1 ***In vitro* effects of *Bifidobacterium lactis*-based synbiotics on human faecal bacteria**

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**Abstract**

Synbiotic supplements contain pre- and probiotics and are used to modulate gut microbiota composition. This study aimed to investigate effects of two synbiotic mixtures on human faecal bacteria *in vitro.* Short chain fructooligosaccharides (FOS) (1% w/v) combined with either *Bifidobacterium lactis* Bb12 or *Bifidobacterium lactis* HN019 ($10^6$ colony-forming units (CFU)/mL)] were added to pH-controlled anaerobic batch cultures inoculated with human faeces. Maltodextrin (1% w/v), FOS (1% w/v) and the probiotic strains were also tested individually. Effects on bacteria, short-chain fatty acids (SCFAs) and branched-chain fatty acids (BCFAs) were assessed over 48 hours. With maltodextrin, FOS and the synbiotic mixtures, there was a significant increase in total bacteria and bifidobacteria numbers, compared to the negative control or probiotics alone. Increases in *Atopobium* cluster and *Clostridium coccoides-Eubacterium rectale* group occurred with FOS and maltodextrin, respectively. Additionally, maltodextrin, FOS and synbiotics resulted in a greater production of acetate and butyrate (SCFAs) compared to the negative control and probiotics alone, whereas concentrations of iso-valerate (BCFA) were lower with these treatments. In conclusion, synbiotic-induced *in vitro* bacterial changes and changes in SCFAs concentrations were not different from those observed with FOS alone. These data suggest that metabolic effects of these synbiotics are largely driven by the prebiotic component.

*Keywords*: Probiotic; Prebiotic; Maltodextrin; Faecal microbiota; Batch culture system; Short-chain fatty acids.

## 1. Introduction

In recent years, studies have indicated that probiotics, prebiotics and their combination (synbiotics) may contribute to the maintenance or improvement of health and prevention of diseases by modifying the gut microbiota (Markowiak & Śliżewska, 2017; Hadi, Mohammadi, Miraghajani, & Ghaedi, 2018; Krumbeck et al., 2018). Probiotics are defined as ‘live microorganisms that, when

49 administered in adequate amounts, confer a health benefit on the host' (Hill et al., 2014). Prebiotics
50 also target gut microbiota with the goal of improving health. Whereas probiotics are live
51 microorganisms, a prebiotic is 'a substrate that is selectively utilized by host microorganisms
52 conferring a health benefit' (Gibson et al., 2017). Thus, prebiotics serve as nutrients for beneficial
53 microorganisms harboured by the host, including administered probiotic strains and indigenous
54 (resident) microorganisms (Gibson et al., 2017). However, in the past few years, prebiotics have no
55 longer seen simply as boosters of bifidobacteria and lactobacilli but are now recognised for their
56 effects on system-wide metabolic and physiological readouts (Sanders, Merenstein, Reid, Gibson,
57 & Rastall, 2019).

58 A number of fermentable carbohydrates have been reported to convey health benefits to the
59 host, but other substances such as polyphenols and polyunsaturated fatty acids might also exert
60 prebiotic effect. In this context, fructooligosaccharides (FOS) and galactooligosaccharides (GOS)
61 currently dominate the prebiotic category (Gibson et al., 2017). These oligosaccharides selectively
62 promote beneficial microorganisms within the gut microbiota and increase production of short-
63 chain fatty acids (SCFAs), among many other benefits (Salazar et al., 2015; Simpson & Campbell,
64 2015). The SCFAs are crucial for intestinal health and can also influence sites distant to the gut,
65 since they may supply energy to colon enterocytes and function as signaling molecules, influencing
66 intestinal permeability. SCFA receptors are not only found in the gut, but are also expressed in
67 immune cells and human white adipose tissue (Gibson et al., 2017; Choque Delgado & Tamashiro,
68 2018).

