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An Enteral Formula Containing Fermented Milk Products and Prebiotics Promotes Glucagon-Like Peptide-1 Secretion via Short Chain Fatty Acid Signaling

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Abstract

An enteral formula containing fermented milk products and prebiotics (prebiotic formula, PF) is known to promote the proliferation of *Bifidobacterium* and the production of short-chain fatty acids (SCFAs) in humans and rats. We studied the effect of PF on the secretion of glucagon-like peptide-1 (GLP-1) and the involvement of SCFAs in this process, using the knockout (KO) mice for the SCFA receptors G protein-coupled receptor 41 (GPR41) and G protein-coupled receptor 43 (GPR43). Wild type (WT) or KO mice were fed either a standard formula (SF) or PF for two weeks, and then were orally administered either PF or SF after overnight fasting and dissected after 0, 30, 60, and 240 minutes. Blood GLP-1 and glucose levels were measured (Experiment 1). Alternatively, mice fed SF or PF for two weeks were dissected after four hours of fasting, and their blood GLP-1 and cecal SCFAs levels were measured (Experiment 2). In Experiment 1, WT and GPR43KO mice showed a significant increase in GLP-1 concentration 30 and/or 60 minutes after formula administration in the PF group compared with that in the SF group. Similarly, WT and GPR43KO mice showed a significant suppression of the increase in glucose levels after formula administration in the PF group compared with that in the SF group. On the other hand, there was no significant difference in GLP-1 concentration or blood glucose levels between the two treatment groups in GPR41KO mice. In Experiment 2, there was a significant increase in cecal SCFA levels in the PF group compared with that in the SF group for all mice, as well as an increase in GLP-1 concentration. PF promotes GLP-1 secretion and SCFAs might contribute to the GLP-1 secretion that occurs directly after ingestion, through GPR41 signal transduction.

Keywords: Enteral nutrition; Fermented milk product; Prebiotics; GPR41; GPR43; GLP-1

Abbreviations: BGS: Bifidogenic Growth Stimulator; EDTA-2Na: Ethylenediaminetetraacetic Acid Disodium Salt; ELISA: Enzyme-Linked Immunosorbent Assay; GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase; GI: Gastrointestinal; GLP-1: Glucagon-Like Peptide-1; GOS: Galacto-Oligosaccharides; GPR41: G Protein-Coupled Receptor 43; GPR43: G Protein-Coupled Receptor 43; HPLC: High-Performance Liquid Chromatography; KO: Knockout; PF: Prebiotic Formula; s.d.: Standard Deviation; SCFAs: Short-chain Fatty Acids; SF: Standard Formula; WT: Wild Type.

Introduction

In recent years, several studies have indicated that short-chain fatty acids (SCFAs) produced by intestinal bacteria are not only used as an energy source but also as signaling molecules that interact with SCFAs receptors such as G protein-coupled receptor 41 (GPR41, also known as FFAR3) and G protein-coupled receptor 43 (GPR43, also known as FFAR2) to carry out various physiological functions [1-3]. L cells in the small intestine express GPR41 and GPR43, and upon activation by SCFAs such as acetic acid, propionic acid, and butyric acid secrete the gastrointestinal hormone glucagon-like peptide-1 (GLP-1) [3,4]. The main function of GLP-1 is to stimulate the secretion of insulin from the pancreas and maintain regular blood glucose levels after eating [5]. GLP-1 also acts on other organs in the body, such as liver, adipose tissue, cardiovascular system, and central nervous system, in order to maintain glucose homeostasis [6]. Additionally, animal studies with GPR41 and GPR43 knockout (KO) mice confirm that SCFAs induce GLP-1 secretion and regulate carbohydrate metabolism via GPR41 and GPR43 [7].

