独立和联合作用乳糖醇、聚醚糖和\textit{Bacteroides thetaiotaomicron} 对高脂肪饮食喂养大鼠的餐后代谢和体重影响

Kaisa Olli\textsuperscript{*}, Markku T. Saarinen\textsuperscript{1}, Sofia D. Forssten\textsuperscript{1}, Mari Madetoja\textsuperscript{2}, Karl-Heinz Herzig\textsuperscript{3,4} and Kirsti Tiihonen\textsuperscript{1}

\textsuperscript{1}DuPont Nutrition and Health, Global Health & Nutrition Science, Kantvik, Finland, \textsuperscript{2}Made Consulting, Turku, Finland, \textsuperscript{3}Medical Research Center Oulu, Institute of Biomedicine and Biocenter of Oulu, Oulu University Hospital, University of Oulu, Oulu, Finland, \textsuperscript{4}Department of Gastroenterology and Metabolism, Poznan University of Medical Sciences, Poznan, Poland

肥胖症与高脂肪饮食中能量密集型食物的摄入以及微生物群的改变有关，其中在瘦体重中或体重减轻后，肠道梭状芽胞杆菌属的丰度会增加。乳糖醇，一种甜味的糖醇，常用于食品中作为糖的替代品。聚醚糖（PDX），一种高度分枝的葡萄糖聚合物，已知能够减少能量摄入。这里，我们测试乳糖醇或PDX与\textit{Bacteroides} \textit{thetaiotaomicron}联合使用是否会对高脂肪（HF）饮食喂养大鼠的餐后代谢产生有益的反应。

一共有175只雄性Wistar大鼠被分为低脂（LF）或高脂（HF）饮食组。\textit{Bacteroides thetaiotaomicron}（10\textsuperscript{10}细菌/动物/天）被以口服方式与或不与乳糖醇（1.6−2 g/动物/天）或聚醚糖（2 g/动物/天）联合使用的方式进行8天的给药。餐后血样、盲肠内容物、粪便在最后一天被收集。

测量内容包括：体重、饲料摄入量、盲肠短链脂肪酸、粪便干物质和热值、血糖、胰岛素、甘油三酯和饱腹感激素浓度。

乳糖醇和PDX在单独使用或与\textit{B. thetaiotaomicron}联合使用时，都能降低平均体重。乳糖醇和PDX能够降低餐后甘油三酯水平。对肠促胰岛素肽（PYY）释放量的测量，乳糖醇和PDX单独使用或与\textit{B. thetaiotaomicron}联合使用时，能够增加PYY的释放，以及0–8 h内面积下曲线（AUC）的测量。

乳糖醇和PDX能够降低餐后胰岛素的AUC（0–8 h）。乳糖醇和PDX在单独使用或与\textit{B. thetaiotaomicron}联合使用时，对餐后血糖、甘油三酯和胰岛素水平的影响较小。

乳糖醇和PDX可能提供额外的手段来调节餐后代谢和体重管理，而\textit{B. thetaiotaomicron}在测试剂量下的影响较小。

关键词：\textit{Bacteroides}, 胰岛素，乳糖醇，肥胖症，聚醚糖，PYY, 饱腹感信号，甘油三酯

缩写：AUC, 作用下曲线；BCFA, 支链脂肪酸；BWG, 体重变化；CCK, 胆囊收缩素；GLP-1, 胰高血糖素样肽-1；HF，高脂；LF，低脂；PDX，聚醚糖；PYY，胰高血糖素样肽；qPCR, 定量聚合酶链反应；SCFA, 短链脂肪酸。
INTRODUCTION

Managing postprandial glucose and lipid responses can decrease the risk of metabolic diseases and influence body weight management. Therefore, low glycemic and satiety-increasing food components, such as non-starch polysaccharides, have been studied for their impact on energy metabolism and satiety (1). Several studies have also demonstrated that the gut microbiota contributes to the control of body weight and energy homeostasis (2, 3).

Lactitol, a sweet-tasting sugar alcohol that consists of galactose and sorbitol, is commonly used in low-calorie products to replace sucrose. Lactitol is not absorbed in the small intestine or hydrolyzed by gastrointestinal tract enzymes but, unlike polydextrose (PDX), it is metabolized rapidly by the gastrointestinal microbes (for review, see Ref. (4)). PDX is a highly branched, randomly bonded glucose polymer and its structural complexity prevents its hydrolysis by mammalian enzymes in the upper gastrointestinal tract. PDX is widely recognized as a soluble fiber (5) and due to its very low energy density, PDX might reduce energy intake and influence the gastrointestinal microbiome. As PDX passes through the small intestine, it is partially metabolized by the colonic microbes (6). A sustained degradation of PDX has been demonstrated also throughout a four-stage colon simulation model (7). In human studies, PDX has already shown to enhance satiety and reduce energy intake during a sequential ad libitum lunch (8–10).

Most of the ingested lactitol and part of the PDX are fermented by the microbiota in the lower gastrointestinal tract producing short-chain fatty acids (SCFAs) (4, 7, 11), which stimulate gastrointestinal peptide secretion, such as peptide tyrosine tyrosine (PYY), glucagon-like peptide-1 (GLP-1), and cholecystokinin (CCK) from enteroendocrine cells (12, 13). These peptides control intestinal secretion and motility, i.e., function as ileal brake, in addition to the central inhibition of food intake. PYY has also shown to increase energy expenditure and fat oxidation in humans and rodents (14). Previous animal studies have shown that lactitol stimulates PYY and GLP-1 secretion (15). Lactitol and synbiotics, such as Lactobacillus delbrueckii subsp. rhhamnosus strain GG, and Bifidobacterium lactis Bp12 have increased plasma PYY concentrations in animals (15, 16). SCFAs injected directly into the rat colon have been shown to increase the release of PYY (17, 18). In humans, the oral intake of SCFAs and colonic delivery of SCFAs via fermentable dietary fiber stimulated PYY and GLP-1 release, although contradictory effects on appetite and satiety sensation have been observed (19, 20).

