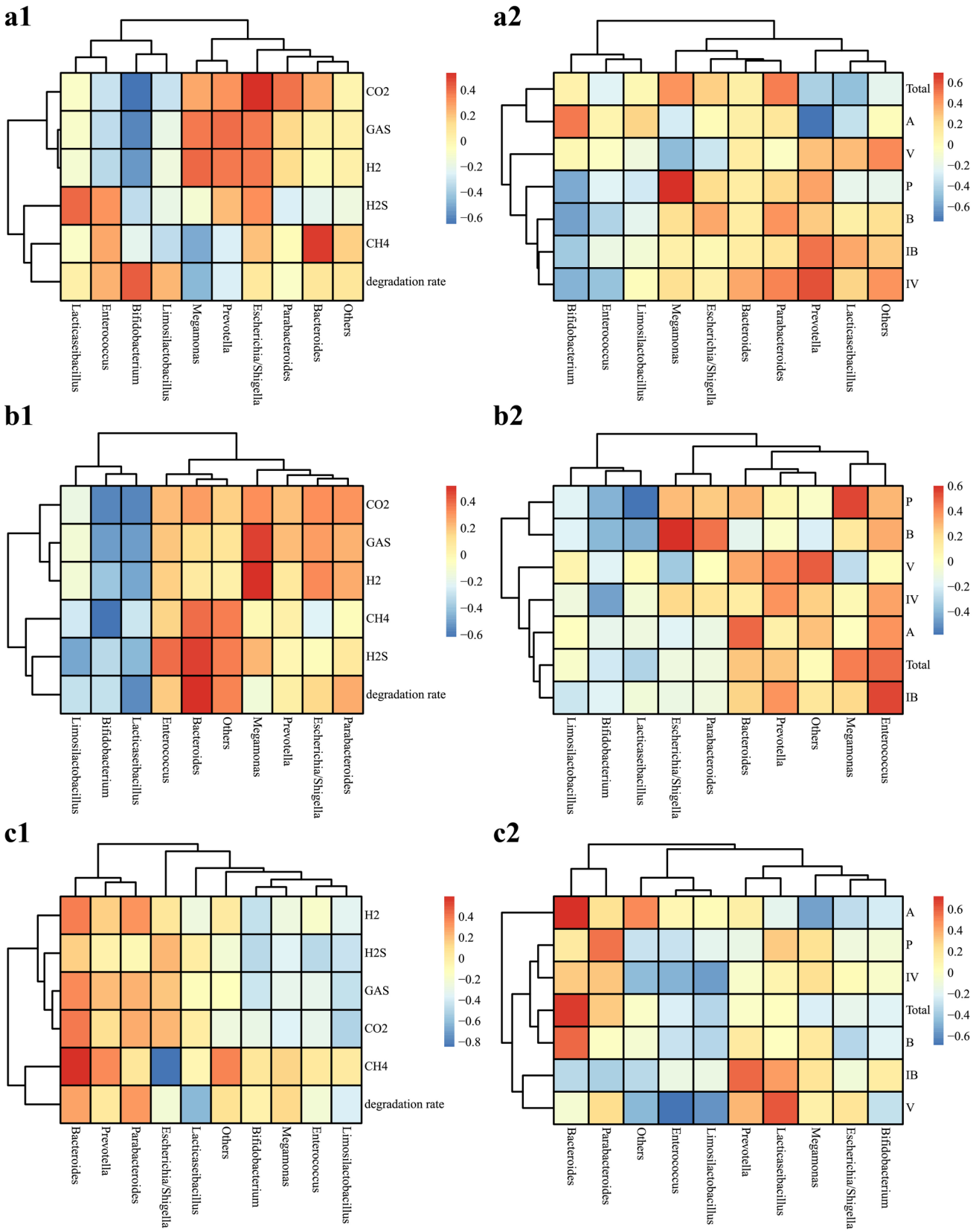

Fig. 5 Spearman correlation heatmaps of microorganisms at GOS + K56 (**a1**, **a2**), XOS + K56 (**b1**, **b2**), PG + K56 groups (**c1**, **c2**) with total, acetic, propionic, butyrate, isobutyric, and isovaleric acid, total gases, H_2, CO_2, CH_4, and degradation rate