In patients where oral administration of nutrients is difficult due to dementia, nutritional deficiency, cerebrovascular disorders, severe injury, or gastrointestinal (GI) tract surgery, nutritional management is conducted via enteral nutrition. Elderly people or patients who are highly susceptible to infection, for example after surgery, produce stress hormones that lead to insulin resistance, resulting in hyperglycemia [8]. In such cases, blood glucose levels need to be managed. Up until now, this has been accomplished by changing the type and amount of carbohydrates used for enteral nutrition. However, very recently, glycemic control using prebiotics and probiotics has been shown to be effective [9]. An enteral prebiotic formula (PF) has been developed and is used in the clinical setting. PF contains products obtained from milk fermentation by *Lactobacillus bulgaricus* and *Streptococcus thermophilus,* prebiotics such as galacto-oligosaccharides (GOS) and bifidogenic growth stimulator (BGS), and more water-soluble dietary fiber than that in standard formula (SF). In human clinical trials, elderly people who received PF as part of their enteral nutrition showed an increase in *Bifidobacterium* counts in their feces and an improvement in stool quality [10,11]. In addition, PF administration was associated with an increase in the number of *Bifidobacterium* in the cecum and SCFA levels in a rat enteral nutrition model [12].

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GOS reach the large intestine without being broken down by human digestive enzymes, and are utilized by the intestinal bacteria, preferentially by *Bifidobacterium* [13]. GOS intake positively affects mouse intestinal flora, especially the amount of *Bifidobacterium* [14]. BGS has a similar intestinal regulatory effect [15,16]. The increase in the amount of *Bifidobacterium* in the intestine promotes the synthesis of lactic acid and acetic acid via the carbohydrate metabolism.

Because PF promotes SCFAs production by intestinal bacteria such as *Bifidobacterium*, and consequently induces the secretion of GLP-1, we hypothesized that PF might be effective in blood glucose management during enteral nutrition.

Thus, we conducted a comparative investigation on the effect of PF on GLP-1 secretion and the involvement of SCFAs in this process. For this purpose, we used wild type (WT), and GPR41KO and GPR43KO mice.

Materials and Methods

Experimental animals

The WT C57BL/6 mice used in this study were purchased from Clea Japan, Inc. (Tokyo, Japan). GPR41KO and GPR43KO mice were generated as described previously [2,17]. During the experiments, all mice were kept on a 12 hours light/dark cycle (lights on at 7 AM), a room temperature of 21 ± 2°C, and a humidity of 55 ± 15%. The mice were free to drink water and eat food, except before dissection.

The study protocol was approved by the Ethics Committee of Meiji Co., Ltd. Animals were handled according to Meiji Co., Ltd. Guidelines for Animal Care and Use.

Experimental diet and experimental methods

Experimental diet: Commercially available SF (Meibalance®1.0, Meiji, Tokyo, Japan) and PF (YH-Flore®, Meiji) were used as diet for the experiments. The experimental diet was powdered after lyophilization, solubilized in water, and orally administered. Fermented milk products were used as a protein source in PF, which also included the prebiotics GOS and BGS. The fermented milk products were prepared by fermenting skim milk with *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*. Furthermore, PF had 1.8 times the dietary fiber content of SF (Table 1). Aside from these differences, the nutrient ratios in SF and PF were similar.

Experiment 1: Blood collection after overnight fasting, and 30, 60, and 240 minutes after oral administration: Male 9- to 10-week-old WT, GPR41KO, or GPR43KO mice with average body weights of 22.7 ±1.0 g, 21.3 ± 1.4 g, and 24.7 ± 2.0 g, respectively, were divided into two groups per strain, with equal average weights. Each group was fed with SF or PF for two weeks. Feeding was stopped before turning off the lights on the day before dissection. On the day of dissection, some animals did not receive formula and were dissected in a fasting state, the remaining were given 12 kcal/kg of the formula corresponding to their treatment group.

		Units	100 kcal (100 mL)	
			Standard Formula	Prebiotic Formula
Macronutrients	Protein[1]	g	4.0	4.0
	Carbohydrate	g	15.5	16.1
	Fat	g	2.8	2.8
Prebiotics	Dietary fibres[2]	g	1.0	1.8
	Galacto-oligo saccharides[3]	g	0	0.40
	BGS[4]	µg	0	1.65

[1]Milk protein and sodium caseinate in Standard Formula and fermented milk products in Prebiotics Formula were used as protein sources.
[2]Dietary fiber: 1.4 kcal/g, [3]Galacto-oligo saccharides: 2 kcal/g
[4]Prebiotic Formula contains 1.65 µg/kcal 1,4-dihydroxy-2-naphthoic acid (DHNA) derived from BGS (Bifidogenic Growth Stimulator).

