

1 **Effects of oat β -glucan consumption at breakfast on *ad libitum* eating, appetite, glycemia,**
2 **insulinemia and GLP-1 concentrations in healthy subjects**

3

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23 **Conflict of Interest:** RES is a part time employee of DSM Nutritional Products, Basel,
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25

26 **Author Contribution:**

27 All authors conceived and designed the study; SMMZ collected the data; SMMZ and RES
28 analysed and interpreted the data, drafted and revised the manuscript; and all authors read and
29 approved the final version of the manuscript.

30

31

32 **Abstract**

33

34 There is evidence that oat β -glucan lowers appetite and *ad libitum* eating; however, not all
35 studies are consistent, and the underpinning mechanisms are not entirely understood. We
36 investigated the effects of 4 g high molecular weight (MW) oat β -glucan on *ad libitum* eating,
37 subjective appetite, glycemia, insulinemia and plasma GLP-1 responses in 33 normal-weight
38 subjects (22 female/11 male, mean age (y): 26.9 ± 1.0 , BMI (kg/m^2): 23.5 ± 0.4). The study
39 followed a randomised double-blind, cross-over design with subjects fed two test breakfasts
40 with and without oat β -glucan followed by an *ad libitum* test meal on two different days. Blood
41 samples and ratings for subjective appetite were collected postprandially at regular time
42 intervals. Oat β -glucan increased feelings of fullness ($p=0.048$) and satiety ($p=0.034$), but did
43 not affect energy and amount eaten at the *ad libitum* test meal. There was a treatment by time
44 interaction for plasma GLP-1, plasma insulin and blood glucose. GLP-1 was significantly
45 reduced at 90 min ($p=0.021$), blood glucose at 30 min ($p=0.008$) and plasma insulin at 30 and
46 60 min ($p=0.002$ and 0.017 , respectively) following the oat β -glucan breakfast when compared
47 with the control breakfast. Four grams of high MW oat β -glucan lowers appetite but not *ad*
48 *libitum* eating and beneficially modulates postprandial glycaemia, it does however, not increase
49 plasma GLP-1 secretion

50

51 **Keywords:** oat β -glucan, energy intake, satiety, GLP-1, glucose, insulin

52 Introduction

53 Obesity is a worldwide epidemic. For example, the proportion of adults in the United Kingdom
54 who are either overweight or obese is around 65%, according to the most recent findings (NCD
55 Risk Factor Collaboration 2017). Not only does obesity significantly increase the risk of Type
56 2 Diabetes Mellitus, it also poses challenges to the management of diabetes after diagnosis (Lin
57 et al., 2015). To combat obesity and its comorbidities from a nutrition perspective, research has
58 focussed on increasing the satiating power of the diet so that individuals feel full with fewer
59 calories consumed (Astrup, 2005).

60

61 A number of studies suggest that high fibre consumption is associated with increased satiation
62 and/or satiety (Wanders et al., 2011; Poutanen et al. 2017), lower body weight (Slavin, 2005),
63 and improved postprandial glycemia (Yuan et al., 2014). There is evidence that increased fibre
64 consumption not only reduces energy density of ingested food (Heaton et al., 1973; Rolls et
65 al., 1999) but exerts a direct inhibitory effect on eating (Wanders et al., 2011; Pereira &
66 Ludwig, 2001; Ibarra et al., 2014). The effect appears to depend on the chemical structure and
67 the physicochemical properties of the fibre type, i.e. fibre viscosity, water-holding capacity and
68 fermentability, rather than on total fibre intake (Wanders et al., 2011). Although the inhibitory
69 effect varies depending on the study population, type, dose and mode of fibre administered as
70 well as the timing of food intake assessment relative to treatment (Zaremba et al., 2017), several
71 studies suggest that fibre viscosity is the dominant characteristic that determines the satiating
72 effect (Clark and Slavin, 2013; Wanders et al., 2011).

73

74 Cereal oat and barley β -glucan consists of high molecular weight polysaccharides that exhibit
75 high viscosity at low concentrations, consumption of which has been shown to effectively blunt
76 glycaemic responses by increasing the viscosity of the contents of the upper gastrointestinal

77 (GI) tract (Wanders et al., 2011), hence, slowing gastric emptying and glucose absorption
78 (Marciani et al. 2001). There is a positive non-linear relationship between molecular weight
79 and viscosity, with the molecular weight of beta-glucan being subject to cultivar variety,
80 growing conditions, processing and storage. The molecular weight of purified oat beta glucan
81 is in the range of 50 – 3000 kDa (Ajithkumar et al., 2005) but is decreased by food preparation
82 such as bread-making or further extrusion that impacts bioactivity of cereal β -glucan (Tosh et
83 al., 2008; Tosh et al., 2010; Wang and Ellis, 2014). The glucose lowering characteristics of β -
84 glucan from oat and barley have been approved by the European Food Safety Authority (EFSA)
85 with a condition of use health claim that 4 g of β -glucan for each 30 g of available carbohydrate
86 be consumed per meal to obtain the claimed effect (EFSA, 2011).

87

88 The evidence that cereal β -glucan lowers appetite and *ad libitum* eating is less conclusive, and
89 the underpinning mechanisms are not entirely understood. While increased oral exposure time,
90 stomach distention and colonic fermentation with increased production of short chain fatty
91 acids (SCFA) may contribute to the satiating effect (Byrne et al., 2015; Wanders et al., 2013,
92 Kristensen and Jensen, 2011), the role of GI hormones with hypothesized roles in appetite
93 (Steinert et a., 2017) remains controversial. Some studies report postprandial reductions in
94 ghrelin (Vitaglione et al., 2009) and increases in cholecystokinin (CCK) and peptide YY (PYY)
95 (Vitaglione et al., 2009; Beck et al., 2009a; Beck et al., 2009b), while others found no effects
96 on PYY (Weickert et al., 2006) and glucagon-like peptide-1 (GLP-1) (Ames et al., 2015a).
97 Moreover, although one study suggested that PYY secretion was increased by more viscous
98 foods (Beck et al., 2009b), another study found that PYY, CCK and GLP-1 responses were
99 lower after a highly viscous oat bran drink compared with an identical test drink with reduced
100 natural viscosity due to β -glucanase treatment (Juvonen et al., 2009).