Discussion

The intestinal microbiota is a potential determinant of the development of obesity [26]. *L. paracasei* K56 may reduce body fat in adults with obesity and increase the abundance of *Bacteroides, Alistipes,* and *Parastutella* in their intestines [27]. Synbiotics based on *L. paracasei* K56 significantly reduced body fat weight compared with either probiotics or prebiotics alone [28]. In this study, we examined the effects of *L. paracasei* K56 and three combinations of prebiotics on the gut microbiota of children with obesity, using an *in vitro* fermentation model. This model has been previously used to study probiotics and prebiotics [29, 30]. GOS, XOS, and their synbiotic combinations produced a higher proportion of acetic acid, lower amounts of propionic and butyric acids, and lower amounts of H_2S and H_2 gases. These results suggest that GOS and XOS are more compatible with K56 than with PG. Additionally, GOS and XOS promoted the abundance of *Bifidobacterium* and combinations of these prebiotics with *L. paracasei* K56 increased the abundance of *Limosilactocobacillus*. Collectively, these results suggest that synbiotics may regulate the abundance of certain species that constitute gut microbiota, providing a foundation for synbiotics-based personalized intervention programs.

Individuals with obesity have a lower gut microbial diversity and abundance of beneficial bacteria than normal individuals. Turnbaugh et al. [31] sequenced the gut microbiota of twins with different degrees of fatness and thinness and found lower gut microbial diversity in the twin with obesity. Zuo et al. [32] analyzed the bacterial colony counts in the feces of obese people in China and found that the contents of *Escherichia coli*, *Lactobacillus*, and *Bifidobacterium* was lower compared with those of individuals with normal body mass. The findings of the present study suggest that GOS and XOS may stimulate the abundance of *Bifidobacterium*, which is consistent with previous findings [33, 34]. Supplementation with probiotics and prebiotics may help restore intestinal microecological balance and boost beneficial bacteria in the gut microbiota of patients with obesity [35, 36]. Additionally, similar to previous studies on in vitro simulated fermentation, short-term in vitro fermentation of prebiotics and probiotics did not change the alpha diversity of intestinal microorganisms [24]. However, prebiotics and probiotics can change the abundance of specific intestinal flora. In our study, synbiotic *Lacticaseibacillus* was significantly enriched following the addition of *L. paracasei* K56. *Lactobacilli* may help reduce weight [18]. Kadeer et al. [27] found that *L. paracasei* K56 reduced the weight, visceral adipose tissue content, and waist circumference of human subjects. *Lactobacillus* may directly reduce the cholesterol content in blood vessels and upregulate the transcription factor peroxisome proliferator-activated receptor in epididymal adipose tissue. The expression of fatty acid-binding protein 4 and carnitine palmitoyl transferase-I stimulated lipid oxidation, thereby delaying obesity [37].

Generally, the metabolic products of prebiotics are usually used as the main indicator for evaluating their effects [30]. Following absorption by gut microbiota, prebiotics are decomposed into SCFAs, including acetic acid, propionic acid, butyric acid, and other organic acids [29]. These organic acids provide energy for the body and reduce the intestinal pH, thereby promoting the growth of beneficial bacteria and creating an unfavorable environment for pathogens [38]. Acetic acid can cause weight loss, and acetic acid produced by gut microbiota enters the peripheral circulation via the veins and crosses the blood–brain barrier, thereby affecting appetite which leads to weight loss [39, 40], and stimulating leptin production via the activation of GPR43 in adipose tissue [41]. Additionally, Araújo et al. [42] reported that acetic acid activates the AMPK/PGC-1α/ PPAR α pathway, which induces fat oxidation by intestinal epithelial cells to promote the consumption of dietary lipids, thereby reducing the binding of dietary lipids with apolipoprotein and releasing them into lymph and blood.