Table 1: Composition of experimental enteral formulae.

Animals were dissected directly after fasting (n=4), or 30 (n=8 or 10), 60 (n=4), or 240 (n=8) minutes after eating. Mice were anesthetized with isofluorane, and blood from the portal vein was collected following a laparotomy. Only animals that were dissected 240 minutes after oral administration of food had their blood collected from the caudal vein in the fasting state, as well as 30, 60, and 120 minutes after fasting. The blood glucose levels in these samples were measured. Moreover, in order to investigate gene expression, distal ileum samples (consisting of 3-6 cm of small intestinal tissue starting at the point where the ileum attaches to the cecum) were collected. The collected tissues were quickly treated with RNA later (Thermo Fisher Scientific, Waltam, MA, USA), and preserved at -80°C.

Experiment 2: Blood collection after four hours of fasting: Male 9-week-old WT, GPR41KO, and GPR43KO mice with average body weights of 22.7 ± 0.5 g, 22.2 ± 0.4 g, and 26.4 ± 1.0 g, respectively, were divided into two groups (n=5) per strain, with equal average weights. One group was fed with SF for two weeks and the other with PF for the same duration. Food was withheld from the animals at 9 AM on the day of dissection, and, after four hours of fasting, the animals were anesthetized with isoflurane, laparotomized, and blood samples were collected from the portal vein and the abdominal portion of the vena cava. Then, the cecum was resected, and its contents were measured. The amount of *Bifidobacterium* in the cecal contents was also measured.

Measurement methods

Blood glucose levels and GLP-1 concentration: The glucose levels in the blood collected from the caudal vein were measured using a glucose oxidase electrode measuring instrument (Breeze2, Panasonic Healthcare, Tokyo, Japan). Blood from the portal vein was collected in a P-800 blood collection system (Nippon Becton Dickinson, Tokyo, Japan) to prevent the degradation of GLP-1. Then, the blood was centrifuged at 1,200 × *g* for 10 minutes at 4°C. The plasma concentration of active GLP-1 was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Active form GLP-1 Assay Kit, Immuno-Biological Laboratories, Fujioka, Gunma, Japan).

SCFAs concentration: Cecal contents (200 mg) were diluted with twice the amount of Milli-Q water, homogenized, and centrifuged at 10,000 × *g* for 10 minutes at 4°C. Next, 2.5 µL of Carrez reagent I (53.5 g $ZnSO_4$*$7H_2O$ in100 mL) and 2.5 µL of Carrez reagent II (17.2 g $K_4[Fe(CN)_6]$*$3H_2O$ in 100 mL) were added to 200 µL of supernatant, and the solution was centrifuged at 10,000 *g* for 10 minutes at 4°C to pellet the proteins [18]. The supernatant was filtered using 0.22 µm filters (UFC30GV00, Merk, Tokyo, Japan), and then used for SCFA analysis.

The SCFA analysis methods used were an improved version of the methods used by Niwa et al. [19]. We used a conductivity detector (CDD-10A, Shimadzu, Kyoto, Japan) to detect the SCFAs by post-column pH buffering electrical conductivity. The two-high-performance liquid chromatography (HPLC) columns used in combination were polymer columns for organic acid analysis (ICSep-ORH-801 6.5 mm × 300 mm, Tokyo Chemical Industry, Tokyo, Japan). The column temperature was

50°C, the mobile phase was 5 mM p-trinitrosulfonic acid, the reaction liquid was a 5 mM p-trinitrosulfonic acid solution and a 20 mM aqueous Bis-Tris solution containing 100 μM ethylenediaminetetraacetic acid disodium salt (EDTA), and the flow rate was 0.5 mL/minute. The content of each cecal SCFA was determined using standard curves. Total SCFA levels were the sum of the acetic acid, propionic acid, and butyric acid levels.