101

102 In order to better understand the satiating capacity of oat β -glucan and its underpinning
103 mechanisms, we aimed to investigate the effect of 4 g of high MW oat β -glucan incorporated
104 into a breakfast meal on *ad libitum* eating following a 150 min intermeal interval as well as on
105 subjective feelings of appetite, postprandial glycemia, insulinemia and plasma GLP-1, the latter
106 because of its central role in both appetite and glycaemic control. We hypothesised that the oat
107 β -glucan containing breakfast would increase fullness and satiety and decrease *ad libitum*
108 eating more than the isocaloric control breakfast, and that this would be accompanied by
109 increases in plasma GLP-1 and reductions in blood glucose and plasma insulin.
110

111 **Materials and methods**

112 *Subjects*

113 A sample size calculation was conducted for the primary outcome measure of energy intake.
114 Comparable cross-over trials showed a decrease in energy intake at *ad libitum* lunches of
115 between 85 to 170kcal, which varied depending on a number of factors, such as dose of ingested
116 β -glucan, inter-meal intervals, subject characteristics, and test-meal compositions. For
117 example, in a study by Vitaglione *et al.* (2009) a 3 g β -glucan intervention at breakfast reduced
118 *ad libitum* lunch energy intake after 3 hours by 170 kcal; whereas Rebello *et al.* (2016a)
119 reported a reduction of 85 kcal at an *ad libitum* lunch following 2.68 g of oat β -glucan
120 consumption. Using an average standard deviation of 200 kcal and assuming a conservative
121 decrease in energy intake of 100 kcal, the resulting expected effect size was 0.5. The resulting
122 minimum sample size was estimated to be n=32-34 (one sample t-test, $\alpha = 5\%$, power of 80%:
123 nQueryAdvisor 7.0).

124

125 Of the 43 subjects enrolled in the study, there were seven withdrawals due to participant time
126 constraints, and these were not included in the analysis. Of the 36 subjects who completed the
127 study, a further three subjects did not adhere to the study protocol, and therefore, were excluded
128 from data analysis (two subjects did not consume all of the test breakfasts and one subject
129 arrived at both study mornings with elevated fasted blood glucose). Of the remaining 33
130 subjects, 22 were female and 11 were male (age 26.9 ± 1.0 years; weight 68.1 ± 2.0 kg; BMI
131 23.5 ± 0.4 kg/m²; waist circumference 78.0 ± 1.5 cm). Before inclusion in the study, potential
132 subjects were briefed and given the opportunity to ask questions. This was followed by a health
133 assessment, including anthropometric measurements, vital signs, and a general health
134 questionnaire which gave details of food allergies, metabolic disease, weight changes and
135 smoking habits. Eating behaviour was determined using the Dutch Eating Behaviour

136 Questionnaire (van Strein et al., 1986). Restrained eaters were not eligible for participation.
137 Those also excluded were breakfast skippers, postmenopausal, pregnant or lactating females,
138 smokers, dieters or those taking medications which may affect appetite. Prior to enrolment,
139 fasted glucose and haemoglobin measurements were checked to exclude subjects with glucose
140 impairment (>5.6 mmol/L) and/or anaemia (<120 g/L for females and <130 g/L for males).
141 Subjects were required to be willing to allow blood collections and not have food allergies to
142 test meal ingredients (gluten, lactose). Ethical clearance was granted by Queen Margaret
143 University Research Ethics Committee, Edinburgh, where the research was conducted.
144 Participants were recruited from Musselburgh, East Lothian and surrounding areas. Written
145 informed consent was obtained from all subjects. The trial was registered on ClinicaTrials.gov
146 with registration number NCT02637388.

147

148 *Experimental design*

149 The study followed a randomised double-blind, cross-over design with subjects fed two test
150 breakfasts with and without oat β -glucan followed by an *ad libitum* test meal on two different
151 days. There was at least one week between individual study sessions and subjects were required
152 to complete both sessions within 4 weeks. Each subject was scheduled to arrive at the same
153 time and on the same day of the week for each treatment and instructed to abstain from
154 strenuous exercise, alcohol and coffee consumption 24 h prior to treatments. Food diaries
155 completed 24 h before each treatment showed no differences in energy intakes the day before
156 study sessions (1845 ± 95 kcal and 1851 ± 115 kcal prior to control and oat β -glucan breakfast,
157 $p=0.94$ respectively). Each participant arrived fasted (for 10 hours) at the laboratory between
158 8:30am and 10:00am during weekdays.

159

160 On each occasion, an antecubital vein catheter was inserted for blood collection (for plasma
161 insulin and GLP-1) while blood glucose was quantified using a finger-prick blood test. Only
162 subjects with complete data sets/blood samples were included in analysis for GLP-1 and
163 insulin. After taking a fasted blood sample, subjects consumed the test breakfast within 10 min.