The relationship between butyric acid, propionic acid, and obesity remains complex and somewhat contradictory. Propionic acid and butyric acid can induce the secretion of glucagon like peptide 1 and peptide YY, thereby increasing energy expenditure, suppressing appetite, and exerting anti obesity effects [43]. But their contents were positively correlated with the degree of obesity in children with obesity. Gyarmati et al. [44] found that the levels of butyric, isovaleric, and propionic acids increased significantly with the severity of obesity. Additionally, Payne et al. [45] reported that the concentrations of butyric and propionic acids were significantly higher in children with obesity. In the present study, propionic and butyric acids were significantly lower in the YCFA + K56, PG + K56, GOS, GOS + K56, XOS, and XOS + K56 groups than those in the blank control group, indicating that they can reduce the production of propionic and butyric acids in the intestines of obese children. Correlation analysis revealed that *Bifidobacterium* was negatively correlated with propionic and butyric acids synthesis, while *Lactobacillus* and propionic acid production were negatively correlated. Ruiz-Aceituno et al. [46] showed that biosynthetic pathways for propionic and butyric acids are non-existent in *Bifidobacterium* species. Although *Lactobacillus rhamnosus* ATCC 53103 secretes acetic acid in brain–heart infusion broth containing 0.1% glucose, propionic acid or butyric acid were not detected in the spent culture supernatant [47]. This may explain the low propionic and butyric acid levels observed in the GOS, GOS + K56, XOS, and XOS + K56 groups.Intestinal microbes utilize

several gases, including CO_2, H_2, CH_4, an H_2S, during fermentation. Due to differences between the compositions of the prebiotics and glycosidic bonds, the gases produced vary. Notably, these gases are detrimental to human health. Excessive gas production leads to flatulence and other gastrointestinal discomforts. Thus, gas production is considered a major adverse event associated with the consumption of prebiotics [48]. In the present study, the GOS, GOS + K56, XOS, and XOS + K56 groups produced the lowest total gas volumes, and consequently the lowest output of H_2, H_2S, and CH_4. Additionally, the GOS and XOS groups exhibited better degradation rates than the PG + K56 group. The degradation rate reflects the consumption of prebiotics by the intestinal microbiota during the fermentation process. Prebiotics are the fermentation substrates of probiotics. A higher degradation rate usually indicates that the intestinal microbiota can better utilize prebiotics [49]. Although the degradation rates of all synbiotic combinations decreased after the addition of *L. paracasei* K56, the differences were not statistically significant. This suggests that GOS and XOS have better absorption effects and lower gas production.

Despite the promising results, this study had some limitations. *In vitro* experiments may not totally capitulate *in vivo* conditions, which may lead to differences between our results and those of any *in vivo* animal experiments. Moreover, there may be differences between the results of *in vitro* studies based on simulated fermentation and those based on the actual environment in the human intestines. Therefore, clinical experiments involving human subjects are necessary to validate the results of this study. Moreover, further studies are necessary to develop optimal synbiotic combinations based on *L. paracasei* K56.

Conclusions

This study showed that GOS and XOS promoted the abundance of *Bifidobacterium* and combinations of these prebiotics with *L. paracasei* K56 increased the abundance of Limosilactocobacillus and reduce the abundance of *Escherichia/Shigella*, thereby increasing the proportion of acetic acid and reducing the amounts of propionic and butyric acids, as well as CO_2, H_2, CH_4, and H_2S in the gut. Additionally, this study provides a theoretical foundation for the development of novel K56-based synbiotics to improve childhood obesity.

Acknowledgements We would like to thank Editage (www.editage.cn) for English language editing.

Author Contributions Conceptualization: J.L. and L.L.; Methodology: J.L.; Software: J.C.; Validation: W.-L.H.; Formal analysis: J.C.; Investigation: Z.Z.; Resources: Q.J., W.-L.H., and W.Z.; Data curation: J.C. and P.Y.; Writing—original draft preparation: X.D., and P.Z.; Writing—review and editing: X.D. and P.Z.; Visualization: Y.Z. and S.Y.; Supervision: J.L.; Project administration: X.D. and C.Y.; Funding acquisition: L.L. All authors have read and agreed to the published version of the manuscript.

Funding This research was funded by the Medical and Technology Project of the Pioneer and "Pioneer" and "Leading Goose" R&D Program of Zhejiang (grant number: 2022C03138). The sponsors had no role in the design, execution, interpretation, or writing of the study.

Data Availability All sequences were submitted to NCBI under SRA accession number SRP469565 and further are available from the corresponding author on reasonable request.

Declarations

Ethics Approval The study was approved by the Ethics Committee of Hangzhou Normal University (May 26, 2020, No. 20190061).

