Comparisons of Effects on Intestinal Short-Chain Fatty Acid Concentration after Exposure of Two Glycosidase Inhibitors in Mice

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Acarbose and voglibose are the most widely used diabetes drugs as glycosidase inhibitors. In this study, the use of these two inhibitors significantly increased the content of starch in large intestine, and altered the concentration of short-chain fatty acids (SCFAs) by affecting the intestinal microbiota. However, there are some differences in the intestinal microbiome of the two groups of mice, mainly in bacteria such as Bacteroidaceae bacteroides and Desulfovibrionaceae desulfovibrio. The productions of acetate and propionate in caecum in voglibose group were significantly higher than those in acarbose group and two kinds of glycosidase inhibitors were close in the production of butyrate in caecum. The Tax4Fun analysis based on Kyoto Encyclopedia of Genes and Genomes (KEGG) data indicated that different productions of acetate and propionate between acarbose group and voglibose group may be related to 2-oxoisovalerate dehydrogenase and pyruvate oxidase. In addition, in-vitro experiments suggested that voglibose had less effect on epithelial cells than acarbose after direct stimulation. According to the recent researches of SCFAs produced by intestinal microbiota, our comparative study shown higher concentration of these beneficial fatty acids in the lumen of voglibose-treated mice, which implied a lower level of inflammation.

Key words acarbose; voglibose; microbiota; short-chain fatty acid; Tax4Fun

With the increasing number of obesity and diabetes around the world, there are more and more different classes of medications to treat diabetes, such as biguanides (metformin), sulfonylureas, insulin, glucagon-like peptide 1 (GLP-1) agonists, dipeptidyl peptidase IV inhibitors, thiazolidinediones and sodium-glucose transporter 2 inhibitors (to name a few). However, acarbose and voglibose as part of α-glucosidase inhibitors (AGIs) are another class of anti-diabetic medications. As first-line hypoglycemic drugs used in patients with type 2 diabetes especially in Asian since their diet meals contain high content of carbohydrates, acarbose and voglibose can typically reduce postprandial glucose level by delaying carbohydrate digestion in the gut, so they can be a useful treatment in the patients who raised basal glucose concentrations slightly and marked postprandial hyperglycaemia. In addition, there will also be some side effects, mainly reflected in gastrointestinal flatulence, loose stool and so on. The main reason for this is that fermentation of undigested carbohydrates by bacteria produced gas in the colon.

Acarbose and voglibose treatment delayed starch digestion in the small intestine but there was compensatory salvage through bacterial fermentation in the large intestine. When carbohydrates (starch, dietary fiber) enter the large intestine, they are degraded by the fermentation of anaerobic flora. Under normal diet situations, the better part of starch was digested and absorbed in the small intestine, therefore, extra starch entering the large bowel changed microbial eco-system to some degree. Some enteric bacterial species own specialized metabolic enzymes, therefore different carbohydrate fermentation contributes diverse influences on the intestinal microbiota. For example, the digestion of resistant starch by intestinal bacteria can have an impact on the structure of the large intestinal microbiota, and resistant starch fermentation often contributes to an increase in Bifidobacterium spp., Parabacteroides spp., Ruminococcus spp., and Eubacterium spp.6,7 According to accumulating evidence, gut microbiota play an intricate role in obesity and diabetes with participating in host metabolism by regulating immune system, increasing energy extraction and altering lipid metabolism.8,9 Some researchers believe that the treatment of diabetes drugs have a certain relationship with gut microbiota. It was showed that in high fat diet (HFD)-fed mice given the prebiotic oligofructose, Bifidobacterium spp. correlated with many indicators significantly and positively.10 Similarly, Shin et al. demonstrated that HFD-fed mice showed a higher level of bacterium Akkermansia after treated with metformin.11 As acarbose is metabolized only within the gastrointestinal tract, Zhang et al. hypothesize that regulation of gut microbiota maybe one of mechanisms of acarbose to diabetes and confirmed the hypothesis through a randomized, double-blind, controlled crossover experiment.12 Moreover, Su et al. showed that the gut microbiota Bifidobacterium longum and Enterococcus faecalis were increased significantly after 4 weeks of anti-diabetic treatment in both diabetes groups.13 In addition, although Blanch et al. revealed that addition of acarbose decreased the ratio of Streptococcus bovis to Megasphaera elsdenii relative to the control group,14 little evidence is available on the alteration of acarbose on microbial community.15