69 In a general sense, probiotic and prebiotic interventions serve to increase the community of
70 beneficial microorganisms and products of their growth and metabolism in the host (Sanders,
71 Merenstein, Reid, Gibson, & Rastall, 2019). In this manner, it is known that microbiota
72 composition can also be successfully modulated by certain synbiotic treatments. *In vivo* and *in vitro*
73 studies have shown the positive effects of specific synbiotics (Panigrahi et al., 2017, Freitas et al.,
74 2018; Kim, Keogh, & Clifton, 2018; Krumbeck, Walter & Hutkins, 2018); however, the

75 development of effective combinations remains a challenging issue. It is currently unknown if it is
76 possible for a probiotic strain to benefit from the presence of a prebiotic substrate in the competitive
77 environment of the human gut (Krumbeck et al., 2018).
78 *Bifidobacterium lactis* Bb12 and *B. lactis* HN019 are widely used bifidobacterial strains in
79 fermented dairy production that may convey health-promoting properties in human hosts (Jungersen
80 et al., 2014; Meng et al., 2016). One approach to enrich for bifidobacteria, increase the number of
81 responders, and enhance their metabolic activity would be to administer a prebiotic together with a
82 select strain or that use the prebiotic as a growth substrate. According to ecological theory, the
83 provision of resources in a microbial community leads to a relaxation of competition and therefore
84 could enhance colonization success of probiotic strains (Walter, Maldonado-Gómez & Martínez,
85 2018). Despite the proven bifidogenic effect of FOS, bifidobacteria ability to use different
86 carbohydrates varies among strains (Milani et al., 2015).
87 The synbiotic combination of *B. lactis* Bb12 and FOS, was used in the INvestigation of
88 SYbiotic TrEatment (INSYTE) study (*www.clinicaltrials.gov* registration number NCT01680640);
89 a unique proof-of-concept double blind, randomised, placebo-controlled trial with a ~12 month
90 intervention in patients with non-alcoholic fatty liver disease (NAFLD) (Scorletti et al., 2018). The
91 aim of the current study was to investigate whether: a) *Bifidobacterium lactis* Bb12 and FOS and b)
92 *B. lactis* HN019 and FOS, were more effective than maltodextrin, FOS or either probiotic alone,
93 using an *in vitro* faecal bacterial culture system. The potential fermentation properties of the two
94 synbiotics were investigated using pH-controlled anaerobic faecal batch cultures. Levels of selected
95 microbial groups and concentrations of SCFAs and BCFAs were then quantified in order to assess
96 relationships between changes in organic acids and variations in microbial populations.
97

98 **2. Materials and methods**

99 *2.1. Substrates*

Maltodextrin and FOS with a degree of polymerization < 10 were supplied by Chr. Hansen, Hørsholm, Denmark. Freeze-dried *Bifidobacterium animalis* subsp. *lactis* HN019 (DuPont-Danisco, Madison, USA) and *Bifidobacterium animalis* subsp. *lactis* Bb12 (Chr. Hansen, Hørsholm, Denmark) were stored at -80°C. Plates of de Man-Rogosa-Sharpe (MRS) agar (Oxoid Ltd, Basingstoke, UK) were inoculated with the bifidobacterial strains and incubated at 37°C in an anaerobic chamber (10% $CO_2$, 10% $H_2$ and 80% $N_2$, Don Whitley Scientific Ltd, Shipley, UK) for 48 hours. After incubation, bottles containing 9 mL of MRS broth were then inoculated with one colony from each plate, and incubated for 24 hours under the same conditions, as mentioned above.

### *2.2. Faecal Sample Preparation*

Faecal samples were collected from three healthy individuals (2 women and 1 man aged between 25 and 35 years) who had not taken antibiotics, probiotics or prebiotics for at least six months before the faecal collection, and who had no history of gastrointestinal disorders. The number of volunteers selected in this study was based on previously batch cultures studies (Ramnani, Costabile, Bustillo, & Gibson, 2015, Monteagudo-Mera et al., 2018, Wang et al., 2019). Faecal samples were collected, on site, on the day of the experiment and placed in an anaerobic jar (AnaeroJar™ 2.5L, Oxoid Ltd), including a gas-generating kit (AnaeroGen™, Oxoid). Samples were prepared as reported by Guergoletto, Costabile, Flores, Garcia and Gibson (2016) and 15 mL of the resulting faecal slurries were immediately used to inoculate the batch-culture systems.

