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DOI:

[10.1016/j.appet.2015.12.011](https://doi.org/10.1016/j.appet.2015.12.011)

*Document Version*

Peer reviewed version

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*Citation for published version (APA):*

Darzi, J., Frost, G. S., Swann, J. R., Costabile, A., & Robertson, M. D. (2016). L-Rhamnose as a source of colonic propionate inhibits insulin secretion but does not influence measures of appetite or food intake. *Appetite*, 98, 142–149. <https://doi.org/10.1016/j.appet.2015.12.011>

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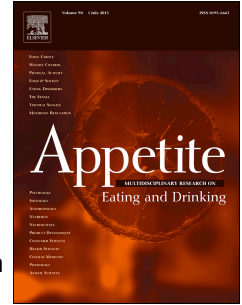
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# Accepted Manuscript

L-Rhamnose as a source of colonic propionate inhibits insulin secretion but does not influence measures of appetite or food intake

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PII: S0195-6663(15)30120-3

DOI: [10.1016/j.appet.2015.12.011](https://doi.org/10.1016/j.appet.2015.12.011)

Reference: APPET 2795

To appear in: *Appetite*

Received Date: 19 August 2015

Revised Date: 4 December 2015

Accepted Date: 14 December 2015

Please cite this article as: Darzi J., Frost G.S., Swann J.R., Costabile A. & Robertson M.D., L-Rhamnose as a source of colonic propionate inhibits insulin secretion but does not influence measures of appetite or food intake, *Appetite* (2016), doi: 10.1016/j.appet.2015.12.011.

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1 **L-Rhamnose as a source of colonic propionate inhibits insulin secretion but does not influence**  
2 **measures of appetite or food intake**

3  
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24  
25 **Running Title:** Darzi *et al.* Inulin and L-Rhamnose effects on appetite

26

27 **Abstract**

28 Activation of free fatty acid receptor (FFAR)2 and FFAR3 *via* colonic short-chain fatty acids,  
29 particularly propionate, are postulated to explain observed inverse associations between dietary  
30 fiber intake and body weight. Propionate is reported as the predominant colonic fermentation  
31 product from L-rhamnose, a natural monosaccharide that resists digestion and absorption reaching  
32 the colon intact, while effects of long-chain inulin on appetite have not been extensively  
33 investigated. In this single-blind randomized crossover study, healthy unrestrained eaters ( $n=13$ )  
34 ingested 25.5 g/d L-rhamnose, 22.4 g/d inulin or no supplement (control) alongside a standardized  
35 breakfast and lunch, following a 6-d run-in to investigate if appetite was inhibited. Postprandial  
36 qualitative appetite, breath hydrogen, and plasma glucose, insulin, triglycerides and non-esterified  
37 fatty acids were assessed for 420 min, then an *ad libitum* meal was provided. Significant treatment x  
38 time effects were found for postprandial insulin ( $P=0.009$ ) and non-esterified fatty acids ( $P=0.046$ )  
39 with a significantly lower insulin response for L-rhamnose ( $P=0.023$ ) than control. No differences  
40 between treatments were found for quantitative and qualitative appetite measures, although  
41 significant treatment x time effects for meal desire ( $P=0.008$ ) and desire to eat sweet ( $P=0.036$ )  
42 were found. Breath hydrogen was significantly higher with inulin ( $P=0.001$ ) and L-rhamnose  
43 ( $P=0.009$ ) than control, indicating colonic fermentation. These findings suggest L-rhamnose may  
44 inhibit postprandial insulin secretion, however neither L-rhamnose or inulin influenced appetite.

45

46 **Highlights:**

- 47 • Postprandial effects of supplementation with inulin and L-rhamnose were investigated
- 48 • Neither inulin nor L-rhamnose influenced subjective or quantitative appetite measures
- 49 • L-rhamnose supplementation did inhibit insulin production postprandially

50

51 **Keywords:** Appetite; satiety; postprandial insulin; inulin-type fructans; short-chain fatty acids

52

53 **Abbreviations:** Area under curve, AUC; Energy intake, EI; Free fatty acid receptor, FFAR;  
54 Glucagon-like receptor-1, GLP-1; Homeostasis Assessment Model, HOMA; Incremental area under  
55 curve, iAUC; L-rhamnose, L-Rha; Peptide YY, PYY; Short-chain fatty acid, SCFA; Visual  
56 analogue scale, VAS

57

58

ACCEPTED MANUSCRIPT

59 **Introduction**

60 Inverse associations between dietary fiber intake and body weight (Du, et al., 2010; Howarth,  
61 Huang, Roberts, & McCrory, 2005), hunger and energy intake (EI) following non-digestible  
62 carbohydrate ingestion in randomized controlled trials (Wanders, et al., 2011), indicate dietary fiber  
63 and other non-digestible carbohydrates may have a role in the prevention and treatment of obesity.  
64 Postulated mechanisms include an increased viscosity of intestinal contents (Kristensen & Jensen,  
65 2011), a reduced energy density due to the bulking effect of non-digestible carbohydrates (Burton-  
66 Freeman, 2000), and an inhibition of EI arising from effects of non-digestible carbohydrate on  
67 satiation and satiety (Burton-Freeman, 2000), possibly mediated by actions of colon derived short-  
68 chain fatty acids (SCFA).

69 Physiological serum SCFA concentrations are low, in the region of 1, 2 and 65  $\mu\text{mol/L}$  for  
70 fasting serum butyrate, propionate and acetate (Fernandes, Vogt, & Wolever, 2011). Postprandially  
71 SCFA concentrations appear to increase significantly in response to ingestion of some non-  
72 digestible carbohydrates including resistant starch (Robertson, Bickerton, Dennis, Vidal, & Frayn,  
73 2005). Physiological SCFA concentrations have been shown to activate two G-protein coupled  
74 receptors, free fatty acid receptor (FFAR) 2 and FFAR3 (Brown, et al., 2003; Le Poul, et al., 2003),  
75 with propionate reported as the most potent agonist (Le Poul, et al., 2003). FFAR2 and FFAR3 are  
76 co-localized in colonic enteroendocrine L-cells with peptide YY (PYY) and glucagon-like peptide 1  
77 (GLP-1) (Karaki, et al., 2006; Karaki, et al., 2008; Tazoe, et al., 2009), both hormones which are  
78 postulated to play roles in the physiological regulation of appetite (Hussain & Bloom, 2013; Lean &  
79 Malkova, 2015). *In vivo* administration of SCFA increases plasma PYY in rats (Cherbut, et al.,  
80 1998; Psichas, et al., 2015) and pigs (Cuche, Cuber, & Malbert, 2000), and of propionate increases  
81 GLP-1 and PYY *via* FFAR2 activation in rodents (Psichas, et al., 2015). *In vitro* and *in vivo*  
82 evidence in rodents further indicates SFCA-induced FFAR2 and FFAR3 activation upregulates  
83 leptin expression in adipose tissue (Covington, Briscoe, Brown, & Jayawickreme, 2006; Xiong, et

84 al., 2004). Thus SCFA, particularly propionate, may be postulated to influence energy homeostasis  
85 and insulin secretion.

