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Ascophyllum nodosum enriched bread reduces subsequent energy intake with no effect on postprandial glucose and cholesterol in healthy, overweight males.

HALL, Anna, FAIRCLOUGH, Andrew, MAHADEVAN, Kritika and PAXMAN, Jenny

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Abstract: The consumption of seaweed isolates (such as alginate) has been shown to successfully reduce energy intake and modulate glycaemic and cholesterolaemic responses. To date, the effect of adding whole seaweed to bread has not been widely investigated. This study aims to investigate the acceptability of Ascophyllum nodosum enriched bread, and measure its effect on energy intake and nutrient absorption in overweight, healthy males. Results from the acceptability study, (79 untrained sensory panellists) indicated that it is acceptable to incorporate seaweed (Ascophyllum nodosum) into a staple food such as bread when up to 20g are added to a 400g wholemeal loaf. A single blind cross over trial (n=12 males, aged 40.1±12.5 years; BMI 30.8±4.4 kg/m²) was used to compare energy intake and nutrient uptake after a breakfast meal using the enriched bread against the control bread. Consumption of the enriched bread led to a significant reduction (16.4%) in energy intake at a test meal 4 hours after breakfast. Differences between treatment arms for area under the curve, peak values, and time of peak for blood glucose and cholesterol were not significant. Further investigation of potential mechanisms of action is warranted.

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Subject: Submission of original article for Appetite

Dear Sirs,

Please find attached the manuscript '***Ascophyllum nodosum* enriched bread reduces subsequent energy intake with no effect on post-prandial glucose and cholesterol in healthy, overweight males**' submitted for publication as an **original article** in **Appetite**.

Here in we present, for the first time, that energy intake can be significantly reduced following the consumption of *Ascophyllum nodosum* enriched bread compared to a control (standard wholemeal) bread. To date, no research has been conducted on the inclusion of whole seaweed in bread and its effect on energy intake, although some work has been published using seaweed isolates such as alginate (Wolf et al., 2002; Williams et al., 2006; Paxman et al., 2008; Hoad et al., 2004; Mattes et al., 2007). We describe how the consumption of bread enriched with *Ascophyllum nodosum* at breakfast, reduced energy intake at a test meal 4 hours later with no apparent effect on glucose, cholesterol, hunger or fullness. Results from this study suggest that the consumption of whole seaweed may be beneficial in reducing short term energy intake, presenting an attractive option for weight loss or weight maintenance. In light of the rising levels of overweight and obesity, manipulating the satiating capacity of food may prove beneficial in the control of food intake, and potentially therefore, weight regulation. With this in mind, we believe this article will be of significant interest to the wider scientific community, particularly to readers of Appetite.

This research was approved via the appropriate University ethics procedures (reference number CFI/2009/RE06).

This manuscript has been prepared in line with the 'Guide for Authors' published on the journal website. I hereby affirm that the content of this manuscript is original. Furthermore, it has been neither published elsewhere fully or partially in any language nor submitted for publication (fully or

partially) elsewhere simultaneously. I also affirm that the all authors have contributed to, seen and agreed to the submitted version of the manuscript and to the inclusion of their names as co-authors. The authors report no conflict of interest.

Yours faithfully,

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Highlights

We investigate the acceptability of *Ascophyllum nodosum* enriched bread.

We measure the effect of *A. nodosum* enriched bread on markers of appetite.

A. nodosum enriched bread was acceptable up to 20g / 400g wholemeal loaf.

A. nodosum enriched bread reduced energy intake but not nutrient uptake at a meal.

1 *Ascophyllum nodosum* enriched bread reduces subsequent energy intake with no effect on post-
2 prandial glucose and cholesterol in healthy, overweight males.

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8 Key Words: Seaweed, Appetite, Energy Intake, Glycaemia, Lipaemia

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23 **Abstract**

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25 energy intake and modulate glycaemic and cholesterolaemic responses. To date, the effect of
26 adding whole seaweed to bread has not been widely investigated. This study aims to investigate
27 the acceptability of *Ascophyllum nodosum* enriched bread, and measure its effect on energy
28 intake and nutrient absorption in overweight, healthy males. Results from the acceptability
29 study, (79 untrained sensory panellists) indicated that it is acceptable to incorporate seaweed
30 (*Ascophyllum nodosum*) into a staple food such as bread when up to 20g are added to a 400g
31 wholemeal loaf. A single blind cross over trial (n=12 males, aged 40.1±12.5 years; BMI
32 30.8±4.4 kg/m²) was used to compare energy intake and nutrient uptake after a breakfast meal
33 using the enriched bread against the control bread. Consumption of the enriched bread led to a
34 significant reduction (16.4%) in energy intake at a test meal 4 hours after breakfast. Differences
35 between treatment arms for area under the curve, peak values, and time of peak for blood
36 glucose and cholesterol were not significant. Further investigation of potential mechanisms of
37 action is warranted.

38

39 Key Words: Seaweed, Appetite, Energy Intake, Glycaemia, Lipaemia

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47 **Introduction**

48 Obesity is described as an excess accumulation of body fat to the detriment of health leading to
49 an increased risk of mortality (Sørensen, Virtue & Vidal-Puig, 2010). Recent UK data suggest
50 that in 2008, 61 % of adults were overweight or obese, with 24 % classified as obese (NHS
51 Information Centre, 2009). It is evident that the increased prevalence of overweight individuals
52 has been accompanied by a parallel rise in numbers of obese individuals (Foresight, 2007).
53 With an increasing body mass index (BMI), comes an increased risk of the development of type
54 II diabetes mellitus, hypertension, general cardiovascular disease, certain cancers (Kopelman,
55 2007) and poor psychosocial well-being (Dixon, Dixon & O'Brien, 2003). The direct and
56 indirect costs of treating overweight and obesity in England are extensive and are anticipated to
57 rise in parallel with average BMIs (Foresight, 2007a). Obesity is a multifactorial disease
58 (Martinez, 2007) and the aetiological factors involved act both independently and dependently
59 (Haskell *et al.*, 2007). In response to the problem, numerous prophylactic and lifestyle
60 approaches have been developed although the majority appear, in the long term, relatively
61 unsuccessful with only an estimated 20% of individuals deemed "successful" in achieving
62 weight loss (Wing & Phelan, 2005).