164

165 The breakfast consisted of Kellogg's Rice Krispies cereal (Kellogg Company, Manchester,
166 UK), with semi skimmed milk (1.8 % fat) and Greek-style yoghurt (Tesco Groceries,
167 Edinburgh, UK). Four grams of high MW oat β -glucan (from 14.6 g of OatWell Original
168 Powder, DSM Nutritional Products Ltd., Kaiseraugst, Switzerland) was split between the cereal
169 and Greek-style yoghurt to improve palatability of the breakfast. For this, 7.3g OatWell powder
170 was mixed with Greek-style yoghurt and 7.3g OatWell powder was mixed with dry Rice
171 Krispies in a bowl before semi-skimmed milk (150 mL) was poured over the Rice Krispies by
172 the subject immediately before commencing the meal. Tosh *et al.* (2010) previously determined
173 the MW of OatWell™ oat β -glucan to be $2.213 \times 10^6 \text{ g mol}^{-1}$.

174

175 A researcher who was not involved in the study was responsible for assigning the order of the
176 two breakfasts (with and without oat β -glucan) using a random number generator (Microsoft
177 Excel) and supervised the subjects whilst eating. Subjects were required to finish the breakfast
178 within 10 minutes and afterwards to rate the palatability of both breakfasts using a VAS. The
179 breakfasts were matched for their protein, fat and carbohydrate contents: 1) in order
180 to accommodate for the energy content of the oat bran powder, the Greek-style yoghurt was
181 reduced by 10 g in the intervention breakfast. In order to adequately match protein and CHO
182 contents of both breakfasts, 28 mL of PROMILK50 (ready-to-drink vanilla protein milk,
183 MyProtein, Cheshire, UK) was added to the control breakfast (**Table 1**).

184

185 After the breakfast, additional blood samples for measurement of plasma insulin and GLP-1
186 and blood glucose were collected at intervals of 30 min (t=0-90) and VAS were completed at
187 intervals of 15 min (t = 0-150 min). At t = 150 min, each subject was then offered an *ad libitum*
188 test meal and allowed to consume as much food and water as desired until reaching comfortable
189 fullness, for a maximum of 30 min (t = 150–180 min).

190

191 The meal consisted of ham sandwiches, made from white sliced bread (approximately 40 g per
192 slice, Hovis medium soft white, High Wycombe, UK), butter (10 g per slice, Countrylife,
193 Surrey, UK) and sliced cooked ham (approximately 45 g per sandwich, Tesco Groceries,
194 Edinburgh, UK). Nutritional composition of the ham sandwiches was 10.3 g of protein, 11.7 g
195 of fat, 23.9 g of carbohydrate with 243 kcal, all per 100 g. The sandwiches were cut into four
196 equal-sized pieces and served in excess to the subject along with water. Subjects were told to
197 eat until they felt ‘comfortably full’ and to complete a food diary for the remainder of the day
198 (i.e. from when they left the laboratory until when they stopped eating at night). Plate waste
199 and water left over were weighed after the subject left the laboratory.

200

201 ***Measurements:***

202 *Appetite and food intake:* Perceptions of hunger, fullness, desire to eat, satiety and prospective
203 food consumption were measured using validated VAS (Blundell et al., 2010). Each VAS was
204 composed of lines 100 mm in length anchored by the descriptors *not at all* to *extremely*. Food
205 intake at the test meal was calculated from the amount of food (g) eaten at the *ad libitum* meal.
206 Energy intake (kcal) and macronutrient composition (expressed as g and % of energy) were
207 then calculated using Nutritics dietary assessment software (version 4.0, Nutritics Ltd., Dublin,
208 Ireland).

209

210 Blood glucose and plasma insulin and glucagon-like peptide (GLP-1): Finger-prick blood
211 glucose measurements were taken using a sterile lancet device (Accu-Chek Safe T Pro Plus,
212 Roche Diagnostics, UK) and quantified by an Accu-Check Aviva glucometer (Roche
213 Diagnostics, UK). Blood samples for total GLP-1 and insulin measurements were collected in
214 lavender capped BD Vacutainer® plastic K2EDTA tubes (BD Diagnostics, US). The tubes
215 were placed on ice and centrifuged at 3,000 x rpm (Thermo Scientific Heraeus Biofuge Primo
216 R) for 15 min at 4 °C. Plasma samples were aliquoted into cryogenic eppendorf tubes and
217 stored at -85 °C until analysis. Plasma concentrations of total GLP-1 (intra-coefficient of
218 variation (CV): <5 %; inter-CV: <12 %; 1.5 pM sensitivity as per Millipore, CAT# EZGLP1T-
219 36K) and insulin (intra-CV: 4.6–7.0 %; inter-CV: 9.1–11.4 %; 1 µU/mL sensitivity as per
220 Millipore, CAT# EZHI-14K) were measured using ELISA kits (Merck, Germany). A
221 quantitative curve fitting program for immunoassays (MasterPlex 2010), which used a 5
222 Parameter Logistic model equation, was used to compute standard curves and determine insulin
223 and total GLP-1 concentrations.

224

225 Test food viscosity: A constant shear rheometer, Bohin Rheometer C-VOR 150 (Malvern Bohin
226 Instruments), fitted with a 4° /40 mm diameter cone and plate geometry, was used for all
227 viscosity measurements. Measurements were carried out at 37°C to mimic stomach temperature
228 and at shear rates ranging from 0.5×10^{-1} to $1.0 \times 10^2 \text{ s}^{-1}$.