According to studies during these years, it was well-known that short-chain fatty acids (SCFAs) might be a key aspect in the prevention and treatment of bowel disorders, metabolic syndrome and certain types of cancer.16–21 Generally, dietary fiber intake results in intestinal fermentation and soluble fiber is the main source.22 Carbohydrate fermentation by gut microbiota produces the byproducts as main source of SCFAs in the body23 and with acarbose treatment more carbohydrates...
for fermentation in colon may support changes in gut microbiota and promote SCFA production.24,25 Acarbose treatment in rats contributes to a higher level of SCFA in the colon,4 and Weaver et al. have also showed increased colonic butyrate production in human studies of acarbose supplementation.25 SCFAs are an indispensable waste product to the microbial community,26 meanwhile for colonic epithelium and immune modulation SCFAs especially butyrate are thought to be beneficial.27,28 The short-chain fatty acid receptors Free Fatty Acid Receptor 2 (FFA2 or GPR43) and Free Fatty Acid Receptor 3 (FFA3 or GPR41) are activated by acetate, propionate and butyrate29 but the metabolic function of them remains controversial. Sleeth et al. indicated that the binding of SCFAs to FFA2 and FFA3 located on L-cells may participate in controlling glucagon-like peptide 1 (GLP-1).30 In contrast, another study reported that SCFAs have no effect on GLP-1 in vivo.31 Besides, Paiva et al. indicated that acarbose treatment did not change the gene expression of sodium glucose cotransporter 1 (SGLT1) and glucose transporter 2 (GLUT2) but increased GLUT1 protein by 75% in middle small intestine.32 Moreover, monocarboxylate transporter 1 (MCT1) and sodium-coupled monocarboxylate transporter 1 (SMCT1), as SCFA transporters, were indispensable for SCFAs33 and butyrate is taken up into colonic enterocytes either by passive diffusion of the free acid or via the apical uptake transporters MCT1 and SMCT1.34 The association between acarbose, SCFAs and genes of these receptors and co-transporters remains further studies.

In summary, it was found that the physiological changes after acarbose exposure, including intestinal microflora and short chain fatty acid concentration, had some preliminary studies. However, reports about effects of voglibose exposure were not much. Therefore, we detect the content of starch, SCFA concentration, microbiota and gene expression in the level of tissue and cell to compare different effects of exposure with acarbose and voglibose through the long-term intragastric administration on ICR mice.

MATERIALS AND METHODS

Animals Male ICR mice (6 weeks old) purchased from the SLAC Laboratory Animal Co., Ltd. (Shanghai, China) were used in this study. Mice were acclimatized for a week before the study and then assigned to three groups. All the mice were housed in cages under the condition: constant temperature (24°C), humidity (40–70%) and a 12 h light/12 h dark cycle before and during the experiment. Mice were allowed access to standard rodent diet and water until the experiment started.

Experimental Design After the acclimatization, mice were divided into 3 groups and treated for two weeks as designed. To mimic the human meals, mice were given two meals a day in the inhibitor-treated assay. Mice were given 300 μL water with or without designed inhibitor 10 min before each meal by intragastric administration, and then free access to the standard chow diet mixed with 1% titanium dioxide for 1 d (two meals). The result was shown in Fig. S1, which indicate that the base amount (1×) was sufficient for the inhibitor to slow down the starch digestion.

Cell Culture Colon carcinoma cell line Caco-2 were cultured in 1640 medium, respectively, with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 units/mL penicillin, 100 units/mL streptomycin in a humidified atmosphere with 5% CO2 at 37°C. The Caco-2 cells were grown in 60 mm polystyrene culture dishes (Corning Inc., Corning, NY, U.S.A.) and medium was exchanged every three days. Both cells were separately treated with low doses and high doses of glucose, acarbose, voglibose, acetic acid, propionic acid and butyrate acid for 24 h, phosphate buffered saline (PBS) was used as control.

Sample Preparation The whole intestine, including small intestine and large intestine, was removed from anesthetized mice. The contents of ileum, cecum and colon segment were all removed and put into the EP tube and placed on the ice. These contents were kept frozen until further processing for testing starch concentration, determination of SCFAs and de...