63

64 As body weight is determined by long term energy balance, manipulating the satiating capacity
65 of food may prove beneficial in the control of food intake, and potentially therefore, weight
66 regulation. The addition of fibre to the diet may be particularly beneficial in this respect
67 (Slavin, 2005; O'Neil *et al.*, 2010; Birketvedt *et al.*, 2000; Howarth, Saltzman & Roberts, 2001;
68 Lui *et al.*, 2003).

69

70 For centuries, seaweed (a source of dietary fibre), has been a traditional part of the Asian diet
71 (Jiménez-Escrig & Sánchez-Muniz, 2000) however consumption is comparatively low in the

72 UK (Rose *et al.*, 2007) where typically, the only consumption of seaweed is as isolated
73 hydrocolloids used in the food industry as thickening and stabilising ingredients (Brownlee *et*
74 *al.*, 2005). However, it is becoming increasingly well recognised for its nutritional properties.
75 Notably, seaweed contains favourable amounts of a variety of polysaccharides, dietary fibre,
76 minerals (iodine and calcium) and polyphenols (Burtin, 2003; MacArtain *et al.*, 2007).
77 Seaweed isolates (for example alginates) used in appetite research have predominantly yielded
78 encouraging results by decreasing free-living energy intake (Paxman *et al.*, 2008), reducing
79 cholesterol absorption in rats (Kimura *et al.*, 1996; Seal & Mathers, 2001) and postprandial,
80 BMI dependent cholesterolaemia in humans (Paxman *et al.*, 2008a), reducing peak glucose
81 (Williams *et al.*, 2004) and glycaemic response (Wolf *et al.*, 2002), increasing feelings of
82 fullness and decreasing feelings of hunger (Hoad *et al.*, 2004). However, not all studies have
83 shown this modulation of appetite markers. Mattes *et al.*, (2007) found that daily consumption
84 of an alginate enriched breakfast bar had no effect on appetite ratings or energy intake over a 5
85 day period.

86

87 Whilst there is growing evidence to suggest the use of seaweed isolates may be beneficial to
88 health, there appears to be a paucity of evidence surrounding the use of whole seaweed as an
89 ingredient. As consumption of seaweed remains highest in Asian populations most
90 observational studies investigating seaweed ingestion have been conducted in this region, where
91 it has been shown longitudinally to reduce the risk of breast cancer (Yang *et al.*, 2010),
92 osteoporosis (Nakayama *et al.*, 2008), cardiovascular mortality (Shimazu, 2007) as well as type
93 2 diabetes and prediabetes (Lee *et al.*, 2010). To date, no appetite research has been conducted
94 using seaweed as a whole food ingredient. However, as the prevalence of overweight and
95 obesity are rife in the UK it seems appropriate to investigate its appetite modulating potential.

96

97 The aim of this study was to assess the acceptability of seaweed-enriched bread, and to
98 determine its effects on human energy intake, appetite sensations, and postprandial glycaemia
99 and cholesterolaemia.

100

101 **Methods**

102 The study took place in two stages: an acceptability study followed by a satiety study. In each
103 phase participants gave full informed written consent and procedures for both phases were
104 approved by the appropriate local ethics committee (reference number CFI/2009/RE06).

105

106 Study 1: Acceptability Study

107 As palatability can modulate food intake (Robinson *et al.*, 2005; Yeomans *et al.*, 2008), it is
108 important to evaluate the sensory acceptability of test foods (Mattes *et al.*, 2005). In this paper,
109 the terms palatability and sensory acceptability have been used interchangeably similar to some
110 previous studies (Archer *et al.*, 2004; Killinger *et al.*, 2004; Pelletier & Dhanaraj, 2006).

111 Seventy nine untrained sensory panellists aged between 18 and 65 years (40 males, aged 18-65)
112 were recruited to assess the sensory acceptability of 5 samples of wholemeal bread containing 0
113 g (control), 5 g, 10 g, 15 g and 20 g *Ascophyllum nodosum* (Seagreens[®] Ltd, West Sussex, UK)
114 per 400 g loaf (Table 1).

115

116 Bread samples were toasted on each side for 1 minute, cut with a pastry cutter (7.5 cm diameter)
117 to remove crusts and topped with scrambled eggs (prepared as described by McCance and
118 Widdowson in The Composition of Food, Food Standards Agency and Institute of Food
119 Research, 2002). Slice depth was kept constant using an industrial slicer. Samples were
120 randomly coded using 3 digit blinding codes and were presented in a random order. In
121 accordance with standard protocol (Mailgaard, Civille, & Carr, 2006), five sensory attributes

122 (appearance, aroma, taste, texture, aftertaste), as well as overall acceptability were evaluated on
123 touch screen operated visual analogue scales with extremes varying from extremely
124 unacceptable (1) to extremely acceptable (9) using industry standard FIZZ software (Version
125 2.10c, Biosystemes, France). A score of 5 was used as a cut off for lower level acceptability
126 (Mexis *et al.*, 2010). A timed break of 1 minute was enforced between samples, during which
127 panellists consumed water (≤ 200 mL, Brontë Natural Spring water LTD (UK)) and crackers
128 (Carr's Water Biscuit, United Biscuits (UK) LTD) to cleanse their palates. Tests were
129 conducted silently in temperature controlled (22-24°C) individual booths, with standardised
130 'natural' lighting, and positive-air flow. Results were analysed using one-way repeated
131 measures ANOVA and Bonferroni *post-hoc* analyses on SPSS V17.0 (SPSS Inc. Chicago,
132 USA).