229

230 ***Data and statistical analysis***

231 Statistical analysis was performed using SPSS software (version 23.0; Chicago, IL, USA).
232 Normality of all data were tested using Shapiro–Wilk statistic. Differences in energy intake
233 between the two treatments were assessed using Students paired samples t-test. **Total area**
234 **under the curves** (AUC) for subjective appetite ratings, blood glucose and hormones were

235 calculated using the trapezoidal method. Subjective appetite ratings were analysed using
236 ANCOVA with baseline values used as co-variate (Blundell et al., 2010). Time x treatment
237 effects for blood glucose and hormones were identified using a two-factor analysis of variance
238 (2 factor-ANOVA) with time and treatment (breakfast) as factors. *Post hoc* comparisons,
239 adjusted for multiple comparisons by Bonferroni's correction, were performed where
240 ANOVAs revealed significant effects to identify differences between treatments across
241 timepoints. All tests were two tailed and significance was set at $p < 0.05$. All values are presented
242 as means \pm standard error of the mean (SEM).
243

244 **Results**

245 There was no effect of treatment on *ad libitum* eating. Total intakes at the test meal were 681
246 ± 46 kcal and 267 ± 18 g with the breakfast containing oat β -glucan and 704 ± 51 kcal and 275
247 ± 20 g with the control breakfast ($t(32)=0.875$, $p=0.388$ and $t(32)=0.846$, $p=0.404$,
248 respectively). The oat β -glucan breakfast also did not detectably affect subjects' energy intake
249 for the remainder of the study day when compared with the control breakfast ($t(31)=-1.70$,
250 $p=0.099$, **Table 2**). There was also no difference in water intake at the *ad libitum* meal ($t(32)=-$
251 0.32 , $p=0.751$, **Table 2**).

252

253 There was a significant effect of oat β -glucan breakfast on total AUC of satiety ratings after
254 controlling for baseline AUC, ($F[1,60]=3.07$, $p=0.034$. Total AUC for satiety following oat β -
255 glucan and control breakfast were 7604 ± 459 mm x min and 6516 ± 427 mm x min,
256 respectively. There was also a significant effect on total AUC of fullness ratings after
257 controlling for baseline AUC, ($F[1,60]=2.98$, $p=0.048$. Total AUC for fullness following oat
258 β -glucan and control breakfast were 7563 ± 428 mm x min and 6505 ± 453 mm x min,
259 respectively (**Figure 1**). There was no effect of oat β -glucan on hunger ($p=0.133$), desire to eat
260 ($p=0.098$) or prospective food consumption ($p=0.213$).

261

262 There were no differences in baseline (fasting) values between study days for total GLP-1
263 ($t(20)=-1.76$, $p=0.09$, blood glucose ($t(32)=0.29$, $p=0.771$), or plasma insulin ($t(20)=-1.40$,
264 $p=0.176$, **Figure 2**).

265

266 For plasma GLP-1, Mauchly's Test of Sphericity indicated that the assumption of sphericity
267 had been violated, $X^2(5) = 9.59$, $p=0.03$, and therefore, a Greenhouse Geisser correction was
268 used. There was a treatment x time interaction ($F[2.3,45.3]=6.62$, $p=0.002$) for GLP-1. Plasma

269 GLP-1 concentrations were significantly reduced at 90 min after the oat β -glucan breakfast
270 when compared with the control breakfast (22 ± 9 pmol/L vs. 17 ± 9 pmol/L, $t(20)=2.50$,
271 $p=0.021$, **Figure 2A**). There was no significant difference for GLP-1 AUCs between treatments
272 ($t(20)=0.59$, $p=0.56$, **Table 3**). Only subjects with complete data sets were included in the
273 analysis for plasma GLP-1 (full data, $n=21$).

274

275 For blood glucose, Mauchly's Test of Sphericity indicated that the assumption of sphericity
276 had been violated, $X^2(5) = 16.78$, $p=0.005$, and therefore, a Greenhouse Geisser correction was
277 used. There was a treatment x time interaction ($F[2.3,72.3]=49.13$, $p<0.001$) for blood glucose.
278 Blood glucose was significantly lower at 30 min after the oat β -glucan breakfast when
279 compared with control (6.0 ± 1.0 mmol/L vs. 6.5 ± 0.9 mmol/L ($t(32)=2.81$, $p=0.008$, **Figure**
280 **2B**)). There was no significant difference for blood glucose AUCs between treatments
281 ($t(32)=1.21$, $p=0.235$, **Table 3**).

282

283 For plasma insulin, Mauchly's Test of Sphericity indicated that the assumption of sphericity
284 had been violated, $X^2(5) = 12.2$, $p=0.033$, and therefore a Greenhouse Geisser correction was
285 used. There was a treatment x time interaction ($F[2.08,44.1]=56.98$, $p<0.001$) for plasma
286 insulin. Plasma insulin was significantly lower at 30 and 60 min after the oat β -glucan breakfast
287 when compared with control (32.4 ± 18 μ U vs. 50.3 ± 23.1 μ U, $t(20)=3.63$, $p=0.002$ and 15.8
288 ± 9.2 μ U vs. 24.4 ± 18 μ U, ($t(20)=2.62$, $p=0.017$, respectively, **Figure 2C**). The AUC for
289 insulin over the 90 min period was also significantly lower following the oat β -glucan breakfast
290 when compared with the control breakfast ($t(20)=3.99$, $p=0.001$, **Table 3**). Only subjects with
291 complete data sets were included in the analysis for plasma insulin (full data, $n=21$).

292

293 The viscosity of the different components of the breakfast containing oat β -glucan was
294 considerably greater than that of the control breakfast, with significant differences in viscosity
295 seen at 50s^{-1} ($p<0.001$), a shear rate representative of gastric conditions (**Figure 3**).

296

297 A significant reduction in palatability ratings for the β -glucan breakfast compared to control
298 breakfast was reported (36.4 ± 4.3 mm and 72.9 ± 3.8 mm, respectively, $p<0.001$). There were,
299 however, no significant differences for aftertaste (61.8 ± 4.2 mm, 64.0 ± 4.5 mm, $p=0.71$) or
300 smell (52.3 ± 3.5 mm, 59.4 ± 3.8 mm, $p=0.07$) between breakfasts.