### *2.3. In vitro Batch Culture Fermentation*

Sterile stirred batch culture fermentation vessels (300 mL working volume) were prepared and aseptically filled with 135 mL of sterile basal nutrient medium, prepared as reported by Guergoletto, Costabile, Flores, Garcia and Gibson (2016). Once in the fermentation vessels, sterile medium was maintained under anaerobic conditions by sparging the vessels with $O_2$-free $N_2$ overnight (15mL/min). The temperature was held at 37°C using a circulating water bath and pH

126 values controlled between 6.7 and 6.9 using an automated pH controller (Fermac 260; Electrolab,
127 Tewkesbury, UK) which added acid or alkali as required (0.5 M HCl and 0.5 M NaOH).
128 Seven vessels (a to g below) containing faecal slurry were prepared for treatments with: a)
129 maltodextrin (1% w/v), b) FOS (1% w/v), c) *B. lactis* Bb12 ($10^6$ CFU/mL), d) *B. lactis* HN019 ($10^6$
130 CFU/mL), e) FOS combined with *B. lactis* Bb12 (Syn 1), f) FOS combined with *B. lactis* HN019
131 (Syn 2) at the same concentrations as when used alone, and g) a negative control (without any
132 added substrate or microorganism). Maltodextrin was tested alone since this carbohydrate is
133 commonly used as placebo/control for studies testing the effects of prebiotics in humans. Seven
134 batch culture fermenters were thus run in parallel and the experiment was performed in triplicate,
135 using one faecal sample from a different donor each time. The probiotics and carbohydrates were
136 added to each vessel just before the addition of 15 mL (1:10, w/v) of fresh faecal slurry. Batch
137 cultures experiments were conducted for 48 h, and 4 mL samples obtained from each vessel at 0, 8,
138 24 and 48 h for analyses.
139
140 *2.4. Enumeration of Bacterial Populations by FISH Analysis*
141 Fluorescence in situ hybridisation (FISH) analysis by flow cytometry was performed as
142 described by Daims, Stoecker and Wagner (2005). Briefly, aliquots (750 µL) of batch culture
143 samples were centrifuged at 1136 × g for 5 min. Pellets were suspended in 375 µL filtered 0.1 M
144 Phosphate-buffered saline (PBS) solution. Filtered 4% paraformaldehyde (PFA) (1125 µl) was
145 added and samples were stored at 4°C for 4 h. Samples were then washed twice with PBS to
146 remove PFA and re-suspended in a mixture containing 300 µl PBS and 300 µl 99% ethanol.
147 Samples were then stored at -20°C, until used for hybridization as described by Wang et al. (2019).
148 The probes used (Sigma Aldrich Ltd, Poole, UK) are listed in Table 1.
149
150 *2.5. Short-chain Fatty Acids (SCFAs) Analysis*

151 SCFAs were measured by gas chromatography as previously reported by Richardson,
152 Calder, Stewart and Smith (1989). Aliquots of 1 mL of sample supernatant were transferred into
153 glass tubes with 50 µL of 2-ethylbutyric acid (0.1 M, internal standard) (Sigma, Poole, UK). 500 µL
154 of concentrated hydrochloric acid (HCl) and 2 mL of diethyl ether (Sigma Aldrich Ltd., Poole, UK)
155 were added to each glass tube and samples vortexed for 1 min. Samples were centrifuged at 2000 x
156 g for 10 min. The diethyl ether (upper) layer of each sample was transferred to a labelled clean glass
157 tube. A second extraction was conducted by adding another 0.5 ml diethyl ether, followed by
158 vortexing and re-centrifugation. The diethyl ether layers were pooled. 400 µl of pooled ether extract
159 and 50 µl N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA) (Sigma-Aldrich,
160 Poole, UK) were added into a GC screw-cap vial. Samples were left at room temperature for 72 h to
161 allow SCFAs in the samples to completely derivatise.