86 L-Rhamnose (L-Rha), a natural monosaccharide that resists digestion and absorption  
87 reaching the colon intact (J.A. Vogt, Pencharz, & Wolever, 2004), shows promise as a suitable  
88 candidate to investigate effects on colonic propionate on appetite. Propionate is reported as the  
89 primary SCFA produced during fermentation of L-Rha *in vitro* (Fernandes, Rao, & Wolever, 2000),  
90 and L-Rha ingestion increased serum propionate concentrations in humans acutely (J. A. Vogt, et  
91 al., 2004) and chronically (J. A. Vogt, et al., 2004). Effects of L-Rha ingestion on metabolic  
92 response have been previously investigated (J. Vogt, Ishii-Schrade, Pencharz, & Wolever, 2004; J.  
93 A. Vogt, et al., 2004); albeit not extensively, however effects on appetite have not.

94 Effects of supplementing with inulin-type fructans on appetite are more extensively  
95 investigated; however, results are contradictory due to variable dosages, differing types of inulin-  
96 type fructans and limitations in study design (reviewed by (Darzi, Frost, & Robertson, 2011)).  
97 While a number of studies have investigated effects on appetite of short-chain inulin-type fructans,  
98 also termed oligofructose and fructooligosaccharides (for example (Hess, Birkett, Thomas, &  
99 Slavin, 2011; Parnell & Reimer, 2009; Pedersen, et al., 2013; Peters, Boers, Haddeman, Melnikov,  
100 & Qvyjt, 2009; Verhoef, Meyer, & Westerterp, 2011)), few investigations of long-chain inulin  
101 effects exist (Archer, Johnson, Devereux, & Baxter, 2004; Karalus, et al., 2012; Tarini & Wolever,  
102 2010). The present study therefore aimed to investigate the acute effects of providing L-Rha or  
103 long-chain inulin, following a 6-d run-in, on postprandial appetite and metabolite concentrations  
104 compared to control (no supplement).

105

106 **Methods**107 *Participants*

108 Healthy, non-smoking unrestrained eaters 18-55 y were recruited via e-mail advertisement at the  
109 University of Surrey and attended the Clinical Investigation Unit (CIU) in a fasted state for  
110 screening. Inclusion criteria were BMI between 19-26 kg/m<sup>2</sup>, fasting blood glucose <6.0 mmol/l,  
111 weight stable for at least 3 months, non-smoker and reported habitual alcohol intake ≤20 units.  
112 Exclusion criteria included following a weight reducing diet, presence of gastrointestinal, endocrine  
113 or cardiovascular disorders, history of depression, eating disorders or substance abuse, pregnancy or  
114 lactation, taking regular medication (except birth control medication), and high dietary restraint  
115 (score ≥3.5 on the Dutch Eating Behaviour Questionnaire restraint scale (Van Strien, Frijters,  
116 Bergers, & Defares, 1986)). The study was conducted according to the Declaration of Helsinki and  
117 all procedures involving human participants were approved by the University of Surrey Ethics  
118 Committee (Ref: EC/2008/53/FHMS). Participants gave written informed consent.

119

120 *Study Protocol*

121 This single-blind 3-way randomized crossover study was conducted from September 2008 to May  
122 2009. Participants commenced 1-wk study periods during which L-Rha, long-chain inulin or control  
123 (no supplement) were consumed an order randomly assigned using [www.randomizer.org](http://www.randomizer.org). The  
124 condition randomized to was concealed by providing participants with ready prepared jelly (Jell-O)  
125 and mousse containing the supplement or control (no supplement) to disguise the treatment. Each  
126 study period comprised a 6-d run-in with a study day at the CIU on Day 7 and were separated by a  
127 washout period of ≥1-wk. Prior to commencing the study, all participants who had not previously  
128 participated in an appetite study attended an initial study morning at the CIU to familiarise with the  
129 techniques being used. To control for effects of hormonal variations throughout the menstrual cycle  
130 (Asarian & Geary, 2013), female participants not using birth control medication attended the study

131 day at approximately the same point of the menstrual cycle for each study day during the mid-  
132 follicular phase (between days 8 and 12). Participants were informed that the study aimed to  
133 compare the effects of two fiber supplements compared to a placebo (no fiber) on appetite and  
134 metabolic response.

135

#### 136 *Six day run-in*

137 The supplement dosages used were based on the reported dose used in previous investigations of L-  
138 Rha (J. A. Vogt, Ishii-Schrade, Pencharz, Jones, & Wolever, 2006; J. A. Vogt, et al., 2004), with  
139 the equivalent inulin dose calculated to be matched by pentose/hexose equivalents. The target dose  
140 of 25.5 and 22.4 g/d for L-Rha and inulin was reached by Day 4, increasing from one-third, half and  
141 two-thirds target dose during Days 1-3. The supplement (inulin or L-Rha) or control (no  
142 supplement) was provided within two portions of jelly (Hartleys Sugar Free Jelly) during run-in to  
143 be consumed alongside participants' usual diet. Sugar free jelly was chosen as the vehicle to  
144 provide the supplement, as it did not contribute greatly to the EI, it disguised the supplement or  
145 control, and made it easy for the participant to consume the supplement, as no additional  
146 preparation was required. The jellies were collected by or were dropped off to participants every  
147 few days. Compliance, gastrointestinal symptoms and the taste of the jellies were assessed using a  
148 daily monitoring diary, and a 4-day food diary was completed from Days 3 to 6 using household  
149 measures. Gastrointestinal symptoms (stomach pain, diarrhoea, constipation, belching, flatulence,  
150 nausea, acid regurgitation, heartburn and bloating) were monitored on a five point scale (1: none, 3:  
151 moderate, 5: debilitating), and taste was assessed on a nine point Likert Scale..

152

#### 153 *Study day (Day 7)*

154 Participants arrived at the CIU in a fasted state after consuming a standard low fiber meal the  
155 previous evening and avoiding alcohol and unaccustomed exercise for 24-h. Participants were

156 required to stay in the CIU for the entire study duration and water was provided *ad libitum*. Upon  
157 arrival anthropometric measurements were taken and an intravenous cannula was inserted into an  
158 antecubital vein. Two fasting blood samples were taken 30 min and 5 min before breakfast, and  
159 hydrogen concentrations in expired breath were measured using a Gastrolyser 2 portable hand held  
160 breath hydrogen monitor (Bedfont Scientific Ltd, Rochester, UK). Following each baseline blood  
161 and breath sample appetite was subjectively assessed by 100 mm visual analogue scale (VAS)  
162 questionnaires for fullness, hunger, prospective food consumption, desire to eat meal / snack / sweet  
163 / savoury / salty / fatty and nausea as previously described (Flint, Raben, Blundell, & A., 2000).  
164 Gastrointestinal symptoms (flatulence, diarrhoea, bloating, belch/burp, stomach discomfort, urge to  
165 defacate and heartburn) were also assessed by 100 mm VAS following the first baseline blood  
166 sample.