301

302 **Discussion**

303 The evidence for oat and barley β -glucans to lower appetite and *ad libitum* eating is
304 contradictory and the underpinning mechanisms, particularly GI satiation peptide secretion,
305 unclear. Here we investigated the effect of high MW oat β -glucan incorporated into a breakfast
306 meal on *ad libitum* eating, subjective appetite, plasma GLP-1 and insulin as well as blood
307 glucose concentrations in 33 healthy subjects. Based on previous studies, we hypothesized that
308 oat β -glucan increases fullness and/or satiety and reduces *ad libitum* eating associated with **by**
309 **increases in plasma GLP-1 and reductions in blood glucose and plasma insulin.** We found that
310 subjects were more satiated and fuller after consuming the oat β -glucan breakfast **when**
311 **compared to the control; however, in contrast to our hypothesis, this did not translate into a**
312 **reduction in food intake either during the *ad libitum* test meal or for the remainder of the day.**
313 There was also no **increase in plasma GLP-1; in contrast, we found** a small but significant
314 decrease 90 min after the breakfast with oat β -glucan. In line with **the literature, we observed**
315 **significant reductions** in postprandial blood glucose and plasma insulin (Tosh 2012).

316

317 The potency of oat and barley β -glucan to modulate appetite has been reported in several
318 studies, although the effect seems to vary depending on the study design, **subject characteristics**
319 **(e.g. BMI, sex)** and the dose and MW of β -glucan consumed (doses range from 2.2 to 9.4 g
320 with varying or unreported MWs (Vitaglione et al., 2009; Beck et al., 2009a; Lyly et al., 2009;
321 Willis et al., 2009; Clegg & Thondre, 2014)). Fullness was increased in 14 healthy overweight
322 subjects following a 3.82 g dose of β -glucan oat bran cereal with a high MW of 1.378×10^6
323 gmol^{-1} consumed at breakfast in a study conducted by Beck *et al.* (2009a), and similarly by
324 Pentikainen *et al.* (2014) following 4 g of high MW β -glucan incorporated into biscuits and
325 juice consumed at breakfast by normal weight female subjects. In contrast, Beck et al (2009a)
326 reported no effect on subjective appetite ratings following a 5.65 g high MW β -glucan

327 containing breakfast. Our findings are in line with the majority of studies and suggest that oat
328 β -glucan beneficially modulates appetite by increasing fullness and satiety.

329

330 The manufacturing process such as baking, cooking or extrusion (Hu et al., 2010; Ames et al.
331 2015b) and other test food characteristics including food matrix and formats may also affect
332 the satiating capacity of oat β -glucan (Rebello et al., 2014; El Khoury et al., 2012). The
333 physicochemical properties of the matrix in which the fibre is delivered in combination with
334 the gut environment play a critical role in determining the hydration or swelling and water-
335 retention capacity of the fibre (Rebello et al., 2016b). In our study, β -glucan was consumed in
336 a semi-solid food matrix, with yoghurt and cereal with milk used as the vehicle to deliver β -
337 glucan. Other studies that have used a semi-solid food matrix also reported increased satiety
338 and fullness with test meals containing 1.6 to 4 g oat β -glucan (Rebello et al., 2014; Rebello et
339 al., 2016a; Geliebter et al., 2015). Juvonen and colleagues (2011), however, found no effect on
340 subjective appetite following a semi-solid semolina-based pudding that contained 5.1 g oat β -
341 glucan, suggesting that the food matrix alone does not determine oat β -glucan's satiating
342 capacity. More research is, thus, warranted to better understand how a fibre's satiating capacity
343 depends on experimental paradigms, population characteristics and fibre/food format features.

344

345 The beneficial effect on subjective appetite did not translate into a decrease in food intake at
346 the *ad libitum* test meal, which is in line with a number of studies that have reported similar
347 dissociations following oat β -glucan consumption (Beck et al., 2009a; Clark and Slavin, 2013).
348 It is important to note that although appetite VAS are generally sensitive to experimental
349 manipulations and are reproducible, they have failed to predict meal size under a number of
350 conditions (Beck et al., 2009a; Stubbs et al., 2007; Flint et al., 2000). The magnitude of
351 differences in self-reported VAS which precede *ad libitum* eating were investigated recently

352 by Sadoul *et al.* (2014) based on a large number of studies that used a wide range of nutrient
353 preloads. They found that a significant difference in energy intake at lunch was likely to be
354 achieved if the difference in satiety VAS (intervention *vs.* control) immediately before the *ad*
355 *libitum* meal was at least 15–25 mm on a 100 mm scale. In our study, differences in satiety
356 VAS at meal onset 150 min after the preload was only about 10 mm which may possibly
357 explain the lack of effect on *ad libitum* eating. Whether a different inter-meal interval or a
358 higher dose of oat β -glucan or another food matrix may have resulted in significant eating
359 effects should be investigated more comprehensively, for example, by using varying time
360 intervals, doses and formats in the same study. Because satiation depends on both gastric and
361 intestinal nutrient stimulation, and their interactions (Steinert *et al.*, 2017), an optimal dose and
362 timing between preload and *ad libitum* test meal is likely crucial to detect an eating-inhibitory
363 effect. Perhaps the best method may be to have participants select the time of the next meal,
364 this approach has been scarcely explored.