133

134 Study 2: Satiety Study

135 12 males, aged between 18 and 65 years (Mean age 40.1 ± 12.5 years) self reported as
136 overweight (BMI ≥ 25 kg/m²) but otherwise healthy were recruited to take part in this study.
137 Consistent with other research in the area of dietary fibre and appetite (Paxman *et al.*, 2008), the
138 following exclusion criteria were applied to the study: individuals suffering from irritable bowel
139 syndrome, inflammatory bowel disease, Cushing's syndrome, dumping syndrome, severe
140 constipation, severe diarrhoea or coeliac disease, type 1 diabetes, food allergies or any serious
141 medical condition. The study had a single blind, cross-over design. A wash out period of 1
142 week was considered appropriate in order to eliminate potential carry over effects.

143

144 Recruitment took place via email, online newsletters, and posters situated in various locations
145 around the University campuses and in community health centres in the local area. The advert
146 was also posted on electronic forums and a social networking site.

147

148 During an initial pre-screen, BMI (previously self-reported) was measured. Height (without
149 shoes) and weight were recorded to the nearest 0.1 cm and 0.1 kg respectively using SECA
150 scales and stadiometer (SECA 709 mechanical column scales with SECA 220 telescopic
151 measuring rod; SECA Birmingham, United Kingdom). Height measurements were made at the
152 point of normal breath inspiration with the head positioned in the Frankfort horizontal plane.
153 Percentage body fat and water were measured using bioelectrical impedance analysis (BodyStat
154 1500; BodyStat Ltd., Isle of Man, British Isles) while the participant was lying in the supine
155 position on non-conducting foam matting in accordance with the manufacturer's guidelines.
156 Participants were asked to complete the Three Factor Eating Questionnaire-R18 (TFEQ-R 18)
157 (Karlsson *et al.*, 2000), an adapted version of the 51-item TFEQ designed by Stunkard &
158 Messick, (1985). The TFEQ-R 18 is a self administered questionnaire used to assess eating
159 restraint, uncontrolled eating and emotional eating. Its validity has been successfully evaluated
160 in both obese (Karlsson *et al.*, 2000) and normal weight populations (Hyland *et al.*, 1989).

161

162 The intervention phase occurred over a period of 3 days. On day 1, participants were required
163 to abstain from physical training activities and alcohol consumption and to fast overnight for 12
164 hours (8pm-8am). At the beginning of day 1, participants started recording a 3 day estimated
165 measures diet diary (guidance was given during the pre-screen session).

166

167 At 8:30 on day 2, anthropometric measurements (as described previously) were taken and
168 baseline capillary blood samples were collected from the finger tip using a single use Accu-
169 chek® Softclix® Pro lancing device (Roche Diagnostics Ltd., West Sussex, UK). 30µL of blood
170 was collected in Microsafe Collection and Dispensing Tubes (Inverness Medical, Cheshire,
171 UK), applied immediately to the sample area of a Reflotron® Cholesterol Test Strip and inserted

172 into the Reflotron[®] dry chemistry analyser. Total blood glucose was measured using a single
173 droplet of capillary blood applied to a OneTouch[®] Ultra[®] Test Strip with FastDrawTM design
174 which was inserted into a OneTouch[®] Ultra[®] Blood Glucose Monitoring System (reference
175 range 1.1 to 33.3 mmol/L; Lifescan Inc., Bucks, UK). Participants were also asked to rate their
176 baseline perceived hunger and fullness, along with 6 other 'distracter ratings' ("how friendly/
177 nauseous/ thirsty/ happy/ energetic/ relaxed do you feel?") on 100 mm visual analogue scales
178 (VAS) with left end points anchored at "not at all" and right end points anchored at
179 "extremely".

180

181 At 09:00 participants were asked to complete a second, identical VAS and consume a breakfast
182 consisting of scrambled eggs on either the *Ascophyllum nodosum* enriched bread (intervention
183 arm; 20g *Ascophyllum nodosum* / 400g loaf), or standard wholemeal bread (control arm) in the
184 specialist feeding facility at the laboratory.

185

186 The feeding facility is temperature controlled (22- 24°C) with standardised 'natural' lighting,
187 and positive-air flow. Participants followed a "silent" protocol in individual booths and were
188 instructed to consume all the food provided. Bread samples were toasted for 1 minute on each
189 side and topped with scrambled eggs, prepared as described by McCance and Widdowson in
190 The Composition of Food (Food Standards Agency and Institute of Food Research, 2002). It is
191 calculated that the bread enriched with 20 g *Ascophyllum nodosum* contained 4.6 g alginate per
192 loaf (1.15 g per serving), compared with the alginate-free control bread. Breads were coded to
193 ensure blinding of conditions and participants were asked to rate the pleasantness of the meal
194 using an electronic VAS on the Sussex Ingestion Pattern Monitor (SIPM version 2.08,
195 University of Sussex).

196

197 Over the subsequent 4 hours, an additional 10 capillary blood samples were taken (09.30, 09.45,
198 10.00, 10.15, 10.30, 10.45, 11.00, 11.15, 11.30, 12.00, 12.30, 13.00) and an equal number of
199 paper-based VAS questionnaires were completed following each blood collection. Blood
200 samples were tested for glucose and cholesterol using procedures described earlier. Participants
201 could drink ≤ 1 litre water *ad libitum* over the course of the morning. Water bottles were
202 weighed prior to distribution and after 13:00 to quantify how much water had been consumed.