Proglucagon mRNA expression levels: The expression of proglucagon in the distal ileum from mice was measured by quantitative PCR (qPCR). Total RNA was extracted from ileum tissues using an RNeasy Mini Kit (Qiagen, Tokyo, Japan), cDNA was synthesized from the total RNA using a PrimerScript RT reagent kit (Takara RR037A, Takara Bio, Kusatsu, Shiga, Japan). qPCR was conducted using the SYBR Primer Ex Taq II (Takara RR820A, Takara Bio) and the Thermal Cycle Dice Real Time System TP800 (Takara Bio). The qPCR primers for proglucagon were 5′-TGGCAGCACGCCCTTC-3′ (forward) and 5′-GCGCTTCTGTCTGGGA-3′ (reverse). The qPCR primers for the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were 5′-TGTGTCCGTCGTGGATCTGA-3′ (forward) and 5′-TTGCTGTTGAAGTCGCAGGAG-3′ (reverse). The expression of proglucagon was calculated using the Comparative Ct method.

Quantification of *Bifidobacterium*: The quantification of *Bifidobacterium* was carried out using qPCR and a 7300 Real Time PCR System (Applied Biosystems, Foster City, CA, USA) following the methods of Nagafuchi et al. [10]. The total DNA template was extracted from the cecal contents using a QIAamp DNA Stool mini kit (Qiagen). The primers 5′-CTCCTGGAAACGGGTGG-3′ (forward) and 5′-GGTGTTCTTCCCGATATCTACA-3′ (reverse) were used to amplify *Bifidobacterium* genomic DNA. A calibration curve was created for *Bifidobacterium longum* OLB6125 using the methods of Matsuki et al. [20], and the number of *Bifidobacterium* per gram of cecal contents was determined.

Statistical analysis

The SPSS statistics software version 23 (IBM, Armonk, NY, USA) was used for statistical analysis. The average values (means) for the SF group and PF group were compared using Student's t-test, with a significance level of $P<0.05$. If the P values were not less than 0.05 but less than 0.1, the values were considered to show significance trends and, therefore, are mentioned in the article. The data are represented as the mean ± the standard deviation (s.d.).

Results

Active GLP-1 concentration in plasma during a fasting state, and 30, 60, 240 minutes after oral administration (Experiment 1)

The relative levels of GLP-1 in each experimental group of animals, when fed with PF or SF at the indicated times after oral administration are shown in Figure 1. There were no differences in plasma GLP-1 levels between the SF and the PF group after an overnight fast. In WT mice, GLP-1 levels 30 minutes after oral administration were significantly higher in the PF group compared with the SF group; similar results were obtained 240 minutes after oral administration. In GPR43KO mice, the levels of GLP-1 in the PF-fed group were significantly higher than those in the SF-fed group 30 and 60 minutes after oral administration. Conversely, in the GPR41KO mice we noticed a slight increase in the levels of GLP-1 of the PF-fed mice, compared with those of the SF-fed mice, only 240 minutes after oral administration ($P=0.064$).