It is now well established that obesity is associated with changes in the microbiome, especially the relative abundance of the two dominant bacterial divisions, the Firmicutes and the Bacteroidetes. The gut microbiota of genetically obese (ob/ob) mice had an increased abundance of Firmicutes over Bacteroidetes (21). An increase in the relative abundance of Bacteroidetes has been correlated to weight loss (22). Evidently, the high-fat (HF)-enriched Western diet provides a competitive advantage to the Firmicutes (23). Cani et al. have proposed that the amount of beneficial bifidobacteria in the gut differ according to the state of obesity, showing lower bacterial counts in obese and higher counts in lean subjects (24). Both lactitol and PDX possess prebiotic properties, and lactitol can increase the quantity of beneficial bacteria, such as lactobacilli and bifidobacteria (25). An increase in the number of these beneficial intestinal bacteria has also been demonstrated in human trials after the use of PDX alone (4 g/day) (26) and in combination with a probiotic bacteria mixture (27) or another prebiotic (28). The effect of Bacteroidetes on satiety is still unclear, although the higher relative abundance of gut Bacteroidetes is repeatedly being reported with lean subjects or after weight loss (29). In a recent study in rats, the addition of prebiotics was able to normalize the reduced amount of Bacteroidetes often reported in obesity (30). Prebiotics have also raised the concentration of plasma PYY and the cecum mRNA levels of PYY (31).

Therefore, we tested the combined effects of two carbohydrates and Bacteroides species in a large animal trial hypothesizing that the supplementation of Bacteroides inoculum might affect the weight gain in rats fed a HF diet. Furthermore, this effect could be modified by the addition of synbiotic effects of lactitol or PDX. The use of a combination of intestinal Bacteroides species, Bacteroides thetaiotaomicron, and two different indigestible carbohydrates, such as lactitol or PDX, has not been studied before.

MATERIALS AND METHODS

Animals and Experimental Design

A total of 175 male Wistar (HsdBrlHan:WIST) rats (Harlan, The Netherlands) were used in this study. The experiments were approved by the Regional State Administrative Agency of Southern Finland and were conducted under the animal license number ESLH-2008-03964. Institutional and national guidelines for the care and use of animals were followed. Animals arrived at the age of approximately 8 weeks were housed in plastic cages (Makrolon 3, Tecniplast, Bayer MaterialScience AG, Leverkusen, Germany), two to three per cage (floor area 814 cm²), and were kept in a conventional animal room (21°C ± 3°C, 55 ± 15% humidity). The animals were randomly assigned to different groups and they were acclimatized with their respective diets for 2 weeks before the first dosage of test items. The animals were trained to consume their food within 5 h from the start of the dark cycle.

The first experiment (Experiment A) studied whether Bacteroides supplementation can affect satiety and how the responses differ between animals fed a low-fat (LF) or a HF diet. The latter experiment (Experiment B) evaluated the effects of lactitol and PDX – administered separately, or together with B. thetaiotaomicron inoculum – on weight gain, satiety signals, and other metabolic parameters in rats fed a HF diet.

Experiment A

The animal trial set-up and the arrangement of different treatment groups are presented in Figure 1.

Animals were randomly allocated into five study groups (I–V), 15 animals in each group. Two different diets were used: RM 1 (E) diet (Rat and Mouse Maintenance diet, product code: 801002, Special Diet Services, Witham, UK) was used for the
study groups I and II and Western RD (P) diet (Western Rat Diet, product code: 829100, Special Diet Services, Witham, UK) for the study groups III–V. The RM 1 diet contained 7.42% calories from fat and the Atwater fuel energy was 13.75 MJ/kg. The Western RD diet contained 42% calories from fat and the Atwater fuel energy was 19.35 MJ/kg. Therefore, the diets were named LF and HF, respectively (Table 1). Study groups I and III were dosed with a vehicle (0.9% NaCl), groups II and IV with B. thetaiotaomicron (10^{10} bacteria/animal/day) in 0.9% NaCl, and group V with lactitol (1.6–1.8 g/animal/day) (Danisco USA Inc., Thomson, IL, USA) in sterile water. B. thetaiotaomicron (DSM 2079) was pre-cultured over-night in cooked Meat Medium (Difco, Le Pont de Claix, France) and then cultivated anaerobically over-night in MRS medium (Lab M Limited, Lancashire, UK). The test items used in conjunction with B. thetaiotaomicron were prepared in advance and stored at −20°C until the dosage. Lactitol was dissolved in sterile water on the day of the dosage. All test items and the control vehicle were dosed per os and administered by oral gavage using Teflon feeding needles (AgnTho's, Lidingö, Sweden) for 8 days with a volume of 2 ml/rat/day. On study days 1–7, the dosage was carried out in the morning just before feeding period (dark cycle), after which the feed was available ad libitum for 5 h. On study day 8, the dosage took place within 2 h before the onset of the dark cycle and the animals were given feed ad libitum immediately after the dosage. The feed consumption was measured from each separate cage for 7 days during an acclimatization period and on study days 1–7 (n = 5, three animals per cage). Some minor spillage of feed might have occurred through the cage bars and was not recorded. Feed intake was calculated as consumption per 100 g of body weight.

### Experiment B

Animals were allocated into four study groups (I–IV), 25 animals in each group. The HF diet (Western Rat Diet (P), 829100, Special Diet Services, Witham, UK) (Table 1) was used for all study groups throughout Experiment B. Study group I was dosed with a control vehicle (0.9% NaCl) and groups II–IV with B. thetaiotaomicron (DSM 2079) (10^{10} bacteria/animal/day) in 0.9% NaCl. In addition to B. thetaiotaomicron, groups III and IV received lactitol (2 g/animal/day) (Danisco USA Inc., Thomson, IL, USA) or PDX (2 g/animal/day) (Litesse® Ultra, Danisco USA Inc., Terre Haute, IN, USA), respectively. The test item preparation and the dosage (2 ml/animal/day) were conducted and feed intake (n = 10, two to three animals per cage) calculated as described in Experiment A. The feed was available for 5 h after the last dose was given.