Table 1. Oligonucleotides Used for Measurement of mRNA Levels

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<tr>
<th>Primer name</th>
<th>Primer sequence (5′→3′)</th>
<th>References</th>
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<td>GAPDH-Human F</td>
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<td>R AAATGGGCCCCAGCTTC</td>
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<td>FFA2-Mouse F</td>
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<td>R TGAAGATGTAGAGGAGCAG</td>
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tection of micro-ecology. The pH of content samples were detected in a 10-fold dilution method as previously described.36)

Intestinal segment tissues of ileum, cecum and colon were removed and cleared of food residue by washing with diethyl pyrocarbonate-containing saline. Each intestinal segment was put into a 2 mL tube, followed by immediate addition of Trizol (15596018, Thermo Fisher, U.S.A.).

Cells stimulated by various substances were collected, centrifuged and then added with Trizol. The samples were stored at −80°C for quantitative PCR analyses.

RT and Real-Time Quantitative PCR Analyses Total RNA was isolated from the tissue samples and cells samples then reverse transcribed with a HiScript Q RT SuperMix for qPCR (R123-01, Vazyme, China), and a PrimeScript™ RT reagent kit with gDNA Eraser (TaKaRa, Shiga, Japan) according to the manufacturer's instruction manuals. Real-time quantitative PCR was conducted with StepOne Real-Time PCR System (4376374, Thermo Fisher). Primers generated in this study were designed by Beacon Designer™ free edition. The primer sequences are shown in Table 1. All reactions for real-time quantitative PCR were carried out with the following parameters: 95°C for 15s, 60°C for 20 s, and 40 cycles of 72°C for 20 s.

Dry Matter and SCFA Measurement The caecum content samples were dried to a constant weight with a constant temperature at 103°C (ISO standard 6496; International Organization for Standardization, 1999). The content samples were thawed and H3PO4 was added, then mixed in tubes with a vortex, and centrifuged at 20000×g for 5 min. The supernatant was diluted 1:1 with isocaproic acid solution. Caecum SCFA concentrations including acetate, propionate and butyrate were measured by gas chromatography (Fisons HRGC Mega 2; CE Instruments) at 190°C. N2 saturated with methanoic acid was determined to be as the carrier gas and isocaproic acid was used as an internal standard.

Total Carbohydrate Assay Starch contents of intestinal samples were measured mainly according to the analysis procedure provided by the Resistant Starch Assay Kit (catalog number K-RSTAR, AOAC Official Method 2002.02 and AACC Method 32–40 (McCleary et al., 2002) Megazyme International Ireland Ltd. Co., Wicklow, Ireland) with the following modification for few contents from small intestine of laboratory animals. In brief, 400 µL of mixture (pancreatic α-amylase, 10mg/mL, and amyloglucosidase, 3 U/mL) was added to each 100mg of sample (in a 1.5 mL tube, T1) and incubated in a shaker with 200 strokes/min for 16 h at 37°C. After incubation, 400 µL of ethanol (99%) was added and stirred vigorously (stop reaction), and then centrifuged at 12000×g for 2 min. Samples were centrifuged at 12000×g for 3 min. Then 10 µL of the supernatant and 300 µL of glucose

![Fig. 1. Determination of Components in Different Intestinal Segments of Male ICR Mice after Two-Week Treated with Acarbose and Voglibose](image-url)

(A) Measurement values of content in ileum. (B) The relative content in ileum. (C) The relative content in caecum. (D) The relative content in colon. The relative content means the value calculated after internal correction with titanium dioxide, which is equal to the ratio of measurement values of target composition to corresponding titanium dioxide. Values are mean±S.E.M., n=5 mice per treatment. * indicates that the content differs significantly from the control group, p<0.05. The small intestine was divided into 5 equal parts and the content of the last segment was measured. The white bar represents control group, the gray bar represents acarbose group and the dark gray bar represents voglibose group.
oxidase-peroxidase-aminoantipyrine (GOPOD, >12000 U/L glucose oxidase; >650 U/L peroxidase; 0.4 mM 4-aminoantipyrin) were added respectively and the mixture was incubated in the water bath at 50°C for 30 min. Absorbance was measured using a 96-well micro-plate reader (Spectra Max 190, Molecular Devices, Sunnyvale, CA, U.S.A.) at 510 nm. Sodium acetate buffer (0.1 M, pH 4.5) were used as a blank, glucose with different concentration (start from 500 mg/mL in 0.2% benzoic acid, end at 0.8 mg/mL) was tested as a standard curve. The glucose concentration of liquid was calculated depend on the standard curve.