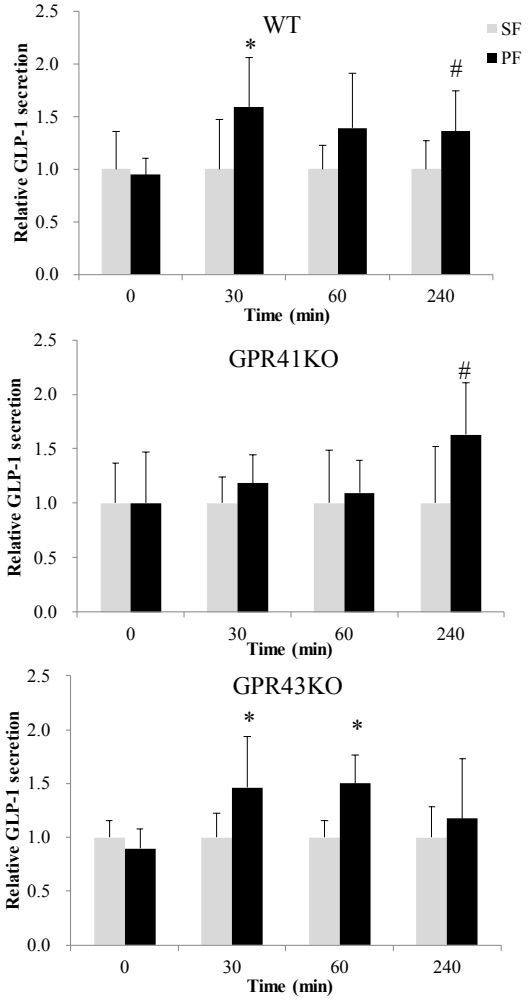

Figure 1: Blood GLP-1 levels after fasting and after oral administration of formula: Relative GLP-1 secretion 0, 30, 60 and 240 min after administration of SF or PF (12 mL/kg) in overnight-fasting WT, GPR41KO, and GPR43KO mice that were fed SF or PF for 14 days (Experiment 1). Data represent the mean ± s.d.; n=3-4 (0, 60 min), n=6-9 (30 min), n=5-8 (240 min). *: $P<0.05$, #: $P<0.1$ *versus* SF using the Student's *t*-test.

Proglucagon gene expression in the distal ileum (Experiment 1)

Next, we investigated the mRNA expression of proglucagon in the distal ileum after the animals were fed for two weeks with the experimental diet and fasted overnight (Figure 2). There were no significant differences between the SF- and the PF-fed animals in any of the experimental groups.

GLP-1 levels in the plasma after two weeks of the experimental diet (Experiment2)

We evaluated GLP-1 levels in plasma collected from the portal vein of mice fed SF or PF for two weeks and fasted for four hours. WT mice fed PF had significantly higher GLP-1 levels than those fed SF (Figure 3). Similar results were obtained in GPR41KO mice. GRP43KO mice showed a similar tendency, but the data were not significant.

Changes in blood glucose levels after oral administration of the experimental diet (Experiment 1)

Next, we monitored blood glucose levels in WT, GPR41KO, and

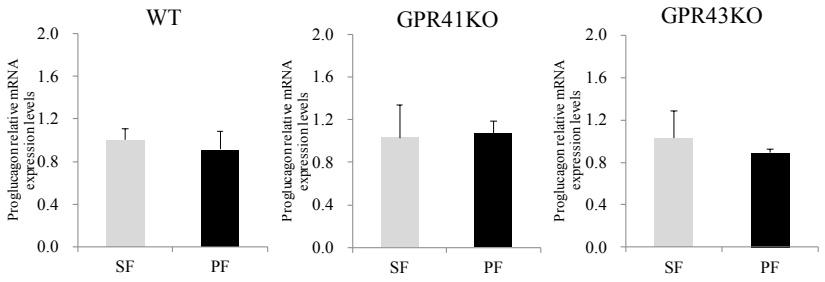

Figure 2: Proglucagon levels during fasting: Proglucagon mRNA expression levels in the distal ileum in WT, GPR41KO and GPR43KO mice fed SF or PF for 14 days and under fasting conditions (Experiment 1). Data represent the mean ± s.d. (n=4). Student's *t*-test indicated that the difference between the SF and PF groups was not significant.

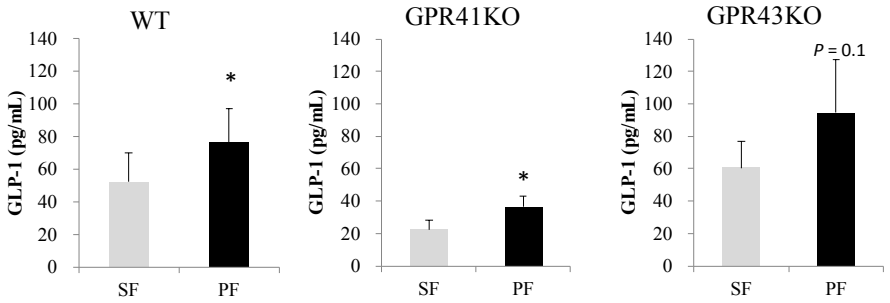

Figure 3: Blood GLP-1 levels after four hours of fasting: Blood was collected through the portal vein and GLP-1 levels were measured after fasting for four hours in WT, GPR41KO and GPR43KO mice fed SF or PF for 14 days (Exp. 2). Data represent mean ± s.d. (n=4-5). *: $P<0.05$ *versus* SF using the Student's *t*-test.