203

204 At 13:00 participants returned to the feeding facility to consume an *ad libitum* meal of Don
205 Mario 100% durum wheat semolina penne pasta (Abbey Foods Ltd, Liverpool, UK) with Sacla
206 Italia Vine-ripened Tomato & Mascarpone Stir Through sauce (Fratelli Sacla, S.p.A., Asti,
207 Italy). This test meal was eaten in the feeding facility and Sussex Ingestion Pattern Monitor
208 (SIPM) software was used to covertly weigh how much food was consumed. SIPM was
209 developed from the Universal Eating Monitor and has subsequently been used in many appetite
210 and sensory studies (Bertenshaw, Lluch & Yeomans, 2008; Yeomans *et al.*, 2008; Yeomans,
211 Weinberg & James, 2005; Yeomans *et al.*, 2009). Upon entering the feeding facility,
212 participants answered a series of “mood ratings” which measured how they were feeling and to
213 further distract them from the true purpose of the study. These ratings were the same as those
214 measured on the paper-based VAS utilised earlier. Following this, participants were provided
215 with the test meal and were instructed to “eat until you are comfortably satisfied” (Flint *et al.*,
216 2007). At 100 g intervals, participants were asked to rate the pleasantness of the food, and then
217 to continue eating. When each participant had consumed 300 g of their meal (i.e. reached the
218 “refill weight”) they were asked to call the experimenter who provided a new bowl of food,
219 identical to the previous serving. At the end of the meal, participants were asked to confirm that
220 they had finished eating, and were again asked to complete the mood ratings. Neither the order
221 of in which rating scales were presented, nor the polarity of these scales were randomised
222 however participants were not able to refer to previous ratings (Flint *et al.*, 2000), reducing

223 carryover and/or memory effects. Following the consumption of lunch, participants left the
224 feeding facility, continued with their usual routine, and continued to complete their food diaries.

225

226 During the follow-up stage (day 3), participants continued to record their estimated measures
227 food diary in a free-living environment. They were contacted by a researcher and asked to
228 recall what they had consumed during the 24 hours immediately post intervention. The
229 Automated Multiple Pass Method (AMPM) was used to collect food intake information over a
230 24 hour period, not including food supplements (Raper *et al.*, 2004). These data were used to
231 cross-check the food diaries for accuracy. NetWISP (version 3.0 for Windows, Tinuviel
232 Software, Warrington, UK) was used to analyse all dietary data.

233

234 Data are presented as means \pm standard deviations and graphs were prepared in Microsoft Excel
235 2007. Blood measurements taken from 0-240 minutes allowed area under the curve (AUC) data
236 to be produced using NCSS (Hintze, 2007. NSCC, PASS & GESS. NCSS. Kaysville, Utah).
237 AUC data were also produced for hunger and fullness ratings over the same time period. Paired
238 samples *t*-tests and Pearson correlation coefficients were carried out using SPSS version 17.0
239 (SPSS Inc. Chicago, USA). In all analyses, the accepted alpha level of significance was $p < 0.05$.

240

241 **Results**

242 Acceptability Results

243 79 untrained sensory panellists (40 males) were recruited; all of whom successfully completed
244 the acceptability tests. Importantly, all the breads were rated by panellists as acceptable overall
245 and for each individual sensory attribute (table 2). The control bread was rated significantly
246 higher than the *Ascophyllum nodosum* enriched bread for overall acceptability ($p=0.002$) and for
247 aftertaste ($p=0.003$), and significantly higher than all but the bread enriched with 15 g

248 *Ascophyllum nodosum* for flavour (p=0.008). *Post-hoc* tests showed no significant differences
249 between any of the enriched breads. Interestingly, the bread containing 20 g *Ascophyllum*
250 *nodosum* was considered slightly more acceptable overall than the product containing 5 g
251 *Ascophyllum nodosum* although this did not reach significance.

252

253 As a result of these findings, the bread containing 20 g *Ascophyllum nodosum* per 400 g loaf
254 was selected for use in the satiety study.

255

256 Satiety Results

257 12 males were recruited for the satiety study (Age 40.1±12.5 years; BMI 30.8±4.4 kg/m²). 1
258 participant was excluded from the dietary analysis due to incomplete diet diaries, and a different
259 participant was excluded from cholesterol analyses as fasted blood results indicated
260 hypercholesterolaemia (>6.5 mmol/L, Musial *et al.*, 2001; Engbers, van Poppel, & van
261 Mechelan, 2007).

262 Energy intake at the test meal following the ingestion of *Ascophyllum nodosum* enriched bread
263 was 747.7 kJ (178.7 kcal), 16.4% lower (p=0.006) than energy intake following the
264 consumption of the control bread (Mean = 3825.0 ± 1590.6 kJ (914.2 ± 380.2 kcal) and 4572.7
265 ± 1927.5 kJ (1092.9 ± 460.7 kcal) respectively).

266

267 A = *Ascophyllum nodosum* enriched bread; C = control bread; Test meal = *ad libitum* lunchtime
268 feed; Post test meal = 24 hour energy intake after test meal (free living environment); Total =
269 test meal + post test meal

270

271 During the 24 hour, free living period that occurred after participants had left the feeding facility
272 (referred to in figure 1 as "post test meal" energy intake was lower in the intervention arm of the
273 trial compared to the control arm (8974.7 ± 3365.2 kJ (2145.0 ± 804.3 kcal) and $10303.1 \pm$
274 2356.8 kJ (2462.5 ± 563.3 kcal) respectively) although this difference of 1326.3 kJ (317 kcal)
275 was not significant ($p=0.133$).