Informed Consent Informed consent was obtained from all subjects involved in the study.

Competing Interests The authors declare no competing interests.

References

1. NCD Risk Factor Collaboration (NCD-RisC) (2016) Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19·2 million participants. Lancet 387:1377–1396. https://doi.org/10.1016/s0140-6736(16)30054-x
2. NCD Risk Factor Collaboration (NCD-RisC) (2017) Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. The Lancet 390:2627–2642. https://doi.org/10.1016/S0140-6736(17)32129-3
3. Sung H, Siegel RL, Torre LA et al (2018) Global patterns in excess body weight and the associated cancer burden. CA: A Cancer J Clin 69:88–112. https://doi.org/10.3322/caac.21499
4. Bradwisch SA, Smith EM, Mooney C, Scaccia D (2020) Obesity in children and adolescents. Nursing 50:60–66. https://doi.org/10.1097/01.nurse.0000718908.20119.01
5. Shamriz O, Leiba M, Levine H et al (2017) Higher body mass index in 16–19 year-old Jewish adolescents of North African, Middle Eastern and European origins is a predictor of acute

myeloid leukemia: a cohort of 2.3 million Israelis. Cancer Causes Control 28:331–339. https://doi.org/10.1007/s10552-017-0863-5
6. Weihrauch-Blüher S, Schwarz P, Klusmann J-H (2019) Childhood obesity: increased risk for cardiometabolic disease and cancer in adulthood. Metabolism 92:147–152. https://doi.org/10.1016/j.metabol.2018.12.001
7. Huh JY, Park YJ, Ham M, Kim JB (2014) Crosstalk between adipocytes and immune cells in adipose tissue inflammation and metabolic dysregulation in obesity. Mol Cells 37:365–371. https://doi.org/10.14348/molcells.2014.0074
8. Maruvada P, Leone V, Kaplan LM, Chang EB (2017) The human microbiome and obesity: moving beyond associations. Cell Host Microbe 22:589–599. https://doi.org/10.1016/j.chom.2017.10.005
9. Jackson VM, Breen DM, Fortin J-P et al (2015) Latest approaches for the treatment of obesity. Expert Opin Drug Discov 10:825–839. https://doi.org/10.1517/17460441.2015.1044966
10. Vallianou N, Stratigou T, Christodoulatos GS et al (2020) Probiotics, prebiotics, synbiotics, postbiotics, and obesity: current evidence, controversies, and perspectives. Curr Obes Rep 9:179–192. https://doi.org/10.1007/s13679-020-00379-w
11. Bäckhed F, Ding H, Wang T et al (2004) The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci 101:15718–15723. https://doi.org/10.1073/pnas.0407076101
12. Borgo F, Verduci E, Riva A et al (2017) Relative abundance in bacterial and fungal gut microbes in obese children: a case control study. Child Obes 13:78–84. https://doi.org/10.1089/chi.2015.0194
13. Gao X, Jia R, Xie L et al (2018) A study of the correlation between obesity and intestinal flora in school-age children. Sci Rep. https://doi.org/10.1038/s41598-018-32730-6
14. Hijova E (2021) Probiotics and prebiotics, targeting obesity with functional foods. Bratislava Med J 122:647–652. https://doi.org/10.4149/bll_2021_104
15. Hill C, Guarner F, Reid G et al (2014) The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 11:506–514. https://doi.org/10.1038/nrgastro.2014.66
16. Swanson KS, Gibson GR, Hutkins R et al (2020) The international scientific association for probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of synbiotics. Nat Rev Gastroenterol Hepatol 17:687–701. https://doi.org/10.1038/s41575-020-0344-2
17. Markowiak P, Śliżewska K (2017) Effects of probiotics, prebiotics, and synbiotics on human health. Nutrients 9:1021. https://doi.org/10.3390/nu9091021
18. Crovesy L, Ostrowski M, Ferreira DMTP, Rosado EL, Soares-Mota M (2017) Effect of *Lactobacillus* on body weight and body fat in overweight subjects: a systematic review of randomized controlled clinical trials. Int J Obes 41:1607–1614. https://doi.org/10.1038/ijo.2017.161
19. Lu H, Zhao W, Liu WH et al (2021) Safety evaluation of Bifidobacterium lactis BL-99 and *Lacticaseibacillus paracasei* K56 and ET-22 in vitro and in vivo. Front Microbiol 12:686541. https://doi.org/10.3389/fmicb.2021.686541
20. Miao Z, Zheng H, Liu WH et al (2023) *Lacticaseibacillus paracasei* K56 attenuates high-fat diet-induced obesity by modulating the gut microbiota in mice. Probiotics Antimicrob Protein 15:844–855. https://doi.org/10.1007/s12602-022-09911-x