167 A standard mixed breakfast and lunch (described below) were provided at  $t=0$  min and  
168  $t=180$  min, and consumed within 15 min. A mousse was provided alongside breakfast and lunch  
169 containing two-thirds and one-third of the daily target supplement dose, respectively, or no  
170 supplement (control). The taste of the mousse was rated after each meal using 100mm VAS.  
171 Following each meal blood was sampled every 15 min for the first hour, then half-hourly, VAS  
172 were completed after blood samples to assess appetite half-hourly and to assess gastrointestinal  
173 symptoms hourly. Breath was sampled hourly for the first hour and half-hourly thereafter.

174 The cannula was removed at  $t=420$  min (240 min following lunch) after which participants  
175 were seated in individual booths at  $t=420$  min and served an *ad libitum* homogenous pasta meal in a  
176 quantity exceeding usual portion sizes and instructed to eat until they were “comfortably full”, as  
177 previously conducted by our group (Bodinham, Frost, & Robertson, 2010; Darzi, Frost, &  
178 Robertson, 2012). Participants were free to leave and asked to complete a food diary for the  
179 remainder of the day which, in combination with intake at breakfast, lunch and the *ad libitum* test

180 meal, was used to determine overall 24 h intake. Dietary analysis was performed using WinDiets  
181 Professional Version 2005 (Robert Gordon University, Aberdeen, UK).

182

### 183 *Test products and study day test meals*

184 The supplements used were Orafit Beneo Inulin HP (DKSH Great Britain Limited, Wimbledon,  
185 UK), a high degree of polymerisation inulin (average degree of polymerisation  $\geq 23$ ), and food  
186 grade 98 % L-(+)-Rhamnose Monohydrate (Vitanutrition Ltd, Co Dublin, Ireland). During the  
187 control leg, only the carrier product was consumed.

188 On study days the supplement under investigation was incorporated into a mousse (Angel  
189 Delight, Premier Foods Group, made using semi-skimmed milk), as used previously by our group  
190 (Bodinham, et al., 2010). Two-thirds of the daily dose (16.8g L-Rha or 14.9 g inulin HP) was  
191 provided at breakfast, and one-third (8.4 g L-Rha or 7.5 g inulin HP) was provided at lunch. For  
192 control, the mousse with no added supplement was provided. To enhance compliance, participants  
193 were given a choice of four mousse flavours and were given the same flavour on each study day.

194 The standard mixed breakfast supplied on the study day comprised croissants (66 g) with  
195 strawberry or plum jam (28 g), the supplement-containing mousse and either water (250 g) or sugar-  
196 free cordial (42 g cordial and 208 g water). The same jam and drink was on each study day. The  
197 breakfast supplied 2074 kJ, 10.5 g CHO, 36.4 g protein and 21.1 g fat when made using strawberry  
198 jam and chocolate mousse.

199 The standard study day lunch comprised ham, chicken or cheese sandwiches, crisps, the  
200 supplement-containing mousse and either water or sugar-free cordial. The same sandwich filling  
201 and drink was supplied on each study day. On the first study day participants were provided eight  
202 sandwich quarters and 20 g crisps and were asked to consume at least 4 full sandwich quarters and  
203 as many crisps as they would like. On subsequent study days participants were required to consume  
204 the same number of sandwiches and amount of crisps as consumed on the first study occasion, as

205 previously reported (Weststrate & van Amelsvoort, 1993) and used by our group (Bodinham, et al.,  
206 2010).

207 All food ingredients were weighed to the nearest 1 g except the Angel Delight and non-  
208 digestible carbohydrate supplements which were weighed to the nearest 0.1 g.

209

#### 210 *Ad libitum test meal*

211 The *ad libitum* test meal comprised a homogenous pasta dish made following a standard recipe of  
212 Tesco Fusilli Pasta Twists (400 g dry weight) mixed with Ragu Original Pasta Sauce (500 g), Tesco  
213 Mild Cheddar (100 g) and Tesco Vegetable Oil (30 g). The dish supplied 9750 kJ, 81.5 g protein,  
214 339.1 g carbohydrate, 70.0 g fat and 15.9 g fiber and had a mean weight of  $1520 \pm 53$  g and energy  
215 density of  $6.4 \pm 0.2$  kJ/g. The weight and energy density varied due to differing amounts of water  
216 absorbed by the pasta during cooking which was accounted for when calculating EI. The dish was  
217 weighed before and after serving to determine intake.

218

#### 219 *Biochemistry*

220 Venous blood samples were centrifuged at 1750 g for 10 min and plasma aliquots were stored at -20  
221 °C until analysis. Samples were batch analysed with samples from the same participant in the same  
222 batch to minimise inter-assay variability. Plasma glucose, TG, non-esterified fatty acids (NEFA)  
223 and total and HDL cholesterol concentrations were measured using commercial kits for the ILAB  
224 650 analyzer (Instrumentation Laboratory, Milan, Italy), with an inter-assay CV of <2 %. Plasma  
225 insulin concentrations were analyzed by radioimmunoassay using a commercial kit (Millipore, St.  
226 Charles, Missouri), with an inter- and intra-assay CV <10 %. Serum SCFA were analyzed by gas  
227 chromatography as previously described (Bodinham, et al., 2014; J. A. Vogt, et al., 2004).

228

#### 229 *Insulin sensitivity*

230 Insulin sensitivity parameters were assessed on the study day (Day 7). Fasting insulin sensitivity  
231 was assessed by Homeostasis Assessment Model (HOMA) using the HOMA2 Calculator Version  
232 2.2 (University of Oxford, Oxford, UK) to estimate steady state  $\beta$ -cell function, insulin sensitivity  
233 and insulin resistance from fasting plasma glucose and insulin concentrations as previously  
234 described (Levy, Matthews, & Hermans, 1998; Matthews, et al., 1985; Wallace, Levy, & Matthews,  
235 2004). Postprandial insulin sensitivity was assessed using the minimal model index method as  
236 described by (Caumo, Bergman, & Cobelli, 2000).

237

### 238 *Calculations and statistical analysis*

239 Area under curve (AUC) for postprandial data (appetite and gastrointestinal VAS ratings, plasma  
240 metabolites and SCFA, and breath hydrogen) was calculated by the trapezoidal rule and incremental  
241 AUC (iAUC) was also determined to allow for baseline concentration differences. Statistical  
242 analyses were conducted using SPSS for Windows (version 21, SPSS Inc, Chicago, IL). Normality  
243 was tested using the Kolmogorov-Smirnov test. Differences in the effects of treatment on dietary  
244 intake, fasting breath hydrogen, AUC and iAUC of postprandial data, gastrointestinal symptom  
245 mean daily ratings, HOMA and postprandial insulin sensitivity estimates were investigated by one  
246 way repeated measures ANOVA with post-hoc Bonferonni or Friedman test with post-hoc  
247 Wilcoxon signed ranks test as appropriate. Postprandial data was also analysed by two-way  
248 (treatment x time) repeated measures ANOVA. Data are presented as mean  $\pm$  SD unless otherwise  
249 stated and differences were considered significant at  $p \leq 0.05$  except post-hoc Wilcoxon, where  
250 significance was set at  $p \leq 0.0167$  ( $=0.05/3$ ).