365

366 Several lines of evidence support the hypothesis that increased gastric volume contributes to
367 satiation (Steinert *et al.*, 2017). Viscous fibres absorb large quantities of water and most studies
368 link ingestion of viscous dietary fibres to delayed gastric emptying (Benini *et al.*, 1995;
369 Bergmann *et al.*, 1992; Marciani *et al.*, 2000, de Graaf *et al.*, 2004), which will increase gastric-
370 volume signals. Bergmann *et al.* (1992), for example, found sensations of satiety and hunger
371 highly correlated with gastric emptying rates following consumption of viscous psyllium fibre
372 ($r=0.989$, $p=0.0001$). We did not measure gastric emptying in the current study; however, there
373 are a few studies that report a slowing of gastric emptying with cereal β -glucans under similar
374 conditions (Juntunen *et al.*, 2002; Geliebter *et al.*, 2015; Yu *et al.* 2014).

375

376 It has been speculated that because viscous dietary fibres increase the viscosity of digesta in
377 the small intestine, they prolong small intestinal transit time and **absorption rate of nutrients,**
378 **which** increases contact time with enteroendocrine cells and, thus, peptide release. In addition,
379 high viscous fibres may disrupt proper mixing of food particles and digestive enzymes,
380 resulting in an increased delivery of unabsorbed nutrients into distal parts of the small intestine
381 where the density of GLP-1 and PYY secreting L-cells is highest (Kristensen & Jensen, 2011;
382 Rebello et al. 2016b). Indeed, Beck and colleagues reported that CCK and PYY increases
383 linearly with increasing amounts of oat β -glucan (Beck et al., 2009a; Beck et al., 2009b). For
384 PYY, there was a significant dose response relationship between grams consumed and PYY
385 AUC ($r^2 = 0.994$, $P = 0.003$). The effect was most pronounced with doses of 4 to 6 grams at a
386 late postprandial phase, as a function of both viscosity and concentration (Beck et al., 2009b).
387 Juntunen et al., (2002) demonstrated an increase in post-prandial GLP-1 at 120 and 150 minutes
388 following 5.4 g β -glucan-containing rye bread. In contrast, a study by Ames et al., (2015)
389 reported no effect of barley fibre enriched tortillas on post-prandial GLP-1 secretion, doses of
390 which ranged from 4.5 g to 11.6 g β -glucan. Moreover, when the natural viscosity of a 300 mL
391 beverage containing 30 g oat bran concentrate (including 5.1 g soluble fibre) was reduced by
392 β -glucanase treatment, CCK, PYY and GLP-1 were increased rather than decreased when
393 compared with the high viscous isocaloric control drink (Juvonen et al., 2009). The high-
394 viscosity beverage was still rated as more filling than control, and although there was no
395 difference in *ad libitum* eating this finding suggests that increased viscosity does not favour
396 CCK, PYY and GLP-1 secretion and that oat bran affects appetite independent of GI peptide
397 secretion. Our findings are in line with the latter study. We found a small but significant
398 decrease in plasma GLP-1 at 90 min with oat β -glucan suggesting that (i) high viscous oat β -
399 glucan does not favor activation of enteroendocrine cells and, thus, GLP-1 secretion and (ii)
400 oat β -glucan beneficially modulates appetite independent of increased plasma GLP-1, at least

401 under our conditions. Assuming that oat β -glucan slowed gastric emptying 0-90 min after meal
402 onset, an explanation for the decrease in plasma GLP-1 is provided by studies showing that the
403 secretion of GLP-1 and other GI peptides is dependent on intestinal caloric load, with higher
404 loads resulting in larger responses (Pilichiewicz et al., 2007). We can, however, not exclude an
405 effect on later phase GLP-1 secretion due to increased delivery of unabsorbed nutrients into
406 distal intestinal parts.

407

408 As expected, consumption of β -glucan at breakfast significantly blunted the post-prandial
409 blood glucose and insulin responses in line with a large number of previous studies (Tosh
410 2012). This is most likely due to a delay in gastric emptying and subsequent glucose absorption,
411 although we did not directly assess this. However, we found that the test meal rich in oat β -
412 glucan showed substantially higher viscosity than the control meal, which supports this
413 hypothesis.

414

415 There are a number of limitations that require consideration. We did not measure plasma GLP-
416 1 (or other satiation peptide) concentrations >90 min after the breakfast meal and thus can only
417 speculate about hormone level at *ad libitum* test meal onset. Caution should also be taken when
418 interpreting data for GLP-1 and insulin due to missing data and, thus, smaller sample size.
419 Moreover, variability in energy intakes may have resulted due to menstrual cycle status
420 (Asarian and Geary, 2013), which was not monitored or controlled in female subjects in our
421 study. Finally, palatability ratings of the β -glucan breakfast were lower than the control
422 breakfast, thus, memory or other cognitive effects may have influenced subsequent eating as
423 suggested by some studies (Johnson and Vickers, 1992; Yeomans et al., 2001).

424

425

426 In conclusion, 4 g of high MW oat β -glucan beneficially modulated appetite with increased
427 feelings of fullness and satiety but with no effect on *ad libitum* eating. This is associated with
428 reduced plasma GLP-1 at 90 min, and a significant reduction in blood glucose and plasma
429 insulin.

430

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440

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616

617 **Table 1.** Ingredients, energy and macronutrient composition of the breakfasts

	Control	β-glucan
Ingredients		
Kellogg’s Rice Krispies (g)	30	30
Semi-skimmed milk (1.8% fat) (mL)	150	150
Greek-style yoghurt (g)	90	80
Protein milk (mL)	28	-
OatWell oat bran (g)	-	14.6
Nutrient content		
Total energy (kcal)	319.8	329.1
Fat g (% of total energy)	11.6 (33)	11.3 (31)
Carbohydrate g (% of total energy)	39.7 (50)	39 (47)
Protein g (% of total energy)	13.8 (17)	13.9 (17)
Fibre (g)	0.3	7.9
β-glucan (g)	0	4
Weight (g)	298	274.5