162 An Agilent/HP 6890 Gas Chromatograph (Hewlett Packard, UK) fitted with a HP-5MS 30
163 m × 0.25 mm column with a 0.25 µm coating (Crosslinked (5%-Phenyl)-methylpolysiloxane,
164 Hewlett Packard, UK) was used for analysis of derivatised SCFAs and BCFAs. Temperatures of
165 injector and detector were 275°C. The column was programmed to increase in temperature from
166 63°C to 190°C at a rate of 15°C $min^{-1}$ and was then held at 190°C for 3 min. Helium was the carrier
167 gas (flow rate 1.7 ml $min^{-1}$; head pressure 133 KPa). A split ratio of 100:1 was used. Quantification
168 of the samples was obtained through calibration curves of lactic, acetic, propionic, butyric, valeric,
169 iso-butyric and iso-valeric acids at concentrations between 12.5 and 100 mM.

170

171 *2.6. Statistical Analysis*

172 Differences within each treatment were evaluated after 8, 24 and 48 h of fermentation
173 compared with baseline (0 h of fermentation) using paired Student's t-tests and were considered
174 significantly different from baseline when P ≤ 0.05. The data were then analysed to compare
175 different treatments at the same time point using the post hoc analysis (Tukey test) and P ≤ 0.05 to

176 indicate significance. Analyses were performed using Statistica software version 10.0 (Statsoft
177 South America).
178
179 **3. Results**
180 *3.1. Bacterial Enumeration by FISH*
181 Bacterial counts in control and treatment groups are shown in Figs. 1 and 2. Increases in
182 total bacterial levels (Fig. 1A) were observed after 8 h for maltodextrin, FOS, Syn 1 and Syn 2
183 compared to probiotics alone (P = 0.014, P = 0.034, P = 0.013 and P = 0.032, respectively). The
184 highest number of bacteria was detected in the fermentation with Bb12 + FOS (Syn 1) at 8 h, $Log_{10}$
185 8.34 ± 0.18 CFU/mL, while at baseline counts were $Log_{10}$ 7.56 ± 0.38 CFU/mL.
186 A significant increase in *Bifidobacterium* spp. numbers (Fig. 1B) was also observed for
187 maltodextrin, FOS, Syn 1 and Syn 2 (P = 0.022, P = 0.038, P = 0.034 and P = 0.023, respectively)
188 compared to probiotics and the negative control. Bifidobacteria counts ranged from $Log_{10}$ 5.64 ±
189 0.32 at baseline to 7.77 ± 0.52 CFU/mL after 8 h of fermentation with Syn 1. Regarding
190 *Lactobacillus-Enterococcus* group (Fig. 1C), a rise was observed, but the increase was not
191 significant at all time points (P > 0.10), the highest number was detected in the FOS culture at 24 h.
192 Levels of *Clostridium coccoides–Eubacterium rectale* group (Fig. 1E) significantly increased
193 at 8 and 24 h of fermentation with maltodextrin (P = 0.028 and P = 0.038, respectively) ($Log_{10}$ 6.61
194 ± 0.23 to 7.63 ± 0.53 CFU/mL). An increase in *Atopobium* cluster (Fig. 2A) was observed with FOS
195 fermentation at 8 h ($Log_{10}$ 5.12 ± 0.59 to 7.02 ± 0.54 CFU/mL) (P = 0.034). Because of high
196 variations among the volunteers at 8 and 24 h, the increase in *Atopobium* cluster was not significant
197 for maltodextrin and synbiotic mixtures compared to baseline.
198 No significant differences were found for the other bacterial groups analysed, including
199 *Bacteroides* spp.-*Prevotella* group, *Roseburia* genus, Clostridial cluster IX populations, *F.*
200 *prausnitzii* group, *Desulfovibrio* genus, *Clostridium histolyticum* group and *Cytophaga-Flexibacter-*
201 *Bacteroides*.

*3.2. SCFAs Concentrations*

Following administration of all treatments, acetate was the main end product of microbial fermentation (Table 2). Acetate concentrations were highest with maltodextrin, FOS and synbiotic mixtures ($P < 0.0002$) at all time points (8, 24 and 48 h) compared to the negative control and probiotics; however, there were no significant differences among these four treatments (maltodextrin, FOS, Syn 1 and Syn 2). Acetate levels ranged from $4.12 \pm 1.57$ mM at baseline to $77.34 \pm 9.14$ mM after 48 h of fermentation with FOS.