### Short-Chain Fatty Acids

Following terminal blood sampling, digesta samples (content of cecum) were taken from all animals, frozen and stored at below...
−18°C. Gas chromatographic analysis of the SCFAs (acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid, 2-methylbutyric acid) and lactic acid in the rat cecal digesta samples was performed using pivalic acid as an internal standard as described previously (32). SCFAs are expressed as micromole/gram wet weight.

**Dry Matter and Heat Value of Feces**

Following terminal blood sampling in Experiment B, fecal samples were collected from the cages (two to three rats per cage), pooled, frozen, and stored at below −18°C. The dry matter of feces was measured gravimetrically after drying the fecal samples in an oven at 105°C for 16 h. The heat value of the dried samples was measured using an adiabatic bomb calorimeter (Parr Instruments, Moline, IL, USA).

**Quantification of Bacteroides spp.**

Microbial DNA was extracted from the cecum digesta (Experiment A) or fecal samples (Experiment B) by a bead beating step before using the QIAamp DNA stool Mini kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Quantitative PCR (qPCR) was used to quantify the genus Bacteroides (including Bacteroides–Prevotella–Porphyromonas) using the SYBR green methodology (Core reagent kit, Applied Biosystems, Foster City, CA, USA) in a total volume of 25 µl containing 10 ng of template DNA and 250 nM of the forward primer gBacter_F and reverse primer gBacter_R (33). The amplification and detection of DNA were performed with an ABI 7500 sequencing detection system (Applied Biosystems). To obtain a standard curve, a 10-fold dilution series ranging from 10 pg to 10 ng of DNA from the B. thetaiotaomicron (DSM 2079) was added to the qPCR assays. For determination of DNA, triplicate samples were used, and the mean quantity per gram wet weight was calculated.

**Blood Analyses**

The studies were designed in a way that enabled several consecutive blood samplings to clarify the kinetics of glucose, triglycerides, insulin, and gut peptides. All blood samples were taken from the lateral tail vein except for the terminal samples that were taken by cardiac puncture under 4% isoflurane anesthesia. On study day, eight blood samples (five animals per group) were taken into the collection tubes (Capiject® or Venosafe™, Terumo® Europe N.V, Leuven, Belgium, EDTA added as an anticoagulant) before the dosage and at 1, 3, 5, and 8 h after the dosage. The only exceptions were the terminal blood samples that were taken to vacuum tubes. The plasma was separated by centrifugation (1600 × g, 15 min, RT), and the plasma samples were frozen and stored at −70°C. Insulin and PYY were analyzed from the plasma samples using the MILLIPLEX™ MAP Rat Gut Hormone Panel kit (#RGT-88K, Millipore, Billerica, MA, USA) according to the kit manufacturer’s instructions. The blood glucose was determined from the complete blood samples (five animals per group) using the HemoCue® Glucose 201+ analyzer (HemoCue AB, Angelholm, Sweden). In Experiment B, the blood serum samples (10 animals per group) were collected for triglyceride analysis, which was performed with Triglycerides GPO-PAP reagent (Roche Diagnostics GmbH, Mannheim, Germany) and the Roche/Hitachi MODULAR ANALYTICS measuring instrument (Roche Diagnostics GmbH). The serum samples were frozen at −20°C after sampling. Blood triglycerides were not measured in Experiment A.

**Statistical Analysis**

The statistical analyses were performed with Prism 5 Version 5.01 (GraphPad Software, Inc., San Diego, CA, USA). One-way ANOVA or two-way ANOVA with Tukey’s multiple comparison test, or unpaired Student’s t-test with two-tailed distribution were used, as indicated in the text. For all tests, p < 0.05 was considered statistically significant. The areas under the curve (AUCs) of insulin and PYY were calculated using the trapezoidal rule and ignoring the peaks that were <10% of the distance from the minimum or maximum Y values.

**RESULTS**

**Body Weight Gain and Feed Intake**

The use of a HF diet is a common way to induce obesity in animal models (34). The fat and energy content of the HF diet used in this study (42% calories from fat) were modified so that it resembled the Western human diet.

Lactitol did not have any effect on the daily total fiber supply. However, PDX is widely recognized as soluble dietary fiber (5) and the commercial PDX used in this study (Litesse®) is considered to have 90% fiber content. The energy value of PDX is generally recognized as being 1 kcal/g; hence, adding in total 2 kcal/animal/day. The energy value of lactitol is 2 kcal/g, which adds in total 3.2–4 kcal/animal/day.

**Experiment A**

During the acclimatization period, the body weight gain (BWG) increased expectedly in all HF groups when compared to LF groups, and the increases in BWG were statistically significant (p < 0.001, one-way ANOVA) when compared to the LF control group (data from acclimatization period not shown). In the lactitol-supplemented HF group, the mean BWG declined by 39% during the 8-day dosing period (p < 0.001, one-way ANOVA), when compared to the HF control group (Figure 2A). The LF group supplemented with B. thetaiotaomicron did not have any significant effect on the mean BWG during the 8-day dosing period.

Lactitol had the biggest effect on feed intake with nearly a 40% decrease in the mean feed consumption after the acclimatization period (p < 0.0001, Student’s t-test). In the HF control group and HF group supplemented with B. thetaiotaomicron, feed intake decreased by 19 and 18%, respectively (p < 0.0001, Student’s t-test). The lactitol-supplemented HF group had the lowest mean feed intake when compared to all other groups (p < 0.0001, Student’s t-test). The two LF groups showed the highest feed intakes among all diet groups; however, there was no
statistically significant difference between the LF control group and B. thetaiotaomicron-supplemented LF group. The mean feed intake in the HF and LF control groups, on the other hand, differed significantly (p < 0.0001, Student's t-test). The actual feed intake values in Experiment A are reported in Figure 2B.