251 The sample size was based on the chronic crossover study of Vogt *et al* in which a  
252 significant increase in serum propionate was found with L-rhamnose supplementation in 11  
253 participants (J. Vogt, et al., 2004). As we wanted to investigate if a rise in serum propionate would  
254 drive effects on appetite, we aimed to recruit at least 11 participants to investigate these

255 mechanisms. A power analysis was performed retrospectively in which we found 13 participants  
256 would have given an 80 % power of detecting a difference in actual food intake of 749 kJ, with a  
257 measured SD of the response to L-rhamnose treatment of 845 kJ. As the actual net effect of the L-  
258 rhamnose ingestion was a reduced food intake of only 150 kJ, with 5/13 participants eating more  
259 following the L-rhamnose than following control, 250 participants would have been required at  
260 80% power in order to achieve statistical significance, which far exceeds other studies of this nature  
261 by several fold. The lack of statistical effect is therefore likely to be due to a lack of biological  
262 effect rather than simply a power issue.

263

264

265 **Results**

266

267 Thirteen participants aged 19-32 y with BMI and DEBQ restraint score ranging from 19.5 to 24.7  
268 kg/m<sup>2</sup> and 1.1 to 3.6 respectively completed the study. Of the eight female participants, seven were  
269 using birth control medication (**Table 1**). Results are presented for the run-in period (days 1 to 6)  
270 and for the study day (day 7).

271

272 *Quantitative appetite assessment*

273 No differences between treatments were found for mean daily energy and macronutrient intake  
274 during the days 3 to 6 of the run-in period, or for 24-h dietary intake on the study day (**Table 2**).  
275 Mean *ad libitum* EI of the homogenous pasta meal served at 420 min on the study day did not differ  
276 between control, inulin or L-Rha (4202 ± 1666, 4089 ± 1680 and 4053 ± 1538 kJ respectively).

277

278 *Qualitative appetite assessment*

279 No treatment or treatment x time effects were found for postprandial VAS appetite ratings on the  
280 study day for hunger (**Figure 1A**), fullness, prospective consumption or desire to eat a snack, and  
281 AUC did not differ between treatments. A treatment x time interaction was found during the  
282 morning (0-180 min) for meal desire (F(12,144) = 2.36, P=0.008) (**Figure 1B**) and during the  
283 afternoon (180-420 min) for the desire to eat sweet (F(16,192) = 1.78, P=0.036), but no difference  
284 between treatments was found by post-hoc analysis.

285

286 *Breath hydrogen concentrations*

287 Significant treatment effects were found for fasting and postprandial AUC breath hydrogen  
288 concentrations ( $\chi^2(2) = 14.3, P=0.001$  and  $\chi^2(2) = 15.9, P<0.001$  respectively) (**Figure 1C**). Fasting  
289 concentrations were significantly higher following inulin than L-Rha (T = 89, r = -0.49, P=0.002)

290 and control ( $T = 82$ ,  $r = -0.41$ ,  $P = 0.011$ ), and the AUC was significantly higher for inulin ( $T = 91$ ,  
291  $r = -0.51$ ,  $P=0.001$ ) and L-Rha ( $T = 83$ ,  $r = -0.42$ ,  $P = 0.009$ ) than control.

292

### 293 *Postprandial metabolites*

294 For postprandial insulin concentrations (**Figure 2A**) treatment effects approaching significance  
295 following breakfast ( $F(2,24) = 3.38$ ,  $P=0.051$ ) and significant treatment x time effects following  
296 breakfast ( $F(16,192) = 1.88$ ,  $P=0.024$ ), lunch ( $F(18,216) = 1.81$ ,  $P=0.026$ ) and during the entire  
297 study day ( $F(34, 408) = 1.71$ ,  $P=0.009$ ) were found. The iAUC following breakfast and during the  
298 entire study day were significantly lower for L-Rha than control ( $T = 9$ ,  $r = -0.41$ ,  $P=0.011$  and  $T =$   
299  $13$ ,  $r = -0.36$ ,  $P=0.023$  respectively) (**Figure 2B**). No treatment or treatment x time interactions for  
300 postprandial glucose or triglycerides and no treatment effects for postprandial NEFA concentrations  
301 were found, although there was a significant treatment x time interaction ( $F(16,192) = 1.72$ ,  
302  $P=0.046$ ) following lunch for NEFA.

303

### 304 *Serum SCFA*

305 No treatment or treatment x time interactions or differences between AUC or iAUC were found  
306 postprandially and no differences between fasting concentrations were found between treatments  
307 for propionate, acetate or butyrate (**Figure 3**).

308

### 309 *Insulin sensitivity*

310 Neither fasting insulin sensitivity, cell function and insulin resistance as estimated by HOMA nor  
311 postprandial insulin sensitivity estimated using the minimal model method were found to differ  
312 between treatments (data not shown).

313

### 314 *Gastrointestinal symptoms*

315 During run-in, the mean daily flatulence ratings during days 4 to 6 (when participants were  
316 consuming the target dose of supplement) were significantly influenced by treatment ( $\chi^2(2) = 8.6$ ,  $P$   
317  $= 0.014$ ). Scores were significantly higher during inulin treatment than control ( $T = 36$ ,  $r = -0.40$ ,  
318  $P=0.012$ ) and L-Rha ( $T = 6$ ,  $r = -0.35$ ,  $P=0.028$ ), with mean scores of  $2.2 \pm 0.9$ ,  $1.4 \pm 0.6$  and  $1.5 \pm$   
319  $0.5$  respectively. On the study day (day 7), the AUC for the urge to defecate was significantly  
320 higher during L-Rha treatment than control ( $T = 84$ ,  $r = -0.43$ ,  $P = 0.007$ ) (data not shown). None of  
321 the other gastrointestinal symptoms was significantly influenced by treatment during run-in or on  
322 the study day.

323

#### 324 *Taste ratings*

325 Mean daily ratings of the taste of the jellies supplied during the run-in period did not differ between  
326 treatments. On the study day, treatment significantly influenced the rated taste of the mousse at  
327 breakfast ( $F(2,24) = 5.49$ ,  $P=0.011$ ), with the lowest VAS score for L-Rha, followed by inulin and  
328 the highest for control ( $58 \pm 21$ ,  $73 \pm 20$  and  $75 \pm 21$  mm respectively), but not at lunch.