618 Nutritional information was taken from nutrient declarations present on product food labels

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623 **Table 2.** Food consumed at the *ad libitum* test meal and for the remainder of the study day
624 following control and β -glucan breakfasts

	Control	β-glucan	p-value
Energy intake at <i>ad libitum</i> test meal (kcal)	704 \pm 51	681 \pm 46	0.388
Food quantity at <i>ad libitum</i> test meal (g)	275 \pm 20	267 \pm 18	0.404
Water intake at <i>ad libitum</i> test meal (mL)	213 \pm 11	218 \pm 15	0.751
Energy intake for the remainder of study day (subsequent 12 h) (kcal)	886 \pm 91	1094 \pm 120	0.099

625 Data are from n=33 subjects except for food intake for the remainder of study days, where
626 one subject failed to return their food record (n=32). Data are means \pm SEM

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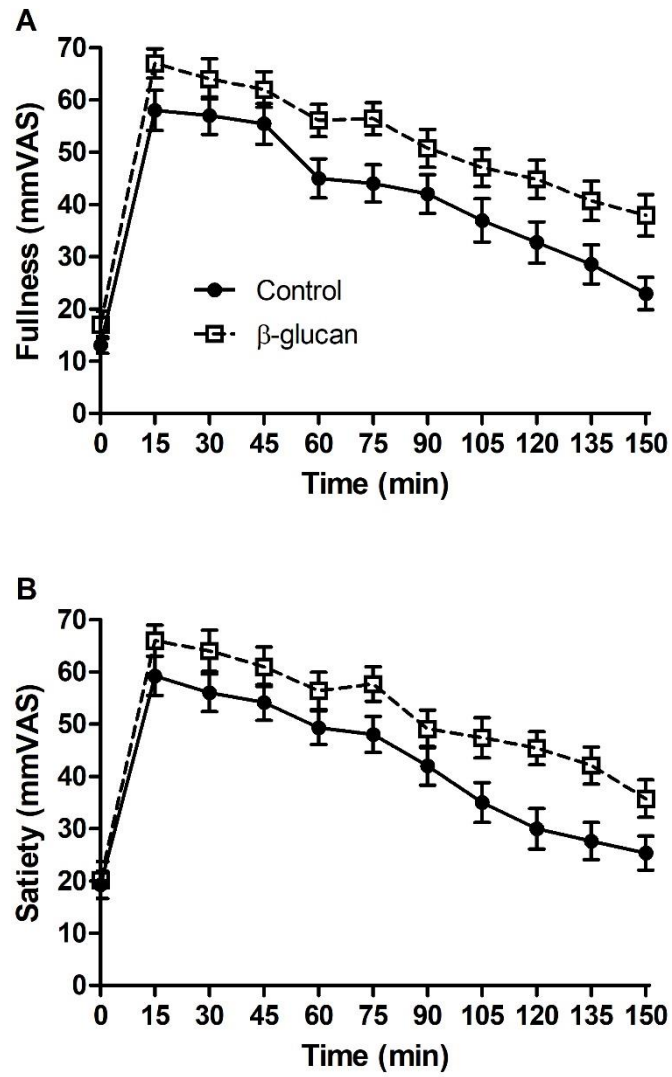
636 **Table 3.** Area under the curves (AUC) for blood glucose and plasma insulin and total GLP-1
637 concentrations following control and β -glucan breakfasts

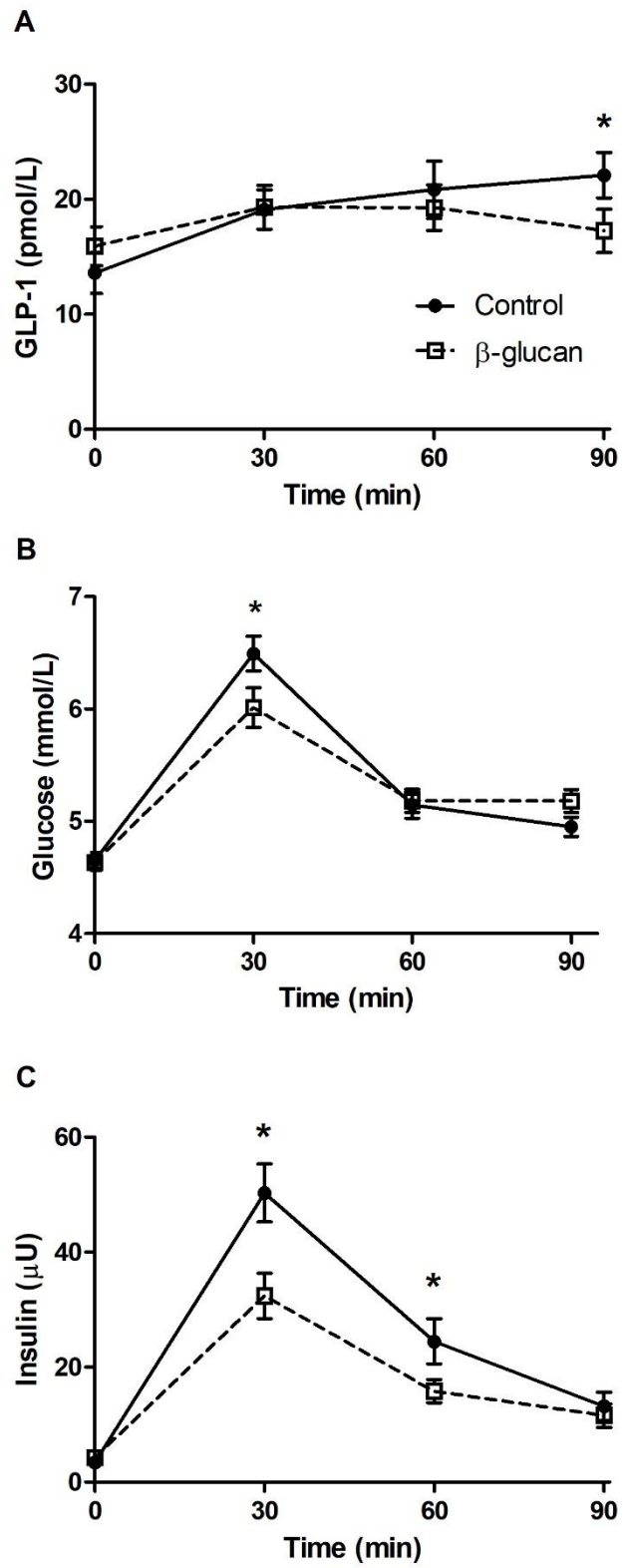
	Control	β-glucan	p-value
Glucose AUC ($mmol \times L^{-1} \times min^{-1}$)	498.2 \pm 47.3	483.0 \pm 49.5	0.235
Insulin AUC ($\mu U \times mL^{-1} \times min^{-1}$)	2491.0 \pm 1211	1682.2 \pm 902.4	0.001
Total GLP-1 AUC ($pmol \times L^{-1} \times min^{-1}$)	1732.7 \pm 713	1654.7 \pm 706.8	0.560