Supplementation with maltodextrin had the largest effect in increasing the concentration of butyrate at 8 h ($1.58 \pm 0.60$ mM), which was significantly higher ($P < 0.011$) than the negative control ($0.36 \pm 0.07$ mM) and other treatments. After 24 h of fermentation, the concentration of butyrate was also higher in vessels with FOS ($1.93 \pm 0.13$ mM, $P < 0.004$), Syn 1 ($2.49 \pm 0.55$ mM, $P < 0.002$) and Syn 2 ($2.56 \pm 1.03$ mM, $P < 0.001$) compared to the negative control ($0.60 \pm 0.09$ mM) and probiotics ($0.59 \pm 0.12$ mM with Bb12 and $0.52 \pm 0.14$ mM with HN019 strain).

Propionate was increased in all vessels compared to baseline ($P < 0.046$); for instance, values ranged from $0.17 \pm 0.07$ to $18.55 \pm 9.54$ mM after 48 h of fermentation with FOS. However, because of high variations among volunteers, increase of this SCFAs in all treatments was not significant compared to the negative control ($P > 0.15$).

On the other hand, isovalerate, a branched-chain fatty acid (BCFA) product of amino acid metabolism was higher in the probiotic vessels ($0.12 \pm 0.05$ mM with Bb12 and $0.11 \pm 0.05$ mM with HN019, $P < 0.009$) and the negative control ($0.17 \pm 0.03$ mM, $P < 0.016$) after 24 and 48 h of fermentation compared to the carbohydrate-containing vessels. Finally, to a lesser extent, valerate and isobutyrate concentrations were higher ($P < 0.033$ and $P < 0.049$, respectively) after all treatments compared to baseline, but no statistical difference was found among treatments ($P > 0.38$).

228 **4. Discussion**

229 In this study, the experimental design compared probiotics and prebiotics alone and in
230 combination in a controlled setting. Our novel results show that there were marked changes in total
231 bacteria and bifidobacterial numbers in human faecal slurry in response to incubation with either
232 FOS, maltodextrin or either synbiotic mixture. Bifidobacteria are recognised as one of the most
233 important bacterial groups associated with human health, providing beneficial effects in the large
234 intestine (Russel, Ross, Fitzgerald & Stanton, 2011) and it is well established that an increase in
235 bifidobacterial numbers is favoured by the presence of (mainly) fermentable carbohydrates. In fact,
236 a simple bifidobacterial community may co-operate between themselves as well as with other
237 members of the gut microbiota in the utilization of specific glycans, by means of cross-feeding
238 activities, so as to provide growth benefits to one or both members of such a community as well as
239 with the other members of the gut microbiota (Turroni et al., 2018).

240 Surprisingly our data also show that there was increased growth of bifidobacteria with
241 maltodextrin supplementation. Whereas FOS (along with GOS) is the dietary prebiotic most
242 extensively documented to confer health benefits in humans, maltodextrin is a maltooligosaccharide
243 not usually considered to be a prebiotic. FOS is preferentially metabolized by bifidobacteria, since
244 its $\beta$ (1→2)-glycosidic bonds can be readily degraded by β-fructanosidase enzyme (prevalent in
245 bifidobacteria) (Gibson et al., 2017). It was postulated that enhanced growth of bifidobacteria in the
246 presence of maltodextrin was probably due to the ability of some bifidobacterial species to produce
247 the enzyme that hydrolyses maltodextrin to glucose for growth (Yeo & Liong, 2010), confirming
248 the bifidogenic activity of maltodextrin *in vitro*. However, it is important to emphasize that
249 maltodextrin is a common placebo for studies testing the effects of prebiotics in humans (Kolida,
250 Meyer & Gibson, 2007; Costabile et al., 2010; Ramnani, Costabile, Bustillo, & Gibson, 2015;
251 Pedersen et al., 2016). Since maltodextrins are partially depolymerized starch granules, digestion of
252 maltodextrin occurs through the same starch digesting enzymes in humans (α-amylase and maltase).
253 The glucose and maltose obtained from maltodextrin digestion is readily absorbed in the small

254 intestine and subsequently used in metabolism (Hofman, Van Buul, & Brouns, 2016), and therefore,
255 no intact substrate reaches the colon to be fermented by the intestinal bacteria such as
256 bifidobacteria.