329

#### 330 **Discussion**

331 Our findings suggest supplementation with 25.5 g/d L-Rha or 22.4 g/d inulin HP significantly  
332 influence postprandial plasma insulin ( $P=0.009$ ) and plasma NEFA ( $P=0.046$ , following lunch)  
333 responses. The lowest response for both was with L-Rha treatment, previously reported to enhance  
334 serum propionate concentrations (J. A. Vogt, et al., 2004), which was to a significant level for  
335 plasma insulin in comparison to control. This was accompanied by significant increases in  
336 postprandial breath hydrogen concentrations ( $P<0.001$ ) following L-Rha and inulin ingestion in  
337 comparison to control, indicating colonic fermentation occurred, although serum SCFA  
338 concentrations were not increased alongside this. However quantitative and subjective appetite

339 measures were not altered with supplementation, except desire to eat ( $P=0.008$ ) during the morning,  
340 suggestive of a lack of effect of these non-digestible carbohydrate supplements on appetite.

341 The observed suppression of postprandial plasma insulin following L-Rha may have arisen  
342 *via* propionate-mediated activation of FFAR2 and/or FFAR3 in colonic mucosa initiating ileal brake  
343 mechanisms via PYY and GLP-1 production (Karaki, et al., 2006; Karaki, et al., 2008; Tazoe, et al.,  
344 2009). However, unlike previous reports (J. A. Vogt, et al., 2004) we found no impact on serum  
345 propionate concentrations following L-Rha ingestion. Previous studies found L-Rha  
346 supplementation did not alter postprandial insulin or NEFA responses (J. A. Vogt, et al., 2006), in  
347 contrast with the present study, nor postprandial plasma glucose and triglyceride responses (J. A.  
348 Vogt, et al., 2006; J. A. Vogt, et al., 2004), in common with the present study. A limitation of  
349 previous investigations is that glucose was used as control, which could confound interpretation,  
350 and blood samples were collected only hourly, therefore potentially missing postprandial effects of  
351 these metabolites, which change rapidly following a meal. By contrast the present study examined  
352 effects of L-Rha on postprandial metabolites with regular blood sampling and an appropriate  
353 control, explaining why our results reveal novel findings in contrast to those before. The effects we  
354 observed did not appear to be related to insulin sensitivity, as we found no influence of L-Rha or  
355 inulin on estimates of fasting and postprandial insulin sensitivity.

356 To our knowledge this is the first study to investigate effects of L-Rha on appetite. In  
357 contrast to the lack of effect on appetite in the present study, colonic delivery of propionate while  
358 tethered to inulin significantly increased postprandial PYY and GLP-1 responses and reduced EI  
359 acutely and weight gain chronically (Chambers, et al., 2014). Possibly greater colonic  
360 concentrations of propionate were achieved with this novel non-digestible carbohydrate than *via* L-  
361 Rha, arguably a natural source of propionate, in the present study. This is supported by our finding  
362 that serum propionate concentrations were not altered by treatment. Further, in contrast to the  
363 present study, the novel non-digestible carbohydrate did not significantly alter postprandial insulin

364 concentrations (Chambers, et al., 2014). Possibly the effects on insulin following L-Rha  
365 supplementation may not be potentiated by the colonic generation of propionate, but by some other,  
366 as yet unknown, mechanism.

367 Relatively few previous investigations have reported on effects on appetite of long-chain  
368 inulin (Archer, et al., 2004; Karalus, et al., 2012; Tarini & Wolever, 2010) like that used in the  
369 present study, with the majority of studies reporting on supplementation with short-chain inulin-  
370 type fructans (for example (Hess, et al., 2011; Parnell & Reimer, 2009; Pedersen, et al., 2013;  
371 Peters, et al., 2009; Verhoef, et al., 2011)). Long-chain inulin has the benefit of being associated  
372 with less adverse gastrointestinal symptoms than the short-chain counterpart (Bonnema, Kolberg,  
373 Thomas, & Slavin, 2010; Bruhwylter, Carreer, Demanet, & Jacobs, 2009), providing a rationale to  
374 investigate effects on long-chain inulin. Indeed in the present study we found that gastrointestinal  
375 symptoms were not adversely affected by long-chain inulin supplementation, except significantly  
376 higher flatulence scores during run-in, and even then the mean flatulence symptom score with inulin  
377 treatment of  $2.2 \pm 0.9$  (scored on a 9-point Likert Scale) remained relatively low.

378 We did not find any significant effects of supplementation with long-chain inulin on  
379 qualitative or quantitative appetite measures, on postprandial metabolites or on serum SCFA  
380 concentrations. The lack of effect on postprandial serum SCFA in conjunction with a higher breath  
381 hydrogen response has been previously reported following consumption of long-chain inulin  
382 (Fernandes, et al., 2011). In common with our findings, a previous trial in 22 unrestrained females  
383 found acute ingestion of 10 g long-chain inulin within a chocolate crisp bar did not alter qualitative  
384 or quantitative appetite measures in comparison to a control bar (Karus, et al., 2012). By contrast  
385 24 g long-chain inulin used as a fat-replacer in sausage patties significantly reduced 24-h EI in  
386 comparison to a full fat patty in an acute meal challenge in healthy participants ( $n=33$ ), although  
387 rated satiety was not altered, and *ad libitum* EI was not investigated (Archer, et al., 2004). However,  
388 the control had a higher fat and therefore energy content than the inulin preload making

389 interpretation difficult (Archer, et al., 2004). In our study the control was energy and macronutrient  
390 matched to the investigative products. Higher plasma GLP-1 concentrations at 30 min and reduced  
391 ghrelin concentrations at 270 and 360 min were reported following an acute meal challenge in 12  
392 healthy participants with 24 g inulin plus 56 g high-fructose corn syrup in comparison to 56 g high-  
393 fructose corn syrup and 80 g high-fructose corn syrup mixed into a drink (Tarini & Wolever, 2010).  
394 However as effects on GLP-1 were seen so early on postprandially this is suggestive the  
395 observation is not linked to colonic fermentation. Overall the evidence does not strongly support a  
396 role for long-chain inulin in influencing appetite, although studies are limited by participant  
397 numbers.

398 The majority of previous studies investigating metabolic effects of long-chain inulin have  
399 investigated fasting rather than postprandial effects, therefore there is a paucity of comparative  
400 studies. In an acute meal challenge, glucose and insulin responses did not differ significantly  
401 following supplementation with 24 g inulin + 56g high-fructose corn syrup mixed into a drink in  
402 comparison to 56 g and 80 g high-fructose corn syrup in healthy participants (n=12) (Tarini &  
403 Wolever, 2010), agreeing with findings from the present study. Similarly there was no difference in  
404 postprandial glucose and insulin in response to an oral glucose tolerance test in men classified at  
405 higher risk of cardiovascular disease (n=10) following consumption of 15 g inulin per day within  
406 bread rolls for 4 weeks in comparison to nutrient-matched control bread rolls (Tripkovic, Muirhead,  
407 Hart, Frost, & Lodge, 2014).