GPR43KO mice. In all mice, blood glucose levels peaked around 30 minutes after oral administration of the experimental diet, then gradually decreased (Figure 4). In WT mice, the PF group had slightly lower blood glucose levels 30 minutes after administration of the experimental diet, when compared with the SF group (P=0.082), and significantly lower levels after 60 minutes. In GPR43KO mice, the PF group had significantly lower blood glucose levels 30 and 60 minutes after administration of the experimental diet than the SF group. Conversely, in GPR41KO mice, no significant difference was found at any time point, between the SF- and the PF-fed groups.

Quantification of *Bifidobacterium* and SCFA levels in the ceca of mice (Experiment 2)

After two weeks of the experimental diet followed by four hours of fasting, we quantified the number of *Bifidobacterium* per gram of cecal contents from the different treatment groups. We found that the amount of *Bifidobacterium* was significantly higher in the PF groups of both WT and GPR41KO mice than in the SF groups (Figure 5). Conversely, in GPR43KO mice, the *Bifidobacterium* content was significantly lower in the PF group than the SF group.

Additionally, the weight of the cecal contents was significantly higher in the PF groups than in the SF groups of WT, GPR41KO, and GPR43KO mice (Table 2). The total cecal SCFA levels were significantly higher in the PF groups than in the SF groups for WT, GPR41KO, and GPR43KO mice. Similar data were obtained when we evaluated the levels of the individual SCFAs such as acetic acid, propionic acid, and butyric acid (Table 2).

Discussion

We hypothesized that the ingestion of fermented milk products and prebiotic-containing PF leads to the increased production of SCFAs through fermentation in the intestines, promoting the secretion of GLP-1. We used WT mice and SCFA receptor KO mice, namely GPR41KO and GPR43KO mice, to evaluate our hypothesis. Previous research has shown that there is an increase in cecal *Bifidobacterium* and total SCFAs in rats fed with PF compared to those fed with SF [12]. We observed a similar effect on *Bifidobacterium* and cecal fermentation in WT mice in response to PF (Figure 5).

First, we considered the effect of oral administration of PF on the early stages of GLP-1 secretion (Experiment 1) and the involvement of SCFA receptors in this process. WT mice were fed the experimental diet for two weeks and fasted for one night; GLP-1 levels in blood collected through the portal vein were measured 30 minutes after formula administration. We found significantly higher levels of GLP-1 in PF- than in SF-fed mice (Figure 1). On the other hand, there was no significant difference in either the levels of GLP-1 or gene expression of the GLP-1 precursor proglucagon in the distal ileum between mice fed with either formula in a fasting state (Figure 2). These results suggest that a rapid GLP-1 secretory response occurs in response to PF ingestion in WT mice. Furthermore, similar experiments in GPR43KO mice showed a significant increase in blood GLP-1 levels 30 and 60 minutes after formula administration in the PF-fed group compared with the SF-fed one; no significant changes between the PF and the SF group were observed in GPR41KO mice treated in the same way (Figure 1). This suggests that SCFAs act as signals for the GPR41 receptor in the early stages after PF ingestion to promote GLP-1 secretion.