**Experiment B**

In animals fed a HF diet, B. thetaiotaomicron supplementation alone did not have any significant effect on the mean BWG. However, when lactitol or PDX were administered together with B. thetaiotaomicron, the BWG declined by 41 and 28%, respectively, when compared with the HF control group (p < 0.001, one-way ANOVA) (Figure 2C).

The mean feed intake was highest in the HF control and B. thetaiotaomicron-supplemented HF groups; however, there was no statistically significant difference between these two groups. The animals administered with the combination of B. thetaiotaomicron and lactitol had the lowest mean feed intake (p < 0.05, Student's t-test, compared to the other HF groups). The PDX-supplemented HF group consumed on average less feed (p < 0.0001, Student's t-test) when compared to the HF control group. The actual feed intake values in Experiment B are reported in Figure 2D.

Some soft feces were noticed in PDX and lactitol-supplemented groups when fecal samples were collected, and one animal per each of these groups had diarrhea during blood sampling.

**SCFAs in Cecum**

In Experiment A, the concentrations of total SCFAs, acetic acid, and butyric acid were 35–65% lower in all HF diet-fed animals compared to the LF control animals, but the changes were not statistically significant (one-way ANOVA) (Table 2). In Experiment B, the total SCFA concentrations decreased by 44 and 45% in lactitol and PDX-treated HF groups, respectively (p < 0.001,
The animals in these groups also received *B. thetaiotaomicron* Lactitol and PDX in combination with Dry Matter and Table 3 one-way ANOVA), due to a reduction of acetic acid (Table 3). The animals in these groups also received *B. thetaiotaomicron* inoculum, but this did not induce any significant change in the concentration of SCFAs when administered alone. The concentration of branched-chain fatty acids (BCFAs), isobutyric acid, and isovaleric acid decreased in both lactitol and PDX-treated HF groups (*p* < 0.001, one-way ANOVA), and 2-methylbutyric acid in lactitol-treated HF group (*p* < 0.05, one-way ANOVA), when compared to the HF control group. These results are presented in Table 3.

**Dry Matter and Heat Value in Feces**

Lactitol and PDX in combination with *B. thetaiotaomicron* decreased the dry matter significantly by 19 and 25%, respectively, when compared to the HF control group (*p* < 0.001, one-way ANOVA). The heat value increased by 10% in the *B. thetaiotaomicron*-fed animals, when compared to the HF control group (*p* < 0.001, one-way ANOVA). These data are presented in Table 3.

**Bacteroides in Cecal Digesta**

The prevalence of *Bacteroides* spp. in the cecal digesta was quantified by qPCR in only the group of LF diet-fed animals (Figure 3). The high amount of fat in the samples from animals fed the HF diet interfered with the microbial DNA extraction, resulting in unreliable microbial counts. However, the quantity of *Bacteroides* spp. in the cecal digesta in the LF group supplemented with *B. thetaiotaomicron* increased significantly by 11-fold when compared

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**TABLE 2 | Cecal concentrations of SCFAs (n = 5) measured 8 h after the last dose in Experiment A.**

| Group | SCFAs (μmol/g) | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
|-------|----------------|------|-----|------|-----|------|-----|------|-----|
|       | Acetic acid    | 28.42| 11.92| 30.66| 10.89| 17.26 | 9.42| 17.70 | 9.66| 11.83 | 2.62|
|       | Proionic acid  | 5.19 | 2.12 | 6.36 | 2.52 | 4.30  | 2.53| 4.73  | 2.79| 6.61  | 2.81|
|       | Butyric acid  | 21.82| 12.35| 19.96| 11.02| 13.07 | 3.49| 13.94 | 4.33| 7.53  | 2.67|
|       | Lactic acid   | 4.88 | 3.03 | 7.46 | 3.57 | 3.84  | 1.88| 1.78  | 0.7 | 13.68 | 7.34|

**TABLE 3 | Cecal concentrations of SCFAs (n = 10) and fecal dry matter (n = 20) and heat value (n = 20) measured 8 h after the last dose in Experiment B.**

| Group | SCFAs (μmol/g) | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
|-------|----------------|------|-----|------|-----|------|-----|------|-----|------|-----|
|       | Acetic acid    | 47.51| 2.42 | 51.28| 2.53| 21.18***| 1.93| 22.83***| 1.99|
|       | Proionic acid  | 11.95| 0.64 | 12.81| 0.8 | 10.85 | 1.14| 9.39  | 0.59|
|       | Butyric acid  | 3.63 | 0.61 | 4.02 | 0.88| 2.74  | 0.46| 1.92  | 0.34|
|       | Lactic acid   | 0.31 | 0.17 | 0.69 | 0.2 | 0.9   | 0.2 | 0.43  | 0.28|

**SCFAs are expressed as μmol/g wet weight.** There were no statistically significant differences between different groups in Experiment I. BCFA, branched-chain fatty acid; B. thet., Bacteroides thetaiotaomicron; HF, high-fat diet; LF, low-fat diet; nd, not detected; SCFA, short-chain fatty acid; SEM, standard error of the mean.

**SCFAs are expressed as μmol/g wet weight.** There were no statistically significant differences between different groups in Experiment I. BCFA, branched-chain fatty acid; B. thet., Bacteroides thetaiotaomicron; HF, high-fat diet; LF, low-fat diet; nd, not detected; SCFA, short-chain fatty acid; SEM, standard error of the mean.
to the LF control group, showing quantities of 9.76 log10 (SEM 9.35 log10) and 8.72 log10 (SEM 7.97 log10) cells per gram cecal digesta (wet weight), respectively (p < 0.05, one-way ANOVA).

**Blood Glucose and Triglyceride Response**
Plasma glucose levels increased shortly after the meal. In the LF control group, all glucose values were below 8 mmol/l (Figure 4A), which is within the normal postprandial level in rats (35). Glucose concentrations tended to be higher in the HF control group compared to the LF diet groups, but the only significant deviation from the LF control group was measured at time point 5 h (p < 0.001, two-way ANOVA), with the glucose concentration peaking at 9.84 mmol/l (SEM = 0.73) in the HF control group (Figure 4A). Within the LF diet groups, blood glucose levels remained low and there was no difference between the LF control group and the LF group supplemented with *B. thetaiotaomicron*. Blood glucose levels did not differ between the HF diet groups (Figure 4B).