276

277 Total energy intake (test meal energy intake + post test meal energy intake) was significantly
278 lower (2117.5 kJ (506.1 kcal); $p=0.007$) following the consumption of SG bread ($12914.3 \pm$
279 4428.3 kJ (3086.6 ± 1058.4 kcal)) compared to the control bread (16538.1 ± 3307.5 kJ
280 (3592.7 ± 790.5 kcal)).

281

282 Differences between treatment arms for AUC, peak values, and time of peak for blood glucose
283 and cholesterol and for hunger and fullness were not significant, although the time at which
284 postprandial peak hunger was reached was considerably delayed following consumption of the
285 *Ascophyllum nodosum* bread compared to the control bread reached (191.3 ± 94.2 v. $115.0 \pm$
286 120.6 minutes; $p=0.055$).

287

288 The pleasantness of the *Ascophyllum nodosum* bread was not significantly different to the
289 control bread suggesting participants were successfully blinded to each treatment. There was no
290 significant difference in the amount of water consumed between meals on each arm of the trial.

291

292 **Discussion**

293 *Ascophyllum nodosum* enriched bread is acceptable

294 Results from the acceptability tests show that 20 g *Ascophyllum nodosum* can be successfully
295 incorporated into a 400 g wholemeal loaf whilst maintaining acceptability. This is encouraging
296 for the food industry, particularly the bakery sector who may wish to incorporate *Ascophyllum*
297 *nodosum* not only as a potentially satiating ingredient, but also as a salt replacer, anti-staling
298 and antimicrobial agent.

299 An analysis of the bread using a combination of the SIGMA and Fibertech methods showed the
300 bread enriched with 20 g *Ascophyllum nodosum* (17.8 g/100 g) contained 4.5 g more dietary
301 fibre/100 g than the control bread (13.3 g/100 g). Thus all samples were classified as high fibre
302 foods. Traditionally, high fibre foods tend to be solid (Slavin & Green, 2007) and have low
303 level palatability, making them less organoleptically appealing than high energy dense
304 alternatives (Burton-Freeman, 2000). However Gomez and colleagues (2003) suggest two
305 reasons for adding dietary fibre to bakery products: firstly, to increase the overall fibre content
306 of the product, and secondly, to decrease the energy density. Dietary fibres have been
307 successfully added to a wide variety of food matrices including bakery products, cereals, pasta
308 noodles and a variety of beverages (Collar *et al.*, 2006; Collar *et al.*, 2007; Santos *et al.*, 2008;
309 Rosell *et al.*, 2006; Brennan & Cleary, 2007, Hall *et al.*, 2010). The addition of lupin kernel
310 fibre to white bread and pasta resulted in no significant differences in overall acceptability
311 (n=44) (Clarke & Johnson, 2002), neither did the addition of carob fibre, inulin or pea fibre to
312 bread (Wang *et al.*, 2002). Similarly, Gomez and colleagues (2003) found the addition of 2 %
313 orange, pea or wheat fibre to flour enhanced textural shelf life and showed no deterioration in
314 palatability. Indeed, Angioloni & Collar (2011) found an increase in overall acceptability after
315 the addition of a binary mixture of cellulose and either fructo-oligosaccharide or gluco-
316 oligosaccharide. This, coupled with maintaining shelf life for 10 days, suggests that dietary
317 fibre can be successfully added to bread from both a physical and sensorial perspective.

318

319 It is easier to maintain the acceptability of fibre enriched foods when fibrous isolates are added
320 to products rather than wholefood ingredients. Previous studies have incorporated sodium
321 alginate into beverages (Paxman *et al.*, 2008; Paxman *et al.*, 2008a; Wolf *et al.*, 2002; Hoad *et*
322 *al.*, 2004) and a few have developed food products such as crispy bars (Williams *et al.*, 2004)
323 and breakfast bars (Mattes *et al.*, 2007). The amounts of alginate used in these studies (1.6 g
324 and 1.1 g respectively) are comparable to those found in the bread containing *Ascophyllum*
325 *nodosum*. Most authors (Paxman *et al.*, 2008; Paxman *et al.*, 2008a; Wolf *et al.*, 2002; Hoad *et*
326 *al.*, 2004; Williams *et al.*, 2004), but not all (Mattes *et al.*, 2007) have reported beneficial health
327 effects at these levels. Alginate (and separately, other hydrocolloids such as carageenan,
328 xanthan, and hydroxypropylmethylcellulose (HPMC)) have also been added to bread (0.1 % and
329 0.5 %), showing a reduced loss of moisture and dehydration rate due to their ability to retain
330 water. A trained sensory panel (n=10) scored all samples as acceptable, with the highest scores
331 from the alginate (0.5 %) and HPMC enriched (0.1 %) samples (Guarda *et al.*, 2004).

332

333 Whilst the addition of marine extracts (such as alginate) to bread and bakery products has been
334 successful, to date, the effect of adding whole seaweed to bread has not been widely
335 investigated. Prabhasankar, Ganesan and Bhaskar (2009) added brown seaweed (*Sargassum*
336 *marginatum*) to pasta, enhancing biofunctional characteristics, and Prabhasankar *et al.* (2009)
337 showed that the addition of *Undaria pinnatifida* (up to 10 %) was sensorially acceptable with no
338 significant differences between the control (0 %) and 5 % breads, or between the 5 % and 10 %
339 breads. Acceptability was significantly reduced at levels greater than 10 %. No studies
340 investigating the potentially satiating effects of whole seaweed in bread have been published to
341 date. The successful incorporation of whole seaweed (*Ascophyllum nodosum*) into bread meant
342 that the bread containing the highest amount of seaweed (20 g/400 g loaf) could be used in our
343 subsequent satiety study.