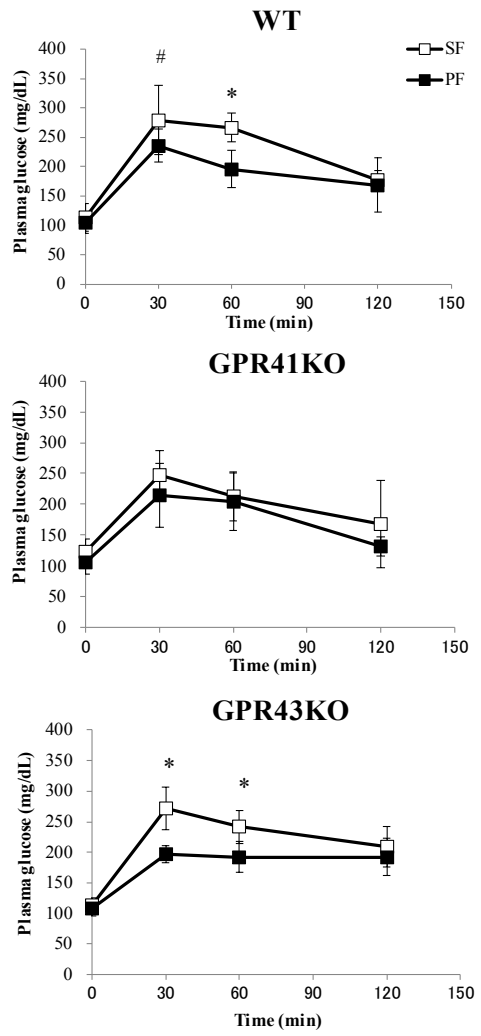

Figure 4: Changes in blood glucose levels after fasting and after oral administration of formula: Time course of blood glucose levels after administration of SF or PF (12 mL/kg) in WT, GPR41KO, and GPR43KO mice fed SF or PF for 14 days (Experiment 1). Data represent the mean ± s.d. (n=7-8). *: $P<0.05$, #: $P<0.1$ *versus* PF using the Student's *t*-test.

GLP-1 acts as a stimulus for incretin and insulin secretion, thus regulating blood glucose levels [5]. A comparison of the changes in blood glucose levels after oral administration of SF and PF showed that there was significant suppression of the increase in blood glucose levels in the PF group compared to the SF group in both WT and GPR43KO mice. However, there was no significant difference in the increase in blood glucose levels after oral administration of either PF or SF in GPR41KO mice (Figure 4). These results strongly suggest that ingestion of PF may contribute to the early stage suppression of the rise in blood glucose levels through GPR41 via stimulation of GLP-1 secretion, consistently with the data discussed above.

SCFAs activate GPR41 and GPR43, which are expressed in the L cells, and induce the secretion of GLP-1 from these cells [4,7]. A suggested mechanism by which oral administration of PF stimulates GLP-1 secretion is that cecal SCFAs, especially propionic acid and butyric acid, increase over the two weeks of PF ingestion. PF contains indigestible oligosaccharides and more water-soluble dietary fiber than SF, and the increase in cecal undigested fermentation substrates may promote the production of SCFAs such as acetic acid, propionic acid, and butyric acid (Table 2). In addition, some researchers have reported the increase in the number of small intestine L cells that express SCFA receptors and in secreted GLP-1 because of the long-term ingestion of fructo-oligosaccharides, a type of indigestible oligosaccharide that produces SCFAs upon intestinal fermentation [21]. A similar change occurs because of the ingestion of PF containing GOS, another type of indigestible oligosaccharide (unpublished data). These findings suggest that histological changes in the intestine, such as an increase in the number of cells secreting GLP-1 and expressing SCFA receptors due to PF ingestion may contribute to increased GLP-1 secretion upon PF administration. However, since the activation of GPR41 is related to propionic acid and butyric acid rather than acetic acid, we cannot explain these differences with the increase in the amount of *Bifidobacterium* we observed. It is possible that other bacterial species are involved in this process. Future studies need to investigate whether species other than *Bifidobacterium* are involved in GRP41 activation.

There are also other possibilities for the mechanism of GPR-41 activation due to PF. The main organic acid produced by *Lactobacillus bulgaricus* and *Streptococcus thermophilus* from PF is lactic acid; however, small amounts of acetic acid and butyric acid are also produced. Propionic acid produced by propionic acid bacteria is also present in BGS [22]. SCFA receptors, especially GPR41, are expressed in gastrointestinal hormone-producing endocrine cells of the stomach and duodenum as well as the jejunum, ileum and colon [23,24]. Propionic acid and/or butyric acid derived from fermented milk products and BGS in PF may promote GLP-1 production in the upper gastrointestinal tract via GPR41.