21. Zhen S, Ma Y, Zhao Z, Yang X, Wen D (2018) Dietary pattern is associated with obesity in Chinese children and adolescents: data from China Health and Nutrition Survey (CHNS). Nutr J 17(1):68. https://doi.org/10.1186/s12937-018-0372-8
22. Wu Q, Pi X, Liu W et al (2017) Fermentation properties of isomaltooligosaccharides are affected by human fecal enterotypes. Anaerobe 48:206–214. https://doi.org/10.1016/j.anaerobe.2017.08.016
23. Duncan SH, Hold GL, Harmsen HJM et al (2002) Growth requirements and fermentation products of Fusobacterium prausnitzii, and a proposal to reclassify it as Faecalibacterium prausnitzii gen. nov., comb. nov. Int J Syst Evol Microbiol 52:2141–2146. https://doi.org/10.1099/00207713-52-6-2141
24. Zhang Q, Zhao W, Zhao Y et al (2022) In vitro study of Bifidobacterium lactis BL-99 with fructooligosaccharide synbiotics effected on the intestinal microbiota. Front Nutr 9:890316. https://doi.org/10.3389/fnut.2022.890316
25. Wang X, Li J, Li N et al (2021) Evolution of intestinal gases and fecal short-chain fatty acids produced in vitro by preterm infant gut microbiota during the first 4 weeks of Life. Front Pediatr 9:726193. https://doi.org/10.3389/fped.2021.726193
26. Álvarez-Arraño V, Martín-Peláez S (2021) Effects of probiotics and synbiotics on weight loss in subjects with overweight or obesity: a systematic review. Nutrients 13:3627. https://doi.org/10.3390/nu13103627
27. Kadeer G, Fu W, He Y et al (2023) Effect of different doses of *Lacticaseibacillus paracasei* K56 on body fat and metabolic parameters in adult individuals with obesity: a pilot study. Nutr Metab (Lond) 20:16. https://doi.org/10.1186/s12986-023-00739-y
28. Min QQ, Sun T, Xu JY et al (2020) Differential modulation of the metabolic effects of diet-induced obesity by probiotic *Lactobacillus paracasei* K56 and prebiotic α-galactooligosaccharides. Curr Top Nutraceutical Re 19:21–28. https://doi.org/10.37290/ctnr2641-452X.19:21-28
29. Topping DL, Clifton PM (2001) Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. Physiol Rev 81:1031–1064. https://doi.org/10.1152/physrev.2001.81.3.1031
30. Yali LI, Mohan W, Wen Z et al (2022) In vitro fermentation characteristics by different combination of probiotics and prebiotics. Food Ferment Ind 48:50–57.https://doi.org/10.13995/j.cnki.11-1802/ts.028781
31. Turnbaugh PJ, Hamady M, Yatsunenko T et al (2009) A core gut microbiome in obese and lean twins. Nature 457:480–484. https://doi.org/10.1038/nature07540
32. Zuo H, Xie ZM, Zhang WW et al (2011) Gut bacteria alteration in obese people and its relationship with gene polymorphism. World J Gastroenterol 17:1076–1081. https://doi.org/10.3748/wjg.v17.i8.1076
33. Li Z, Summanen PH, Komoriya T, Finegold SM (2015) In vitro study of the prebiotic xylooligosaccharide (XOS) on the growth of Bifidobacterium spp and Lactobacillus spp. Int J Food Sci Nutr 66:919–922. https://doi.org/10.3109/09637486.2015.1064869
34. Liu F, Li P, Chen M et al (2017) Fructooligosaccharide (FOS) and galactooligosaccharide (GOS) increase Bifidobacterium but reduce butyrate producing bacteria with adverse glycemic metabolism in healthy young population. Sci Rep 7:11789. https://doi.org/10.1038/s41598-017-10722-2
35. Nicolucci AC, Hume MP, Martínez I et al (2017) Prebiotics reduce body fat and alter intestinal microbiota in children who are overweight or with obesity. Gastroenterology 153:711–722. https://doi.org/10.1053/j.gastro.2017.05.055
36. Fehlbaum S, Prudence K, Kieboom J et al (2018) In vitro fermentation of selected prebiotics and their effects on the composition and activity of the adult gut microbiota. Int J Mol Sci 19:3097. https://doi.org/10.3390/ijms19103097
37. Kim DH, Jeong D, Kang IB (2017) Dual function of Lactobacillus kefiri DH5 in preventing high-fat-diet-induced obesity: direct reduction of cholesterol and upregulation of PPAR-α in adipose tissue. Mol Nutr Food Res 61:1700252. https://doi.org/10.1002/mnfr.201700252
38. Tan J, McKenzie C, Potamitis M et al (2014) The role of short-chain fatty acids in health and disease. Adv Immunol 121:91–119. https://doi.org/10.1016/B978-0-12-800100-4.00003-9