257 The impact of bifidobacteria in the breakdown of dietary carbohydrates is crucial for the
258 establishment and reinforcement of trophic relationships among members of the gut microbiota
259 (Turroni et al., 2018). In particular, bifidobacteria possess a range of cell-associated and
260 extracellular glycosidases and specific transport systems enabling them to rapidly assimilate low-
261 molecular weight sugars (Rivière, Selak, Geirnaert, Abbeele & De Vuyst, 2018). On the other hand,
262 other microorganisms are adept at breaking down high molecular weight polysaccharides and this
263 pathway from a polysaccharide to a SCFA is a complex and indirect network of metabolism
264 (Sanders, Merenstein, Reid, Gibson & Rastall, 2019). Since lactate and acetate are utilized by other
265 microorganisms to produce propionate and butyrate, probable ecological networks involved in the
266 metabolism of carbohydrates have been elucidated (Rivière, Selak, Lantin, Leroy & De Vuyst,
267 2016; Scott, Martin, Duncan & Flint, 2014), although the extent to which they operate in the gut is
268 not clear at the present time. In this respect, lactate and acetate produced by bifidobacteria may be
269 substrates for butyrate-producing colon bacteria such as *Eubacterium rectale* (Moens, Verce &
270 Vuyst, 2017). Numbers of *Clostridium coccoides*-*Eubacterium rectale* group were increased in the
271 maltodextrin vessel, possibly due to a cross-feeding between species. *E. rectale* is recognised as one
272 of the most prolific butyrate producers in the human colon. Coinciding with the increase in *E.*
273 *rectale* numbers, the highest concentration of butyrate after 8 h was found in the vessel with
274 maltodextrin. FOS and synbiotic mixture fermentations also increased butyrate concentrations after
275 24 and 48 h. Butyrate also has an important role in the colon because of its beneficial effects in the
276 colonic epithelium (Canani, Costanzo, Leone, Pedata, Meli & Calignano, 2011).

277 Another group of bacteria stimulated by FOS was *Atopobium,* commonly isolated from
278 healthy human faeces, which belongs to the *Collinsella* genus (Thorasin, Hoyles & McCartney,
279 2015). From the present study, it is not possible to distinguish whether the *Atopobium* group can

280 ferment FOS, although it is able to metabolize fructose (Moore, Cato & Holdeman, 1971) or if the
281 increase in numbers is due to cross-feeding between different bacterial groups. An increase in this
282 group has already been reported *in vitro* with FOS (Saulnier, Gibson & Kolida, 2008); but the role
283 of *Atopobium* in the human colon is still unclear.

284 Although *B. lactis* fermentation in probiotic vessels had no significant effect upon relevant
285 microbial populations at the level of genus, we can speculate that an interplay between *B. lactis* and
286 other strains may have occurred. This outcome is plausible due to the highly competitive
287 environment that favours autochthonous strains (Walter, Maldonado-Gómez & Martínez, 2018)
288 and/or the numbers of probiotic bacteria that were added was possibly too low to have a discernible
289 effect. Since most commercial probiotic strains belong to species that are allochthonous to the
290 human gastrointestinal tract (such as *B. lactis*), and lack the required traits to successfully colonize
291 gut ecosystems, the potential of using autochthonous members of the human microbiome to develop
292 next-generation probiotics and bio-therapeutics is increasingly recognized (O'Toole, Marchesi &
293 Hill, 2017).

294 FOS, maltodextrin and synbiotic mixtures induced modulation of faecal bacteria to increase
295 acetate and butyrate production. Acetate is produced mainly through the fructose-6-phosphate
296 phosphoketolase pathway by bifidobacteria (Miller & Wolin, 1996) and along with butyrate is
297 considered beneficial to the human gut (Gibson et al., 2017). In this manner, the greater
298 concentration of acetate is most likely related to the increase of bifidobacteria in the FOS,
299 maltodextrin and synbiotic vessels and their production of acetate from carbohydrates (Rivière,
300 Selak, Geirnaert, Abbeele & De Vuyst, 2018).