408 The present study had a number of limitations which need to be acknowledged when  
409 interpreting findings. Ideally the study would have been double blinded, however as there was only  
410 a single investigator (JD) to conduct the study this was not possible. The study included relatively  
411 few participants, however effects on food intake were so negligible retrospective power analysis  
412 suggested 250 participants would be required for statistical significance, which is far greater than in  
413 other studies of this nature. Thus the lack of effect is likely due to a lack of biological effect rather

414 than simply a power issue. We chose to include female participants in order to be more  
415 representative of the general population. However whilst every effort was made to control for  
416 hormonal fluctuations, this will likely have added some variability. Participants were informed of  
417 the broad purpose of the study, which may have impacted behavior, however every effort was made  
418 to maintain a uniform protocol for each condition. We did not assess if participants were aware of  
419 the study hypotheses or if they were able to accurately report any differences in the protocol  
420 according to the condition they were randomised to. As water was supplied *ad libitum* to be more  
421 reflective of free-living conditions, this may have impacted on appetite, an effect we could not  
422 assess as water intake was not measured.

423 In conclusion, the present investigation found neither inulin HP nor L-Rha influenced  
424 appetite, and inulin HP did not influence postprandial metabolic responses. However L-Rha  
425 appeared to inhibit postprandial insulin secretion and also NEFA, possibly *via* propionate-mediated  
426 colonic FFAR2 and/or FFAR3 actions, although serum propionate was not significantly altered and  
427 insulin was not inhibited following ingestion of propionate tethered to inulin. This suggests that the  
428 mechanism underlying our findings may not be mediated by propionate as originally hypothesized,  
429 which warrants further investigation.

430

#### 431 **Conflict of interest disclosure**

432 None to declare.

433

#### 434 **Acknowledgements**

435 We are grateful to all the volunteers who participated in this research and also to Dr Shelagh  
436 Hampton, John Wright and Nicola Muirhead for medical assistance and cannulation expertise. JD  
437 was supported by an educational fellowship from Premier Foods and inulin was supplied by DKSH  
438 Great Britain Limited.

439

440 **Author contributions**

441 JD, MDR and GSF designed the research. JD conducted the research, analysed the data and wrote  
442 the paper. MDR and GSF refined the paper. JS and AC analysed the serum samples for SCFA  
443 concentrations and refined the paper. All authors read and approved the final manuscript.

444

ACCEPTED MANUSCRIPT

445 **References**

- 446 Archer, B. J., Johnson, S. K., Devereux, H. M., & Baxter, A. L. (2004). Effect of fat replacement by  
 447 inulin or lupin-kernel fibre on sausage patty acceptability, post-meal perceptions of satiety  
 448 and food intake in men. *Br J Nutr*, *91*, 591-599.
- 449 Asarian, L., & Geary, N. (2013). Sex differences in the physiology of eating. *Am J Physiol Regul*  
 450 *Integr Comp Physiol.*, *305*, R1215-1267.
- 451 Bodinham, C. L., Frost, G. S., & Robertson, M. D. (2010). Acute ingestion of resistant starch  
 452 reduces food intake in healthy adults. *Br J Nutr*, *103*, 917-922.
- 453 Bodinham, C. L., Smith, L., Thomas, E. L., Bell, J. D., Swann, J. R., Costabile, A., Russell-Jones,  
 454 D., Umpleby, A. M., & Robertson, M. D. (2014). Efficacy of increased resistant starch  
 455 consumption in human type 2 diabetes. *Endocrine Connections*, *3*, 75-84.
- 456 Bonnema, A. L., Kolberg, L. W., Thomas, W., & Slavin, J. L. (2010). Gastrointestinal tolerance of  
 457 chicory inulin products. *J Am Diet Assoc*, *110*, 865-868.
- 458 Brown, A. J., Goldsworthy, S. M., Barnes, A. A., Eilert, M. M., Tcheang, L., Daniels, D., Muir, A.  
 459 I., Wigglesworth, M. J., Kinghorn, I., Fraser, N. J., Pike, N. B., Strum, J. C., Steplewski, K.  
 460 M., Murdock, P. R., Holder, J. C., Marshall, F. H., Szekeres, P. G., Wilson, S., Ignar, D. M.,  
 461 Foord, S. M., Wise, A., & Dowell, S. J. (2003). The Orphan G protein-coupled receptors  
 462 GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *J*  
 463 *Biol Chem.*, *278*, 11312-11319.
- 464 Bruhwiler, J., Carreer, F., Demanet, E., & Jacobs, H. (2009). Digestive tolerance of inulin-type  
 465 fructans: a double-blind, placebo-controlled, cross-over, dose-ranging, randomized study in  
 466 healthy volunteers. *Int J Food Sci Nutr*, *60*, 165-175.
- 467 Burton-Freeman, B. (2000). Dietary fiber and energy regulation. *J Nutr*, *130*, 272S-275S.
- 468 Caumo, A., Bergman, R. N., & Cobelli, C. (2000). Insulin sensitivity from meal tolerance tests in  
 469 normal subjects: a minimal model index. *J Clin Endocrinol Metab.*, *85*, 4396-4402.
- 470 Chambers, E. S., Viardot, A., Psichas, A., Morrison, D. J., Murphy, K. G., Zac-Varghese, S. E.,  
 471 MacDougall, K., Preston, T., Tedford, C., Finlayson, G. S., Blundell, J. E., Bell, J. D.,  
 472 Thomas, E. L., Mt-Isa, S., Ashby, D., Gibson, G. R., Kolida, S., Dhillon, W. S., Bloom, S. R.,  
 473 Morley, W., Clegg, S., & Frost, G. (2014). Effects of targeted delivery of propionate to the  
 474 human colon on appetite regulation, body weight maintenance and adiposity in overweight  
 475 adults. *Gut*, 10.1136/gutjnl-2014-307913.
- 476 Cherbut, C., Ferrier, L., Rozé, C., Anini, Y., Blottière, H., Lecannu, G., & Galmiche, J. P. (1998).  
 477 Short-chain fatty acids modify colonic motility through nerves and polypeptide YY release  
 478 in the rat. *Am J Physiol*, *275*, G1415-1422.
- 479 Covington, D. K., Briscoe, C. A., Brown, A. J., & Jayawickreme, C. K. (2006). The G-protein-  
 480 coupled receptor 40 family (GPR40-GPR43) and its role in nutrient sensing. *Biochem Soc*  
 481 *Trans.*, *34*, 770-773.
- 482 Cucho, G., Cuber, J. C., & Malbert, C. H. (2000). Ileal short-chain fatty acids inhibit gastric motility  
 483 by a humoral pathway. *Am J Physiol Gastrointest Liver Physiol.*, *279*, G925-G930.
- 484 Darzi, J., Frost, G. S., & Robertson, M. D. (2011). Do SCFA have a role in appetite regulation?  
 485 *Proc Nutr Soc*, *70*, 119-128.
- 486 Darzi, J., Frost, G. S., & Robertson, M. D. (2012). Effects of a novel propionate-rich sourdough  
 487 bread on appetite and food intake. *Eur J Clin Nutr.*, *66*, 789-794.
- 488 Du, H., van der A, D. L., Boshuizen, H. C., Forouhi, N. G., Wareham, N. J., Halkjaer, J.,  
 489 Tjønneland, A., Overvad, K., Jakobsen, M. U., Boeing, H., Buijsse, B., Masala, G., Palli, D.,  
 490 Sørensen, T., Saris, W. H., & Feskens, E. J. (2010). Dietary fiber and subsequent changes in  
 491 body weight and waist circumference in European men and women. *Am J Clin Nutr*, *91*,  
 492 329-336.