344

345 *Ascophyllum nodosum* enriched bread decreases energy intake at a test meal

346 This study has shown for the first time that *Ascophyllum nodosum* enriched bread, consumed
347 within a composite breakfast meal of scrambled eggs on toast, can significantly lower energy
348 intake at a meal served 4 hours later in overweight but otherwise healthy males. Mean energy
349 intake was significantly lower (747.7 kJ (178.7 kcal); $p=0.006$) following the consumption of
350 the *Ascophyllum nodosum* enriched bread compared to the control bread. Other laboratory
351 based studies have found a reduction in energy intake following the consumption of lupin fibre
352 enriched bread (Lee *et al.*, 2006) and an alginate-pectin combination fibre (Pelkman *et al.*,
353 2007) reduced energy intake by approximately 10 % ($p=0.11$). However, similarly to the
354 current study, these were acute, laboratory based feeding studies which do not emulate free
355 living situations well. The current study was small and well controlled, with high internal, yet
356 low external validity. While laboratory based studies such as this enable rigorous control and
357 considerable precision while allowing little influence from external factors, they are too short to
358 make definitive statements about long term energy balance (Stubbs *et al.*, 1998). These acute
359 feeding studies are suitable precursors to longer term, free living experiments although there
360 appear to be relatively few examining the relationship between fibre and energy intake. Paxman
361 *et al.* (2008) report a daily energy deficit of 135 kcal in adults ($n=68$) while consuming an
362 alginate based beverage (1.5 g alginate) for 7 days. Similarly, Cani and colleagues (2006)
363 report a daily energy intake reduction of 120 kcal with the consumption of 8 g oligofructose a
364 day in a small pilot study, and Pasman *et al.* (1997) fed large amounts of guar gum (40 g/day) to
365 17 participants, reporting a substantial daily energy deficit of 310 kcal/ day. No previous acute
366 laboratory based studies, or long term, free living studies have examined the relationship
367 between whole seaweed and appetite. A free living study is warranted; a daily energy deficit of
368 ~100 kcal may help prevent weight gain (Hill *et al.*, 2003; Lean, Lara & Hill *et al.*, 2006), and
369 whilst we have shown this to be eminently achievable in a laboratory setting, the application of
370 these findings to the general, free-living population is limited.

371

372 Total energy intake (energy intake from test meal combined with 24 hour energy intake) was
373 significantly lower (2117.5 kJ (506.1 kcal); $p=0.007$) following the treatment compared to the
374 control bread. A habitual energy reduction of ~500 kcal/ day may be beneficial in long term
375 sustained weight loss (Astrup, 1999) which may reduce the risk of type II diabetes mellitus
376 (Moore *et al.*, 2000) and hypertension in overweight and obese individuals (Moore *et al.*, 2005).

377

378 *Ascophyllum nodosum* enriched bread has no effect on nutrient uptake

379 There were no significant differences in AUC glucose or cholesterol following the consumption
380 of the *Ascophyllum nodosum* enriched bread compared to the control. Peak glucose values of
381 6.9mmol/l were reached at 75 minutes for both treatments and there were no significant
382 differences in cholesterol levels at any time point throughout the intervention. In a small (n=14)
383 yet well controlled pilot study, Paxman *et al.* (2008a) showed that compared to a control
384 (containing no alginate), the consumption of a beverage containing 1.5 g sodium alginate
385 significantly ameliorated the increased glucose and cholesterol uptake found in individuals with
386 a higher body fat percentages compared to those with lower body fat percentages. Wolf *et al.*
387 (2002) (n=30) also added alginate (3.6 g) to a beverage and while no difference was seen in
388 peak glucose, a significant ($p<0.01$) decrease in AUC glucose was apparent when compared to
389 the control. Torsdottir *et al.* (1991) saw a reduced rise in glucose ($p<0.02$) and a slower rate of
390 gastric emptying ($p<0.05$) in 7 diabetic males following the consumption of 5 g sodium alginate
391 compared to a control. Each of these studies used sodium alginate, a seaweed isolate, and
392 suggested that the modulated glycaemic response was due to gelation of alginate causing a
393 slower rate of gastric emptying and possible nutrient encapsulation. One study used whole red
394 seaweed (Nori) in a capsule form (3 g) and measured the postprandial glucose response to white
395 bread consumed 15 minutes later. The authors concluded that Nori seaweed significantly
396 ($p\leq 0.05$) reduced AUC glucose, and again, postulate that delayed gastric emptying was the

397 mechanism of action (Gõni *et al.*, 2000). Previous studies have described how the inclusion of
398 dietary fibre, may reduce blood cholesterol levels, and various mechanisms have been described
399 (Braaten *et al.*, 1994; Sola *et al.*, 2010; Brown *et al.*, 1999; Ripsin *et al.*, 1992; Gunness,
400 Flanagan & Gidley, 2008; Jeminez-Escrig & Sanchez-Muniz, 2000; van Horn *et al.*, 1991;
401 Behall *et al.*, 2004). However it is evident that these benefits did not occur in the present study.
402 Nutrient uptake in the current study was neither slowed nor reduced, suggesting neither gelation
403 nor nutrient encapsulation occurred. A more likely mechanism here is that the seaweed acted as
404 a bulking agent, increasing gastric stretch to a greater extent than standard wholemeal bread. It
405 is also possible that an altered gut peptide response mediated enhanced satiety or brought about
406 premature satiation at the subsequent test meal. The mechanism(s) of action for the observed
407 effects warrant further investigation.