Additionally, we evaluated the effect of PF on later stages of GLP-1 secretion; specifically, we investigated GLP-1 secretion in mice PF- or SF-fed for two weeks and fasted for four hours (Experiment 2). PF administration promoted GLP-1 secretion in WT and GRP41KO mice. In addition, GPR43KO mice had a tendency of higher GLP-1 levels after PF ingestion. Therefore, we believe that neither of the SCFA receptors investigated has an exclusive effect on the late stages of GLP-1 secretion. It has been reported that GRP41 and GRP43 can compensate each other in mice deficient for one of them [7]. This effect would explain our results. Moreover, GPR119 [25] and TGR5 [26], which are receptors for molecules other than SCFAs, may work to promote GLP-1 secretion in our experimental system.

Our results showed that PF promotes the early stages of GLP-1 secretion through the GPR41 receptor. This effect may be useful for blood glucose management in patients who require enteral nutrition. In addition, SCFAs are known to act on the cardiovascular and central nervous systems through GPR41 to help maintain homeostasis in the body [17]. Therefore, we believe that PF might have clinical applications beyond blood glucose management.

Conclusion

PF containing fermented milk products and prebiotics promotes GLP-1 secretion. SCFAs might act as signal transduction molecules via GPR41 in a G protein-coupled-receptor signaling pathway that leads to GLP-1 secretion after oral administration. PF may be effective in regulating blood glucose level in patients that require enteral nutrition.

Acknowledgments

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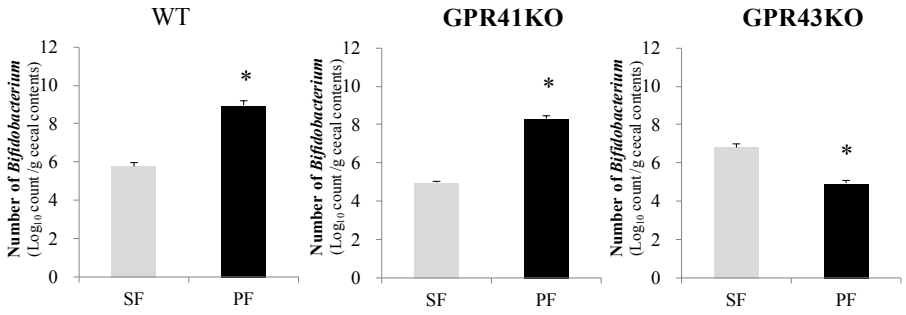

Figure 5: Quantification of *Bifidobacterium* in the cecal contents, four hours after fasting: The number of *Bifidobacterium* per gram of cecal content was measured in WT, GPR41KO, and GPR43KO mice fed SF or PF for 14 days and fasted for four hours (Exp. 2). Data represent the mean ± s.d. (n=5). *: $P<0.05$ *versus* SF using the Student's *t*-test.

	Unit	WT		GPR41KO		GPR43KO	
		SF	PF	SF	PF	SF	PF
Cecal contents	g	0.13 ± 0.00	0.13 ± 0.05*	0.17 ± 0.04	0.33 ± 0.03*	0.26 ± 0.04	0.35 ± 0.04*
Total SCFA	μmol/cecum	2.50 ± 0.56	5.73 ± 1.24*	4.21 ± 0.85	7.48 ± 2.02*	0.13 ± 0.00	7.87 ± 1.30*
Acetic acid	μmol/cecum	1.79 ± 0.40	4.15 ± 0.87*	2.90 ± 0.58	5.28 ± 1.50*	3.56 ± 0.63	5.56 ± 0.88*
Propionic acid	μmol/cecum	0.47 ± 0.09	1.02 ± 0.25*	0.85 ± 0.16	1.41 ± 0.34*	1.05 ± 0.23	1.49 ± 0.31*
Butyric acid	μmol/cecum	0.25 ± 0.07	0.56 ± 0.16*	0.46 ± 0.12	0.80 ± 0.21*	0.58 ± 0.11	0.81 ± 0.16*

Data represent mean ± s.d (n=5). *: $P < 0.05$ *versus* SF using Student's *t*-test.

Table 2: Cecal contents in the different treatment groups. Cecal content weight and amount of total short chain fatty acids (SCFA) and each SCFA per cecum. Mice were fed SF or PF for two weeks and fasted for four hours (Experiment 2).

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