301 In contrast to these findings, the presence of maltodextrin and FOS inhibited production of
302 the BCFA isovalerate perhaps due to the increase of carbohydrate availability which reduces the
303 relative protein availability for bacterial fermentation. Since the end products of proteolytic
304 fermentation include toxic metabolites (such as certain phenolic compounds, amines, and ammonia)
305 (Gibson, 2004), this beneficial effect may be mediated by decreased microbial proteolysis.

306 Moreover, it is important to emphasize that it is also possible for prebiotics, such as FOS, to exert
307 microbiota-independent effects (Bindels et al., 2017; Wu et al., 2017). The hypothesis underlying
308 much research on prebiotics (and barrier function and inflammation) is that fermentation products
309 such as SCFA probably mediate the beneficial effects through several mechanisms (Sanders,
310 Merenstein, Reid, Gibson, & Rastall, 2019).

311 Finally, the synbiotics tested in this study did not have superior efficacy *in vitro*, compared
312 to the prebiotic component alone, in modulating either the faecal microbiota towards a purportedly
313 healthy composition or the production of SCFAs. Therefore, our findings are of value to science
314 and society since it was possible to establish that the improvement of outcomes in the synbiotics
315 tested was induced by the prebiotic component. However, that said, it is plausible that there could
316 be strain-specific changes and other health benefits beyond SCFA production that we have not
317 measured. Given that no synergism was observed between FOS and the probiotic strains, future
318 trials may explore higher doses of probiotics in synbiotic combinations. Additionally, *in vivo* effects
319 of the synbiotic supplement (*B. lactis* Bb12 and FOS) on changes in gut microbiota through 16S
320 rRNA gene sequencing have been looked at (Scorletti et al., 2018). Hopefully together with the
321 results showed here, this will help to better understand effects of this specific synbiotic on
322 dysbiosis. Synbiotic treatment is safe and well tolerated; therefore if it can be shown that these
323 agents have efficacy to ameliorate liver fat issues, they could be used in primary care settings to
324 treat patients with NAFLD who are in the early stages of liver disease.

325 Our data highlights the need for a careful assessment of specific combinations, dosages and
326 outcome measures used in synbiotic investigations of health and disease-related outcomes, since
327 overall interventions of synbiotics showed mixed findings (Freitas et al., 2018; Kim, Keogh &
328 Clifton, 2018; Krumbeck, Walter & Hutkins, 2018). Further well-designed randomised controlled
329 trials are needed, testing individual components and probiotic/prebiotic combinations.

330

331 **5. Conclusion**

332 In the current study, maltodextrin, FOS and synbiotics led to changes in relevant microbial
333 populations and SCFAs concentrations, compared with the *B. lactis* strains alone. Beneficial
334 modulations were observed in terms of higher levels of bifidobacteria and *Clostridium coccoides*-
335 *Eubacterium rectale* group combined with significant increases also in acetate and butyrate
336 concentrations. Neither bacterial changes, at the level of bacterial genus, nor SCFAs production
337 were different with the synbiotics compared with the prebiotic alone. These data suggest that
338 metabolic effects of the synbiotics tested (FOS combined with either *B. lactis* Bb12 or *B. lactis*
339 HN019) are largely induced by the prebiotic component.

340

341 **Declaration of interest**

342 Materials were provided at no cost by Chr. Hansen Holding A/S, Boege Alle 10-12, 2970
343 Hoersholm, Denmark. Chr. Hansen had no input into any aspect of study design or into data
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356

357 **Author contributions**

358 FCHB was responsible for laboratory analyses, recruitment of donors and writing the
359 manuscript. XW was responsible for laboratory analyses and interpretation of the results. GNC,
360 WAS and LHSM were responsible for interpretation of the results and writing the manuscript. GRG
361 was responsible for the original concept of the study, the study design and for supervising the work.
362 ES, PCC, CDB and GRG made extensive restructuring and revisions of the manuscript.

363

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