16S Ribosomal RNA (rRNA) Gene Sequencing of the Gut Microbiota

The gut microbiota was investigated using 16S rRNA gene sequencing in intestinal content samples. DNA was isolated from every content sample from one group, using a PowerSoil DNA Isolation Kit (MO BIO Laboratories) according to the manufacturer’s instructions, and the resultant DNA was quantified and stored at −80°C until further analysis. When sequencing, every three DNA from the same treated group were joined together as the same ration. The V4 region in the 16S rRNA gene was targeted using the universal primers 515 (5′-GTG CCA GCMGCCGCGGTAA-3′) and 806 (5′-GGA CTA CHVGGGTWTCTAAT-3′). For each sample, 1 ng of the purified DNA was used as a template for amplification and then barcoded with specific indexes. The amplified products were then normalized, pooled and sequenced by an Illumina MiSeq (Lianchuan, Hangzhou, China). The data were then analyzed using quantitative insights into microbial ecology (QIIME, version 1.9.1).37 The functional genes of bacterial communities based on the 16S rRNA sequencing data was analyzed by Tax4Fun, which provide a good approximation to the gene profiles obtained from metagenomic shotgun sequencing methods. The results from Tax4Fun were further analyzed using Statistical Analysis of Metagenomic Profiles (STAMP) (version 2.1.3).38

Statistical Analysis

All data were expressed as the mean±standard deviation (S.D.). Figures were analyzed using Origin 2017 software. One-way ANOVA and Dunnett’s multiple comparison post-hoc tests were performed for all results. p values <0.05 were considered statistically significant.

RESULTS

Acarbose and Voglibose Inhibited Starch Digestion in Different Intestinal Segments

At the beginning of the experiments, we had set a one day’s pre-experiment for the ileum samples to test the suitable concentration of inhibitors as mentioned above (Fig. S1), besides, we have also detected the starch digestion of the ileum samples at the third day during the two weeks’ assay (Fig. S2). Both one day’s and three days’ results ensured that the un-digestion starch would enter the lager intestine in our experiment.

After two weeks of intervention with acarbose and voglibose (treated group), the content of total sugars in the ileum was analyzed using the method described above. As expected, the content of starch in the treated groups was significantly
higher than that in the control group (Fig. 1A). Moreover, the content of oligosaccharide in the treated groups was slightly lower than that in the control group. In order to exclude the interference of water in the content on the result, the contents of different intestinal segments were detected and internal corrected with titanium dioxide. It was shown that the relative contents of glucose, oligosaccharide and starch in the ileum shared the similar trend (Fig. 1B). In the ileum or caecum, the relative content of oligosaccharide showed no significant difference between treated group and control group (Figs. 1B, C), but that of treated group was much higher than control group in the colon (Fig. 1D). The relative content of starch in treated groups was significantly higher than that in the control group in different intestinal segments including ileum, caecum and colon. This result demonstrated that the digestion of starch is greatly inhibited in the intestine.

**Effects of Acarbose and Voglibose on SCFA Concentrations in the Caecum**

It was known that the higher content of carbohydrates, induced by pectin or resistant starch, enhanced the fermentation of SCFA in the large intestine by the intestinal microbiota. We first detected the pH of content sample from each group, the results are as follows: water (6.99±0.12), acarbose (6.45±0.20), voglibose (6.32±0.13). It seems like that the rising carbon source lower the lumen pH. Then, the SCFA concentrations were measured in the caecum contents collected at section. In the acarbose or voglibose treated group, it was shown that the content of total SCFA was significantly higher than that in the control group (Fig. 2D), but the variation of different components was inconsistent. The butyrate concentration was significantly higher than that in the control group after intervention of acarbose and voglibose (Fig. 2C). However, voglibose significantly increased the concentrations of acetate and propionate while there was no significant difference in acarbose group compared to the control group (Figs. 2A, B).

**Effects of Acarbose and Voglibose on Microbiota in the Caecum**

The relative abundance of bacteria showed significant difference after glycosidase inhibitors treated for two weeks, and we selected eight genera which were representative. As shown in Fig. 3, the relative abundances of Bacteroidaceae bacteroides, Rikenellaceae alstipes and Lachnospiraceae blautia were significantly higher than the control group after two-week treated with glycosidase inhibitors. The relative abundances of Desulfovibrionaceae desulfovibrio, Ruminococcaceae ruminiclostridium 6 and Ruminococcaceae ruminococcaceae UCG-005 were significantly lower than the control group after two-week treated with glycosidase inhibitors. It is worth noting that the relative abundances of Ruminococcaceae ruminococcus 2 and Family XIII family XIII UCG-001 decreased after two-week treated with acarbose while increased after two-week treated with voglibose. This result indicates that acarbose and voglibose, both belong to glycosidase inhibitors, significantly changed the gut microbiota but they had very different effects on some microbes.