Serum triglycerides decreased significantly (p < 0.05, two-way ANOVA) in the groups supplemented with the combination of *B. thetaiotaomicron* and lactitol or *B. thetaiotaomicron* and PDX (Figure 5) at time points 3 and 8 h, respectively. In the HF control group, serum triglyceride concentrations peaked earlier (3 h) and were higher than with the *B. thetaiotaomicron* or lactitol-treated groups, which both peaked at 5 h time point. In the PDX-supplemented group, serum triglyceride concentrations peaked also at the 3 h time point. There were no significant differences in the triglyceride concentrations at time point 0.

**Plasma Insulin Response**
No significant differences in the fasting insulin levels (time point 0 h) were observed between different groups. Plasma insulin levels increased shortly after the meal initiation in the HF and LF control groups. In the HF groups with *B. thetaiotaomicron* or lactitol supplementation, the acute insulin responses were attenuated, showing decreased insulin levels at time point 1 h, when compared to the fasting values of the same group [p = 0.0004 and p = 0.012, respectively (Student’s t-test)]. Insulin levels in the *B. thetaiotaomicron* and lactitol-supplemented HF groups were lower than in the HF control group at time point 1 h [p < 0.01 and p < 0.001, respectively (two-way ANOVA)] (Figure 6A). When the insulin AUC (0–8 h) was examined, the HF group administered with lactitol showed significantly lower values than the HF control group or the HF group supplemented with *B. thetaiotaomicron* [p = 0.002 and p = 0.048, respectively (Student’s t-test)] (Figure 6B).

In comparison to the HF control group, the plasma insulin levels decreased 8 h after the meal initiation in animals administered with either *B. thetaiotaomicron* alone, or together with lactitol or PDX [p < 0.05, p < 0.001, and p < 0.001, respectively (two-way ANOVA)] (Figure 6C). No significant differences were seen between the groups at other time points and no significant peaks appeared. The insulin AUC (0–8 h) decreased significantly in lactitol and PDX-enriched groups, compared to the HF control.
The potential effect of *B. thetaiotaomicron* on the gut microbiota or through microbial metabolites. However, the fecal heat value of animals that were given *B. thetaiotaomicron* with lactitol or PDX did not increase. The percentage of fecal dry matter decreased in lactitol and PDX-supplemented animals with more moist feces in these groups.

The heat value of feces increased in animals supplemented with *B. thetaiotaomicron*, when compared to other groups, indicating that more energy was present in the feces. This would support the initial hypothesis that high amounts of intestinal Bacteroidetes could help to maintain a low body weight. This effect may be mediated through changes in the composition of the gut microbiota or through microbial metabolites. However, the fecal heat value of animals that were given *B. thetaiotaomicron* with lactitol or PDX did not increase. The percentage of fecal dry matter decreased in lactitol and PDX-supplemented animals with more moist feces in these groups.

The potential effect of *Bacteroides* as a probiotic together with fermentable fibers was studied in an animal model. Previously, the symbiotic effects of lactitol with another microbial strain, *Lactobacillus* NCFM®, have been successfully tested in *vitro* and in human clinical trials with proven beneficial effects on the gut microbiota and its activity (39, 40). Studies with *B. thetaiotaomicron* have revealed that co-colonization with other microbial species can enhance the efficiency and change the specificity of bacterial polysaccharide fermentation (41). In the present study, the increased fermentation of indigestible carbohydrates was not detected with *Bacteroides* supplementation. In addition, *B. thetaiotaomicron* increased fecal energy value. On the other hand, the BWG and plasma triglyceride concentrations were decreased by lactitol and PDX, when supplemented with *B. thetaiotaomicron*. The dietary fat content is also known to affect the microbiome; i.e., reduced levels of Bacteroidetes are reported in mice fed a HF diet and linked to an increased risk of obesity (23, 42). In our study, the impact of *B. thetaiotaomicron* on the detected higher quantities of *Bacteroides* spp. was significant in LF diet-fed animals, indicating that the dose of *B. thetaiotaomicron* had successfully passed through the gastrointestinal tract. The method of gavaging *B. thetaiotaomicron* has been demonstrated previously as a reliable and efficient way to ensure colonization (41). A limitation of our study, however, was with the use of only one strain of *Bacteroides* spp., while the Bacteroidetes phylum comprises a wide range of strains with different properties. Therefore, the administration of one single strain of *Bacteroides* spp. over a relatively short time proved not to be sufficient enough to counteract the effects of a HF diet.

The HF diet resulted in a marginally lower concentration of total SCFAs compared to a LF containing diet. In animals fed the HF diet, lactitol and PDX decreased the total cecum SCFAs, mostly due to the highly reduced level of acetic acid. Decreased
SCFA concentrations with lactitol and PDX have been reported previously in rodents (32, 43). The concentration of BCFAs, including isobutyric and isovaleric acid, was decreased by both lactitol and PDX, indicating less protein fermentation in these groups. A similar decrease in the relative concentration of BCFAs, isobutyric acid and isovaleric acid, by the addition of lactitol and PDX has been noted previously (32). However, no significant effect of the tested supplements on the absolute concentration of butyric acid was seen in the present study. Interestingly, in vitro studies with a semi-continuous four-stage colon simulator model have demonstrated that PDX has great potential to increase SCFAs in the gut (7, 44). One explanation to the low SCFA concentration detected in the present study could be the “snapshot” nature of the analysis, demonstrating the state of measured SCFAs at a particular time point (8 h after the last dosage) in only a single sample of digesta per animal. Also, an increase of the cecal volume could be one explanation for the reduced SCFA concentrations, as suggested before (32). However, cecal weight was not measured in the present study.