408

409 The discordance between the nutrient uptake findings from the present study and others in the
410 published literature base may be explained by the small amount of alginate present in the
411 *Ascophyllum nodosum* enriched bread consumed (participants in the present study consumed
412 100g of bread, containing an estimated 1.15 g of alginate). This amount is not dissimilar to that
413 used by Mattes *et al.* (2007) who incorporated 1.1 g sodium alginate into a breakfast bar and
414 suggested that the lack of effect of the product on appetite ratings and energy intake over 5 days
415 was due to the low amounts of alginate used, which lead to poor gelation in the stomach. It is
416 also possible that in this study, alginate was entrapped within the seaweed particles. Amounts
417 of alginate in the current study are estimates based on the nutritional profile of *Ascophyllum*
418 *nodosum*, and it is unlikely that intra-gastric gelation occurred.

419

420 *Ascophyllum nodosum* enriched bread does not alter hunger and fullness ratings

421 There were no significant differences for total AUC hunger or hunger at any time point
422 throughout the intervention between the *Ascophyllum nodosum* enriched bread and control
423 bread. Interestingly, peak hunger was reached over 1 hour (76 minutes) later after the
424 consumption of the enriched bread v control with borderline significance (p=0.055). This delay
425 in peak hunger could potentially have contributed to the reduced energy consumed at the test
426 meal. Fullness was not significantly affected at any time point in the current study.

427

428 *Compliance*

429 While the sample size was small, the study was well controlled. Compliance to the protocol
430 was high; one participant consumed a small amount of alcohol (5.3 % of total energy intake) at
431 lunchtime on day 1, and a different participant took part in training type activities on the
432 morning of day 1. It is unlikely that these activities had an effect on the overall outcome of the
433 study. As instructed, all participants consumed the breakfast provided in its entirety.
434 Participants were blind to the treatment they received, and did not report any significant
435 differences in the pleasantness, or other flavour attributes of the bread suggesting that they were
436 unaware of which treatment they received. From the debrief session it became apparent that
437 participants were unaware of the weighing scales concealed within the feeding facility, ensuring
438 the food consumed during the test meal was covertly weighed.

439

440 In conclusion, this study has shown for the first time that the incorporation of *Ascophyllum*
441 *nodosum* into bread significantly reduces subsequent energy intake both at a test meal and
442 beyond (test meal + 24 hour period post intervention). However no significant differences were
443 seen in AUC glycaemia or cholesterolaemia which suggests that neither delayed gastric
444 emptying nor nutrient encapsulation occurred. There were also no significant differences in
445 AUC hunger or fullness. Further investigation of potential mechanisms of action is warranted.

446

447 This study was an acute feeding trial. Incorporating *Ascophyllum nodosum* into a long term,
448 appropriately powered, free living intervention study involving the substitution of “normal”
449 bread for *Ascophyllum nodosum* bread, would help to establish the potential for seaweed
450 enriched bread to reduce habitual energy intake longitudinally with potential to favourably
451 affect BMI or body composition.

452

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457

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745 **Table 1: Ingredients in the control and enriched bread**

	Control bread	<i>Ascophyllum nodosum</i> enriched bread
Wholemeal flour	280 g	280 g
Water	160 g	160 g
<i>Ascophyllum nodosum</i>	0 g	5, 10, 15, 20 g
Sugar	6 g	6 g
Butter	15 g	15 g

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758 **Table 2: Sensory characteristics of bread containing *Ascophyllum nodosum***

Amount of	0	5	10	15	20					
<i>Ascophyllum nodosum</i>										
per 400 g loaf (g)										
Estimated amount of	0	1.15	2.3	3.45	4.6					
alginate per 400 g loaf										
(g)										
	M	SD	M	SD	M	SD	M	SD	M	SD
Appearance	6.42 ^a	1.80	6.46 ^a	1.58	6.41 ^a	1.38	6.58 ^a	1.38	6.45 ^a	1.39
Aroma	6.38 ^a	1.55	6.14 ^a	1.45	6.06 ^a	1.53	6.30 ^a	1.55	6.09 ^a	1.44
Flavour [*]	6.31 ^b	1.83	5.56 ^a	1.74	5.50 ^a	1.74	5.67 ^{ab}	1.65	5.52 ^a	1.75
Aftertaste [‡]	6.34 ^b	1.67	5.58 ^a	1.59	5.63 ^a	1.59	5.70 ^a	1.50	5.54 ^a	1.70
Texture	6.44 ^a	1.80	5.94 ^a	1.62	6.14 ^a	1.62	5.92 ^a	1.72	6.00 ^a	1.71
Overall Acceptability [§]	6.60^b	1.68	5.79^a	1.52	5.95^a	1.52	5.93^a	1.59	5.86^a	1.64

759 Data are presented as means and standard deviations. Different letters in the same row

760 denote means that are significantly different to one another (* p =.008, ‡ p=.003,

761 §p=.002). Cut off for overall acceptability was 5 (Mexis et al., 2010).