- 493 Fernandes, J., Rao, A. V., & Wolever, T. M. (2000). Different substrates and methane producing  
494 status affect short-chain fatty acid profiles produced by In vitro fermentation of human  
495 feces. *J Nutr*, *130*, 1932-1936.
- 496 Fernandes, J., Vogt, J., & Wolever, T. M. (2011). Inulin increases short-term markers for colonic  
497 fermentation similarly in healthy and hyperinsulinaemic humans. *Eur J Clin Nutr.*, *65*,  
498 1279-1286.
- 499 Flint, A., Raben, A., Blundell, J. E., & A., A. (2000). Reproducibility, power and validity of visual  
500 analogue scales in assessment of appetite sensations in single test meal studies. *Int J Obes  
501 Relat Metab Disord.*, *24*, 38-48.
- 502 Hess, J. R., Birkett, A. M., Thomas, W., & Slavin, J. L. (2011). Effects of short-chain  
503 fructooligosaccharides on satiety responses in healthy men and women. *Appetite*, *56*, 128-  
504 134.
- 505 Howarth, N. C., Huang, T. T., Roberts, S. B., & McCrory, M. A. (2005). Dietary fiber and fat are  
506 associated with excess weight in young and middle-aged US adults. *J Am Diet Assoc*, *105*,  
507 1365-1372.
- 508 Hussain, S. S., & Bloom, S. R. (2013). The regulation of food intake by the gut-brain axis:  
509 implications for obesity. *Int J Obes (Lond)*. *37*, 625-633.
- 510 Karaki, S., Mitsui, R., Hayashi, H., Kato, I., Sugiya, H., Iwanaga, T., Furness, J. B., & Kuwahara,  
511 A. (2006). Short-chain fatty acid receptor, GPR43, is expressed by enteroendocrine cells and  
512 mucosal mast cells in rat intestine. *Cell Tissue Res.*, *324*, 353-360.
- 513 Karaki, S., Tazoe, H., Hayashi, H., Kashiwabara, H., Tooyama, K., Suzuki, Y., & Kuwahara, A.  
514 (2008). Expression of short-chain fatty acid receptor, GPR43, in the human colon. *J Mol  
515 Histol.*, *39*, 135-142.
- 516 Karalus, M., Clark, M., Greaves, K. A., Thomas, W., Vickers, Z., Kuyama, M., & Slavin, J. (2012).  
517 Fermentable fibers do not affect satiety or food intake by women who do not practice  
518 restrained eating. *J Acad Nutr Diet*, *112*, 1356-1362.
- 519 Kristensen, M., & Jensen, M. G. (2011). Dietary fibres in the regulation of appetite and food intake.  
520 Importance of viscosity. *Appetite*, *56*, 65-70.
- 521 Le Poul, E., Loison, C., Struyf, S., Springael, J. Y., Lannoy, V., Decobecq, M. E., Brezillon, S.,  
522 Dupriez, V., Vassart, G., Van Damme, J., Parmentier, M., & Detheux, M. (2003).  
523 Functional characterization of human receptors for short chain fatty acids and their role in  
524 polymorphonuclear cell activation. *J Biol Chem.*, *278*, 25481-15489.
- 525 Lean, M. E., & Malkova, D. (2015). Altered gut and adipose tissue hormones in overweight and  
526 obese individuals: cause or consequence? *Int J Obes (Lond)*. 10.1038/ijo.2015.1220 [Epub  
527 ahead of print].
- 528 Levy, J. C., Matthews, D. R., & Hermans, M. P. (1998). Correct homeostasis model assessment  
529 (HOMA) evaluation uses the computer program. *Diabetes Care.*, *21*, 2191-2192.
- 530 Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F., & Turner, R. C.  
531 (1985). Homeostasis model assessment: insulin resistance and beta-cell function from  
532 fasting plasma glucose and insulin concentrations in man. *Diabetologia*, *28*, 412-419.
- 533 Parnell, J. A., & Reimer, R. A. (2009). Weight loss during oligofructose supplementation is  
534 associated with decreased ghrelin and increased peptide YY in overweight and obese adults.  
535 *Am J Clin Nutr*, *89*, 1751-1759.
- 536 Pedersen, C., Lefevre, S., Peters, V., Patterson, M., Ghatei, M. A., Morgan, L. M., & Frost, G. S.  
537 (2013). Gut hormone release and appetite regulation in healthy non-obese participants  
538 following oligofructose intake. *Appetite*, *66*, 44-53.
- 539 Peters, H. P., Boers, H. M., Haddeman, E., Melnikov, S. M., & Qvyjt, F. (2009). No effect of added  
540 beta-glucan or of fructooligosaccharide on appetite or energy intake. *Am J Clin Nutr*, *89*, 58-  
541 63.