Fermentation of indigestible carbohydrates has been shown to affect glycemic responses and satiety (45). The modulation of the postprandial lipid concentrations by indigestible carbohydrates has not been widely investigated. In the present study, lactitol had already lowered the serum triglycerides 3 h after the last dose was given, when it was used in combination with \textit{B. thetaiotaomicron}. With lactitol, the postprandial triglyceride response was lower and peaked later than with the control group, therefore, weakening the postprandial rise of triglycerides. PDX supplemented with \textit{B. thetaiotaomicron} reduced the serum triglyceride concentration significantly 8 h after the last dose was given. PDX also marginally lowered the immediate triglyceride response. Hence, our data suggest that lactitol and PDX potentially have an attenuating effect on the postprandial triglyceride response in rats fed a HF diet. Previous human studies have also shown that a combination...
of PDX and lactitol (46), or PDX alone (47) have reduced the level of postprandial serum triglycerides. Since triglycerides are an energy source during metabolism, the reduction of triglycerides in circulation with dietary supplements, such as lactitol or PDX, could be beneficial for weight management and reducing the risk of cardiovascular disease. Even though rat is a commonly used model for metabolic disease studies, conclusions concerning triglyceride responses in humans should be drawn with the great care (48). In addition, differences in postprandial triglyceride responses between genders have been reported in rat studies (49), and this has to be taken into consideration when evaluating the triglyceride effects of potential food ingredients in rats.

Dietary proteins and fibers are known to affect insulin secretion (50, 51). PDX has shown to maintain low postprandial blood glucose levels (26) and lactitol has a low glycemic and insulimemic index (52). In the present study, a HF diet increased glucose levels, when compared to a LF diet that validates the model used in this study. Here, we showed that the pre-meal ingestion of both lactitol and PDX significantly decreased insulin AUC (0–8 h), but had no effect on glucose levels. Interestingly, in both experiments the postprandial plasma PYY levels consistently increased 3 h after the ingestion of lactitol when in combination with _B. thetaiotaomicron_. A similar effect with lactitol has been demonstrated previously (15). However, no direct effect of _Bacteroides_ or PDX on PYY levels was noted in the present study. According to previous animal studies, the addition of prebiotic fibers in HF diets fed to rats was able to increase satiety hormone levels and reduce BWG (53). Therefore, managing postprandial glucose and insulin responses with prebiotic supplements could help decrease the risk of metabolic diseases and also play a role in body weight management in humans.

In conclusion, the HF diet had detrimental effects on glucose metabolism and body weight but the two indigestible carbohydrates tested in this study, lactitol and PDX, may provide an
additional means of regulating weight management and post-prandial metabolism, especially via the triglyceride response. In addition, lactitol significantly increased the PYY satiety hormone levels. However, the oral administration of a single strain of *B. thetaioaotiamicon* had a minor or no effect on metabolism.

**AUTHOR CONTRIBUTIONS**

KT planned and supervised the study. K-HH contributed to the design of the study and supervised the PYY analysis. KO analyzed the data and was responsible for writing the manuscript. MS conducted the SCFA, dry matter, and heat value analyses, and contributed to the manuscript. SF supervised the microbial analysis and participated in production of the manuscript. MM analyzed the data and was responsible for writing the manuscript.