The top 40 genus of each treatment mice in caecum can be found in the supplemental information (Fig. S3), and the detailed data (relative abundance of each microbiome) was also included in a supplemental excel file (genus_list.xlsx).
Different Effects on Microbiota between Acarbose and Voglibose through the Tax4Fun Analysis Based on Kyoto Encyclopedia of Genes and Genomes (KEGG) Data

Whether the changes in gut microbiome composition were associated with functional perturbations of the gut bacteria was analyzed by Tax4Fun. Indeed, a number of bacterial functional genes were enriched in glycosidase inhibitors-treated mice, and different enrichments were predicted between acarbose and voglibose group. For example, a pyruvate oxidase (K00158), related to acetate metabolism, was highly elevated in voglibose-treated mice compared to the control group, but there is no significant difference between the acarbose and voglibose group. For example, a pyruvate oxidase (K00158), related to acetate metabolism, was highly elevated in voglibose-treated mice compared to the control group, but there is no significant difference between the acarbose and voglibose group as shown in Figure; three subunit of 2-oxoisovalerate dehydrogenase, K00166, K00167 and K09699, related to propionate metabolism were predicted significantly increased after voglibose treatment compared both control and acarbose group (Fig. 4). It was worth noting that multiple genes related to butyrate metabolism showed no significant difference among the control, acarbose and voglibose groups (Fig. 4), although the concentration of butyrate were different between the three groups.

The Expression of Genes Related to Induction and Transport of Glucose and SCFA in Intestinal Tissues

In order to verify the results of genetic changes at the tissue level, the same work was undertaken at the cellular level based on Caco-2 cell line. No significant modulation was found in MCT gene expression after stimulation with different substances (Fig. 6). In addition, the stimulation with low dose created no change to SMCT gene expression (Fig. 6B), when it comes to high dose, the expression of SMCT in propionate group and butyrate group was significant higher than that of in control group (Fig. 6A). As shown in Fig. 6A, the stimulation of with high dose led to great impacts on genes related to glucose transport at section. The expression of SGLT1 in acarbose, propionate and butyrate group was much lower than that in the control group. Similarly, the expression of GLUT2 in acarbose group and acetate group was much lower than that in the control group, but much higher in butyrate group compared with the control group. Moreover, the stimulation of acarbose, voglibose and propionate with low dose also inhibit the expression of GLUT2. Understandably, acarbose and voglibose, as glycosidase inhibitors, delayed the digestion and absorption of starch and it was also consistent with the result. However, the effect of short chain fatty acids on the expression of genes related to glucose transporter needs further study.

DISCUSSION

In the present study, we compared differences between the intestinal microflora of acarbose and voglibose after long-term treatment...
exposure, and the changes caused by the concentration of SCFA, and differences in expression of genes related to SCFA metabolism at the level of transcription in vivo and in vitro.

The concentrations of SCFA, including acetate, propionate and butyrate in intestinal tract increased in varying degrees after extra addition of slowly digested carbohydrates in daily diets, such as resistant starch, guar gum, pectin, inulin and so on. In this study, acarbose and voglibose significantly increased butyrate concentration (Fig. 2C) and voglibose significant increased concentrations of acetate and propionate but acarbose did not (Figs. 2A, B). We hypothesized that the two inhibitors had different effects on the fermentation of SCFA in the intestinal microenvironment. In the comparative analysis of micro-ecological community related to SCFA fermentation, we find that two major microbiome, Desulfovibrionaceae desulfovibrio, and Bacteroidales S24-7 group, showed significant difference between the two inhibitor-treated groups. The relative abundance of Desulfovibrionaceae desulfovibrio was lower in inhibitor-treated groups, while the Bacteroidales S24-7 group was enhanced by the treatment, which seems to promote the production of total SCFA as shown in Fig. 2. There was no significant difference in the production of butyrate between two inhibitor-treated groups, but the voglibose group had the higher production of acetate and propionate. We speculated the different abundance of the Bacteroidales S24-7 group between two inhibitor groups was closely related to the different production of acetate and propionate. It is worth noting that the relative abundance of Bacteroidaceae bacteroides, which rose in the inhibitor treated groups, is higher in voglibose group. The Bacteroidaceae bacteroides became one of the top5 genera in the voglibose-treated mice, which indicated a relationship to the higher production of acetate and propionate. The ratio of R. ruminococcus 2 and Family XIII family XIII UCG-001 in voglibose group is significantly higher than that in acarbose group. The ratio of R. ruminococcus 2 and Family XIII family XIII UCG-001 in acarbose group is significantly higher than that in control group. Gut microbiota was changed with the altered pH. In our study, the caecum microbiota of inhibitor-treated mice was affected by at least three reasons: 1) the more carbon source; 2) the lowered lumen pH; and 3) the present of acarbose or voglibose molecules. Depend on our data, it remains unclear what is the dominant reason that altered the gut microbiota. Further research should test the inhibitor molecules in a mimic gut fermentation tank in-vitro.