39. Braniste V, Al-Asmakh M, Kowal C et al (2014) The gut microbiota influences blood-brain barrier permeability in mice. Sci Transl Med 6:263ra158. https://doi.org/10.1126/scitranslmed.3009759
40. Frost G, Cai Z, Raven M, Otway DT, Mushtaq R, Johnston JD (2014) Effect of short chain fatty acids on the expression of free fatty acid receptor 2 (Ffar2), Ffar3 and early-stage adipogenesis. Nutr Diabetes 4:e128. https://doi.org/10.1038/nutd.2014.25
41. Zaibi MS, Stocker CJ, O'Dowd (2010) Roles of GPR41 and GPR43 in leptin secretory responses of murine adipocytes to short chain fatty acids. FEBS Lett 584:2381–2386. https://doi.org/10.1016/j.febslet.2010.04.027
42. Araújo JR, Tazi A, Burlen-Defranoux O (2020) Fermentation products of commensal bacteria alter enterocyte lipid metabolism. Cell Host Microbe 27:358-375.e7. https://doi.org/10.1016/j.chom.2020.01.028
43. Fan Y, Pedersen O (2021) Gut microbiota in human metabolic health and disease. Nat Rev Microbiol 19(1):55–71. https://doi.org/10.1038/s41579-020-0433-9
44. Gyarmati P, Song Y, Dotimas J, Yoshiba G, Christison A (2021) Cross-sectional comparisons of gut microbiome and short-chain fatty acid levels among children with varied weight classifications. Pediatr Obes 16:e12750. https://doi.org/10.1111/ijpo.12750
45. Payne AN, Chassard C, Zimmermann M et al (2011) The metabolic activity of gut microbiota in obese children is increased compared with normal-weight children and exhibits more exhaustive substrate utilization. Nutr Diabetes 1:e12. https://doi.org/10.1038/nutd.2011.8
46. Ruiz-Aceituno L, Esteban-Torres M, James K (2020) Metabolism of biosynthetic oligosaccharides by human-derived Bifidobacterium breve UCC2003 and Bifidobacterium longum NCIMB 8809. Int J Food Microbiol 316:108476. https://doi.org/10.1016/j.ijfoodmicro.2019.108476
47. Calvigioni M, Bertolini A, Codini S (2023) HPLC-MS-MS quantification of short-chain fatty acids actively secreted by probiotic strains. Front Microbiol 14:1124144. https://doi.org/10.3389/fmicb.2023.1124144
48. Probert HM, Gibson GR (2002) Investigating the prebiotic and gas-generating effects of selected carbohydrates on the human colonic microflora. Lett Appl Microbiol 35:473–480. https://doi.org/10.1046/j.1472-765X.2002.01223.x
49. Zhang Q, Zhao W, He J et al (2024Jul) (2024) Effect of Lacticaseibacillus paracasei K56 with galactooligosaccharide synbiotics on obese individuals: an in vitro fermentation model. J Sci Food Agric 104(9):5042–5051. https://doi.org/10.1002/jsfa.13359