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768 **Figure Legends**

769 Figure 1: Energy intake at various time points during and post-intervention

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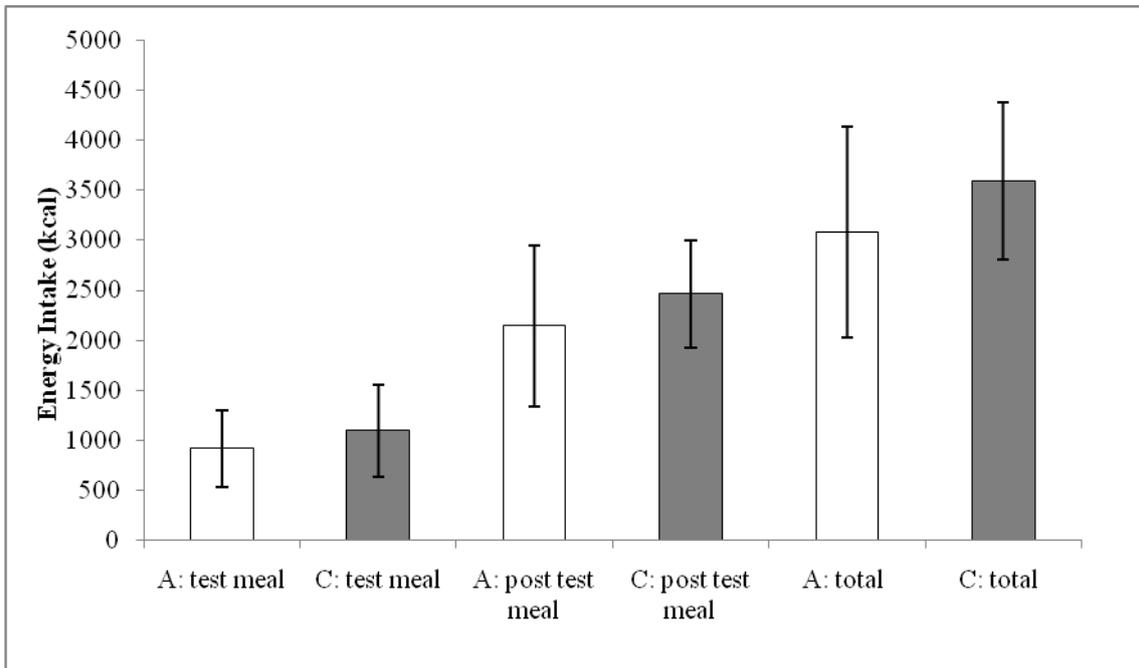
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790 **Figure 1**



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792 A = *Ascophyllum nodosum* enriched bread; C = control bread; Test meal = *ad libitum* lunchtime

793 feed; Post test meal = 24 hour energy intake after test meal (free living environment); Total =

794 test meal + post test meal

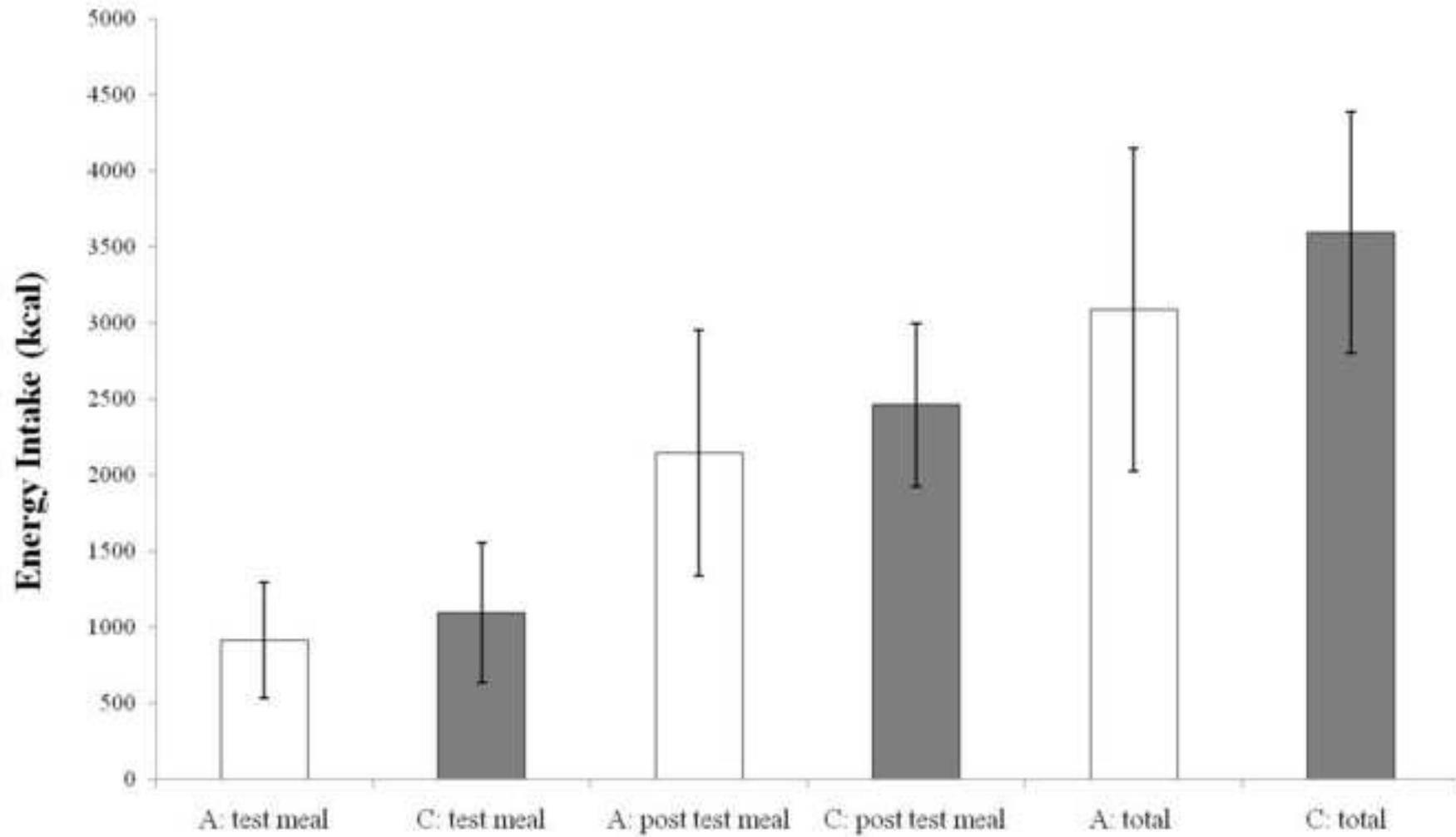


Figure 1