**REFERENCES**

1. Kumar V, Sinha AK, Makkar HPS, de Boeck G, Becker K. Dietary roles of non-starch polysaccharides in human nutrition: a review. *Crit Rev Food Sci Nutr* (2012) 52(10):899–935. doi:10.1080/10408398.2010.512671
2. Cani PD, Delzenne NM, Amar J, Burcelin R. Role of gut microflora in the development of obesity and insulin resistance following high-fat diet feeding. *Pathol Biol* (2008) 56(5):355–9. doi:10.1016/j.patbio.2007.09.008
3. Nieuwdorp M, Gilijamse PW, Pai N, Kaplan LM. Role of the microbiome in energy regulation and metabolism. *Gastroenterology* (2014) 146(6):1525–33. doi:10.1053/j.gastro.2014.02.008
4. Drakoularakou A, Hasselwander O, EDMUND M, Ouwehand AC. Lactitol, an emerging prebiotic: functional properties with a focus on digestive health. *Food Sci Tech Bull Funct Foods* (2007) 3(7):71–80. doi:10.1016/j.gast.2007.11.14685
5. Raninen K, Lappi M, Mykkänen H, Poutanen K. Dietary fiber type reflects physiological functionality: comparison of grain fiber, inulin, and polydextrose. *Nutr Rev* (2011) 69(1):9–21. doi:10.1111/j.1753-4887.2010.00358.x
6. Craig SA, Holden JF, Khaled MY, Craig SA, Holden JF, Khaled MY. Determination of polydextrose as dietary fiber in foods. *J AOAC Int* (2000) 83(4):1006–12.
7. Mäkeläinen HS, Mäkivuokko HA, Salminen SJ, Rautonen NE, Ouwehand AC. The effects of polydextrose and xylitol on microbial community and activity in a 4-stage colon simulator. *J Food Sci* (2007) 72(5):M153–9. doi:10.1111/j.1750-3841.2007.00350.x
8. Hull S, Re R, Tühonen K, Viscione L, Wickham M. Consuming polydextrose in a mid-morning snack increases acute satiety measurements and reduces subsequent energy intake at lunch in healthy human subjects. *Appetite* (2012) 59(3):706–12. doi:10.1016/j.appet.2012.08.004
9. Ranawana V, Muller A, Henry CJK. Polydextrose: its impact on short-term food intake and subjective feelings of satiety in males—a randomized controlled cross-over study. *Eur J Nutr* (2013) 52:885–93. doi:10.1007/s00394-012-0395-4
10. Ibarra A, Astbury NM, Olli K, Alhoniemi E, Tühonen K. Effects of polydextrose on different levels of energy intake. A systematic review and meta-analysis. *Appetite* (2015) 87(0):30–7. doi:10.1016/j.appet.2014.12.099
11. Probert HM, Apajaheli HT, Rautonen N, Stowell J, Gibson GR. Polydextrose, lactitol, and fructo-oligosaccharide fermentation by colonic bacteria in a three-stage continuous culture system. *Appl Environ Microbiol* (2004) 70(8):4505–11. doi:10.1128/AEM.70.8.4505-4511.2004
12. Karhunen LJ, Juvenen KR, Huotari A, Purhonen AK, Herzig KH. Effect of protein, fat, carbohydrate and fibre on gastrointestinal peptide release in humans. *Regul Pept* (2008) 149(1–3):70–8. doi:10.1016/j.regpep.2007.10.008
13. Herzig KH. Cholecystokinin- and secretin-releasing peptides in the intestine—a new regulatory enterodocrine mechanism in the gastrointestinal tract. *Regul Pept* (1998) 73(2):89–94. doi:10.1016/s0167-0115(97)02062-8
14. Karra E, Chandarana K, Batterham RL. The role of peptide YY in appetite regulation and obesity. *J Physiol (Lond)* (2009) 587(1):19–25. doi:10.1113/jphysiol.2008.164269
15. Gee JM, Johnson JT. Dietary lactitol fermentation increases circulating peptide YY and glucagon-like peptide-1 in rats and humans. *Nutrition* (2005) 21(10):1036–43. doi:10.1016/j.nut.2005.03.002
16. Lesniewska V, Rowland I, Cani PD, Neyrinck AM, Delzenne NM, Naughton PJ. Effect on components of the intestinal microflora and plasma neuropeptide levels of feeding *Lactobacillus delbrueckii*, *Bifidobacterium lactis*, and inulin to adult and elderly rats. *Appl Environ Microbiol* (2006) 72(10):6333–8. doi:10.1128/AEM.00915-06
17. Fu-Cheng XM, Anini Y, Charoit J, Voisin T, Galliche JP, Rosé C. Peptide YY release after intraaduodenal, intraileal, and intracolonial administration of nutrients in rats. *Pfluegers Arch* (1995) 431(1):66–75. doi:10.1007/bf00374378
18. Cherbut C, Ferrier L, Roze C, Anini Y, Blottiere H, Lecannu G, et al. Short-chain fatty acids modify colonic motility through nerves and polypeptide YY release in the rat. *Am J Physiol Gastrointest Liver Physiol* (1998) 275(6):G1415–22.
19. Conterno L, Fava F, Viola R, Tuohy KM. Obesity and the gut microbiota: does up-regulating colonic fermentation protect against obesity and metabolic disease? *Genes Nutr* (2011) 6(3):241–60. doi:10.1007/s12263-011-0230-1
20. Darzi J, Frost GS, Robertson MD. Do SCFA have a role in appetite regulation? *Proc Nutr Soc* (2011) 70(1):119–28. doi:10.1017/S0033812410004039
21. Levy RE, Bakhéed F, Verburnh P, Louzupone CA, Knight RD, Gordon JJ. Obesity alters gut microbial ecology. *Proc Natl Acad Sci U S A* (2005) 102(31):11070–5. doi:10.1073/pnas.0504978102
22. Levy RE, Verburnh PJ, Klein S, Gordon JJ. Microbial ecology – human gut microbes associated with obesity. *Nature* (2006) 444(7122):1022–3. doi:10.1038/nature44102a
23. Verburnh PJ, Bakhéed F, Fulton L, Gordon JJ. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host Microbe* (2008) 3(4):213–23. doi:10.1016/j.chom.2008.02.015
24. Cani PD, Neyrinck AM, Fava F, Knauf C, Burcelin RG, Tuohy KM, et al. Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. *Diabetologia* (2007) 50(1):2374–83. doi:10.1007/s00125-007-0791-0
25. Chen C, Li L, Wu Z, Chen H, Fu S. Effects of lactitol on intestinal microflora and plasma endotoxin in patients with chronic viral hepatitis. *J Infect* (2007) 54(1):98–102. doi:10.1016/j.jinf.2005.11.013
26. Jie Z, Bang-yao L, Ming-jie X, Hai-wei L, Zu-kang Z, Ting-song W, et al. Studies on the effects of polydextrose intake on physiologic functions in Chinese people. *Am J Clin Nutr* (2000) 72(6):1503–9.
27. Tühonen K, Suomalainen T, Tynkkynen S, Rautonen N. Effect of prebiotic supplementation on a probiotic bacteria mixture: comparison between a testinal tract. *Regul Pept* (1998) 73(2):89–94. doi:10.1016/s0167-0115(97)02062-8

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rat model and clinical trials. *Br J Nutr* (2008) 99(4):826–31. doi:10.1017/ s0007114507825141

28. Beards E, Tuohy K, Gibson G. A human volunteer study to assess the impact of confectionery sweeteners on the gut microbiota composition. *Br J Nutr* (2010) 104(5):701–8. doi:10.1017/S0007114510001078

29. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* (2006) 444(7122):1027–31. doi:10.1038/nature05414

30. Parnell JA, Reimer RA. Prebiotic fibres dose-dependently increase satiety hormones and alter Bacteroidetes and Firmicutes in lean and obese JCR:LA-cp rats. *Br J Nutr* (2012) 107(4):601–13. doi:10.1017/S000711451003163X

31. Forssten SD, Korczyńska MZ, Zwijsen RML, Noordman WH, Madetoja M, Ouwehand AC. Changes in satiety hormone concentrations and feed intake in rats in response to lactic acid bacteria. *Appetite* (2013) 71:16–21. doi:10.1016/j. appetite.2013.06.093

32. Peuranen S, Tiitinen K, Apajalahi J, Kettunen A, Saarinen M, Rautionen N. Combination of polydextrose and lactitol affects microbial ecosystem and immune responses in rat gastrointestinal tract. *Br J Nutr* (2004) 91(6):905–14. doi:10.1079/BJN20041114