638 Data are from n=33 subjects for blood glucose and n=21 for plasma insulin and total GLP-1.

639 AUC based on 0-90 min data. Data are means \pm SEM

640

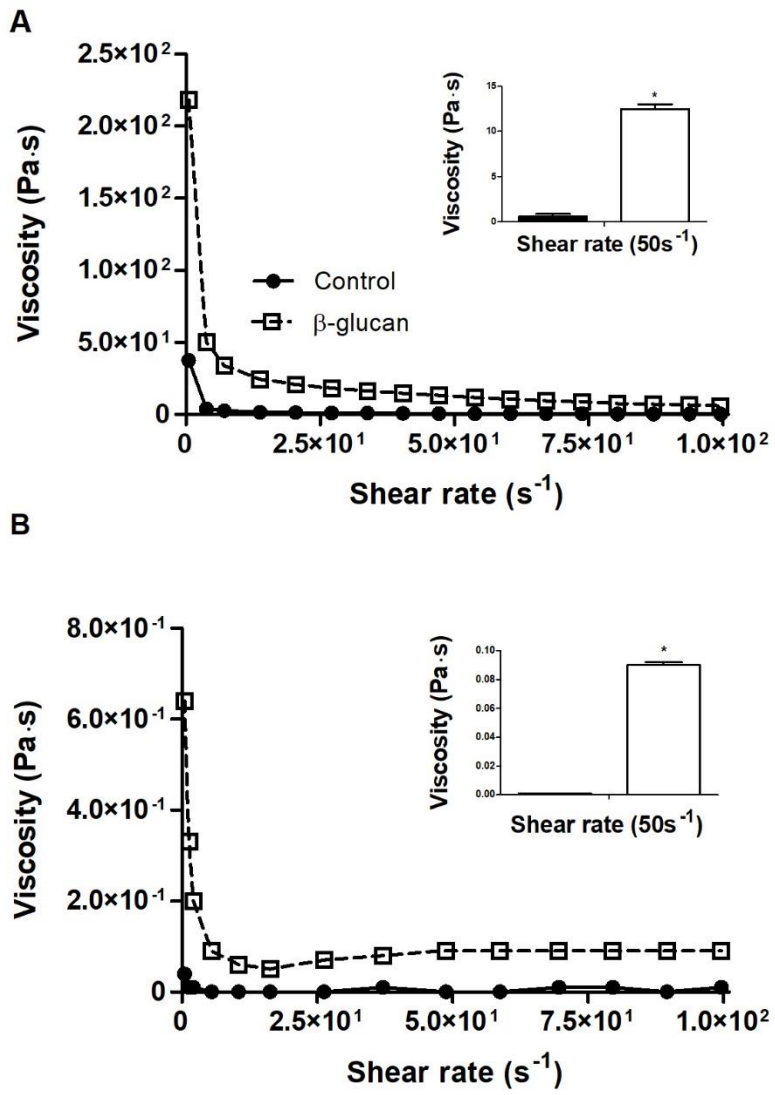




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651 **Figure legends:**

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653 **Figure 1** Visual analogue scales (VAS) for subjective ratings of fullness (A) and satiety (B)
654 during the 150-min postprandial period following control (●) and β-glucan (□) breakfast
655 consumption. Data were analysed by ANCOVA using baseline value as co-variate. Data are
656 means ±SEMs (n=31, two subjects were excluded from data analysis as they misunderstood
657 the VAS questionnaires).

658

659 **Figure 2** Plasma glucagon-like peptide-1 (A), blood glucose (B), and plasma insulin (C)
660 concentrations during the 90-min postprandial period following control and β-glucan breakfast
661 consumption. Data were analysed with two-factor ANOVA, with treatment and time as factors.
662 In case of significant differences, post hoc comparisons, adjusted for multiple comparisons by
663 Bonferroni correction, were performed to determine significant differences between the control
664 (●) and β-glucan (□) breakfasts. *p<0.05. Data are means ±SEMs (A, n=33; B and C, n=21, 12
665 subjects were excluded from the analysis due to incomplete data sets).

666

667 **Figure 3** Viscosity of both, the yoghurt (A) and milk (B) component of the test meal containing
668 high-molecular weight oat β-glucan (□) or control (●) across different shear rates ranging from
669 0.5×10^{-1} to $1.0 \times 10^2 \text{ s}^{-1}$. Inserts depict a shear rate of 50 s^{-1} , representative of gastric conditions.
670 *p<0.05