After analyzing the diversity of intestinal microflora, the strength of related enzymes in SCFA fermentation pathway was predicted based on KEGG database and the result indicated that K00158, K00166, K00167 and K09699 could be key enzymes which determine the different production of SCFA between acarbose group and voglibose group. Pyruvate oxidase and 2-oxoisovalerate dehydrogenase are important enzymes for the fermentation of acetate and propionate respectively, and the strength of these two enzymes in voglibose group was significantly higher than acarbose group and control group. Fortunately, this prediction agrees with the actual production of SCFA. It is worth noting that in our KEGG prediction results, no accurate approaches were found to explain the reason of acarbose and voglibose group significantly improving the production of butyrate. This suggests that (1) the KEGG function prediction based on the Tax4Fun system has its limitations. (2) There may be a more complex pathway for the fermentation of butyrate. The result that acarbose in-

Fig. 5. Changes of Relative Gene Expression of FFA2, FFA3, MCT, SMCT, SGLT1 and TIR3 in Different Intestinal Segments of Male ICR Mice after the Treatment with Acarbose and Voglibose for Two Weeks, as Determined by qRT-PCR

(A) Changes of relative gene expression in ileum tissue samples. (B) Changes of relative gene expression in caecum tissue samples. (C) Changes of relative gene expression in colon tissue samples. Bars from left to right represent the control group, acarbose group and voglibose group in order. Values are mean±S.E.M., n=5 mice per treatment (*p<0.05).
creased butyrate production in this study was consistent with the previous conclusion, however, that voglibose increased butyrate production was reported for the first time in this paper.

The expression of genes related to induction and absorption of glucose and SCFA was detected in different intestinal tissues of mice, including ileum, caecum and colon. Effects of acarbose and voglibose on the above genes were specific to location, more concretely, it is related to the fermentation site of microbes. MCT1 and SMCT1, related to SCFA transport, were significantly affected in ileum where microorganism content is not high and this may be associated with the abnormal occurrence of SCFA. Moreover, acarbose group and voglibose group shared same trend. In the position of the caecum and colon, expression of MCT1 and SMCT1 seems to be not associated with the exposure of acarbose and voglibose, but the expression of SCFA-induced genes, FFA2 and FFA3, changed significantly. It is worth noting that the expression of FFAR3 (GPR41), which was highly specific with acetate and propionate, changed significantly compared to control group and acarbose group, and this is also consistent with test data of SCFA above in voglibose group. In Caco-2 cells, we simulated the exposure of SCFA in intestinal tract. High dose propionate had a significant effect on the expression of SMCT1, which is consistent with Itagaki's work. The direct effects of acarbose and voglibose on Caco-2 cells were different from each other, and voglibose seemed to have no significant effect on epithelial cells while acarbose significantly decreased the expression of SMCT1, SGLT1 and GLUT2, and this is not similar to the results reported previously. The differences may
be attributed to different carriers, the work was carried out in vivo but Caco-2 cells were stimulated directly with acarbose and voglibose in this study. Interestingly, the expression of genes related to SCFA transport showed the opposite result at the level of tissue and cell and it is unable to explain the possible reasons at present. We speculated that vitro experiments still cannot simulate the complex regulation of the body, especially the signal transmission between different types of cells.

It was found that the production of SCFA varied from acarbose group to voglibose group, mainly reflected in the productions of acetate and propionate. It is clear that acetate can affect the fermentation of *Escherichia coli,* propionate is associated with liver clearance, and butyrate may be associated with inflammatory reaction. We can not decide which inhibitor is better because there is no suitable criterion, but in our opinion, compared with acarbose-treated group, voglibose-treated group would maintain a lower level of inflammation with the same anti-digestion effect of starch.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Materials

The online version of this article contains supplementary materials.

REFERENCES