33. Rinttilä T, Kassinen A, Malinen E, Krogius L, Palva A. Development of an extensive set of 16S rDNA-targeted primers for quantification of pathogenic and indigenous bacteria in faecal samples by real-time PCR. *J Appl Microbiol* (2004) 97(6):1166–77. doi:10.1111/j.1365-2672.2004.02409.x

34. Buerter R, Scholnerich J, Bollheimer LC. High-fat diets: modeling the metabolic disorders of human obesity in rodents. *Obesity* (2007) 15(4):798–808. doi:10.1038/oby.2007.608

35. Strubbe JH, Steffens AB. Blood glucose levels in portal and peripheral circulation and their relation to food intake in the rat. *Physiol Behav* (1977) 19(2):303–7. doi:10.1016/0031-9384(77)90342-0

36. Vrang N, Madsen AN, Tang-Christensen M, Hansen G, Larsen PJ. PY(3-36) reduces food intake and body weight and improves insulin sensitivity in rodent models of diet-induced obesity. *Am J Physiol Regul Integr Comp Physiol* (2006) 291(2):R367–75. doi:10.1152/ajpregu.00726.2005

37. Akiyama T, Tachibana I, Shirohara H, Watanabe N, Otsuki M. High-fat diet and their relation to food intake in the rat. *Diabetes Res Clin Pract* (1996) 31(1–3):27–35. doi:10.1016/0168-8227(96)01205-3

38. Cani PD, Neyrinck AM, Maton N, Delzenne NM. Oligofructose promotes satiety in rats fed a high-fat diet: involvement of glucagon-like peptide-1. *Obes Res* (2005) 13(6):1000–7. doi:10.1038/oby.2005.117

39. Mäkivuokko H, Forssten S, Saarinen M, Ouwehand A, Rautionen N. Synergistic effects of lactitol and *Lactobacillus acidophilus* NCFM in a semi-continuous colon fermentation model. *Benef Microbes* (2010) 1(2):131–7. doi:10.3920/RM2009.0033

40. Björkånd M, Ouwehand AC, Forssten SD, Nikkilä J, Tiitinen K, Rautionen N, et al. Gut microbiota of healthy elderly NSAID users is selectively modified with the administration of *Lactobacillus acidophilus* NCFM and lactitol. *Age (Dordr)* (2012) 34(4):987–99. doi:10.1007/s11357-012-9924-5

41. Samuel BS, Gordon J. A humanized gnotobiotic mouse model of host-archaeal-bacterial mutualism. *Proc Natl Acad Sci U S A* (2006) 103(26):10011–6. doi:10.1073/pnas.0602187103

42. Hildebrandt MA, Hoffmann C, Sherrill-Mix SA, Keilbaugh SA, Hamady M, Chen Y-Y, et al. High-fat diet determines the composition of the murine gut microbiome independently of obesity. *Gastroenterology* (2009) 137(5):1716–24. doi:10.1053/j.gastro.2009.08.042

43. Islam MS, Sakaguchi E, Kashima N, Hoshi S. Effect of sugar alcohols on gut function and body composition in normal and cecocectomized rats. *Exp Anim* (2004) 53(4):361–71. doi:10.1538/expanim.53.361

44. Mäkivuokko H, Nurmi J, Nurminen P, Stowell J, Rautionen N. In vitro effects on polydextrose by colonic bacteria and caco-2 cell cyclooxygenase gene expression. *Nutr Cancer* (2005) 52(1):94–104. doi:10.1207/s15327944nc5201_12

45. Nilsson AC, Ostman EM, Hoist JF, Bjorck IME. Including indigestible carbohydrates in the evening meal of healthy subjects improves glucose tolerance, lowers inflammatory markers, and increases satiety after a subsequent standardized breakfast. *J Nutr* (2008) 138(4):732–9.

46. Shimomura Y, Maeda K, Nagasaki M, Matsuo Y, Murakami T, Rajotte G, et al. Attenuated response of the serum triglyceride concentration to ingestion of a chocolate containing polydextrose and lactitol in place of sugar. *Biosci Biotechnol Biochem* (2005) 69(10):1819–23. doi:10.1271/bbb.691819

47. Tiitinen K, Rautionen N, Alhorniemi E, Ahotupa M, Stowell J, Vasankari T. Postprandial triglyceride response in normal lipopidemic, hyperlipidemic and obese subjects – the influence of polydextrose, a non-digestible carbohydrate. *Nutr J* (2015) 14:23. doi:10.1186/s12957-015-0009-0

48. Russell JC, Proctor SD. Small animal models of cardiovascular disease: tools for the study of the roles of metabolic syndrome, dyslipidemia, and atherosclerosis. *Cardiovasc Pathol* (2006) 15(6):318–30. doi:10.1016/j.carpath.2006.09.001

49. Panzoldo NB, Urban A, Parra ES, Oliveira R, Zago VS, da Silva LR, et al. Differences and similarities of postprandial lipemia in rodents and humans. *Lipids Health Dis* (2011) 10:86. doi:10.1186/1476-511x-10-86

50. Davy BM, Melby CL. The effect of fiber-rich carbohydrates on features of Syndrome X. *Am J Diet Assoc* (2003) 103(1):86–96. doi:10.1053/jada.2003.50005

51. Venn BJ, Mann JL. Cereal grains, legumes and diabetes. *Eur J Clin Nutr* (2004) 58(11):1443–61. doi:10.1038/sj.ejcn.1601995

52. Natah SS, Hussien KR, Tuominen JA, Koivisto VA. Metabolic response to lactitol and xylitol in healthy men. *Am J Clin Nutr* (1997) 65(4):947–50.

53. Reimer RA, Maurer AD, Eller AK, Hallam MC, Shaykhutdinov R, Vogel HJ, et al. Satiety hormone and metabolic response to an intermittent high energy diet differs in rats consuming long-term diets high in protein or prebiotic fiber. *J Proteome Res* (2012) 11(8):4065–74. doi:10.1021/pr300487s

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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