- 542 Psichas, A., Sleeth, M. L., Murphy, K. G., Brooks, L., Bewick, G. A., Hanyaloglu, A. C., Ghatei,  
543 M. A., Bloom, S. R., & Frost, G. S. (2015). The short chain fatty acid propionate stimulates  
544 GLP-1 and PYY secretion via free fatty acid receptor 2 in rodents. *Int J Obes.*, *39*, 424-429.
- 545 Robertson, M. D., Bickerton, A. S., Dennis, A. L., Vidal, H., & Frayn, K. N. (2005). Insulin-  
546 sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose  
547 tissue metabolism. *Am J Clin Nutr*, *82*, 559-567.
- 548 Tarini, J., & Wolever, T. M. (2010). The fermentable fibre inulin increases postprandial serum  
549 short-chain fatty acids and reduces free-fatty acids and ghrelin in healthy subjects. *Appl*  
550 *Physiol Nutr Metab.*, *35*, 9-16.
- 551 Tazoe, H., Otomo, Y., Karaki, S., Kato, I., Fukami, Y., Terasaki, M., & Kuwahara, A. (2009).  
552 Expression of short-chain fatty acid receptor GPR41 in the human colon. *Biomed Res.*, *30*,  
553 149-156.
- 554 Tripkovic, L., Muirhead, N. C., Hart, K. H., Frost, G. S., & Lodge, J. K. (2014). The effects of a  
555 diet rich in inulin or wheat fibre on markers of cardiovascular disease in overweight male  
556 subjects. *J Hum Nutr Diet.*, 10.1111/jhn.12251.
- 557 Van Strien, T., Frijters, J. E. R., Bergers, G. P. A., & Defares, P. B. (1986). The Dutch eating  
558 behaviour questionnaire for assessment of restrained, emotional and external eating  
559 behaviour. *Int J Eat Disord.*, *5*, 295-315.
- 560 Verhoef, S. P., Meyer, D., & Westerterp, K. R. (2011). Effects of oligofructose on appetite profile,  
561 glucagon-like peptide 1 and peptide YY3-36 concentrations and energy intake. *Br J Nutr.*,  
562 *106*, 1757-1762.
- 563 Vogt, J., Ishii-Schrade, K. B., Pencharz, P. B., & Wolever, M. S. (2004). L-Rhamnose increases  
564 serum propionate after long-term supplementation, but lactulose does not raise serum  
565 acetate. *Am J Clin Nutr*, *80*, 1254-1261.
- 566 Vogt, J. A., Ishii-Schrade, K. B., Pencharz, P. B., Jones, P. J., & Wolever, T. M. (2006). L-  
567 rhamnose and lactulose decrease serum triacylglycerols and their rates of synthesis, but do  
568 not affect serum cholesterol concentrations in men. *J Nutr*, *136*, 2160-2166.
- 569 Vogt, J. A., Pencharz, P. B., & Wolever, M. S. (2004). L-Rhamnose increases serum propionate in  
570 humans. *Am J Clin Nutr*, *80*, 89-94.
- 571 Wallace, T. M., Levy, J. C., & Matthews, D. R. (2004). Use and abuse of HOMA modeling.  
572 *Diabetes Care.*, *27*, 1487-1495.
- 573 Wanders, A. J., van den Borne, J. J., de Graaf, C., Hulshof, T., Jonathan, M. C., Kristensen, M.,  
574 Mars, M., Schols, H. A., & Feskens, E. J. (2011). Effects of dietary fibre on subjective  
575 appetite, energy intake and body weight: a systematic review of randomized controlled  
576 trials. *Obes Rev*, *12*, 724-729.
- 577 Weststrate, J. A., & van Amelsvoort, J. M. (1993). Effects of the amylose content of breakfast and  
578 lunch on postprandial variables in male volunteers. *Am J Clin Nutr*, *58*, 180-186.
- 579 Xiong, Y., Miyamoto, N., Shibata, K., Valasek, M. A., Motoike, T., Kedzierski, R. M., &  
580 Yanagisawa, M. (2004). Short-chain fatty acids stimulate leptin production in adipocytes  
581 through the G protein-coupled receptor GPR41. *Proc Natl Acad Sci USA.*, *101*, 1045-1050.
- 582
- 583

585 **TABLE 1** Baseline characteristics of participants at screening. Data shown as mean  $\pm$  SD and n  
 586 (%).

587

	Overall (n=13)	Male (n=5)	Female (n=8)
Age / y	23 $\pm$ 4	23 $\pm$ 3	23 $\pm$ 4
BMI / kgm <sup>-2</sup>	22.1 $\pm$ 1.6	23.0 $\pm$ 1.5	21.5 $\pm$ 1.5
Waist circumference / cm	75.5 $\pm$ 6.9	82.9 $\pm$ 3.1	70.5 $\pm$ 2.3
Body fat / %	21.9 $\pm$ 6.5	15.5 $\pm$ 3.4	26.0 $\pm$ 4.1
Systolic BP / mmHg	115 $\pm$ 9	120 $\pm$ 7	110 $\pm$ 8
Diastolic BP / mmHg	68 $\pm$ 8	67 $\pm$ 6	69 $\pm$ 9
Fasting blood glucose / mmol/L	4.3 $\pm$ 0.5	4.4 $\pm$ 0.5	4.2 $\pm$ 0.4
DEBQ Restraint Score	2.1 $\pm$ 0.8	2.2 $\pm$ 0.9	2.1 $\pm$ 0.7
Using birth control / n (%)			7 (88 %) <sup>#</sup>

588

589 Abbreviations: DEBQ, Dutch Eating Behaviour Questionnaire Restraint Score, BMI, body mass  
 590 index, BP, blood pressure.

591 <sup>#</sup> 5 using combined pill, 1 using contraceptive implant, 1 using progesterone-only pill

592

593

594 **TABLE 2** (A) Mean daily intake during last 4-d of run-in period, and (B) 24-h intake on study day,  
 595 including all provided foods during study and additional intake for the remainder of the day in 13  
 596 healthy men and women during supplementation with inulin, L-Rhamnose (L-Rha) or control. No  
 597 differences were found between treatments. Analyses carried out by one-way within participants  
 598 repeated measures ANOVA or Friedman's as appropriate. Data shown as mean  $\pm$  SD.  
 599

	(A) Daily intake during run-in			(B) 24-h intake on study day		
	Control	Inulin	L-Rha	Control	Inulin	L-Rha
Energy / kJ	9078 $\pm$	8730 $\pm$	8691 $\pm$	12368 $\pm$	11667 $\pm$	12383 $\pm$
	1857	2232	2131	3128	1930	2656
Energy excluding alcohol / kJ	8822 $\pm$	8266 $\pm$	8138 $\pm$	11964 $\pm$	11210 $\pm$	11500 $\pm$
	1901	1985	1734	2162	1765	1245
Fat / % E	33.5 $\pm$ 5.9	31.9 $\pm$ 5.6	33.0 $\pm$ 4.7	31.6 $\pm$ 3.3	30.3 $\pm$ 2.9	32.9 $\pm$ 2.6
Protein / %E	14.7 $\pm$ 3.1	14.1 $\pm$ 2.5	14.3 $\pm$ 2.4	13.9 $\pm$ 1.8	13.8 $\pm$ 1.3	13.6 $\pm$ 1.5
Carbohydrate / %E	49.0 $\pm$ 5.2	49.3 $\pm$ 7.2	47.4 $\pm$ 7.8	52.4 $\pm$ 3.5	53.2 $\pm$ 3.6	51.1 $\pm$ 3.9
Alcohol / %E	2.9 $\pm$ 4.0	4.7 $\pm$ 5.8	5.3 $\pm$ 7.9	2.0 $\pm$ 6.2	2.8 $\pm$ 5.9	2.3 $\pm$ 3.7

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604

605 **FIGURE LEGENDS**

606

607 **FIGURE 1:** Postprandial appetite ratings for (A) desire to eat a meal, (B) hunger, and (C)  
608 postprandial breath hydrogen in 13 healthy men and women after consuming a mixed breakfast and  
609 lunch that included inulin (■), L-rhamnose (▲) or control (●) following a 6-day run-in period.  
610 Values are mean with error bars representing the SEM.

611

612 **FIGURE 2:** Postprandial plasma insulin (A) timecourse curve and (B) incremental area under  
613 curve in 13 healthy men and women after consuming a mixed breakfast and lunch that included  
614 inulin (■), L-rhamnose (▲) or control (●) following a 6-day run-in period. Values are mean with  
615 error bars representing the SEM.

616

617 **FIGURE 3:** Postprandial serum (A) propionate, (B) acetate and (C) butyrate in 13 healthy men and  
618 women after consuming a mixed breakfast and lunch that included inulin (■), L-rhamnose (▲) or  
619 control (●) following a 6-day run-in period. Values are mean with error bars representing the SEM.

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